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A REVIEW OF THE FEEDING AND GROWTH OF POSTLARVAL ABALONE

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ABSTRACT Survival and growth of abalone postlarvae are greatly influenced by food quality and quantity. Abalone larvae settle on coralline algae, some diatoms films, and conspecific mucous trails. These substrata provide a wide range of potential food sources, which are used in different ways as postlarvae develop. We identify three major transitions in feeding and growth: (1) at metamorphosis, a shift from lecithotrophy/dissolved organic matter (DOM) absorption to particle feeding, with an overlap in nutrition sources; (2) at about 600–800 μm shell length (SL), postlarvae become responsive to the "digestibility" of diatom diets and grow more rapidly on efficiently digested strains; (3) at around 5–10 mm SL juveniles shift from a biofilm-dominated diet to a macroalgae-dominated diet. We can make some tentative generalizations about growth rates of postlarvae fed ingestible diatoms. Rapid growth (20–30 μm/day) in the first 10 days postsettlement often falls to 10–20 μm/day in the following 1–2 wk, perhaps reflecting the exhaustion of nutrition from yolk/DOM. Postlarvae of 0.8–2 mm SL grow ~40–60 μm/day on "digestible" diatoms and 15–30 μm/day on "indigestible" ones. Rapidly growing juveniles over 3 mm SL will grow at 100 μm/day or more, whereas poor diets can halve these growth rates. Growth rates of individual abalone will vary widely on both good and poor diets. Diatoms that are emerging as key species for abalone culture include *Cylindrotheca closterium* (and needle-shaped *Nitzschia* spp.), *Cocconeis* spp., and *Achnanthes* spp., but caution is needed in extrapolating from the limited data avilable. Hatcheries need to maintain a suitable quantity of ingestible food, as well as consider food quality. Many aspects of postlarval feeding and growth require further research.

KEY WORDS: postlarval abalone, feeding, nutrition, growth, benthic diatoms

INTRODUCTION

Abalone are commercially important gastropods around the world. The culture of abalone species has been stimulated by the decline of natural stocks and has made great advances. Abalone have been successfully reared from the egg to the adult under artificial conditions (e.g., Seki 1980, Ebert and Houk 1984, Uki and Kikuchi 1984, Hahn 1989). However, research on their feeding habits in the early life stages has been limited (Kawamura 1996), and control of the initial food is still one of the most critical problems in hatchery seed production (Hahn 1989, Seki 1997). Survival rates in many abalone hatcheries are low and variable, especially in first few months (Searcy-Bernal et al. 1992).

Benthic diatoms have traditionally been used as initial foods for postlarvae in abalone hatcheries (Seki 1980, Ebert and Houk 1984, Hahn 1989). Most hatcheries have used diatom films that developed naturally or pure strains of unknown dietary value. Many hatcheries in Japan use the pregrazing method (Seki 1980, Hahn 1989). This method relies on the grazing pressure of juvenile or adult abalone to select for strongly adhesive, solitary diatoms that are suitable for settlement (Suzuki et al. 1987, Kawamura and Kikuchi 1992). The pregrazed plate method is used to ensure consistently high settlement (Seki 1980, Slattery 1992), but it also supplies good food sources for postlarvae (Takami et al. 1997a).

By understanding the feeding and growth of young abalone in hatcheries, we can improve growth and survival of postlarvae. This is important to achieve consistent and efficient production and to minimize the proportion of slow-growing animals.

In natural habitats, larval abalone settle largely on crustose coralline algae (CCA) (e.g., Saito 1981, Morse and Morse 1984, Shepherd and Turner 1985, McShane and Smith 1988, McShane 1992, McShane 1995) and grow there for at least several months (McShane and Naylor 1995, Shepherd and Daume 1996, McShane 1996). The feeding, growth, and survival of abalone postlarvae on CCA are therefore critical in understanding natural recruitment in abalone fisheries.

In this article, we review data on feeding and growth of postlarvae abalone. We focus especially on diatoms and on our recent progress with *Haliotis discus hannai* Ino 1953 in Japan, and *Haliotis iris* Gmelin 1791 in New Zealand. The review covers data from laboratory experiments, abalone hatcheries, and natural habitats and is divided according to major food categories. We have not included data presented without description of methodology (e.g., many of the data in Hahn 1989). Artificial diets are not included, because data from current trials with postlarvae (elg., Fleming et al. 1996) are not yet available.

We include some data on juvenile feeding, particularly to discuss the transition from microalgal to macroalgal diets, and the size at which diatoms no longer provide adequate nutrition for abalone. There is a no clear boundary defined between postlarval and juvenile abalone. The formation of the first respiratory pore (~2 mm SL) or the size at which the first respiratory pore is sealed with shell (~3–5 mm in *H. iris* and *H. discus hannai*) (Takahashi and Obata 1986, Seki 1997, R. D. Roberts, unpublished data) may be useful reference points.

After reviewing the relevant published literature, we make some tentative generalizations about growth rates and key diatom species. We discuss the importance of maintaining a suitable food quantity in hatcheries, and we identify gaps in current knowledge of postlarvae nutrition.

MAJOR TRANSITIONS IN POSTLARVAL FEEDING

As background to the review that follows, it is useful to describe the major transitions that are apparent in postlarval feeding

and growth. Three major transitions can be recognized. The first is the transition from lecithotrophy to particle feeding. Abalone larvae are lecithotrophic and do not have a functional mouth or digestive tract. Within a day of the velum being shed, the mouth forms and active feeding begins (Crofts 1937, Seki and Kan-no 1981a, Norman-Boudreau et al. 1986, Ohashi 1993, Kawamura and Takami 1995). In terms of the abalone's nutrition, there appears to be overlap between lecithotrophy and early ingestion. A second transition is evident at around 600–800 μm shell length (SL). Postlarvae below this size grow at similar rates regardless of diatom strain, provided they receive an adequate supply of biofilm material. In contrast, larger postlarvae grow much more rapidly on certain diets, such as highly digestible diatoms. The final transition is from a biofilm-dominated diet to a macroalgae-dominated diet. Data from natural habitats and hatcheries suggest that this change occurs at around 5–10 mm SL. Below, we review the published data on growth and feeding of postlarvae abalone in relation to these various feeding stages.

LARVAL YOLK SUPPLY AND ABSORPTION OF DISSOLVED ORGANIC MATTER

Abalone species have planktonic larval stages lasting several days (e.g., Seki and Kan-no 1977, Hahn 1989, McShane 1992). Larvae carry a yolk supply derived from the egg. This energy source could fuel larval life and metamorphosis but can also be preferentially supplemented by the uptake of dissolved organic matter (DOM) by both larvae and early postlarvae (Jaeckle and Manahan 1989a, Jaeckle and Manahan 1989b, Manahan and Jaeckle 1992, Shilling et al. 1996).

Yolk supply and DOM absorption can support extended larval life. In *Haliotis rufescens* Swainson 1822, larvae can live for 20–30 days before their yolk supply is exhausted (Morse 1985). In static water with antibiotics, larvae of *Haliotis virginea* Gmelin 1791 and *H. iris* can continue swimming for ~40 days at 17°C (Roberts and Lapworth unpubl.). Although larvae deprived of settlement cues can survive for protracted periods, their postsettlement survival is presumably compromised beyond a certain age. Work with *H. iris* suggests that larvae that are older than ~25 days (at 17°C) at metamorphosis show poor survival as postlarvae (Roberts and Lapworth unpubl.).

Larval yolk and DOM uptake provide sufficient nutrition for early postlarval development. Peristomal shell formation in the absence of particulate food is observed when metamorphosis is induced with γ-aminobutyric acid (Morse et al. 1979, Barlow and Truman 1992, Roberts and Nicholson 1997). For example, *H. iris* postlarvae grew 30–120 μm of peristomal shell during 10 days of incubation, with most of the growth occurring in the first 4 days (Roberts and Nicholson 1997). In *H. rufescens*, normal development can proceed without feeding for up to 14 days postfertilization at 15°C (Morse et al. 1980). Yolk reserves were visible in *Haliotis discus discus* Reeve 1846 until 530 μm SL, 6 days after settlement (Ohashi 1993). However, Asano and Kawamura (unpubl. data) observed that yolk supply was diminished at the time of metamorphosis in *H. discus hannai* by an immunological technique. We are not aware of any published work on the effect of yolk quality/quantity on metamorphic success and early postsettlement survival.

DIATOMS

Diatoms for Postlarvae <600–800 μm SL

Only a handful of studies have examined the growth of abalone postlarvae reared on unialgal benthic diatom strains (Table 1). Some diatom strains pass through the postlarval abalone gut intact, whereas others are ruptured and lose their cell contents. In this review, we use "digestibility" to refer to the proportion of diatom cells that lose cell contents when ingested and passed through the postlarval gut. We recognize that other factors will contribute to the overall digestibility of a diet. Diatom strain makes relatively little difference to growth rates of postlarvae less than about 600–800 μm SL. Kawamura and Takami (1995) reported that early postlarval growth was not significantly higher on a digestible diatom than on two largely indigestible diatoms. Similarly, recent studies with *H. iris* showed little divergence of growth curves for different diets until postlarvae reached about 500–600 μm SL (Roberts et al. unpubl.).

The lack of a clear growth response to diatom strain in very young postlarvae could be due to the residual benefit of a nutritionally complete yolk supply. As micronutrients from the yolk become depleted, postlarvae may require access to diatom cell contents to maintain rapid growth. Alternatively, very young postlarvae may lack the digestive capabilities to fully use diatom cell contents. The possibility that postlarvae will show delayed benefits from access to cell contents in their first few weeks has not been examined.

Postlarvae feeding on largely indigestible diatoms probably get much of their nutrition from the extracellular secretions of diatoms, which consist mainly of polysaccharides (Hoagland et al. 1993). The degree to which bacteria or other nondiatom microbes contribute to the nutritional value of the film remains largely unknown.

Diatoms for Postlarvae 0.8–4 mm SL

Many diatoms pass through the gut of postlarval *H. discus hannai* and *H. iris* alive and unharmed (Kawamura et al. 1995, Kawamura et al. 1998). Kawamura et al. (1995) measured the "digestion efficiency" of diatom strains for postlarvae by counting the percentage of intact cells that lose their cell contents when eaten. Postlarval *H. discus hannai* over ~1 mm SL fed on four diatom strains with high digestion efficiencies (64–100%) grew significantly faster than did postlarvae fed on five strains with low digestion efficiencies (4–17%) (Table 1; Kawamura et al. 1995). Similar results were obtained with *H. iris*—postlarvae grew significantly faster on two highly digestible strains than on six inefficiently digested strains (Table 1; Kawamura et al. 1998). Postlarvae over about 800 μm SL seem to require high levels of absorption of diatom cell contents for rapid growth, whereas smaller postlarvae grow at similar rates on diatoms with high and low digestibility.

Factors Controlling Diatom Digestibility

The factors that control the digestibility of a diatom strain are complex. In general, the digestibility of a strain is stable over time, but it can change dramatically within a couple of weeks (Roberts et al. unpubl.). Release of cell contents often involves physical rupture of the frustule and/or girdle elements. Rupture frequently results from the action of the radula but could also occur within the

TABLE 1.

Growth rates of postlarval abalone fed unialgal diatom diets in laboratory experiments.

Abalone Species	Diatom Strain	Daily Growth Rate (μm SL/day)	Initial Abalone Size (μm SL)	Final Abalone Size (μm SL)	Digestion Efficiency* (%)	Temperature (°C)	Reference
H. discus hannai	*C. scutellum*	14.4	280	510–865	ND†	20	Kawamura and Takami 1995
	C. closterium‡	27.2	280	735–1,177	High		
	Navicula ramosissima	16.6	280	548–841	Low		
	Stauroneis constricta	21.6	280	724–865	Low		
H. discus hannai	*C. closterium*‡ growing on the chlorophyte *U. lens*	27	280	820	—§	22	Seki 1997
H. discus hannai	*Cocconeis* sp.	30.6	280	800	—§	17–18	Oghai et al. 1991
	C. closterium‡	24.1	280	690	—§		
	Navicula ramosissima	22.9	280	670	—§		
H. gigantea	*Actinocyclus tenuissimus*	12.8	321	552	—§	19	Ishida et al. 1995
	Cocconeis sp.	32.6	340	927	—§		
	Melosira moniliformis	12.8	352	518	—§		
	Navicula ramosissima	28.0	333	836	—§		
H. iris	*A. longipes*-1	33.7	570	1,245	93.5	17	Kawamura et al. 1998
	A. longipes-2	11.3	549	788	ND†		
	C. pseudomarginata	11.8	570	830	94.6		
	N. britannica	16.4	555	896	4.3		
	Navicula ramosissima	17.0	606	938	1.8		
	Navicula sp.	15.1	603	902	18.6		
	Nitzschia ovalis	15.4	568	886	32.3		
	Nitzschis sp.	35.3	553	1,280	92.7		
H. discus hannai	*A. brevipes*	57.2	1,230–1,540	1,960–2,470	64.3	20	Kawamura et al. 1995
	A. longipes	47.8	1,420–1,540	1,860–2,360	71.5		
	Amphora angusta	29.0	1,120–1,420	1,450–1,770	8.2		
	C. scutellum	46.4	1,050–1,310	1,460–1,950	84.3		
	C. closterium‡	50.1	920–1,300	1,270–2,190	100		
	Navicula ramosissima	21.1	1,120–1,590	1,470–1,790	16.5		
	Nitzschia sp.	13.6	1,120–1,600	1,260–1,750	8.5		
	Pleurosigma sp.	24.2	1,330–1,600	1,420–2,100	3.8		
	Synedra investiens	20.5	980–1,270	1,140–1,610	5.6		

* Digestion efficiency is the proportion of live cells that lose cell contents when eaten (Kawamura et al. 1995).

† Not determined: for these diatom strains, there were insufficient diatom cells in the feces to accurately assess digestion efficiency.

‡ *C. closterium* is often called *Nitzschia closterium*.

§ Data not available.

stomach. Further work is needed to know whether diatoms can also be digested by membrane disruption, with the frustule intact.

We know several of the factors that affect diatom digestibility for postlarval abalone. Attachment strength of the diatom strain is important. Very tightly attached diatoms such as *Cocconeis scutellum* (Kawamura et al. 1995), *Cocconeis pseudomarginata* (Kawamura et al. 1998), *Achnanthes brevipes* (Kawamura et al. 1995), and *Achnanthes longipes* (Kawamura et al. 1995, Kawamura et al. 1998) require considerable force to be detached from substrata and are usually ruptured if dislodged. In contrast, many diatom strains with low adhesive strength are ingested without cell rupture, and the majority of ingested cells pass through the gut alive and unbroken (Kawamura et al. 1995, Kawamura et al. 1998).

However, not all weakly adhering diatoms have low digestibility. *Cylindrotheca closterium* (Kawamura et al. 1995) and *Nitzschia* sp. (Kawamura et al. 1998), which had low attachment strengths, produced high digestion efficiencies and relatively rapid growth of postlarvae. These results could be because of their structurally weak silica frustule, as suggested by Reimann and Lewin (1964) for *C. closterium*. Takami et al. (1996) suggested that the structural strength of diatom cells may depend on factors such as culture conditions and diatom growth phase. This could explain temporal variation in digestibility of certain strains.

Diatom cell size and growth form can limit digestibility by precluding ingestion. Two strains of *A. longipes* with different cell sizes and stalk lengths produced different growth and survival rates of *H. iris* postlarvae (Kawamura et al. 1998). The postlarvae could not ingest the larger celled/longer stalked strain up to at least 1,300 μm SL, and the postlarvae fed on this strain had slow growth and low survival compared with those fed the smaller celled/shorter stalked strain (Kawamura et al. 1998). Large mobile cells (such as loosely attached *Pleurosigma* spp. or long-stalked *Achnanthes* spp.) are often brushed aside by the shell or snout, so ingestion is very inefficient (R. Roberts pers. obs.).

The size of diatom cells has been seen as a critical factor determining the dietary value of diatoms, especially for very small postlarvae (Seki and Kan-no 1981a, Hahn 1989, Fleming et al. 1996). The postlarval mouth is initially only 10 μm across (Seki

and Kan-no 1981a), and diatoms ingested by newly metamorphosed postlarvae are mostly less than 10 μm in width (Norman-Boudreau et al. 1986). However, ingestibility of diatom cells may not be critical for young postlarvae. Postlarval abalone below about 600–800 μm SL appear to gain adequate nutrition from biofilm components other than diatom cells (such as extracellular secretions and possibly bacteria). Once postlarvae require diatom cell contents for continued rapid growth, they can readily ingest much larger diatom cells. Thus, we suspect that the effect of cell size on the growth of small (<600–800 μm SL) postlarvae will only be critical when access to other biofilm components is limited. For larger postlarvae (>800 μm SL), access to cell contents becomes important, so any effect on ingestibility can affect growth. Diatom cell size can affect the efficiency with which the radula passes food into the mouth (small cells are generally handled more efficiently; R. Roberts pers. obs.). In the case of very large diatoms, cell size could preclude ingestion even for large postlarvae.

When diatom digestibility becomes important for postlarval growth, the biochemical composition of diatom cell contents may affect growth rates. For example, fatty acids profiles are a critical component in aquaculture feeds and are known to vary among diatom species (Dunstan et al. 1994).

Growth on Cocconeis *Species*

The diatom genus *Cocconeis* represents interesting and complex study in relation to early postlarval feeding. *Cocconeis* species are dominant on the pregrazed plates, common on CCA, and highly effective settlement inducers, so postlarval feeding on these diatoms has received considerable attention. In laboratory experiments with *H. discus hannai*, postlarvae grew well on *Cocconeis scutellum* var. *parva* for the first 10 days postsettlement (~25 μm/day at 20°C), reaching ~500 μm SL. The growth rate then dropped dramatically over the next 14 days, to only 7.5 μm/day, whereas significantly higher growth rates were maintained on three other diatom strains (Kawamura and Takami 1995). On other occasions, *C. scutellum* has produced good growth of small postlarvae *H. discus hannai* (Takami and Kawamura unpubl. data) and *H. iris* (Roberts et al. unpubl.). Other *Cocconeis* species also produced relatively good growth of *H. discus hannai* (Ohgai et al. 1991) and *Haliotis gigantea* Gmelin 1791 (Ishida et al. 1995) (Table 1).

H. discus hannai <800 μm SL do not efficiently detach *C. scutellum* var. *parva* cells, probably because of the diatom's very high attachment strength and/or its low profile (Kawamura and Takami 1995, Takami et al. 1997a). *H. iris* postlarvae less than ~850 μm SL detached few *C. pseudomarginata* cells, whereas larger postlarvae (850–1,200 μm SL) ingested many (Kawamura et al. 1998). Daume et al. (1997) reported that postlarval *Haliotis rubra* Leach 1814 began grazing *C. scutellum* from CCA 18 days after settlement. *H. iris* ingested very low numbers of *C. scutellum* cells 10 days postsettlement and became increasingly efficient at removing them over the following 3 wk (Roberts et al. unpubl.). A similar pattern was observed with *Haliotis midae* Linnaeus 1758 grazing on *Cocconeis sublittoralis* in mixed diatom films (Matthews and Cook 1995).

From these examples, it appears that *Cocconeis* species support good growth once postlarvae reach a size where they can efficiently ingest the diatom. Growth rate can decline dramatically in postlarvae >500 μm SL if postlarvae are still unable to ingest *Cocconeis* efficiently, suggesting that the postlarvae may come to require diatom cell contents for rapid growth. This may be due to the depletion of stored micronutrients from the yolk, as discussed above. Factors that may influence whether growth tails off include the amount of extracellular secretion in the culture (Kawamura and Takami 1995), the nature of associated microbial communities, and the attachment strength of the diatom.

Diatoms for Juveniles >4 mm SL

Juveniles of *H. discus discus*, *H. discus hannai* (10–20 mm SL), and *H. midae* selectively ingested three-dimensional colonies and loosely attached diatom species, leaving behind prostrate diatoms with high attachment strength such as *Cocconeis* spp. (Ioriya and Suzuki 1987, Suzuki et al. 1987, Matthews and Cook, 1995). Thus, *Cocconeis* spp. are often dominant on pregrazed plates (Seki 1980).

Takami et al. (1996) compared growth rates of juvenile *H. discus hannai* of about 30 mm SL fed on three species of diatoms (*A. longipes*, *C. scutellum* and *Navicula britannica*) and on fronds of a brown alga *Laminaria japonica*, which is listed as the one of the best diets for the growth of juveniles (Uki 1981). The juvenile growth rate on *A. longipes* was higher than that on *Laminaria*, whereas growth rates on *C. scutellum* and *N. britannica* were significantly lower. Changes in muscle glycogen and midgut gland triglyceride content (the main energy sources during the initial period of starvation; Takami et al. 1995) showed that juveniles fed on *N. britannica* were starving (Takami et al. 1996). Digestion efficiency of juveniles fed on *N. britannica* was low (9.6%). Similarly poor results were found with postlarvae fed on *N. britannica* (Table 1). In contrast, Uki and Kikuchi (1979) reported rapid growth (about 200 μm/day at 20°C) of juvenile *H. discus hannai* (8–10 mm SL) fed on four loosely attached benthic diatom species including *N. britannica*. Uki and Kikuchi (1979) did not report the digestion efficiency of *N. britannica*. The digestibility of this diatom can vary over time (Roberts et al. unpubl.).

Juveniles of 30 mm SL fed on *C. scutellum* grew slowly despite the high digestion efficiency (86.6%) of this diatom. A larger, three-dimensional diatom, *A. longipes*, with slightly lower digestion efficiency (67.5%) produced more rapid growth (Takami et al. 1996). This apparent conflict is explained by the volume of cells ingested, which was seven times higher for *A. longipes* than on the prostrate, low-volume *C. scutellum* (Takami et al. 1996). It appears that juvenile abalone do not graze *Cocconeis* species if more favorable foods are available. Further, these results suggest that the energetics of *Cocconeis* ingestion become insufficient to support rapid growth, but the size at which this occurs has not been determined.

CCA

Survival and Growth of Postlarvae on CCA

CCA without appreciable quantities of microbial biofilm can support postlarval abalone growth. Garland et al. (1985) reported that CCA with negligible microalgae sustained growth of *H. rubra* for 13 wk after settlement, but postlarval growth rate was low, especially for the first 6 wk (about 10.5 μm/day at 15–17°C). These authors considered that postlarval *H. rubra* depended mostly on the cuticle and epithallial contents of CCA for nutrition. Shep-

herd and Daume (1996) also suggested that a CCA, *Sporolithon durum*, which had no surface biofilm, supplied enough food for postlarval *Haliotis laevigata* Donovan 1808 and *Haliotis scalaris* Leach 1814 from settlement to 1–2 mm SL. Growth rates were not reported. Kitting and Morse (1997) reported on postlarval *H. rufescens* feeding on the CCA *Lithothamnion californicum*. During the first 10 days postsettlement, the postlarvae did not ingest "cellular solids" and grew very slowly (5 μm/day at 15°C).

Takami et al. (1997b) have shown that the presence of diatoms on CCA produces more rapid growth. Postlarval *H. discus hannai* reared on a CCA, *Lithophyllum yessosense*, without diatoms or trail mucus grew to 500 μm as rapidly as did those on CCA with diatoms. However, growth to 1 mm SL was slower on CCA without diatoms (24.4 μm/day at 20°C) than on CCA with diatoms (53.4 μm/day) (Takami et al. 1997b). Older postlarvae (initial SL, 1.5 mm) showed the same pattern (Takami et al. 1997b). The growth pattern on CCA without diatoms mirrors that seen on some *C. scutellum* strains, where growth slows markedly after about 500 μm SL (Kawamura and Takami 1995, Takami et al. 1997a). Seki (1997) reported a similar pattern with growth of postlarval *H. discus hannai* on a CCA *Titanoderma* sp., with growth slowing after 15 days postsettlement. The surface biofilm was not described.

McShane and Naylor (1995) settled *H. iris* larvae onto "conditioned" CCA-covered rocks and transferred them to the sea after 1 wk. Postlarvae grew at an average of only 17 μm/day over 35 days and 30 μm/day over 135 days postsettlement. Growth rates may have been influenced by the very high density of larvae at settlement (1,000/20-cm-diameter rock) or the "conditioning" of rocks presettlement. Sasaki and Shepherd (1995) used size frequency modes to estimate daily growth rates of *H. discus hannai* postlarvae, which settled naturally on CCA. The authors suggested average growth rates of 42 μm/day over 79 days and 32 μm/day over 59 days, in two separate years.

Shepherd and Daume (1996) reviewed survival rates of postlarvae on CCA in natural habitats. The five known studies reported highly variable mortality of postlarvae with mean monthly M values ranging from 0 to 2.7 (Shepherd and Daume 1996). Very high mortality occurs in the first couple of weeks postsettlement (Sasaki and Shepherd 1995), so the exact sampling period explains some of the variation in reported M values.

Food sources for postlarvae on CCA include the alga's surface polysaccharide and epithallial cell contents, and biofilm components such as diatoms and bacteria plus their extracellular products (Garland et al. 1985, Shepherd and Daume 1996, Takami et al. 1997b). Trail mucus of gastropods inhabiting CCA is a further possible food source in the natural habitat (Takami et al. 1997b). On the basis of the limited data available, it appears that food sources from CCA themselves can keep postlarvae alive but are not adequate to support rapid growth.

Most studies report mean growth rates, but there is usually wide variation in the performance of individuals, at least in experimental and hatchery conditions. For example, a postlarva occasionally grows very rapidly despite poor food conditions. The reasons for this are unknown but may include physiological, behavioral, and genetic factors. Such exceptional individuals may have a much higher chance of surviving to adulthood in natural habitats. There are limited studies on the feeding ecology of postlarval and early juvenile abalone in their natural habitats. More work is needed to understand and enhance natural recruitment.

Diatoms on CCA in Natural Habitats

Little work has been done on diatom communities on CCA surfaces in natural habitats. We know that these communities can vary enormously, but there is evidence that they are often dominated by *Cocconeis* spp. Diatoms with strongly adhesive prostrate forms such as *Cocconeis* spp. were dominant on artificial substrata in areas dominated by CCA at several sites in northeastern Japan (Kawamura et al. 1992, Kawamura 1994), and dense patches of *Cocconeis* spp. were observed on CCA in nearby abalone habitat (Takami and Kawamura unpubl. data). *Cocconeis* spp. tend to dominate benthic diatom communities when there is high grazing pressure from gastropods (Kesler 1981, Steinman et al. 1989, Kawamura et al. 1992, Kawamura 1994), including juvenile and adult abalone (Ioriya and Suzuki 1987, Suzuki et al. 1987). Given that high densities of grazing gastropods are often found on CCA (Ayling 1981, Choat and Schiel 1982, Kawamura 1997), we might expect *Cocconeis* to be dominant. However, diatom colonization can also be influenced by sloughing of coralline epithallial cells (e.g., Masaki et al. 1984, Johnson and Mann 1986, Daume et al. 1997). To determine the role of diatoms in the nutrition of postlarval abalone on CCA in natural environments, we need to carry out further field experiments, or to simulate field conditions and diatoms densities more closely in the laboratory.

TRAIL MUCUS OF CONSPECIFIC ABALONE

Trail mucus of conspecific abalone is a possible food source for small postlarval abalone both on pregrazed plates and on CCA in natural habitats. *H. discus hannai* postlarvae were grown on plates containing *C. scutellum*, pregrazed *C. scutellum*, and conspecific trail mucus without diatoms. Postlarvae on the pregrazed *Cocconeis* film grew well to reach 1,400 μm SL by the end of the experimental period (4 wk postsettlement). Postlarvae grown on *Cocconeis* alone and mucous trails alone reached only ~500 and ~700 μm average SL, respectively, over the same period (Takami et al. 1997a). Trail mucus left by juveniles during grazing, supplemented with extracellular mucus from diatoms, was suggested as the main food source for small postlarvae until about 800 μm SL, when they began detaching *Cocconeis* cells (Takami et al. 1997a). Juvenile trail mucus was not a sufficient food source for *H. discus hannai* larger than about 700 μm SL.

In natural habitats, trail mucus from gastropods other than abalone could be a food source for small postlarval abalone. However, trail mucus of three gastropods (*Monodonta labio, Haloa japonica*, and *Cantharidus jessoensis*) failed to induce settlement of *H. discus hannai* larvae, whereas settlement was induced by trail mucus of four *Haliotis* species (*H. discus hannai, H. discus discus, H. gigantea*, and *Haliotis sieboldii* Reeve 1846) (Seki and Kan-no 1981b).

BACTERIA

The diatom strains, trail mucus, and CCA used in postlarval feeding experiments (reviewed above) were not axenic. Bacteria associated with those food materials are a possible food source for abalone.

Mucous trails of marine gastropods provide a good medium for bacterial growth (Horn 1986, Peduzzi and Herndl 1991), and CCA surfaces are colonized by bacteria in the natural environment (Lewis et al. 1985, Johnson et al. 1991). Garland et al. (1985) reported that postlarval *H. rubra* ingested bacteria on CCA. They

suggested that bacteria may perform metabolic activities in the gut of abalone that are significant to the host's development.

The role of bacteria in postlarval abalone feeding requires further research. Bacteria are no doubt consumed by postlarvae, but their contribution to nutrition has not been studied. Bacterial cells may be largely indigestible (like many diatoms), so their extracellular products could be the main source of nutrition. Gut bacteria contributed to polysaccharide degradation in juvenile (35 mm SL) *H. midae* (Erasmus et al. 1997).

MACROALGAE OTHER THAN CCA

Ulvella lens

Pregrazed plates dominated by *Ulvella lens* (a radially growing prostrate green alga) and *Cocconeis* spp. have been used as settlement surfaces in northern Japan (Takahashi and Koganezawa 1988). However, *Ulvella* itself does not support rapid growth of postlarvae. Newly metamorphosed postlarval *H. discus hannai* did not grow well when fed on *U. lens* and the upright diatom *Synedra tabulata* (Uki et al. 1981). Seki (1997) reported *U. lens* without diatoms or trail mucus sustained growth of postlarvae of *H. discus hannai* for 20 days after settlement, but produced slower growth than *Ulvella* plus *C. closterium*, especially after 15 days postsettlement. Growth of *H. discus hannai* (1.5 mm initial SL) was significantly lower on *U. lens* (Table 2; Kawamura 1994) than on four digestible diatom strains (Table 1; Kawamura et al. 1995).

Juvenile Stages of Macroalgae

Juvenile stages of various macroalgae supported moderate to rapid growth (90–138 μm/day) of 3–4 mm SL *H. discus discus* (Table 2; Yotsui 1978, Maesako et al. 1984). Comparable growth was recorded on a diatom, *Navicula* sp. (Maesako et al. 1984). Smaller postlarvae (1.3–2 mm SL) grew poorly on juvenile *Myrionema* sp. (Table 2; Yotsui 1978).

Undaria pinnatifida

H. discus hannai (1.3 mm initial SL) grew only 42 μm/day over 4 mo when fed on *Undaria pinnatifida*, softened by freezing (Sakai 1976). Salted *Undaria* produced low to moderate growth rates for *H. discus discus* (3.4 mm initial SL) and *H. gigantea* (3.1 mm initial SL) (Table 2; Fujii and Yotsui 1989, Fujii 1996).

Blue-Green Algae

Maesako et al. (1984) examined growth of *H. discus discus* of 3–4 mm SL fed on six species of blue-green algae. Two of them, *Entophysalis deusta* and *Plectonema golenkiniatum*, produced good growth (comparable to a diatom, *Navicula* sp., and juvenile macroalgae—see above). Four other blue-greens gave slower growth (0–60 μm/day), and one (*Aphanocapsa litoralis*) was toxic (Maesako et al. 1984).

Transition to Macroalgal Diets

Evidence from both natural habitats and hatcheries suggests that abalone feeding becomes increasingly macroalgae dominated

TABLE 2.

Growth rates of young abalone reared on macroalgae and blue-green algae.

Abalone species	Algal Diet	Daily Growth Rate (μm SL/day)	Initial Abalone Size (mm SL)	Growth Period (days)	Temperature (°C)	Comments	Reference
H. discus discus	*Myrionema* sp.	23.3	1.3–2.0	30	—*	An early developmental stage which has disc shaped prostrate form was used in this experiment	Yotsui 1978
H. discus hannai	*U. pinnatifida*	42	1.3	107	11.6–13.8	Sliced fronds of *Undaria* were used.	Sakai 1976
H. discus hannai	*U. lens*	26.1	1.4–1.5	16	20	Green alga with a radially spreading prostrate form.	Kawamura 1994
H. discus discus	*Myrionema sp.*	138	3.7	30	16.9–21.0	Brown alga with a radially spreading prostrate form	Maesako et al. 1984
H. discus discus	*Colpomenia sinuosa*	109.7	3.5	30	14.0–19.3	Macroalga germlings	Maesako et al. 1984
	Ectocarpus siliculosus	107.2	3.6	30	16.9 21.0		
	Endarachne binghamiae	88.0	3.4	30	14.0 19.3		
	Enteromorpha spp.	110.0	3.7	30	16.9–21.0		
H. discus discus	*U. pinnatifida*	72	3.4	50	—*	The *Undaria* fronds were salted before they were fed to the abalone.	Fujii and Yotsui 1989
H. gigantea	*U. pinnatifida*	92	3.1	120	12.5–21.5	As above	Fujii 1996
H. discus discus	*A. litoralis*	0.0	3.5	30	14.0–19.3	Blue green algae	Maesako et al. 1984
	E. deusta	96.7	3.6	30	14.0–19.3		
	Plectonema calothrichoides	58.7	3.4	30	14.0 19.3		
	P. golenkiniatum	86.7	3.5	30	14.0–19.3		
	Phormidium fragile	60.3	3.6	30	14.0–19.3		
	Spirulina subsalsa	31.0	3.6	30	14.0 19.3		

* Data not available.

as juveniles grow. However, the size at which this transition occurs is highly variable and probably depends on local food supply.

In the natural environment, the diet of *H. discus hannai* shifted from predominantly diatom in 5–10 mm SL juveniles to predominantly macroalgae and seagrass in 10–20 mm SL animals (Tomita and Tazawa 1971). Fragments of CCA dominated gut contents of 5–10 mm SL *H. laevigata* and *H. scalaris*, but the diet shifted from CCA to drift macroalgae as juveniles grew to 20 mm SL (Shepherd and Cannon 1988).

In hatcheries, abalone of 3–4 mm SL fed and grew well on early juvenile macroalgae (Maesako et al. 1984). They also fed on artificially softened macroalgal fronds, although reported growth rates were generally lower than on juvenile macroalgae (Sakai 1976, Fujii 1996, Fujii and Yotsui 1989). Juvenile *H. discus hannai* began feeding on soft algae such as *Ulva* spp. at about 4–5 mm SL (Seki 1997). Juveniles over 13 mm SL fed on macroalgal fronds such as *Laminaria* and *Eisenia* (Seki 1997), which were reported as having high dietary values for juveniles of 24–34 mm SL (Uki 1981, Uku et al. 1986). In a laboratory experiment, *H. discus discus* over 5 mm SL grew faster on CCA than on benthic diatoms (Ino 1952). In those studies, the relative contributions of macroalgal material and surface microbiota are unknown. Biofilm may still be an important component of the diet during weaning onto macroalgae.

Enzyme production may provide clues to diet changes. A marked increase in the activity of macroalgal polysaccharide-degrading enzymes was seen in *H. discus hannai* at about 2 mm SL, when fed on a diatom, *C. scutellum* (Takami et al. 1998). Other studies of abalone digestive enzymes have all related to larger juveniles and adults (e.g., Erasmus et al. 1997).

A shift in the feeding habit from microalgal feeding to macroalgal feeding seems to occur at around 5–10 mm SL. There are limited detailed data on the feeding habits of young juvenile abalone and on the relative contributions of microbial and macroalgal components—the diet around the time of weaning is probably a combination of both. Juvenile macroalgae may be important in both hatcheries and natural habitats.

DISCUSSION

Generalizations About Growth Rates

Most of the data in this review relate to temperate abalone species, which have similar juvenile growth patterns. The first respiratory pore forms at around 2 mm SL (e.g., Hahn 1989) and this pore is filled with shell at about 3–5 mm SL (at least in *H. discus hannai* and *H. iris*). We can make tentative generalizations about growth rates of these postlarvae when fed on ingestible diatom diets. In the first 10 days after settlement, growth rate is generally between 20 and 30 μm/day. Growth rate often falls in the following 1–2 wk. This drop may reflect the loss of supplementary energy from residual yolk and/or DOM absorption. Diatom digestibility makes little difference during this period, and growth rates are typically 10–20 μm/day. Above ~800 μm SL, growth rates respond strongly to diet. Postlarvae of 0.8–2 mm SL fed on highly digestible diatom diets grow at about 40–60 μm/day, whereas those fed poor diatom diets will generally grow at ~15–30 μm/day. Rapidly growing juveniles over 3 mm SL will grow at 100 μm/day or more, whereas poor diets can halve these growth rates.

These generalizations are based on mean growth rates. The ranges for individual animals will be much greater on both good and poor diets. We also assume that postlarvae are ingesting material on poor diets. Growth rates of postlarvae without adequate food intake will be much lower than those eating relatively poor diets. Hypothetical growth curves for temperate abalone postlarvae reared for 60 days on good and poor diatom diet are shown in Figure 1. They illustrate the influence that diet quality can have on growth, even in the first 2 mo.

Research Needs

Further research is needed on the digestibility of diatoms, and particularly on temporal changes in digestibility, and the factors

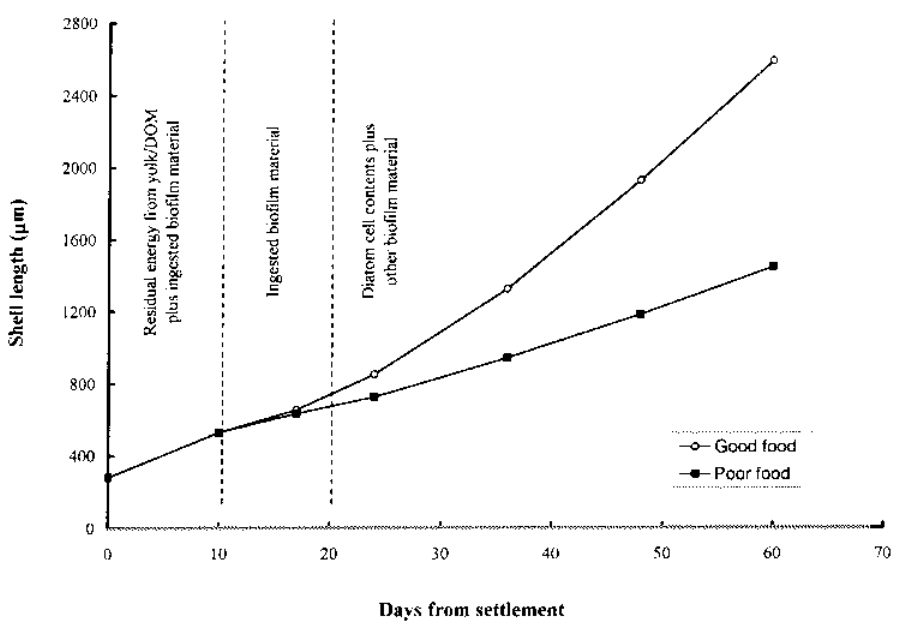

Figure 1. Hypothetical growth curves for postlarval abalone fed unialgal diatom films, illustrating the effect of food quality on growth rate. The "good food" curve is based on data from highly digestible diatoms, and the "poor food" curve is based on data from largely indigestible diatoms. Likely sources of nutrition during three postlarval feeding stages are shown.

controlling it. From our limited experience, we know that the digestibility of a strain can change from low to high and then remain high over at least several weeks (with weekly subculturing). The ability to increase the digestibility of cultures would benefit abalone hatcheries.

Studies on digestive enzymes may contribute to our knowledge of the digestibility of food sources and their real contribution to postlarval nutrition. The development of the radula and digestive apparatus has not been studied in detail in relation to postlarval nutrition.

The precise biochemical dietary requirements of postlarval abalone are not known, and there are limited data on the biochemical composition of diatoms, especially benthic species. Diatom species do differ in important dietary components such as fatty acid profiles (Dunstan et al. 1994), but the implications of this in terms of food value and growth rates of abalone are not known. Rather than analyze a wide range of benthic diatoms, it may be more productive to analyze key components of key species and examine the way in which biochemical food value can be enhanced by manipulating growing conditions.

The role of behavior in feeding success has been neglected for abalone (Fleming et al. 1996), and postlarvae are no exception. We know of some passive interactions between the postlarva and food that can affect ingestion efficiency, but the role of active postlarval behavior has not been studied.

The role of bacteria in postlarval nutrition is poorly known. Bacteria will be ingested with most foods, but they may be largely indigestible and provide little nutrition. Their potential role in gut function should be studied further.

There are very few data on the feeding and survival of postlarvae and small juveniles in natural habitats. These studies are a critical part of understanding natural recruitment. Diatom communities on CCA and the role they play in postlarval nutrition are poorly known. Competition for food between abalone postlarvae and other microalgal grazers has not been studied in detail. Survival studies must take into account the immediate postlarval period if they are to accurately estimate mortality of settled larvae. The role of exceptionally robust or fast-growing postlarvae should also be considered in relation to survival in natural habitats.

Key Diatoms for Abalone Postlarvae

A number of diatom species/genera are emerging as useful candidates for postlarval abalone culture. We need to be cautious, however, about extrapolating from the few studies published to date. Differences may occur between abalone species, between growing systems/conditions, and between strains of the same species/genus. Certainly, generalizations should not be made across diatom genera without clear identification of the critical properties of the diatom. The identification of CSIRO *Nitzschia* and *Navicula* strains by Fleming et al. (1996) should not be misinterpreted as identifying particularly useful species—there are numerous species in each of these genera, and we know that their food value can vary widely.

Mindful of the dangers of generalizing, some discussion of useful diatom species is warranted. *C. closterium* (= *Nitzschia closterium*) and other so-called "needle *Nitzschia*" species are highly digestible, even for postlarvae less than 500 μm SL. Their digestibility appears to be due to structural weakness of the frustule. Cultures generally have rapid growth and high mobility until they near stationary phase. In our experience, "needle *Nitzschia*" species are seldom good settlement inducers (and they can have negative effects) so are best added after settlement. At least some "needle *Nitzschia*" species can be bulk cultured in suspension by techniques developed for planktonic algae (R. Roberts pers. obs.).

Cocconeis species are generally very tightly attached, low-profile cells. They often become dominant in hatcheries, but are not very fast growing. The cells are nearly always broken during ingestion (so digestibility is high), but postlarvae <600–800 μm SL often do not ingest *Cocconeis* cells efficiently. On pregrazed plates, the combination of mucous trails and dense *Cocconeis* appears to provide suitable food for all stages of postlarval abalone. *Cocconeis* films probably become energetically inadequate as juveniles grow, but the size at which this occurs has not been established. The food value of pregrazed plates and the consistently high settlement they induce make them a favorable candidate for the initial stages of abalone culture. The technique is not widely used outside Japan, despite evidence of its effectiveness for *H. rufescens* (Searcy-Bernal et al. 1992, Slattery 1992). *Cocconeis* spp. can be cultured on large scales, but they grow relatively slowly and most cells are damaged during harvest. Novel growing systems are required.

Some *Achnanthes* species are firmly tethered to the substratum by a stalk, so most cells are broken if ingested. Stalk length and cell size vary greatly and can limit ingestibility for young postlarvae. *Achnanthes* spp. attaching strongly to the substratum by a stalk may be useful as food for large postlarvae and juveniles, which can ingest them efficiently, and grow rapidly. Three-dimensional *Achnanthes* populations provide a much higher biomass per unit area than do low-volume, two-dimensional *Cocconeis* films. *Achnanthes* cells are likely to be broken during harvest, and dense *Achnanthes* populations can interfere with settlement. Starter cultures could be added to postlarval tanks some time after settlement, to grow to high densities by the time postlarvae are large enough to ingest them.

Food Quantity

Food quality can have a major influence on growth rates. However, in abalone hatcheries, the ability to maintain a suitable *quantity* of *accessible* food is also critical and probably has a great influence on both growth and survival of postlarvae. Ingestion rates have been estimated by measuring the size of cleared patches in diatom films (Martines-Ponce and Searcy-Bernal 1998) and by measuring fecal volume (Roberts et al. unpubl.). Ingestion rates increase exponentially as postlarvae grow (Roberts et al. unpubl.), so rapid clearance of diatom films from plates is not uncommon and may lead to heavy mortalities. Increased postlarval densities reduce percent survival and growth rates (e.g., Oomori et al. 1995).

Food accessibility complicates the issue of food quantity. Postlarvae ingest some diatoms much more efficiently than others, which leads to selective feeding. This is partly due to passive physical interactions between the abalone and the food. On the microscopic scale of a postlarva, a young, thin, and mobile diatom film differs from an old, thick, and stable film. Old films often peel away as sheets, and postlarvae may dislodge a large proportion of the biomass rather than ingesting it. Biofilm thickness and age will also affect the water quality in the diffusive boundary layer that the postlarva occupies (Searcy-Bernal 1996).

Abalone farmers want predictable production of healthy, fast-growing juveniles. The challenge is to maintain a supply of readily ingestible and digestible food, in the face of rapidly increasing grazing pressure. This is not a simple task.

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GRAZING RATES OF RED ABALONE (*HALIOTIS RUFESCENS*) POSTLARVAE FEEDING ON THE BENTHIC DIATOM *NAVICULA INCERTA*

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ABSTRACT This article describes attempts to estimate grazing rates of haliotid postlarvae. Two similar experiments with *Haliotis rufescens* postlarvae of different ages (2–30 days) were conducted. Postlarvae were placed into 10-mL containers previously inoculated with the benthic diatom *Navicula incerta*. Two hours later, video recordings at different magnifications on an inverted microscope were performed. Images were analyzed digitally to estimate grazed areas and diatom density, which were combined to calculate grazing rates. Individual grazing rates ranged from 20 to 724 cells/hour during the first month of postlarval life. Grazing activity was minimal during the first week but increased abruptly after the second or third week after a shell length of 0.47 mm was attained. The potential application of the quantitative assessment of grazing rates for optimizing abalone postlarval culture is discussed.

KEY WORDS: abalone, *Haliotis rufescens*, postlarvae, grazing rates

INTRODUCTION

In abalone (*Haliotis* spp.) seed production, most mortality (ca. >90%) occurs during the first 2 mo after larval settlement, probably because of poor metamorphosis induction and/or postlarval culture conditions (Hahn 1989, Morse 1992, Searcy-Bernal et al. 1992a, Seki and Taniguchi 1996). High losses (ca. >70%) may occur even after successful metamorphosis (Searcy-Bernal et al. 1992a, Searcy-Bernal et al. 1992b) because of postlarval overgrazing of the benthic biofilms supplied as food. On the other hand, when grazing pressure is below optimal, these biofilms may become overdeveloped, which also results in high postlarval mortalities due to adverse ecological conditions (Ebert and Houk 1984, Hahn 1989, Tong and Moss 1992, Searcy-Bernal 1996).

Among several research topics currently addressed on these early abalone stages, special interest has been recently devoted to postlarval feeding, including the determination of the onset of feeding on benthic diatoms, the initial postlarval feeding requirements, and the effect of different diatoms strains on early survival and growth (Norman-Boudreau et al. 1986, McBride 1990, Matthews and Cook 1995, Kawamura and Takami 1995, Kawamura 1996, Daume et al. 1997, Kawamura et al. 1997, Kitting and Morse 1997, Takami et al. 1997). However, the quantitative assessment of postlarval grazing rates has not yet been addressed, despite its importance for the adequate management of culture systems.

This article describes experiments to estimate grazing rates of haliotid postlarvae. Two similar trials with *Haliotis rufescens* (Swainson) postlarvae of different ages (2–30 days) feeding on the benthic diatom *Navicula incerta* are described. Grazing rates were estimated by the digital analysis of video-recorded images.

METHODS

Two similar experiments were conducted in September 1995 and June 1996. Early veliger larvae of the red abalone *H. rufescens* were donated by a commercial hatchery (Abulones Cultivados, Eréndira, México). Further larval culture and metamorphosis induction with γ-aminobutyric acid (GABA) were performed at the Instituto de Investigaciones Oceanológicas (Ensenada, México) in 20-L containers, following methods described elsewhere (Searcy-Bernal et al. 1992a, Searcy-Bernal et al. 1992b).

The cultured diatom *N. incerta* was added as food 2 days after settlement induction. This small benthic diatom (ca.13 μm length) was isolated from local coastal waters and is currently used in research and commercial operations in México. Seawater (cartridge filtered to 1-μm-pore-sized, ultraviolet-treated, 10 mg/L of chloramphenicol) was changed every other day during the first 2 wk, and a gentle flow of filtered seawater (5 μm pore size) was provided thereafter. Constant fluorescent illumination (ca. 50 μE/s/m^2) was supplied. The average water temperatures were 17 ± 1 and 14 ± 1°C in the first and second experiments, respectively.

At different times after settlement induction (2, 4, 6, 12, and 30 days in the first experiment and at Days 4, 6, 10, 12, 18, and 25 in the second), postlarvae were collected for the determination of grazing rates. Six postlarvae of each age were introduced into each of the six wells of a sterile culture chamber (Corning Mod. 25810, 10-mL capacity and 9.4-cm^2 bottom area), which were previously (24–36 h) inoculated with *N. incerta* following standardized criteria such as immersion of batch culture containers in an ultrasound bath for 3–5 min to disperse cells before counting and the addition of a uniform inoculum to all wells (ca. 200 cells/mm^2).

Two hours after the introduction of abalone postlarvae, video recordings were performed on an inverted microscope, at different magnifications, for the determination of postlarval length, grazed areas, and the density of diatoms in adjacent ungrazed surfaces by digital image analysis (NIH Image 1.57 program in a PowerPC Macintosh computer). If required, estimates of grazed areas were corrected by subtracting inner subareas occupied by diatoms that were not removed by postlarvae. Grazing rates were estimated by multiplying grazed areas by diatom densities and dividing these products by 2 h. Previous experience has shown that postlarvae start feeding almost immediately after their introduction into vessels (ca. in less than 2 min) and that *N. incerta* growth and recolonization of cleared areas are negligible during the 2-h experimental period (Martínez-Ponce 1997).

RESULTS

Tables 1 and 2 provide the data used to estimate average grazing rates for different postlarval ages in Experiments 1 and 2, respectively. Postlarval growth data for both trials are displayed graphically in Figure 1, which shows an overall higher growth in

TABLE 1.

Means of the different variables used to estimate individual grazing rates of *H. rufescens* postlarvae feeding on the diatom *N. incerta* in Experiment 1 (September 1995; average temperature, 17 ± 1°C).

Age (days)	No. of Postlarvae	Shell Length (mm)	Grazed Area (mm^2/h)	Diatom Density (cells/mm^2)	Grazing Rate (cells/h)
2	16	0.290	0.122	156	20
4	23	0.323	0.588	108	53
6	36	0.378	0.727	149	110
12	17	0.470	1.497	448	662
30	25	1.326	1.165	632	724

the first trial than in the second (37 and 11 μm/day, respectively), although this variable was similar during the first 12 days. Slopes of linear regressions of length versus time in Experiments 1 (0.32) and 2 (0.15) were significantly different (t = 5.556, p = 0.001).

Average grazing rates are plotted in Figure 2. In the first trial, individual rates ranged between 20 and 724 cells/h, with lowest values during the first week and a sharp increase between ages 6 and 12 days. In the second experiment, these values ranged between 74 and 292, with lowest figures in the first 2 wk and an increase between 12 and 18 days. At ages 4 and 6 days, grazing rates were not significantly different between experiments (df = 1,11; F = 4.67; p = 0.06 and F = 1.73, p = 0.22, respectively), but a significant difference was found at 12 days of age (df = 1,11; F = 7.47, p = 0.02).

Figure 3 shows the relationship between grazing rates and postlarval lengths in both trials. Grazing on *N. incerta* increased abruptly after postlarvae reached 0.47 and 0.58 mm in the first and second experiments, respectively.

DISCUSSION

The digital analysis of video-recorded images has proved to be a useful tool in studying abalone postlarval feeding. This method allows the quick and reliable determination of grazed areas, diatom densities, and postlarval length, which are basic data required for the estimation of grazing rates, postlarval growth, benthic diatom production, etc. Handling of abalone is minimized, and images can be stored as a permanent record for verification of data or for performing new observations or measurements not previously considered. In particular, the use of an inverted microscope allows the direct observation of the ingestion of food particles (Kawamura 1996) and of the development and functioning of postlarval feeding structures (e.g., mouth and radula). Image analysis has also been recently used to record the frequency of feeding movements of abalone postlarvae (Kitting and Morse 1997), in studies on larval metamorphosis (Searcy-Bernal et al. 1997), and to assess standing crop in an abalone commercial growout facility (Vilchis-Guajardo 1997).

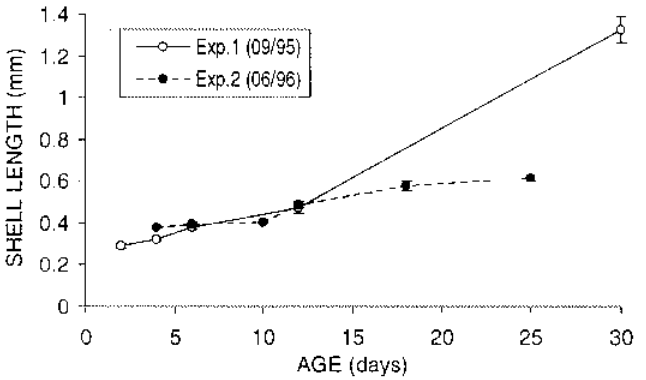

Figure 1. Growth of red abalone (*H. rufescens*) postlarvae in two independent experiments. Data are means of six replicates, and vertical bars are standard errors.

The method used may produce overestimates of grazing rates under certain conditions. For instance, clumps of diatoms are usually formed in high biofilm densities, which can be removed from the substrate by the shell movements of postlarvae and not by feeding activity. This situation may also result from suboptimal separation of diatom cells by ultrasound during the preparation of experimental vessels. Diatoms can also be removed by the radula and not ingested, or ingested and regurgitated. Although these situations were occasionally observed, their effect would not invalidate the general inferences from the experiments discussed below.

In both experiments, grazing rates of *H. rufescens* postlarvae on *N. incerta* were minimal during the first days after metamorphosis. This result is consistent with studies on gut and feces contents in several abalone species including *Haliotis kamtchatkana* (Norman-Boudreau et al. 1986), *Haliotis discus hannai* (Seki and Taniguchi 1996, Kawamura and Takami 1995, Kawamura 1996), *Haliotis midae* (Matthews and Cook 1995), *Haliotis rubra* (Daume et al. 1997), *Haliotis iris* (Kawamura et al. 1997), and *H. rufescens*

TABLE 2.

Means of the different variables used to estimate individual grazing rates of *H. rufescens* postlarvae feeding on the diatom *N. incerta* in Experiment 2 (June 1996; average temperature, 14 ± 1°C).

Age (days)	No. of Postlarvae	Shell Length (mm)	Grazed Area (mm^2/h)	Diatom Density (cells/mm^2)	Grazing Rate (cells/h)
4	23	0.380	0.261	288	74
6	24	0.392	0.254	293	74
10	24	0.407	0.162	388	61
12	21	0.498	0.160	498	76
18	28	0.579	0.633	463	292
25	27	0.615	0.388	476	173

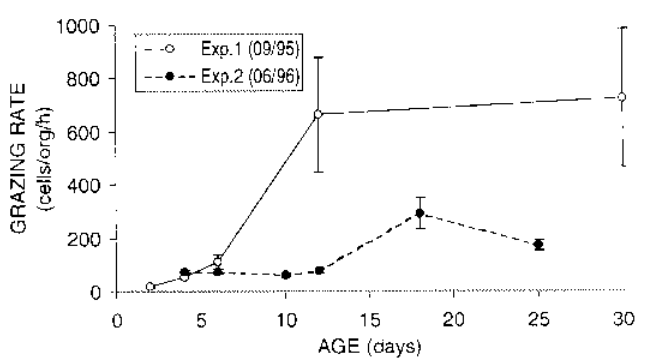

Figure 2. Estimated grazing rates of *H. rufescens* postlarvae at different ages in the two experiments. Data are means of six replicates, and vertical bars are standard errors.

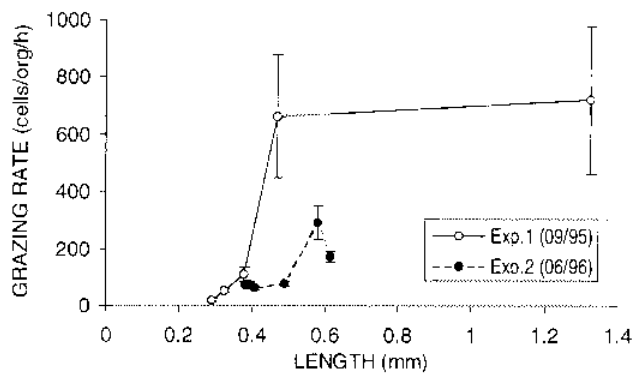

Figure 3. Relationship between grazing rates of *H. rufescens* postlarvae and their shell length in two independent experiments.

(Kitting and Morse 1997). During these early postlarval days, most energy requirements may be derived from the use of yolk reserves and dissolved organic matter (Manahan and Jaeckle 1992, Shilling et al. 1996) or the ingestion of diatom extracellular products, mucus, bacteria, and other components of biofilms (Kawamura 1996, Seki and Taniguchi 1996).

Postlarval growth rates were also similar between experiments during the first days postsettlement, but significant differences developed after Day 12, resulting in an average growth rate three times higher in Experiment 1 than in Experiment 2 (37 and 11 μm/day, respectively). This difference might reflect variations in culture and experimental conditions or between larval batches. Most conditions were similar between trials (e.g., postlarval density, feeding, irradiance, etc.), but temperature differed widely and may have influenced postlarval growth (Tables 1 and 2). Average growth rates of *H. rufescens* postlarvae reported here are within the range of values at similar temperatures found in previous studies (Leighton 1974, Ebert and Houk 1989, Flores-Aguilar 1989, McBride 1990). However, despite the temperature differences, shell growth was similar during the first 12 days in both trials. This suggests that the effect of temperature on growth was especially important after postlarvae became dependent on diatoms. The lower temperature in Experiment 2 might have limited diatom growth and biofilm development, resulting in poor food quality or abundance for postlarvae in culture containers, although this possibility was not explored.

There was a striking difference between experiments regarding the onset of vigorous feeding. In Experiment 1, it occurred between Days 6 and 12, whereas in Experiment 2, it occurred between Days 12 and 18 after settlement (Fig. 2). However, the ingestion of diatoms was observed on the inverted microscope from Day 4 in both experiments. Vigorous feeding started at a larger size in Experiment 2 after a shell length of 579 μm was attained, compared with 470 μm in Experiment 1 (Tables 1 and 2; Fig. 3). These results suggest that in Experiment 2, postlarvae were able to reach higher developmental stages, mainly on the basis of the utilization of energy reserves, dissolved organic matter, or other nondiatom food sources, delaying the dependence of feeding on *N. incerta*. Although this pattern may be related to the lower temperature in this trial, nutritional or genetic differences between larval batches cannot be ruled out.

The initial feeding on diatoms may be influenced by several factors, including diatom size and adhesive strength. Kawamura (1996) reported that *H. discus hannai* postlarvae can start consuming loosely adhered small diatoms at a shell length of 350 μm; however, diatoms with high adhesive strength, like *Cocconeis* spp. are consumed after postlarvae attain a shell length of 800 μm and are strong enough to break their cell walls. Similar results have been reported for *H. midae* (Matthews and Cook 1995) and *H. rubra* (Daume et al. 1997). In this study, two trials with the same abalone and diatom species resulted in very different patterns of initial postlarval feeding, suggesting that environmental conditions and differences in larval batches may also be important factors controlling the onset of abalone postlarval feeding.

The quantitative assessment of grazing rates during abalone postlarval development under culture conditions and of their relationship with biological and environmental factors provides important guidelines for controlling and optimizing this difficult culture stage. Postlarval densities and feeding strategies might then be managed to maximize production.

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INCREASING THE YIELD OF RED ABALONE WITH THE ALGA, *MICROCLADIA COULTERI*

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ABSTRACT High growth rate is a key to viability in the abalone aquaculture industry, and an excellent diet is required to achieve it. A basic *Macrocystis* diet might be greatly improved by supplementing it with another algal species. We observed that the epiphytic red alga, *Microcladia coulteri*, was rapidly consumed by 8–16 mm abalone. The purpose of this study was to evaluate this alga for its ability to stimulate growth rate in juvenile abalone under production conditions. A pairwise design with eight cages and 33,000 8-mm abalone was used to compare a control *Macrocystis* diet with a *Microcladia*-supplemented diet over a 4-mo period. The supplemented cages gained weight 2.6 times as rapidly as did the controls. Shell growth and survival were significantly higher in the supplemented populations as well. Thus, the *Microcladia* had a pronounced effect on the yield.

KEY WORDS:

INTRODUCTION

Because of the long production cycles in commercial abalone (*Haliotis* spp.) culture operations, profitability is closely tied to the growth rates that are sustained. Periods of slow growth need to be identified and eliminated in order to speed up the production process.

One such slow growth period often occurs at approximately 8 mm, when the abalone are weaned off of the natural and formulated nursery feeds and are placed on the macroalga *Macrocystis pyrifera* (Linnaeus) Agardh. These 5- to 8-month-old juveniles often grow at mean rates of only 1.0 mm/mo during the first 4 mo on *Macrocystis*. For instance, Trevelyan et al. (1995) compared *Macrocystis*-fed populations of juvenile red abalone with baskets receiving both *Macrocystis* and a supplement of the red alga *Palmaria mollis* Smith. It was found that mean growth rates in the unsupplemented controls averaged 1.1 mm/mo, whereas baskets supplemented with *P. mollis* grew significantly faster, averaging 1.4 mm/mo. Thus, growth during the early weaning period was slow but could be improved by dietary changes. Methods for culturing *P. mollis* were developed by Levin (1991), and its use as an abalone feed was first discovered at the Hatfield Marine Science Center (Dr. C. J. Langdon pers. comm.).

This discovery suggested that other red algal species might also stimulate growth of juvenile abalone. Tons of a diverse mixture of red algae drift up onto the beaches near our facility each year. While testing various species from this mixture, we noticed that one abundant species, *Gigartina exasperata* Harvey and Bailey, often supported a lush growth of a delicate red epiphyte, *Microcladia coulteri* Harvey, and that this epiphyte was rapidly consumed by the abalone. It appeared possible to collect enough *Microcladia* to use commercially, at least during the weaning period. Thus, the purpose of this study was to evaluate *Microcladia* for its value as a supplemental feed for 8–16 mm abalone.

MATERIALS AND METHODS

On May 25, 1994, eight floating mesh baskets were stocked with 4,200 abalone (8.26 mm in mean length) each at the Abalone Farm Inc. (AFI) facility in Cayucos, CA. These baskets were divided by location into four side-by-side pairs of baskets. Each of the baskets in each pair was randomly assigned to receive one of the two different feeding treatments. Thus, a randomized, pairwise design was used in this study.

All eight baskets were initially filmed with live benthic diatoms, and all baskets received 1–4 kg of fresh *Macrocystis* per week. This was enough to maintain a constant, *ad libidum* feed resource for these baskets and also to form a canopy that shaded the populations. One basket in each pair also received a supplement of 0.2–1.0 kg of *Microcladia* per week. The feedings were conducted 3 times per week. *Microcladia* was collected from the beach wrack below the AFI facility, away from the seawater effluent discharges. Most of the *Microcladia* was collected from fronds of *G. exasperata*, which is a large red alga sometimes called "Turkish towel" that is often abundant in the local drift algae. The *Microcladia* was identified as *M. coulteri* by the descriptions in Abbott and Hollenberg (1976). *M. coulteri* is the largest of the three *Microcladia* species occurring in California, and ranges from Vancouver to Baja California. However, we may have also collected and fed some *Microcladia californica*, because as Abbott and Hollenberg (1976) point out, the vegetative limits of the two species are confused.

After 124 days (4.1 mo), the baskets were harvested. Algal debris was removed, and the whole population from each basket was weighed. A sample (80 g) was taken from each population and was weighed and counted. Shell lengths of 50 abalone from this sample were measured to the nearest 0.1 mm with vernier calipers. From these data were calculated the monthly percent weight gain, survival, and the mean shell growth rate (millimeters per month) occurring in each basket. In addition, the coefficient of variation (CV) for final shell length was calculated for each basket population. CV equals the ratio of the variance over the mean, and is expressed as a percent. Pairwise *t*-tests were performed to test for significant treatment effects.

RESULTS

Feeding Rate

Microcladia was consumed rapidly. The abalone appeared to prefer the *Microcladia* over *Macrocystis*, consuming all of the former before consuming much of the latter. The abalone initially consumed 14 g of *Microcladia* per basket per day. This equaled a daily *Microcladia* feeding rate of 4% of biomass per day. This

TABLE 1.

Monthly percent weight gain.

Treatment	Pair 1	Pair 2	Pair 3	Pair 4	Mean
Control	57.9	54.1	41.3	51.0	51.1
Microcladia	139.2	141.3	107.2	153.2	135.2

ration was gradually increased to 142 g/day by the end of the experiment. At this time, the daily *Microcladia* ration was 6.8% of biomass. Uneaten *Microcladia* began to rot after 3 days in the baskets. Therefore, it had to be fed 3 times per week in order to maintain a constant supply.

Weight Gain

Table 1 shows the monthly percent weight gain data for each of the eight baskets. Each *Microcladia* basket gained weight 2.4–3.0 times faster than did its respective control basket. The *t*-test showed that baskets receiving *Microcladia* gained weight significantly ($p = 0.002$) faster than did control baskets. The monthly percent weight gain of 153% observed in the *Microcladia* basket of Pair 4 was the fastest that we had ever measured in the basket system.

Survival

Table 2 shows the survival rate observed in each of the eight baskets. Survival was good in all baskets but was especially good in the *Microcladia* baskets. In fact, the *t*-test showed that *Microcladia* significantly increased the survival rate ($p = 0.015$). Thus, the increased population weight gain (Table 1) observed in the *Microcladia* baskets was partly due to increased survival.

Shell Growth

However, most of the increased weight gain in these *Microcladia* baskets was due to increased growth rates of the surviving abalone, as Table 3 demonstrates. The feeding treatment had a highly significant effect ($p = 0.00006$) on the mean shell growth rate occurring in a basket.

The *Microcladia*-fed populations grew at mean rates of 1.58–2.01 mm/mo, depending on the pair. The *Microcladia* basket in Pair 3 had the slowest growth of the four *Microcladia* baskets. This basket had one small *Cancer* crab in it for several weeks. This crab caused some of the abalone to crawl out of the water every night. Removal of the crab remedied the situation. The relatively slow growth in this basket may have been caused by the presence of this crab. Overall, however, the *Microcladia*-fed baskets had rapid growth, averaging 1.79 mm/mo. This growth rate was 74% faster than that of the controls.

TABLE 2.

Survival.

Treatment	Pair 1	Pair 2	Pair 3	Pair 4	Mean
Control	92.9	91.4	90.0	86.2	90.1
Microcladia	98.8	97.5	102.5	98.0	99.2

TABLE 3.

Shell growth (mm/mo).

Treatment	Pair 1	Pair 2	Pair 3	Pair 4	Mean
Control	1.01	1.09	0.79	1.21	1.03
Microcladia	1.71	1.87	1.58	2.01	1.79

Uniformity of Growth

Another important parameter is the CV of the final shell lengths (Table 4). This measures how uniformly the populations grew. The CV of the *Microcladia* baskets averaged slightly less than did that of the controls, although this difference was not significant.

Cost-Benefit Calculation

Each *Microcladia* basket received a total of 11.6 kg of *Microcladia* during the 4 mo of the experiment. Approximately 1 man hour was needed to collect 1 kg of *Microcladia*. Thus, at $6.00/h, the *Microcladia* cost about $70.00 per basket. If we assume that the abalone were worth 2.0 cents/mm, then we can estimate the dollar value of the increased production occurring in each basket receiving the *Microcladia*. On average, a *Microcladia* basket yielded 17,747 more mm of abalone than did the average control basket. This number of millimeters was worth approximately $355.00. Thus, the benefit greatly exceeded the cost.

DISCUSSION

This study demonstrated that a *Microcladia* supplement strongly enhanced the growth, survival, and overall productivity of juvenile red abalone populations in our commercial basket system. The supplement produced a rate of population weight gain equal to 2.6 times that of the conventional *Macrocystis* controls. This red algal supplement resulted in shell growth averaging 1.8 mm/mo, which was 74% faster than that of the controls (1.0 mm/mo). *Microcladia* is therefore the best supplement found thus far for our 8–15 mm abalone. Recent work in our nursery suggests that *Microcladia* is an excellent alga for younger (5–8 mm) abalone as well.

The *Microcladia* supplement caused the abalone to produce shells with the same bright color, predominantly red, that is characteristic of wild *Haliotis rufescens*. Although red algae are known to be preferred and superior to brown algae by abalone from the southern hemisphere, *H. rufescens* is thought to prefer brown algae such as *Macrocystis* (Shepherd and Steinberg 1992). Perhaps red algae such as *Microcladia*, although usually a small component of the diet of *H. rufescens*, nevertheless play a disproportionately important role in this species' health and nutrition.

One potential problem of using *Microcladia* in production systems was seen in Pair 3 of this study: the inadvertent introduction of young predatory crabs with the algae. A method needs to be developed to ensure that the alga is free of such pests. To this goal,

TABLE 4.

CV in final shell lengths.

Treatment	Pair 1	Pair 2	Pair 3	Pair 4	Mean
Control	45.39	55.09	48.88	37.46	46.71
Microcladia	51.16	38.92	22.14	40.84	38.27

rinsing the alga in fresh water might be tested. Another problem is that *Microcladia* abundance fluctuates seasonally and can at times be difficult to collect. Culturing this species could potentially solve both of the above problems. Work therefore needs to be done to develop culture methods for this species.

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ROTATION DIETS: A METHOD OF IMPROVING GROWTH OF CULTURED ABALONE USING NATURAL ALGAL DIETS

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ABSTRACT The effect of rotating different algae in the diet of cultured South African abalone *Haliotis midae* was investigated. A diet rotation feeding scheme was designed to use less abundant algae, as well as the abundant kelp *Ecklonia maxima*. Rotation diets entailed feeding abalone on *Ecklonia* for 11 days of a 2-wk rotation cycle and on one of the less abundant algae species, or a mixture of the less abundant algae species, for the remaining 3 days. Six rotation diets and six single-species control diets were tested simultaneously over a 6-mo period. Shell length and body weight growths were ranked highest for the single-species diet *Porphyra*, but rotation diets were placed in the subsequent four ranking positions. Shell length growth rates varied between 27 and 38 μm day^{-1} for abalone fed rotation diets and between 15 and 53 μm day^{-1} for those fed single-species diets. Body weight growth rates varied between 34 and 55 mg day^{-1} for abalone fed rotation diets and between 9 and 74 mg day^{-1} for those fed the control diets. Abalone fed on rotation diets, generally, had a better body weight-to-shell length ratio.

KEY WORDS: abalone, diet rotation, algae, growth

INTRODUCTION

Dietary research on abalone has focused on the natural diet of wild abalone, single-species diets in culture, and more recently, the production of an artificial diet. Research into the natural diet of the South African abalone, for example Barkai and Griffiths (1986) has shown that abalone feed on a broad selection of algae, normally with at least two species being found in the gut content at any time. Preferences certainly do exist, with red algae usually being favored by a number of different abalone species (Tutschulte and Connell 1988, Shepherd and Steinberg 1992, Stepto 1993, Fleming 1994).

Originally, research focused on single-species diets in an attempt to isolate algae species producing the highest growth rates and feed conversion efficiencies. Sakai (1962) fed abalone on 12 single-species algal diets. Kikuchi et al. (1967) fed 20 different algae to abalone. Uki et al. (1986) carried out the largest algal dietary study, testing the dietary value of 57 species of algae, and grouped the algae into four ranks on the basis of the results of their study and previous studies.

Other studies, testing the feasibility of abalone culture, have also focused on single-species diets. A few have added a mixed diet to compare with single-species diets (Owen et al 1984, Cook and Claydon 1991, Day and Fleming 1992, Fleming 1995). The general trend from those studies indicates that mixed diets produce better growth rates than do single-species diets. Day and Fleming (1992) state that although an alga may not support sustained growth when fed singly, it may be of great value when part of a mixed diet, providing essential nutrients to the diet. Mixed diets are expected to sustain growth rates, whereas growth rates on single-species diets tend to decrease over time (Day and Fleming, 1992). This study was designed to investigate the possibility of using many different algae in the diet of cultured *Haliotis midae*, instead of just the most abundant algae.

MATERIALS AND METHODS

Abalone were fed for 6 mo by a diet rotation scheme. The rotation scheme entailed feeding a primary alga (the most abundant kelp species) for approximately 80% of the time and one of five secondary algae (less abundant species) for the remainder of each 2-wk rotation period. A rotation diet with a mixture of secondary algae was also tested. Control diets were used to determine the effect of secondary alga diets when fed as single-species diets. The decision to use a rotation scheme instead of mixing the algae is based on the argument that in this way a farmer has greater control over the time or amount of feeding done on a particular alga.

Test Animals

Four hundred eighty hatchery-reared animals of approximately 2 y of age, 43.68 (±0.59) mm shell length, and 13.24 (±0.55) were used to test the growth response of abalone fed on six diet rotation schemes and the six concurrently run control diets. Groups of 20 unmarked abalone were housed in 5-L transparent plastic jars, which were supplied with temperature-controlled (18.5 ± 1 C), fresh, sand-filtered water (12 L h^{-1}). The water was aerated vigorously. The six rotation diets and six control diets were tested in duplicate ($n = 2$).

The abalone were measured every 2 mo over a period of 6 mo. Shell length was measured with a Sylvac digital vernier caliper, and body weight was measured with a Sartorius balance.

Growth rates were calculated by the following equations:

$$\text{BWGR} = (W_1 - W_0)/t$$

$$\text{SLGR} = (L_1 - L_0)/t$$

where W_0 = mean initial weight, W_1 = mean final weight, L_0 = mean initial length, L_1 = mean final length, and t = time in days.

Diets

Ecklonia maxima was selected as the primary alga species because it is found in abundance along the coast of South Africa (Simons 1990), making it the most economical feed for commercial abalone culture. The secondary algae species are less abundant but are present in sufficient quantity to make up a small portion of

the diet of cultured abalone. The secondary algae were as follows: *Laminaria pallida, Porphyra capensis, Ulva* spp., *Aeodes orbitosa,* and *Gracilaria verrucosa.*

The diet rotation scheme cycle was repeated every 14 days. The primary species was fed for 11 days, and secondary species were fed for 3 days. Secondary species were fed singly or in a mixture. When a mixture was used, it was composed of *Porphyra, Ulva* and *Aeodes*, supplied in equal mass amounts. Feeding occurred every 3 or 4 days, at which time, old algae were removed and the containers were cleaned. Each algal species used in the rotation diets was fed as a single-species diet in the controls.

Feed Conversion Efficiency, Consumption, and Mass/Length Ratio

Feed consumption was measured with rotation diets and the control diets. Feed consumption was measured over three-night (Friday to Monday) and four-night (Monday to Friday) periods to reduce variation resulting from erratic feeding patterns (pers. obs.). Daily feed consumption was calculated as a percentage of body weight.

Feed conversion efficiency (FCE) was only calculated for abalone groups fed single-species diets. It is not meaningful to calculate FCEs on rotation diets because at least two different algae were consumed.

Feed consumption and conversion efficiency were calculated by the following equation:

$$\text{FCE} = (\text{growth/ration}) * 100$$

where growth is the wet body weight gain per day and the ration is the wet feed intake per day, calculated from consumption of diets over three- and four-night periods.

The body weight-to-shell length ratio (BW/SL) was calculated for control diets and rotation diets. For each diet trial, the final mean mass was divided by the final mean length. The BW/SL ratio gives an indication of the flesh volume per unit shell length resulting from feeding on a particular diet treatment.

Statistical Analysis

The effect of the interaction between diet and time on mean shell length was analyzed by a two-way analysis of variance (ANOVA) for the effects of diet and time (Sokal and Rohlf 1969, Zar 1984). A Tukey's HSD multiple range test was applied to test for differences between mean abalone size (shell length) on different diet treatments at each time and growth rate.

ANOVA was performed on consumption rates. Consumption rates for control and rotation diets were analyzed separately because of the different method of feeding.

RESULTS

A significant increase in shell length and body weight was detected, over time, for all diets, by the two-way ANOVA. All diets showed positive growth rates (Figs. 1 and 2), with the exception of *Ulva* and *Gracilaria*, which yielded a decrease in body weight over the first 2-mo period, after which their growth rates became positive.

Variation in growth was low among rotation diets but high among the single-species diets. Shell length growth rates ranged between 0.027 and 0.038 mm day^{-1} on rotation diets and between 0.015 and 0.053 mm day^{-1} on the single-species diets. Body weight growth rates were between 43 and 55 mg day^{-1} on rotation diets and between 9 and 74 mg day^{-1} on the single-species algal diets (Table 1).

Statistical analysis of shell length by a two-way ANOVA could only detect differences between treatments ($p < 0.05$) after 4 mo. At 4 mo, mean abalone shell length for the single-species diet of *Ulva* was significantly less than that on most rotation diets, with the exception of the rotation diets *Ecklonia* with *Laminaria* and *Ecklonia* with *Ulva*. Mean shell length of abalone fed *Ulva* was also significantly lower than that for abalone fed single-species diets of *Porphyra* and *Ecklonia* (Fig. 1). After 6 mo, the single-species diet of *Ulva* was significantly different from all diets except the combination of *Ecklonia* and *Laminaria* and the single-species diet of *Laminaria*. Shell length and body weight growth rates, over the 6-mo period, were ranked from highest (1) to lowest (12) growth rate (Table 1). Generally, the rotation diets produced higher growth rates (Table 1; Figs. 1 and 2) than did single-species diets. The exception to this was the single-species algal diet *Porphyra*, which produced the highest growth rates for both shell length (0.053 mm day^{-1}) and body weight (74 mg day^{-1}). The best rotation diets were *Ecklonia* with *Porphyra* (0.038 mm day^{-1} for shell length and 55 mg day^{-1} for body weight) and *Ecklonia* with

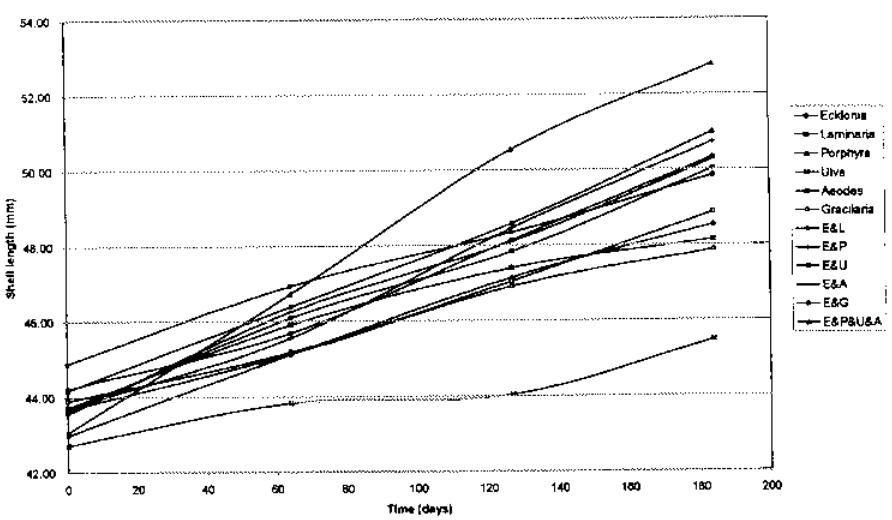

Figure 1. Shell length growth for all diet treatments over the 6-mo period.

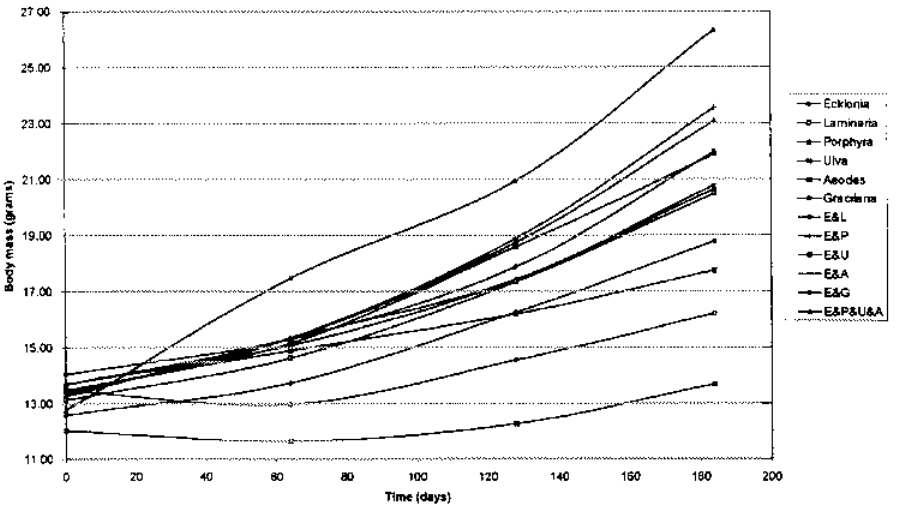

Figure 2. Body weight growth for all diet treatments over the 6-mo period.

a mixture of *Porphyra*, *Ulva*, and *Aeodes* (0.037 mm day^{-1} for shell length and 51 mg day^{-1} for body weight).

Consumption of the single-species diets ranged from 2.25% body weight day^{-1} for *Porphyra* to 5.44% body weight day^{-1} for *Ecklonia* (Table 2). *Porphyra* was consumed in significantly smaller amounts ($p < 0.05$) than *Laminaria* (4.32% body weight day^{-1}), *Gracilaria* (4.21% body weight day^{-1}) and *Ecklonia*. *Ecklonia* was also consumed in larger amounts than *Aeodes* (2.98% body weight day^{-1}) and *Ulva* (2.92% body weight day^{-1}).

Consumption of *Ecklonia* in the rotation diets was similar for all rotation treatments and the single-species treatment. Consumption rates of secondary algae varied significantly ($p < 0.05$). *Laminaria* (5.94% body weight day^{-1}) was consumed in larger amounts than all other secondary algae. Consumption of *Ulva* (0.52% body weight day^{-1}) was lower than consumption of *Porphyra* (1.53% body weight day^{-1}) and *Aeodes* (1.16% body weight day^{-1}) in the rotation diet with the mixture of secondary algae.

FCEs were calculated for single-species diet treatments only. FCE ranged from 0.025 for abalone fed on *Gracilaria* to 0.17 for those fed on *Porphyra*. The feeding scheme of rotation diets, with two or more algae being eaten, does not allow for meaningful calculation of FCE ratios.

The BW/SL ratio was calculated for each diet trial using the final mean body weight and shell length. In general, BW/SL ratios were higher for rotation diets than for the single-species diets (Fig. 3). The diet treatment of *Porphyra* had the highest BW/SL ratio, whereas the diet treatment of *Ulva* had the lowest BW/SL ratio. The range of single-species diet BW/SL ratios (0.301–0.499 g mm^{-1}) was greater than the range of rotation diet BW/SL ratios (0.387–0.464 g mm^{-1}).

DISCUSSION

E. maxima is the most abundant algae species found along the Southwest and West Coast of southern Africa (Simons 1990). This alga forms extensive kelp beds, extending up to 3 km off shore, and has a biomass turnover three times per annum (Field et al. 1977). Because of this abundance, *Ecklonia* would be the most likely source of feed for any abalone farms developed along this area of the coast. However, *Ecklonia* produces abalone growth rates that are acceptable but not exceptional (Cook and Claydon 1991, Simpson 1992). Numerous other species of algae occur along the coast but are not available in sufficient quantity for use as a single-species diet on an abalone farm (Simons 1990).

TABLE 1.

Mean initial size (mm, g) and daily growth rate (mm day^{-1}, g day^{-1}) over the 6-mo growth period, rank, and BW/SL ratio for rotation and control diets.

Diet Description	Mean Initial Size (SD) (mm) (n = 40)	Mean Final Size (SD) (mm) (n = 40)	Growth Rate (mm day^{-1})	Tukey HSD Test	Rank BW/SL	Final	Mean Initial Size (SD) (g) (n = 40)	Mean Final Size (SD) (g) (n = 40)	Growth Rate (g day^{-1})	Tukey HSD Test	Rank
Ecklonia	44.23 (4.01)	50.33 (4.60)	0.033	bcd	6	0.410	13.70 (4.28)	20.58 (5.37)	0.037	cde	8
Laminaria	43.90 (4.51)	48.87 (4.99)	0.027	abcd	8	0.420	13.13 (4.12)	20.56 (6.03)	0.040	cde	6
Porphyra	43.07 (3.64)	52.80 (4.85)	0.053	e	1	0.499	12.78 (3.27)	26.33 (7.14)	0.074	e	1
Ulva	42.72 (3.69)	45.48 (4.02)	0.015	abcd	12	0.301	12.04 (3.40)	13.69 (3.83)	0.009	a	12
Aeodes	43.72 (3.79)	48.14 (4.33)	0.024	abcd	10	0.368	13.48 (4.47)	17.74 (5.63)	0.023	abc	10
Gracilaria	43.67 (4.07)	47.87 (4.26)	0.023	abcd	11	0.338	13.47 (3.97)	16.20 (4.24)	0.015	abc	11
Ecklonia + *Laminaria*	42.99 (3.58)	48.54 (4.83)	0.030	bcd	7	0.387	12.59 (3.28)	18.78 (4.94)	0.034	bcd	9
Ecklonia + *Porphyra*	43.71 (4.39)	50.73 (5.37)	0.038	d	2	0.464	13.36 (4.51)	23.55 (6.83)	0.055	ef	2
Ecklonia + *Ulva*	43.61 (4.77)	50.02 (5.54)	0.035	bcd	5	0.438	13.43 (4.45)	21.89 (7.17)	0.046	de	4
Ecklonia + *Aeodes*	43.56 (6.50)	50.26 (5.04)	0.036	bcd	4	0.414	13.27 (4.21)	20.62 (6.06)	0.040	cde	6
Ecklonia + *Gracilaria*	44.89 (3.17)	49.84 (6.14)	0.027	abcd	8	0.441	14.05 (3.41)	21.98 (4.96)	0.043	de	5
Ecklonia + *Porphyra/Ulva/Aeodes*	44.19 (3.06)	50.99 (4.07)	0.037	cd	3	0.453	13.69 (3.39)	23.08 (6.09)	0.051	de	3

a–e =

TABLE 2.

Consumption rates (wet weight) in grams per abalone per day and as a percentage of body weight per day, and FCE, expressed here as the ratio (single-species diets only). Rotation diet consumption rates are given for the primary (*Ecklonia*) and secondary diets in different columns.

Diet Description	Consumption (g abalone^{-1}/ day^{-1})	Standard Deviation	Consumption (% Body Weight/ day^{-1})	FCE	*Eklonia* Consumption (g abalone^{-1}/ day^{-1})	Standard Deviation	Consumption (% Body Weight/day $^{-1}$)	Other alga Consumption (g abalone^{-1}/ day^{-1})	Standard Deviation	Consumption (% Body Weight/ day $^{-1}$)
Single-species diets										
Ecklonia	0.875	(0.244)	5.44	0.042						
Laminaria	0.701	(0.221)	4.32	0.057						
Porphyra	0.434	(0.117)	2.55	0.17						
Ulva	0.348	(0.059)	2.92	0.026						
Aeodes	0.453	(0.129)	2.98	0.051						
Gracilaria	0.588	(0.279)	4.21	0.025						
Rotation diets										
Ecklonia + *Laminaria*					0.907	(0.046)	6.6	0.929	(0.24)	5.94
Ecklonia + *Porphyra*					0.956	(0.09)	6.23	0.726	(0.083)	4.19
Ecklonia + *Ulva*					1.048	(0.138)	6.92	0.36	(0.066)	2.06
Ecklonia + *Aeodes*					0.818	(0.108)	5.31	0.298	(0.05)	1.79
Ecklonia + *Gracilaria*					0.954	(0.147)	6.3	0.449	(0.234)	2.56
Ecklonia + *mixture*					0.785	(0.103)	5.13			
+ *Ulva*								0.089	(0.03)	0.52
+ *Porphyra*								0.258	(0.021)	1.53
+ *Aeodes*								0.197	(0.022)	1.16

Previous studies on other *Haliotis* species, as well as other molluscs, have shown that mixed diets produce better growth rates than do single-species diets (e.g., Owen et al. 1984, Enright et al. 1986, Day and Fleming 1992, Fleming 1994a). The rotation diets tested in this study are similar to mixed algal diets. The difference is that in rotation diet schemes, the different algae are provided separately. This technique results in easier regulation of the feeding by abalone on each alga. The single implementation of each alga is important because abalone usually only consume preferred algae in a mixed diet.

Stepto (1993) found that *E. maxima* was the least preferred of the three algae fed to *H. midae* and suggested that this may be because of high phenolic levels. In a mixed diet, it is likely that *Ecklonia* would be avoided, which would negate the purpose of feeding *Ecklonia* as part of the diet.

Growth rates of abalone fed on rotation diets were generally higher than those of abalone fed single-species diets. The exception to this is *Porphyra*, which produced higher growth rates than any other diet. After ranking of growth rates on all diet treatments, the better rotation diets were placed in Positions 2–5 for both shell length and body weight growth rates.

The single-species diets, *Ulva*, *Aeodes*, and *Gracilaria*, produced poor growth rates, whereas *Porphyra* and *Ecklonia* produced good growth rates. The growth rates achieved on rotation diets were similar between diets, and mean shell length could not be shown to differ significantly ($p > 0.05$) between diets after 6 mo.

Duncan and Klekowski (1975) stated that essential substances might become limiting when animals are fed one type of food in long-term experiments. This results in decreased growth rates.

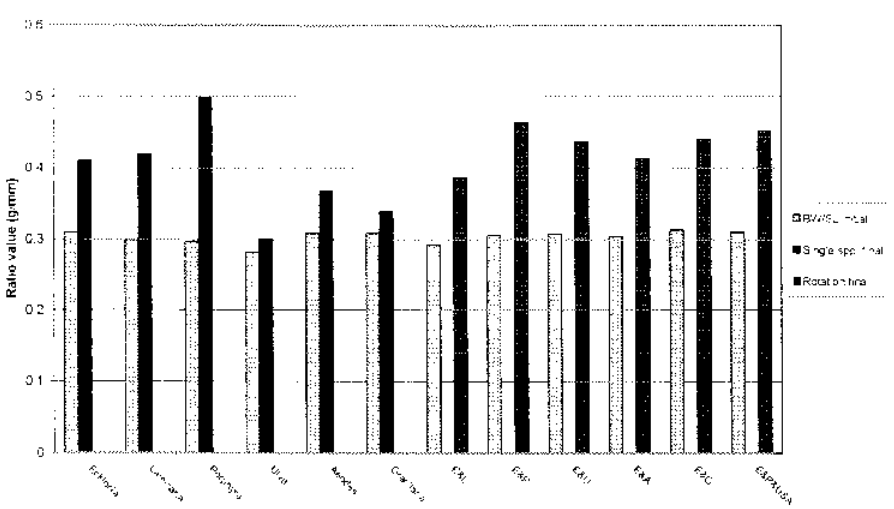

Figure. 3. BW/SL ratios for all diet treatments at the end of the 6-mo diet trial.

Growth rates on single-species diets remained constant throughout the 184-day period. Continued single-species feeding would likely have resulted in decreased growth rates (Day and Fleming 1992). Feeding trials on *Haliotis rubra* using single species of dried algae revealed that abalone cease to grow after a period ranging from 50 to 200 days (Day and Fleming 1992). The rapid effects shown by *H. rubra* may be due to the drying of algae before feeding. It is possible that this study was conducted for an insufficient time for dietary deficiencies on single-species diets to materialize.

Duncan and Klekowski (1975) stated that it is rare for an animal to feed on the same food for its entire life. Wile *H. midae* were found with a variety of algae in their guts (Newman 1968, Barkai and Griffiths 1986, Barkai and Griffiths 1987). This suggests that they obtain the required nutrients for growth by selecting a mixed diet (Day and Fleming 1992). Fleming (1994a) suggested that preference for certain algae might be due to the presence of essential nutrients not available in other algae.

Although certain algal species (for example, *Ulva, Aeodes*, and *Gracilaria*) constitute a poor diet when supplied singly, they may be of great value when supplied as part of a mixed diet, thereby supplying essential nutrients to the diet (Day and Fleming 1992). This was observed for the algae *Ulva* and *Aeodes*, which produced better growth rates when fed as part of a rotation diet. Results demonstrated that combinations of low-quality algae often produce good growth rates.

Shell length growth rates of abalone fed on rotation diets remained constant over time, but the body weight growth rates showed a definite increase over time. The exponential increase in body weight was not as pronounced on single-species diets.

Rotation diets appear to have a greater ability to increase the ratio of body weight to shell length. BW/SL rations are important in that they indicate the mass of abalone per unit shell length. Thus, at marketable size (e.g., 80 mm), the value of an abalone, priced by weight, will be dependent on the BW/SL ratio. Certain diets will, therefore, produce more valuable abalone.

Previous studies on other *Haliotis* species, where mixed diets have been tested, have usually consisted of three or more algae species (Owen et al. 1984, Fleming 1994a). One of the rotation diets tested in this study consisted of three secondary algae species, which were fed together. Shell length and body weight growth rates on this diet were higher than on other rotation diets (except *Ecklonia* rotated with *Porphyra*), but there was no significant difference ($p > 0.05$). Abalone fed the rotation diet with a mixture of secondary algae species were able to select preferred algae. This was observed by the difference in the consumption rate of *Porphyra, Ulva*, and *Aeodes* in this treatment. The high growth rate suggests that the abalone may have selectively fed on particular algae to fulfill their nutritional balance requirements. Fleming (1994b) found that *H. rubra* selected foods that maximize the intake of nitrogen and subsequently lead to maximum growth rates.

In rotation diets, it is likely that the level of consumption of the secondary algae is related to the nutritional deficiencies present in the primary algae. The secondary alga, *Porphyra*, is eaten at 4.19% body weight day^{-1} when part of a rotation diet, twice that when fed as a single-species diet. It is possible that these increased quantities consumed make up for nutritional deficiencies in the primary algae (*Ecklonia*). *Ulva, Aeodes*, and *Gracilaria* are consumed at lower levels as part of a rotation diet, suggesting that these algae are not nutritionally as good as *Ecklonia*. This is supported by the low growth rates of abalone fed on single-species diets of *Ulva, Aeodes*, and *Gracilaria* and the low FCEs on *Ulva* and *Gracilaria*. Although *Ulva, Aeodes*, and *Gracilaria* are inferior diets, they provide essential nutrients when fed as part of the rotation diet. Thus, growth rates of abalone fed on rotation diets with *Ulva, Gracilaria*, and *Aeodes* were higher than growth rates of abalone fed either of these, or the primary alga, singly.

Growth rates achieved on the rotation diet of *Ecklonia* and *Laminaria* were low. This is probably because of the similar nutrient content of these algae, which are both *Phaeophyta*. This suggests that secondary algae in rotation diets should be from a different family from the primary alga. For example, brown algae should be fed in combination with red or green algae and not other brown algae.

Ideally, in a rotation diet, the poorer diet should be fed for a shorter period of time. Poorer algae should be fed for sufficient time for uptake of essential nutrients but not long enough to retard growth rates. *Ulva, Aeodes*, and *Gracilaria* should be fed for a short period of time. On the other hand, the period of feeding on *Porphyra* should be extended, for as long as supplies allow, to increase growth rates on that particular rotation diet.

CONCLUSIONS

This study demonstrates that the rotation diets tested generally produce higher growth rates than do their component single-species diets. Single-species diets are often limiting in one or more nutrients. Feeding on single-species diets for long periods of time often results in decreased growth rates (Day and Fleming 1992). Rotation diets (and mixed diets) provide a wider base for the uptake of essential nutrients. By using the different nutrient compositions of different algae, rotation diets ensure that abalone receive a balance of the required nutrients. Growth rate can be improved by regulating the intake of each alga. This is done by controlling the length of time abalone are fed on each alga.

Ecklonia is the most abundant alga along the South to West Coast of South Africa and is likely, therefore, to form the major part of cultured abalone diets in this area. Growth rates on *Ecklonia* can be improved by addition of either *Porphyra, Ulva*, or *Aeodes*, or a mixture of all three, in a rotation diet.

Growth rates on a particular algal combination may vary with location (pers. obs.). For farming purposes, it is important that trials of different diets be undertaken at all new locations. It is also likely that altering the levels of the primary and secondary algae in a rotation diet will produce different growth results. Hence, having isolated the best rotation mixture, different levels of primary and secondary algae should be tested.

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COMPLEMENTARY ADDITIVITY OF THE DIGESTIBILITY COEFFICIENTS OF FEED INGREDIENTS FED TO JUVENILE GREENLIP ABALONE (*HALIOTIS LAEVIGATA*)

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ABSTRACT When least-cost feeds are formulated, it is assumed that when a range of ingredients are combined in a compound feed, they will behave as if they were fed separately, that is, it is assumed that their nutritive value is additive. This is the basis of least-cost linear computer programs. However, experiments conducted on pigs and rats have revealed that the inclusion of dietary fiber in manufactured feeds may result in a depression of the digestibility coefficients of the constituent ingredients. Thus, feeds that are formulated by adding the digestibility coefficients of each constituent ingredient, derived by the direct measurement of the digestibility of each ingredient when incorporated into base diets, may lead to an overestimation of the digestibility of nutrients in the manufactured feed. A series of isonitrogenous diets containing semolina, fishmeal, barley, lupin kernel meal, and various combinations of these ingredients were fed to juvenile greenlip abalone (*Haliotis laevigata*) to determine the apparent fecal digestibilities of N, amino acids, and gross energy. The digestibility coefficients of the single-ingredient diets were used to predict the digestibility of N, amino acids, and gross energy in casein in the mixed diets. The calculated digestibility values for casein did not differ significantly between diets. Thus, the apparent fecal digestibilities of N, amino acids, and gross energy of the constituent ingredients were not affected by varying ingredient combinations in the diets. Apparent fecal digestibility values for the chosen ingredients are additive when they are incorporated into compound feeds for abalone and so are good descriptors of nutritive value and can be used with confidence in diet formulations.

KEY WORDS: abalone, manufactured feeds, digestion, fiber, amino acids, energy

INTRODUCTION

Emphasis is placed on the efficiency of production of farmed animals to ensure commercial viability. From a nutritional perspective, maximum efficiency is attained by matching diet specifications as closely as possible to the animal's requirements for the least possible cost. In the case of abalone, this must be achieved while maintaining a diet form that is stable underwater. Commercial nutritionists generally undertake the following procedures in an attempt to achieve maximum production efficiency: (1) the nutritional requirements of the animal are defined, (2) the nutritive value of individual ingredients to be used in the compound feeds is defined by applying previously determined digestibility or availability coefficients to estimates of the chemical composition, and (3) least-cost diets are formulated, by linear programming techniques, on the basis of the above information. The nutritive value of ingredients is often expressed in terms of digestible amino acids (when available amino acid values are not available) and digestible energy (although net energy is a more accurate estimate). Thus, when formulating feeds, digestibility coefficients for nutrients in feedstuffs are usually given for each ingredient separately. Definition of the digestibility of N, gross energy, and some amino acids in ingredients commonly used in manufactured feeds for the Australian greenlip abalone (*Haliotis laevigata*) has enabled diet costs to be significantly reduced and the growth performance of abalone to be enhanced. Diet mixtures, however, in most cases contain two or more ingredients, and the question is, therefore, whether digestibility of these mixtures can be calculated on the basis of the digestibility coefficients of the individual components. The basic assumption is made that individual diet ingredients will have the same nutritive value when they are combined in mixed diets as when they are fed individually—that is, it is assumed that their nutritive value is additive.

The significant effect of dietary fiber, specifically the nonstarch polysaccharides (NSP), on the efficiency of use of the protein and energy components of monogastric diets has been well documented for chickens (Annison 1990, Annison 1991, Annison 1992, Choct and Annison 1992), growing rats (Hansen et al. 1991, Zhao et al. 1996), growing pigs (Bach Knudsen and Jensen 1993a, Bach Knudsen et al. 1993b, van Barneveld 1996), and prawns (Sarac et al. 1993). NSP are the major components of fiber in feedstuffs, with cellulose being the most abundant of these. Other forms of noncellulosic NSP can be classified as hemicellulose, pectic substances, and a third group of more water-soluble polysaccharides including gums and mucilages. The absence of endogenous NSP-degrading enzymes and the low density of microorganisms in the

small intestine of monogastrics mean that NSP pass to the hind gut for degradation by microorganisms. Apart from the low digestibility and utilization of NSP by monogastrics, the viscous nature of soluble NSP and their physiological and morphological effects on the digestive tract are thought to be detrimental to the digestion of nutrients (Choct et al. 1992). The mechanisms include altered intestinal transit time (Gohl and Gohl 1977) and modification of the intestinal mucosa (Johnson and Gee 1981, Ikegami et al. 1990).

Hansen et al. (1991) compared the digestibility coefficients of various ingredients fed individually and in combination when fed to growing rats. They found that digestibility coefficient values in mixtures could be calculated with high precision from digestibility coefficients from individual ingredients, but cautioned that mixtures containing high levels of soluble dietary fiber, such as peas and barley and peas and wheat, produced significantly higher values for the calculated digestibility coefficient compared with the measured digestibility coefficient. This suggests that combinations of ingredients with high levels of soluble NSP may result in a further reduction in the digestibility of specific nutrients, including protein and energy. When different sources of fiber are combined in mixed diets, it appears that the properties of the resulting combination of fiber do not necessarily resemble those of the constituents. Laplace et al. (1989) investigated the associative effects between wheat bran and soybean hulls in semipurified diets on the ileal and overall digestibilities of amino acids, energy, and cell-wall components in growing pigs. The overall digestibility of crude protein and all amino acids was significantly less in diets containing wheat bran and soybean hulls compared with those that contained either wheat bran or soybean hulls.

If combinations of NSP have different properties than do their constituents, resulting in a further depression of amino acid and energy digestibility, it is likely that the cause for reduced digestibility is increased gut digesta viscosity rather than a change in microbial activity. van Barneveld et al. (1995) reported that growing pigs fitted with ileal cannulas and fed diets containing graded levels of a lupin NSP isolate experienced a significant linear decrease in lysine and energy digestibility. This coincided with a significant linear increase in digesta viscosity, but there was no significant effect on the microbial activity in the small intestine, the large intestine, or the cecum.

In Australia, investigations are currently underway to compile a comprehensive database on the nutritive value of a broad range of local, readily available ingredients that have potential for inclusion in abalone diets, particularly grains and legumes. It is hypothesized that when NSP from legumes is combined with NSP from various cereals, the effects on the digestibility of N, amino acids, and energy will be emphasized and will vary depending on the ingredients. To examine these effects, digestibility experiments were conducted on the basis of diets consisting of a NSP-containing cereal (barley) mixed with a high-NSP legume (kernel of *Lupinus angustifolius*) and low-NSP protein sources (fishmeal and semolina). Thus, the aim of this experiments was to test the assumption that the digestibility of (1) N, (2) amino acids, and (3) gross energy derived from single-ingredient diets is additive when these ingredients are mixed in formulated diets for abalone.

MATERIALS AND METHODS

Diets

Eleven test diets were formulated (Table 1). Five single-ingredient diets contained either barley, semolina, fishmeal, *L. angustifolius* (lupin kernel meal), or casein as the sole protein source. The inclusion level of each ingredient was adjusted to ensure that the single-ingredient diets were isonitrogenous. Four mixed-ingredient diets contained either barley or semolina mixed with either fishmeal or lupin kernel meal. Two mixed diets contained barley and semolina mixed with either lupin kernel meal or fishmeal. The level of casein in the mixed diets was adjusted to

TABLE 1.

Composition of experimental diets (g/kg, air-dry basis).

	Single Diets					Mixed Diets					
Ingredient	**1**	**2**	**3***	**4***	**5***	**6**	**7**	**8**	**9**	**10**	**11**
Barley†	951.8					400		400		200	200
Semolina		766.9					400		400	200	200
Fishmeal			700 (117.5)			300	300			300	
Lupin kernel meal‡				700 (221.8)				300	300		300
Casein					700 (88.4)	51.7	42.8	158.0	149.0	47.4	153.5
Pregelled starch	12.4	197.3	264.2 (846.7)	264.2 (742.4)	264.2 (875.8)	212.5	221.4	106.2	115.2	216.9	110.7
Jack Mackerel Oil	20	20	20	20	20	20	20	20	20	20	20
Mineral premix§	2	2	2	2	2	2	2	2	2	2	2
Vitamin premix§	3	3	3	3	3	3	3	3	3	3	3
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E¶	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium alginate	5	5	5	5	5	5	5	5	5	5	5
Chromic oxide	5	5	5	5	5	5	5	5	5	5	5
n-Hexatriacontane	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

* Diets 3, 4, and 5 were reformulated after the first collection period because inadequate feces were produced. Values presented in parentheses are the inclusion levels before reformulation.

† Clean chebec barley.

‡ *L. angustifolus* cv. *gungurru*.

§ Vitamin and mineral premixes as described by Uki et al. (1985).

¶ Microvit E Promix 50 (encapsulated).

‖ CVCF-90 Aquaculture.

ensure that they were isonitrogenous. Pregelled starch was used in all diets as an inert, low-fiber filler and also acted as a binder. Chromic oxide and *n*-hexatriacontane were included as indigestible markers in all diets for use in subsequent digestibility calculations. *n*-Hexatriacontane was added by dissolving in warm oil before addition to the diet. Vitamin and mineral premixes were as described by Uki et al. (1985), and sodium alginate was included as a diet binder. All ingredients were finely ground when received. All diets were produced by Adam & Amos Pty. Ltd. in the form of flat, well-bound "chips" as described by Coote et al. (1996). Samples of each diet (100 g) and samples of each ingredient (100 g) were bagged and frozen at −30°C for later analysis. The proximate analysis, total amino acid content, and gross energy content of the ingredients and diets are presented in Tables 2 and 3, respectively. The diets were stored at −30°C, and each week a sufficient quantity was thawed and stored at room temperature.

Abalone Management and Feces Collection

Diet allocation

Each dietary treatment was allocated to one of 11 digestibility tanks over three separate collection periods to provide three replicates per diet based on a completely randomized design. To randomize tank effects, diets were randomly assigned to digestibility tanks but were not assigned to a tank if it already contained that diet in a previous collection period. The collection periods were between 16 and 20 days with each collection period beginning within a few days of the previous one being completed. Animals feeding on the Single Diets, 3, 4, and 5 produced very little feces during the first collection period, and so these diets were reformulated to reduce the amount of pregelled starch and increase the level of the single-protein source to 700 g/kg (Table 1).

Tanks and Collection System

Conical tanks were fitted with fecal collection and drainage plumbing (Fig. 1). A bucket (20-L capacity) with a mesh bottom (4-mm-pore-size mesh) was used to contain the abalone while permitting feces to drop into the collection tube. A number of 25-cm lengths of 80-cm polyvinylchloride pipe with numerous holes were supplied as shelters. Attached to the bottom of each digestibility tank was a screw-on collection tube (11 cm long, 15 mm in diameter) and a tap used to empty the tank before cleaning. Seawater flowed into the top of each tank at a rate of about 2 L/min. Seawater was filtered to 30 μm by primary sand filters and then to 10 μm by secondary composite sand filters. The outlet was positioned about 5 cm below the top edge of each tank. Aeration was supplied (0.5 L/min) to each tank at all times by an air stone. The water temperature during the experiment was 18 ± 0.2°C. The lights were turned off at 6 p.m. and on at 6 a.m. (12D:12L).

Abalone and Feeding

Each bucket contained between 60 and 80 juvenile (shell length, 40–60 mm, 70 g wet weight) *H. laevigata*. These abalone

TABLE 2.

Proximate analysis, total amino acid content (g/kg, air-dry basis), and gross energy content (MJ/kg, air-dry basis) of barley, semolina, fishmeal, lupin kernel meal, casein, and pregelled starch used in experimental diets.

Component	Diet Ingredient					
	Barley*	Semolina	Fishmeal	Lupin Kernel Meal†	Casein	Pregelled Starch
Dry matter	901.9	863.3	899.9	908.4	893.3	939.9
Crude protein (N × 6.25)	78.8	97.8	638.3	338.1	832.2	5.5
Ash	23.7	2.8	151.0	27.8	3.1	0.40
Ether extract (bp. 40–60° C)	24.7	7.2	98.3	61.0	7.5	7.7
Fiber extract						
Crude	27.8	15.1	13.6	73.1	14.6	—
Acid detergent	90.7	21.0	10.9	123.3	—	—
Neutral detergent	305.4	18.8	350.7	217.4	4.9	—
Gross energy (MJ/kg)	16.44	15.60	18.56	18.54	21.48	16.22
Amino acids						
Aspartic acid	3.21	3.40	51.05	20.14	38.37	—
Threonine	1.98	2.71	27.07	7.85	25.11	—
Serine	2.32	4.43	24.55	11.16	34.81	—
Glutamic acid	12.34	30.12	85.64	49.85	133.11	—
Proline	4.77	8.05	22.38	8.88	55.03	
Glycine	2.07	2.92	32.25	8.03	9.63	—
Alanine	1.52	1.61	22.37	4.94	11.91	—
Valine	3.07	4.16	31.27	9.63	40.75	—
Isoleucine	2.33	3.70	29.53	10.45	32.41	—
Leucine	3.77	6.06	42.59	14.35	50.67	
Tyrosine	1.12	2.25	17.42	5.81	24.37	—
Phenylalanine	2.03	3.87	21.75	6.22	21.2	-
Lysine	2.23	1.85	44.94	10.26	46.11	- -
Histidine	0.87	1.61	15.51	4.19	12.08	
Arginine	2.51	2.94	32.73	20.88	19.04	—

* Clean chebec barley.

† *L. angustifolius* cv. *gungurru*.

TABLE 3.

Proximate analysis, total amino acid content (g/kg, air-dry basis), and gross energy content (MJ/kg, air-dry basis) of experimental diets.

	Single Diets					Mixed Diets					
Component	1	2	3	4	5	6	7	8	9	10	11
Dry matter	936.0	896.2	936.1	907.1	922.6	931.8	887.2	932.4	913.8	931.3	910.7
Crude protein (N × 6.25)	95.2	82.1	463.9	236.7	602.0	282.0	259.6	281.3	271.6	281.7	274.6
Ash	30.5	13.4	115.5	28.8	17.5	60.2	54.1	27.6	19.2	58.4	24.5
Ether extract (bp. 40–60° C)	45.7	22.1	85.3	66.1	24.9	52.3	31.7	28.9	25.2	33.8	27.3
Fiber extract:											
Crude	29.0	8.2	8.7	60.2	8.7	15.0	7.0	42.1	17.4	2.4	25.2
Acid detergent	63.4	25.2	43.8	73.2	13.3	29.7	14.5	54.9	40.7	27.4	48.0
Neutral detergent	86.7	182.4	434.1	180.0	20.4	251.8	178.5	154.7	106.6	184.7	118.6
Gross energy (MJ/kg)	16.82	15.76	18.61	17.52	20.26	17.77	16.78	18.09	17.66	17.59	17.62
Chromic oxide	4.5	4.8	2.1	3.7	3.4	4.4	4.6	4.5	4.3	4.4	5.1
Amino acids											
Aspartic acid	3.07	1.73	26.22	13.63	27.51	14.83	12.60	13.38	12.66	12.96	12.63
Threonine	1.93	1.41	14.07	5.42	18.27	8.36	6.70	7.33	6.94	7.26	6.94
Serine	2.35	2.40	12.58	8.21	24.90	8.42	7.05	10.31	9.96	7.68	9.97
Glutamic acid	12.02	16.41	41.98	34.00	96.57	31.51	30.35	40.41	42.84	28.80	41.47
Proline	4.32	4.41	11.48	6.39	34.90	9.99	9.45	14.36	14.04	9.21	13.11
Glycine	1.92	1.46	15.70	5.44	7.06	8.04	7.09	4.90	4.47	7.16	4.56
Alanine	1.45	0.90	13.07	3.30	8.52	6.88	5.79	4.10	3.68	5.97	3.74
Valine	2.97	2.16	16.18	6.13	29.90	11.23	9.59	11.25	10.04	9.16	9.91
Isoleucine	2.17	1.97	15.28	6.62	24.40	9.69	8.35	9.97	9.26	8.26	8.36
Leucine	3.68	3.17	21.95	9.86	37.25	14.01	12.25	14.73	13.81	12.36	13.09
Tyrosine	1.10	1.07	7.40	4.01	15.64	4.53	4.49	6.10	5.60	4.58	5.62
Phenylalanine	1.92	1.71	9.77	4.37	15.03	6.41	5.70	6.70	6.30	5.37	6.10
Lysine	2.05	1.05	24.22	6.95	32.89	13.37	11.17	10.93	10.20	11.20	9.95
Histidine	0.83	0.66	6.29	2.59	18.67	3.60	3.41	3.64	3.42	3.35	3.16
Arginine	2.40	1.67	14.72	13.69	15.08	8.22	7.20	10.19	10.00	8.12	8.68

had been raised on diatoms from settlement to about 10 mm in shell length and then grown on manufactured abalone feed containing 26% crude protein and 18 MJ/kg of gross energy. The abalone were preconditioned for 1 wk on the test diet assigned to that tank. During both the preconditioning and the experimental periods, animals were fed to slight excess daily at about 5 p.m.

Fecal Collection

On each day of fecal collection, the buckets containing the abalone were removed, the digestibility tanks were drained of water, and all fittings were cleaned of feces and uneaten feed. Abalone were out of water for about 8 min. After the cleaning, the tanks were refilled and the buckets were replaced. Collection tubes were fitted and placed on ice by 9 a.m. The feces were collected from the tubes at about 4 p.m. by gently pouring the contents onto a 1-mm-pore-size gauge mesh. Fines were discarded because of concerns that they may have leached excessively. The mesh was then placed into a petri dish and frozen at −30°C. The following day, the frozen fecal sample was scraped off the mesh, pooled into a composite sample, and frozen at −30°C before chemical analysis. The duration of each collection period (between 16 and 20 days) was determined by the time required to collect sufficient quantities of feces (about 4 g) for analysis. Before analysis, the samples were freeze dried for 3 days and ground with a mortar and pestle.

Chemical Analyses

Chemical analysis for proximates (i.e., dry matter, light petroleum extract, crude, neutral-detergent and acid-detergent fiber, and ash) were undertaken by the methods of the Association of Official Analytical Chemists (1984). Gross energy was determined by adiabatic bomb calorimetry. Chromic oxide was determined by atomic absorption spectroscopy on the basis of a modification of the methods described by Hillebrand et al. (1953). The modified methodology involved preliminary ignition of the sample at 500°C to

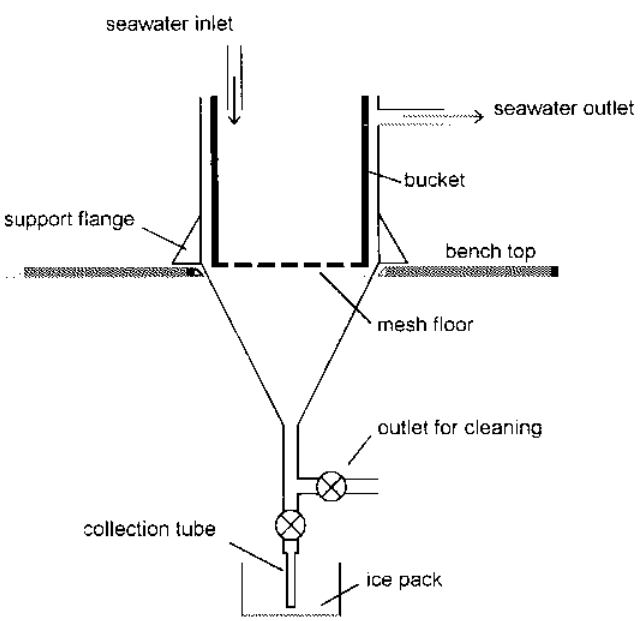

Figure 1. Diagram of the digestibility tank.

remove organic material and the dissolution of the sample in hydrochloric acid instead of sulfuric acid (M. Frith pers. comm., University of Tasmania, Launceston, Australia). Amino acids in the ingredients, manufactured diets, and abalone feces were separated by ion-exchange chromatography after a 24-h hydrolysis at 110°C with a constant boiling point 6N HCL under N_2 and were measured after reaction with ninhydrin. Norleucine was used as an internal standard, with accepted recoveries falling between ±0.025 g/kg of the batch mean. Digestibility coefficients for methionine and cystine and the content of these amino acids in the ingredients have not been reported because preoxidation of the samples with performic acid before hydrolysis was not completed (so that methionine and cystine could be reported as methionine sulfone and cysteic acid, respectively).

Digestibility Determination

The apparent digestibilities of nutrients in the single-ingredient diets were calculated with the following formula (Hardy 1997):

$$\text{Apparent Digestibility} = 1 - \frac{\text{Cr}_{\text{diet}} \times \text{Nutrient}_{\text{feces}}}{\text{Cr}_{\text{feces}} \times \text{Nutrient}_{\text{diet}}}$$

where Cr is chromium content and Nutrient is nutrient or energy content of the diet. The calculation of digestibilities in the mixed diets was based on the values obtained when the feedstuffs were given individually. In this calculation, the relative proportion from each feedstuff of the various nutrients had to be considered. This was calculated using the following formula based on van Barneveld et al. (1997):

$$\text{AD} = \frac{(\text{IL} \times \text{Nutrient}_{\text{Ingredient 1}} \times \text{AD}_{\text{Ingredient 1}}) + (\text{IL} \times \text{Nutrient}_{\text{Ingredient 2}} \times \text{AD}_{\text{Ingredient 2}})}{(\text{IL} \times \text{Nutrient}_{\text{Ingredient 1}}) + (\text{IL} \times \text{Nutrient}_{\text{Ingredient 2}})}$$

where IL is the inclusion level, AD is the apparent fecal digestibility, and Nutrient is the nutrient or energy content of the individual ingredients. These calculated digestibilities were compared with the measured digestibility coefficients for the mixed diets.

Statistical Analysis

The results were analyzed by analysis of variance of the completely randomized design, with a general linear model, and the treatment means were separated by least significant difference. Before analysis, data were analyzed by a univariate procedure and normal plot to establish that the data were in fact normally distributed, which was the case. The presence of outlying measurements was assessed by the RANK procedure in SAS (SAS Institute Inc. 1988), and normal scores were computed from the ranks based on the Blom (1958) version. Because no outlying measurements were detected by the RANK procedure, all were used in the calculation of digestibility estimates for all parameters, respectively.

RESULTS

Fecal digestibility coefficients were determined by chromic oxide analysis alone because insufficient feces were available for *n*-hexatriacontaine analysis (Table 4). Because of the need to reformulate Diets 3 and 4, only two replicates were collected for these treatments. Because of inadequate production of feces by abalone feeding on the reformulated Diet 5, no data were collected for the digestibility of nutrients in casein. Consequently, the apparent digestibility of casein was estimated with the following formula:

$$\text{AD}_{\text{casein}} = \frac{(\text{AD}_{\text{diet}} \times \text{Nutrient}_{\text{diet}}) - \Sigma(\text{IL} \times \text{Nutrient}_{\text{other ingredients}} \times \text{AD}_{\text{other ingredients}})}{\text{IL}_{\text{casein}} \times \text{Nutrient}_{\text{casein}}}$$

TABLE 4.

***H. laevigata*. Apparent fecal digestibility coefficients of N, gross energy, and amino acids in experimental diets containing single ingredients (Diets 1–5) or combinations of ingredients (Diets 6–11) fed to juvenile greenlip abalone**

	Single Diets					Mixed Diets						
Component	**1**	**2**	**3**	**4**	**5†**	**6**	**7**	**8**	**9**	**10**	**11**	**SE**
N	0.54	0.71	0.43	0.91	—	0.58	0.44	0.84	0.86	0.52	0.85	1.42
Gross energy	0.25*	0.25*	0.51*	0.80*	—	0.58	0.41	0.61	0.57	0.53	0.60	0.67
Amino acids												
Aspartic acid	0.43	0.54	0.37	0.91	—	0.48	0.34	0.84	0.86	0.40	0.84	1.83
Threonine	0.39	0.62	0.36	0.89	—	0.49	0.33	0.83	0.85	0.42	0.83	2.08
Serine	0.46	0.81	0.37	0.91	—	0.54	0.42	0.87	0.88	0.50	0.87	1.91
Glutamic acid	0.52	0.77	0.43	0.94	—	0.61	0.55	0.88	0.91	0.57	0.89	1.67
Proline	0.40	0.71	0.20	0.89	—	0.58	0.52	0.85	0.88	0.49	0.86	3.60
Glycine	0.45	0.67	0.41	0.90		0.50	0.38	0.79	0.81	0.44	0.79	1.88
Alanine	0.43	0.63	0.42	0.88	—	0.50	0.36	0.79	0.81	0.41	0.78	2.13
Valine	0.43	0.69	0.33	0.88		0.53	0.40	0.85	0.86	0.44	0.84	2.02
Isoleucine	0.30	0.58	0.33	0.86	—	0.48	0.34	0.83	0.84	0.40	0.80	1.95
Leucine	0.40	0.65	0.36	0.88	—	0.51	0.41	0.84	0.86	0.46	0.83	1.93
Tyrosine	0.44	0.75	0.31	0.92	—	0.49	0.45	0.85	0.79	0.51	0.84	3.02
Phenylalanine	0.41	0.70	0.34	0.89		0.53	0.44	0.84	0.86	0.43	0.83	1.90
Lysine	0.45	0.61	0.42	0.91	—	0.52	0.39	0.87	0.88	0.44	0.86	1.94
Histidine	0.45	0.68	0.48	0.91	—	0.57	0.53	0.88	0.89	0.58	0.87	1.54
Arginine	0.51	0.71	0.37	0.95	—	0.49	0.35	0.87	0.90	0.46	0.85	2.01

* The digestibility of gross energy in barley, semolina, fishmeal, and lupin kernel meal was calculated from the digestibility values presented here by subtracting the amount of digestible energy contributed from the oil and pregelled starch in each diet.

† No data were collected because of inadequate fecal collection.

where AD is apparent fecal digestibility, Nutrient is the nutrient or energy content of the diet or ingredients, and IL is inclusion level. To test the assumption of additivity of nutrients in the mixed diets, the estimates of the apparent fecal digestibility of nutrients in casein in Diets 8–11 were compared statistically. Diets 6 and 7 were not included in the analysis because the low inclusion level of casein in these diets (5.2 and 4.3%, respectively) meant that casein was only contributing a small proportion of the nutrients and hence the data were not reliable or meaningful. Diet 10 was included despite its low inclusion level of casein (4.7%) because the data appeared to be meaningful.

If ingredients are additive, the estimates of nutrient digestibility in casein will not differ significantly between the diets used in the calculation. The estimates of the fecal digestibility of N, serine, glutamic acid, proline, glycine, valine, isoleucine, leucine, phenylalanine, and arginine in casein were not significantly different between diets ($p > 0.05$). The estimate of the fecal digestibility of gross energy, aspartic acid, threonine, alanine, tyrosine, lysine, and histidine in casein in Diet 10 differed significantly from those in Diets 8, 9, and 11 ($p < 0.01$; Table 5). Thus, the fecal digestibilities of all nutrients in casein were statistically similar between Diets 8, 9, and 11 ($p > 0.01$).

DISCUSSION

Because the estimates of the fecal digestibility of nutrients in casein in Diet 10 were higher than in the other diet in some cases and lower in others, it can be assumed that the results from Diet 10 were not a result of nonadditivity of ingredients but rather random variation. This is not surprising, given the low inclusion level of casein in Diet 10 of 4.7%. In Diets 8, 9, and 11, where the inclusion level of casein was about 15%, ingredients were additive, irrespective of dietary fiber levels. Thus, combining ingredients with high NSP levels in manufactured abalone diets does not affect the fecal digestion of dietary N, amino acids, or gross energy contributed from constituent ingredients. Unlike other monogastric species, such as pigs, rats, and chickens, abalone appear to have the digestive capability to use nutrients from high-fiber diets. This is supported by recent work by a number of researchers that demonstrates that abalone have abundant carbohydrases, in particular, the polysaccharide hydrolases (reviewed in Fleming et al. 1996), and have digestive tract bacteria that play a key role in digestion (Erasmus et al. 1994, Erasmus 1996) and are capable of processing dietary fiber, including cellulose (M. T. Viana pers. comm.). Because dietary NSP does not appear to hinder the digestion of other nutrients, the results suggest that fecal digestibility coefficients of ingredients are additive when incorporated into manufactured feeds for abalone and so are good descriptors of nutritive value before ingredient inclusions in diets. The high protein digestibility of mixed diets containing combinations of lupin meal and barley also suggests that cereals and legumes can be used in manufactured feeds as a cheap source of protein and energy in abalone diets.

The endogenous N content of the feces can have a considerable effect on the values for the apparent protein digestibility of certain feed ingredients for abalone (Coote 1997). Initially, all diets were formulated to be isonitrogenous, but reformulation of Diets 1–5 after the first collection period meant that maintaining equal crude protein contents was not possible. Care must be used when inter-

TABLE 5.

***H. laevigata*. Calculated apparent fecal digestibility coefficients of N, gross energy, and amino acids in casein incorporated into diets containing various mixtures of barley, fishmeal, lupin kernel meal, and semolina.**

Component	8 (Barley and Lupin)	9 (Semolina and Lupin)	10 (Barley, Semolina, and Fishmeal)	11 (Barley, Semolina, and Lupin)	Statistics SE	p	Significance‡
N	0.85	0.86	0.90	0.87	5.09	0.935	NS†
Gross energy*	0.95[b]	0.98[b]	2.46[a]	1.06[b]	19.28	0.000	§
Amino acids							
Aspartic acid	0.86[a]	0.87[a]	0.67[b]	0.85[a]	2.91	0.004	
Threonine	0.88[a]	0.89[a]	0.68[b]	0.84[a]	2.51	0.000	§
Serine	0.90	0.88	0.90	0.88	2.74	0.920	NS
Glutamic acid	0.93	0.96	0.96	0.94	5.22	0.950	NS
Proline	0.94	0.94	1.14	0.93	10.45	0.465	NS
Glycine	0.82	0.77	0.62	0.75	6.42	0.252	NS
Alanine	0.84[a]	0.81[a]	0.15[b]	0.79[a]	6.54	0.000	§‖
Valine	0.92	0.90	0.90	0.88	10.04	0.996	NS
Isoleucine	0.91	0.91	0.79	0.84	8.38	0.677	NS
Leucine	0.90	0.91	0.90	0.87	3.22	0.806	NS
Tyrosine	0.87[b]	0.74[b]	1.36[a]	0.84[b]	9.12	0.006	
Phenylalanine	0.97	0.92	0.75	0.88	5.27	0.163	NS
Lysine	0.91[a]	0.90[a]	0.52[b]	0.88[a]	3.72	0.000	§
Histidine	0.93[b]	0.96[b]	1.31[a]	0.92[b]	2.75	0.000	§
Arginine	0.82	0.87	1.24	0.73	14.12	0.132	NS

* The digestibility values presented have been adjusted to account for the contribution of digestible energy from the oil and pregelled starch in the diets.

† NS, not significant.

‡Values in column with different superscripts differ significantly ($p < 0.05$).

§ $p < 0.001$.

‖ $p < 0.01$.

preting the results, particularly for diets with low protein levels and/or for diets with low digestibilities. However, the similarity of the estimates of the apparent digestibility of protein in casein (about 0.87) obtained in this study with those reported by others (Fleming et al. 1996, Coote 1997) suggests that our data were not significantly biased as a result of the endogenous contributions of N.

The results from this experiment demonstrate that apparent fecal digestibility values are additive. Similar research is required to assess the additivity of true fecal digestibility values for N and amino acids in feed ingredients. This is particularly important because endogenous N losses in abalone are significant (Coote 1997). It is possible that the digestibility values of ingredients currently used in commercial manufactured feeds for Australian abalone are underestimating their true nutritive value. Thus, commercial feed companies may be oversupplying these ingredients in their diets. By determining true digestibility values of feed ingredients and assessing their additivity, diet cost could be further reduced and diets could be formulated to more closely meet nutritional requirements.

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INFLUENCE OF DIETARY OIL TYPE AND OIL INCLUSION LEVEL IN MANUFACTURED FEEDS ON THE DIGESTIBILITY OF NUTRIENTS BY JUVENILE GREENLIP ABALONE *(HALIOTIS LAEVIGATA)*

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ABSTRACT Previous experiments aimed at defining optimum protein-to-energy ratios in manufactured abalone feed revealed that high energy diets resulted in depressed abalone growth rates. Subsequent investigations showed the higher energy density of the diets was achieved through the addition of fish oil. Given abalone's low requirement for lipids and the small amount of lipase in their gut, it was hypothesized that high levels of dietary lipid depresses the digestibility of other nutrients, such as amino acids, by restricting the action of digestive enzymes. A series of isonitrogenous diets containing 30, 60, or 90 g/kg of either jack mackerel oil or olive oil were formulated and fed to juvenile *Haliotis laevigata* to determine the apparent fecal digestibilities of dry matter, gross energy, nitrogen (N), and amino acids. Inclusion of jack mackerel oil in manufactured abalone feeds at levels above 60 g/kg decreased the digestion of dietary N and amino acids, and inclusion at levels above 30 g/kg decreased gross energy digestion. Oil type also influenced the apparent fecal digestibility of N, amino acids, and gross energy. Olive oil inclusion had significantly different effects on the digestion of these parameters when compared with jack mackerel oil; however, these differences were minimal up to inclusion levels of 30 g/kg. It was concluded that the addition of marine or vegetable oils to manufactured abalone diets should be limited to 30 g/kg if the digestibility of N, amino acids, and gross energy is not to be affected and that the addition of oils to increase dietary energy levels in abalone diets may not be a cost-effective strategy and may be detrimental to both energy and protein supply.

KEY WORDS: digestibility, amino acids, energy, oil, manufactured feeds, abalone

INTRODUCTION

The inclusion level of oil in manufactured diets for abalone, and the type of oil, may effect the digestion of energy and amino acids. Coote (1997) conducted an experiment investigating the effects of different digestible protein to digestible energy ratios on the growth and body composition of juvenile greenlip abalone (*Haliotis laevigata*) over a 61-day period. In this experiment, two pairs of diets were formulated to contain 10.6 MJ digestible energy (DE)/kg and 12.7 MJ DE/kg, respectively. Each pair consisted of one diet high in dietary protein and one with negligible protein, and these pairs were blended to provide 10 levels of dietary protein at each energy level, respectively. Based on the concept of protein- and energy-dependent phases in protein deposition (Campbell 1988, Bikker 1994), juvenile abalone fed diets with a higher energy density were expected to grow faster at higher dietary protein levels. However, contrary to expectations, abalone fed the high energy diets grew significantly slower than those fed the low energy diets, at all protein levels. In addition, it was found that the digestibility of protein in the high energy diets diminished as the dietary protein level increased.

Examination of the experimental diets revealed that the main difference between the high energy and the low energy diets was the oil inclusion level. The high energy diets contained approximately 50 g/kg more oil than the low energy diets. Because the pairs of diets were blended to produce the respective dietary protein levels at a set level of digestible energy, and because the higher protein diet in the pair had a higher oil content (90 g/kg vs. 55 g/kg), it indicates that as the protein content of the blends increased, so did the dietary oil level. This suggests that higher levels of oil inclusion in abalone diets may be affecting the ability of abalone to digest other nutrients. As oil is an essential addition to abalone diets to supply essential fatty acids, it is important to define the level at which oil inclusion starts to interfere with the digestion of other nutrients. It is also worthwhile defining whether oil type has any influence on abalone digestion mechanisms. The objectives of this experiment were to determine the influence of oil inclusion level and oil type on the fecal digestibility of N, amino acids, and gross energy in juvenile greenlip abalone (*Haliotis laevigata*).

MATERIALS AND METHODS

Dietary Oils

Jack mackerel oil (Triabunna Fish Oils, Triabunna, Tasmania, Australia) and olive oil (Aceites Borges Pont, S.A. Tarrega, Spain) were the two oils compared in this experiment. Jack mackerel

(*Trachurus declivis*) oil was selected on the basis of being a marine oil with high levels of 20:5(n-3) (~7.6% total fatty acids) and 22:6(n-3) (~3.1% total fatty acids), and it was the oil used by Coote (1997). In contrast, olive oil is characterized by a lack of highly unsaturated fatty acids (HUFAs) and polyunsaturated fatty acids (PUFAs, Tacon 1990). The jack mackerel oil is also likely to have a higher digestibility than the olive oil because of a higher level of unsaturated fatty acids, which are better digested than monounsaturated or saturated fatty acids (B. Glencross, pers. comm., CSIRO Marine Research, Cleveland, Australia). For this reason, the presence of jack mackerel oil might be expected to have a smaller influence on the digestibility of other components of the diet when included at high levels.

Diets

Seven diets were formulated to undertake this experiment (Table 1). The basal diet (diet 1) contained semolina, pre-gelled starch, casein, fish meal, and defatted soyflour (Table 2). No oil was added to the basal diet used as the control diet for the experiment. Test diets contained 30, 60, or 90 g/kg of jack mackerel oil (diets 2–4) or olive oil (diets 5–7), respectively, included in the basal diet at the expense of kaolin (filler). All other ingredient levels were kept constant in the test diets to preserve the crude protein and amino acid levels and to ensure that lipid supplied from other dietary ingredients did not vary. Vitamin and mineral premixes were as described by Uki et al. (1985) and sodium alginate was included as a diet binder. Chromic oxide and n-hexatriacontane were included as indigestible markers in all diets for use in subsequent digestibility calculations. n-Hexatriacontane was added by dissolving in warm oil prior to addition to the diet. For the control diet (diet 1), this marker was ground in a mill with a 0.5-mm screen and then mixed thoroughly with the semolina in a mixer before addition, because no oil was added to diet 1. Diets were produced in the form of "flat chips" as described by Coote et al. (1996).

Abalone Management and Feces Collection

Diet Allocations and Abalone

Diets were randomly allocated to two of 14 digestibility tanks over two separate collection periods to provide four replicates per diet based on a completely randomized design. Each digestibility tank contained between 60–80 juvenile *H. laevigata* (shell length 40–60 mm). These abalone had been raised on diatoms from settlement to about 10 mm in shell length and then grown on manufactured abalone feed containing 26% crude protein and 18 MJ/kg of gross energy.

Tanks and Collection System

Conical-shaped tanks were fitted with fecal collection and drainage plumbing as described by Coote (1997). A bucket (20-L capacity) with a mesh bottom (4-mm mesh) was used to contain the abalone within the tank while permitting feces to drop into the collection tube, which was packed with ice during fecal collection. Seawater, filtered to 10 μm by a combination of primary and composite sand filters, was continuously supplied to each tank at a rate of 2-L/min. Water temperature was maintained at 18°C and aeration (0.5 L/min) was provided by one air stone per tank.

Feeding

A diet adaption/acclimation period of 7 days was allowed before feces collection, during which time, the abalone were fed every night, and the tanks were cleaned each morning, but no feces were collected. Abalone were fed to excess (i.e., abalone were supplied with more feed than they could consume between tank cleaning and feeding each day, but not enough to foul the tanks) at 1700 h each day. Tanks were cleaned each morning to ensure all uneaten food was removed in an attempt to prevent diet mixing with feces.

Fecal Collection

Fecal collection occurred every day until 5–6 grams of dry fecal matter had been collected from each tank. This took approximately 19 days for the first collection period and 25 days for the second. Cleaning and tank setup took place between 0730 and 0900 each morning, with fecal collection completed between 1600 and 1700 each afternoon. This meant that feces were in the collection tubes for no longer than 8 h. Levels of leaching of nutrients from the feces were not measured, but it was assumed that any leaching that did occur was the same across all treatments. In

TABLE 1.

Composition of experimental diets (g/kg, air-dry basis).

Ingredient	Diet						
	1	2	3	4	5	6	7
Semolina	190.0	190.0	190.0	190.0	190.0	190.0	190.0
Pre-gelled starch	410.0	410.0	410.0	410.0	410.0	410.0	410.0
Casein	140.0	140.0	140.0	140.0	140.0	140.0	140.0
Fish meal	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Defatted soyflour	60.0	60.0	60.0	60.0	60.0	60.0	60.0
Jack mackerel oil	—	30	60	90	—	—	—
Olive oil	—	—	—	—	30	60	90
Kaolin (filler)	154.8	124.8	94.8	64.8	124.8	94.8	64.8
Sodium alginate	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix[a]	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral premix[a]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chromic oxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0
n-Hexatriacontane	0.2	0.2	0.2	0.2	0.2	0.2	0.2

[a] Vitamin and mineral premixes as described by Uki et al. (1985).

TABLE 2.

Proximate analysis, and total amino acid content (g/kg, air-dry basis), and gross energy content (MJ/kg, air-dry basis) of semolina, casein, pre-gelled starch, fishmeal, and defatted soyflour used in experimental diets.

	Diet Ingredient				
Component	**Semolina**	**Casein**	**Pre-gelled Starch**	**Fishmeal**	**Defatted Soyflour**
Dry matter	863.3	897.7	939.9	899.9	877.8
Crude protein (N × 6.25)	97.8	848.8	5.5	638.3	477.0
Ash	2.8	3.1	0.40	151.0	62.8
Ether extract (bp 40–60°)	7.2	7.5	7.7	98.3	10.6
Fiber extract:					
Crude	15.1	14.6	---	——	49.8
Neutral-detergent	18.8	4.9			120.3
Acid-detergent	21.0	——	——	——	79.9
Amino acids					
Aspartic acid	3.40	54.50	——	51.05	26.22
Threonine	2.71	35.91	–	27.07	10.81
Serine	4.43	48.10	——	24.55	12.73
Glutamic acid	30.12	181.18	——	85.64	49.61
Proline	8.05	65.57		22.38	9.60
Glycine	2.92	14.89	---	32.25	9.76
Alanine	1.61	16.22		22.37	6.54
Valine	4.16	55.08	——	31.27	12.76
Isoleucine	3.70	44.97	----	29.53	12.11
Leucine	6.06	71.27	——	42.59	17.54
Tyrosine	2.25	41.82	——	17.42	7.98
Phenylalanine	3.87	35.79	——	21.75	10.53
Lysine	1.85	61.45	——	44.94	14.75
Histidine	1.61	19.82	——	15.51	6.30
Arginine	2.94	28.65	——	32.73	16.62
Gross energy (MJ/kg)	15.51	21.54	16.22	19.16	17.06

addition, because all the feces collected from all treatments were in the form of discrete, intact pellets, it was assumed that the level of leaching was minimized.

On each day of fecal collection, the buckets containing the abalone were removed, the digestibility tanks were drained of water and all fittings were cleaned of feces and uneaten feed. Abalone were out of the water for 1–2 min while this was completed. Following cleaning, the tanks were refilled, the buckets replaced, and collection tubes were fitted. A small esky was placed underneath each collection tube and filled with ice to keep the collecting feces cold and thus minimize any subsequent enzymic or microbial degradation. Approximately 8 h later (i.e., each afternoon), the feces were collected from the tubes by gently pouring the contents onto a 1-mm mesh screen. The mesh was then placed in a petri dish and frozen at −20°C. The following day, the frozen feces were scraped off the mesh, pooled into a composite sample for each tank, and returned to the freezer before chemical analysis. Immediately before chemical analysis, the feces were freeze-dried and then ground with a mortar and pestle.

Chemical Analyses

Chemical analyses for proximates (i.e., crude protein, dry matter, ether extract, crude fiber, neutral-detergent fiber, acid-detergent fiber, and ash) were undertaken using the methods of the Association of Official Analytical Chemists (1984). Gross energy was determined by adiabatic bomb calorimetry. Chromic oxide was determined using atomic absorption spectroscopy based on a modification of the method described in Hillebrand et al. (1953). The modified methodology involved preliminary ignition of the sample at 500°C to remove organic material and the dissolution of the sample in hydrochloric acid instead of sulphuric acid (M. Frith, pers. comm., University of Tasmania, Launceston, Tasmania, Australia). Amino acids in the ingredients, manufactured diets, and abalone feces were separated by ion-exchange chromatography following a 24-h hydrolysis at 110°C with constant boiling point 6N HCl under N_2 and measured after reaction with ninhydrin. Norleucine was used as an internal standard with accepted recoveries falling between ±0.025 g kg^{-1} of the batch mean. Digestibility coefficients for methionine and cystine and the content of these amino acids in the ingredients have not been reported, because preoxidation of the samples with performic acid before hydrolysis was not completed (so that methionine and cystine could be reported as methionine sulphone and cysteic acid, respectively).

Statistical Analysis

The results were analyzed by analysis of variance (ANOVA) of the completely randomized design, utilizing a general linear model, and the treatment means were separated by least-significant difference (LSD). Before analysis, data were analyzed using a univariate procedure and normal plot to establish that the data were, in fact, normally distributed, which was the case. The presence of outlying measurements was assessed using the RANK procedure in SAS (SAS Institute Inc., 1988), and normal scores were computed from the ranks based on the Blom (1958) version. Because no outlying measurements were detected using the RANK procedure, all were used in the calculation of digestibility esti-

mates for all parameters, respectively. Digestibility data for diets 1–4 and diets 1, 5–7 were separated and analyzed in two parts to assess the linear responses of abalone fed diets containing each oil type.

RESULTS

Feces were successfully collected (5–6 g fecal dry matter/tank containing 80–100 abalone) for all treatments during both collection periods. No abalone mortalities were recorded for any tank in any treatment. Digestibility coefficients were determined using chromic oxide analysis alone, because insufficient faeces were available for n-hexatriacontane analysis.

Graded levels of jack mackerel oil inclusion significantly increased ($p < .05$) dry matter digestibility (DMD; Table 3). In contrast, olive oil inclusion had little effect on DMD. Both oils significantly decreased ($p < .05$) N digestibility. N digestibility was significantly lower ($p < .05$) in diets containing 90 g/kg of jack mackerel oil; whereas, a significant decrease was evident in diets containing more than 30 g/kg of olive oil. The digestibility of N in diets containing olive oil at levels of 60 g/kg (0.79) and 90 g/kg (0.78) was also lower than those containing jack mackerel oil at the same level (0.83 and 0.81, respectively). Gross energy digestibility was significantly reduced in diets containing more than 30 g/kg of either jack mackerel or olive oil as compared to the basal diet; however, as with N digestibility, the magnitude of the decrease was greater in diets containing olive oil.

The apparent fecal digestibility of all reported amino acids was significantly lower ($p < .05$) in diets containing 90 g/kg of jack mackerel oil as compared to the control diet, with the exception of isoleucine digestibility, which remained unchanged (Table 3). With the exception of proline and valine, the digestibility of amino acids in diets containing 60 g/kg of jack mackerel oil were not significantly different from the control diet.

A significant decrease ($p < .05$) in the apparent fecal digestibility of threonine, serine, glutamic acid, proline, valine, tyrosine, lysine, and alanine was observed in diets containing more than 30 g/kg of olive oil (Table 3). The digestibility of aspartic acid, threonine, leucine, phenylalanine, and histidine was significantly lower ($p < .05$) in diets containing 60 g/kg of olive oil compared to diets containing 90 g/kg of olive oil.

With the exception of tyrosine and arginine, the apparent fecal digestibility of amino acids in diets containing 60 g/kg of olive oil were significantly lower ($p < .05$) than amino acids in diets containing the same amount of jack mackerel oil. There was no difference ($p > .05$) in the digestibility of amino acids in diets containing 90 g/kg of either jack mackerel oil or olive oil, with the exception of phenylalanine, the digestibility of which was significantly lower in the diet containing jack mackerel oil.

Contrasting the apparent fecal digestibility of dry matter, N, gross energy, and amino acids in the control diet against diets containing graded levels of jack mackerel oil and olive oil, respectively, revealed a linear decrease in digestibility of N, gross en-

TABLE 3.

Fecal digestibility coefficients of dry matter, N, gross energy, and amino acids in manufactured diets containing graded levels of jack mackerel oil (diets 2–4) or olive oil (diets 5–7) fed to juvenile greenlip abalone *(Haliotis laevigata)*.

Diet→	1	2	3	4	5	6	7		
	Oil Inclusion Level (g/kg)								
		Jack Mackerel Oil			Olive Oil			Statistics	
Component	0	30	60	90	30	60	90	p > F	SEM
Dry matter	0.62^{a}	0.62^{a}	0.65^{b}	0.69^{c}	$0.60^{a,d}$	0.58^{d}	0.62^{a}	***	0.010
N	0.85^{a}	0.84^{a}	0.83^{a}	0.81^{b}	0.83^{a}	$0.79^{b,c}$	0.78^{c}	***	0.008
Gross energy	0.84^{a}	0.82^{b}	0.79^{c}	0.79^{c}	$0.83^{a,b}$	0.75^{e}	0.73^{f}	***	0.007
Amino acids									
Aspartic acid	0.85^{a}	0.84^{a}	0.82^{a}	0.78^{b}	$0.81^{a,b}$	0.71^{c}	0.78^{b}	***	0.013
Threonine	0.85^{a}	0.85^{a}	0.83^{a}	0.79^{b}	0.83^{a}	0.69^{c}	0.78^{b}	***	0.013
Serine	0.88^{a}	0.88^{a}	0.88^{a}	0.84^{b}	0.87^{a}	0.82^{b}	0.84^{b}	**	0.010
Glutamic acid	0.91^{a}	0.91^{a}	0.90^{a}	0.88^{b}	0.91^{a}	0.88^{b}	0.86^{b}	***	0.006
Proline	0.94^{a}	$0.93^{a,b}$	$0.91^{b,c}$	$0.89^{c,d}$	$0.93^{a,b}$	0.88^{d}	0.87^{d}	***	0.008
Glycine	$0.75^{a,c}$	0.76^{a}	0.76^{a}	0.69^{b}	$0.74^{a,c}$	0.67^{b}	$0.71^{b,c}$	**	0.014
Alanine	$0.79^{a,c}$	$0.79^{a,c}$	0.83^{a}	0.73^{b}	$0.79^{a,c}$	$0.74^{b,c}$	$0.76^{b,c}$	*	0.017
Valine	0.88^{a}	0.87^{a}	0.75^{b}	0.83^{c}	0.87^{a}	0.82^{c}	0.82^{c}	***	0.007
Isoleucine	0.82	0.85	0.83	0.80	0.80	0.78	0.80	NS	0.016
Leucine	0.86^{a}	$0.84^{a,b,f}$	0.85^{a}	$0.82^{b,c,d,f}$	$0.84^{a,e,f}$	$0.80^{d,e}$	$0.82^{c,e,d}$	**	0.010
Tyrosine	$0.84^{a,d,f}$	$0.86^{a,e,f}$	$0.85^{a,e,d,f}$	$0.82^{b,e,g}$	0.87^{e}	$0.83^{d,e,h}$	$0.84^{f,g,h}$	**	0.009
Phenylalanine	0.87^{a}	0.86^{a}	0.85^{a}	$0.83^{b,d}$	0.86^{a}	0.81^{d}	0.89^{e}	***	0.008
Lysine	0.88^{a}	0.88^{a}	0.86^{a}	0.80^{b}	0.86^{a}	0.83^{c}	0.79^{b}	***	0.008
Histidine	0.89^{a}	0.89^{a}	0.88^{a}	$0.85^{b,c}$	0.88^{a}	0.83^{c}	$0.87^{a,b}$	**	0.009
Arginine	$0.82^{a,c}$	0.84^{a}	$0.81^{a,c}$	$0.77^{b,d}$	0.85^{a}	$0.78^{b,c}$	0.74^{d}	***	0.014

NS, not significant.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

SEM, standard error of the mean.

[a b] Diets in a row with different superscripts differ significantly ($p < .05$).

ergy, and all reported amino acids in diets containing increasing levels of jack mackerel oil, with the exception of alanine and isoleucine (Table 4). A significant linear decrease in apparent fecal digestibility was also evident for all amino acids in diets containing graded levels of olive oil, with the exception of alanine, isoleucine, tyrosine, and phenylalanine.

Oil type significantly influenced the diet digestible energy content (Table 5). A significant linear increase ($p < .001$) was observed in diets containing graded levels of jack mackerel oil; whereas, no linear response was observed in diets containing olive oil. Significant differences ($p < .01$) were detected between diets containing graded levels of olive oil, but a significant increase was only observed for diets 5 and 7, with no change in the digestible energy content of diet 6. The magnitude of the increase in diet digestible energy content observed in diet 7 was also lower than the increase detected for diet 4, which contained 90 g/kg of jack mackerel oil.

DISCUSSION

Inclusion of jack mackerel oil in manufactured abalone diets at levels above 60 g/kg will decrease the digestion of dietary N and amino acids when fed to juvenile *H. laevigata*. Similarly, inclusion of jack mackerel oil in manufactured diets at levels above 30 g/kg will decrease gross energy digestion. Oil type can also influence the apparent fecal digestibility of N, amino acids, and gross energy. Olive oil inclusion had significantly different effects on the digestion of these parameters when compared with jack mackerel oil; however, these differences were minimal up to inclusion levels of 30 g/kg.

In a study investigating the effect of dietary protein and energy level on growth and body composition of the South African abalone, *Haliotis midae*, Britz and Hecht (1997) demonstrated that dietary lipid level influenced growth rate, thus supporting the findings of the current experiment. Britz and Hecht (1997) tested three dietary fat levels (2, 6, and 10%) and concluded that a dietary lipid level of 10% was too high for *H. midae* to maintain maximum growth.

The results from the current experiment suggest that the poor growth response of juvenile *H. laevigata* observed by Coote (1997) when they were fed high-oil diets (55–90 g/kg) was attributable, in part, to a decrease in the apparent fecal digestibility of N and amino acids. In addition, as the oil content of the diets increased, the gross energy digestibility would have decreased. Hence, the digestible energy content of the diets would have been higher than the low-oil diets, but the magnitude of the difference would have been lower than expected.

The results of the current experiment are consistent with the findings of Wee et al. (1992), who reported a high efficiency of lipid digestion by *H. laevigata* feeding on diets containing 3.4% lipid. The current experiment gives no indication of the efficiency of lipid digestion at higher oil inclusion levels; however, the decrease in the digestion of other nutrients at higher oil inclusion levels suggests that dietary oil addition should be limited to 30 g/kg. The increase in dry matter digestibility with increasing addition of jack mackerel oil also suggests that a significant amount of added fish oil is passing into the feces undigested. This is further supported by Uki et al. (1985), who reported that growth rate and feed conversion efficiency of *H. discus hannai* were maximal at 5% lipid in the diet. It seems that not only are abalone feed formulators oversupplying lipid in their diets (up to 6%, Fleming et al. 1996), but this oversupply is affecting the utilization of other nutrients.

Fleming et al. (1996) suggested that further investigations are required to determine whether abalone lipid requirements are met using added fish oils to artificial diets. Because many algae commonly eaten by abalone contain very low levels of lipid, and because abalone have high levels of 22:5(n-3) and 20:4(n-6) in their muscle (Dunstan and Volkman 1994), which is opposite to most other marine animals, abalone may benefit little from the addition of fish oils to the diet. Furthermore, the Japanese Nihon Nosan Kogyo K.K. diet used as a reference by Fleming et al. (1996), because of its superior promotion of growth and intake, contained the lowest level of total lipid (1.5%) of all 12 diets reviewed. Wee et al. (1992) and Maguire et al. (1993) also reported that the requirement for lipid is very low for abalone, and their ability to utilize digested exogenous lipid is high. For these reasons, coupled with the negative effects of fish oil addition above 30 g/kg observed in the current experiment, fish oil contained in fish meal added to manufactured feeds may be sufficient to meet the n-3 fatty acid requirements of abalone, and hence, additional fish oil supplementation may be unnecessary.

Vegetable oils, such as olive oil, used in conjunction with fish oils bound in fish meal, may represent a useful way to improve the fatty acid profile of the diet, while reducing the cost of diet manufacture. Uki et al. (1986) demonstrated a requirement for n-3 and n-6 polyunsaturated fatty acids using a diet containing soybean oil and pollack liver oil in the ratio 3:2, and Fleming et al. (1996) suggested that abalone feed formulators should attempt to match the ratio of fatty acids in the Japanese Nihon Nosan Kogyo K.K. diet, which contains n-3 fatty acids at levels of 0.3% in the diet (i.e., 20% of the lipid) and n-6 fatty acids at levels of 0.04% in the diet. In the current experiment, inclusion of olive oil in diets at

TABLE 4.

Statistical analysis of diets 1–4 (jack mackerel oil) and 1, 5–7 (olive oil), respectively, for linear responses to graded level of oil inclusion.

	Jack Mackerel Oil	Olive Oil
Component	**Linear ($p > F$)**	**Linear ($p > F$)**
Dry matter	***	NS
N	**	***
Gross energy	***	***
Amino acids		
Aspartic acid	**	**
Threonine	***	**
Serine	**	**
Glutamic acid	***	***
Proline	**	***
Glycine	**	*
Alanine	NS	NS
Valine	***	***
Isoleucine	NS	NS
Leucine	**	**
Tyrosine	*	NS
Phenylalanine	**	NS
Lysine	***	***
Histidine	*	*
Arginine	**	**

NS, not significant.
* $p < .05$.
** $p < .01$.
*** $p < .001$.

TABLE 5.

Influence of oil type and inclusion level on the digestible energy content (MJ/kg, air-dry basis) of manufactured diets fed to juvenile greenlip abalone (*Haliotis laevigata*).

	Oil inclusion level (%)				Statistics		
Oil Type	0	3	6	9	p > F	Linear	SEM
Jack mackerel oil	12.21[a]	12.63[b]	13.29[c]	14.36[d]	***	***	0.084
Olive oil	12.21[a]	12.96[b]	12.00[a]	12.84[b]	**	NS	0.129

** $p < .01$.

*** $p < .001$.

SEM, standard error of the mean.

[a–d] Values in a row with different superscripts differ significantly ($p < .05$).

levels of 30 g/kg had no effect on the digestibility of dry matter, N, gross energy, or amino acids, suggesting no negative consequences of adding this oil on the digestion of other nutrients. Diets containing 30 g/kg olive oil also had a significantly higher digestible energy content, and in the case of olive oil, addition beyond 30 g/kg did not improve digestible energy content. Addition of low levels of vegetable oils, such as olive oil, may be a cost-effective means of supplying fatty acids and energy in abalone feeds.

The digestibility of the majority of reported amino acids was lower in diets containing 60 g/kg of olive oil as compared to the same level of jack mackerel oil. As suggested previously, this difference may be attributable to a higher level of unsaturated fatty acids in the jack mackerel oil as compared to olive oil, making it more digestible and, hence, less influential on the digestion of other dietary nutrients. Alternatively, this difference may be attributable to the limited exposure of these abalone to vegetable proteins and energy sources. The manufactured diets fed before experimentation were based on fish oils only. Taylor (1994) demonstrated that *H. kamtschatkana* has a capacity for enzymic adaption to various lipid levels in manufactured feeds, and Erasmus et al. (1994) reported that gut bacteria play a significant role in the digestive tract of abalone. In this instance, the bacterial population would need to be significantly modified to digest vegetable oils adequately, as compared to fish oils. The fact that there was no difference between the olive oil and jack mackerel oil when included in diets at 90 g/kg, suggests that at higher levels, no amount of adaption in lipase secretion or the bacterial population will improve nutrient digestion.

CONCLUSIONS

Addition of marine or vegetable oils to manufactured abalone diets should be limited to 30 g/kg if the digestibility of N, amino acids, and gross energy is not to be affected. Higher inclusion of oils will reduce the digestibility of these nutrients and is likely to result in significant reductions in the growth performance of abalone.

The findings of the current experiment, coupled with a low requirement for lipids by abalone, questions the need for supplementary marine oil in manufactured diets, apart from fish oil retained in fish meal that is essential for the supply of n-3 fatty acids. The addition of vegetable oils, such as olive oil up to 30 g/kg, has no negative effects on the digestion of other nutrients in the diet, improves the digestible energy content, may improve the balance of n-3 to n-6 fatty acids, and could represent one mechanism to reduce the cost of abalone diets. Further research is required to define the performance of abalone fed manufactured diets containing reduced levels of supplementary marine oil and mixtures of marine oils and vegetable oils.

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THE EFFECT OF TEMPERATURE AND ARTIFICIAL DIETS ON GROWTH RATES OF JUVENILE *HALIOTIS TUBERCULATA* (LINNAEUS, 1758)

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ABSTRACT Growth rates of *Haliotis tuberculata* were evaluated at three different temperatures (15, 18, and 22°C) when provided two artificial diets (fish meal and casein meal). Juveniles fed the fish meal diet and cultured at 22°C produced higher growth rates on shell length and body weight (134.81 μm day^{-1} and 3.205 μg day^{-1}), respectively. In addition, the soft tissue weight/shell weight ratio was found to be the highest in these juveniles. No differences in proportions of crude protein, crude lipids, and moisture content were found between diets. Survival of juveniles during the feeding trial was not affected by the dietary treatments. The feed conversion ratio did differ significantly between low (15°C) and highest (18 and 22°C) temperatures.

KEY WORDS: abalone, temperature, artificial diets, growth, *Haliotis tuberculata*

INTRODUCTION

The ormer (*Haliotis tuberculata*), the most commercially important European abalone, is a cold-water specie. In the natural environment, it grows slowly, reaching a mean shell length of 45 mm in a minimum of 3 y (Forster 1967, Clavier and Richard 1986). Abalone stocks have been declining around the world with a concomitant increase in the price of this important delicacy. Previous works have examined the culture of the ormer in Europe (Koike et al. 1979, Hayashi 1980, Hayashi 1982, Culley 1981, Culley and Peck 1981, La Touche et al. 1993, Mgaya and Mercer 1994, Mgaya and Mercer 1995). To date, the only published research concerning nutritional requirements of *H. tuberculata* has been conducted by Peck (1989), using natural food, and by Mercer et al. (1993) and Mai et al. (1994, 1995, and 1996), using natural and artificial food. However, recent research demonstrated that the growth rate of *H. tuberculata* fed with the alga *Ulva* sp. and cultured at temperatures above 20°C was higher than reported in the literature (Shpigel et al. 1996a, Shpigel et al. 1996b). The economic viability of abalone aquaculture depends on good growth rates. In culture, feed conversion ratio (FCR) is greatly influenced by factors such as quality of feed, feed intake, and water temperature (Hahn 1989). In particular, temperature is the most important environmental factor that influences metabolic rate and energy expenditure (Fry 1971). Dixon (1992) and Britz et al. (1997) have determined growth rates of *Haliotis midae* using artificial diets at different temperatures of culture. They concluded that abalone growth increased significantly with temperature. Artificial diets have been evaluated; they can improve the growth rates of juveniles and young adults (e.g., Uki et al. 1985, Nie et al. 1986, Hahn 1989, Uki and Watanabe 1992, Viana et al. 1993, Viana et al. 1996, Mai et al. 1994, Mai et al. 1995, López and Viana 1996, Knauer et al. 1996). This study was designed to assess growth rates (length and weight gain), FCR, and soft tissue weight/shell weight (Stw/Sw) ratio of abalone with two artificial diets with different sources of protein (fish meal [FM] and casein [CA]). Three different temperatures were used in conjunction with the diet treatments.

MATERIALS AND METHODS

Abalones

Four-month-old juveniles of *H. tuberculata* used in this experiment were obtained by semiartificial spawning with a gradual increase in seawater temperature at the Rocquaine Shellfish Pounds, in Guernsey, Channel Islands, at the end of July 1996. Two months after fertilization, juveniles were shipped (by air) to the Southampton Oceanography Centre (within 1 h) and placed in the rearing system for acclimatization. Two batches of 99 animals were randomly taken from a lot of 500. Mean length and weight were 3.22 ± 0.81 mm and 15.98 ± 6.88 mg, respectively. During acclimatization, temperature was gradually raised to that used in the experiments: 15, 18, and 22°C.

Preparation of Diets

Formulations of the two experimental diets are presented in Table 1. Diets were prepared with different sources of protein, one based on casein (CA) and the other based on fish meal (FM). In order to obtain the same proximate content in the two diets, it was necessary to add to each different percentages of ingredients (FM, CA, corn meal, and cod oil). Vitamin and mineral mixtures were formulated as recommended by Special Diets Services (SDS) Company, England. All ingredients were individually ground with a Waring blender, passed through a mesh with a 240-μm pore size, and then mixed to obtain a homogeneous paste. The paste was flattened with a kitchen roller to a thickness of 1.5–2.0 mm. Pieces of 5 × 5 mm were cut and stored at −20°C until required.

A dried seaweed (*Palmaria palmata*; 16.5% protein) was used to feed the control group of the juvenile abalone. *P. palmata*, one of the main diets of *H. tuberculata* in the natural environment, was harvested, dried, and stored in summer to be used during winter

TABLE 1.

Composition of two artificial diets for juvenile abalone *H. tuberculata*.

Ingredients	FM-Based Diet	CA-Based Diet
Fish meal*	35	
Casein meal*		25
Seaweed meal†	20	20
Corn meal‡	10	18
Soybean meal*	8	8
Cellulose*	5	5
Sodium alginate*	8	8
Gelatine*	4	4
Vitamins mix§	3	3
Minerals mix§	5	5
Cod oil*	2	4

All ingredients expressed as percent dry weight.

* Sigma Chemical Co., United Kingdom, *P. palmata* from the English Channel Islands.

† Maseca, produced in Mexico.

‡ Mix based on the requirements for fish (SDS), mg/100 g diet.

§ Vitamins: A, 4.9; B_1, 13; B_2, 8; B_6, 18; B_{12}, 0.015; C, 40; D, 0.30; E, 5.0; H, 0.025; K, 2.25; choline chloride, 15; PABA, 35; Folic acid, 2.75; Inositol, 500. Minerals: calcium, 1600; cobalt, 0.05; copper, 0.9; iodine, 0.01; ferric citrate, 60; potassium, 3.5; selenium, 0.05; zinc, 9.

(December to April, feeding trial), when the fresh seaweed is not available on the coasts of the English Channel Islands.

Proximate Analysis

All analyses were carried out with five replicates (0.5–1.5 g each) for composition of diets and for samples of ormer (soft body tissue) at the beginning and at the end of the experimental period. Moisture content was determined by weighing samples before and after drying at 70°C for 24 h. Ash content was measured with the remaining dried samples by incinerating at 500°C in a muffle furnace for 8 h. Crude protein content was analyzed by the Kjeldahl method for nitrogen content of the samples. The value obtained was then multiplied by 6.25 to estimate crude protein. Fat content was determined by a column procedure with methanol-chloroform/water as the eluting solvent (Bligh and Dyer 1959). The percentage of carbohydrate was determined by the difference where % carbohydrate = 100– (% protein + % lipid + % ash + % moisture).

Experimental Procedure

Groups of 11 juveniles between 2.42 and 4.02 mm in length and 9.1 and 22.86 mg in weight were transferred to 2,000-mL plastic containers. Three replicates per diet were held in a constant temperature bath (±0.5°C) at each of the following temperatures: 15, 18, and 22°C. Ultraviolet-sterilized, filtered, and heated seawater was changed every morning for 105 days. Aeration was provided, and every container was covered to prevent animals from escaping. The light/dark sequence was 12 h/12 h, and tanks were covered with a 4-mm-pore-size mesh net to reduce ambient light. Salinity was 32 ± 3 ppt. The experimental group was fed every afternoon, and any uneaten food was collected the following morning to estimate the feed consumed (Fc) and the FCR. Growth was measured monthly as gain in weight and length. Weights were obtained with an electronic balance (to 0.1 mg). A light microscope with a camera lucida and a digitizing tablet was used to measure the shell length in the smallest abalone.

Food stability is defined as the amount of dry matter lost in seawater. This evaluation was conducted at intervals through a 35-day period before the experiment, with containers that did not contain abalone, under the same conditions as those of the growth experiments. Food was collected after having remained in the aquaria for 12 h. The dry weight (samples dried at 70°C for 24 h) for each trial (15, 18, and 22°C) was measured.

The Fc was calculated in terms of dry weight with the following Equation 1:

$$Fc = (GS/100) - R \quad (1)$$

where G is the weight of food offered per animal per day (in milligrams); S is the percentage of food recovered indicating the stability of the pellet, obtaining a factor for each diet and for each temperature (from the controls without abalone); and R is the remaining food (in grams) after the abalone had fed.

The FCR was calculated in the following manner (Equation 2):

$$FCR = Fc/W \quad (2)$$

where Fc is grams of food (dry basis) consumed per animal per day and W is grams of body weight gained (wet basis) per animal per day, as in Britz (1996). At the end of this phase, 3 animals from each replicate (54 animals in total; 9 animals from each temperature, from each diet) were processed to find the Stw/Sw, wet basis.

Statistical Analyses

Data for experimental replicates were pooled because no significant differences were found between them by a one-way analysis of variance at a significance level of $p = 0.05$. In proximate analysis, a two-sample *t*-test was used to compare the means for diets and soft tissue of abalone. A two-way analysis of variance was used in order to compare the shell length between diets and temperatures, followed by a multiple comparison of the means (SNK, Student-Newman-Keuls method). The same analysis was performed for the data on the body weight. A one-way analysis of variance was used to assess the food stability between diets and temperatures, followed by comparison of the means by the SNK method. The daily growth rates were analyzed by the Kruskal-Wallis one-way analysis of variance on ranks. All statistics were calculated with the SigmaStat package (1996).

TABLE 2.

Proximate analysis of the two diets used and soft tissues of juvenile abalone *H. tuberculata*.

Sample	Crude Protein	Crude Lipid	Carbohydrate	Ash	Moisture
FM diet	31 (0.8)	5.6 (0.7)	21.54 (0.7)	12.1 (0.5)	29.2 (0.9)
CA diet	36 (0.7)	4.9 (0.4)	19.06 (0.82)	8.1 (0.9)	31.5 (0.8)
Soft tissue*	9.2 (0.6)	2.7 (0.5)	1.96 (0.1)	4.8 (0.5)	82.3 (0.7)
Soft tissue†	10.7 (0.7)	4.6 (0.5)	2.82 (0.1)	6.0 (0.5)	76.7 (0.5)

Data are expressed as percent dry weight. Standard errors are in parentheses, n = 3.

* Soft body tissue from juveniles before the start of the experimental trial.

† Soft body tissue from juveniles at the end of the experimental trial.

TABLE 3.

Survival of the juveniles during the feeding trial as a percentage.

Diet	°C	S (%)	Survival (%)	Stw/Sw Ratio	Fc (mg)	W (mg)	FCR
CA	15	95.4 (0.2)	90	1.04 (0.5)	0.16 (0.01)	0.08 (0.03)	3.52
	18	92 (0.3)	100	1.77 (0.4)	0.95 (0.10)	0.87 (0.08)	0.84
	22	87.2 (0.55)	100	2.49 (0.2)	1.57 (0.16)	1.80 (0.27)	0.76
FM	15	97.4 (0.75)	90	1.1 (0.5)	0.19 (0.01)	0.12 (0.02)	3.56
	18	93.5 (0.63)	100	1.9 (0.2)	1.32 (0.12)	1.66 (0.09)	0.91
	22	89.5 (0.85)	100	2.6 (0.4)	1.62 (0.14)	1.97 (0.20)	0.76

Average values of Stw/Sw. Stability (S) of each diet (dry matter lost in seawater without abalones over 12 h) is given as a percentage. Fc and average weight gain (W) are estimated. FCR indicated as feed consumed/weight gain. Standard errors are in parentheses.

RESULTS

Diets were similar in crude protein ($t = -10.7$, $df = 8$, $p = 0.0001$), crude lipid ($t = 2.05$, $df = 8$, $p = 0.0074$), and moisture ($t = -4.01$, $df = 8$, $p = 0.0039$) content, whereas ash ($t = 9.27$, $df = 8$, $p = 0.0001$) content was different between diets (Table 2). Analysis of the soft tissue of *H. tuberculata* juveniles showed no differences between samples for crude protein ($t = -3.58$, $df = 8$, $p = 0.0072$), crude lipid ($t = -6.29$, $df = 8$, $p = 0.0002$), and ash content ($t = -3.81$, $df = 8$, $p = 0.0051$). However, significant differences were found for moisture ($t = 14$, $df = 8$, $p = 0.0001$) content (Table 2). The food stability test indicated that a significantly greater ($F[5,30] = 184.1$; $p < 0.0001$) mean dry matter loss occurred at 18 and 22°C compared with 15°C for the CA and FM diets, after 12 h of submersion in seawater (Table 3). Survival of juvenile *H. tuberculata* during the feeding trial was not affected by dietary treatment and averaged 90% for animals cultured at 15°C and 100% for animals cultured at 18 and 22°C. The relationship of Stw/Sw ratio is shown in Table 3. Significantly greater differences ($F[5,12] = 10.4$; $p = 0.0005$) were found between temperatures (15, 18, and 22°C). FCR was related to temperature ($p < 0.05$), decreasing from 3.52 and 3.56 at 15°C to 0.76 and 0.76 at 22°C for the CA diet and the FM diet, respectively (Table 3).

Shell Growth

Significant effects of diets ($F[9,946] = 466.1$; $p < 0.0001$) and temperatures ($F[2,946] = 407.2$; $p < 0.0001$) were found on the shell length. During the first month, the mean growth of abalone was low (Fig. 1a); mean values were 3.25, 3.62, and 3.58 mm mo^{-1} for the CA diet and 3.21, 3.78, and 3.81 mm mo^{-1} for the FM diet in abalone cultured at 15, 18, and 22°C, respectively. After January, the mean length increased markedly; the maximum value at the end of the 4-mo experiment was 14.16 mm for abalone on the FM diet at 22°C, about 2.5 times the value for the same diet at 15°C (Fig. 1a).

Body Weight

A two-way analysis of variance indicated highly significant effects of diets ($F[9,946] = 312.3$; $p < 0.0001$) and temperatures ($F[2,946] = 485.9$; $p < 0.0001$). During the first month, mean growth on both diets was similar ($p < 0.05$; Fig. 1a and b). After January, animals cultured at 18 and 22°C grew faster than did animals cultured at 15°C ($p < 0.05$), showing significant differences between temperatures by April ($p < 0.05$; Fig. 1b). By the end of the experimental trial, mean weights of the groups on the FM diet were 173.95 and 222.85 mg for 18 and 22°C, respectively. For the CA diet, means were 131.15 and 203.84 mg at the same temperatures. Juveniles cultured at 15°C on the CA diet exhibited a significantly ($p < 0.05$) lower weight gain (0.08 mg day^{-1}) compared with juveniles cultured at 18°C (1.1 mg day^{-1}) and 22°C (1.8 mg day^{-1}). The same tendency was observed for juveniles fed the FM diet. Mean length and weight increased over time (Fig. 1a and b) on both diets at each of the three temperatures (15, 18, and

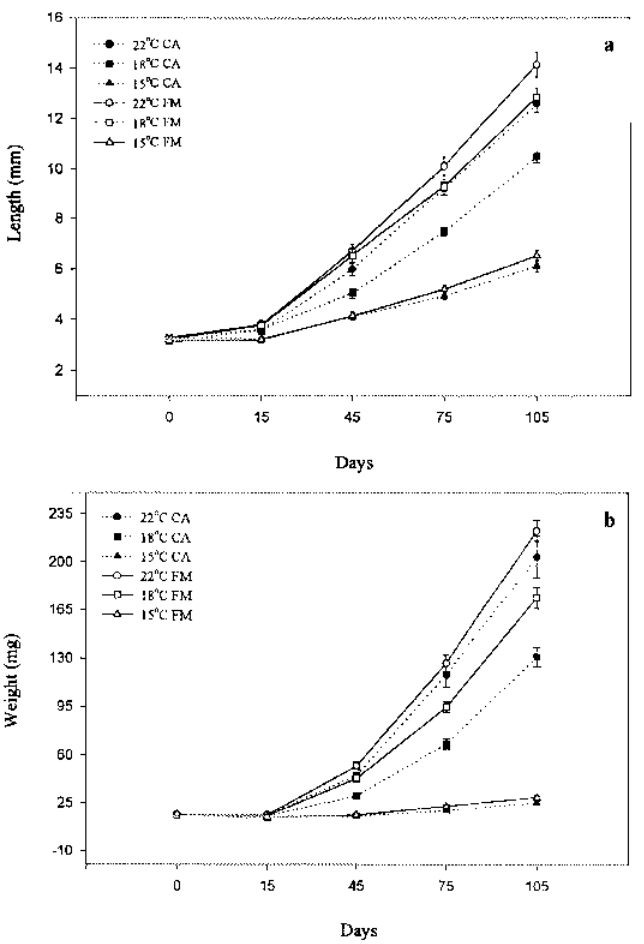

Figure 1. Mean growth of *H. tuberculata* provided during 105 days (December to April) with two artificial diets (CA and FM) and cultured at 15, 18, and 22°C. Bars indicate standard errors. (a) Shell length means. (b) Body weight means.

TABLE 4.

Daily growth rates of juvenile *H. tuberculata* fed for 105 days (December to April) on two artificial diets, FM and CA, and cultured at 15, 18, and 22°C.

Temperature °C	Months	Growth Rates			
		CA ($\mu m\ day^{-1}$)	FM ($\mu m\ day^{-1}$)	CA ($\mu g\ day^{-1}$)	FM ($\mu g\ day^{-1}$)
15°	January	0.04 (14.7)	3.82 (12.6)	−176.6 (122)	−167.5 (130)
	February	29.09 (7.1)	31.60 (6.6)	55.1 (6)	62.4 (59)
	March	26.32 (6.6)	36.64 (8.5)	133.5 (78)	211.1 (69)
	April	39.82 (5.5)	43.92 (8.9)	196.4 (101)	223.7 (67)
18°	January	23.69 (14.8)	36.6 (14.8)	−55.4 (104)	−20.4 (105)
	February	48.23 (9.1)	91.44 (10.41)	514.8 (52)	921.4 (76)
	March	80.29 (7.3)	92.62 (15.4)	1,256.2 (121)	1,721 (175)
	April	100.6 (9.9)	118.5 (15.9)	2,103.8 (274)	2,644 (321)
22°	January	29.98 (4.7)	34.12 (14.7)	13.8 (10)	33.1 (101)
	February	80.56 (8.5)	96.39 (12.1)	962 (79)	1,201.4 (95)
	March	107.5 (10.5)	113.5 (15)	2,436 (315)	2,485 (199)
	April	112 (13.3)	134.81 (19.7)	2,854 (514)	3,205 (322)

Standard errors in parentheses.

22°C). At the beginning of the experiment, the average length and weight of juvenile *H. tuberculata* comprising experimental groups did not differ significantly ($p > 0.05$; Fig. 1a and b). After January, these differences became significant ($p < 0.05$) between temperatures, reaching a maximum at 22°C.

Significant differences occurred between diets and temperatures on the daily growth rates of the shell (Kruskal-Wallis method, $H = 212.9$, $df = 23$, $p \leq 0.0001$) and body weight ($H = 442.9$, $df = 23$, $p \leq 0.0001$) on animals cultured at 18 and 22°C (Table 4). The growth rates for animals cultured at 15°C were low, with no growth in length and loss of weight at the beginning (0.04 $\mu m\ day^{-1}$ and −176.6 $\mu g\ day^{-1}$), but reaching highest values in April (39.82 $\mu m\ day^{-1}$ and 196.4 $\mu g\ day^{-1}$), which are still low compared with the other treatments. Low growth rates were found consistently at low temperature (Table 4).

The control group of juveniles fed dry *P. palmata*, after 75 days of the experimental trial, reached up to 85% (15°C), 73% (18°C), and 64% (22°C) mortality in all replicates. Under those circumstances, after the third measurement, this group was removed from the study.

DISCUSSION AND CONCLUSIONS

H. tuberculata provided an FM diet (as a main source of protein) and, cultured at 18 and 22°C, produced superior growth rates for shell length and body weight compared with juveniles fed a CA diet and cultured at 18 and 22°C. Although the CA diet contained a slightly higher protein content than did the FM diet, a significantly greater growth rate was observed in juveniles fed the FM diet. Our results differ from those reported by Uki et al. (1985), who found that a CA diet resulted in better growth than an FM diet in *Haliotis discus hannai*. Diet palatability is an important consideration when formulating diets for aquaculture species. Abalone show a preference for certain ingredients, resulting in better food acceptance and consumption (Harada 1992, Sakata and Ina 1992, Shepherd and Steinberg 1992, Viana et al. 1994, Viana et al. 1996). However, the nutritional value of a diet is of major importance and is implied by the content of those elements necessary to give a proper balance between energy and growth. In this study, fish meal was more efficient than CA, resulting in better overall growth rates.

Water temperature is one of the most important environmental factors influencing metabolic rate and energy expenditure. Strict comparison of our results on the effects of temperature and diets on growth with data on other species is difficult because experimental conditions vary widely, including environmental factors and the initial sizes used. Nevertheless, growth rates of *H. tuberculata* have been shown to be much higher when cultured at temperatures between 18 and 22°C (Peck 1989, Shpigel et al. 1996a, Shpigel et al. 1996b).

The daily growth rates for animals cultured at 15°C were low, with no measurable length increase and loss of weight at the beginning. Highest values for this group occurred in April, which are still low compared with the other treatments. According to several authors (Peck 1989, Mercer et al. 1993, Mai et al. 1995), *H. tuberculata* fed natural, mixed, and formulated diets, cultured at temperatures of 12–15°C, produced acceptable rates of growth for shell length and body weight. We found low growth rates at low temperature (15°C). Animals during winter have lower food intake rates and also have reduced metabolism and energy requirements. Most of the energy absorbed sustains metabolic needs, and any deficit is drawn from its own nutrient reserves (Widdows 1973). Individuals would be expected to lose weight (Peck 1989).

From the beginning of the experimental trial, the animals in the control group fed dry *P. palmata* began to lose weight with no shell length increase. Mortalities were high, reaching the highest levels (up to 85%) by the third month. We believe that the dry seaweed did not supply adequate nutrients. Dried and rehydrated marine algae often are not as acceptable and supportive of growth as are fresh (living) plants (Leighton pers. comm.). We were not able to use fresh seaweed in this experiment because *P. palmata* is a seasonal species, disappearing during the coldest months of the year. Indeed, *P. palmata* is one of the major constituents of the natural diet of *H. tuberculata*, which also includes a variety of delicate seaweeds, such as *Ulva lactuca* and *Enteromorpha intestinalis*, and coarser ones, such as *Laminaria* spp., *Chondrus crispus*, encrusting coralline algae, and sessile diatoms (Culley and Peck 1981, Mercer et al. 1993). Combinations of these algal foods should provide all nutrients necessary for maximum growth.

It is well known that seaweeds are, in general, relatively low in protein and lipid (cf. Mai et al. 1994, Mai et al. 1996). Hahn (1989)

reported observations that abalone fed on seaweed supporting colonies of bryozoa or hydroids grew faster than did those fed the same species without such growths. These metazoans could play an important role in abalone nutrition, as a supplementary source of protein and lipid. In our control group, the use of dried seaweed cleaned of all fouling organisms may have reduced its nutritive value and contributed to its poor performance as a food. The high mortalities may be related to bacterial growth and degradation of the rehydrated alga.

A mixed algal diet may have been more appropriate for our study. Among other researchers, Day and Fleming (1996) and Viana et al. (1993 and 1996) found that single species of seaweeds, provided as exclusive food items, did not sustain growth over extended periods, failing to provide a nutritionally balanced diet and thus reflected in low growth rates in *Haliotis rubra* and *Haliotis fulgens*. Furthermore, some recent studies on artificial diets (e.g., Britz 1996, Britz et al. 1997) have not included a seaweed-fed control group. This could be a matter of choice after finding low growth rates and high mortalities when offering dried seaweed under the same experimental conditions as maintained in feeding trials with formulated diets.

Our results clearly show a significant increase in body and shell growth rate at higher temperatures. FCRs also improved markedly at higher temperatures. The diet providing protein from FM supported better growth in *H. tuberculata* than did the CA-based diet. We would recommend that those involved in culture of this abalone control seawater temperature to remain in the range of 18–22°C and provide an FM-based artificial diet.

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GROWTH OF JUVENILE ABALONE, *HALIOTIS DISCUS HANNAI* INO 1953 AND *HALIOTIS RUFESCENS* SWAINSON 1822, FED WITH DIFFERENT DIETS

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ABSTRACT The objective was to determine the growth rate of juvenile *Haliotis discus hannai* (Japanese abalone) and *Haliotis rufescens* (red abalone) with four different diets: three diets based on fresh algae (*Lessonia trabeculata, Macrocystis integrifolia* [MA], and *Ulva rigida*) and one artificial diet (PE). The results obtained for both species are not comparable because the initial size and densities used were different. The best growth rate for Japanese abalone was obtained with the artificial diet (PE), 0.24 and 0.82 %/day, and for red abalone, it was obtained with algae (MA), 0.15 and 0.57 %/day, for the length and weight, respectively. Factors affecting abalone growth are discussed with special reference to those factors influencing the rate of ingestion and the use of the diets.

KEY WORDS: *Haliotis discus hannai, Haliotis rufescens,* feeding and nutrition, molluscs, diets, growth

INTRODUCTION

Juvenile abalone start feeding on algae at about 10 mm in size, and they can consume 5–30% of their body weight per day (Hahn 1989, Uki and Watanabe 1989). This high feeding rate is due to the high content of water and relatively low content of protein in the algae. Thus, the abalone require large amounts of fresh algae, mainly laminarian algae. These algae are expensive because of their extensive use in the production of alginates and other compounds widely used in industry. The use of these algae becomes one of the bottlenecks in the cultivation of abalone. For this reason, it is necessary to consider a reliable and inexpensive supply of fresh algae to maintain a commercial abalone culture at a reasonable cost. Accordingly, it was decided to include in the study the alga *Ulva* sp., which has no commercial value but is rich in protein content and is accepted by the abalone. The growth rate of abalone fed with algal diets is low and variable over time. Therefore, it was decided to include an artificial diet made of fish meal, which contains protein, fat, and algae meal as an attractive element.

To establish the control of feeding efficiency, it was decided to determine the growth rate through measurement of the indices of feeding, such as feed conversion (FC) and feeding rate (FR), which are very important when cultivating abalone commercially. In the study, these indices were applied to Japanese abalone and red abalone fed on four different diets, based on three different species of algae, *Lessonia trabeculata, Macrocystis integrifolia,* and *Ulva rigida,* and one based on the artificial pellet. The alga *L. trabeculata* was selected as the control for the other three diets, because this alga is commonly used to feed the abalone grown in the hatchery of the Universidad Católica del Norte (UCN) in Coquimbo, Chile.

MATERIALS AND METHODS

Experimental Design

The study was carried out at the university between October 1995 and July 1996. The data are related to 9 mo of study, considering that the first month was necessary to condition the abalone to the culture facilities.

A total of 180 juvenile abalone were furnished by the UCN hatchery and distributed in 24 culture baskets: 120 Japanese abalone in 12 baskets and 60 red abalone in the remaining 12 baskets (in both cases, three replicates for four different diets). The average length of the abalone was 21.06 (±0.04) and 35.94 mm (±0.93) for *H. discus hannai* and *H. rufescens,* respectively. Because of the different sizes and densities for each species at the beginning of the study, the results for both species are not comparable.

Animal Rearing

The number of abalone for the experiment was determined by the availability of abalone at the UCN hatchery at the time the study was initiated. However, this amount was lower than the theoretical number of abalone for the available surfaced area in the baskets (carrying capacity was 75 Japanese and 25 red abalone for each basket). The baskets were submerged in a fiberglass tank of 1,000-L capacity. The baskets were labeled LE (*L. trabeculata*) UL (*U. rigida*), MA (*M. integifolia*) and PE (artificial diet).

The water flow rate in the tank was 20 L/min of seawater, filtered to 60 μm, with a water change rate greater than once every hour. Constant aeration and flow provided more than 7 mL of O_2/L, a level recommended by Uki and Kikuchi (1975) based on the weight of the animals and the average temperature of the seawater.

Sampling Methods

Measurements of length, width, and weight were taken monthly on each individual abalone. Growth rate of abalone larger than 2 cm in length of shell was slow (1–2 mm/mo), and we wished not to expose the animals to more frequent handling (measuring).

Growth Measurements

Weight, width, and length were recorded once per month to obtain the growth rate per day. With this data, the specific growth rate (SGR) in length and weight was determined with the formulae described by Martinez (1987) and Mai et al. (1995):

$$\mathrm{SGR}(\%/\mathrm{day}) = 100 \times \frac{(\ln(\mathrm{Wf}) - \ln(\mathrm{Wi}))}{\mathrm{days}};$$

$$\mathrm{SGR}(\%/\mathrm{day}) = 100 \times \frac{(\ln(\mathrm{Lf}) - \ln(\mathrm{Li}))}{\mathrm{days}}$$

where Wi and Li are the initial weight and length, respectively, and Wf and Lf are the final weight and length of the abalone, respectively.

Food Consumption

Every week, the wet weight of food supplied to each basket was recorded, and the unconsumed food was removed from the baskets and also recorded. The experimental feeding protocol was given separately for each diet to three replicate groups in separate baskets containing 10 Japanese abalone and 5 red abalone (3 replicates × 4 diets = 12 × 2 species of abalone = 24 baskets). The abalone were fed *ad libitum* with 50 g of algae (an amount much higher than the real needs of the abalone) of the three different species. The algae were placed in each basket once each week and 10 g of pellets were placed twice each week. The pellets contained 42.1% carbohydrates, 36% protein, 5.7% ash, 5% fat, 1.2% crude fiber, and 10% water containing 1,490 kJ/100 g of energy.

The algae used as food were harvested weekly to ensure freshness. A control basket divided in three sections was included in the experiment (without abalone) in which 50 g of the three different species of algae was placed weekly to determine the variation in weight. These algae were placed in and removed from the control basket at the same time as the regular feeding schedule. To estimate the FC and the FR, the equation used was as described in Grenier and Takekawa (1992), including some modifications by the authors, in accordance with the preliminary experimental results (Ip).

FC: $$FC = \frac{W_i}{W_g};$$

FR: $$FR(\%/\text{day}) = \frac{(F_1 - F_2) * 100}{t * w}$$

Food consumption (AC): $$AC = [F_1 - (F_2 - I_p)]$$

where F_1 = initial food weight (g), F_2 = final food weight (g), Ip = weight gain of algae due mainly to water absorption, t = number of days, w = mean abalone weight (g), W_i = weight ingested by the abalone (g), and W_g = weight gain by the abalone (g).

STATISTICAL ANALYSIS

Data from each different treatment were analyzed by a one-way analysis of variance (ANOVA) test and Tukey test to determine differences of means. Statistical analysis was performed with the software Statgraphics 6.0®.

RESULTS

Growth of Abalone

At the beginning of the experiment (approximately the first 4 mo), a significant difference between mean length and weight of the abalone for each diet was not observed ($p < 0.05$). After 4 mo, the differences increased and were significant, specifically in the diets with the best results: PE and UL for Japanese abalone and PE and MA for red abalone. This could indicate that, during this period, the abalone adapted physiologically to a diet change.

The growth in length and weight for each diet is significant over time ($p < 0.00001$). The best growth in length, width, and weight for Japanese abalone was obtained with PE and UL, and for red abalone, the best growth was obtained with MA and PE (Table 1).

SGR

The ANOVA showed that there was not a significant difference in mean SGR for any of the replicate diets, for both species (ANOVA, $p > 0.05$). Applying the Tukey test to SGR (Tukey test $p < 0.05$) for the Japanese abalone, the existence of a significant difference in means was shown between the SGR of the abalone fed with the diets, with the exception of the juveniles fed with LE and MA. For red abalone, the same analysis revealed that, in general, there was not a significant difference in mean SGR for the different diets, except between LE and MA and MA and UL (Fig. 1).

Food Utilization Indices

The average FR expressed as percentage of body weight per day for the Japanese abalone fed with LE was 10.14 (±5.55); with MA, it was 15.84 (±8.15), and with UL, it was 9.84 (±7.24) (Fig. 2). The ANOVA indicated that there was no significant difference between the FRs on LE and UL.

The average FR (%) for red abalone for the different algae (LE, MA, UL) was 2.78 (±1.19), 4.26 (±2.30), and 2.48 (±1.42), respectively (Fig. 3). An ANOVA was performed and revealed that there were significant differences among the diets for the feeding rate of red abalone ($p = 0.00001$).

The ANOVA for Japanese abalone revealed that there were no significant differences ($p = 0.1381$) between the conversion ratios for the different diets (Fig. 4). In the case of the red abalone, negative values were not observed (Fig. 5). Applying the ANOVA to the factors of diet and temperature, no significant differences were revealed between the FC of the LE and UL diets (diets, $p = 0.0389$; temperature, $p = 0.0315$; interaction, $p = 0.0110$).

DISCUSSION

Abalone Growth Performance

For the Japanese abalone, the best growth in length, width, and weight was obtained with the artificial diet (PE), having an average

TABLE 1.

Growth of juveniles of *H. discus hannai* and *H. rufescens* fed with four different diets.

	H. discus hannai					*H. rufescens*				
Diet	**N**	**L_1**	**L_2**	**W_1**	**W_2**	**N**	**L_1**	**L_2**	**W_1**	**W_2**
LE	30	21.12 ± 0.87	9.87	1.02 ± 0.14	2.47	15	34.51 ± 1.05	11.1	5.91 ± 0.39	8.26
MA	30	21.03 ± 0.69	9.78	1.07 ± 0.69	2.38	15	33.70 ± 0.94	15.02	5.47 ± 0.46	14.30
UL	30	21.07 ± 0.73	13.59	1.08 ± 0.47	4.09	15	34.88 ± 0.86	6.28	5.83 ± 0.97	5.01
PE	30	21.03 ± 1.01	16.73	1.03 ± 0.18	6.31	15	35.94 ± 1.44	12.09	6.65 ± 0.92	13.74

L_1, initial length (mm); L_2, length gain (mm); W_1, initial weight (g); W_2, weight gain (g).

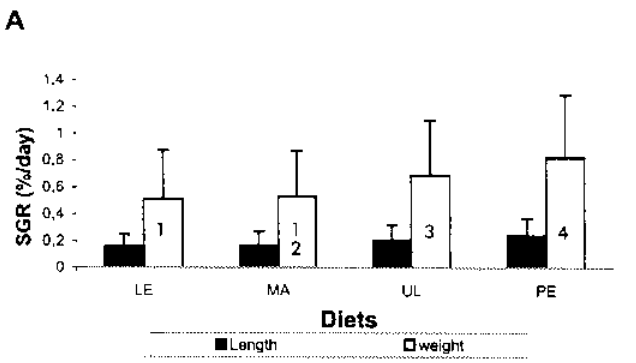

Figure 1. SGR for *H. discus hannai* (A) and *H. rufescens* (B) fed with four types of diets. Each bar represents the average of three replicates with standard deviations. The bars sharing the same number are not different significantly.

growth rate 24% higher than that obtained with natural diets (algae). This is in accordance with the findings made by Hahn (1989), who stated that the growth of juveniles fed with artificial diets is usually higher, because these formulations contain the appropriate concentration of each of the necessary elements (basic food stuffs, vitamins, and minerals) for the abalone.

For the Japanese abalone, the growth obtained with the three natural diets in length, width, and weight was best with UL, with a growth rate of 0.69%/day. This result is in agreement with those obtained by Uki and Watanabe (1986) and Uki and Watanabe (1991), who described a growth rate for *H. discus hannai* fed with *Ulva* sp. of 0.71%/day. Although the SGR in length of the abalone fed with LE is similar to the SGR of the abalone fed with UL, it is important to mention that color, calcification, and thickness of the shell were better in the abalone fed with UL. This could be related to the high percentage of protein and carbohydrates present

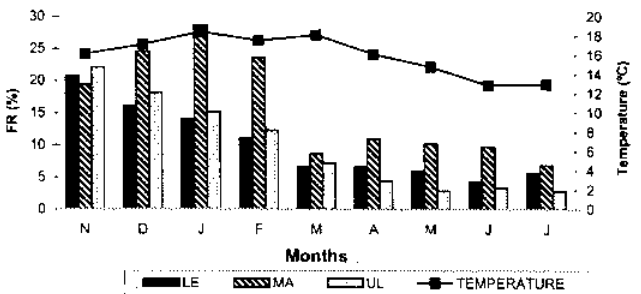

Figure 2. Mean FR calculated for juvenile Japanese abalone in relation to the mean monthly temperature.

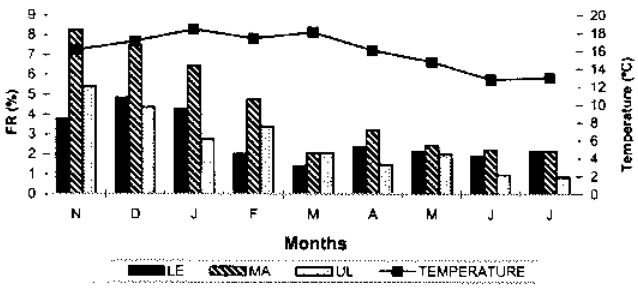

Figure 3. Mean FR calculated for juvenile red abalone in relation to the mean monthly temperature.

in UL compared with that in the other two species of algae. *Ulva* sp. contains 27% more protein and 100% more carbohydrate than do Lessonia sp. Mai et al. (1995) conducted a comparative study of nutrition in two species of abalone (*Haliotis tuberculata* and *H. discus hannai*) examining growth for several levels of lipids in the diet. They found poor growth rate for juveniles of *H. discus hannai* with a diet containing a higher percentage of lipids. An earlier study by Uki and Watanabe (1991) found reduced growth when lipids were greater than 5% of their artificial diet.

The results of growth obtained in the red abalone are in agreement with those of others. Owen et al. (1984) noted that the best results were obtained with diets based on the alga MA, in comparison with other local algae like *L. flavicans*, L. nigrescens, and diets of diatoms (microalgae). Furthermore, the diet with MA surpassed the SGR of the abalone fed with an artificial diet, 10 and 21% in length and weight, respectively. The lowest growth in length, width, and weight of the red abalone in this study was obtained with UL, which in spite of its leafy morphology (abundant frond), soft texture, and ease of grazing for the abalone, was not a good food source for this species because it was consistently low in conversion and utilization. This was observed in the SGR and is in accordance with the results obtained by Stuart and Brown (1994) for *Haliotis iris*, who tested several natural diets, obtaining the lowest growth rate with *Ulva*. This alga supplied the red abalone with sufficient energy only for basal metabolism (maintenance) because the growth rate was 0.1%/day, relatively similar to that obtained in this study of 0.3%/day.

Food Utilization Indices

Abalone appear to have erratic feeding habits, sensitive to a number of environmental and physiological influences. In this study, the value of FR for *H. discus hannai* was strongly associated to the hardness or softness of the laminarin algae, *L. trabeculata* is more robust than *M. integrifolia*. Therefore it may be more difficult

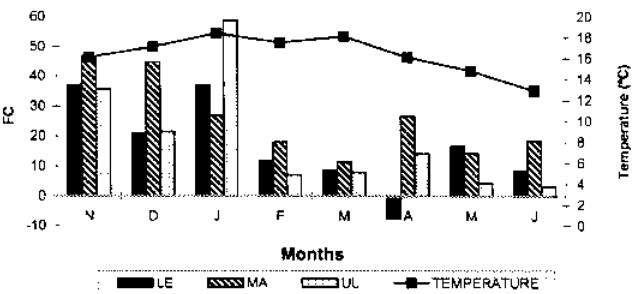

Figure 4. FC calculated for juvenile Japanese abalone in relation to the mean monthly temperature.

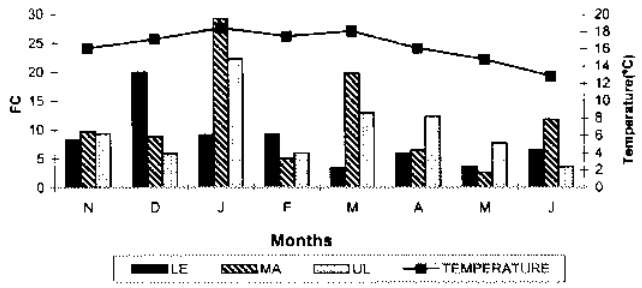

Figure 5. FC calculated for juvenile red abalone in relation to the mean monthly temperature.

to graze for the juvenile abalone, according to the report of Shepherd and Steinberg (1992) for three species of Australian abalone. Shepherd and Steinberg (1992) identified three factors that affect selection of natural algal foods by abalone: (1) the chemical metabolites present in the alga; (2) algal morphology, referring to the toughness of the algae; and (3) nutritive value of the alga, relative to its contribution to the organism at its stage of development.

PRACTICAL APPLICATIONS

Referring to the algal diets and the artificial diet used in this study, and to the cost of feeding in abalone culture, the cost of acquisition and storage of the algae must be considered. For this reason, and in accordance with the results of this study, a feeding plan for juvenile Japanese abalone should be based on a diet of *Ulva* sp. in combination with an artificial diet (amounting to 1%/day of the weight of the abalone being fed). The artificial component should be considered as a nutritive supplement to lessen the cost in food requirements. At the same time, it is recommended that a locally produced pellet be developed, mainly for the juvenile phase. This could include *Ulva* sp. meal as an algal protein base and attractant element. For the red abalone, a diet based on *Macrocystis* and an artificial diet with the same characteristics mentioned above is recommended.

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THE EFFECT OF CELLULOSE ON THE GROWTH AND CELLULOLYTIC ACTIVITY OF ABALONE *HALIOTIS FULGENS* WHEN USED AS AN INGREDIENT IN FORMULATED ARTIFICIAL DIETS

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ABSTRACT This work demonstrated that when cellulose was substituted for sodium alginate in an artificial diet, there was no significant change in growth of juvenile *Haliotis fulgens*. An increase in cellulolytic activity was observed in stomach fluid when cellulose was present in the diet (19%), compared with a noncellulose diet (3.24 vs. 1.87 U, respectively), suggesting enzymic adaptation. Growth in length and weight of abalone provided diets with and without cellulose was similar ($p < 0.05$). The feed conversion efficiency ratios were higher for the feed with cellulose (1.21 vs. 1.04), although differences were not statistically significant. Abalone fed the high-cellulose diet had associated greater activity of both cellulase and alginase. The possible role of microorganisms in the production of carbohydrases like alginase and cellulase is discussed.

KEY WORDS: Abalone, nutrition, cellulose, cellulases, digestion

INTRODUCTION

Artificial diets have been shown to produce better growth rates in juvenile abalone than do natural algal diets (Uki et al. 1985, Viana et al. 1993a). Recent research on artificial diets for abalone has focused on improvements in nutritional quality and stability in seawater (Gorfine and King 1991, Lopez and Viana 1995). Although abalone are regarded as herbivores, better growth has been observed when they are fed artificial diets compounded to approximate their organic composition with a relatively high content of protein and carbohydrate and a low content of lipid and fiber (Fleming et al. 1996). This finding is curious because the abalone natural diet is mainly composed of brown kelps, which are rich in structural carbohydrates (fiber) and low in protein. Because kelp and other macroalgae comprise the bulk of the natural diet, abalone may be expected to digest complex carbohydrates such as the alginates, laminarin, and cellulose. Several Japanese studies on composition of artificial foods for abalone have yielded diets supporting good growth rates (Fleming et al. 1996) that contain high amounts of sodium alginate (Bardach et al. 1975, Hahn 1989). Abalone are able to digest sodium alginate (and other salts) by the enzyme alginase (or algin depolymerase), chiefly in the stomach (Oshima 1931, Nakada and Sweeny 1967, Leighton 1968, Uki and Watanabe 1992). Interestingly, Fleming et al. (1996), in a review, pointed out that cellulases reported for various abalone species exhibited different levels of activity with variation between species and types of cellulose hydrolyzed. On the contrary, Uki et al. (1985) reported that when cellulose was added as an ingredient in the diet from 0 to 20%, a decrease in growth rate, FCE, and PER was found with increasing cellulose content, indicating that the abalone could not easily digest the substrate or that its presence interfered with digestive function. Cellulases, enzymes that hydrolyze cellulose, are typically found in specific bacteria. Cellulases are not known to be produced by digestive tissue in higher animals. However, cellulolytic enzymes are reported either to be existent as endogenous secretions or formed by microflora in many mollusks (Stone and Morton 1958, Leighton 1968, Livingstone and de Zwaan 1983). Ruminants depend on bacteria and protozoa in the rumen to digest cellulosic plant fiber, and it is known that for these mammals, diet composition is of importance to provide an optimal environment in the rumen to regulate and maintain an appropriate microbial population, resulting in optimal enzyme production.

Cellulose is the most widely distributed structural plant polysaccharide and is one of the most abundant and chemically resistant of all substances elaborated by living cells (Gortner 1950). Cellulose is a linear chain of 100–200 glucose units mutually joined by β-1, 4-linkages. The polysaccharides from seaweed comprise three groups: the water-soluble sulfuric acid esters (agar, carrageenan and fucoidan), the water-soluble reserve carbohydrates such as laminarin, and the alkali-soluble polyuronides represented by algin. Alginic acid $(C_6H_8O_6)_n$ is composed chiefly of D-mannuronic acid units (and a lesser portion of guluronic acid residues) that yield 2,3-dimethyl D-mannuronide on methylation and hydrolysis. The polymer is characterized by β-1, 4-linkages that are similar to the geometric pattern of cellulose rather than starch. Alginic acid is a principal carbohydrate component of *Macrocystis pyrifera* (Chapman and Chapman 1973), a common natural food of *Haliotis fulgens*.

The aim of this work is to examine the digestibility of cellulose and, when substituted for sodium alginate in a formulated diet, its effect on growth. Enzyme activity in the stomach (alginase and cellulases) is compared between treatments.

MATERIALS AND METHODS

Diet Preparation

Silage was made as described by Viana et al. (1993b). In summary, chopped abalone viscera was mixed with 2.6% phosphoric acid, 2.6% citric acid, and 0.1% sodium benzoate as a preservative. The mixture was blended to obtain a homogenate and left for 60 days in covered plastic buckets. The silage was neutralized with

sodium carbonate and calcium carbonate (2% each) before diet preparation. The ingredients for both diets are shown in Table 1. They were based on the constituents recommended by Uki and Watanabe (1992). Vitamin and mineral mixtures were those recommended by Hahn (1989). Gelatin was used as the primary binder. Sodium alginate was needed in order to bind the diet fully, although in one diet, it was used minimally. For Diet I, sodium alginate was used at 20% **(DA),** and Diet II contained 19% cellulose and 1% sodium alginate **(DC).** All ingredients were blended until a completely homogenized mixture was obtained. A sufficient amount of calcium existed in the mineral mixture and the neutralized acid silage to convert the sodium alginate to the calcium alginate gel. The diet was then rolled flat to a thickness of 2 mm, and pieces of 2 cm in diameter were cut and frozen at −25°C until needed. The pieces of formulated diet were tested for their 12-h stability in seawater. This was done routinely by leaving two pieces in each control bucket without animals, one for each diet, to measure the daily weight loss (dry basis) throughout the experiment.

Proximate Analysis

Percent dry weight was calculated from the residue weight of triplicate samples (4–6 g) of each diet after being dried to constant weight at 100°C. Total nitrogen was determined in triplicate samples analyzed by the Kjeldahl method (AOAC 1990). Crude protein was calculated as %N × 6.25. Total lipid was determined in triplicate samples (10 g) by a column procedure with methanol-chloroform/water as the eluting solvent (Bligh and Dyer 1959). Carbohydrate was found by the difference. Ash was determined after heating duplicate samples (3 g) of each diet to 600°C for 4 h.

TABLE 1.

Percent composition of the artificial diets (DA and DC) tested in this study, given as a percentage of dry matter.

Ingredients	20% Sodium Alginate (DA)	19% Cellulose (DC)
Sodium alginate*	20.0	1.0
Cellulose†	0.0	19.0
Silage‡	30.0	30.0
Soybean meal§	10.0	10.0
Corn meal	10.0	10.0
Starch (rice)	5.0	5.0
Kelp meal¶	9.7	9.7
Gelatin#	6.0	6.0
Vitamin mixture**	3.0	3.0
Mineral mixture**	6.0	6.0
Choline chloride	0.08	0.08
Methionine	0.2	0.2

* Kelgin MV, kindly supplied by Kelco.

† Alphacel purchased from ICN.

‡ Abalone viscera kindly supplied by the fishery cooperative Emancipación, given as dry matter basis.

§ Fifty percent protein, kindly supplied by the American Soybean Association.

Maseca, produced in Mexico.

¶ Kindly supplied by CRIP (Centro Regional de Investigaciones Pesqueras, Mexico).

Commercial gelatin with 50 blooms.

** As recommended by Hahn (1989), using Stay-C (Ascorbyl polyphosphate) from Roche.

Experimental Procedure

Two hundred twenty-two specimens of 8-mo-old *H. fulgens* of 18.9 mm (SE = 0.10) and 0.9 g (SE = 0.02) in average shell length and weight, respectively, were obtained from the laboratory of Bahia Tortugas B.C.S. The abalone were held in running aerated seawater controlled at the rate of 300 mL min^{-1}. Seawater was pumped from the ocean, filtered, and delivered to tanks in a flow-through system. Average daily temperature varied between 19 and 23°C throughout the feeding trials. Oxygen, pH, and ammonium content were monitored (data not shown). Feeding experiments were conducted after 21 days of acclimation to laboratory conditions. During this preliminary period, abalone were fed a common artificial diet with 10% sodium alginate.

Experimental animals were held in 20-L plastic containers (three replicates per treatment) with 37 abalone per container under the same conditions described above. Diets were given for 12 h at nighttime (*ad libitum*) every night. Each morning, remaining food was carefully collected for drying and weighing. This was done throughout the first 9 wk. After that time, diets were offered continuously, being changed every 24 h until Week 15. Algae growing on the inside walls of the containers were removed twice a week with a soft brush. Whole-body weight was measured with an electronic balance (±0.001 g), and shell length was determined with an electronic digital caliper (±0.03 mm) every 3 wk.

Specific growth rate (SGR % day^{-1}) was estimated according to Espe (1993) (Equation 1). When a net weight loss occurred, no absolute values were considered for Equation 1:

$$\text{SGR} = 100\ \{(\ln \text{final weight} - \ln \text{start weight})\ \text{day}^{-1}\text{s of experiment}\} \quad (1)$$

Consumption (Equation 2) and food conversion efficiency ratios (Equation 3) were estimated during the first 9 wk of the experiment, as recommended by Uki and Watanabe (1992):

$$F = (GS/100) - R \quad (2)$$

where G represents the offered food, S is the percent recovered food from the control buckets, and R is the food remaining after the abalone have fed.

FCE was calculated as follows:

$$\text{FCE} = \text{wet weight gain (g)/dry weight of food consumed (g)} \quad (3)$$

Note: This measure of food utilization is related to conversion efficiency, but is in fact the ratio of animal live or wet weight gain to the amount of dry diet consumed. In practice, this method is more useful to the culturist than is knowledge of true conversion efficiency (%).

Enzyme Activity

In order to evaluate the effect of substitution of cellulose for sodium alginate, the enzyme activity of 12 representative abalone selected in a random manner from each treatment was analyzed at the end of the experiment. Alginase activity was estimated according to Mody and Chauhan (1993) by incubating a mixture of sodium alginate, potassium chloride, and stomach extract. The reaction was stopped after 8 min of incubation at 37°C by heating the solution and was measured in a spectrophotometer at 485 nm

after the addition of a solution with phenol-H_2SO_4 as indicator. One unit of activity was defined as the amount equivalent to produce 1 μg of glucose in 8 min of incubation per gram of viscera (wet weight).

Cellulase activity was estimated according to Worthington (1979) by incubating a solution of 1% cellulose with the enzyme mixture (stomach fluid). After 2 h at 37°C, the fluid was mixed with anthrone-H_2SO_4 solution and percent transmittance was measured at 625 nm. One unit of activity was defined as the amount required to produce 1 mg of glucose after 2 h of incubation at 37°C per gram of viscera (wet weight).

Statistical Analyses

A one-way analysis of variance (ANOVA) was used at specific times in the course of the trials to compare mean growth rate in length (μm/day) and SGR (SGR %/day) among diet treatments. A one-way ANOVA was also used for comparisons of growth rate in length and SGR over time, followed by a multiple comparison of the means (SNK = Student-Newman-Keuls test). A Kruskal-Wallis test, followed by the nonparametric Dunn's multiple comparisons (Zar 1984), was used when violations of homogeneity in the data were obtained. The software Sigma-Stat for Windows (Jandel 1994) was used in the statistical analyses.

RESULTS

Both diets (**DA** and **DC**) were similar in content of protein (23.1–24.7%), lipid (1.3–1.1%), and minerals (17.1–15.1%) (Table 2) and produced similar growth in length ($p > 0.005$) at each observation (Fig. 1). However, **DC** resulted in higher growth in weight than that observed for **DA** at the end of the experiment ($p < 0.005$) (Table 3). The growth rate in length for both treatments showed that growth was higher at the beginning of the experiment (80 μm/day), with values dropping as low as 30 μm/day by Week 9. During this interval, feed was offered 12 h a day. When diets were offered 24 h a day, the growth rate increased to levels almost as high as those observed at the beginning of the experiment (Table 3). A similar effect was reflected for total growth (Fig. 2) and SGR, with high values at the beginning of the experiment (0.90%/day and 1.01%/day for **DA** and **DC**, respectively), followed by a decrease until diets were offered for 24 h. Then, the SGR increased for both treatments. At the end of the experiment, **DC** gave a significantly higher SGR of 1.2%/day, compared with 0.80%/day for **DA** ($p = 0.019$) (Table 3). Stability (as dry matter loss after 12 h of immersion in seawater) was not significantly different for both treatments, with 35.1 ± 7.0% for **DC** and 20.1 ± 3.6% dry matter loss for **DA**.

Over the course of the trials, food intake was significantly higher for **DA** (average, 11.52 mg/day) compared with **DC** (7.4 mg/day) ($p = 0.003$). However, the FCE ratios for both diets were not significantly different ($p = 0.617$; Table 4), with a higher value of 1.21 for **DC** compared with 1.04 for **DA**.

Digestive enzyme activity differed significantly between treatments. Alginase and cellulase activities were higher in the groups fed cellulose (**DC**); levels of alginases were 0.04 U (**DA**) and 0.26 U (**DC**), whereas levels of cellulases were 1.87 U (**DA**) and 3.24 U (**DC**) (Table 5).

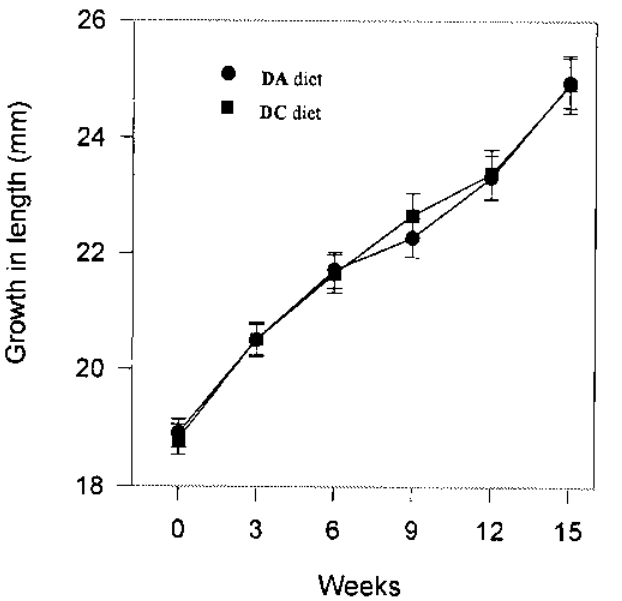

Figure 1. Total growth in length (mm) of abalone fed on a diet containing 20% sodium alginate (DA) and 19% cellulose (DC). Means and standard errors given.

DISCUSSION

Although the two diets supported similar growth during the experiment, **DC** became significantly higher in SGR by the end of the experiment. The growth rate for both diets was low at Week 9 but increased toward the end of the experiment when feed was offered for 24 h. This behavior is similar to that observed earlier

TABLE 2.

Proximate analysis of the diets DA and DC (20% sodium alginate and 19% cellulose, respectively).

Component	DA (%)	DC (%)
Crude protein*	23.1	24.7
Total lipid*	1.3	1.1
Ash*	17.1	15.1

* Given as dry matter basis.

TABLE 3.

Growth rates of juvenile abalone fed for 15 wk with feed containing 20% sodium alginate (DA) and 19% cellulose (DC).

Growth rate	No. of Weeks	DA	DC	p Value
μm/day	0	—	—	
	3	77.1 (0.66)	73.9 (4.64)	0.528
	6	60.3 (3.96)	55.4 (0.90)	0.295
	9	33.5 (3.52)	45.5 (11.8)	0.384
	12	44.2 (6.65)	42.8 (5.84)	0.882
	15	61.8 (8.11)	75.3 (1.35)	0.176
SGR* %/day	0	—	—	
	3	0.97 (0.09)	1.01 (0.05)	0.683
	6	0.78 (0.06)	0.74 (0.02)	0.660
	9	0.62 (0.15)	0.50 (0.15)	0.618
	12	0.34 (0.15)	0.37 (0.10)	0.869
	15	0.80 (0.05)	1.20 (0.09)	0.019

Standard errors are indicated in parentheses.

* SGR = 100 {(ln final weight-ln initial weight)/days of experiment}.

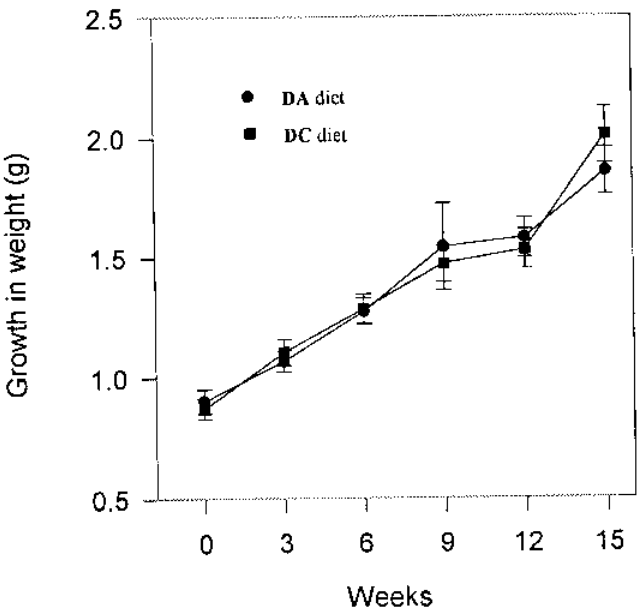

Figure 2. Total growth in weight (g) of abalone fed on a diet containing 20% sodium alginate (DA) and 19% cellulose (DC). Means and standard errors given.

(Viana et al. 1996), where abalone showed lower growth when feed was collected daily from the experimental buckets after a 12-h feeding period. That result may in part be due to stress from manipulation, because when the animals were no longer disturbed daily, an improved growth rate was observed. FCE was higher for the feed with cellulose (1.21 vs. 1.04), although the differences between the diets were not significant. A possible reason for the high variability could be the broad range of daily intake related to differences in pellet stability. Stability *in situ* for Diet **DC**, which was rich in cellulose, was 35.1 ± 7.0% dry matter loss after 12 h of water immersion, compared with 20.1 ± 3.6% for **DA.**

In this work, it was observed that abalone grew well on an artificial diet in which cellulose is the principal carbohydrate instead of sodium alginate. Likewise, enzyme activity in the stomach fluids of abalone fed with the **DC** diet containing 1% sodium alginate and 19% cellulose resulted in significantly greater concentrations or activities of both enzymes, a result that could be regarded as a digestive advantage. It is suggested that cellulose in some way stimulates the production of these enzymes. Alginase activity was relatively low in both groups **DA** (0.04 U) and **DC** (0.26 U) compared with cellulase. It is possible that the low values reflect the short reaction period adopted for the assay of alginase activity. A unit of alginase activity was defined as the amount

TABLE 4.

Food intake (mg/day), FCE, and pellet stability of juvenile abalone fed for 15 wk with diets containing 20% sodium alginate (DA) and 19% cellulose (DC).

Parameter	DA	DC	p Value
Food intake (mg/day)	11.52 (0.96)	7.54 (0.64)	0.003
FCE	1.04 (0.30)	1.21 (0.15)	0.617
Stability	20.1 (3.6)	35.1 (7.0)	0.074

Standard errors are indicated in parentheses.

TABLE 5.

Enzyme activity of alginases and cellulases in the stomach of juvenile abalone *H. fulgens* fed on DA and DC diets.

Component	DA	DC	p Value
Alginases	0.04 (0.01)	0.26 (0.02)	0.001
Cellulases	1.87 (0.15)	3.24 (0.31)	0.003

Standard errors are indicated in parentheses.

equivalent to produce 1 μg of glucose in 8 min of incubation per gram of viscera (wet weight), compared with the interval used to assess cellulolytic activity (2 h of incubation).

In several reports that concern digestive enzymes in marine gastropods, it is suggested that many marine snails, including *Haliotis*, appear to produce endogenous cellulases and other polysaccharidases (Stone and Morton 1958, Leighton 1968, Livingston and de Zwaan 1983). Production of cellulases in these animals may represent an evolutionary relic (Agnisola et al. 1981). Only a few reports consider the role of bacteria. Erasmus (1996) isolated bacteria from the abalone digestive system with cellulolytic activity, suggesting that those bacteria could be responsible for some of the cellulose degradation observed in stomach fluid.

Where present in higher animals, cellulases are found not to be produced endogenously (Schmidt-Nielsen 1981). This would suggest that no higher animal, herbivore or omnivore, could by itself digest the principal structural carbohydrate of plants, cellulose. It is well known that in ruminant mammals, digestion of this material is always brought about through a symbiotic association between the herbivore and microorganisms contained in its digestive tract. It is these microorganisms that are the actual agents for digestion of the cellulose (Flint and Forsberg 1995, Sauvant and van Milgen 1995, Van Soest et al. 1995). In fish, two herbivorous species (*Kyphorus spp.*) have a pouch-shaped extension of the intestine near the rectum that contains large quantities of bacteria. A high level of volatile fatty acids in the pouch indicates bacterial fermentation of plant cellulose (Schmidt-Nielson 1981, Withers 1992).

At present, we are trying experimentally to clear the abalone digestive tract bacteria in order to indicate whether cellulolytic activity in *H. fulgens* is in part or completely due to the activity of bacteria. We have been successful in isolation of certain bacteria with cellulolytic activity. Studies of plant cell wall degradation by pure cultures of rumen microorganisms have shown that only cellulolytic strains cause appreciable degradation. Noncellulolytic bacteria that possess hemicellulase or pectinase activity have been considered to contribute to the digestion of cellulosic substance, mainly through their interactions with cellulolytic species, suggesting that there may be a synergy between different microorganisms in the degradation of plant material at several different levels (Flint and Forsberg 1995). Until now, such a relationship between cellulose and algin degradation has not been studied, and it is premature to suggest a synergism between them, but the fact that, in this work, activities of both enzymes increased in the presence of cellulose, indicates that there is a possibility such synergistic processes are present.

If abalone require microorganisms to degrade the structural carbohydrate in the diet, it will be very important to provide a proper feed that promotes appropriate bacterial growth and thus increases the FCE. Studies of the metabolic products of relevant

microorganisms may advance our knowledge of abalone nutritional physiology and lead to the design of more efficient artificial diets.

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GROWTH CHARACTERISTICS OF THE HYBRID BETWEEN PINTO ABALONE, *HALIOTIS KAMTSCHATKANA* JONAS, AND EZO ABALONE, *H. DISCUS HANNAI* INO, UNDER HIGH AND LOW TEMPERATURE

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ABSTRACT Rearing experiments have been done to compare growth rates in pinto abalone, *Haliotis kamtschatkana* Jonas, and ezo abalone, *H. discus hannai* Ino. The growth rate in shell length of juvenile pinto abalone was superior to that of ezo abalone at water temperatures below 7°C. However, the growth rate declined above 17°C and was lower than that of ezo abalone. The hybrid cross between the female of ezo abalone and the male of pinto abalone was obtained but with low fertilization (20%). The reciprocal cross was never successful. The growth rate of the hybrid was superior to the parental species below 18°C and moderate between parental species under 8°C. Isozymic analysis confirmed that genetic hybridization occurred in this cross, on the basis of the results of electropherograms of the hybrid and its parental species.

KEY WORDS: abalone, *Haliotis kamtschatkana*, *Haliotis discus hannai*, hybrid growth, electrophoresis

INTRODUCTION

Recently, the abalone resource in Hokkaido has been decreasing (Momma 1990). Artificial abalone culture has been carried out in many culture systems. However, the growth rate of abalone in Hokkaido is much lower than that in Honshu because of the prevailing lower water temperatures. An abalone strain that exhibits higher growth rate in low water temperature is desired.

Interspecific hybrids of abalones have been produced among Californian species (Leighton and Lewis 1982) and among Japanese species (Koike et al. 1988, Hara 1992). Heterotic effects in the hybridization of abalones have been reported on growth (Leighton and Lewis 1982, Koike et al. 1988, Hara 1992) and survival (Leighton and Lewis 1982).

Possibly, a heterotic effect in the interspecific hybridization of abalone would be expressed in increased growth under low water temperature. The hybrid between the pinto abalone, *Haliotis kamtschatkana* Jonas, and the ezo abalone, *Haliotis discus hannai* Ino, have been studied, and some information has been obtained on the growth of the hybrid (Momma 1990). In this study, the hybridization between the two species was confirmed genetically by isozymic analysis, and a comparison of daily growth was made of the juvenile hybrids and parental species under artificial conditions.

MATERIALS AND METHODS

Hybridization and Rearing Experiments

Adult pinto abalone used in this study were collected at Sitka, southwestern Alaska. Ezo abalone were collected at Rebun Island and Okushiri Island on the west of Hokkaido, Japan. These adult abalone were kept in tanks and fed kelp, *Laminaria japonica* Areschoug, after marking with tags glued to their shells.

The juveniles of parental species and their hybrids were produced at the Hokkaido Institute of Mariculture. There, spawning was induced with ultraviolet-irradiated seawater and heat shock, common methods used in the abalone hatcheries in Japan. The sperm concentration was 0.5–1 million cells/mL. The juveniles for the rearing experiment concerning growth characteristics of parental species were produced by the mating of several females and males. The juveniles for the rearing experiments to study growth characteristics of the hybrids were produced by pair mating.

Young abalone were fed microalgae that grew on the collector surface until their shell length was over 8 mm. After that, they were kept in a cage made from netting with a mesh opening size of 4 mm and fed an artificial pellet. The juveniles that reached a shell length of about 20 mm were selected for the rearing experiment. A tag with a printed number was attached on the shell with a bond by the method of Hara (1989).

The experimental period was 37–99 days, depending on the temperature group. All groups or species were kept in the same cage and fed the same artificial food in sufficient quantity. The shell length was measured with a digital caliper to 0.01 mm, and the daily growth was calculated according to the following formula:

$$\mu m/d = \frac{Lt - Lo}{d} \times 1{,}000$$

where Lo is initial shell length (mm), Lt is final shell length (mm), and d is the number of days.

Water temperature was controlled at 5, 7, 12, 15, and 17°C in the rearing experiment for the growth characteristics of pinto abalone. The actual average temperatures were 4.9, 7.4, 12.2, 15.5, and 17.2°C. Similarly, water temperature for the rearing experiments for the hybrid was controlled at 8 and 18°C; the actual average temperatures were 9.3 and 18.6°C.

Isozymic Analysis

Pieces of foot muscle of parental species and hybrids were excised and placed into microtubes (1.5 mL). These microtubes were kept under −15°C. The fluid extracted from the muscle with thawing was used for the isozymic analysis by the method of Fujio (1984). A total of nine enzymes were examined by starch gel electrophoresis. Band identification and nomenclature of alleles were followed as by Ikeda et al. (1994).

RESULTS

Growth Characteristics of Parental Species

Figure 1 shows the daily growth in shell length for pinto abalone compared with ezo abalone. The average growth rate of pinto abalone was 46.1 μm/day at 17°C, 76.7 μm/day at 15°C, 71.8 μm/day at 12°C, 25.1 μm/day and 23.0 μm/day at 7°C, and 25.4 μm/day and 14.9 μm/day at 5°C. The growth rates of ezo abalone under the same temperature conditions were 72.0–129.6 μm/day at 17°C, 70.0–106.3 μm/day at 15°C, 46.7–80.1 μm/day at 12°C, 4.8–21.9 μm/day at 7°C, and 2.7–12.4 μm/day at 5°C.

The growth rate of pinto abalone was lower than that of ezo abalone at 17°C. The values were almost the same as for ezo abalone at 15 and 12°C. However, the growth rate of pinto abalone was superior to that of ezo abalone at 7°C and significantly higher ($p < 0.05$, Dunn's Multiple Comparisons Test) at 5°C.

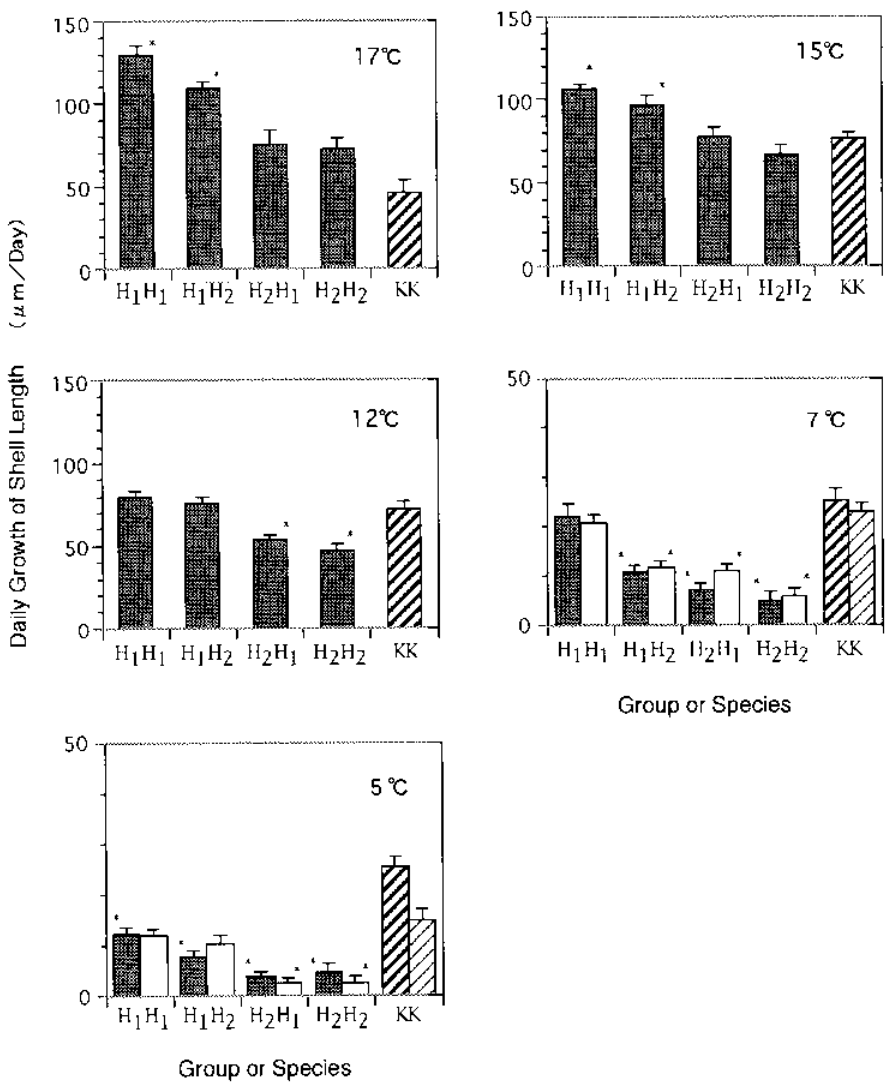

Figure 1. Daily growth of shell length in juvenile of ezo abalone *H. discus hannai*, and pinto abalone, *H. kamtschatkana*, at several water temperatures. H1, *H. discus hannai* collected from Rebun; H2, *H. discus hannai* collected from Okushiri; K, *H. kamtschatkana* collected from Sitka, AK. Combination represents female (first) and male (last), respectively. For example, H1H2 means the cross of female *H. discus hannai* from Rebun and male from Okushiri. There are two lots at 5 and 7°C. The vertical bar indicates the standard error. An asterisk indicates that the means are significantly different from KK ($p < 0.05$, Dunn's Multiple Comparisons Test).

Figure 2 summarizes the results of the rearing experiment. Growth of the pinto abalone was superior to that of the ezo abalone at low water temperature. However, the difference was less at water temperatures above 17°C. Maximum growth rates were observed in the range from 12 to 15°C.

Hybridization

The fertilization rate of parental species and their hybrid was examined 2 or 3 h after fertilization. The hybrid resulting from the cross between the female ezo abalone and the male pinto abalone was obtained. However, the reciprocal hybrid cross was not successful. The fertilization rate in the homologous cross was higher than 80%; that of the hybrid was only 20%.

The hybrid's shell color was very similar to that of ezo abalone. The hybrid in this case showed the maternal character in shell coloration.

Electrophoresis

The electropherogram of several enzymes from the muscle of parental species and hybrid individuals was used to confirm genetic hybridization in this cross. A total of 41 juveniles of the hybrid between pinto abalone and ezo abalone were examined by genotypic analysis with the electropherogram.

Figure 3 shows the electropherogram of the enzyme glucose phosphate isomerase (GPI) for the two species (not including the parents of these hybrids) and the hybrid individuals. Ezo abalone had the allele *C* only. Pinto abalone had the alleles *A* and *B* only. The hybrid had a heterotic combination of parental species, as *AC*.

Pinto abalone samples possessed the alleles *B* and *C* in *AAT**, *A* and *B* in *GPI-2**, *B* in *IDH-1**, *C* in *MDH-1**, *C* in *MDH-2**, and *C* in *ME**. Ezo abalone possessed the allele *A* in *AAT**, *C* in *GPI-2**, *A* in *IDH-1**, *A* in *MDH-1**, *B* in *MDH-2**, and *A* in *ME**. Genotypes of the hybrid had the heterotic alleles as *AB* and *AC* in *AAT**, *AC* in *GPI-2**, *AB* in *IDH-1**, *AC* in *MDH-1**, *AB* and *BC* in *MDH-2**, and *AC* and *BC* in *ME**, respectively (Table 1).

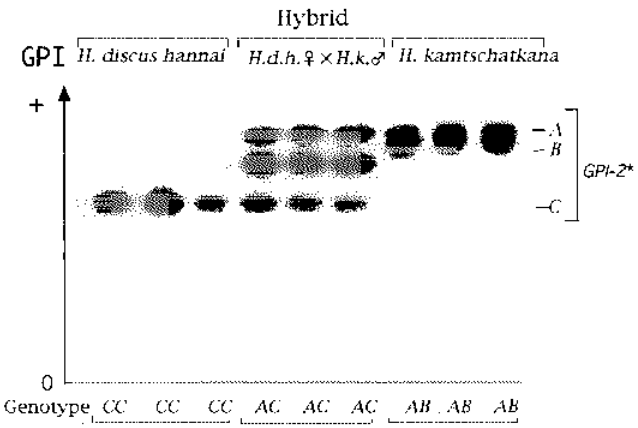

Figure 3. Electropherogram of enzyme GPI-2* for ezo abalone, *H. discus hannai*, pinto abalone, *H. kamtschatkana*, and their hybrid.

Growth Characteristics of the Hybrid

In order to examine the growth characteristics of the F1 hybrid at high and low temperature, observations on the growth rate of the hybrid were studied at two water temperatures, 18 and 8°C, respectively.

Figure 4 shows the daily growth in shell length for ezo abalone, pinto abalone, and the hybrid at 18°C. The values for two groups of the hybrid were 55.6 and 33.4 μm/day and significantly higher than those for the pinto abalone (from 6.5 to 10.9 μm/day)($p < 0.001$). The growth rate of the hybrid also was significantly higher than that of the ezo abalone ($p < 0.05$). At this relatively high temperature, the hybrid shows the tendency of higher growth compared with the parental species. This result may reflect a heterotic

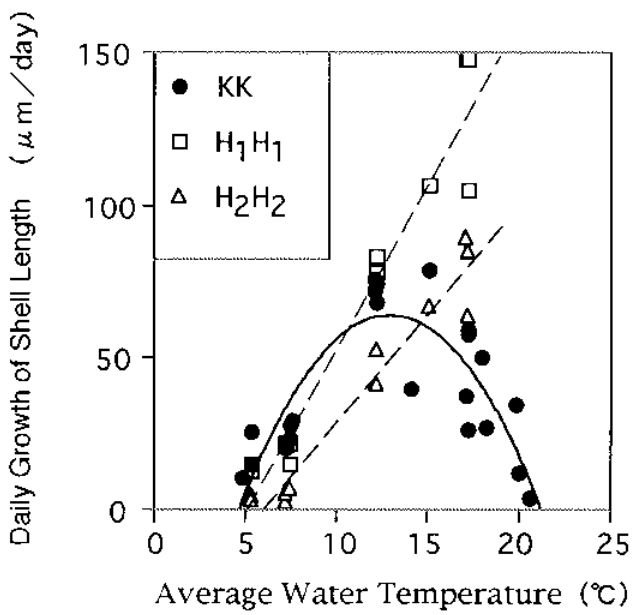

Figure 2. Relation between the water temperature and growth rate of juvenile abalone. *H. discus hannai* and *H. kamtschatkana*. KK, *H. kamtschatkana*; H1H1, *H. discus hannai* collected from Rebun; H2H2, *H. discus hannai* collected from Okushiri.

TABLE 1.

Izosyme analysis on the hybrid between a female *H. discus hannai* and a male *H. kamtschatkana*.

Locus	Genotype	Number of Individuals: Observed	Number of Individuals: Expected	χ^2	Estimated Parental Genotype
Pair No. 1					
*AAT**	*AB*	8	6	1.333	*AA* × *BC*
	AC	4	6		
*GPI-2**	*AC*	20	—	—	*AA* × *CC*
*IDH-1**	*AB*	20			*AA* × *BB*
*MDH-1**	*AC*	20	—		*AA* × *CC*
*MDH-2**	*BC*	20	—		*BB* × *CC*
*ME**	*AC*	3	4.5	1.000	*AB* × *CC*
	BC	6	4.5		
Pair No. 2					
*AAT**	*AC*	8			*AA* × *CC*
*GPI-2**	*AC*	21	—	—	*AA* × *CC*
*IDH-1**	*AB*	21	—	—	*AA* × *BB*
*MDH-1**	*AC*	21			*AA* × *CC*
*MDH-2**	*AB*	10	10.5	0.047	*AB* × *CC*
	BC	11	10.5		
*ME**	*AC*	21	—	—	*AA* × *CC*

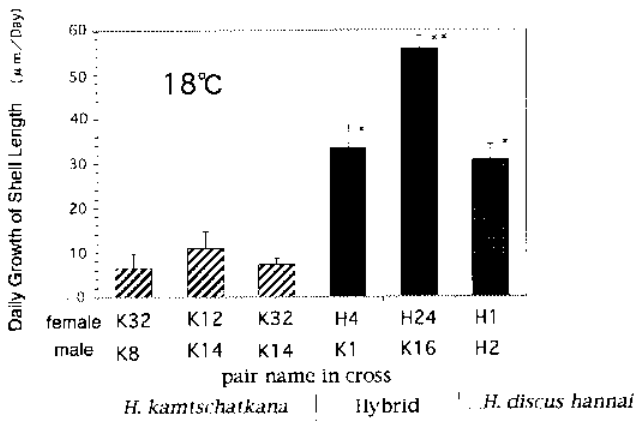

Figure 4. Daily growth of shell length in juvenile ezo abalone, *H. discus hannai*, pinto abalone, *H. kamtschatkana*, and their hybrid at 18°C. The letters K and H in pair name represent the parent's species as *H. kamtschatkana* and *H. discus hannai*, respectively. The number or symbol after the letter represents the individual number of females and males. The bars indicate standard errors. *Significant difference from *H. kamtschatkana*. **Significant difference from both parental species.

effect on growth at high water temperature in the case of the F1 hybrid between the two species.

The daily growth at 8°C for the hybrids is shown in Figure 5. The growth rate of the hybrid ranged from 1.7 to 7.2 μm/day. The values of daily growth were from 6.3 μm/day to 21.9 μm/day in the pinto abalone and were 0.5 to 1.4 μm/day in the ezo abalone. Therefore, the growth rates of the hybrid were intermediate between parental species in all three cage experiments. Use of hybrids could be valuable for improving the growth compared with native species reared at low water temperatures, such as exist seasonally in Hokkaido.

DISCUSSION

Paul and Paul (1981) studied the growth of juvenile pinto abalone at 5.5, 8.5, 11.5, and 13.5°C in tanks. According to their results, shell growth of pinto abalone was inhibited at 5.5°C and maximum growth rates were observed at 13–14°C. This tendency is in agreement with the results of this study. The temperature range of maximum growth rates for the pinto abalone reported by Paul and Paul (1981) is almost the same as that of the water temperature in the summer season at Sitka, southwestern Alaska.

Leighton and Lewis (1982) suggested that sperm concentration should be 10 times greater than that of homologous crosses and eggs must be freshly spawned to obtain the highest fertilization rates in abalone hybrid formation. The concentration of sperm used in this study ranged from 0.5 to 1.0 million cells/mL. The optimal sperm concentration in the fertilization of ezo abalone is considered to be more than 0.1 million/mL and less than 1.9 million/mL (Kikuchi and Uki 1974a). In that study, the egg membrane dissolved in 1.93 million sperm cells/mL and embryos showed abnormal development at 4.83 million/mL. Higher concentrations of sperm have a countereffect to the high fertilization rate by increasing the rate of membrane dissolution in the egg.

The eggs we used in the hybrid formation were fertilized 1 or 2 h after spawning. This time interval was the same as in the fertilization of parental species. Observations on the duration of fertility of spawned eggs and sperm of ezo abalone done by Kikuchi and Uki (1974b) demonstrated that the high fertility lasts for only 1–3 h after spawning at normal temperatures. It would be necessary to obtain the highest fertilization rates in hybridization to use freshly spawned eggs (Leighton and Lewis 1982).

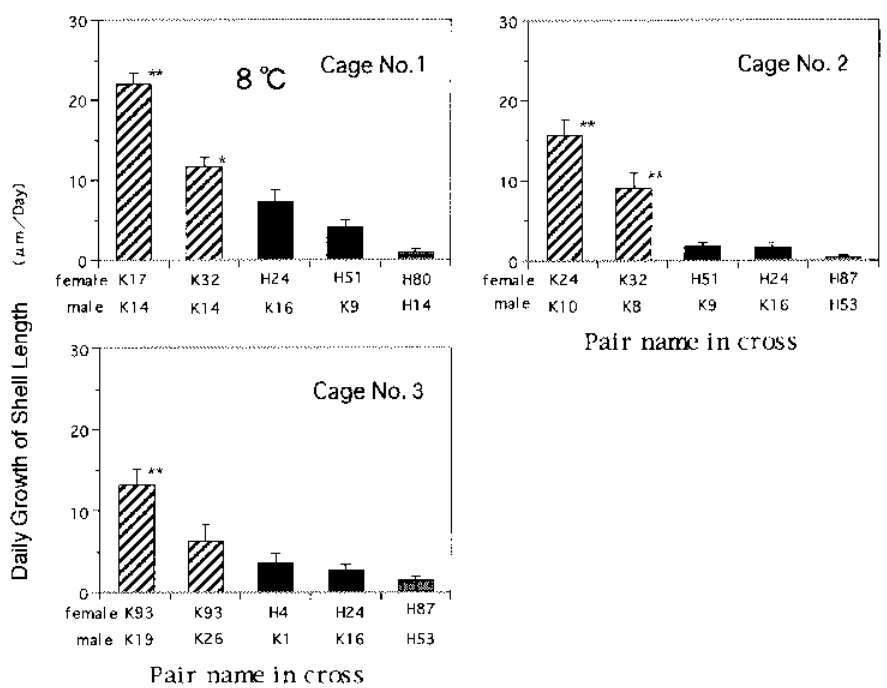

Figure 5. Daily growth of shell length in the juvenile ezo abalone, *H. discus hannai*, pinto abalone, *H. kamtschatkana*, and their hybrid at 8°C. The letters K and H in a pair name indicate the parent's species as *H. kamtschatkana* and *H. discus hannai*, respectively. The number after the letter represents the individual number of females or males. The error bar indicates standard error. *Significant difference from *H. discus hannai*. **Significant difference from hybrid and *H. discus hannai*.

A total of 13 loci in nine enzymes was examined, and different alleles were observed between two species in the 6 loci, *AAT**, *GPI-2**, *IDH-1**, *MDH-1**, *MDH-2**, and *ME** Ikeda et al. (1994) examined the allele frequency of the pinto abalone and ezo abalone using the electropherogram. According to that report, the allele *C* in *GPI-2**, *B* in *MDH-1**, and *A* in *MDH-2** of pinto abalone were observed. These were also found in this study. Similarly, *D* in *GPI-2** and *B* in *ME** of ezo abalone were also recorded. Consequently, all of the estimated parental genotype indicators corresponded with the observed genotype of the parental species described in that report. The fact that the hybrid showed alleles originating from both parental species in some enzymes indicates that genetic hybridization was accomplished in the cross between the female *H. discus hannai* and the male *H. kamtschatkana*.

Juveniles of the hybrid between *Haliotis rufescens* Swainson and *Haliotis fulgens* Philippi and also between *H. rufescens* and *Haliotis sorenseni* Bartsch displayed superior growth. Survival rates of the hybrid between *H. rufescens* and *Haliotis corrugata* Wood were higher than that for *H. corrugata* (Leighton and Lewis 1982). Similarly, Koike et al. (1988) reported that daily feeding rates and monthly growth rates of the hybrid between female *Nordotis madaka* and male *Nordotis gigantea* Gmelin were superior to those of the parental species. The growth rates of the juvenile hybrids between female *N. gigantea* and male *N. madaka*, female N. gigantea and male *Nordotis discus discus* Reeve, and female *N. madaka* and male *N. discus discus* were higher than those of their respective paternal species (Koike et al. 1988). The juvenile hybrid between a female ezo abalone and a male *H. gigantea* also showed growth superior to that of its parental species (Hara 1992).

A heterotic effect of hybridization on growth was observed under high water temperature (18°C) only in this study. Under low temperature, the heterotic effect on growth rate was not observed in this study. However, the value is high compared with that of ezo abalone. Momma (1990) mentioned that the hybrid observed in a field experiment showed improved growth rate compared with ezo abalone in cold water.

The shell color of the hybrid produced in this study was very similar to that of *H. discus hannai*. Producing the reciprocal hybrid will be necessary to determine whether the shell color is determined by the maternal effect or if it is a dominant effect.

From the results of this study, a breeding strategy using hybrids will be available for future abalone culture that is especially applicable to cold water areas. The traits showing heterotic effects would be adopted in F1 hybrids, and the traits intermediate between parents would be useful for selective breeding material. We are continuing the study and will attempt to produce the reciprocal hybrids and also to produce back cross individuals with the goal of defining an abalone combination or strain that will exhibit higher growth rates at low water temperatures.

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IMAGE ANALYSIS APPLIED TO ABALONE STANDING CROP ASSESSMENT IN AN OCEAN-BASED ABALONE FARM

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ABSTRACT Assessment of the standing crop of abalone in a large-scale, ocean-based facility is often difficult. In order to produce a size-frequency distribution that will accurately reflect the entire cultured population, many measurements are necessary. Furthermore, animals from as many holding pens as possible should be sampled. Together, these factors result in time-consuming and labor-intensive operations. Typically, length-frequency data are gathered by manually measuring animals with a vernier caliper. The use of digital image analysis of photographed groups of individuals provides an alternative method of sampling that reduces field efforts and dramatically increases potential sample sizes. In order to compare the relative efficacy of the two methods, abalone lengths were obtained by (1) manual measurements with vernier calipers in the field and (2) measurements from digitized photographs of the same abalone by digital image analysis. Ten direct comparisons were done, each with equal and progressively larger sample sizes. The mean lengths and length-frequency distributions generated by the two methods revealed no significant differences, whereas the image analysis method proved to be markedly faster and more efficient. In addition, this method of sampling exposes abalone to less stress, because time out of the water is minimized. Sampling and measuring abalone with photography and image analysis, respectively, proved to be an excellent alternative of obtaining length measurements of abalone in an open-ocean farm.

KEY WORDS: abalone, open-ocean aquaculture, photography, digital image analysis, stock assessment, precision

INTRODUCTION

Traditional methods of obtaining length measurements of cultured abalone become difficult when trying to assess the standing crop and growth rates of a population that surpasses one million organisms. Large and well-distributed samples are needed to generate precise estimates of parameters being measured, such as length-frequency distributions and modal length changes. However, these types of samples are difficult to obtain. With large cultures of abalone, the sampling process becomes time consuming and labor intensive. Time is of the essence in a growout operation, especially in an ocean-based one, where the entire operation is subject to weather conditions and the priority is to keep the animals fed.

Photography and video techniques have been previously applied in marine ecology to try to reduce time and effort in the field. Photographic sampling techniques have been used to survey percent cover, community composition, spatial patterns, recruitment, and mortality (e.g., Bohnsack 1976, Wethey 1984, Sutherland 1990, Foster et al. 1991, Meese and Tomich 1992). Leonard and Clark (1993) and Whorff and Griffing (1992) have used video frames to measure percent cover of organisms, the latter applying computer-aided image analysis. Sampling techniques such as these have yet to be applied to abalone culture. Photography and computer-aided digital image analysis offer an alternative method for measuring shell lengths of abalone, which can greatly reduce field efforts and dramatically increase the sample size without increasing the time and effort needed to obtain the sample. Photography is preferable to video because the resolution obtained via photography is higher and the process of analysis is faster. Leonard and Clark (1993) concluded in their study that lack of resolution was the limiting factor in the use of video technology to analyze their images.

The aim of this study was to compare shell-length measurements of abalone taken manually, with a vernier caliper (M-VC), with measurements obtained with photography, by computer-aided digital image analysis (P-DIA). Results of direct comparisons of mean lengths and length-frequency distributions generated by both measuring methods are presented. Furthermore, the relative efficacy of measuring abalone by both methods serves to determine and validate the feasibility of applying P-DIA to measure abalone in an open-ocean abalone farm.

MATERIALS AND METHODS

Abalone were sampled at the ocean-based growout facility of Abulones Cultivados. This facility is located 13 km west of Ensenada, Baja California, Mexico, in the Todos Santos Islands, which mark the western boundary of the bay that bears their name. The species of abalone cultivated here is red abalone *Haliotis rufescens* Swainson. The growout consists of cages suspended at midwater from longlines, on the leeward side of the islands.

Sampling for this study was conducted by bringing selected cages to the surface with a work raft. Each cage contained 15 culture substrates, corrugated polyvinylchloride plates (hereafter, "plates"). One plate from each selected cage was chosen at random, dislodged, and removed from the cage. Plates were 60 × 90 cm and had anywhere from 50 to 500 abalone attached to each side, depending on the size and density of the animals in the cage. Selected plates were set horizontally on the floor of the work raft below an apparatus that held a camera perpendicular to the plate (Fig. 1). This was done to minimize the potential for parallax error in the photograph. Before taking the photograph, a scale of 100 mm and a label were set in the middle of the plate, making sure they were lying flat. Photographs were taken as soon as possible after removing the plates from the cages. In a few cases, when the selected cage had abalone of lengths of less than 25 mm and was out of food, the abalone on the plate were clustered and attached to each other. In these cases, the plate was left for several seconds

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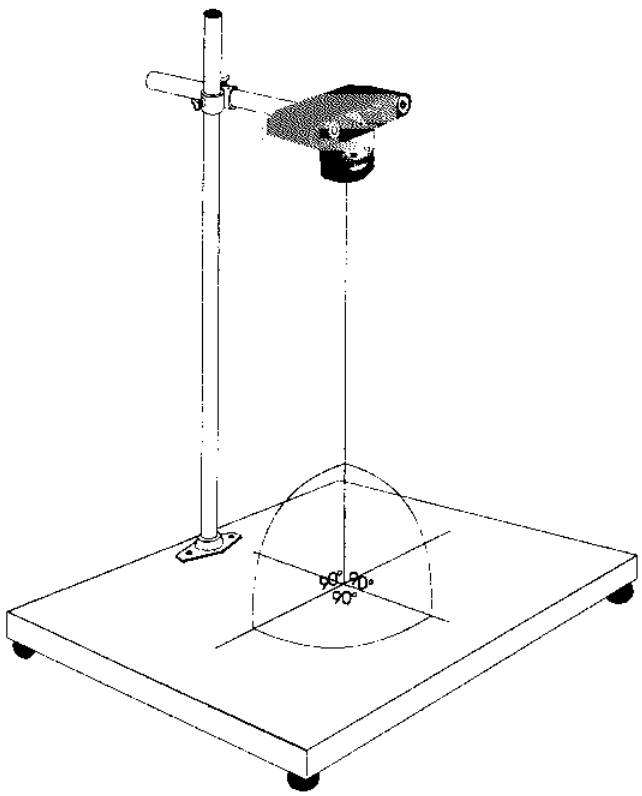

Figure 1. Camera-mounting apparatus.

in the sunlight, which caused the abalone to disperse over the plate. The photograph was then taken.

Photographs were taken with a Nikon 35-mm N70 camera, with a 35-mm f-3.5 auto Nikkor prime lens, using 200 ASA color film. The camera was set in automatic program mode, which focuses the picture and sets the appropriate shutter speed and lens aperture automatically before taking the photograph, thus ensuring high-quality photographs. Sunlight was blocked from the picture frame, and an electronic flash was used in all photographs taken, in order to avoid shadows.

After photographing the plate, all of the abalone attached were dislodged from the plate and measured by M-VC. Recorded manual measurements were then taken to a computer laboratory and entered into a computer spreadsheet. Photographic prints were commercially developed and then digitized to PICT or TIFF file formats into a computer with a 4C Hewlett Packard color scanner, using the program Desk Scan II version 2.2. While digitizing, each image was given a memory size of 3 megabytes, which made the image of high resolution. The computer used was a Macintosh Power PC 7600/132, with 64 megabytes of random access memory, and a Sony triniton multiscan 100 sx high-resolution color monitor. The digitized images were analyzed in the computer by digital image analysis with the public domain program NIH Image version 1.61. Images were opened in the analyzing program, and the scale of the program was set according to the scale of the actual image. Each abalone in the photograph was then selected and measured with the measuring tool of the program. The length measurements obtained from the images were exported to a spreadsheet for statistical comparison with the manual measurements. The entire process was replicated 10 times, increasing in each instance the sample size and including abalone of all sizes.

The difference between mean lengths generated by both measuring methods was determined by using a two-tailed *t*-test. Length-frequency distributions generated by both measuring methods were compared by a heterogeneity χ^2 analysis. A 5% level of significance was used as a criterion for both of the statistical tests applied. Length measurements gathered via M-VC were considered the standard against which the accuracy of measurements obtained with P-DIA were compared.

RESULTS

Mean lengths generated by both sampling methods revealed no significant difference. All 10 two-tailed *t*-tests had $p > 0.05$ (Table 1). Also, the 10 heterogeneity χ^2 analyses concluded that the length-frequency distributions generated by the M-VC and P-DIA measuring methods were not heterogeneous, with all Heter. χ^2 $p > 0.05$. Results of heterogeneity χ^2 analyses are shown along with corresponding superimposed histograms of the length-frequency distributions generated by both methods in Figure 2. In this figure, it can be observed how both distributions show similar patterns. Furthermore, there seems to be no bias with respect to size of abalone in comparison to length measurements of M-VC; the length measurements of P-DIA are equally consistent for small and large abalone. P-DIA does not underestimate or overestimate lengths.

DISCUSSION

From the results of the *t*-tests and heterogeneity χ^2 analyses, it can clearly be observed that both measuring methods produce similar length measurements. Given that the length measurements generated by both measuring methods are equivalent, the practical advantages of applying P-DIA in an open-ocean abalone farm are numerous. Two very important advantages include the speed and ease with which samples can be obtained and the potential for very large samples to be processed. When sampling with photography, a relatively rapid procedure, handling times are decreased and time in the field is not limiting. Also, the number of cages selected to obtain samples can be increased dramatically, thus reducing potential bias resulting from the selection of small numbers of cages.

In most cases, around 300 abalone are attached to each plate of a cage, approximately 150 on each side. Each photograph can be taken in less than 1 min. With two photographs taken by one person, all of these abalone can be sampled and later measured in the computer. In contrast, it would take well over 20 min for two

TABLE 1.

Exact probabilities of two-tailed *t*-tests and descriptive statistics of the two measuring methods.

	M-VC			P-DIA			
Comparison	Mean	±SD	n	Mean	±SD	n	p Value
1	65.76	5.34	34	65.98	5.51	34	0.87016
2	15.77	2.98	56	15.04	3.14	56	0.21195
3	63.39	5.68	103	63.12	5.92	103	0.74009
4	58.96	6.63	125	59.07	6.81	125	0.90173
5	35.45	6.74	210	35.43	6.62	210	0.97438
6	45.07	6.64	213	44.57	7.18	213	0.45969
7	66.13	7.29	450	65.92	8.21	450	0.68110
8	39.54	7.66	469	38.95	7.20	469	0.22822
9	44.02	6.26	503	43.60	6.34	503	0.29100
10	25.90	10.69	505	26.18	10.92	505	0.68170

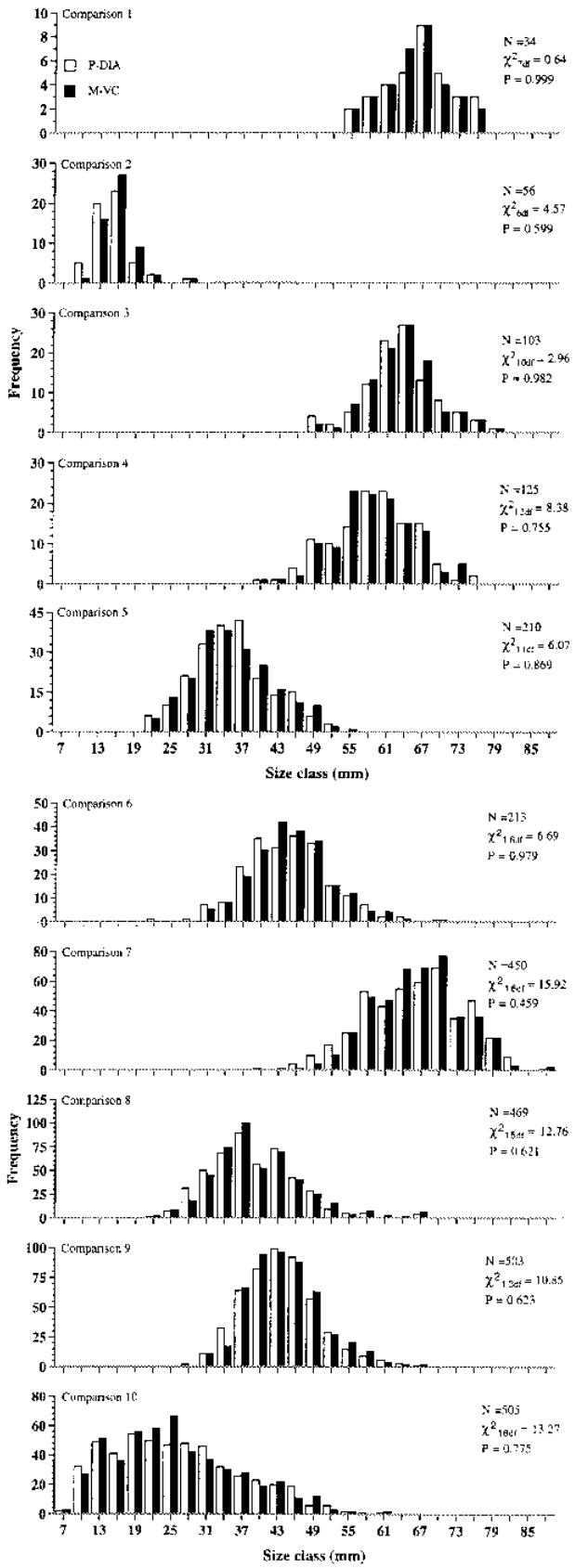

Figure 2. Length-frequency distributions generated by P-DIA and M-VC methods. P-DIA stands for photography and digital image analysis, and M-VC stands for manual vernier caliper.

people to manually measure the abalone, one taking measurements and the other recording the readings for future entrance into the computer. Under normal conditions, while measuring abalone with M-VC under time restraints, not all of the abalone of the selected plate are measured, and the worker selects the abalone to measure, increasing the chance of a biased sample.

Film development can be done quickly by any commercial developing center. Typically, a photograph can be digitized and analyzed in approximately 10 min. Images with fewer animals take less time to analyze. When analyzing images, the measurements obtained are directly exported to the statistical program in which the measurements will be analyzed and do not need to be reentered by hand into the computer. Furthermore, by sampling photographically, a permanent visual record of the abalone sample is kept. Photographs do not need to be digitized and analyzed right away; they can be analyzed at leisure with the sample frozen in time. Additionally, the percent cover of the abalone can also be obtained with the image analysis program, and the farmer can use this as a criterion of when to lower the densities of the cages. Last, and of importance for the health of the animals, abalone are left relatively undisturbed and are exposed to less stress because the time they are kept out of water is minimized and they do not need to be detached for measurement.

As with all sampling techniques, P-DIA has potential limitations. Sampling in small quantities is expensive. Large samples need to be taken in order for the process to be cost efficient. Although most farming facilities already have a computer, and the cost of the image-analyzing program is insignificant, photographic and scanning equipment need to be obtained. Last, abalone that are heavily infested with the sabellid polychaete pest known to most South Africa and California abalone farmers have begun to show a deformed domed appearance because of the pest. Shell length therefore is not a good estimate of growth because in these animals, there is not a linear extension of the shell.

With the application of P-DIA, larger, more representative samples of the cultured population are obtained in a much shorter time period. Furthermore, the amount of cages selected to obtain samples is not restricted by time, so the probability of each abalone of the cultured population being sampled is increased. A parameter estimated from a large sample is more precise than an estimate of the same parameter from a small sample, because the standard error will decrease as the sample size increases (Zar 1996). This large and well-distributed sample of the population will generate more precise estimates of the parameters of the cultured population being measured and will more adequately represent the true cultured population. By using P-DIA, the farmer will obtain robust and precise estimates of length-frequency distributions, modal-length change, mean lengths, standing crop, and available harvest.

The results of this study are applicable to cultures of abalone of any species grown in the ocean, which need large sample sizes to generate precise estimates the dynamics of the population. P-DIA could also be applied to cultures of abalone grown on land-based facilities (e.g., in raceways), with some modification of the photographic sampling technique. Finally, photographic or video-sampling techniques along with computer-aided digital image analysis could also be used to quantify and measure abalone in other stages of development. Overall, applying P-DIA to a large culture operation is a fast and economic way of obtaining large quantities of length measurements with numerous advantages over the traditional way of measuring abalone.

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EFFECTS OF CHRONIC EXPOSURE OF GREENLIP ABALONE, *HALIOTIS LAEVIGATA* DONOVAN, TO HIGH AMMONIA, NITRITE, AND LOW DISSOLVED OXYGEN CONCENTRATIONS ON GILL AND KIDNEY STRUCTURE

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ABSTRACT After chronic, sublethal bioassays of juvenile greenlip abalone, *Haliotis laevigata* Donovan, to reagent ammonia, nitrite, and low dissolved oxygen, tissue samples were dissected for histological analysis. Exposure to the highest ammonia treatment (0.188 mg of free ammonia-nitrogen ([FAN]L^{-1})) resulted in little difference to the gills of these abalone, relative to the controls (0.006 mg of FAN L^{-1}), whereas at this concentration, the right kidney showed decreased tubule definition and enlarged tubule lumen. Exposure to 7.8 mg of NO_2-N L^{-1}, resulted in gill lamellar thickening and epithelial lifting along with a proliferation of mucous cells. The proportion of kidney cell contents occupied by granules increased at this nitrite concentration. Associated with this change in kidney structure was an increase in basally located eosinophilic cytoplasm. Gill mucous cells from abalone exposed to depressed dissolved oxygen levels (55% oxygen saturation) exhibited more intense staining, indicative of a change in mucous composition. Some necrosis of gill epithelium was evident, either as a result of or in association with the occurrence of ciliates (Ancistrocomidae) between the gill lamellae. Right kidney tissue did not exhibit any obvious changes in relation to exposure to low dissolved oxygen levels. Chronic exposure to slight oxygen supersaturation (117%) caused no apparent effects on gill or kidney structure.

KEY WORDS: abalone, *Haliotis laevigata*, ammonia, nitrite, oxygen, histology

INTRODUCTION

The present expansion of abalone aquaculture (Hone and Maguire 1996) brings with it the likelihood of encountering suboptimal water quality, especially where recirculating aquaculture systems are used (Jirsa et al. 1997). The culture of animals in nonoptimal environments may result in deaths, as a direct result of one or more components of the environment or from infectious diseases activated indirectly by suboptimal environments, or in decreased productivity (Tomasso 1996).

The external environment can, if suboptimal, produce deleterious changes in aquatic animals. The overt signs of toxicity are nearly always preceded by biochemical, physiological, and/or morphological changes in the organism (Meyers and Hendricks 1985). Often, the gills are among the organs most affected by waterborne pollutants (Mallat 1985), because the respiratory surface provides an extensive interface with the aquatic environment. In many fish, the kidney often forms a site of histological changes in response to toxicants (Russo 1985). Abalone are diotocardians, possessing two kidneys of differing functions. The role of the right kidney is believed to be in nitrogen excretion because of the presence of excretory vacuoles (Andrews 1985). Some resorption of solutes, which is also the main function of the left kidney, is also believed to occur, because of the presence of coated pits opening between microvilli (Voltzow 1994). The role of the right kidney in nitrogen excretion and protein turnover suggests a possible role with the toxicants considered in this study.

The purpose of this study was to bring together histological observations of juvenile greenlip abalone, *Haliotis laevigata*, from three chronic, sublethal bioassay studies. Specifically, the effects of sublethal exposure for 2–3 mo to high ammonia, nitrite, or low dissolved oxygen on gill and right kidney histological structure were investigated.

MATERIALS AND METHODS

Juvenile greenlip abalone were sampled after chronic sublethal exposure for 58–82 days to ammonia (as NH_4Cl) (Harris et al. 1998), nitrite (as $NaNO_2$) (Harris et al. 1997), or low dissolved oxygen (Harris et al. in press). The experimental ranges were 0.006–0.188 mg free ammonia-nitrogen (FAN), L^{-1} (0.237–9.04 mg total ammonia-nitrogen L^{-1}), 0.024–7.80 mg NO_2-N L^{-1} and 8.9–4.2 mg of dissolved oxygen L^{-1} (117–57% dissolved oxygen saturation).

Five abalone were sampled from two of the triplicate bioassay tanks for each treatment and bled, via an incision in the foot, for 2–3 min before being dissected to remove the posterior portion of the viscera containing the gills and kidney. This tissue was fixed in phosphate-buffered formalin at room temperature (15–18°C) and then dehydrated through a graded ethanol series to xylene in a Tissue-Tek II tissue processor. Dehydrated tissue samples were embedded in paraffin resin on a Shandon Histocentre 2 and sectioned on a Microm HM 340 microtome at 4 μm. Routine Harris' hemotoxylin and eosin staining was carried out on all tissues processed with a Shandon Linistain GLX automatic tissue stainer. All sections were mounted in DPX and examined under a light microscope.

RESULTS

Exposure to the highest ammonia treatment (0.188 mg of FAN L^{-1}) resulted in little difference to the gills of these abalone, rela-

tive to the controls (0.006 mg of FAN L^{-1}) (Fig. 1). At this treatment level, however, the right kidney of all sampled abalone showed less definition in the tubules, and the lumen of the tubules appeared enlarged (Fig. 2). At 0.110 mg of FAN L^{-1}, 10% of the sampled abalone showed both reduced right kidney definition and an enlarged lumen, whereas 20% of abalone showed reduced right kidney definition and 20% of abalone exhibited enlarged right kidney lumen. Typical kidney structure for abalone not exposed to elevated levels of ammonia or nitrite or to low dissolved oxygen is shown in Figure 3.

From exposure to 7.8 mg of NO_2-N L^{-1}, thickening of the lamellae and epithelial lifting of gills were evident in all observed abalone, along with a proliferation of mucous cells at the junction of the lamellae and central gill axis (Fig. 4). Mucous cells, common at the distal tip of the lamellae, also extended further toward the base of the lamellae than for other treatments and control abalone. These mucous cells are evident both as complete and apparently discharged cells with adhered mucous, often giving a ragged appearance to the lamellae and contributing to the poor brush border definition observed here (Fig. 5). Abalone exposed to concentrations less than 7.8 mg of NO_2-N L^{-1} showed typical gill structure including the principal gill filament and lamellar junction (Fig. 6) and lamellar tip (Fig. 7). At 4.15 mg of NO_2-N L^{-1}, 20% of sampled abalone showed thickened gill lamellae, 10% showed lifting of gill epithelium, and 40% showed a proliferation of gill mucous cells. At the highest nitrite concentration (7.8 mg of NO_2-N L^{-1}), the overall height of the kidney tubule cells was increased. This was due to an increase in both the amount of pigment granules in the supranuclear region (toward the lumen surface) and the amount of eosinophilic (protein rich) cytoplasm located in the subnuclear region toward the base membrane (Fig. 8).

Gill mucous cells from abalone exposed to depressed dissolved oxygen levels (55% oxygen saturation) exhibited more intense staining, indicative of a change in the composition of mucous. Necrosis of gill epithelium was evident in all sampled abalone at this dissolved oxygen concentration, either as a result of or in association with the occurrence of ciliates (family Ancistrocomidae; D. Lynn pers. comm.) between the gill lamellae (Fig. 9), observed in 80% of sampled abalone at this concentration. At 63% of oxygen saturation, necrosis of gill tissue was evident in 20% of sampled abalone, whereas ciliates were observed in 40% of the abalone. Right kidney tissue did not exhibit any obvious changes in relation to exposure to low dissolved oxygen levels. One treatment was maintained at 117% oxygen saturation, and no adverse effects on gill or kidney structure were evident.

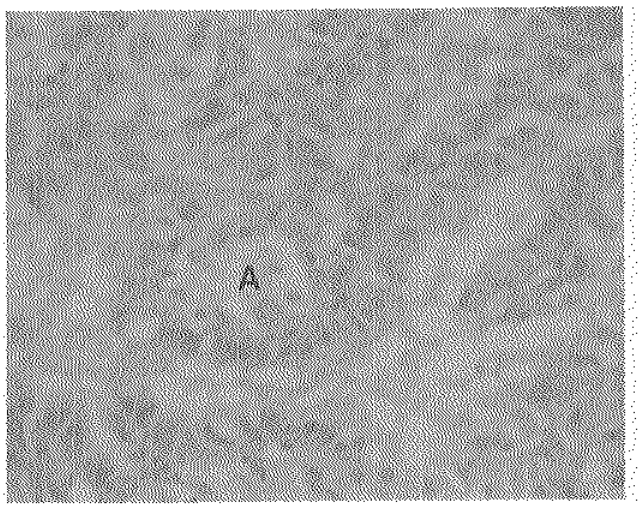

Figure 2. Right kidney of abalone exposed to 0.188 mg of FAN L^{-1}. Magnification, ×400. (A) Enlarged lumen of kidney tubule.

DISCUSSION

Some effects of ammonia on the histological structure of fish have been documented (see Russo 1985), although data for invertebrates are less common. FAN levels of 0.04–0.4 mg L^{-1} have been shown to induce inflammation and degeneration of gills and kidneys for a variety of fish species (Russo and Thurston 1991). The swollen, rounded secondary lamellae observed in rainbow trout after long-term exposure to ammonia (Smart 1976) were not observed in this study. However, among fish, the effects of ammonia are varied, because not all authors found hyperplasia and/or other degenerative changes to the gill structure. Sublethal ammo-

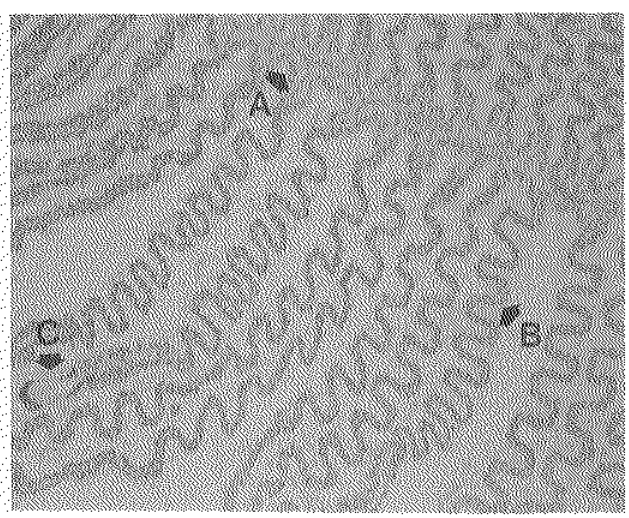

Figure 1. Gills of control abalone from ammonia bioassay. Magnification, ×100. (A) principal filament; (B) gill lamella; (C) distal tip of gill lamella.

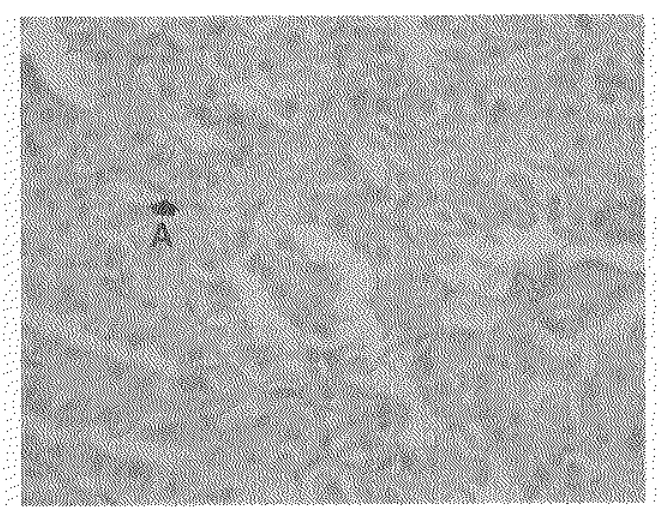

Figure 3. Right kidney of control abalone from nitrite bioassay. Magnification, ×400. (A) Exterior perimeter of normal kidney tubule.

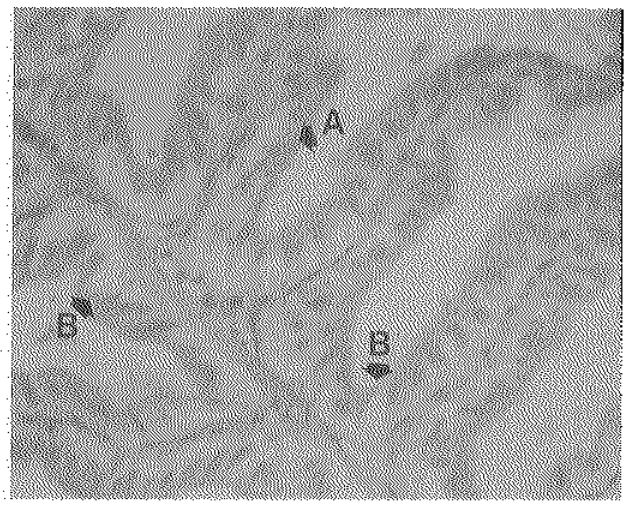

Figure 4. Distal tip of gill lamellae exposed to 7.80 mg of NO_2-N L^{-1}. Magnification, ×400. (A) Lifting of epithelium; (B) proliferation of mucous cells.

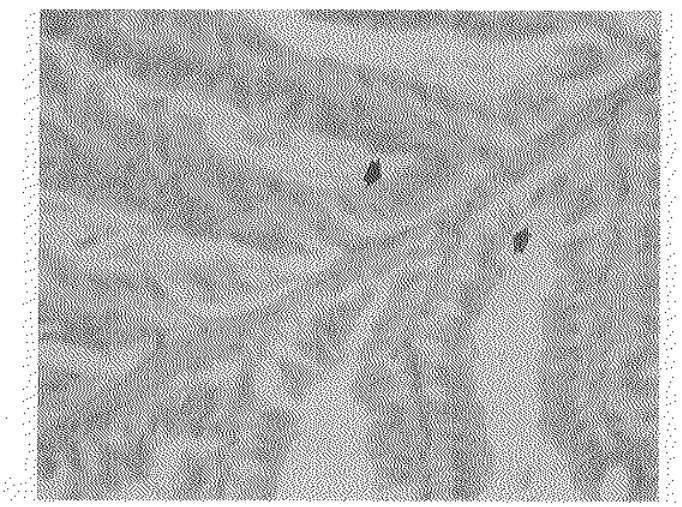

Figure 6. Junction of gill lamellae and principal filament of control abalone from nitrite bioassay. Magnification, ×400.

nia levels are also known to cause histological changes in the kidneys of many fish (Colt and Armstrong 1981). The observed differences in cell definition indicate that external ammonia had some effect on kidney structure in greenlip abalone, even though survival at this concentration was under 50% for a minimum of 58 days of exposure (Harris et al. 1998).

The long term effects of nitrite on histological structure have not been well documented for aquatic invertebrates. Nitrite is known to bioaccumulate in gill, liver, brain, and muscle tissue of fish (Margiocco et al. 1983) and to increase susceptibility to diseases (Hanson and Grizzle 1985). Michael et al. (1987) reported gill hypertrophy and hyperplasia in *Clarias lazera*, with some degree of gill epithelial lifting and necrosis. Gill degeneration, observed in rainbow trout within 3 wks of exposure to nitrite, was noted to disappear with increasing exposure time (Wedemeyer and Yasutake 1978). The observed changes in number and location of mucous cells in greenlip abalone gills suggest a hypersecretion of mucous as a response to chronic sublethal nitrite exposure at 7.8 mg of NO_2-N L^{-1} for a minimum of 82 days of exposure. Although these changes were more evident than for exposure to ammonia, a survival rate of 73% indicates that these changes may not affect survival as much as growth rate (Harris et al. 1997). The observed increase in right kidney pigment and granule deposition may be a reflection of increased kidney protein, and hence cell, turnover. Arillo et al. (1984) hypothesized that tissue hypoxia, as a result of nitrite exposure, was the contributing factor to acute toxicity for rainbow trout. The tissue hypoxia of fish is mediated through production of methemoglobin from the respiratory pigment hemoglobin. Because this pigment does not occur in abalone, not surprisingly, the lesions seen with nitrite toxicity differ from those seen with anoxia.

The effects of low dissolved oxygen on abalone in this study have some parallel in the literature. Histopathological effects of oxygen supersaturation to the red abalone, *Haliotis rufescens* (Elston 1983, Elston and Lockwood 1983) included the presence of

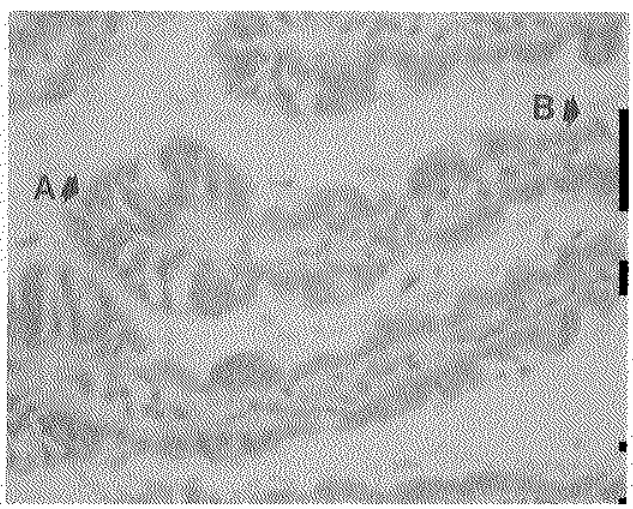

Figure 5. Junction of gill lamellae and principal filament of abalone exposed to 7.80 mg of NO_2-N L^{-1}. Magnification, ×400. (A) Complete mucous cell; (B) discharged mucous cell with adhered mucous.

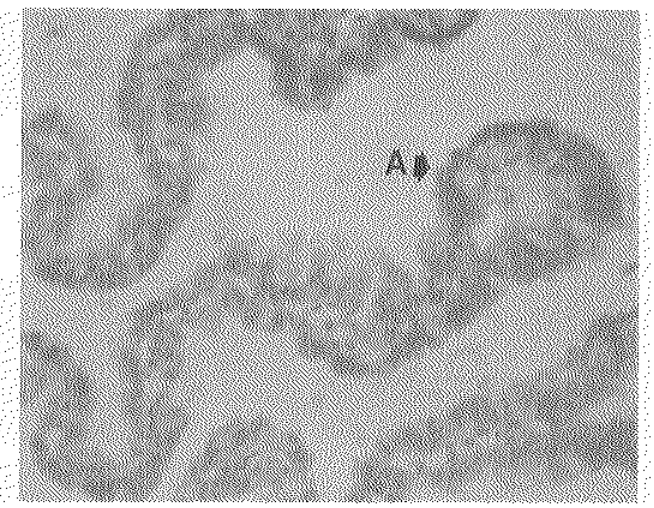

Figure 7. Distal tip of gill lamellae from control abalone from nitrite bioassay. Magnification, ×400. (A) Normal gill lamellar tip showing mucous cells.

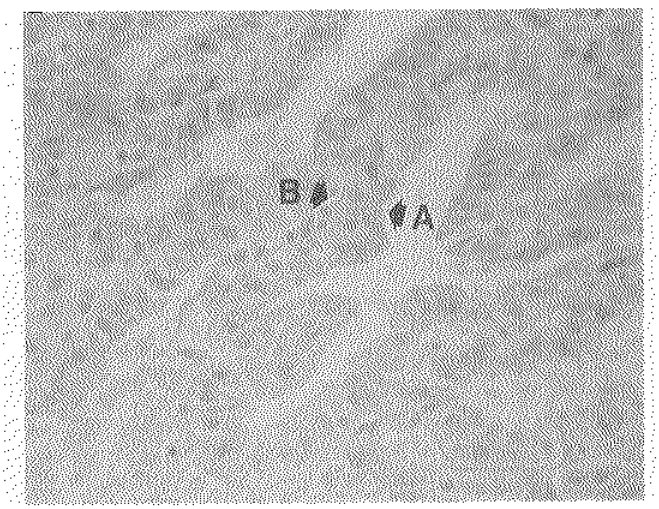

Figure 8. Right kidney of abalone exposed to 7.80 mg of NO_2-N L^{-1}. Magnification, ×400. (A) Pigment granules in supranuclear region of kidney tubule cell; (B) eosinophilic cytoplasm in subnuclear region of kidney tubule cell.

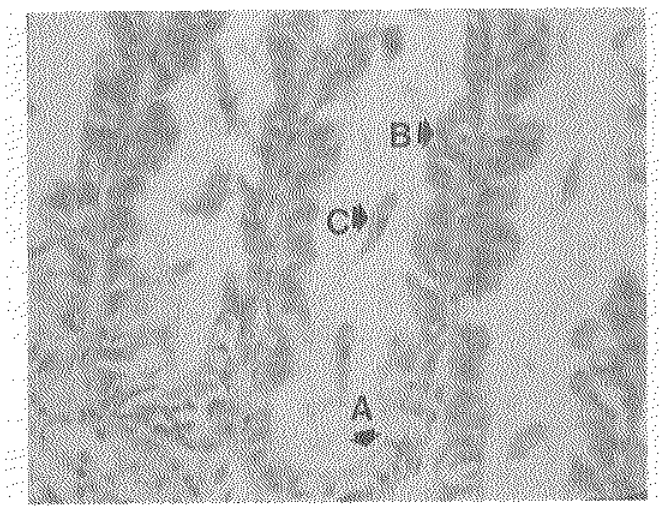

Figure 9. Junction of gill lamellae and principal filament of abalone exposed to 55% oxygen saturation. Magnification, ×400. (A) Intensely stained gill mucous cells; (B) necrotic tissue; (C) ciliate between gill lamellae (family Ancistrocomidae).

gaseous emboli in epipodial, oral, and pedal tissues. Elston (1983) reported the formation of these emboli at 150% oxygen saturation, whereas at 117% oxygen saturation, no emboli were evident in the abalone sampled in this study. Leitman (1992) reported increased bacterial counts from effluent water of abalone tanks at 143% oxygen saturation. The occurrence of ciliates at the 55% oxygen saturation treatment suggests an increased susceptibility to disease at depressed oxygen saturation. Walters and Plumb (1980) determined that low dissolved oxygen levels increase the susceptibility of channel catfish to bacterial infection. Survival of abalone also decreased to 59% of controls at this concentration (Harris et al. in press).

Some of the responses seen to the toxicants in this study are representative of the situation for many other aquatic animals. Mallat (1985) reviewed the literature on structural changes in fish gills induced by toxicants and determined that histopathological gill lesions are largely nonspecific in nature, with changes in gill epithelium, bulbing or fusing of gill lamellae, hypersecretion and proliferation of mucocytes, and changes in chloride cells and gill vasculature common to many different exposure conditions. Mallat (1985) also determined that the frequency of gill lesions is greater from acute rather than sublethal exposure and in freshwater situations rather than marine. This may explain the lack of effect of ammonia on the abalone gills and the subtle nature of changes has occurred during sublethal exposure of greenlip abalone to ammonia, nitrite, and low dissolved oxygen.

In this study, we have identified different histological changes for each environmental stressor, but the effects with low dissolved oxygen may have been dependent on ciliate infestation. Future research will involve bioassays for combinations of stressors and also a broadening of stress attributes to include biochemical changes. The overall aim is to establish a set of stressor-specific changes that can be used for diagnostic purposes.

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BACTERIAL WATER QUALITY IN ABALONE FARMS OF BAJA CALIFORNIA

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ABSTRACT Pathogenic bacteria may enter intensive husbandry systems by four routes: seawater system, air-borne contaminants, broodstock, and food supply. Sand and cartridge filters, as well as ultraviolet (UV) systems, are used in Baja California abalone (*Haliotis* spp.) farms to reduce and control the bacterial community.

Viable heterotrophic bacteria (VHB) and *Vibrio*-like bacteria (VLB) have been enumerated in seawater systems, culture tanks, and diatom cultures used at red abalone (*Haliotis rufescens*) and green abalone (*Haliotis fulgens*) farms. In successful hatchery operations, bacterial water quality after the UV system was excellent, with concentrations as low as 10^2 VHB/mL and 2 VLB/100 mL. These low numbers differ radically from those found in microbial food mats covering postlarval tank surfaces, where bacterial concentrations as high as 10^6 VHB/cm^2 and 10^5 VLB/cm^2 can be reached.

KEY WORDS: abalone culture, *Vibrio* spp., bacteriological water quality, microbial food mats

INTRODUCTION

Good bacterial water quality is always considered a crucial condition for successful achievements in hatchery operations. In order to reduce bacteria in the water system, most aquaculturists use mechanical filtration (pressurized sand, diatomaceous earth, and cartridge filters), and ultraviolet (UV) or ozone treatments as well as antibiotics. The use of bacterial counts in culture media has been proposed as a useful tool to evaluate the efficiency of the different water treatments among aquacultural systems (Lizárraga-Partida et al. 1997) because they provide a direct measure of the organisms that are targeted to be reduced or eliminated. Paradoxically, only few hatcheries perform bacterial counts routinely to monitor water quality.

Counts in culture media, in contrast to direct counts of bacteria stained with acridine orange or DAPI (4'6-diamino-2-phenylindole), give a clear indication of the viable heterotrophic bacteria (VHB), and selective media such as TCBS could be used for the enumeration of organisms related to *Vibrio* spp. This bacterial genus includes species that have been reported as pathogens of fish, crustaceans, and molluscs (Elston and Leibovitz 1980, Brown 1981, Jeffries 1982, Sindermann and Lightner 1988, Baticados et al. 1990, Lavilla-Pitogo 1995, among others). Regarding the abalone (*Haliotis* spp.), Elston and Lockwood (1983) have reported pathogen vibrios in cultured juvenile red abalone *Haliotis rufescens* Swainson. Ebert and Houk (1984) have reported larval mortalities associated with vibrios, and Anguiano et al. (in press) have confirmed the pathogenicity of *Vibrio alginolyticus* on larvae and postlarvae of the red abalone. These studies indicate that *Vibrio* spp. could develop epizootic events in larvae, postlarvae, and juvenile abalone. Li et al. (1997) report a pustule disease in *Haliotis discus hannai* Ino related to *Vibrio fluvialis* biotype II. In this article, we have studied VHB as well as bacteria growing in TCBS media, nominated by *Vibrio*-like bacteria (VLB), in coastal areas, seawater supply systems, and postlarval culture tanks at two Mexican abalone farms located in Baja California.

MATERIALS AND METHODS

The farms that were sampled are located in Ejido Eréndira B.C. and at Puerto Nuevo B.C.S. The first is operated by Abulones Cultivados, a private company dedicated to the commercial production of red abalone. The second is operated by Sociedad Cooperativa Emancipación and is devoted to seed production of green abalone (*Haliotis fulgens* Philippi).

Both farms are located in pristine environments in small fishing communities where the major water quality problems occur during dinoflagellate blooms (Flores-Aguilar et al. 1997). In Eréndira, water is pumped from the coast, passed through three pressure sand filters (Stark Aquatic Systems, maximum flow MF = 3785 L/min), and then conducted to reservoir tanks. From this last point, it is sent to three pressure sand filters (Jacuzzi Model ST33, MF = 450 L/min), three diatomaceous earth filters (Jacuzzi Model EW150, MF = 530 L/min), and then to a central UV irradiation system (Aquanetics Systems, Model Q720IL, MF = 750 L/min) for its utilization in postlarval culture tanks. A second line from the reservoir tanks supplies the broodstock tanks. For hatchery opera-

tions (spawning, fertilization, larval culture, etc.), supplementary filtration of UV-treated water is accomplished with a series of seven 1-μm-pore-size cartridge filters, and finally the water is passed through another UV sterilization unit (Aquanetics Systems, Model 30IL, MF = 30 L/min) (Fig. 1). Postlarvae are reared in 250-L fiberglass tanks that receive, during the first 45 days, periodic inoculations of a cultured diatom, *Navicula incerta*. After this period, the postlarval diet consists of artificial food together with microbial food mats developed from the seawater supply. Tanks are drained and washed periodically to remove feces and food wastes.

In Puerto Nuevo, water is pumped from the sea to a reservoir, passed through a pressure sand filter (Jacuzzi, model ST33, MF = 450 L/min), and then conducted to broodstock and postlarval tanks. For hatchery operations, water is further filtered with two 1-μm-pore-size cartridge filters and passed through a UV sterilization system (Aquanetics Systems, Model 30IL, MF = 30 L/min). Postlarvae are cultured by the Japanese system in 1,000-L tanks with racks of vinyl plates and are fed mixed wild diatoms.

Coastal water was collected at Eréndira in May, June, August, September, and December 1996, and in January, March, May, June, July, and August 1997. Critical points of the seawater system were sampled on February 1996, and in July 1997, in Eréndira and in January 1997, at Puerto Nuevo. Microbial food mats were sampled in November 1997, at Eréndira.

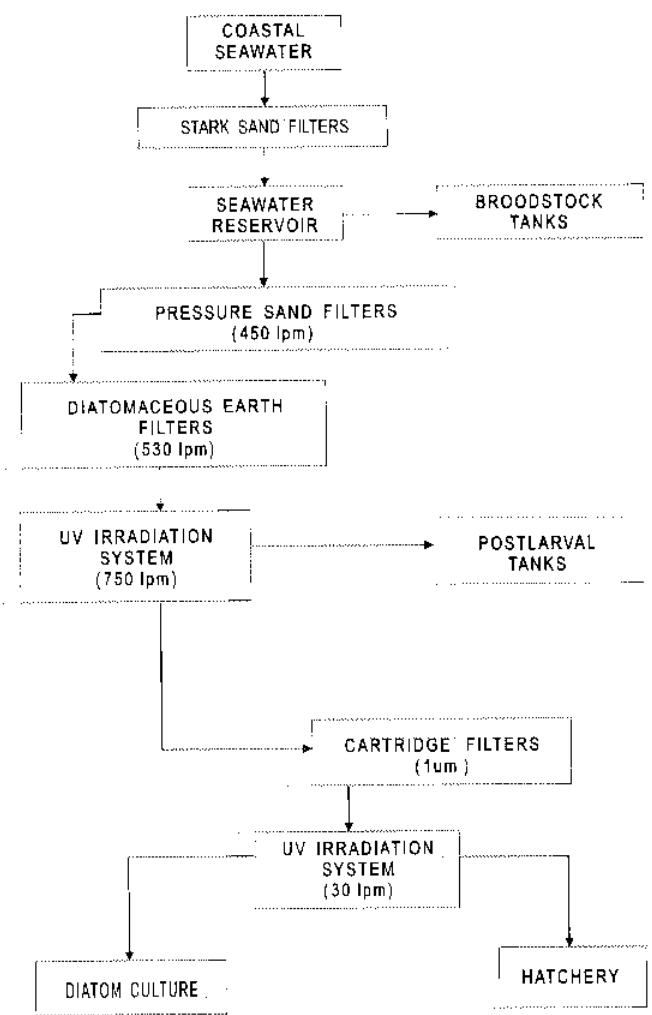

Figure 1. Flowchart of the water supply system at the Abulones Cultivados farm (Eréndira). lpm, liters per minute.

Water samples were collected in sterilized screw-capped bottles and processed immediately. Microbial food mats were sampled inside a 100-cm^2 frame with a sterilized glass slide and suspended in 100 mL of saline solution (0.9% NaCl). In all of the samples, 10-fold dilution series with saline solution were applied. When low concentrations of bacteria were expected, 100 mL of the water sample was filtered through a 0.45-μm-pore-size Millipore filter. These filters were seeded as well as the dilutions onto ZoBell 2216-E medium (Oppenheimer and ZoBell 1952) for VHB counts and TCBS medium (Difco) for VLB counts. The inoculated plates of ZoBell and TCBS media were incubated at 28 ± 2°C. After 48 h, the colony-forming units (CFU) were counted.

RESULTS AND DISCUSSION

In Figure 2, the time series of VHB and VLB average values for coastal water at Eréndira are presented. Tables 1 and 2 summarize the bacteriological results for the seawater systems of Eréndira and Puerto Nuevo, respectively. Finally, Table 3 shows the bacterial concentrations in microbial food mats.

The time series of VHB in coastal waters at Eréndira (Fig. 2) shows higher values (10^4–10^5 CFU/mL) in May to June 1996, and June 1997, which correspond with red tide events (Flores-Aguilar et al. 1997). These results suggest that dinoflagellate blooms may support an increase in the numbers of VHB in coastal waters. Kodama (1990) suggested that bacteria may be the primary source of paralytic shellfish poison (PSP) toxins normally attributed to toxic dinoflagellates. More recently, Doucette and Trick (1995) report an autonomous bacterial PSP toxigenesis. These authors identified the PSP producers as members of the *Alteromonas/Pseudomonas* group. Therefore, the epizootic events of abalone postlarvae reported by Flores-Aguilar et al. (1997), attributed to red tides, may require careful consideration of bacteria associated with the dinoflagellates of this area. VLB concentrations were highest in January and June to July 1997 (Fig. 2), but only in the second case were they possibly associated with red tide events.

In Table 1, results of bacterial concentrations recorded in July 1997 (20 days after a red tide event) show low values of VHB in coastal and reservoir samples, but an increase of these after filtration and UV irradiation, reflecting a bacterial saturation of sand filters that could not be eliminated by the standard UV treatment. Bacteria growing on TCBS (VLB) were present in low concentra-

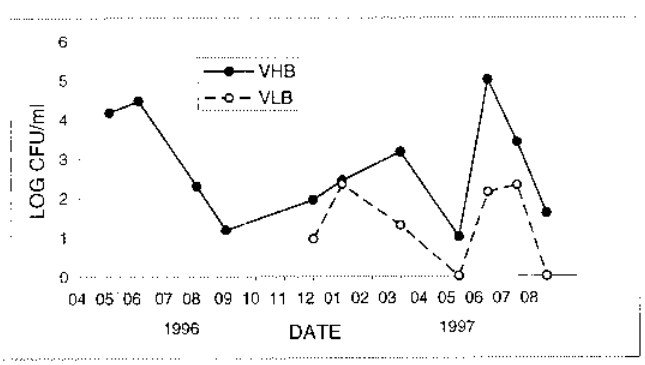

Figure 2. Time series of VHB and VLB in CFU/mL in coastal waters adjacent to Abulones Cultivados farm at Eréndira, Baja California, México.

TABLE 1.

Results of bacteriological samples of the seawater system at Abulones Cultivados farm (Erendira, Mexico).

	February 1996		July 1997		Averages	
Sampling Point	VHB	VLB	VHB	VLB	VHB	VLB
Coast	450	32	500	10	475	21
Reservoir	310	7	190	10	250	8.5
Broodstock	300	5	1240	4	770	4.5
Sand filters	280	17	3350	8	1815	12.5
UV system	30	0.32	2300	0.9	1165	0.61
Postlarvae	150	0.5	1920	1.4	1035	0.95
Hatchery	240	0.02	110	0.001	175	0.01

Each value is an average of two counts in CFU/mL.

tions in all samples, especially after UV treatment (0.001–10 CFU/mL).

The bacterial concentrations recorded in coastal waters at Puerto Nuevo in January 1997 (Table 2), correspond to values reported by Lizárraga-Partida and Vargas-Cárdenas (1996) for Baja California coastal waters free of fecal bacterial contamination. Water used in the Puerto Nuevo hatchery was similar in bacteriological quality to that in Eréndira. Water used in postlarval tanks had values of VHB close to those found at Eréndira, but VLB values were higher. Results of VHB recorded after sand filters and UV treatment indicate that free-living bacteria (Kogure 1989) are not completely eliminated by mechanical filtrations or UV irradiation. VLB counts seem to reflect, as well, the numbers of bacteria attached to suspended particles, a characteristic of pathogenic vibrios (Colwell et al. 1984), because their numbers are greatly reduced by pressure sand filters. UV irradiation seems to be very effective in the elimination of bacteria that grow on TCBS medium, as has been indicated before (Lizárraga-Partida et al. 1997), because their concentration was reduced by two or three orders of magnitude after the UV treatment.

The magnitude of these bacterial populations in culture systems has been reported to depend on the frequency of sand filter backwashing (Lizárraga-Partida et al. 1997), but it may also depend on the frequency of replacement of filter cartridges and the UV lamps. The establishment of such control measures are specific to each farm according to water flow rate, but usually sand filters have to be backwashed daily and cartridge filters have to be cleaned at least once a day. UV lamp replacement is determined by the rate of reduction in UV transmission caused by a process known as "solarization" (Wheaton 1987). The scheduling of these operations could be established by different methods, but our results show that bacterial plate counts are a powerful tool in this regard.

TABLE 2.

Results of bacteriological samples of the seawater system at Puerto Nuevo farm.

Sampling Point	VHB	VLB
Coast	240	5
Reservoir	330	0.1
Broodstock	5000	20
Sand filters	600	0.1
UV system	55	0.1
Postlarvae	700	0.1
Hatchery	60	0.1

Each value is an average of two counts in CFU/mL. Sampling date = 1/97.

TABLE 3.

Results of bacteriological samples of microbial food mats at Abulones Cultivados farm (Erendira, Mexico) in tanks of *H. rufescens* of different ages.

	3-wk-old Postlarvae			3-mo-old Juveniles	
Tank	VHB	VLB	Tank	VHB	VLB
45	60	0.09	69	1,250	200
46	80	0.1	71	1,140	134
47	20	0.1	73	1,240	105
			85	860	56
			86	3,000	180
			93	2,000	400
Averages	53	0.10		1,582	179

Tank numbers correspond to codes used at the farm. Each value is an average of two counts in CFU × 1,000/cm^2.

Food is generally the main source of bacteria in aquacultural systems, as is the case for diatom cultures and artificial food in postlarval tanks. The concept of "microbial food mats" is proposed because of the magnitude of the VHB biomass associated with *N. incerta* films, reaching concentrations between 10^4 and 10^6 CFU/cm^2. These bacteria may form part of the abalone diet (Kawamura 1996), although some could be pathogenic, as suggested by the VLB counts ranging from 10^1 to 10^5 CFU/cm^2 (levels two to five orders of magnitude higher than in the UV seawater supply).

Bacterial populations were much higher in tanks of 3-mo-old abalone compared with those in tanks of 3-wk-old postlarvae (Table 3). Food sources may be partially responsible for this result, because young postlarvae are fed with *N. incerta* cultures, whereas early juveniles receive an artificial food.

High postlarval mortality in Eréndira has been recorded during red tide events and has also been empirically associated with the development of a "red spot" in the bottom of the tanks. Brown (1974) has reported that concentrations above 10^3 CFU/mL of a red-pigmented pseudomonad could produce either retardation of embryonic development or mortality in hard clams (*Mercenaria mercenaria*). Our attempts to isolate red-pigmented bacteria from the bottom of abalone postlarval tanks, following Brown's procedures, remain unsuccessful. Understanding the species composition of microbial food mats and their pathogenic or beneficial effects on different abalone postlarval stages would be useful in developing strategies to improve their survival and growth.

ACKNOWLEDGMENTS

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SABELLID INFESTATIONS IN THE SHELLS OF SOUTH AFRICAN MOLLUSCS: IMPLICATIONS FOR ABALONE MARICULTURE

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ABSTRACT Infestations of sabellid polychaetes were found in South African–farmed abalone in 1994. Growth experiments confirmed that infested abalone had reduced growth rates. Surveys of both the intertidal and the subtidal regions at various locations around the South African coastline revealed that the sabellid was endemic to the region. It occurs in a range of mollusc species, but some species are more susceptible than others. The fact that some mollusc species become infested with worms only when exposed to them in the laboratory suggests that environmental factors may play a role in controlling natural levels of infestation. Larvae disperse by crawling and settle at the growing edge of the shell underneath the mantle. Although the larvae are benthic, there is limited transfer of larvae through the water column, which can result in export of larvae from infested tanks. The risks of dispersal are discussed in a farm management context. Abalone kept in more hygienic laboratory conditions tend to grow faster than they do in farm tanks and have lower levels of infestation by sabellid larvae. This may be because the sabellids are less fecund under these conditions, which also has implications for management.

KEY WORDS: sabellids, South Africa, molluscs, larval dispersal, distribution, management

INTRODUCTION

While conducting growth trials at an abalone farm on the West Coast of South Africa in 1994, we noticed that one group of animals exhibited virtually zero growth and experienced higher mortalities than other groups. A closer examination revealed that the shells of these animals were exceptionally weak and prone to breakage, which presumably lead to the mortalities. The shells also had slightly abnormal shapes, the characteristic fluting of the shell being absent. The weakening of the shell was associated with numerous tiny holes, occupied by a polychaete worm. The growing edge of the shell was infested by tiny, larval worms. Initially, it was thought that this was caused by *Polydora*, a documented shell borer in abalone (Blake and Evans 1973), but closer examination of the worm suggested that it was the same animal that had been recently discovered in Californian abalone farms (Oakes and Fields 1996).

The worm is a sabellid polychaete of a previously undescribed genus (Fitzhugh pers. comm). It has been causing severe damage to the abalone industry in California, and there has been some release of the sabellid into the environment around abalone farms (Culver and Kuris unpubl.). A project was initiated in California to study the threat posed both to farms and to the natural environment (Culver et al. 1997), the latter being necessary because the sabellid is not native to California (Lafferty and Kuris 1996). A similar project was initiated in South Africa by the Abalone Farmers Association of South Africa to assess the risk to abalone farms and to determine whether it is endemic to South Africa, and if so, to determine the distribution of the sabellid worm in wild stocks.

A brief description of the worms' life cycle follows, based on personal observation and that of researchers in California (Kuris and Culver unpubl., Oakes and Fields 1996, Fitzhugh and Rouse unpubl.). Like many other sabellids, it is a simultaneous hermaphrodite, producing both eggs and sperm at the same time. The adults brood the eggs in the tube until they are viable larvae, which then emerge as fairly large (~600 μm in length), crawling larvae possessing two eyespots and sensory tentacles at the anterior end. Five pairs of setae and a broad ciliial band, the neurotroch, are used in locomotion and anchorage. Larvae are very active and crawl over the surface of the host shell until they reach the mantle or shell-secreting portion of the host, or a neighboring animal. They locate themselves between the mantle and shell and secrete a mucous or proteinaceous tube around themselves. The tubes, which are longer than the larvae, are then covered by newly deposited shell of the host animal. The larva maintains an opening to the external environment at its anterior end. A feeding crown is formed after metamorphosis, and as the worm grows, it proceeds to fill the tube. There is also evidence of burrow enlargement. The longevity is unknown at present. Age of reproductive maturity lies between 1 (Kuris and Culver unpubl.) and 3 (pers. obs.) mo. This article discusses the effect that the worm has on abalone growth, the distribution of the sabellid on the South African coastline, the dispersal abilities of the worm, and its implications for management of infestations on abalone farms.

METHODS

Growth

The worm was first noticed in South Africa during a growth experiment involving three cohorts of abalone. Three replicate baskets of each cohort: A (11–13 mm), B (20–25 mm), and C (35–41 mm) were held in three aquaria. Only one cohort (C) was found to be heavily infested with sabellid worms. The baskets were staggered in each aquarium to eliminate tank effects. Each basket contained 20 abalone, which were measured and weighed each month.

A second growth experiment was conducted to confirm the effect of the sabellids on growth. In this case, animals of the same size were used and either mixed together with five heavily infested abalone (sabellid effect) or kept isolated (sabellid free). This was a relative measure because it was not possible to find animals that were totally sabellid free. Three replicates, containing 10 abalone each, were used in each case. Animals were weighed and measured

at monthly intervals for 3.5 mo. Larval counts were performed on a subsample of five animals at 17 days and then at each sampling interval. For this, the abalone was held upside down under a dissecting microscope and the mantle was carefully pushed aside to expose the growing edge of the shell. Larvae at the growing edge, between the spire and the last respiratory pore, were counted (Fig. 1). The length of this edge was measured with a vernier caliper. The number of larvae per centimeter of shell edge was then calculated. Control animals were not sabellid free but had low levels of infestation, and thus, it was necessary to include sabellid counts for control animals.

Distribution

In order to determine whether the sabellid occurred in wild stocks and, if so, the range and geographical distribution of infested species, a range of locations were sampled around the South African coastline, extending from Port Nolloth on the West Coast to Gans Bay on the South Coast (Fig. 2). Molluscs of as many species as possible were collected both subtidally and intertidally, preference being given to areas where abalone occur naturally. Molluscs collected were measured (shell length) and examined for the presence of sabellid larvae at the growing edge only. This method was chosen because it provided a means to quantify large numbers of animals quickly.

Once the field work was completed, laboratory experiments were undertaken to assess the susceptibility of different molluscan species to infestation. A range of species were kept in aquaria together with heavily infested abalone. Three replicate tanks were used, each with four to six individuals of each species, kept together with six heavily infested abalone. The introduced animals were measured, and their sabellid infestations were estimated at the start of the experiment, and again after 2 wk, by counting the number of sabellid larvae per linear centimeter of shell. The experiment was conducted twice.

Dispersal

Larval dispersal is a very important factor to abalone farmers, because this controls the spread of infestations through farms and between farms. The behavior and physiology of larvae were observed in petri dishes under the light microscope, and the larvae were also prepared for scanning electron microscopy. An experiment was conducted to test the dispersal abilities of the worms in a laboratory situation. Twenty uninfested abalone in the 15–23 mm size range and 10 heavily infested abalone in the 50–60 mm size range were used for experiments. Three scenarios were created in aquaria kept at a constant temperature of 17°C with filtered, flow-

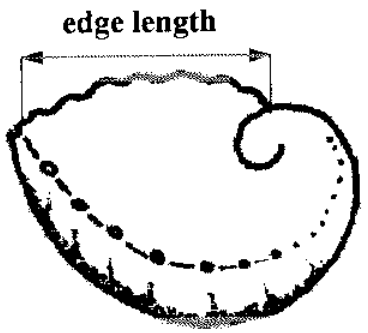

Figure 1. A diagram of an abalone shell to illustrate the region sampled for larval counts.

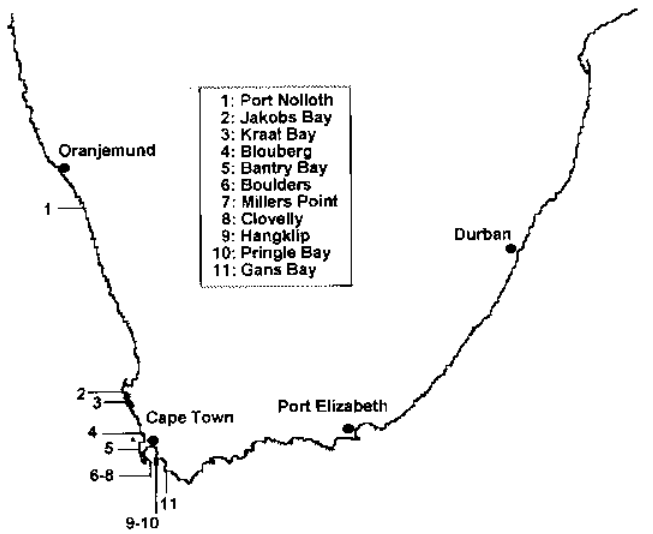

Figure 2. A map of the coastline of South Africa to show sampling sites.

through seawater. First, uninfested abalone were allowed to mix with the infested abalone on the base of the aquarium. Second, uninfested abalone were completely separated from infested abalone by a suspended basket system. The baskets were not touching each other or the sides of the aquarium, so the only way for larvae to reach the other basket was through the water column. In the third scenario, the uninfested abalone were again separated from the infested animals by baskets, but in this case, the baskets rested on the base of the aquarium so that larvae were free to crawl along the base of the aquarium between baskets. The entire experiment was conducted twice with the same baskets. The uninfested abalone were examined at approximately 20-day intervals. Shell lengths were recorded, and a subsample of 10 abalone was used for larval counts as described above. At the end of the experiment, the newly infested abalone from the mixed treatment were separated and further monitored (growth and larval counts) for a period of 2 mo.

Possible export of larvae from culture tanks was tested by filtering water from the outflows of culture raceways that contained infested stocks of abalone through a series of sieves of different mesh sizes. Sieves with pore diameters of 100, 58, 45, and 32 μm were used in sequence. First, water was allowed to flow freely for a period of a few hours, but in a second attempt to quantify the results, a sample of 500 L was passed through the sieves and examined under a dissecting microscope for the presence of larvae.

Sabellid Fecundity

It was noticed that abalone tended to grow faster after some time in laboratory conditions (low densities and cleaner tanks) in comparison to the stock animals in raceways from which they had been selected. At the same time, the worm count at the growing edge dropped. Thus, it was decided to compare the condition of the sabellid worms as follows. Six heavily infested abalone from the second dispersal experiment were sacrificed, and the entire shell was crushed with a pair of pliers. Three abalone from their counterpart stocks in the culture raceways were also crushed. The fragments were placed in seawater in a petri dish, marked with 1-cm gridlines. Adult worms (defined as those worms of any size pos-

sessing feeding crowns); eggs, and larvae were counted in a fixed area. The ratios of eggs and larvae relative to adults were recorded. The experiment was repeated at another farm where animals had also been separated and kept in laboratory conditions.

RESULTS

Growth

Growth rates of abalone from the initial growth experiment are presented in Figure 3. To allow comparisons between abalone of different sizes, growth was expressed as length increments. During the first 6 wk of the experiment, the animals in Size Class C showed virtually no growth. In fact, there was even negative growth in some aquaria, presumably due to shell breakage. The shells become very fragile and prone to breakage when infested. After Day 75, the animals in Size Class C started to grow faster. This may have been an effect of laboratory conditions, as will be discussed later.

The results of the second growth experiment, in which animals of the same starting size were used, are summarized in Figure 4. Both length and weight data are given, along with larval counts at the growing edge of the shell. Because the control animals were found to have low levels of infestation, their larval counts are included with the last two measurements. The experiment must therefore be considered as a comparison in growth between moderately and lightly infested abalone. Significant differences in growth for both weight and length (*t*-test of means, $p < 0.05$, $df = 4$) were recorded. Higher counts of larvae do occur in culture situations (up to 25/cm; see Fig. 6), and these abalone are likely to exhibit even slower growth than represented here.

Distribution

The results of the dive surveys conducted on the West and South Coasts of South Africa were used to calculate the prevalence of the sabellid in natural populations (Table 1). Prevalence is defined as the number of individuals of a host species infected, divided by the number of hosts examined (Margolis et al. 1982). The sabellid is widespread and was found in varying degrees of abundance at each location sampled. It is clear from the table that the sabellid is not limited to abalone but can infest various unrelated genera. Some species such as *Haliotis midae*, *Turbo cidaris*, *Turbo sarmaticus*, and *Burnupena* spp. are very prone to infestation, with a high proportion of the population exhibiting the presence of sabellids. A number of limpet species—*Patella miniata*, *Patella longicosta*, *Patella oculus*—and a winkle, *Oxystele tigrina*, were found with low levels of infestations. However, these were all sampled near the outfall of an abalone farm at Gans Bay, containing infested abalone. The data are presented both with and without the Gans Bay site to show the anomalous pattern (Table 1). It is possible that the export of larvae from the farm creates a situation atypical of that which normally occurs in the wild and allows these species to become mildly infested. In fact, the majority of molluscan species sampled are not very susceptible to infestation by the sabellid in the wild. There are two special cases worth mentioning. Mussels appeared to be generally unsusceptible to sabellid infestation. As an extra test, 26 individuals of *Mytilus galloprovincialis* ranging in size from 24 to 40 mm were taken from a basket at an abalone farm that also contained many heavily infested abalone, but none of the mussels were infested with any stages of sabellids. Similarly, *Crepidula porcellana* also appeared unsusceptible, even though all of the individuals sampled were epibionts of the highly infested *Turbo cidaris* individuals.

There may be various reasons why some species are more prone to infestation than others, and the laboratory experiment was designed to investigate some of the possible reasons. The results are shown in Figure 5, which records prevalence and the density of sabellid larvae at the growing edge of the shell, this being a measure of the intensity of infestation.

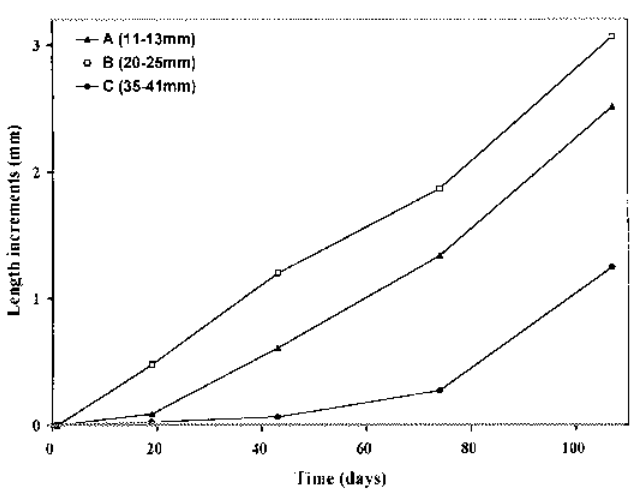

Figure 3. Growth increments over time of three cohorts of abalone in a growth study. Cohort C was found to be heavily infested by sabellids.

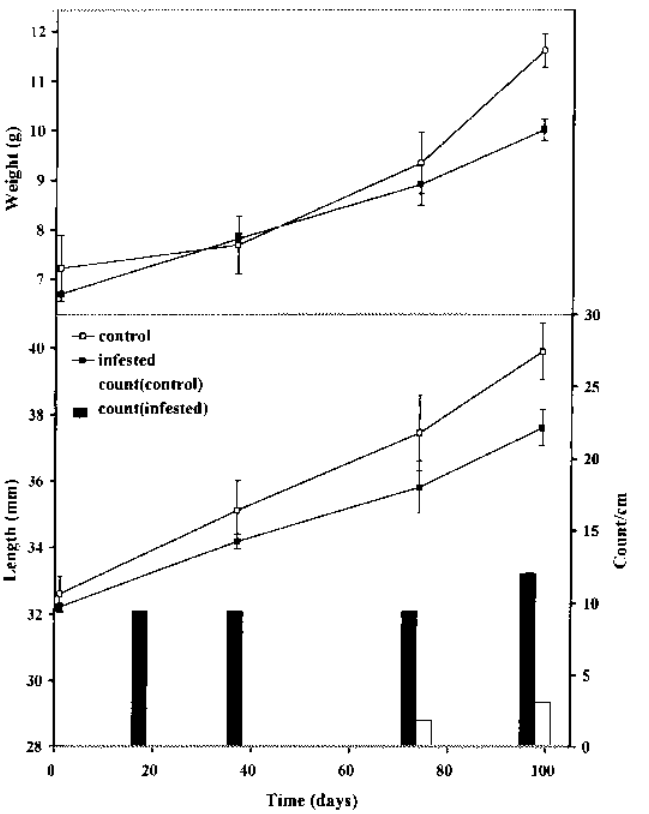

Figure 4. Growth trial to determine the effect of sabellids on growth. The histogram indicates the numbers of larvae at the growing edge of the shell. The control groups were found to have low levels of infestation, which are included as clear bars. Error bars are one standard deviation of the mean.

TABLE 1.

Species sampled on the coastline of South Africa from Port Nolloth on the West Coast to Gans Bay on the South Coast; the number sampled, number infested, and percentage of individuals with sabellid infestations on the growing edge of the shell are shown.

	Excluding Gans Bay			Total		
Species	Sample Size	With Sabellids	Prevalence (%)	Sample Size	With Sabellids	Prevalence (%)
Abalone						
H. midae	140	84	60	173	114	66
H. spadicea	19	0	0	19	0	0
Haliotis parva	2	0	0	2	0	0
Limpets						
P. miniata	32	0	0	71	3	4
P. longicosta	15	0	0	54	10	19
P. oculus	22	1	5	58	8	14
Patella barbara	64	0	0	82	0	0
Patella compressa	56	0	0	92	1	1
Patella argenvillae	26	0	0	26	0	0
Patella cochlear	91	0	0	91	0	0
Patella granatina	72	0	0	72	0	0
P. granularis	81	0	0	102	0	0
C. porcellana	91	0	0	91	0	0
Whelks						
Burnapena papyracea	79	44	56	79	44	56
Burnapena spp.	231	83	36	249	97	39
Argobuccinum pustulosum	48	2	4	48	2	4
Fasciolaria lugubris lugubris	5	0	0	5	0	0
Mussels						
Aulacomya ater	9	0	0	9	0	0
Choromytilus meridionalis	11	0	0	11	0	0
Winkles						
O. nigrina	39	0	0	109	8	7
O. sinensis	45	0	0	126	1	1
O. variegata	74	0	0	110	0	0
Turban shells						
T. sarmaticus	17	14	82	41	35	85
T. cidaris	91	81	89	124	113	91
Other						
Cymatium cutaceum	1	0	0	1	0	0
Clionella sinuata	6	0	0	6	0	0
Marginella nebulosa	1	0	0	1	0	0
Nucella squamosa	9	0	0	9	0	0

Almost all species that exhibit very low or zero infestation in the wild were infected when exposed to larvae in the laboratory. The levels of larval colonization, however, are still lower than those of the host species commonly infested in the wild. Whether this is due to larval preference or host resistance is unclear. An exceptional case was the winkle *Oxystele sinensis*, which never acquired any infestations in the experiments. Some of the high-shore species, such as *Patella granularis* and *Oxystele variegata*, spent a large proportion of the time above the water line in the aquaria, reducing their exposure to the larvae. Had they been forced to remain submersed, it is possible that the counts at the end of the experiment may have been higher. Even though *T. sarmaticus* and *H. midae* exhibited high counts at the start of the experiment (as would be expected from the results in Table 1), the counts increased markedly when they were exposed to the infested, farm-grown abalone in the aquarium. This suggests that the source abalone from the farm are more severely infested than are wild abalone. In a separate experiment, the susceptibility of *Haliotis spadicea* to infestation was tested in the laboratory. Individuals placed in tanks with infested *H. midae* were readily infested by sabellid larvae within a few days.

Dispersal

Larvae observed in petri dishes were found to be very active, with a rapid crawling motion of about 1.5 body lengths per second. They resist being dislodged by water currents by adhering to the substratum on a string of mucus. They possess two eyespots and what seem to be sensory tentacles at the anterior end. It appears from preliminary experiments that larvae preferentially settle under pieces of abalone tissue as opposed to dead shell, but when this is not available, they will settle on the glass of a petri dish, produce a tube, and even metamorphose to produce a feeding crown. Further development in the absence of a host has not been studied.

Even though conditions were different in each repeat of the dispersal experiment, a similar pattern occurred. Results are presented in Figures 6 and 7. Abalone in the mixed tank acquired infestations very quickly. At the first measurement (which oc-

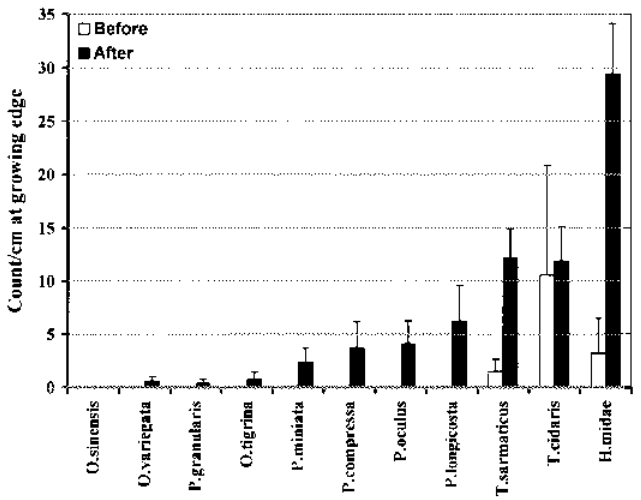

Figure 5. Results of the mixed-species experiments. Bars indicate the density of sabellid larvae at the growing edge of the shell before and after being exposed to heavily infested abalone. Time period was 14 days with n = 6 (4–6). Error bars are one standard deviation of the mean.

curred between 18 and 22 days after initiation), there were already substantial numbers of larvae at the growing edge. In the first dispersal experiment, there were high counts of sabellid larvae and the levels remained high. The growth rate of the mixed animals was lower than those from the other treatments (see Fig. 7). In the second dispersal experiment, the initial counts were much lower and declined further toward the end of the experiment. Thus, even though the growth rate appeared slower initially, it increased toward the end of the experiment to become similar to that of the other two treatments. In both experiments, there were no noticeable differences between the suspended basket treatment and the base basket treatment. This was true for both counts and growth rates. This implied that larvae were not effective at crossing the short distance separating the two baskets. There was a build up of detritus on the base of the aquarium, which may have restricted the

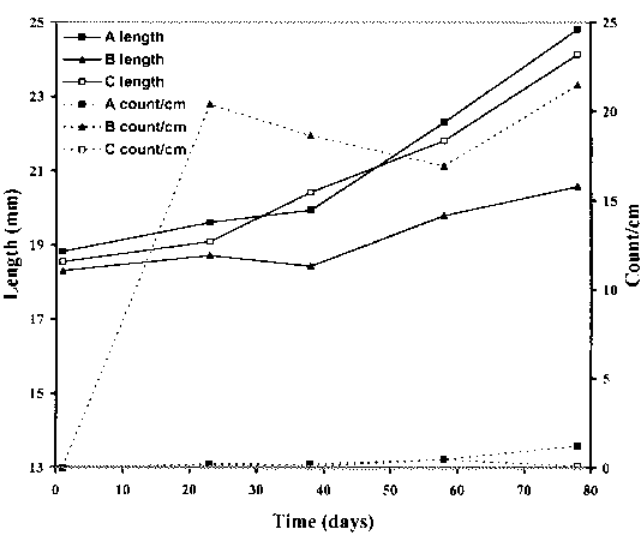

Figure 6. Dispersal Experiment 1. Growth rates and larval counts at the growing edge of the shell of the three dispersal treatments. Treatment A, suspended baskets; Treatment B, mixed animals; Treatment C, baskets on the bottom of the tank.

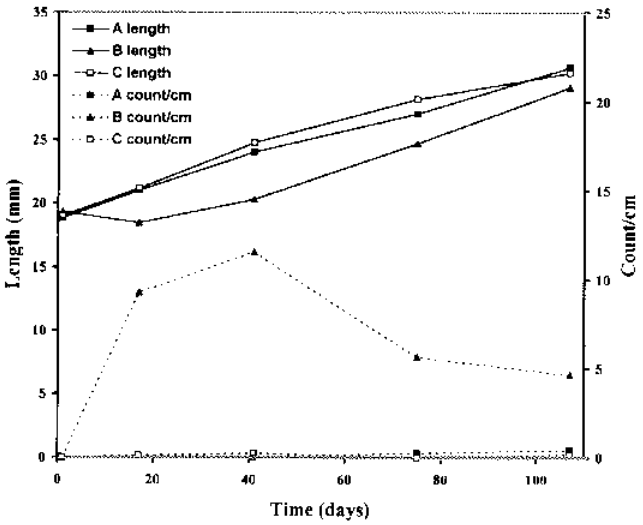

Figure 7. Dispersal Experiment 2. See Figure 6 legend for details.

benthic movement of larvae. It is interesting that there was transfer of larvae from infested to uninfested animals in the suspended basket treatment, implying that the larvae passed through the water column. The levels of transfer were very low, however, and would be unlikely to influence the growth rates in the short term.

Figure 8 illustrates the progress of the abalone from the mixed treatment in the first dispersal experiment after they were removed from the source of infestation. There was a sudden disappearance of larvae at the growing edge of the shell, coupled with an acceleration in growth. Only after Day 150 did larvae appear at the growing edge of the shell, originating from these new infestations. These levels were sustained for some time, and the growth rate decreased. From these data, it was possible to estimate the time taken for the sabellid population to become reproductive from the larval stage. Assuming the first sabellids (which colonized the shell during Days 1–10) were the first ones to produce larvae sometime after Day 120, then it took approximately 4 mo to complete the cycle at 17°C.

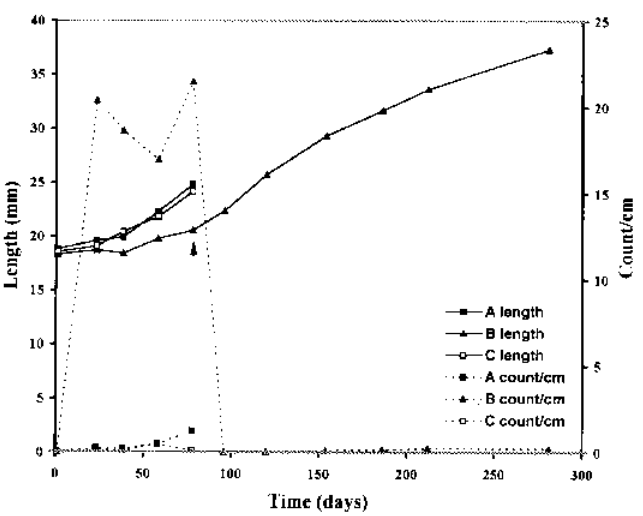

Figure 8. Progress of newly infested animals from Treatment B in Dispersal Experiment 1 after termination of experiment (indicated by arrow).

TABLE 2.

Larval counts from filters placed at the outflows of engrossing raceway tanks containing infested abalone.

	Larval Count with Pore Size of (μm)			
Sample	**100**	**58**	**45**	**32**
Timed (Farm 1)	0	22	0	0
5001 (Farm 2)	0	3	1	0

Table 2 presents the results of the filtering experiment. A fairly high degree of export of larvae from infested raceway tanks occurred. The tanks contained in the region of 1,000 heavily infested abalone in the 50–60 mm size class. Most larvae were caught in the 58-μm-pore-size mesh.

Fecundity

Figure 9 compares animals from the laboratory with their counterparts in typical high-density, raceway culture conditions. The count of larvae per centimeter at the growing edge is shown above the bars. It is clear that the animals in the laboratory had lower infestation rates than did the animals from the raceway, presumably because the worms become less reproductive in the laboratory. This suggestion is supported by the observation that in the raceway there were far more larvae per adult, and eggs outnumbered adults.

DISCUSSION

When the sabellid was first discovered in California in 1993, concern was expressed about its origins and how it came to be on the abalone farms. The general consensus was that it had been introduced from elsewhere because it could not be found in wild abalone stocks in California (Kuris and Culver unpubl., Lafferty and Kuris 1996). This study records the discovery of sabellids in South African abalone farms, which may have remained unnoticed had it not been for the pronounced effect that sabellid infestation has on growth rates of farmed abalone. Furthermore, the surveys of populations of molluscs on the South African coastline reveal that the sabellid is endemic to South Africa. It has now been found at every site sampled along the coastline from Port Nolloth, on the West Coast, to Port Elizabeth on the East Coast (pers. obs.). Its range limits are yet to be determined. Although the polychaete is not host specific, some mollusc species seem less prone to infestation, presumably because of environmental factors that do not favor the transfer of larvae. For example, *P. granularis* and *O. variegata*, which are high-shore species and spend a considerable portion of time exposed during tidal cycles, have low infestation rates. Species that are gregarious, living in semisheltered locations under boulders or in crevices, such as species of *Burnupena*, *Turbo*, and *Haliotis*, seem to be most susceptible to infestation. Both *T. cidaris* and *T. sarmaticus* can be found in association with juvenile *H. midae*, creating the possibility of larval transfer between species.

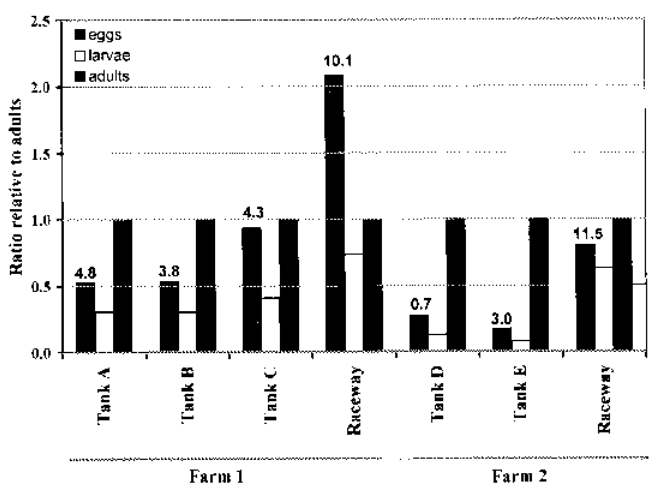

Figure 9. The ratio of eggs and larvae relative to adults is recorded for abalone, which had been used as sabellid-source animals in Dispersal Experiment 2 (Tanks A, B, and C). These are compared with animals of the same cohort that had remained in the culture raceways. Similar data are recorded from another farm where some animals had been moved into laboratory conditions. Values above the bars indicate the larval counts/cm.

Some species appear to have resistance to infestation that cannot be completely explained by environmental factors. *O. sinensis*, for example, can be found in association with *T. cidaris* and juvenile *H. midae*, but does not acquire infestations, even when exposed in a laboratory situation. The reasons for this have not yet been determined.

Dispersal of larvae is an important consideration for farmers who wish to prevent larvae from entering the natural environment. It is also important in the prevention of intrafarm and interfarm transfer. Results from the dispersal experiment have shown that transfer of infestation is very rapid when animals are mixed. If animals are separated by baskets, or similar containers, the rate of larval transfer can be reduced, but does still occur. This implies that larvae are able to pass through the water column, presumably after being dislodged by water movement. Larvae were never observed swimming naturally, but could be induced to do so when dropped into seawater, after which they sometimes "swim" at the surface attached to a mucous thread until they reach the side of the container. This suggests that transfer of larvae through the water column is possible, but that the chances of successful transfer of larvae may be reduced if baskets are well separated.

Growth rate studies confirmed that sabellid infestations reduce growth rates of farmed abalone. Growth is influenced because of the interference caused by larvae at the mantle–shell interface, but low levels of infestation do not appear to slow growth rates. Presumably, this is because the shell deposition can continue relatively unhindered when there are only small, isolated pockets of disturbance. On the other hand, when the entire shell margin is covered by larvae, the normal process of linear shell deposition is not possible. When the source of larvae is removed, the abalone can revert to normal growth almost immediately.

In managing South African abalone farms, an important consideration should be to prevent transfer of infestations within and between farms. Although it is unknown how many larvae may enter through inflows, a filter would limit the introduction of sabellids. All abalone produced on farms start off sabellid free (pers. obs.), and it should be the aim to keep them this way for as long as possible. For this reason, infested animals should be kept in separate raceways. If animals are subdivided within baskets, then transfer of larvae between baskets can be reduced. It is common practice on abalone farms to mix animals from various cohorts to

effectively use space during the size grading of animals. This practice could be harmful in terms of sabellid transfer if not managed very carefully. A useful tool when transferring animals is to clean tanks with a freshwater rinse, to kill all larvae (Culver et al. 1997).

Good tank hygiene is also important. When tanks are kept clean, there is less particulate matter in the water for the sabellids to feed on. Thus, even though there may be many adult sabellids in the shells, they are less reproductive.

Should farmed abalone become infested by sabellids, a treatment methodology to kill them when they reach high numbers would be useful. At present, we are developing a treatment using microencapsulation technology, which exploits the difference in the feeding mechanisms of abalone and sabellids. The sabellids are filterfeeders, whereas the abalone are grazers. It may be possible to encapsulate a toxin, in particulate form, that will only target the sabellids. Results have been promising, and we believe that a treatment should be available in the near future.

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CONTROL OF SABELLID INFESTATION IN GREEN AND PINK ABALONES, *HALIOTIS FULGENS* AND *H. CORRUGATA*, BY EXPOSURE TO ELEVATED WATER TEMPERATURES

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ABSTRACT The majority of the abalone hatcheries in California are now plagued by an introduced shell-infesting sabellid polychaete, which in extreme cases, causes extensive shell deformities and greatly retards body growth. In addition to the adverse effect on the abalone aquaculture industry, there is concern that this pest may become established among native stocks. Efforts to exterminate mature and immature stages of the sabellid have focused on the use of water-soluble toxic substances, coating shells with wax or other substances to smother the worms, or altering the osmotic environment without causing irreversible injury to the abalone. These approaches have not provided a dependable method for control of the polychaete. Exposure of sabellid-infested green and pink abalones (*Haliotis fulgens* and *Haliotis corrugata*) to temperatures near but below their upper thermal limits has produced the desired results. A 48-h exposure to 28.5°C has effectively killed all life stages of the sabellids but caused only minor mortality among the abalone. An LD_{50} 48-h upper limit for *H. fulgens* was confirmed at 31.5°C; that for *H. corrugata* was established at 30.3°C. Treated abalone placed in optimally circulated seawater and fed the standard food, *Egregia menziesii*, resumed normal growth and thenceforth displayed no further symptoms of sabellid infestation. The method is fully compatible with life requirements of the green and pink abalones, but would not be suitable for treatment of species (e.g., *Haliotis rufescens*) known to have upper lethal limits well below 28 C.

KEY WORDS: sabellid control, abalone disease, thermal limits, abalone aquaculture

INTRODUCTION

The majority of abalone hatcheries in California have been plagued by a yet undescribed shell-infesting sabellid polychaete. This ectoparasite, thought to have been introduced in the late 1980s to a California aquaculture facility on shells of imported South African abalone, *Haliotis midae* Linnaeus, 1758, has been spread to other hatcheries by sharing of abalone stock (Culver and Kuris 1995). The minute worm (1–3 mm) establishes itself as a larva on the abalone host immediately between the mantle and the interior nacreous surface, selecting a location near the growing margin of the shell or elsewhere at a site of active nacre deposition (e.g., the respiratory pores). Larval worms appear to become ensheathed, perhaps through induction of the mantle to deposit thin layers of nacreous material. In severe cases, wherein a 2–3 cm juvenile abalone may harbor dozens of individual sabellids, normal deposition of shell material (aragonitic nacre and calcitic ostracum) is hindered. Nacreous layers continue to be deposited, creating a thickened shell, but marginal increment is curtailed. Under these conditions, growth of body tissue appears also to be greatly decreased or stopped altogether.

Sabellid infestation has become a major concern among culturists who have experienced drastic reduction in productivity and marketability of cultured products as abalone growth rate declines and shell morphology becomes grossly abnormal. The possibility of introduction of this pest into native populations of abalone (and other mollusks) is a serious threat. The polychaete and its associated epidemiology have become the center of several investigations in California, ranging from biological and life history studies to searches for methods to eradicate the worm (Culver and Kuris 1995, Oakes and Fields 1994, Trevelyan 1995). Taxonomic study and description of this annelid are in progress by Kirk Fitzhugh, Los Angeles County Museum of Natural History (Culver and Kuris 1995).

Efforts to exterminate mature and immature stages of the sabellid have focused on use of water-soluble toxic compounds, coatings of shell with paraffin, wax, and other materials to smother the worms (Oakes and Fields 1994, Trevelyan et al. 1994, Trevelyan 1995), or alteration of the osmotic environment without causing stress or irreversible injury to the abalone. These approaches have not provided a dependable method for control of the polychaete. Generally, the worms have proved more resistant than the abalone.

The possibility for control by noninvasive approaches (no toxic therapeutants) may exist wherein an environmental factor is changed that exerts a more stressful action on the worm than on the abalone. In this category are temperature and salinity. This study examines the effect of warm seawater on both ectoparasite and host.

Earlier study of temperature tolerance in young stages of California abalones has shown the green abalone, *Haliotis fulgens* Philippi, 1845, to be tolerant of temperatures exceeding 28°C (Leighton 1974) with an LD_{50} (48 h) for juveniles of 31.5°C (Leighton et al. 1981, Leighton 1985). Preliminary observations on thermal tolerance of adult sabellids suggested exposure to temperatures above 29°C to be lethal to the polychaetes if extended over a 1–2 day period. Accordingly, a series of tests were carried out with heavily infested juvenile and young adult green abalone to establish an effective thermal exposure sublethal for the abalone but lethal to all life stages of the sabellid (adults, immature tentacled stages, larvae, and eggs). Although this study focused on the green abalone, a limited number of infested juvenile pink abalone, *H. corrugata* Wood, 1828, became available and were subjected to heat treatments. This abalone is also tolerant of water temperatures in the range 28–30°C (Leighton 1974).

The presence of sabellid polychaete worms was first detected in abalone production raceways of Carlsbad Aquafarm in December 1995. Attempts were made promptly to place all infested stock into isolation tanks well away from the primary nursery and hatchery

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tanks. The latter were then sterilized with sodium hypochlorite, followed by dechlorination with sodium thiosulfate. The isolation tanks served as a source of experimental material with which to conduct this study.

MATERIALS AND METHODS

Treatment of Infested Abalone

Groups of juvenile and young adult abalone produced at the Carlsbad Aquafarm hatchery and visibly infested with the sabellid were placed in a pen of 12-mm-pore-size polyethylene mesh, which was in turn immersed in a tank holding 20 L of seawater. Temperature was controlled with a submersible aquarium heater (Jager, EB 150-W, holding water temperature constant within ±0.1°C). Temperature settings ranged from 27 to 32°C for this series. A registering maximum/minimum immersible thermometer was routinely used to reveal any unexpected water temperature changes; none occurred throughout the study. Aeration was vigorous. Depending on abalone size, numbers per group typically ranged from 20 (abalone larger than 3 cm) to 200 (abalone 1–3 cm). Most thermal exposure experiments were of 48–72 h duration but some were extended to 144 h and longer. Water was partially exchanged once daily. In some runs, shells of infested abalone were removed to be exposed to elevated temperatures in the absence of live abalone tissue ("shells only" series).

Samples of abalone were taken at the beginning of each run and at regular daily intervals throughout each experiment. The effectiveness of the thermal treatments was assessed by the condition of sabellids on microscopic examination of shells freshly separated from soft body parts. This was done because observations were greatly facilitated with nonmoving subjects. Both interior and exterior surfaces of shells were examined immersed in seawater under a high-magnification stereoscopic dissection microscope equipped with fiberoptic illumination. Moribund polychaetes that had been exposed to the high temperatures were generally lethargic, presented contorted tentacles, rarely withdrew into tubules, and could be teased out of tubules with a "hog's eyelash," a toothbrush bristle mounted on a thin applicator stick. As a more invasive procedure, a ceramic tile clipping tool was used to fragment samples of shell material for detailed study of intact sabellid adults, larvae, and eggs.

After heat treatments, abalone remaining (not sacrificed for samples) were consistently placed in separate tanks with flowing ambient seawater for observations to confirm sabellid mortality (including destruction of eggs) over intervals of 1–2 mo. Longer term monitoring was done routinely on all treated stocks and involved many hundreds of samples taken over a period exceeding 18 mo.

An accompanying series of tests was run with shells freshly removed from infested abalone. These "shells only" observations sought to determine the temperature tolerance of sabellid worms without the possible influence of fluids eminating from stressed or moribund abalone.

Treatment of Broodstock (6–12 cm)

Cultured abalone 2–3 y of age retained as broodstock were also subjected to heat exposure treatments to ensure their sabellid-free status. These groups were subjected to spawn induction procedures 2–4 mo after treatment to establish the effect of the heat treatments on gametogenesis and gonad maturation.

Observations on Extracted Live Sabellids

Thermal tolerance in larval, immature, and adult sabellids was examined with a constant temperature block (Temp-blok Module Heater, Scientific Products, Inc.) accommodating a series of test tubes (10 × 100 mm). Larvae (600–800 μm) were collected by pipette after being teased from crushed shell fragments immersed in seawater in a petri dish. These individuals were admitted singly into tubes to constitute groups of four to five. More advanced sabellid stages were usually included in fragments of shell. Typically, five tubes were used; at intervals throughout an experiment, contents of separate tubes were examined in seawater under a high-power dissection microscope equipped with dual fiberoptic illuminators. Exposure time to death was recorded; the endpoint was judged by cessation of motion, development of abnormal morphology or disintegration, and finally, lack of recovery after holding at ambient temperature for 12 h. Temperature effects on mortality of extracted sabellids were observed at a series of temperature settings from 30 to 36°C.

RESULTS

Treatment of Infested Abalone

Over the course of 18 mo, over 50 group treatments were conducted. Collectively, this included over 2,250 juvenile and young adult *Haliotis fulgens* and about 100 juvenile *H. corrugata*. Data on heat treatments for sabellid infestations in *H. fulgens* indicate that exposure of 48 h to 28.5°C was sufficient to cause 100% mortality to the polychaetes. Shorter exposures to higher temperatures (e.g., a 24-h exposure to 29.5°C) were also fully effective. Results for observations over the range 27–30°C are summarized in Table 1 and presented graphically in Figure 1.

In these thermal exposure experiments, *H. fulgens* displayed high tolerance to temperatures up to 30°C, but an increasing intolerance to temperatures above 30°C. Under the conditions of these experiments, rapid immersion of abalone in seawater at temperatures 32–33°C caused excessive mucous production, which soon fouled the water and increased abalone mortality. Thus, longer term exposure to lower temperatures was indicated to be most practical for heat treatments. Routinely, at the onset of each

TABLE 1.

Condition of sabellid polychaetes after consecutive 24-h exposures to a series of temperatures.

Temperature Range (°C)	No. of Observations (Total Abalone)	Hours					
		24	48	72	96	120	144
27.0–27.4	2 (45)	1	1	1	2	3	4
27.5–27.9	5 (301)	1	1	2	3	4	
28.0–28.4	4 (255)	2	3	4			
28.5–28.9	6 (300)	3	4				
29.0–29.4	4 (335)	3	4				
29.5–29.9	4 (175)	4					

Condition of polychaetes was established by microscopic examination. Results were graded as follows: 1, sabellids appeared and behaved normally; 2, relaxed, slow to withdraw into tubules on probing; 3, no response to probing, tentacles swollen with tightly curled tips, worms easily extracted from tubules with a fine probe; 4, sabellids dead, deformed, and decomposing.

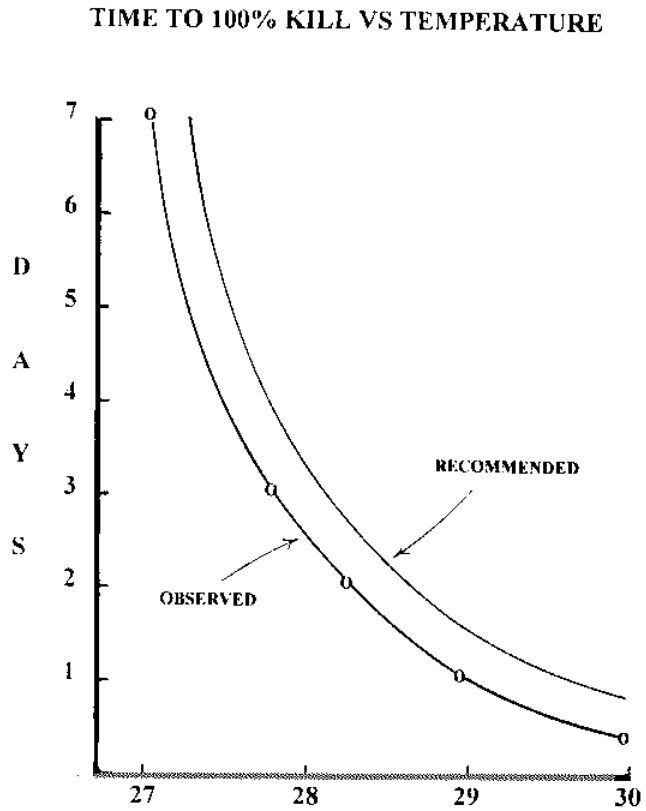


Figure 1. Time to total mortality of sabellid polychaetes as a function of temperature in the range 27–30°C. A recommended treatment interval is indicated as a safety margin to ensure eradication of sabellid adults, larvae, and eggs.

treatment observation, temperature was brought up to the desired level from ambient over a period of about 2 h.

Observations with *H. corrugata* were more incidental to the focus on *H. fulgens*, owing to the few infested juveniles of the former available during the course of this study. Pink abalone often survived exposures comparable to those imposed on green abalone. Groups (n = 4–8) of cultured juvenile pink abalone 2–4 cm held at 29.8°C for 48 h all survived. Those held for 48 h at 31.5°C died. Observations on groups held at 30–31°C recorded some mortality. By interpolation, an LD_{50} (48 h) of 30.3°C is defined for juvenile *H. corrugata*.

Observations on Extracted Sabellids

To examine the effect of short-term exposure of the polychaetes to temperatures above 30°C, individual sabellid larvae and immature and adult stages were placed by pipette or in small fragments of infested shell in a series of tubes in a constant temperature block. At intervals of 30 min (35–36°C) to 2 h (30–31°C), contents of tubes were examined under a high-power dissection microscope. At the highest temperatures, sabellids quickly became lethargic and displayed stress symptoms. Irreversible effects occurred within 30 min at 36°C. Comparable conditions occurred at 1–2 h at 33–34°C and at 2–6 h in the range 30–32°C (Table 2).

Treatment of Broodstock

Five groups (n = 20–30) of young adult green abalone (5–15 cm) subjected to heat treatment at 28.5–28.9°C for 72 h were placed in a broodstock maintenance tank for spawning at a later date. A first attempt to induce spawning in these animals 1 mo after heat treatment yielded active and apparently viable sperm, but ova were distinctly abnormal. Zygotes displayed atypical development, and larvae did not survive. A second spawn induction 3 mo after treatment was successful: larvae were entirely normal, and postlarvae completed metamorphosis with development progressing through juvenile stages.

TABLE 2.

Observations on extracted sabellids exposed to temperatures above 30°C.

Temperature Range (°C)	No. of Observations (Total Worms)	Hours							
		0.5	1	2	3	4	5	6	7
30.0–30.9	5 (24)			2		3	3		4
31.0–31.9	5 (20)		2	2	3			4	
32.0–32.9	5 (22)	1	2	2		3	4		
33.0–33.9	5 (20)	2	3		4	4			
34.0–34.9	5 (18)	3	4	4					
35.0–35.9	5 (16)	3	4						
36.0–36.9	5 (18)	4	4						

Scores as defined in Table 1.

Acclimation Effects

Observations during fall and winter months when ambient water temperature was in the range 14–18°C indicated that cold-acclimated abalone were more sensitive to temperatures above 29°C. Survival of *H. fulgens* and *H. corrugata* subjected to heat treatments in the range 28–30°C was 80–90% (48 h) during late fall, winter, and early spring, but was 95–100% during the warm season. Upper thermal tolerance limits appeared to be lowered by 1–2°C when compared with summer results (ambient temperature range, 20–24°C). Drawing on data gained in this study, upper limits (in terms of LD_{50}, 48 h) for green and pink abalone during the cold season appeared to be approximately 30°C for *H. fulgens* and 29°C for *H. corrugata*. The subject of thermal acclimation will be explored in our laboratory throughout the next year.

No remarkable change in sensitivity of the sabellid with season was detected. Its LD_{50}, 48-h limit is indicated to be 28.3 ± 0.2°C.

Monitoring for the Effectiveness of Heat Treatments

Treated abalone were routinely placed after heat exposure into a series of isolated tanks provided optimally circulated seawater at ambient temperatures and fed the standard dietary element, the kelp *Egregia menziesii* Areschoug, 1876. These abalone, observed over a period of 1–2 mo, resumed normal growth and thenceforth displayed no further symptoms of active sabellid infestation (Fig. 2). Long-term observations on shells of formerly treated abalone involved examination of many hundreds of individuals in raceway and suspended barrel culture units at the Carlsbad Aquafarm. Throughout the full 18-mo study, not a single incidence of active (live) sabellid infestation was found in abalone that had been subjected to thorough heat treatment at the dosages defined in this report. This is considered to constitute unequivocal evidence supporting the success of the heat treatments in eliminating eggs as well as larvae and adults of this sabellid.

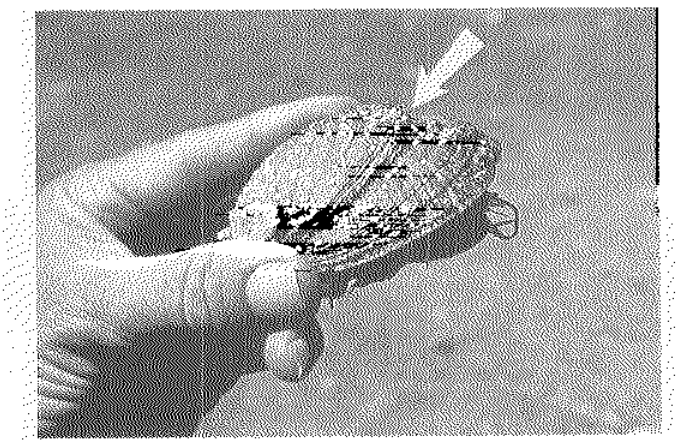
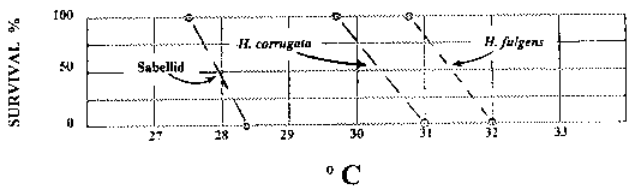


Figure 2. Heat-treated sabellid-infested *H. fulgens* with signature of recovery growth. The arrow marks the point of heaviest sabellid infestation at the time of heat treatment. Note crowding of growth lines and "downward" growth of shell during infestation. Recovery of normal growth was evident within 1 wk after treatment. Subsequent growth (18 mm, marginal increment) occurred over a 3-mo period (spring 1997).



ESTIMATED LD $_{50}$ (48 hr) FOR SABELLID AND ABALONES

Figure 3. Estimated LD_{50} (48 h) for the sabellid polychaete and two species of abalones. Upper thermal limits for abalone from data on summer-acclimated animals.

DISCUSSION

Infestation by the introduced sabellid polychaete has reached epidemic proportions in several red abalone culture facilities in California. Efforts to isolate the ectoparasite within the aquaculture system have been costly but essential to curb further spread and regain the level of productivity necessary to support the enterprise. New regulations by the California Department of Fish and Game pertaining to this problem require that those facilities affected by the sabellid dispose of infested stock and sterilize tanks and water-conducting elements (pumps, water lines, filters, etc.) Furthermore, to prevent establishment of the worm among native biota, effluent lines and discharge areas must be treated to exterminate any remaining sabellids.

It was the goal of this study to develop a means to control this sabellid species by exceeding its thermal tolerance limits while maintaining temperatures still within a thermal range compatible with normal health of these abalone species. It was considered of primary importance to define the fully effective levels for thermal exposure for destruction of the sabellid (adults, larvae, and eggs) and yet produce the least mortality to the abalone. Data were gathered for exposures to produce a complete kill (100% mortality) of the polychaetes.

The upper thermal physiological limit (expressed as exposure for 48 h) for this species of sabellid polychaete appears to fall between 28.0 and 28.5°C. This is about 3°C below the limit for *H. fulgens* and about 2°C below that for *H. corrugata* (Fig. 3). Although close, the tolerance limits for the polychaete are exceeded before those for these two species of abalones. Accordingly, thermal exposure at levels sublethal for green and pink abalones can be used to selectively destroy the sabellids when infesting these abalones. This approach would not be appropriate for treatment of sabellid infestations in cold water abalone such as *H. rufescens* Swainson, 1822, which are injured by water temperatures above 25°C if prolonged over several hours. However, application of the method to control sabellid infestations in tropical abalone species in culture (e.g., *Haliotis asinina* Linnaeus, 1758 and *Haliotis diversicolor supertexta* Lischke, 1870) should be highly effective.

Heat treatments carried out during warm water periods (May to October) produced significantly lower mortality at 29–30°C in both abalone species than were found during the cold water months (November to April). This result may be attributed to elevated temperature acclimation in *H. fulgens* and *H. corrugata*. However, it should be stated that as the supply of sabellid-infested stock became exhausted by midspring 1997, abalone subjected to thermal tolerance runs to establish or confirm upper lethal limits were normal and healthy specimens. The possibility remains that physiological stress resulting from sabellid infestation may in some manner affect thermal tolerance by the abalone. Lower survival rates found for *H. fulgens* and *H. corrugata* given exposures of 24–48 h to 28–29°C may be an effect of both low temperature acclimation and high sabellid infestation levels. Resolution of this problem will require additional research.

CONCLUSIONS

This shell-infesting polychaete may be controlled by exposure to seawater warmed to 28–29°C for a period of 48 h. It is recommended that in practice an additional half-day be given this treatment period as a margin of safety. The interval may be reduced as yet higher temperatures are used. However, exposure of only a few hours to temperatures above 32°C will adversely affect both green and pink abalones.

Observations on thermal limits over short exposure intervals for the sabellid worm indicated that 1 h at 35°C or 2 h at 33°C were lethal. Pink and green abalone juveniles, however, have not survived these exposures. Sensitivity of abalone to elevated temperatures appears to increase during cold water months. I did not find evidence for temperature acclimation in sabellids. It is recommended that thermal treatment for afflicted abalone during summer be maintained at 29–30°C (at least 48 h), and for winter at 27–28°C (at least 48 h), to effect a 100% kill of sabellids but produce the least stressful conditions for the abalone. The described thermal treatment is fully effective for control of this sabellid in infested green and pink abalones and other relatively warm water species, but it is an inappropriate therapy for use with cold water abalones such as *Haliotis rufescens*.

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STUDIES ON THE PUSTULE DISEASE OF ABALONE (*HALIOTIS DISCUS HANNAI* INO) ON THE DALIAN COAST

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ABSTRACT Since first observed in the summer of 1993, a serious pustule disease has spread among several abalone hatcheries in the Dalian area. It affects different growth stages of the abalone. Mortality has been as high as 50–60%. Three strains of bacteria (D, T, and N) were obtained, isolated, and purified from the Aquacultural Company of Dalian (D), the Aquacultural Company of Precious Sea Food of the Pacific Ocean (T), and the Dalian New Harbor Aquacultural Company (N). Observation by electron microscopy showed that the three strains were all short rod bacteria with a single polar flagellum. They all grew in 1% tryptone water in the ranges, 15–42°C; salinity, 0–70‰; and pH, 5.5–11. Physiological and biochemical analyses gave the same result as in an earlier study by the authors. The bacteriophage of the D strain, which was isolated by the authors (reported elsewhere) could propagate and replicate on the N and T strains. Thus, the three strains are concluded to belong to the same species of bacteria, one earlier identified as *Vibrio fluvialis*-II. Results of experimental trials indicated that the pustule disease is transmitted through lesions in the foot. Infection quickly followed intramuscular injection. Infection did not result when bacteria were included in the food or when present in the surrounding seawater.

KEY WORDS: *Haliotis discus hannai*, pustule disease, *Vibrio fluvialis*-II

INTRODUCTION

In September 1993, both wild and cultured abalone (3–8 cm shell length) held at the Dalian Aquacultural Company were found to have an infection that has become known as the pustule disease. Mortality has exceeded 60%. We found only one strain of bacteria by isolation (Liu et al. 1995) and identified the pathogen at the Institute of Microbiology, Chinese Academy of Sciences, as *Vibrio fluvialis*-II (a second type of *V. fluvialis*). In 1994 and 1995, many companies along the Dalian coast became infected with this disease and suffered substantial economic hardships. Although the new cases of abalone pustule disease have the same clinical symptoms as those from the previous case (Li et al. 1997c), the three strains of bacteria recently isolated are now resistant to commonly used antibiotics and have different sensitivities to a series of antibiotics (Li et al. 1996). In an effort to determine whether the causative bacterium has become resistant to treatment or the disease has multiple causes, we have designed experiments to aid in confirmation of the identity of the bacterial strains. Furthermore, we needed to demonstrate transmissibility of the disease by these strains. We have carried out extensive studies concerning diagnosis, prevention, and control of this disease (Li et al. 1996, Li et al. 1997a, Li et al. 1997b). Another "pustule disease" has been reported in abalone in South Australia (Lester et al. 1981, Lester 1986, Lester et al. 1990, Goggin et al. 1995), but in that instance, the disease was caused by an apicomplexan protist parasite, *Perkinsus* sp., and the clinical signs involved yellow pustules, not white pustules.

METHODS

Animals

Both healthy and diseased abalone (320 specimens, 3–8 cm shell length) were obtained from the Dalian Aquacultural Company (D), the Aquacultural Company of Precious Sea Food of the Pacific Ocean (T), and the Dalian New Harbor Aquacultural Company (N). Abalone were held in 10-L aquaria and received natural seawater filtered by sand and maintained at 20°C. Abalone were fed daily with an artificial diet prepared by the authors.

Bacterial Isolation

Hemolymph was withdrawn by syringe from the diseased abalones' hearts which had been washed with sterile 2% physiological saline 3 or 4 times, and then washed with 70% alcohol. In addition, pieces of foot (healthy and diseased), mantle and digestive tissues were excised, rinsed in sterile 2% saline and homogenized. The homogenates were diluted 10-fold before inoculation on a seawater-tryptone-beef broth-agar medium (SWTBA medium, chemical reagent store of Dalian). Plates were incubated at 24–37°C for 2–4 days. The most prevalent colony types were subcultured in beef broth and tryptone liquid medium and lyophilized for storage. All media were augmented with 2% NaCl.

Characterization of Bacterial Growth

In order to evaluate the influence of temperature (15–42°C), pH (6–11), and salinity (0–7% NaCl) on growth of the bacterium, the isolate was cultured in 1% tryptone broth ±2% NaCl for 24 h.

Bacterial growth was estimated by temporal comparisons of absorbance readings at 560 nm (A_{560}) (Nie et al. 1995).

Experimental Transmission of the Three Strains

The following experiments were conducted to determine the mode of infection and infectivity of the three strains. Experimental and control groups (A and B) were held separately in 10-L tanks (10 or 12 abalone/tank) supplied with filtered seawater as described above.

Transmission by Inoculation

One hundred eighty abalone, divided into three groups for the three strains and five subgroups for each strain, were injected intramuscularly at one site on the foot with a total volume of 0.1 mL of physiological saline containing 2.0×10^1, 2.0×10^2, 2.0×10^3, 2.0×10^4, or 2.0×10^5 of the three strains of bacterial cells, respectively. The control group (20 abalone) was injected with the same volume of sterilized seawater. Injected groups of abalone were placed in 10-L seawater tanks. The abalone were examined everyday, and dead individuals were removed, examined, and recorded. The seawater in each tank was changed daily (Hervio et al. 1996).

Transmission Through Cuts or Lesions

Thirty abalone (divided into three groups) were cut (1 mm deep, 3 mm long) at five or more sites on the foot. The wounded abalone were placed by group in a seawater tank containing about 2×10^5 cells/mL in a suspension of tested bacteria. The suspension of bacteria was added daily after a seawater change and maintained for 7 days. The incubation conditions and examinations were similar to those used for the injection tests. Replicate tanks holding groups of abalone with cuts on the foot but without introduction of the bacteria suspension were set up for these experiments as control groups.

Transmission by Diet

Thirty abalone (divided into three groups) were placed in three tanks containing filtered seawater. The abalone were fed daily with an artificial diet infused by 2×10^7 bacterial cells/mL (D, N, and T strains). Maintenance procedures were similar to those used for the above tests. The control group was fed the standard diet without infusion of bacteria.

Transmission by Bath Exposure

Thirty healthy abalone (divided into three groups) were placed in three seawater tanks containing 2.0×10^5 cells/mL in the bacteria suspension and maintained for 7 days. The incubation conditions, and the examination and maintenance procedures, were similar to the other experiments. Ten abalone were placed in a seawater tank without the bacteria as a control group.

The bacterial suspension was diluted with sterilized seawater until there were approximately 10^5 cells/mL, as determined with a hemocytometer. Each day, the abalone were examined with a dissecting microscope. Any moribund abalone were removed, and their foot tissues were examined for pathogenic bacteria with both a light microscope and a transmission electron microscope. After the abalone were examined, the seawater in each tank was changed. Monitoring of abalone in the various experiments was continued for at least 30 days.

RESULTS

Isolation of V. fluvialis*-II*

The isolation procedures indicated that using the SWTBA medium was the best and simplest method for isolating large numbers of viable and infective bacteria. Early in the infection period, there were no obvious pustules on the foot of the abalone; the foot tissue was the best location for isolating the bacteria. After 1 mo or more, we could isolate the *V. fluvialis*-II from any tissue of diseased abalone. The isolation of *V. fluvialis*-II has allowed us to conduct preliminary studies on the mode of transmission, other analyses, and maintenance of the bacteria in the laboratory. The bacteria isolated from the abalone taken from D company were marked as "D strain"; in the same way, the other two strains were designated as the "T strain" and the "N strain."

Characterization of Bacterial Growth

Size

The mean size of the three strains cultured in SWTBA medium remained almost the same during growth. Formalin fixation did not have a measurable effect on the morphology of the cultured *V. fluvialis*-II. A sample of 100 specimens from each strain had measurements of 0.6–0.7 × 1.2–2.5 μm.

Temperature

Maximum growth of the three strains, as indicated by absorbance (A_{560}) after culture overnight, occurred at an incubation temperature of 37°C (Fig. 1). Although growth was slower at 30°C, the absorbance was only slightly less than that at 37°C. *V. fluvialis*-II grew well at temperatures above 15°C, but growth was substantially reduced at 40–42°C, and virtually halted above 43°C.

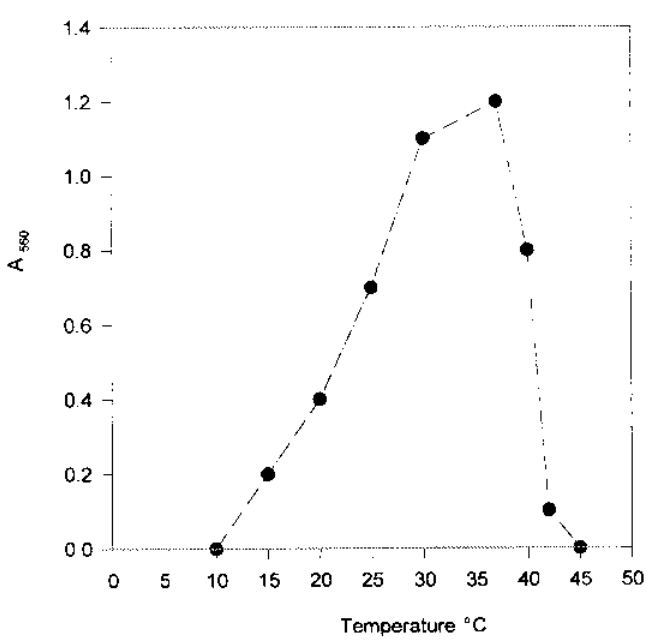

Figure 1. The effect of temperature on bacterial growth (light absorbance in a 5-cm cuvette at 560 nm).

Salinity

After overnight incubation at 37°C, cultures of the three strains in SWTBA medium made up with 20–30‰ seawater yielded the best growth (Fig. 2). There was a reduction in growth in SWTBA cultures made up with 10 and 70‰ seawater, and growth was not measurable in the cultures made up with 0‰ NaCl.

pH

V. fluvialis-II grew well at 37°C in SWTBA cultures, making the medium neutral or slightly alkaline (Fig. 3). The cultures remained in a condition of low growth when the pH value of the medium was <6 and >11. Growth was always prolific while the medium was alkaline (pH 6–11). The cultures showed little or no growth when the medium became acidic.

Mode of Transmission of V. fluvialis-*II*

Transmission of infection by *V. fluvialis*-II was tested by several approaches. The vital and infective bacterium *V. fluvialis*-II could be demonstrated throughout the year in abalone in the laboratory by exposing wounded abalone to water containing the bacterial suspension or by inoculating abalone intramuscularly with the isolated bacteria.

Transmission by Inoculation

In the inoculation trials, the prevalence of the infection of the three strains ranged between 50 and 100%. From 50 to 100% of the inoculated abalone were dead within 7 days. The abalone exposed to the highest bacteria concentrations (2.0×10^4 and 2.0×10^5 cells/mL) became heavily infected and died early without developing pustules. Abalone exposed to lower bacterial concentrations (2.0×10 cells/mL) were still alive after 1 y, and the obvious pustules occurred in the foot of inoculated abalone at 6 mo. In these trials, the mortality of abalone in three groups, inoculated with three strains, was the same, but the survival time of the abalone did differ. That may be the result of differences in the viability and infectiousness of the bacteria present in each inoculation. It is also possible that the susceptibility of individual abalone to infection with *V. fluvialis*-II may be different. The mortality of the control group was 0%.

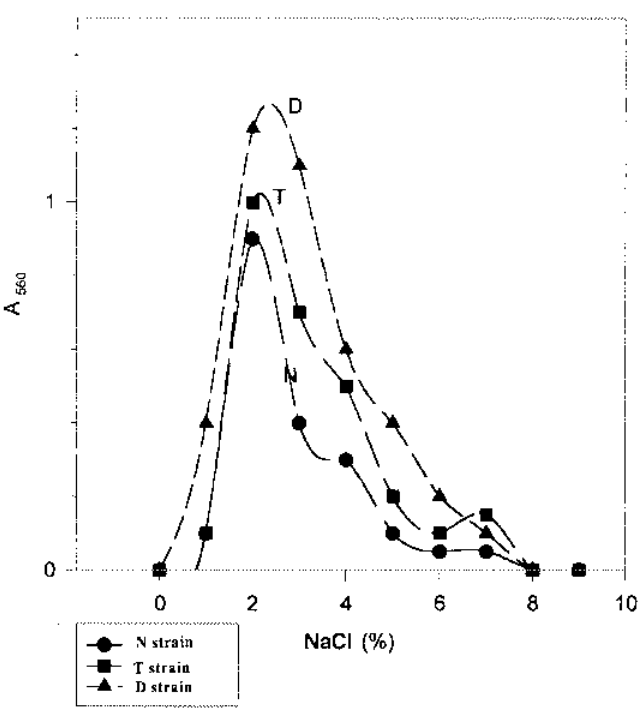

Figure 2. The effect of salinity on bacterial growth.

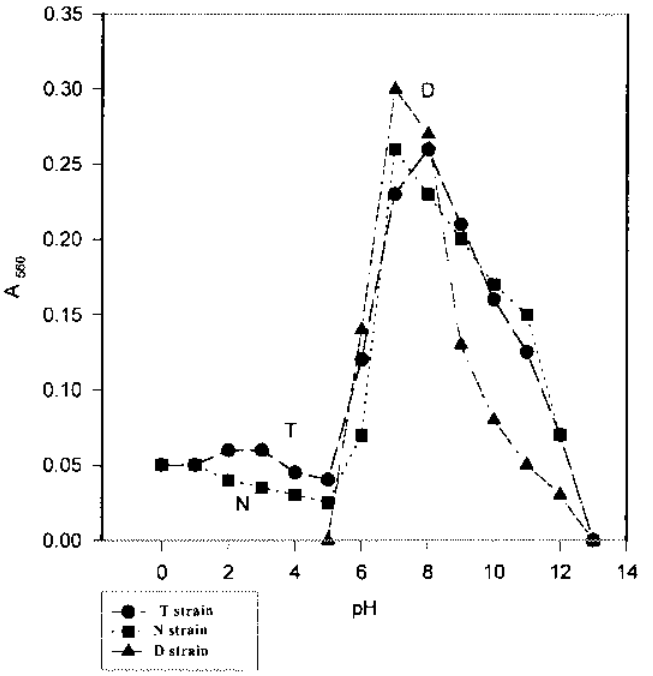

Figure 3. The effect of pH on bacterial growth.

Transmission Through Wounds or Lesions

The results of this experiment are similar to the inoculation result. Abalone with minor cuts on the foot showed clinical signs of the disease. The low intensity of infection was similar to that observed in naturally infected abalone collected from the field in spring. The prevalence of infection at 20°C was initially low (30% at 7 days), but became higher when incubation was prolonged (60–100% at 6 mo). Prevalence (60–100%) was comparable to infection rates (60%) reported for naturally infected beach-cultured abalone on the Dalian Coast (Li et al. 1996). The mortality of abalone in the control group was 0%.

Transmission by Diet

In this trial, the mortality of test and control group abalone was 0%, and the results indicated that the infection of pustule disease was not introduced by diet. Possibly *V. fluvialis*-II cannot invade the tissues of the digestive system.

Transmission by Bath Exposure

The prevalence of infection in bath-exposed abalone held at 20°C was 0%, even though the time of incubation was extended to 6 mo. This indicated that *V. fluvialis*-II cannot invade the body of healthy abalone to manifest the pustule disease.

DISCUSSION

All of the abalone were from D, T, and N Companies. As such, these samples of abalone had been cultured in different ecological

environments, on different scales of culture, and by different culture methods.

The D company is situated near Dalian city, has cultured abalone for many years, and uses seawater that is often polluted. The abalone with pustules included juvenile hatchery abalone and parental abalone that were both wild and cultured animals. This company often used chloramphenicol to control the bacteria disease.

The T company was established only 3 y ago. It is located in the Lu Shun Kou region and has relatively pristine seawater. The culture scale of this facility is the largest of the three companies included in this study. Most of the parental abalone were collected from Chang Hai County and Shandong Province. Because the company cultured over 20,000 parental abalone each year, many of these had pustules on the foot. The high density of culture leads to a high infection rate. Mortality was very high (80%). The gonads of most survivors seldom matured, and consequently, these individuals did not spawn. *V. fluvialis*-II was abundant in their gonads. This company often used chloramphenicol over a long period and at rather large doses.

The N company was founded in 1995; culture methods were either cage culture or pool culture on the beach where water is exchanged only when the tide is flowing. In the latter case, water was seldom changed thoroughly or frequently. Chances of infection among the abalone were great. Control of infection was difficult. Because abalone were reared under relatively open conditions, this company could not administer antibiotics effectively.

On average, there were more than three pustules on the foot of each abalone from the T company. As time went on and the temperature rose, the disease of the abalone became more and more serious. The bacteria gradually moved from the foot to the mantle, alimentary canal, liver, gonads, hemolymph, and other organs. When the bacteria had invaded almost all of the organs of the abalone, they refused to eat and gradually died. The 100 ppm chloramphenicol used earlier became ineffective. Cultured parental abalone failed to spawn. They developed swollen gonads and eventually died. Abalones reared in large cages and in high densities are difficult to monitor to remove the diseased and the dead ones. Infection spread, leading to high mortality. All in all, the prevention and the treatment of the pustule disease have become very important concerns.

The results of biochemical studies, growth characterization, and transmission analysis indicate that the pustule disease of abalone on the Dalian Coast is being caused by the same bacterium, *V. fluvialis*-II. The shorter incubation period and the greater prevalence and intensity of infection in inoculated abalone suggest that the number of bacteria administered to the abalone by inoculation was greater than the number of bacteria that invade the tissues via lesions. Although infection by inoculating is not the natural avenue for *V. fluvialis*-II invasion of host tissues, injection of the pathogen could be used to investigate further the manifestation of the disease, determine its host specificity of the bacterium, and possibly allow for its genetic analysis.

We have referred to the three strains as D, N, and T bacteria. Samples of abalone from these three hatcheries have in common the following features. There were pustules on their feet that contained milk-white pus. There was only one kind of bacterium in the pus. Experiments on the abalone infected with the three strains showed that all of the three are infectious. The three strains were identified at the Institute of Microbiology, Chinese Academy of Sciences, and were reported as *V. fluvialis*-II.

In our earlier studies, we tested only the transmission of the disease by inoculation, lesions, and diet. The physiological and biochemical analyses were carried out at the Institute of Microbiology, Chinese Academy of Sciences. All results of tests we used in this study correspond with our earlier work.

The sensitivity of *V. fluvialis*-II was determined by placing paper discs saturated with specific antibiotics on medium in plates holding the vibrio bacteria and then measuring the diameter of the ring formed by bacterial inhibition (Li et al. 1996). The results showed that different strains have different sensitivities to a series of antibiotics. The D strain was sensitive to ciprofloxacin, cotrimoxazole, furazo lidone, norfloxacin, chloramphenicol, gentamycin, and erythromycin. The N strain was sensitive to chloramphenicol, cotrimoxazole, ciprofloxacin, norfloxacin, gentamycin, and furazo lidone. The T strain was sensitive to norfloxacin, ciprofloxacin, gentamycin, penicillin, and erythromycin. The antibiotics listed above would appear to be better drugs for control of the pustule disease in these three hatcheries, respectively. We recommend that the hatcheries using these antibiotics should use them for short periods and alternately, or resident bacteria may develop resistance, as was the case with chloramphenicol use by the T Company.

The resistance of the bacteria to antibiotics is the result of use of these substances over a long period. The mechanism of resistance of *V. fluvialis*-II has been studied on a molecular level. The total DNA of the three strains was extracted and digested by restriction enzymes, *Eco*RI. Electrophoretic patterns revealed that the three strains had many different bands. It was suggested that the mechanism of resistance of the bacteria was related to gene mutation and that the antibiotics provided the necessary condition for the development of bacterial resistance (Li et al. 1996).

CONCLUSIONS

Comparative characterization of isolates of bacteria associated with pustules on the foot and epipodia of abalone from three aquaculture facilities on the Dalian coast show all cases of the disease to be the consequence of infection by one pathogen, *V. fluvialis*-II. Vibriosis has become rampant in these facilities and has also spread to wild populations along the local coast. Transmission occurred most rapidly through lesions cut in the foot of experimental abalone. The pustule disease was not transmitted through diet or normal contact with the surrounding water.

This malady has become epidemic and has spread despite antibiotic prophylaxis in routine treatment, largely with chloramphenicol at 100 ppm. Antibiotic-resistant strains of *V. fluvialis*-II now established at these aquaculture facilities have caused extensive economic loss. Alternative treatments (biological control) are now being sought.

From our results, we would recommend that the Dalian Coast abalone farms improve husbandry practices by providing improved conditions to abalone in culture. Seawater quality should be maintained by filtration and by ultraviolet sterilization. Water should be aerated constantly. Abalone should be fed fresh algae and held at reduced densities. Conditions should be provided to minimize abrasion to abalone foot tissue. Where appropriate, culture equipment should be cleaned and sterilized with HCl or potassium permangenate. New kinds of antibiotics should be used for short periods, and treatments should be changed to include other kinds of antibiotics. We expect that following this strategy will reduce the extent of infection by vibriosis on the Dalian Coast.

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SEASONALITY IN DIGESTIVE-GLAND SIZE AND METABOLISM IN RELATION TO REPRODUCTION IN *HALIOTIS KAMTSCHATKANA*

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ABSTRACT . A novel method of isolating digestive gland cells in abalone was used to provide information on the metabolic activity of this gland in *Haliotis kamtschatkana*. Activity, expressed as percent change in Vo_2 of isolated cells before and after the addition of glucose and amino acid substrates, was studied in relation to sex and to seasonal changes in gonad and digestive gland indices. At 3- to 4-mo intervals between May 1995 and July 1996, five collections of 10 adult abalone (equal sexes) were made from the West Coast of Vancouver Island, British Columbia. Each animal's live mass (without shell) was recorded, and its gonad was aspirated from the digestive gland into a known volume (and mass) of seawater. The digestive gland was sliced free of its attachment, weighed, and related to shell-less body mass as percent digestive gland index (DGI). The gonad live mass was determined from the mass of aspirated mix of gonad and seawater, and related to shell-less body mass to give a percent gonad index (GI). Digestive gland cells were prepared and maintained in a special buffer, and their Vo_2's measured in microrespirometers. There was no sex effect on seasonal DGI, but significant seasonal differences in DGIs correlated perfectly with metabolic activity of digestive-gland cells with glucose substrate. Thus, when digestive glands were largest relative to body size, metabolic activity of their cells was greatest. GIs were significantly higher for males than females. There was a significant seasonal effect on GIs, with values being high in springtime before spawning (April to May, 10–11%) and low in winter (December, 6%), but with statistical overlap between these and summer values (July to August, 7–8%). Metabolic response of the digestive gland cells was highest with glucose substrate (75% increase over presubstrate resting levels as compared with 4% for amino acid substrate), reflecting the carbohydrate-based metabolism of abalone.

KEY WORDS: *Haliotis kamtschatkana*, abalone, digestive gland, isolated cell suspension, metabolism, reproductive cycle

INTRODUCTION

Attempts to study the role of the digestive gland in the nutrition and growth of marine molluscs *in vivo* is hampered by slow growth rates of the test animals, by difficulty in gaining access to the organ, and by multiplicity of function in digestion and energy metabolism of the gland. Added to this in abalone and other gastropods is the intimate juxtaposition of digestive gland and gonad, which tends to confound study of either organ separately. We have side-stepped some of these problems by examining digestive gland function of abalones *in vitro*, using an isolated-cell suspension similar to that used with mammals (Buck et al., 1992) and decapod crustaceans (Lallier and Walsh 1992, Toullec et al. 1992). In this approach, which we believe to be unique for molluscs, the digestive gland is freed from its attachment to the gonad and the rest of the body, and the cells separated and maintained in suspension in a buffer solution formulated especially for this purpose. The cell suspension can then be used as a bioassay system to monitor the metabolic activity of the digestive gland in relation to gametogenetic and other seasonal events and to study the effects of different substrates or other conditions on energy metabolism of the gland.

Reproductive cycles in temperate-latitude abalone generally follow the pattern: gametogenesis during winter and early spring, maximally ripe gonads in late spring and early summer, and spawning during summer and early autumn (Boolootian et al. 1962, Webber and Giese 1969, Poore 1973, Shepherd and Laws 1974, Hayashi 1980, Shepherd and Hearn 1983). However, considerable variation in this cycle may exist within and between species and also geographically and temporally (Boolootian et al. 1962, Poore 1973, Shepherd and Laws 1974, Giorgi and DeMartini 1977, Sloan and Breen 1988). Although *Haliotis kamtschatkana* generally has the highest gonad indices (GIs) in late spring to early summer, with spawnings in mid- to late summer (Breen and Adkins 1980), there is evidence in some populations of "dribble-type" spawning throughout the year (Emmett and Johnstone 1985).

The large foot muscle acts as a glycogen store for use during gametogenesis (Webber and Giese 1969, Webber 1970). Hayashi (1983) showed in *Haliotis tuberculata* that the glycogen content of the foot tissue decreases from 40% to 0 as gonad maturation proceeds, and Webber and Giese (1969) and Webber (1970) showed in *Haliotis cracherodii* a drop in the total dry mass of polysaccharide (= glycogen) in the foot from 23% to 6%, the lowest value coinciding with late-summer spawning. In *H. cracherodii*, as polysaccharides are drawn from foot stores, so their concentration increases significantly in the gonad, somewhat more in males than females (males: 5–9% dry mass increase; females: 4.5–7% dry mass increase; Webber 1970). Because the digestive gland is the chief organ involved in these energy transformations in abalone, its greatest relative size and metabolic activity would be expected concomitantly with active gametogenetic processes.

This study examines the seasonal effects of glucose and amino acid substrates on metabolism of the digestive gland cells and correlates this metabolic activity with the relative size of the gonad and digestive gland. The specific hypotheses to be tested are: (1) that glucose substrate will be metabolically used over amino acid substrate, as suggested by the carbohydrate-based nutrition of abalone, and (2) that metabolic activity, as measured by oxygen consumption of isolated cells, will correlate seasonally with the reproductive cycle. Thus, we predict that the digestive gland will be largest and most metabolically active when gametogenetic activity is greatest.

MATERIALS AND METHODS

At 3- to 4-mo intervals between May 1995 and July 1996, five collections of 10 adult *H. kamtschatkana* of equal sex ratio were made from the West Coast of Vancouver Island, British-Columbia, near the Bamfield Marine Station. During the few days between collection and their use in experiments, the animals were kept in a recirculating seawater system (12°C, 32‰) under ambient light conditions and fed on kelp.

Estimates of gonad and digestive gland masses were made as follows. An abalone was kept on ice for 1 h; then, its shell was removed and its live tissue mass was measured. The membrane enclosing the gonad was slit in several places with fine scissors. The exposed gonadal tissue was aspirated from the surface of the digestive gland with a suction tube connected to a vacuum pump and collected into a known volume (and mass) of seawater. Virtually all of the gonad could be collected in this way, leaving the digestive gland intact. We believe this method to give a much more accurate measure of GI than the area-slice method commonly used with abalone (Boolootian et al. 1962, Poore 1973, Hayashi 1980, and others). The combined mass of the aspirated gonad and seawater, after subtracting the mass of the latter, yielded the fresh mass of gonad. The digestive gland was cut from the animal, weighed, and immersed immediately in chilled buffer formulated specially for the maintenance of abalone digestive gland cells (Taylor and Carefoot unpubl.). The digestive gland and gonad live masses, expressed as percentages of original total body mass without shell, respectively, gave digestive gland index (DGI) and GI for each animal.

Details of the buffer formulation and preparation of the isolated cell suspensions can be found in Taylor and Carefoot (unpubl.). However, briefly, the digestive gland was minced apart with razor blades to free the individual cells and the resulting cell-buffer slurry was filtered through successively finer nylon meshes (to 73 μm pore size), then washed, and centrifuged three times. After the final spin, the cells were resuspended in fresh buffer and rested overnight at 3°C. The overnight buffer differed from the preparation buffer in its lack of glucose and bovine serum albumin. The next morning, a resuspension of the cells in the second buffer provided the stock material for use in the bioassay metabolism experiments. Trypan blue staining tests of these suspensions (Buck et al. 1992) showed +99% viability of the digestive gland cells.

Tests of metabolic response of the cell suspension were done in a 2-mL volume Gilson oxycell (Middleton, WI) equipped with a Clark type O_2 electrode (Yellow Springs, OH). A known mass of cells in fresh buffer was allowed to equilibrate at 15°C for 1 h and was then placed in the oxycell. Baseline Vo_2 of the cells at 15°C was measured over an initial 15-min period, then a nutrient substrate was added, and Vo_2 was measured for an additional 5 min. The difference in before and after rates, expressed as μg min $^{-1}$g live cells^{-1}, gave the measure of metabolic response to a certain substrate. The two substrates tested were glucose and a mixture of amino acids, administered in 50-μL volumes (glucose, 100 mM; amino acids, saturated solution of casein amino acids: ICN, Inc.). Each test of a substrate was done in triplicate.

RESULTS

Seasonal changes in DGIs and GIs are shown in Figure 1. Because no sex effect on digestive gland size was evident ($F[1,49] = 0.53$, $p = 0.47$, analysis of variance [ANOVA]), the values presented for this organ represent males and females combined. A strong seasonal effect on DGI ($F[4,49] = 9.59$, $p < 0.001$, ANOVA) resolved into a generally low value in winter (December 1995; 7.4%) and higher values in summer (August 1995 and July 1996, 9.4 and 12.2%, respectively), although some statistical overlap was present between these and spring values ($p < 0.05$, Newman-Keuls Multiple Comparison Tests). GI also showed a strong seasonal effect ($F[4,35] = 4.41$, $p = 0.005$, ANOVA), with lowest values being found in spawned-out animals in late summer through winter (July, August, December, 6.2–8.6%) and highest values in spring before spawning (April to May, 10.0–11.3%; $p < 0.005$, Neuman-Keuls Tests). Figure 1a also shows a strong sex effect on GI ($F[1,49] = 9.72$, $p = 0.004$, ANOVA). Males with mean GIs of 10% were significantly higher than females with mean GIs of 7% ($p < 0.05$, Neuman-Keuls Tests). There was no significant correlation, either positive or negative, of DGI and GI values over time ($r_s < 0.3$, $p > 0.20$, Spearman Rank Correlation Analysis).

Figure 2 shows the seasonal effect of different substrates on the metabolism of isolated cell suspensions of digestive glands of *H. kamtschatkana*. Sex had a strong effect on the metabolic response of the cells ($F[1,99] = 23.9$, $p < 0.0001$), with females exhibiting the greatest response (overall increases of 78 and 54 μg of O_2 min^{-1} g cells^{-1} over resting rates, respectively, for females and males; $p < 0.05$, Neuman-Keuls Tests). The cells responded strongly to glucose substrate (75 μg of O_2 min^{-1} g cells^{-1} overall increase over resting rate) and only weakly to the amino acid mix

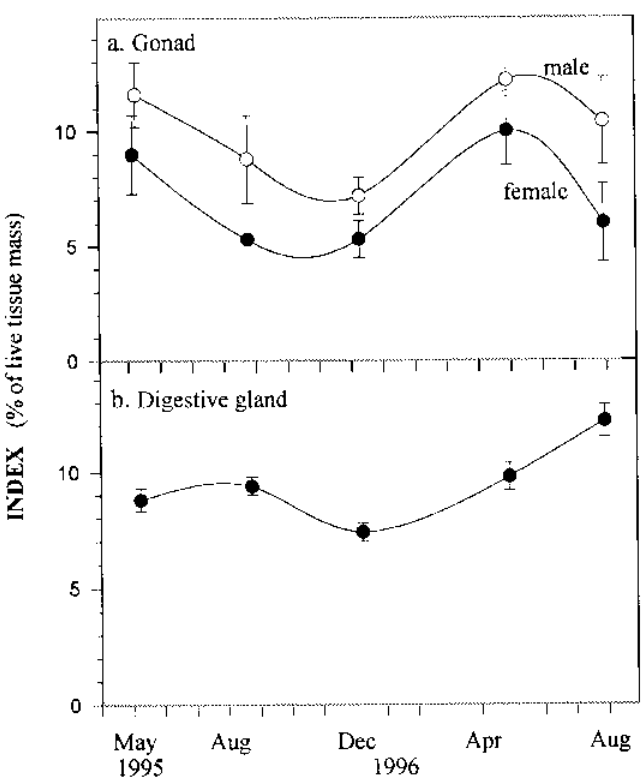

Figure 1. Change in (a) GIs and (b) DGIs over the period May 1995 to August 1996 in *H. kamtschatkana*. Values represent means ± SE of 5 individuals for gonad and 10 individuals for digestive gland. The error bars for August 1995 female GI are hidden within the dimension of the point.

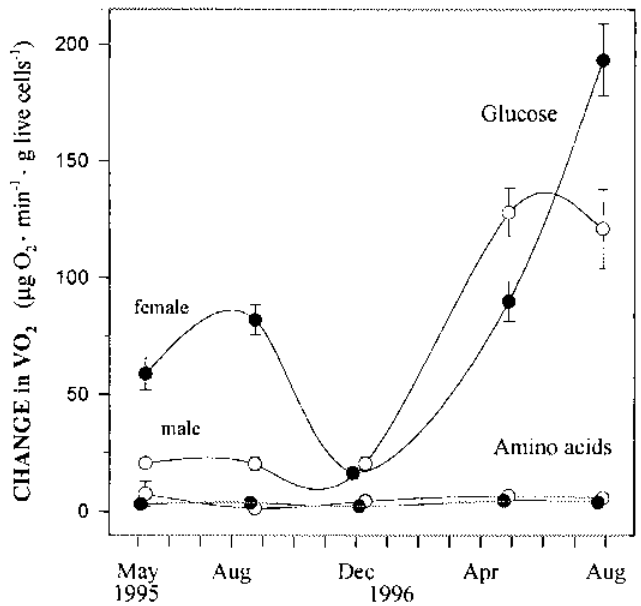

Figure 2. Change in VO_2 of isolated cell suspensions of *H. kamtschatkana* digestive glands from baseline levels when given either glucose or an amino acid mix as substrate. Closed circle, female; open circle, male. Values represent means ± SE of five individuals. Error bars for several of the points are too small to extend beyond the dimension of the points.

(4 μg of O_2 min^{-1} g cells^{-1} increase), with the difference being highly significant ($F[1,99] = 48.2$, $p < 0.0001$, ANOVA). The cells were seasonally least responsive in winter and most responsive in spring and summer ($F[4,99] = 87.0$, $p < 0.0001$, ANOVA), especially spring and summer 1996. These last values formed a statistically segregated grouping from both the winter 1995 values and the spring/summer 1995 values (Fig. 2, $p < 0.05$, Neuman-Keuls Tests).

A perfect correlation was demonstrated between size of digestive gland (DGI) and metabolic response of the cells to glucose ($r_s = 1.0$, Spearman Rank Correlation Analysis). Thus, when digestive glands were smallest (in winter 1995), metabolic response was least, and when the digestive glands were largest (in summer 1996), metabolic response was greatest.

DISCUSSION

GIs in reproductively mature abalone range generally from 12 to 20% of the soft body mass (Webber and Giese 1969, Webber 1970, this study). Although some species spawn more or less completely, leading to indices of essentially zero (e.g., *Haliotis cyclobates* and *Haliotis laevigata* in Australia: Shepherd and Laws 1974), others show less dramatic or no significant seasonal changes (e.g., *Haliotis rufescens* in California: Young and DeMartini 1970, *Haliotis ruber* in Australia: Shepherd and Laws 1974), and GI for these species is of little use in monitoring gametogenetic or spawning activity (Young and DeMartini 1970). In this study on *H. kamtschatkana*, GIs for both males and females showed summer lows, corresponding with spawning, but values remained above about 6%. Because seasonal highs were only 9 and 12% for females and males, respectively, spawning was neither complete nor of high magnitude during 1995 to 1996 for this species.

The greater metabolic responsiveness of female digestive gland cells as compared with those of males in this study cannot be explained simply on the basis of greater gametogenetic growth in females, because gonads in each sex increased by about the same amount during spring/summer 1996. However, Webber (1970) has shown that immediately before spawning the female gonad in *H. cracherodii* consists of about 35% dry mass lipids, as compared with only about 10% for males. In *H. kamtschatkana*, this is reflected in a higher caloric content of female gonads as compared with males (Donovan and Carefoot unpubl.). Lipids represent a much more energetically costly substance to produce than either protein or carbohydrate, and this may at least partly explain the different metabolic activity of the digestive glands in the two sexes.

Our results did not show a clear relationship between digestive gland size and gonad size in *H. kamtschatkana*. Indeed, the few past studies in which DGIs and GIs were measured simultaneously offer somewhat varying views of their interrelationship. Thus, Boolootian et al. (1962) found a strong inverse correlation of digestive gland size with gonad size in *H. cracherodii* and a less evident but nonetheless reciprocal relationship in *H. rufescens*. Webber (1970) also showed DGIs and GIs to be inversely correlated in *H. cracherodii*, but to a much lesser extent than shown by the data of Boolootian et al., and we showed no relationship in *H. kamtschatkana*. Boolootian et al. (1962) interpreted their data for *H. cracherodii* to mean that the digestive gland stockpiles nutrients for gametogenesis, but this was later shown by Webber (1970) not to be the case for this same species, nor would it make sense based on our data for *H. kamtschatkana*. There are two possible explanations for these discrepancies. The first, proposed initially by Boolootian et al. (1962), suggests that the extent and timing of interrelationships of digestive gland and gonad are governed by the seasonal preciseness of gametogenesis and spawning in a species. Thus, *H. cracherodii*, with a distinct seasonal spawning cycle, would have a more marked interrelated cycling of digestive gland and gonad than *H. rufescens* or *H. kamtschatkana*, which exhibits much less distinct spawning cycles (this study, see also Young and DeMartini 1970). The second, less an explanation than a comment, reminds us that digestive gland size and activity will be related not only to gametogenesis, but also to other processes of nutrition and growth. In temperate-latitude species, especially, summer will be a time of optimal feeding and growth, with maximal elaboration of enzymes and allocation of energy for digestion, processing of nutrients, and functioning of transport systems and metabolic pathways involved in all growth, including gametogenesis. Garnering of glycogen and other nutrients required for later gametogenesis may actually be competitive with needs for somatic growth, including that of the shell. Thus, it may be necessary to factor in all growth and activity needs to get a clear picture of seasonal DGI and GI interrelationships in abalone.

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STUDIES ON POSTPRANDIAL CHANGES OF DIGESTIVE STATUS AND FREE AMINO ACIDS IN THE VISCERA OF *HALIOTIS DISCUS HANNAI* INO

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ABSTRACT Experiments were conducted to study the postprandial changes of digestive status and free amino acids (FAA) in the viscera of *Haliotis discus hannai*. A natural pigment in the algal diet, *Palmaria palmata*, served as an indicator to determine the digestive status of the abalone. The changing concentration of the pigment in the viscera indicates that very active digestion took place during the first 10 h and that the total food passage time was about 44 h for the abalone after a meal at 15°C water temperature. Visceral FAA levels were examined at 0, 4, 10, 16, 22, 28, 36, 44, 52, 62, 72, and 96 h after a meal. The mean level of free essential amino acids (FEAA) attained an obvious peak at 4 h. It increased gradually from 4 to 36 h and then maintained at a relatively constant level till 96 h postfeeding. The mean level of free nonessential amino acids (FNEAA) had a pattern of postprandial change similar to that of FEAA. However, greater variation and fluctuation in concentration were observed among the individual FNEAA than the individual FEAA. A significant correlation was established between essential amino acids in the dietary protein and the FEAA in the abalone viscera that was sampled at 4, 22, 28, 36, 44, 52, 62, and 72 h after feeding. The highest correlation coefficient was obtained at 36 h. Both the algal pigment and the FEAA in the viscera are effective indicators reflecting the digestive status of abalone after a meal.

KEY WORDS: abalone, *Haliotis discus hannai*, digestion, free amino acids, nutrition, mollusks

INTRODUCTION

Maintaining an efficient feeding frequency for optimal food intake by cultural animals is essential for a successful aquaculture operation. To design an appropriate feeding regimen, the properties of appetite, feeding, digestion, and absorption of cultural animals should be fully understood. A few approaches have been developed to study the food consumption, total food passage time, and digestive capacity of commercially important species (Talbot 1985, Smith 1989). These approaches may roughly be divided into two groups: direct and indirect measurements. For most species, indirect methods are usually more simple than the former. Natural pigments in diet or artificial colorants incorporated into diet are often used as indicators in indirect methods (e.g., Bayne et al. 1987, Dam and Peterson 1988, Lawrence et al. 1989, Johnston et al. 1994).

For abalone species, a few studies have been conducted to study their stomach contents, food preferences, digestive status, and digestive capacity (Tomita 1972, Yamasaki 1991, Wee et al. 1992, Maguire et al. 1994, Day and Cook 1995, Fleming 1995). Tomita (1972) fed *Haliotis discus hannai* with *Ulva pertusa* and then examined the stomach contents at 1-h intervals for 6 h under light microscopy. It was found that active digestion in the stomach took place from 3 to 6 h after feeding at 14.2°C. By 6 h after feeding, the ingested green alga was further broken down and liquefied. Afterwards, most contents in the stomach had moved into the intestine, although some undigested algal cells were still observed. On this basis, it may be inferred that gastric evacuation time (GET) is at least longer than 6 h for *H. discus hannai*, but no conclusion can be made for the total digestion time of a meal by this abalone, because digestion continues in the intestine (McLean 1970).

When determining the digestibility of *Haliotis rubra*, an Australia abalone, using Cr_2O_3 as a marker, Wee et al. (1992) reported that *H. rubra* took 7 days to void all digesta through the gut after a meal at 10–16.5°C, judging from fecal collection. More recently, Britz et al. (1996) investigated the digestive activity and gut evacuation time in the abalone *Haliotic midae* fed a formulated diet by examining the gut fullness. They found that the abalone processed the bulk of a meal within 24 h at 20°C.

These results reported by different authors were obtained under different conditions, by different methods and abalone species. Hence, it is difficult to compare them with each other. More studies need to be done to fully understand the digestive characteristics and total food passage time in the digestive tract of abalone.

Artificial colorants, radioactive tracers, and radiographic markers are often used as indicators in studying the consumption, digestion, and absorption of artificial foods by aquatic animals (Talbot and Higgins 1983, Mai et al. 1988, Storebakken and Austreng 1988, Lawrence et al. 1989, Johnston et al. 1994). However, when the consumption, digestion, and absorption of natural foods are investigated, natural pigments used as indicators have advantages over the artificial markers. Natural pigments are usually evenly and chemically bound with other dietary components, and they can synchronously move in the digestive tract with other dietary components. Especially when a natural pigment is also one of the digestible dietary components, such as protein, lipid, and carbohydrate, it will be an effective indicator to reflect the movement and digestive status of the ingested diet.

A red pigment in *Palmaria palmata*, a preferred natural feed of abalone (Mercer et al. 1993), can be precipitated by cold acid–acetone (Mai pers. obs.), indicating that the pigment is probably a protein. Additionally, the red color in the viscera of abalone fed with *P. palmata* gradually disappears with the time after feeding (Mai pers. obs.). This suggests that the red pigment can be digested by abalone and can be used as a good indicator for observing the digestive status of abalone after a meal. Hence, this experiment was designed to study the digestive status of *H. discus hannai* after feeding on *P. palmata*, using the natural pigment in the red alga as an indicator. The postprandial changes of free amino acids (FAA) in the viscera were observed to study the relationship between the changing pattern of the dietary pigment and that of the FAA.

MATERIALS AND METHODS

Feeding and Sampling

H. discus hannai of similar sizes (3.5 cm in shell length) were selected from a laboratory-hatched and reared population (n = 150). They were held in three circular air-powered upwelling columns (20 cm in diameter × 35 cm in height) that were provided with shelters of plastic rain water guttering and corrugated plastic plates. Water temperature was maintained at 15°C, and the columns were suspended in a 3-m^3 glass fiber tank. Photoperiod was maintained on a 12L:12D (6:00 am to 6 pm) cycle of natural light. Abalone was fasted for 5 days before being fed with the red alga, *P. palmata* for 2 h from 6 pm to 8 pm. Four animals were randomly collected from each column at each sampling time (0, 4, 10, 16, 22, 28, 36, 44, 52, 72, and 96 h) after the meal. All of the samples were stored below −20°C until analysis. About 100 g of *P. palmata* was sampled and freeze dried.

Extraction of Pigment and FAA

Freeze-dried *P. palmata* was ground into fine powder with a mortar and pestle. An aliquot weighing 100 mg was homogenized with 10 mL of distilled water for 10 min in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 20,000 rpm for 20 min. The supernatant was collected for the absorption spectrum scanning of the red pigment in *P. palmata*.

The frozen abalone was slightly thawed, and then its visceral mass was removed. All visceral tissues of the four animals collected from each column were pooled and homogenized as above. An aliquot weighing 1.0 g was further homogenized with 10 mL of distilled water for 10 min and then centrifuged in the same way as the alga. The supernatant from the unfed animals (0 h) was also used for absorption spectrum scanning, and those from other sampling times were used for absorbance analyses after an optimal absorption peak was selected.

A 200-mg aliquot of homogenized viscera was used for FAA extraction. Protein was precipitated by 1.2 mL of acid–acetone (Ozols 1990). The supernatant was collected, evaporated, and then redissolved in 0.5 mL of 50 mM $NaHCO_3$ (pH 8.1) for FAA analysis.

Determinations of Pigment and FAA in Visceral Tissues

To select a suitable absorbance wavelength for the pigment analysis, both supernatants of *P. palmata* and the visceral tissue of the abalone sampled at 0 h (unfed) were used for absorption spectrum scanning. The spectrum scanning was performed with an ultraviolet (UV)-vis spectrophotometer (Lambda 14) with a range of wavelength from 200.0 to 900.0 nm and an absorbance from 0.00 to 1.50.

Five hundred microliters of FAA supernatant was diluted with an equivalent volume of 2 mM dabsyl chloride freshly prepared in acetonitrile. Derivatization took place at 60°C for 10 min (Kamp 1990). The separation of dabsyl derivatives was achieved on RPLC-(8)-HPLC (column, 250 × 46 mm; particle size, 5 mm). Detection was accomplished with a UV-vis variable wavelength monitor set at 455 nm (Mai et al. 1994).

Statistical Analysis

Data were analyzed by one-way analysis of variance, Tukey test, and correlation where appropriate. Statistical analysis was performed with Systat (SYSTAT 1992).

RESULTS

Selection of Wavelength for Pigment Determination

The absorption spectra of supernatants of *P. palmata* and the viscera of unfed abalone are presented in Figure 1. The red pigment in *P. palmata* had three absorbance peaks, at 317, 500, and 557 nm. The pigment in the viscera of unfed abalone had only one obvious peak, at 293 nm. To avoid the influence of the background color in viscera, the 500-nm wavelength was used for the concentration estimation of algal pigment in abalone viscera.

The postprandial change of algal pigment level in the abalone viscera is shown in Figure 2. The absorbance of supernatant from the viscera of the unfed abalone (0 h) was the lowest (0.185). There was a sharp increase in absorbance after abalone were fed with *P. palmata*. The maximum absorbance was observed for the first sampling at 4 h after the algal meal. A sudden decrease was recorded during the period from 4 to 10 h after feeding. From then on, a short plateau in the level change of algal pigment appeared until 22 h postfeeding. Subsequently, there was a rapid decrease in algal pigment level in the abalone viscera from 22 to 36 h. Then, the absorbance gradually returned to the basal level by 44 h.

Postprandial Changes of FAA in Abalone Viscera

In Table 1, the visceral FAA levels before and at various intervals after feeding of the algal diet are shown. The values of free essential amino acids (FEAA) and free nonessential amino acids (FNEAA) are presented graphically in Figures 3 and 4, respectively. The mean level of FEAA showed an obvious peak at 4 h after feeding. It increased gradually from 10 to 36 h and then maintained at a relatively stable level until 96 h postfeeding. The total levels of FEAA at 36, 44, 62, and 96 h after feeding were not significantly different ($p < 0.05$). However, some fluctuations in the mean level of FEAA were observed during the period from 36 to 96 h.

There were rapid increases in most FEAA levels during the first 4 h postfeeding except for histidine, methionine, and tryptophan, which barely responded to feeding until 16 h after feeding. The levels of the FEAA that attained the peak at 4 h returned nearly to

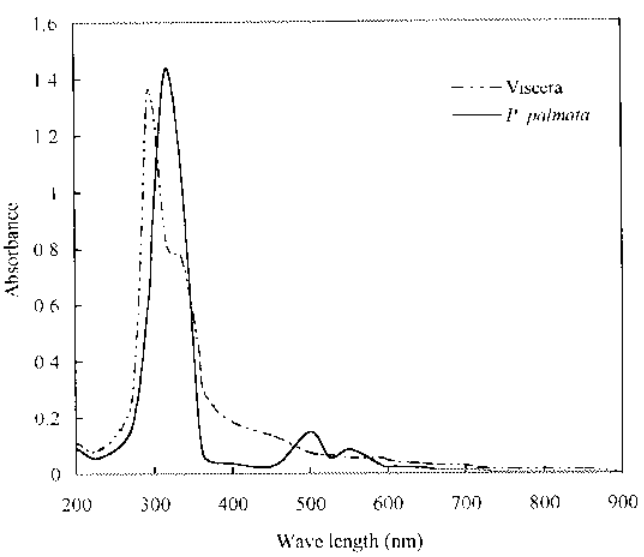

Figure 1. Absorption spectrum of the red pigment in *P. palmata* and the pigment in the viscera of *H. discus hannai* fasted for 5 days.

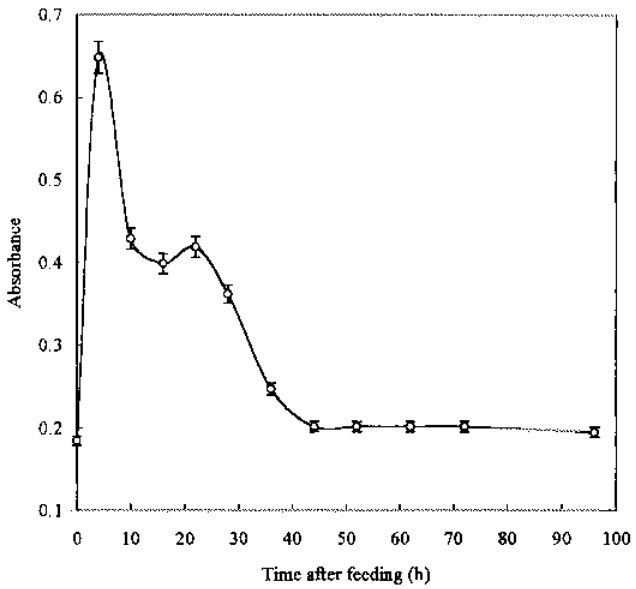

Figure 2. Postprandial change of algal pigment in the viscera of *H. discus hannai*. Vertical bars, standard error.

basal or prefeeding levels between 10 and 16 h postfeeding. Subsequently, all FEAA levels gradually increased up to 36 or 44 h (Arg and Val) and declined to a valley again by 52 h. From 52 to 62 h, the concentrations of all FEAA increased, except for arginine, which continued to decline until 72 h postfeeding. Decreases were observed for the concentrations of almost all FEAA from 62 to 72 h. Tryptophan, methionine, leucine, and phenylalanine continued to drop to the prefeeding levels by 96 h. The level of lysine slightly declined during the last 34 h, but it was still higher than the basal level. Meanwhile, obvious rises were recorded for the concentrations of valine, threonine, isoleucine, and arginine in the period from 72 to 96 h postfeeding, and their final concentrations were significantly higher than the basal levels. As a whole, the total level of all FEAA in viscera at 96 h postfeeding was significantly higher than the prefeeding level (Fig. 3).

The postprandial change pattern of the mean level of FNEAA in abalone viscera was largely similar to that of FEAA (Fig. 4). However, greater variation in the change patterns was observed among the individual FNEAA than individual FEAA. The abundant amino acids, Asx (aspartic acid plus asparagine), Glx (glutamic acid plus glutamine), alanine, and glycine showed greater fluctuations than other amino acids during the 96-h sampling period. The maximal level of FNEAA was attained by 96 h, when taurine was not taken into account. It was twice higher than that of prefeeding. Additionally, the total level of FNEAA was always higher than that of FEAA (Table 1).

It can be seen from Table 1 that taurine, the most abundant amino acid, had a very different change pattern compared with other amino acids during the 96-h period. Its maximal level was measured prefeeding (starved for 5 days), but it suddenly dropped by 62% after feeding. Then, it maintained relatively steady and low levels for the rest of the experimental period. The total concentration of FAA (including taurine) in the viscera attained three peaks during the 96-h experimental period. It appeared at 0 (prefeeding), 36, and 96 h after feeding. The initial and final levels of total FAA in the viscera were not significantly different ($p < 0.05$).

Figure 4 also depicts the change in the level of ammonia after feeding the red alga, *P. palmata*. During the first 52 h, the ammonia level had a change pattern similar to the mean level of FEAA. However, it steadily increased from 52 to 96 h postfeeding.

Correlation Between Dietary Essential Amino Acids and Visceral FEAA

The correlations between the essential amino acids (EAA) in dietary protein (Mai et al. 1994) and the FEAA in the viscera at the different sampling times were established by correlation analysis. The results are shown in Table 2. The FEAA (not including tryptophan) in the viscera are shown to be positively and significantly correlated ($r = 0.58 \sim 0.87$, $p = 0.018 \sim 0.0002$) to the level supplied by the algal diet *P. palmata* at 4, 22, 28, 36, 44, 52, 62, and 72 h postfeeding. No significant correlation was observed prefeeding (0 h) and at 10, 16, and 96 h postfeeding. It can be seen from the correlation coefficients (r) that the best correlation (highest r value) was obtained at 36 h postfeeding, followed by those at 62 and 72 h after feeding.

DISCUSSION

According to the time course of algal pigment in the viscera after feeding in this study (Fig. 3), it can be inferred that most active digestion took place during the first 10 h after the meal. Considering the report by Tomita (1972), this digestive activity is likely to be carried out in the stomach. The sudden change in pigment concentration before and after 10 h postfeeding indicates that the undigested food in the stomach probably was moved into the intestine around this time. It means that the GET in *H. discus hannai* is about 10 h after a meal. This GET is a little longer than that of 3–6 h observed by Tomita (1972). However, GET can be affected by various factors, such as water temperature, dietary properties, age of animal, starvation, meal size, etc. Judging from the recognizability of algae ingested by *H. rubra*, Foale and Day (1992) reported that digestion speed of this abalone is affected by algal toughness, polyphenol level, and prior starvation. The red alga, *P. palmata*, used in this study is obviously tougher than the green alga, *U. pertusa*, used by Tomita (1972). Toughness is likely to be one of the causes for longer GET observed in this experiment.

From 10 h postfeeding, the red pigment in the viscera was maintained at a constant level until 22 h and then gradually reduced to near zero level by 44 h. This suggests that digestion in the intestine lasts longer (34 h) than that in the stomach (10 h). The disappearance of the algal pigment in viscera suggests that either it can almost be broken down to smaller chemical substances that are not able to produce red color, or it can almost be egested by 44 h postfeeding. Using the algal pigment as an indicator for monitoring the digestive status of abalone, it can be speculated that the total food passage time in *H. discus hannai* fed with *P. palmata* at 15°C is about 44 h.

The gut evacuation time of *H. midae* fed a formulated feed is approximately 18–24 h by means of gut fullness examination (Britz et al. 1996). Wee et al. (1992) reported that *H. rubra* took 7 days to void all digesta through the gut after a meal at 10–16.5°C, judging from fecal collection. There appeared to be three types of feces voided during this period: (1) discrete whole pellets, (2) clumps of amorphous materials, and (3) long, thin, dark strings. Toward the end of this period, Type 3 feces dominated. Wee et al. (1992) proposed that the composition of the different fecal types should be reexamined and the reason for the production of different types of feces in the abalone should be investigated. In *Penaeus*

TABLE 1.

Postprandial changes of FAA in the viscera of *H. discus hannai* fed with *P. palmata* (nmol/100 mg of tissue; means [SD], n = 3).

Amino Acids	Time After Feeding (h)												ANOVA
	0	4	10	16	22	28	36	44	52	62	72	96	(p=)
Arg	194 (27)^{a}	318 (16)^{bc}	214 (7)^{ab}	240 (59)^{ab}	291 (83)^{abc}	315 (38)^{abc}	332 (39)^{bc}	385 (14)^{cd}	273 (8)^{abc}	234 (6)^{ab}	215 (38)^{ab}	459 (49)^{d}	0.000
His	18 (3)^{a}	25 (3)^{ab}	19 (1)^{ab}	23 (6)^{ab}	37 (8)^{bcd}	30 (4)^{abc}	47 (5)^{cde}	44 (5)^{cd}	36 (4)^{abc}	55 (10)^{de}	54 (6)^{de}	62 (11)^{e}	0.000
Ile	119 (9)^{a}	149 (26)^{ab}	123 (26)^{ab}	136 (19)^{ab}	172 (26)^{abc}	198 (8)^{abcd}	272 (41)^{de}	266 (42)^{cde}	217 (19)^{bcd}	284 (32)^{de}	264 (63)^{cde}	351 (44)^{e}	0.000
Leu	39 (2)^{a}	154 (19)^{b}	58 (5)^{a}	83 (14)^{ab}	185 (37)^{bc}	222 (7)^{bcd}	299 (49)^{cde}	271 (40)^{cde}	231 (20)^{bcd}	338 (47)^{e}	279 (63)^{cd}	32 (3)^{a}	0.000
Lys	181 (14)^{ab}	223 (24)^{abc}	168 (6)^{ab}	146 (24)^{a}	222 (48)^{abc}	270 (37)^{abcd}	362 (42)^{d}	344 (39)^{cd}	289 (26)^{bcd}	384 (74)^{d}	358 (63)^{d}	357 (61)^{d}	0.000
Met	25 (18)^{ab}	25 (8)^{ab}	25 (19)^{ab}	26 (17)^{ab}	34 (6)^{ab}	43 (10)^{abc}	72 (6)^{c}	53 (7)^{abc}	57 (16)^{bc}	73 (11)^{c}	45 (11)^{abc}	21 (3)^{a}	0.000
Phe	20 (3)^{a}	67 (10)^{abc}	34 (3)^{ab}	38 (3)^{ab}	88 (19)^{bcd}	96 (7)^{cd}	167 (33)^{e}	136 (24)^{de}	112 (7)^{cde}	164 (26)^{e}	139 (41)^{de}	26 (10)^{a}	0.000
Thr	95 (34)^{a}	162 (9)^{ab}	124 (10)^{a}	137 (27)^{a}	174 (55)^{ab}	183 (40)^{abc}	252 (7)^{bc}	250 (17)^{bc}	240 (50)^{bc}	277 (32)^{c}	261 (37)^{bc}	395 (47)^{c}	0.000
Trp	5 (2)^{a}	12 (2)^{ab}	5 (2)^{a}	7 (2)^{ab}	19 (4)^{an}	10 (2)^{ab}	31 (16)^{b}	24 (12)^{ab}	11 (3)^{ab}	22 (6)^{ab}	24 (20)^{ab}	10 (2)^{ab}	0.011
Val	165 (15)^{a}	244 (26)^{abc}	179 (8)^{a}	180 (41)^{a}	267 (44)^{abc}	295 (18)^{bc}	340 (17)^{bc}	392 (76)^{cd}	337 (15)^{bc}	396 (79)^{cd}	334 (47)^{bc}	485 (21)^{d}	0.000
Ala	338 (15)^{a}	559 (49)^{abc}	459 (17)^{abc}	425 (22)^{ab}	423 (78)^{ab}	421 (61)^{ab}	607 (100)^{abc}	547 (118)^{abc}	464 (13)^{abc}	634 (160)^{bc}	673 (218)^{bc}	718 (52)^{c}	0.001
Asx	124 (7)^{a}	328 (95)^{c}	179 (7)^{ab}	149 (31)^{ab}	238 (43)^{abc}	165 (45)^{ab}	504 (13)^{e}	528 (57)^{e}	266 (33)^{bc}	475 (10)^{de}	542 (50)^{e}	352 (53)^{cd}	0.000
Cys	16 (6)^{a}	18 (2)^{a}	12 (4)^{a}	16 (8)^{a}	25 (11)^{ab}	22 (10)^{ab}	29 (2)^{ab}	47 (7)^{bc}	30 (12)^{ab}	30 (2)^{ab}	68 (10)^{c}	48 (21)^{bc}	0.000
Glx	481 (23)^{ab}	420 (115)^{ab}	499 (20)^{ab}	445 (95)^{ab}	450 (87)^{ab}	479 (132)^{ab}	306 (8)^{a}	329 (34)^{a}	554 (70)^{b}	426 (10)^{ab}	305 (29)^{a}	588 (88)^{b}	0.001
Gly	423 (43)^{ab}	375 (73)^{a}	359 (55)^{a}	429 (26)^{ab}	450 (24)^{ab}	707 (71)^{bc}	571 (130)^{ab}	543 (145)^{ab}	443 (44)^{ab}	614 (147)^{abc}	530 (178)^{ab}	906 (174)^{c}	0.000
Pro	113 (6)^{a}	260 (57)^{cde}	144 (18)^{abc}	121 (5)^{ab}	194 (11)^{abcd}	201 (4)^{abcd}	290 (58)^{de}	255 (39)^{cde}	244 (13)^{bcd}	316 (59)^{de}	300 (90)^{de}	378 (46)^{e}	0.000
Ser	158 (28)^{a}	213 (6)^{ab}	163 (30)^{a}	168 (50)^{a}	230 (73)^{abc}	255 (68)^{abc}	316 (21)^{bc}	317 (20)^{bc}	263 (47)^{abc}	311 (15)^{bc}	332 (40)^{cd}	443 (22)^{d}	0.000
Tyr	58 (6)^{a}	97 (5)^{ab}	65 (2)^{a}	64 (11)^{a}	108 (27)^{b}	120 (18)^{bc}	167 (10)^{d}	157 (16)^{cd}	130 (15)^{bcd}	161 (5)^{cd}	150 (20)^{cd}	122 (5)^{bc}	0.000
Tau	6151 (1022)^{b}	2352 (282)^{a}	2526 (58)^{a}	2550 (591)^{a}	1672 (449)^{a}	2192 (443)^{a}	2241 (199)^{a}	2278 (31)^{a}	1643 (167)^{a}	2199 (15)^{a}	2766 (228)^{a}	2489 (265)^{a}	0.000
NH_3	243 (5)^{a}	351 (37)^{abc}	302 (22)^{ab}	310 (37)^{ab}	320 (52)^{abc}	357 (24)^{abc}	453 (53)^{cd}	496 (67)^{d}	427 (61)^{bcd}	493 (10)^{d}	526 (79)^{d}	705 (30)^{e}	0.000
ΣFEAA	860 (30)^{a}	1379 (62)^{bc}	948 (33)^{a}	1015 (115)^{ab}	1490 (98)^{cd}	1566 (43)^{cd}	2176 (142)^{efg}	2166 (152)^{efg}	1802 (9)^{de}	2226 (170)^{g}	2006 (311)^{ef}	2197 (33)^{fg}	0.000
ΣFNEAA	1712 (29)^{a}	2270 (210)^{abc}	1880 (69)^{a}	1817 (134)^{a}	2120 (208)^{ab}	2370 (148)^{abc}	2790 (291)^{bcd}	2723 (363)^{bc}	2394 (94)^{abc}	2966 (355)^{cd}	2899 (610)^{bcd}	3554 (213)^{d}	0.000
ΣFAA	8723 (1067)^{cd}	6001 (546)^{abc}	5354 (160)^{a}	5383 (828)^{ab}	5283 (738)^{a}	6128 (576)^{abc}	7208 (632)^{abcd}	7168 (530)^{abcd}	5838 (259)^{ab}	7391 (537)^{bcd}	7671 (1149)^{cd}	8240 (511)^{d}	0.000

Abbreviations: ΣFEAA, total FEAA; ΣFNEAA, total FNEAA excluding taurine; ΣFAA, total FAA; ANOVA, analysis of variance. Means in the same row sharing the same superscript letter were not significantly different as determined by Tukey test ($p > 0.05$).

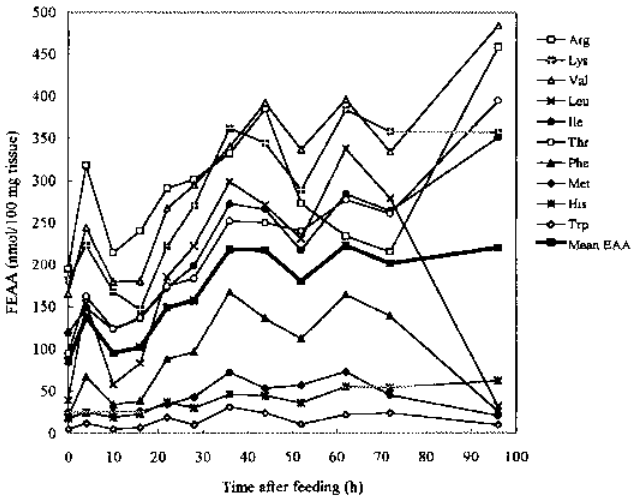

Figure 3. Postprandial change of FEAA in the viscera of *H. discus hannai*.

chinensis, the feed movement in its digestive tract can easily be seen from its dorsal side. Interestingly, the prawn continued to void some long, thin, and colored egesta, which are similar to Type 3 feces of abalone, even after there was no observable food movement in the digestive canal (K. Mai pers. obs.). This means that the long, thin, colored strings could probably consist of mucus and digestive fluid rather than the undigested diet. Additionally, abalone may ingest fecal matter and pedal mucus during the fasted period (Britz et al. 1996). Therefore, the real time for *H. rubra* to complete the digestion of a satiation meal may be shorter than the total time for completing fecal collection.

Although the total food passage times in abalone reported by different authors are fairly discrete, they are longer than those of most fish, where the total food passage times range from 1.5 to 8 h for herbivorous fish and 5 to 26 h for carnivorous fish (Smith 1989). The total food passage time (gut-clearance time) of the algivorous sea urchin, *Paracentrotus lividus*, was about 5 days (Lawrence et al. 1989). However, when the echinoids were fed food containing carmine and then fed nonmarked food on the following days, the gut residence time of the marked food ranged

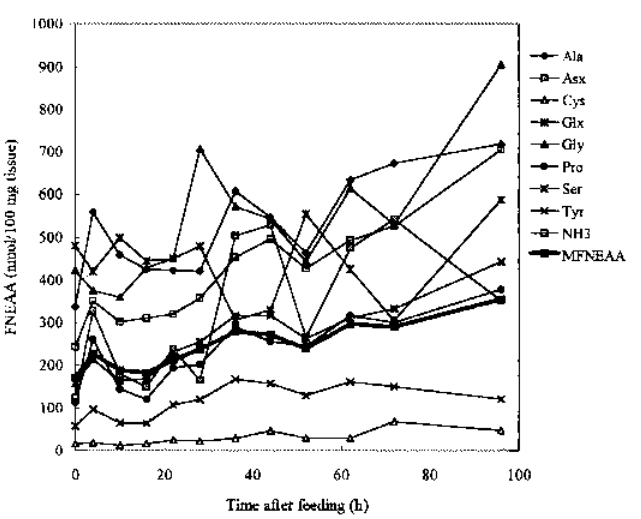

Figure 4. Postprandial change of FNEAA in the viscera of *H. discus hannai*. MFNEAA, mean level of FNEAA.

TABLE 2.

Summary of correlation analyses between the EAA in dietary alga *P. palmata* and the mean values of FEAA in the viscera of *H. discus hannai* sampled at different times after feeding.

Sampling Time (h) Postfeeding	*df*	*r*	*F*	p	Significance
0	7	0.395	4.576	0.069	
4	7	0.570	9.272	0.018	*
10	7	0.407	4.797	0.065	
16	7	0.380	4.285	0.077	
22	7	0.666	13.981	0.007	**
28	7	0.761	22.246	0.002	**
36	7	0.858	42.226	0.000	***
44	7	0.738	19.688	0.003	**
52	7	0.740	19.945	0.003	**
62	7	0.845	38.143	0.000	***
72	7	0.815	30.931	0.001	***
96	7	0.221	1.981	0.202	

The EAA data of *P. palmata* was based on the report by Mai et al. (1994), and Trp was not included in the correlation analyses.

from only 8 to 50 h. Interestingly, their results indicate that the time for a meal to pass the digestive tract is affected by feeding regimen but not by food quality (Lawrence et al. 1989).

It can been seen from the comparison between the postprandial change of dietary pigment in the viscera and that of FEAA that both the pigment and the FEAA are effective indicators reflecting the status of digestion and absorption in abalone after a meal. Evidently, the levels of FEAA are mainly a result of food digestion, because highly significant correlations were established between the EAA in the diet and the FEAA in the viscera at certain sampling times (Table 2). The changing pattern of total FEAA in the viscera and the correlation coefficient (r) are surprisingly similar between 22 and 72 h after feeding. The first significant correlation was established at 4 h postfeeding, indicating that the stomach digestion was mainly carried out during the first 4 h postfeeding. However, the r value at 4 h was the lowest of the correlations between dietary EAA and FEAA in the viscera. This suggests that only preliminary digestion was in progress in the stomach for the first 4 h and that only the amino acids that are in a free state or relatively easily broken down are released during the initial period of stomach digestion. The correlation coefficients gradually increased from 10 to 36 h and then stayed at high and significant levels until 72 h postfeeding. The highest r value was obtained at 36 h. This addresses the question raised by Mai et al. (1994) of the most suitable sampling time for calculating the index, balance, and adequacy of free EAA in viscera to evaluate the nutritional values of protein in natural feeds for herbivores or algivores. The fact that correlation coefficients and the total FEAA level in viscera were maintained at higher levels from 22 to 72 h implies that digestion and absorption possibly continue until 72 h after feeding. However, this may represent the rhythmic digestion and intracellular digestion of the absorbed small protein molecules and peptides during the period from 44 to 72 h postfeeding, which is common in certain mollusks (Morton 1983). This may also indicate that absorbed amino acids are stored in the visceral pools for a long period, because abalone tissues are usually rich in FAA (Mai et al. 1994). Britz et al. (1996) found a slight increase in the gut volume and solid content of *H. midae* during the second dark period, when no food was offered. They suggested that the abalone ingested

other material, like fecal matter and pedal mucus. This phenomenon can probably explain the slight increases on the visceral FAA during the second (24–36 h), third (48–60 h), and fourth (72–84 h) dark periods in this study (Figs. 3 and 4). However, further investigations are needed to fully understand the real causes for the fluctuations of FEAA and FNEAA in the viscera during the digestive process.

From 72 h to the end of the experiment, there were two trends for the levels of the FEAA in the viscera. Lysine, leucine, phenylalanine, methionine, and tryptophan dropped, even below the prefeeding levels at 96 h, except for lysine. Meanwhile, valine, threonine, isoleucine, arginine, and histidine increased again, even up to the highest levels, except arginine. The trend during the last 24 h was perhaps mainly due to the catabolism of tissue protein, because these abalone had been fasted for 3 days after the experimental meal. This hypothesis is supported by the evidence that the lowest correlation coefficient was observed at 96 h. Additionally, the rapid rise of ammonia level in the viscera after 52 h (Fig. 4) may be an indicator of deamination for meeting their energy requirement in a starving state. However, the real reason for the increasing NH_3 level in the viscera remains unknown.

From the discussion above, it is proposed that the gastric emptying time of *H. discus hannai* feeding on *P. palmata* at 15°C is about 10 h, and its total food passage time is about 44 h, with the algal pigment as an indicator. However, its amino acid absorption may last longer.

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DEVELOPMENT OF POLYSACCHARIDE DEGRADATION ACTIVITY IN POSTLARVAL ABALONE *HALIOTIS DISCUS HANNAI*

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ABSTRACT Changes in the activity of the digestive enzymes against carboxymethylcellulose (CMC), alginate, laminarin, and fucoidan in postlarval abalone *Haliotis discus hannai* were measured from 7 days after settlement (Day 7) to 46 days after settlement (Day 46) every 5–10 days. Shell length (SL) and protein per individual showed a rapid increase after Day 37. Enzyme activity was not detected on Day 7 (0.52 ± 0.01 mm SL, mean ± standard error) but by Day 17 (0.97 ± 0.02 mm SL), there was detectable activity from all enzymes. This indicates that a suite of enzymes for brown algal feeding is produced even by the postlarval stage. Changes in the total activity of CMCase, alginate lyase, and laminarinase showed a pattern similar to the growth rate, with a rapid increase observed after Day 37 (1.59 ± 0.04 mm SL). However, specific activities did not show the marked changes evident in the total activities, suggesting that the rapid increase of total activities observed after Day 37 was mainly caused by increased production of digestive enzyme with growth. Despite the negligible contents of cellulose and alginate in food of postlarvae, Days 37 and 46 postlarvae showed strong enzyme activity against these polysaccharides, suggesting that the appearance of enzyme activity during postlarval development does not require the presence of a substrate.

KEY WORDS: postlarval abalone, feeding, digestive enzyme, *Haliotis discus hannai*

INTRODUCTION

Information on the feeding habits of the early life stages of *Haliotis discus hannai* Ino 1953 has markedly increased in recent years (e.g., Kawamura and Takami, 1995, Kawamura et al. 1995, Kawamura et al. 1998b, Seki 1997, Takami et al. 1997a, Takami et al., 1997b). Benthic diatoms have been recognized as the main food source of early life stages of abalone. The dietary value of specific benthic diatoms varies between diatom species (and even strains) and also with the developmental stage of the abalone (Kawamura and Takami 1995, Kawamura et al. 1995, Kawamura et al. 1998a). In Japanese abalone hatcheries, plastic plates dominated by the benthic diatoms *Cocconeis* spp. are used for rearing postlarvae (Seki 1980). *Cocconeis* spp. are suitable foods for favorable growth of postlarval abalone of more than ~1 mm shell length (SL) (Kawamura et al. 1995, Takami et al. 1997a). However, it has been suggested that juvenile abalone of more than 10 mm SL do not prefer grazing on these benthic diatom species if other more favorable foods are available (Ioriya and Suzuki 1987, Suzuki et al. 1987, Kawamura et al. 1998b), because *Cocconeis* is not an efficient food source for these large juveniles (Takami et al. 1996).

It has been previously reported that juvenile *H. discus hannai* begin to feed on macroalgae at about 5 mm SL (Seki 1997). Further, *Haliotis discus discus* Reeve 1846 of 3–4 mm SL shows a rapid growth rate feeding on juvenile macroalgae (Maesako et al. 1984). Large juvenile and adult *H. discus hannai* prefer to feed on brown algae of the order Laminariales (Sakai 1962, Kikuchi et al. 1967, Uki 1981) and show rapid growth rates feeding on these algal species (Kikuchi et al. 1967, Uki 1981, Uki et al. 1986). Thus, a shift in the feeding habit from diatom feeding to macroalgal feeding appears to occur at 5–10 mm SL (Kawamura et al. 1998b). However, there has been limited research on the development of enzyme activity allowing abalone to digest macroalgae.

Macroalgae of the order Laminariales contain significant amounts of polysaccharides such as cellulose, alginate, and laminarin. These polysaccharides could be important energy sources for abalone (Leighton and Boolootian 1963), in which case it is necessary to produce polysaccharide-degrading enzymes in the gut because algal polysaccharides are not digestible without specific enzymes. Indeed, adult abalone have higher enzyme activities against polysaccharides from brown algae than those from green and red algae (Onishi et al. 1985, Anzai et al. 1991).

Thus, the activities of digestive enzymes reflect the feeding habit of the abalone. In this study, we measured the ability of postlarval *H. discus hannai* (7–46 days postsettlement) to degrade brown algal polysaccharides.

MATERIALS AND METHODS

Rearing of Postlarvae

Larval abalone were hatched in June 1997 by the procedures described by Uki and Kikuchi (1984) at the Iwate Sea Farming Association (Iwate, Japan). Four days after fertilization at 20°C, the veliger larvae were transported to the Tohoku National Fisheries Research Institute (Miyagi, Japan) within 4 h. The method of transportation of veliger larvae used in this study did not affect larval behavior (Kawamura and Kikuchi 1992).

Plastic water tanks (diameter, 28 cm; height, 14 cm) were used for rearing of postlarvae. The inside of the tanks was covered with a unialgal benthic diatom *Cocconeis scutellum* Ehrenberg 1838 that had previously been grazed by juvenile abalone (2 cm SL, 15–16 individuals per tank) overnight, ensuring that trail mucus was available. *Cocconeis* spp. coated with trail mucus of juvenile abalone have a high potential for the induction of settlement and metamorphosis of larvae and provide a suitable food source for all stages of postlarval abalone (Takami et al. 1997a).

Five-day-old larvae, which were ready to metamorphose (Seki and Kan-no 1981), were placed in the tanks at a density of approximately 5,000 larvae per tank. This initiation of settlement was termed Day 0. Forty-eight hours after the introduction of larvae to the tanks, the remaining swimming larvae were removed, and the settled and metamorphosed larvae (approximately 70% of the ini-

tially introduced swimming larvae) were reared for 46 days in the tanks containing filtered seawater (0.45-μm-pore-size Millipore filter) at 20°C, 6,000 lux with a 12L:12D photoperiod. All of the seawater was exchanged every 4 or 5 days. On Days 17 and 37 postsettlement, the postlarvae were transferred to new tanks, the insides of which were covered with a monocultured benthic diatom *C. scutellum*, enabling postlarvae to always have access to sufficient food during the experimental period.

Sample Preparation

Postlarval abalone for enzyme assays were taken on Days 7, 17, 22, 28, 37, and 46. Individuals were carefully removed from the tank walls with a water jet from a pipette and were rinsed well with filtered seawater (0.45-μm-pore-size Millipore filter) to remove any diatom cells and feces attached to their bodies. Because of the difficulty of measuring low enzyme levels, 10–50 individuals were pooled in 10-ml centrifuge tubes (six tubes per sampling day). The SL of 20 individuals selected at random from these samples on each sampling day was measured to the nearest 0.01 mm with an inverted optical microscope. Samples were frozen at −80°C until the enzyme assay.

Assay of Enzymes

Frozen postlarvae were homogenized in 1 mL of 100 mM potassium phosphate buffer that had been set on ice, pH 6.9, with a glass hand homogenizer (10-mL centrifuge tube and a tissue grinder). The tip of the tissue grinder was rinsed with 1 mL of homogenization buffer, and the vials, containing 2 mL of homogenate, were centrifuged at 2,250 *g* for 30 min. The supernatant was used for the assays of enzyme activities and protein content.

Carboxymethylcellulase (CMCase), alginate lyase, laminarinase, and fucoidanase activities were measured on each sampling date. Carboxymethylcellulose (CMC) (Sigma, Ref. C-5678), sodium alginate (Sigma, Ref. A-2158), laminarin (Sigma, Ref. L-9634), and fucoidan (Sigma, Ref. F-5631) were used as substrates for CMCase, alginate lyase, laminarinase, and fucoidanase activities, respectively. The assay system of enzymatic activities consisted of 450 μL of 3% (wt/vol) substrate solution dissolved in buffer and 50 μL of the crude enzyme extract. Mixtures were incubated for 2 h at 37°C. Reactions were stopped by placement in a boiling water bath for 3 min. After centrifugation (2,250 *g* for 15 min), supernatants were used for the analysis of reducing sugars (RS). RS were determined spectrophotometrically by the method of Nelson (1944) as modified by Somogyi (1952), with glucose, galacturonic acid, glucose, and fucose as standards for CMCase, alginate lyase, laminarinase, and fucoidanase, respectively. Results were corrected by subtraction of the corresponding blank. Enzyme activities were expressed as micrograms of RS produced per hour per individual (total activities) or per microgram of protein (specific activities).

Enzyme activities measured in this study are activities for the whole body because even the individuals sampled on Day 46 were too small to dissect and remove the digestive tract. A preliminary experiment with juvenile *H. discus hannai* (5–10 mm SL) showed that for eviscerated specimens, the CMCase, alginate lyase, and laminarinase activities were negligible in comparison to specimens with intact digestive tracts. In this study, therefore, we suggest that the polysaccharide hydrolase activities measured in the whole-body specimens can be effectively considered as the digestive enzyme activities.

Protein Determination

Measurement of the soluble protein content was carried out by Peterson's modification of the micro-Lowry method (Peterson 1977). Protein concentration was calculated with bovine serum albumin as a standard.

Statistical Analyses

All statistical analyses were carried out with the JMP (SAS Institute Inc., Cary, NC) statistical computer package.

RESULTS

Postlarvae grew well during the experimental period and reached a mean SL of 2.40 ± 0.1 mm (mean ± standard error) on Day 46. The growth in SL was not linear: the postlarvae showed a markedly higher growth rate during Days 37–46 (89.9 μm/day) than that during Days 7–37 (36.0 μm/day) (analysis of covariance [ANCOVA]; $p < 0.01$; Fig. 1). Soluble protein per individual also increased dramatically after Day 37 (1.59 ± 0.04 mm SL) (ANCOVA, $p < 0.01$; Fig. 2), reflecting a rapid increase in body mass.

Total CMCase activity (μg of RS/individual per h) was first detected on Day 17 (0.97 ± 0.02 mm SL) and increased with age (Fig. 3A). However, the relationship between the enzyme activity and the number of days after settlement was not linear. The total activity increased slowly from Day 17 to Day 37 and then rose sharply between Days 37 and 46 (ANCOVA, $p < 0.01$; Fig. 3A). Similarly, alginate lyase and laminarinase activity was first detected on Day 17 and increased rapidly after Day 37 (ANCOVA, $p < 0.01$; Fig. 3B and C). Although fucoidanase activity was also detected on Day 17, activity did not show a clear increase with age and remained at low levels in comparison with other enzyme activities (Fig. 3D).

In CMCase and fucoidanase, there was no clear trend between the specific activities (μg of RS/μg of protein per h) and the age of postlarvae ($r = 0.28$, $p = 0.16$ for CMCase and $r = 0.27$, $p = 0.15$ for fucoidanase, respectively; Fig. 4A and D). In contrast, specific activities of alginate lyase and laminarinase increased with age ($r = 0.55$, $p < 0.01$ for alginate lyase and $r = 0.63$, $p < 0.01$ for laminarinase, respectively; Fig. 4B and C). However, the marked increase that occurred in the total activity after Day 37 was not observed in the specific activities of alginate lyase and laminarinase.

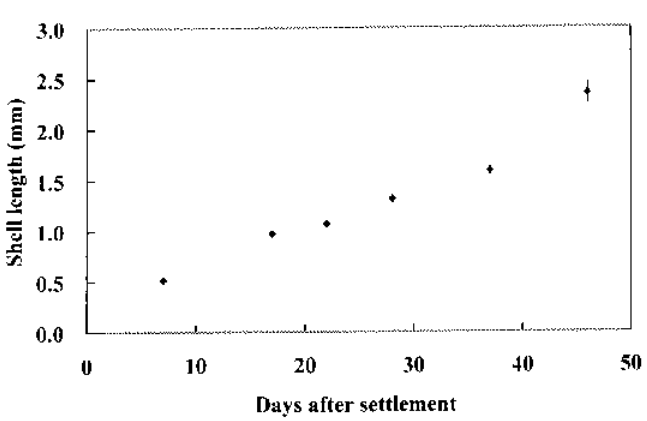

Figure 1. Postlarval growth of *H. discus hannai* in SL. Data indicate the mean ± SE (n = 20).

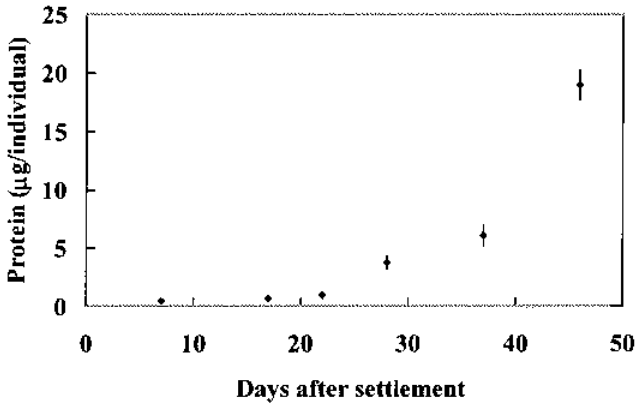

Figure 2. Postlarval growth of *H. discus hannai* in protein content. Data indicate the mean ± SE (n = 6).

DISCUSSION

Postlarval growth in SL and protein content significantly increased after Day 37 (Figs. 1 and 2). A similar growth pattern was reported on postlarval *H. discus hannai* by Takami et al. (1997b); rapid growth was observed from ~ 1.5 mm SL. Further, *H. discus discus* showed a sharp increase during the formation of respiratory pores (~2 mm SL) (Ino 1952). In this study, all of the postlarvae sampled on Day 46 had one or two respiratory pores. Total activities of CMCase, alginate lyase, and laminarinase also increased dramatically after Day 37 (Fig. 3A–C), but the specific activities of these enzymes did not show any marked changes during this period (Fig. 4A–C). Thus, the large changes observed in total activities were mainly caused by the rapid growth of postlarvae; changes of total activities were accompanied by a large increase in SL and protein content.

In this study, postlarvae were fed on the benthic diatom *C. scutellum* with juvenile trail mucus from just after metamorphosis to Day 17 (0.97 ± 0.02 mm SL) and only *C. scutellum* from Day 17 to Day 46. The feeding regimen during this experimental period can be considered to be comparable to that on conditioned (pregrazed) plates used in abalone hatcheries to rear postlarvae (Takami et al. 1997a). The newly metamorphosed stage (from just after metamorphosis to ~0.8 mm SL) of postlarvae cannot detach diatom cells of *Cocconeis* spp. with their radula (Kawamura and Takami 1995, Takami et al. 1997a, Daume et al. 1997, Kawamura et al. 1998a). The extracellular mucus of diatoms (Kawamura and Takami 1995, Takami et al. 1997a) and trail mucus left by juveniles during grazing (Takami et al. 1997a) are thought to be the main food sources for newly metamorphosed postlarvae on these conditioned plates. Postlarvae of more than approximately 0.8 mm SL can dislodge *Cocconeis* spp., breaking cells and accessing the cell contents (Kawamura 1996, Takami et al. 1997a, Kawamura et al. 1998b). In this study, the mean SL of postlarvae on Day 17 was 0.97 ± 0.02 mm. Thus, it appears that postlarvae were already capable of ingesting diatom cell contents by Day 17. The principal storage polysaccharide in diatoms is chrysolaminarin, the structure of which is similar to that of laminarin; both are β-1, 3-glucans, with a small degree of branching at C-6, containing 16–34 glucose units (Beattie et al. 1961, Ford and Percival 1965, Darley 1977). It is known that chrysolaminarin is hydrolyzed by laminarinase (= β-1, 3-glucanohydrolase) activity (e.g., Hassett 1994). Because laminarinase activity was detected on Day 17 (Fig. 4C), postlarvae might possess the digestive ability to break down the storage polysaccharide, chrysolaminarin, of *Cocconeis* cells by that time.

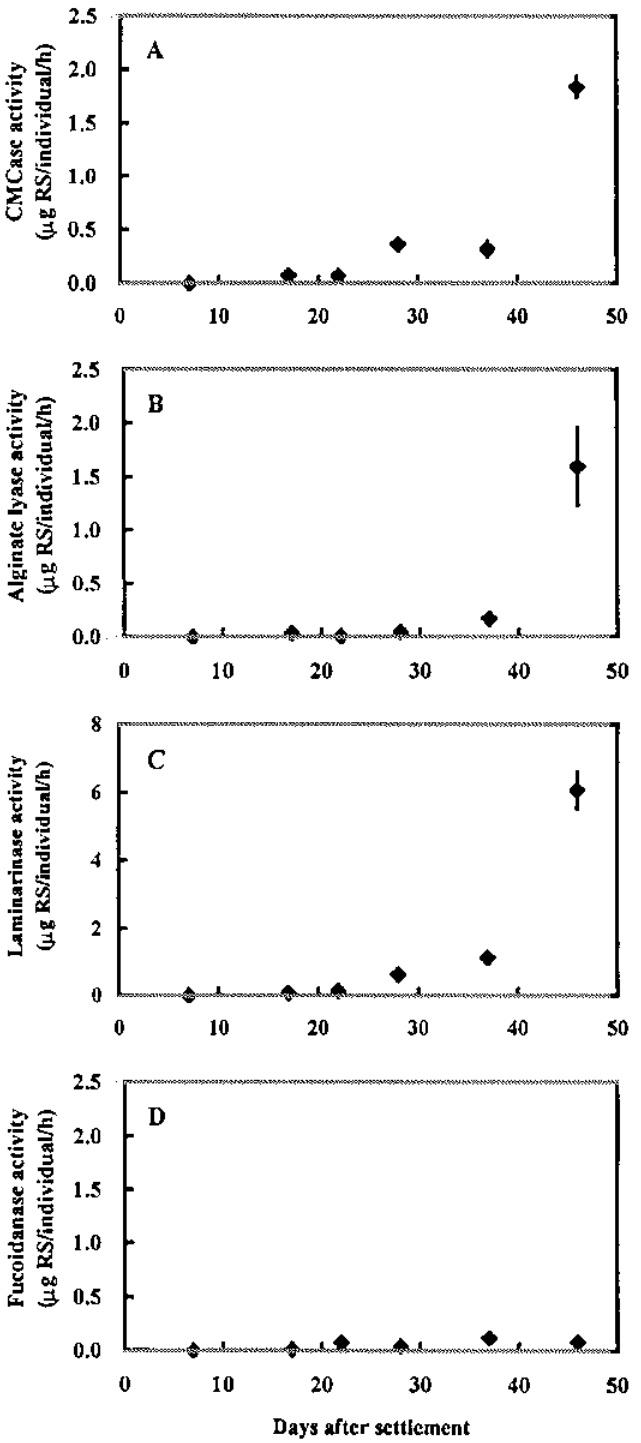

Figure 3. Relationship between total digestive enzyme activities (μg of RS/individual per h) and age (in days) of postlarval *H. discus hannai*. Data are for the mean ± SE (n = 6). (A) CMCase activity, (B) alginate lyase activity, (C) laminarinase activity, (D) fucoidanase activity.

The hepatopancreas of adult abalone produces endogenous enzymes to digest algal polysaccharide (Nakada and Sweeny 1967, Erasmus et al. 1997). However, Garland et al. (1985) observed that bacteria in the fecal material of 13-wk-old *Haliotis rubra* Leach 1814 (2.4 mm SL) remained viable, and they suggested that bacteria could perform metabolic activities in the gut that were highly significant to the development of the host. Viable bacteria have

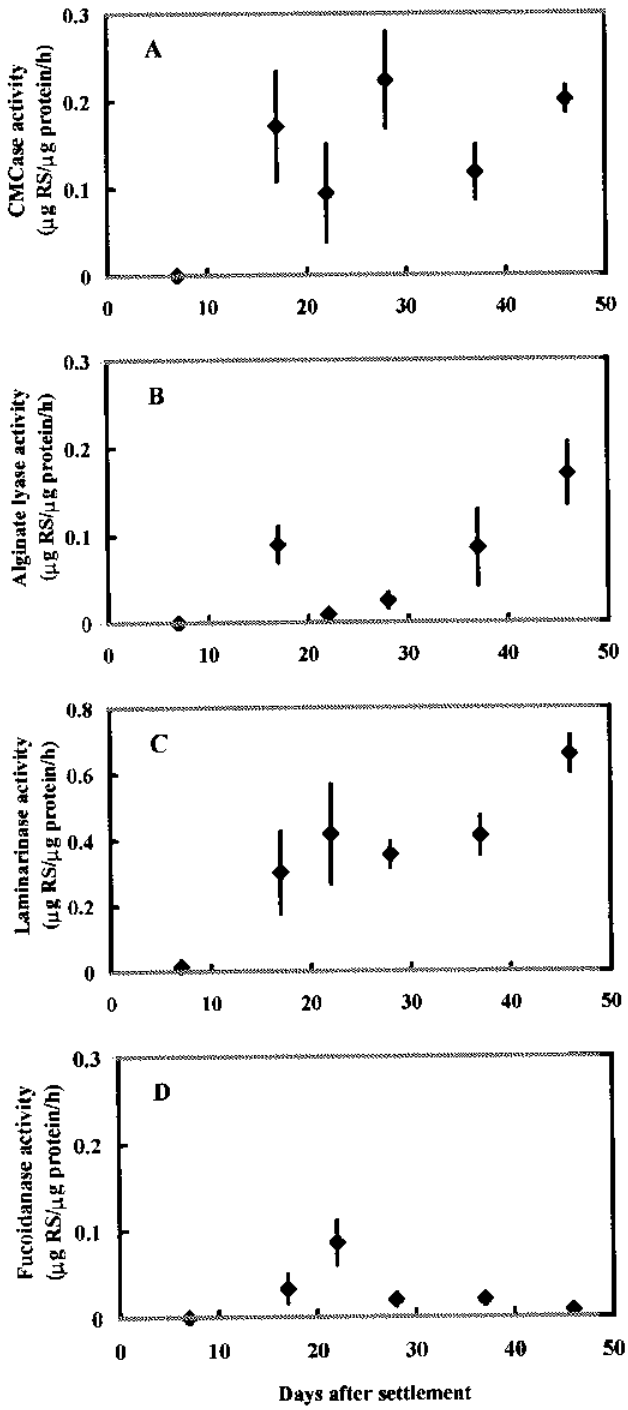

Figure 4. Relationship between digestive enzyme specific activities (μg of RS/μg of protein per h) and age (in days) of postlarval *H. discus hannai*. Data are for the mean ± SE (n = 6). (A) CMCase activity, (B) alginate lyase activity, (C) laminarinase activity, (D) fucoidanase activity.

also been identified in the digestive tract of the *H. discus hannai* (Sawabe et al. 1995) and *Haliotis midae* Linnaeus 1758 (Erasmus et al. 1997). The studies of Sawabe et al. (1995) and Erasmus et al. (1997) showed that bacteria resident in the abalone digestive tract could hydrolyze polysaccharides from algae. Gut bacteria may contribute to the ability of the host to digest algal polysaccharides. Postlarvae in this study were not under axenic conditions. The enzyme activities might be influenced by those of gut bacteria.

The digestive enzyme composition observed in individuals sampled on Days 37 and 46 were consistent with previous findings reported for adult abalone. Japanese *Haliotis* spp. possess strong digestive enzyme activities against polysaccharides that occur in brown algal cells such as cellulose, alginate, and laminarin (Onishi et al. 1985, Nakagawa and Nagayama, 1988, Yamaguchi et al. 1989, Anzai et al. 1991), with the exception of fucoidan (Onishi et al. 1985, Anzai et al. 1991). The presence of food in gut is one of the main stimuli for the secretion of digestive enzymes. Further, activity of digestive enzymes is influenced by the chemical composition of food (Knauer et al. 1996, Erasmus et al. 1997). Adult *H. midae* Linnaeus 1758 can alter their digestive enzyme composition to best use brown or red algae (Erasmus et al. 1997). In this study, the appearance and increase of laminarinase activity may have been partly due to the diatom (chrysolaminarin-rich) diet. However, despite the negligible contents of cellulose and alginate in diatom cells, the enzyme profiles of Days 37 and 46 postlarvae also showed strong enzyme activity against these polysaccharides (Fig. 3A and B). This suggests that the appearance of enzyme activity during postlarval development does not require the presence of a substrate. Alternatively, activities of these enzymes could be caused by gut bacteria. Erasmus et al. (1997) showed that most of the bacterial strains isolated from the gut of adult *H. midae* synthesized two or three carbohydrases. It is possible that chrysolaminarin-degrading bacteria in the postlarval gut possessed CMCase and alginate lyase in addition to laminarinase. It would be interesting to identify the enzyme activities of bacteria resident in the digestive tract of postlarvae.

Changes of total activities of CMCase, alginate lyase, and laminarinase were accompanied by postlarval growth. However, an increase of these activities did not merely reflect an increase in body mass. Gradual increase in specific activity of alginate lyase and laminarinase (Fig. 4B and C) may be due to a qualitative change in the digestive system as well as a quantitative one. It is possible that the activities measured in this study are the result of different isozymes that become active during postlarval development. For example, two kinds of alginate lyase were purified from hepatopancreas of adult abalone (Nakada and Sweeny 1967, Boyen et al. 1990).

H. discus hannai of 4–5 mm SL begin to feed on macroalgae (Seki 1997), and *H. discus discus* of 3–4 mm SL show a rapid growth rate feeding on juvenile macroalgae (Maesako et al. 1984). Abalone may begin to effectively use macroalgae from these developmental stages. This study shows that a suite of enzymes useful for digesting brown algal polysaccharides is produced even by postlarval abalone (~1 mm SL). This suggests that postlarvae may use brown algal polysaccharide if they can ingest either the algal cells or their surface biofilm. The morphological development of feeding apparatus such as the radula (Daume et al. 1997, Roberts et al. unpubl.) also needs further investigation to elucidate the developmental changes of the digestive ability against macroalgae.

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EFFECT OF ACTIVITY ON ENERGY ALLOCATION IN THE NORTHERN ABALONE, *HALIOTIS KAMTSCHATKANA* (JONAS)

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ABSTRACT The effect of activity, in the form of increased respiratory energy expenditure and secretion of mucus, on the summer and winter energy budgets of *Haliotis kamtschatkana* was assessed. Abalone exhibited seasonal variations in field activity with 20% of all individuals observed crawling during June to October, compared with <5% during December to February. In the laboratory, abalone exhibited diurnal as well as seasonal variation in activity. The laboratory activity budget showed that an average abalone spends 9.8 h day^{-1} quiescent, 12.0 h day^{-1} alert, 0.7 h day^{-1} feeding, and 1.5 h day^{-1} crawling during the summer, and 15.8 h day^{-1} quiescent, 5.5 h day^{-1} alert, 2.3 h day^{-1} feeding, and 0.4 h day^{-1} crawling during the winter. Videotapes of abalone made over 24-h periods revealed that abalone usually crawl at a rate of one shell length min^{-1}. Locomotion is not continuous; rather, abalone stop and then start again, on average twice per meter. Components of the energy budget, $C = F + U + P_g + P_r + R + M$ were measured during summer and winter months. None of the slopes of regressions of $\log_{10}$energy (J day^{-1}) on $\log_{10}$mass (g) was significantly different between summer and winter for any of the energy budget components, except those of somatic growth on mass. Summer *y*-intercepts were all significantly higher than winter *y*-intercepts, indicating that energy consumption and expenditure were higher during the summer. Respiratory energy expenditure was the largest component of both summer and winter budgets. Activity accounted for 23% of total consumed energy during the summer and 13% during the winter.

KEY WORDS: abalone, activity, energy budget, *Haliotis kamtschatkana*, secretion of mucus

INTRODUCTION

Increasing world demand for abalone has caused severe declines in most populations, including those of British Columbia's *Haliotis kamtschatkana* (Emmett and Jamieson 1988). Efforts have been made to manage this resource, including closure of the fishery (1990), farming, and reintroducing abalone into depopulated areas. Studies have shown that transplanting *H. kamtschatkana* from exposed habitats to more sheltered habitats leads to increased growth and ultimately greater population density (Breen 1986, Emmett and Jamieson 1988), suggesting that this may be a feasible strategy to enhance depleted British Columbia stocks. Successful reintroduction and stock enhancement will depend on identification of suitable habitat, which will in turn depend on complete knowledge of the biology of the abalone. Emmett and Jamieson (1988) point out that *H. kamtschatkana* do not grow to marketable size in high wave–exposure areas, but also note that the cause of the decreased growth is not known. Suggestions of inadequate food supplies or high rates of mortality illustrate the need for more information about the energy balance of *H. kamtschatkana*.

A poorly understood aspect of abalone biology and, indeed, of gastropods in general, is the energetic cost of activity. Abalone must crawl in order to forage, escape predators, find adequate refugia, and reproduce. Several studies have documented the movements of individual abalone and have shown that abalone vary widely in their motility (Momma and Sato 1969, Poore 1972, Shepherd 1973). Diurnally, abalone crawl mostly at night, and the amount of movement depends on size (Shepherd 1973, Sloan and Breen 1988), availability of food or shelter (Momma and Sato 1969, Poore 1972, Shepherd 1986, Sloan and Breen 1988), and type and degree of predation (Schiel and Welden 1987).

Activity is energetically costly to gastropods because of both increased metabolic rate and secretion of mucus. Many studies have shown a rise of oxygen consumption (Vo_2) during activity in gastropods (Newell and Roy 1973, Calow 1974, Fitch 1975, Crisp 1979). Cost of transport, or the amount of energy needed to transport a unit mass over a unit distance, has also been measured (Denny 1980, Houlihan and Innes 1982, Innes and Houlihan 1985, Donovan and Carefoot 1997). Calow (1974) estimated that 20% of "routine metabolism" of the pulmonate snail *Planorbis contortus* was devoted to activity. Likewise, several authors have pointed out the importance of mucus as a contribution to molluscan energy expenditure (Paine 1971, Calow 1974, Horn 1986, Davies et al. 1990), and Denny (1980) attributes the relatively high energetic cost of gastropod crawling to production of mucus. Calow (1974) estimated production of mucus as 13–32% of absorbed energy in *P. contortus*, and Carefoot (1967) estimated that mucus accounted for 15% of the energy budget of the opisthobranch *Archidoris pseudoargus*.

Metabolic rates of marine organisms depend on a myriad of internal and environmental factors that interact in different ways at different times of the year (Newell 1973). Seasonal temperature differences affect both oxygen consumption (see Bayne and Newell 1983, Carefoot 1987) and activity (Newell 1969, Poore 1972, Newell and Kofoed 1977). Newell and Pye (1971) showed interaction between activity level and temperature in *Littorina littorea* in that the active rate of respiration was more temperature dependent than the standard rate, suggesting that activity would have a different effect on a gastropod energy budget at different times of the year. Indeed, Widdows and Bayne (1971) found that both filtration rate and oxygen consumption in the mussel *Mytilus edulis* were affected by acclimation to high and low temperatures, which in turn, changed the animal's energy allocations. Evidence

for effects of temperature on secretion of mucus is more scarce, but Kideys and Hartnoll (1991) found that secretion of mucus in the whelk *Buccinum undatum* decreased at low temperatures. Changes in secretion of mucus at different temperatures and during different seasons would cause further changes in the effect of activity on an energy budget.

From observations of abalone, it is evident that activity and locomotion play important roles in their daily lives, yet there has been no study on the effects of activity on respiratory energy loss, secretion of mucus, and energy balance of abalone, and these are the bases of this study. We determined time-energy budgets for *H. kamtschatkana* for both summer and winter in order to assess the effect of activity on them.

MATERIALS AND METHODS

Energy budgets were calculated for *H. kamtschatkana* by measuring all components of the energy budget $C = F + U + P_g + P_r + R + M$. The components not directly affected by activity, consumption (C), production of feces (F), nitrogen excretion (U), somatic growth (P_g), and reproductive growth (P_r) were measured once during summer (June to August 1995) and once during winter (November 1995 to January 1996), except for P_g, which was measured monthly (March 1995 to July 1996), and P_r, which was measured once at the end of the experiment (August 1996). The two components directly affected by activity, respiration (R) and secretion of mucus (M), were estimated by developing summer (June 1995) and winter (December 1995) time budgets and then integrating amounts of time spent in each activity state with energy equivalents for each state.

Collection of Animals

Abalone were collected in Barkley Sound near the Bamfield Marine Station, Bamfield, British Columbia, and transported to the Shannon Point Marine Center, Anacortes, WA. They were held in a large tank with a constant supply of fresh seawater and fed *ad libitum* on *Nereocystis luetkeana*, a preferred kelp food (Paul et al. 1977).

Time Budgets

Activity states of *H. kamtschatkana* were monitored in both laboratory and field, and time budgets were determined from the amount of time spent in each different activity state. In total, five states that appeared important to the energetics of *H. kamtschatkana* were identified: (1) quiescent (shell held tightly to the substratum, cephalic and mantle tentacles retracted), (2) alert (shell raised off the substratum, tentacles extended), (3) active (back and forth movements in a small area without moving any appreciable distance), (4) crawling (moving an appreciable distance in one direction), and (5) feeding. In addition to laboratory and field observations, videotapes were made of crawling abalone.

Laboratory

Abalone were placed in a large open-air tank exposed to natural light and with a constant supply of fresh seawater. They were observed hourly, and the number of abalone in each of four states (quiescent, alert, crawling, and feeding) was recorded. This experiment was conducted in summer (June 1994; n = 105) and winter (January 1996; n = 70). Daily activity budgets were calculated from these summer and winter data.

Field

Activity states (quiescent, alert, and crawling) of field abalone were recorded during daytime SCUBA dives in Barkley Sound, near the Bamfield Marine Station. Dives were made between 9 am and 12 pm, in alternate months from April 1994 to April 1995. Divers followed 100-m transects and recorded the activity state of all abalone seen (n = 52–203 for each outing). These data were used to compare the amount of activity in the laboratory and the field.

Videotaped Crawling Activity

Abalone (n = 15; 70–120 g live mass) were placed three at a time in a glass aquarium (30 × 50 × 15 cm) with an adequate flow of fresh seawater (2 L min^{-1}) and were videotaped over a 24-h period. Videotapes were analyzed for (1) rate of crawling, (2) total distance moved during crawling, and (3) number of crawling bouts.

Energy Budgets

Because we were initially interested in potential gender differences in energy budget parameters, 10 females and 10 males were used for each component of the energy budget (unless otherwise stated). The animals ranged in live mass from 13 to 175 g so the effect of mass on energy budget parameters could also be investigated.

Consumption (C)

Abalone were kept in plastic mesh cages and fed pieces of kelp of known mass each day at 3 pm over a 4-day period. Uneaten kelp was removed each following day at 3 pm and weighed. Each day, three pieces of kelp were placed in empty cages as controls and change of mass was recorded. The mass of the uneaten kelp from each abalone's cage was subtracted from the initial mass of the piece, and the result was corrected for any difference in mass exhibited by the mean of the controls to determine the wet mass of kelp consumed.

To determine the energy content of food eaten, samples of kelp were weighed fresh and then dried at 60°C to constant mass. Samples of dried kelp were combusted in a Phillipson microbomb calorimeter to determine their energy content. Average daily energy consumption (J day^{-1}) for each abalone was calculated by multiplying the daily wet mass of consumed kelp by the energy content per gram wet mass of the kelp.

Feces Production (F)

Abalone (n = 5; 20–128 g live mass) were held individually in 1-L aerated plastic containers filled with filtered (5 μm pore size) seawater at ambient temperature over a 4-day period. Kelp of known mass was fed to each animal on the first day, and uneaten remnants were removed and weighed on the following day. The abalone were held in the containers for three more days, during which feces were collected daily. The feces were dried at 90°C to constant mass and then combusted in a microbomb calorimeter. The mean energy value for the feces was used to calculate F in the energy budget.

Nitrogen Excretion (U)

Individual abalone were placed in sealed containers and maintained at ambient temperature. Duplicate 1-mL aliquots of the

water in the containers were collected after 1 h and analyzed for (Solorzano 1969). Because nitrogen excretion by *H. kamtschatkana* does not fluctuate daily (Taylor and Carefoot unpubl.), nitrogen excretion was measured at 9 am and the values were extrapolated to a 24-h period. Energy costs (J day^{-1}) were calculated from micrograms of ammonia excretion by multiplying by 24.83 J mg of NH_4^{-1} (Elliot and Davison 1975).

Somatic Growth (P_g) and Reproductive Growth (P_r)

Mass and length of each abalone were recorded monthly for the duration of the experiment (16 mo). At the end of the experiment, when the animals were ready to spawn (August 1996), they were weighed a final time and removed from their shells. Each abalone was separated into five components: (1) shell, (2) pedal and adductor muscles with head and tentacles, (3) visceral mass including stomach, digestive gland, and gills, (4) gonad, and (5) hemolymph, mucus, and mantle water that drained off of the abalone during dissection. During dissection, the visceral mass was separated from the large pedal muscle and the gonad was removed from around the digestive gland by aspirating it into a clean glass vial. Wet mass of shell, muscle, viscera, and gonad was recorded for each animal. The soft tissues from each animal were homogenized individually and dried, and energy content was determined by combustion in a microbomb calorimeter. Shell caloric content was estimated from Paine (1971), assuming that abalone shell was 1.1% protein and protein has an energy value of 23.83 J mg^{-1}. Because it proved impossible to collect enough mucus and hemolymph from each animal for analysis, and lacking an estimate for the energy value of hemolymph, this mucus and hemolymph portion was assumed to be similar in energy value to pedal mucus (23.97 J mg^{-1}; Calow 1974).

Somatic growth was determined by regressing monthly mass of each abalone on time, with the slope of the regression being then a measure of growth (g mo^{-1}). This method was used, as opposed to subtracting final mass from initial mass and dividing by time, because there was considerable monthly fluctuation in wet mass. Mass change during winter (November to March) was compared with mass change during summer (April to October). Daily gain in live mass was converted to energy gain (J day^{-1}) by partitioning total gain in live mass into gain in mass of individual body parts (shell, muscle, and viscera) estimated from the proportion of whole mass that each of these tissues constituted. Each component was then multiplied by the energy content of the respective tissue.

An attempt was made to spawn the animals during summer (August 1995) with hydrogen peroxide (Morse et al. 1977). However, because only one animal spawned, energy devoted to reproduction (P_r) was estimated from gonad mass (including gametes) at the end of the experiment. Reproductive growth (J day^{-1}) was determined for each abalone by multiplying gonad mass by its energy content.

Respiration (R)

On the basis of the time budget, four states of activity were identified as those most often exhibited by *H. kamtschatkana* (quiescent, alert, feeding, and crawling). Because we were unable to induce abalone to feed in the respirometer, we assumed that energy expended during the feeding state was equivalent to that expended during the active state, which could be measured.

To assess the extent of increase in oxygen consumption from quiescent to alert and alert to active states, abalone ($n = 21$; 13–144 g live mass) were placed in round, Perspex respirometry chambers. Temperature was maintained at 10°C, and oxygen consumption was monitored continuously with a polarographic oxygen electrode connected to a computerized data acquisition system (Datacan; Sable Systems, Inc.). The state of the animal (quiescent, alert, active) was recorded every 2 min during the duration of the trial. Often, each animal did not exhibit all states during one trial, so animals were placed in the respirometer multiple times over a period of several days (separated by at least 48 h). Thus, oxygen consumption for each state was measured 2–4 times, permitting an average for each state to be calculated. Energy costs (J h^{-1}) were calculated from oxygen consumption (μL of O_2 h^{-1}) by multiplying by an oxygen equivalent (Q_{ox}) of 20.88 J mL of O_2^{-1} (Elliot and Davison 1975). This represents a weighted value for the catabolism of carbohydrate, protein, and fat based on the proportion of each found in *N. luetkeana*.

Summer and winter quiescent metabolic rates of abalone ($n = 20$; 13–175 g live mass) were measured individually over 1-h periods in respirometers as described above. The extents of increase in oxygen consumption from quiescent to alert and alert to active, determined above, were then applied to these summer and winter quiescent rates to estimate energy expenditure during different activity states at different times of the year. Energy expenditure during crawling was determined from Donovan and Carefoot (1997).

Production of Mucus (M)

Secretion of mucus was measured for two aspects of activity: adherence to the substratum and locomotion. The amount of mucus needed for substratum adherence during summer and winter was determined by allowing individual abalone ($n = 20$; 13–175 g live mass) to attach to a clean glass plate immersed in a tank supplied with fresh seawater. After an abalone had been stationary for 10 min after adherence, it was removed quickly from the plate. A 10-min period was chosen because Davies (1993) found that stationary limpets stop producing mucus within 10 min after attachment. The plate was then rinsed with distilled water to remove salt residues and dried at 60°C for 30 min. The dried mucus was carefully scraped from the plate, and its carbon content was determined (NA-1500 Elemental Analyzer; Carlo Erba Strumentazione). Mass of carbon (μg) was converted to dry mass of mucus (μg) by assuming that gastropod pedal mucus is 24.5% carbon (Peck et al. 1993). Dry mass of mucus was converted to energy (J), assuming a conversion of 23.97 kJ g^{-1} of mucus (Calow 1974). Secretion of mucus during crawling was determined from Donovan and Carefoot (1997).

RESULTS

Time Budgets

Laboratory Activity

During the summer, definite diurnal trends were seen, with greater locomotion during the night (18%) and increased quiescence during the day (only 1–2% crawling; Fig. 1, top). Peak quiescence occurred during daytime, with usually 50% or more abalone being in this state. The abalone fed steadily throughout the day because kelp was plentiful in the tank.

During the winter, abalone were more quiescent and less active than in the summer, and there was less of a diurnal trend (Fig. 1, bottom). Peak locomotion was generally from 7 pm to 12 am, but the percent crawling was no greater than 2.6%. Throughout the day, 60% or more of the abalone were quiescent, with an increase in alertness occurring during the period from 5 pm to 11 pm. Time

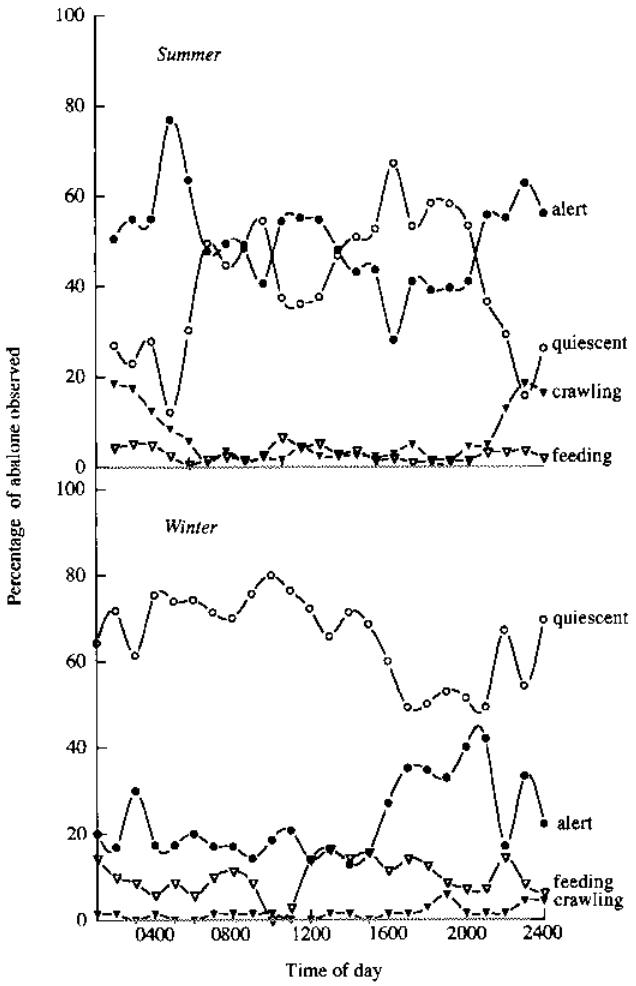

Figure 1. Seasonal differences in activity of *H. kamtschatkana* in the laboratory.

spent by an average laboratory-held abalone at each activity during summer and winter is shown in Table 1.

Field Activity

Abalone exhibited more activity in the warm summer months (Fig. 2). For example, about 20% of all abalone were observed to be crawling during June, August, and October. Few animals were crawling during the winter months; instead, they were most often quiescent or alert. No animals were observed feeding.

Videotaped Crawling Activity

Locomotion was not continuous, and the average abalone stopped and started again twice for every meter moved. Average rate of crawling was 1 body length min^{-1}.

TABLE 1.

Average amount of time spent each day by *H. kamtschatkana* in different activity states during summer and winter.

	Time (h)				
Season	**Quiescent**	**Alert**	**Feeding**	**Crawling**	**Totals**
Summer	9.8	12.0	0.7	1.5	24
Winter	15.8	5.5	2.3	0.4	24

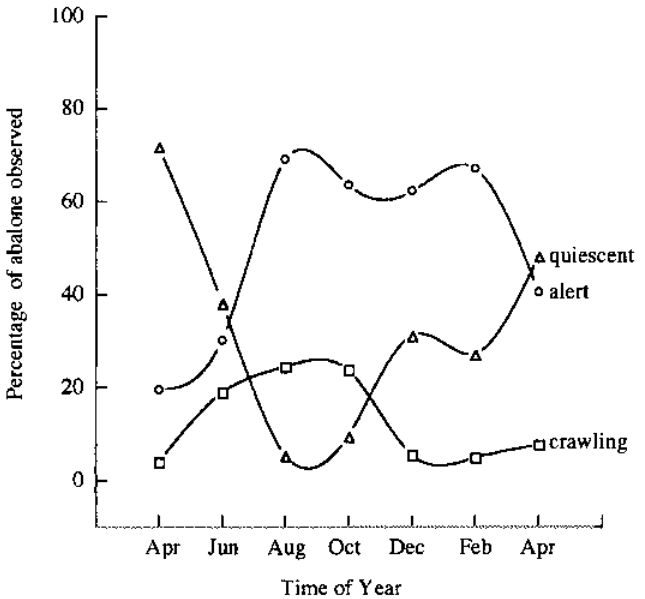

Figure 2. Field activity of *H. kamtschatkana* during daytime in Barkley Sound, British Columbia from April 1994 to April 1995.

Energy Budgets

There were no differences in values for any of the energy budget components between male and female abalone (all $t < 2.0$, all $p > 0.06$), save for some aspects of reproduction. Gonad energy content was higher in females than in males (females, 23.4 J mg of dry gonad $mass^{-1}$ ± 0.9 SE; males, 20.0 ± 0.5; $t = 3.21$, $p = 0.01$). However, there was no gender difference in total gonad mass (females, 10.3 mg of dry gonad mass g live abalone $mass^{-1}$ ± 1.7 SE; males, 9.4 ± 1.6; $t = 0.38$, $p = 0.71$) and ultimately no difference in yearly reproductive energy expenditure (females, 4.2 kJ y^{-1} ± 0.7 SE; males, 2.8 ± 0.3; $t = 1.89$, $p = 0.09$). Thus, values for both males and females were combined for the regressions of energy budget components on mass.

Regression equations for the five energy budget components not directly affected by activity (C, F, U, P_g, and P_r) are presented in Table 2. None of the slopes of the summer regression equations were significantly different from the winter regressions (all $t < 0.66$, all $p < 0.10$), except for the slopes of somatic growth on mass ($t = 2.02$, $p < 0.05$). However, all y-intercepts of the summer regressions were significantly higher than those of the winter regressions (all $t > 3.44$, all $p < 0.005$). Thus, except for somatic growth, the scaled relationship between energy and size remained constant between summer and winter, but summer values were greater than winter values.

Oxygen consumption increased with activity level over a wide range of abalone mass (Fig. 3). The slopes of the $\log_{10}$-$\log_{10}$ transformed regressions of oxygen consumptions on mass for quiescent, alert, and active abalone were not significantly different ($F_{[0.05(2),2,56]} = 0.43$, $p > 0.05$; analysis of covariance [ANCOVA]), but the y-intercepts were ($F_{[0.05(2),2,56]} = 32.7$, $p < 0.001$; ANCOVA). For a 50-g abalone, then, oxygen consumption increased 33% from quiescent to alert, and by a further 29% from alert to active.

Regressions of $\log_{10}$ respiratory energy on $\log_{10}$ mass during summer and winter for quiescent abalone are described by the equations $\log_{10}R = 0.34 + 0.74 \log_{10}m$ (r^2 0.77, $t = 7.70$, $p <$

TABLE 2.

Regression statistics for the components of summer and winter energy budget components that are not directly affected by activity for the abalone *H. kamtschatkana* (n = 20).

Energy Budget Component (*Y*)	log*a*	*b*	r^2	*t*	p*
Summer					
Consumption (C)	2.19	0.64	0.59	5.15	<0.001
Feces (F)	1.45	0.64	0.59	5.15	<0.001
Nitrogen (U)	−1.19	0.61	0.21	2.11	0.050
Somatic growth (P_g)	1.08	0.56	0.33	2.72	0.016
Reproductive growth (P_r)	0.45	0.79	0.60	4.20	0.001
Winter					
Consumption (C)	2.00	0.55	0.46	3.68	0.002
Feces (F)	1.11	0.55	0.46	3.68	0.002
Nitrogen (U)	−2.03	0.91	0.27	2.56	0.020
Somatic growth (P_g)	−2.39	1.33	0.57	3.99	0.002

Regression statistics are for the equation $\log Y = \log a + b \log m$, where Y is an energy budget component in J day^{-1} and m is mass in g.

* Values are derived from student's *t*-tests.

0.001) and $\log_{10}R = 0.05 + 0.78 \log_{10}m$ ($r^2 = 0.73$, $t = 6.97$, $p < 0.001$), respectively, where R represents respiratory energy (J h^{-1}) and m represents mass (g). When respiratory energy expended by a 50-g abalone, calculated from these equations, is combined with increases measured in respiratory energy for the different activity states, it can be seen that during the summer, a 50-g abalone will expend 40 J h^{-1} in the quiescent state, 53 J h^{-1} in the alert state, and 68 J h^{-1} in the active state. Likewise, during the winter, a 50-g abalone will expend 24 J h^{-1} in the quiescent state, 32 J h^{-1} in the alert state, and 41 J h^{-1} in the active state. Additionally, respiratory energy expenditure during crawling can be estimated from the cost of transport of *H. kamtschatkana*. The total cost of transport of a 50-g *H. kamtschatkana* crawling at one shell length·min^{-1} (7.1 cm min^{-1}) is 169 J kg^{-1}m^{-1} (calculated from the multiple regression of $\log_{10}$ total cost of transport on $\log_{10}$ mass and $\log_{10}$ speed; Donovan and Carefoot 1997). Thus, a 50-g abalone uses 8 J m^{-1} while crawling.

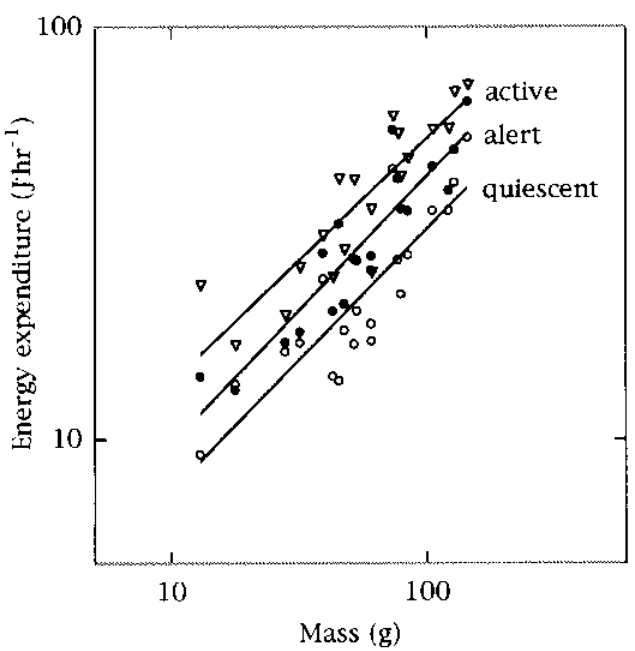

Figure 3. Regressions of $\log_{10}$ energy expenditure (J h^{-1}) on $\log_{10}$ mass for abalone in three activity states. Regression equations are: quiescent $\log_{10}$energy = −2.82 + 0.67 $\log_{10}$mass; alert $\log_{10}$energy = −2.76 + 0.70 $\log_{10}$mass; active $\log_{10}$energy = −2.53 + 0.63 $\log_{10}$mass. All regressions were significant (all $t > 11.26$, all $p < 0.001$).

Amounts of energy lost as secretion of mucus on adherence to the substratum are described by the regression equations $\log_{10}M = -0.17 + 0.86 \log_{10}m$ ($r^2 = 0.46$, $t = 3.67$, $p = 0.002$) and $\log_{10}M = -0.65 + 0.85 \log_{10}m$ ($r^2 = 0.27$, $t = 2.60$, $p = 0.018$), for summer and winter animals, respectively, where M represents energy from secretion of mucus (J adherence^{-1}) and m represents mass (g). Thus, a 50-g abalone will expend 20 J of energy adhering to the substratum in summer and 6 J in winter. Abalone secrete 0.12 μg of dry mucus cm^{-2} foot area for every centimeter they crawl (Donovan and Carefoot 1997). A 50-g (71-mm) abalone has a foot area of 18 cm^2 (Donovan and Carefoot, 1997), which yields a secretion rate of mucus of 2.2 μg of dry mucus cm^{-1} crawled. This converts to 5 J m^{-1} lost as mucus during crawling, which can be added to the respiratory energy expenditure during crawling of 8 J m^{-1} noted above to get an overall energy cost of 13 J m^{-1}.

Values for daily respiratory energy expenditure and secretion of mucus for a 50-g abalone during summer and winter are presented in Table 3. These calculations combine the time budgets from Table 1 with the energetic costs of the different activity states. In turn, these respiration and secretion of mucus values are presented with values for the other energy budget components (calculated from the regressions in Table 2 for a 50-g abalone) in Table 4.

DISCUSSION

In order to asses the effect of activity on the seasonal energy budgets of *H. kamtschatkana*, daily respiratory energy expenditure and secretion of mucus in the absence of activity must be estimated. For respiratory energy, this can be accomplished by extrapolating summer and winter quiescent energy equivalents (40 and 24 J h^{-1}, respectively) over a 24-h period. Thus, a 50-g abalone would expend 960 J day^{-1} during summer and 576 J day^{-1} during winter if it were completely quiescent. Daily secretion of mucus in the absence of activity can be estimated by assuming that the abalone adheres to the substratum once per day and then remains still. In that case, a 50-g abalone would expend 20 J day^{-1} during summer and 6 J day^{-1} during winter. This estimate of respiratory energy expenditure for inactive abalone would then represent 51% of total daily energy consumption in summer, compared with 59% when activity is accounted for (Table 4), a difference of 8%. Likewise, the estimate of secretion of mucus for an inactive abalone in summer would represent only 1% of daily consumption, rather than 16%, a difference of 15%. Thus, activity accounts for 23% of summer energy consumption. In the same manner, activity accounts for 13% of winter energy consumption.

In fact, because abalone crawl less in the laboratory than they do in the wild, we have probably underestimated the activity component of the energy budget of field *H. kamtschatkana*. Abalone are known to increase foraging activity when food is scarce (Poore 1972, Shepherd 1973, Sloan and Breen 1988), which did not occur in the laboratory. Likewise, field abalone must crawl to find refugia and escape predation. Shepherd (1986) has shown that motility of *Haliotis laevigata* is related to crevice space, with abalone increasing the amount they crawl until they find a suitable crevice.

TABLE 3.

Total daily respiratory and energy expenditures of mucus for a 50-g abalone.

Parameter	Time (h)	Energy Equivalents (J h^{-1})	Distance Moved (m day^{-1})	Cost of Transport (J m^{-1})	Total Energy (J day^{-1})		No. of Adherences per Day	Energy Equivalents (J $adherence^{-1}$)	Distance Moved (m day^{-1})	Energy Equivalent (J m^{-1})	Total Energy (J day^{-1})
Respiration (R)						Mucus (m)					
Summer						Summer					
Quiescent	9.8	40			392	Adherence	14	20			280
Alert	12.0	53			636	Crawling			6	5	30
Feeding	0.7	68			48						310
Crawling			6	8	48						
					1,124						
Winter						Winter					
Quiescent	15.8	24			379	Adherence	4	6			24
Alert	5.5	32			176	Crawling			2	5	10
Feeding	2.3	41			94						34
Crawling			2	8	16						
					665						

The daily time budget (Table 1) was integrated with energetic costs of each activity. Summer and winter quiescent respiratory energy rates (J h^{-1}) were calculated from regressions of respiratory energy on mass (see Text for regression equations). Alert and feeding rates (the latter assumed to be equivalent to active rates) were calculated from increases over quiescent rates determined by measuring oxygen consumption during different activity states. Distance moved (m day^{-1}) was estimated from average time spent crawling (1.5 h in summer, 0.4 h in winter; Table 1) and average crawling rate (7.1 cm min^{-1}; videotape data). Energetic cost of this movement (cost of transport) was estimated from Donovan and Carefoot (1997). Number of adherences per day was estimated from number of crawling bouts per meter over total distance (videotape data), and energy equivalent of mucus was estimated from Donovan and Carefoot (1997).

Predators of *H. kamtschatkana* include octopus, crabs, fish, and seastars (Sloan and Breen 1988), and *H. kamtschatkana* exhibits a dramatic crawling escape response in the presence of the seastar *Pycnopodia helianthoides*. Our comparison of activity levels in laboratory and field also supports the idea that field abalone are more active than laboratory-held abalone. During observations of field abalone by SCUBA divers in June, 19% of all abalone observed were crawling during daytime. This was not the case during the daytime summer observations in the laboratory, where only 2–5% of abalone were crawling. Winter daytime values were closer to each other, with 5% of abalone crawling in the field in both December and February and 0–2% crawling in the laboratory.

As expected, values of summer and winter energy budget components differed for *H. kamtschatkana*, much of it due to differences in activity level. For a representative 50-g abalone, winter consumption was only 45% of summer consumption, with nearly all consumed energy going toward maintenance (R, M, and U; Table 4). The largest component of the winter energy budget was respiration, accounting for 77% of all consumed energy. Respiration was also the largest component of the summer budget, but the proportion of consumed energy going toward this component was only 59%, owing to increases in somatic and reproductive growth. Costs of mucus were nine times greater in summer than winter, and mucus accounted for 16% of consumed energy during summer. The proportions of energy lost as feces and nitrogenous waste remained relatively constant between summer and winter. For the summer budget, all energy consumed was accounted for (actually, overestimated by 3%), and for the winter budget, 94% was accounted for.

Two other energy budgets for abalone, one by Peck et al. (1987) for the European abalone *Haliotis tuberculata* and one by Barkai and Griffiths (1988) for the South African abalone *Haliotis midae*, are shown in Table 5. Our summer energy budget (Table 4) can be compared with that of Peck et al. (1987), who appear to have determined a summer energy budget for their species on the

TABLE 4.

Values for each component of summer and winter energy budgets for a representative 50-g *H. kamtschatkana*.

	Energy (J day-1)								
Season	C	F	U	P_g	P_r	R	M	Total	% Total of C
Summer	1,894	345	<1	108	62	1,124	310	1,949	
% of C		18	<1	6	3	59	16		103
Winter	860	111	<1	1	0	665	34	811	
% of C		13	<1	<1	0	77	4		94

Values for components not directly affected by activity (C, F, U, P_g, and P_r) were calculated from regressions from Table 2. Values for respiration (R) and mucus (M) are from Table 3.

TABLE 5.

Energy budgets for three species of abalone expressed as percentages of C.

Species	F	U	P_g	P_r	R	M	Total % of C
*H. kamtschatkana** (summer)	18	<1	6	3	59	16	103
H. tuberculata†	18	1	13	4	27	26	89
H. midae‡	63	<1	5		8		76

Percentages of respiration and mucus from this study were calculated from the summer activity budget and summer energy budget. The percentages for *H. tuberculata* were calculated from regression equations in Peck et al. (1987) for animals of a size similar to the representative 50-g abalone used for this study. The proportions for *H. midae* have no entry of mucus and P_g and P_r were estimated as a single value.

* This study.

† Peck et al. (1987).

‡ Barkai and Griffiths (1988).

basis of their data for reproduction. *H. kamtschatkana* has a much higher respiration component than does *H. tuberculata* (59% of consumed energy compared with 27%, respectively), whereas *H. tuberculata* diverts more energy to growth and production of mucus. Interestingly, mucus was as large a component for *H. tuberculata* as was respiration, accounting for 26% of consumed energy, whereas it was less for *H. kamtschatkana* (16%). Barkai and Griffiths (1988) found that *H. midae* loses 63% of consumed energy to feces, a much larger proportion than measured for either *H. kamtschatkana* or *H. tuberculata* (both 18%). This is most likely because of the different kelp species used as food in the different studies. Kelp vary widely in morphological and physiological defenses to herbivory (Hay and Steinberg 1992, Lobban and Harrison 1997), resulting in digestibility differences for the herbivores. The respiration component for *H. midae* was only 8% of consumed energy, but the authors note that it would be higher if they had incorporated activity into their measurements. All three energy budgets indicate little energy lost to nitrogen excretion.

This study has shown that activity has potentially large effects on both summer and winter energy budgets of the abalone *H. kamtschatkana*. In the field, it is likely that more energy would be expended on activity, especially in areas where food is scarce or predation intense, and this has potential ramifications to the dynamics of abalone populations. In the case of predators, such as seastars, eliciting a crawling escape response, intense predation would not only affect population dynamics by increasing mortality, but could also cause decreased somatic growth and reproductive effort as energy is diverted from these energy budget parameters to respiratory and mucous components.

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CLASSIFICATION OF NEUROSECRETORY CELLS, NEURONS, AND NEUROGLIA IN THE CEREBRAL GANGLIA OF *HALIOTIS ASININA* LINNAEUS BY LIGHT MICROSCOPY

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ABSTRACT The gross anatomical study of the nervous system of *Haliotis asinina* reveals that it comprises a pair of cerebral ganglia, a buccal ganglion, a pleuropedal ganglion mass, and a visceral ganglion, connected together by nerve commissures and connectives. There are eight types of nerve cells in the cerebral ganglia on the basis of their histological characteristics and stain affinity: two types of neurosecretory cells, three types of neurons, and three types of neuroglia. The neurosecretory cells are large and occur along the periphery of the ganglia. They contain neurosecretory granules in the cytoplasm that stained deep violet with paraldehyde-fuchsin. The neurons are the most numerous cell type and occur in various parts of the cortex. The neuroglia are small cells and contain spindle-shaped nuclei.

KEY WORDS: abalone, cerebral ganglia, *Haliotis asinina*, histological study

INTRODUCTION

The abalone is one of the most primitive gastropods in form and structure. The central nervous system of abalone is of the streptoneurous type, with no concentration of neuronal mass, and it consists of several ganglia connected by connectives and commissures (Bullock 1965, Joosse 1979). Most ganglia are elongated and flattened, and sheaths of ganglion cells usually extend to form flat commissures and connectives linking ganglia together (Crofts 1929).

The cerebral ganglia are the most anterior ganglia in the head. They are paired, lie above the esophagus or buccal mass, and are connected by a long commissure (Dorsett 1986). The cerebral ganglia have connectives to the buccal, pleural, and pedal ganglia, and they send nerves to innervate the eyes, statocysts, and head tentacles (Crofts 1929, Bullock 1965), thus playing an important role in guiding the animals around their habitat.

The cerebral ganglia of several temperate abalone species have been extensively studied., e.g., *Haliotis tuberculata* Linnaeus, *Haliotis lamellosa* Lamarck, *Haliotis cracherodii* Leach, *Haliotis rufescens* Swainson, *Haliotis discus hannai* Ino, and *Nordotis discus* Reeve (Crofts 1929, Miller et al. 1973, Hahn 1994a, Yahata 1971). Several types of neurons and neurosecretory cells have been described in the ganglia (Miller et al. 1973, Yahata 1971, Hahn 1994a). Yahata (1971) and Hahn (1994a) reported that there were four types of neurons in the cerebral ganglia of *N. discus* and *H. discus hannai*, which were designated as types A, B, C, and D. Type A and Type B cells were believed to be neurosecretory cells. This article reports on the gross anatomy of the nervous system and the classification, based on the histological characteristics of neurons and other cell types in the cerebral ganglia of *Haliotis asinina* Linnaeus, which is a tropical abalone species native to Thailand.

MATERIALS AND METHODS

Anatomical Study

Mature abalone, *H. asinina*, with a shell length of 4–5 cm were obtained from the Coastal Aquaculture Development Center, Klong Wan, Prachuap Khiri Khan Province, Thailand. These animals were reared in a land-based aquaculture system in well-circulated and aerated seawater. They were given appropriate algal food *ad libitum* and kept under normal daylight. Abalone were anesthesized with 5% MgCl, after which their shells were removed. They were then placed on a layer of paraffin wax poured on an enamel pan and immersed in 70% alcohol. The dissections were made under a stereomicroscope, which was also used to make drawings with the aid of a camera lucida.

Histological Study

The cerebral ganglia were dissected out and fixed in Bouin's fluid in 0.14 NaCl for 12 h. Tissues were dehydrated through a graded series of ethanol, infiltrated with dioxane, and embedded in paraffin. Sections were cut at 5–6 μm thickness and stained with hematoxylin-eosin (H&E), chrome-hematoxylin-phloxine (CH-P) (Gomori 1941), and paraldehyde-fuchsin (PF) (Gomori 1950). Neurons and cells in the cerebral ganglia were observed and evaluated for their cell size and shape, nuclear size and shape, and staining affinity under an Olympus Vanox light microscope.

RESULTS

Anatomical Study

The nervous system of *H. asinina* consists of a pair of cerebral ganglia, a buccal ganglion, a pleuropedal ganglion mass, and a visceral or abdominal ganglion. These ganglia are connected by nerve commissures and connectives (Fig. 1). The cerebral ganglia are connected by dorsal and ventral cerebral commissures. Thus, the ganglia and commissures surround the anterior esophagus. The middle part of the ventral cerebral commissure swells into a small buccal ganglion. Two major nerves, i.e., the optic and tentacle nerves, join the cerebral ganglia separately (Fig. 1). Optic nerves innervate the eyes, whereas tentacle nerves innervate the tentacles. The cerebropleural and cerebropedal connectives leave the ventroposterior part of the cerebral ganglia and merge into a pleuropedal ganglion mass. Arising from this mass are two loops of nerve cords: the visceral cord and the paired pedal nerve cords. The visceral cord twists into a figure 8 around the visceral mass. At its posterior end is a single visceral ganglion that gives off many nerves going to digestive and reproductive organs. The paired pedal nerve cords run parallel along the midline of the foot muscle (Fig. 1). The two pedal cords are connected at several intervals by pedal cord commissures. From each pedal cord, many nerves arise to innervate the foot muscles (Fig. 1).

Histological Study

The paired cerebral ganglia are elongated and flattened (Fig. 2A). The ganglia are composed of two parts: the outer cortex and the inner medulla (Fig. 3A). Each ganglion is surrounded by a loose connective tissue rich in collagen-like fibers and capillaries (Fig. 2B). In the cortex, there are numerous neurons, neurosecretory cells, and neuroglia. The outermost cell layer of the cortex is in close contact with the basement membrane of the ganglion capsule. Most of the medial part of the cortex is relatively thin and contains only two to three layers of cells, with only a few neurosecretory cells. In contrast, the dorsal, ventral, and lateral parts of the cortex of each ganglion are thick and contain four to five cell layers (Fig. 3A, see Fig. 5A). Although more neurosecretory cells are dispersed throughout these regions, there are especially high concentrations of these cells in the dorsal and ventral parts, or "horns" of the ganglion (Fig. 3A).

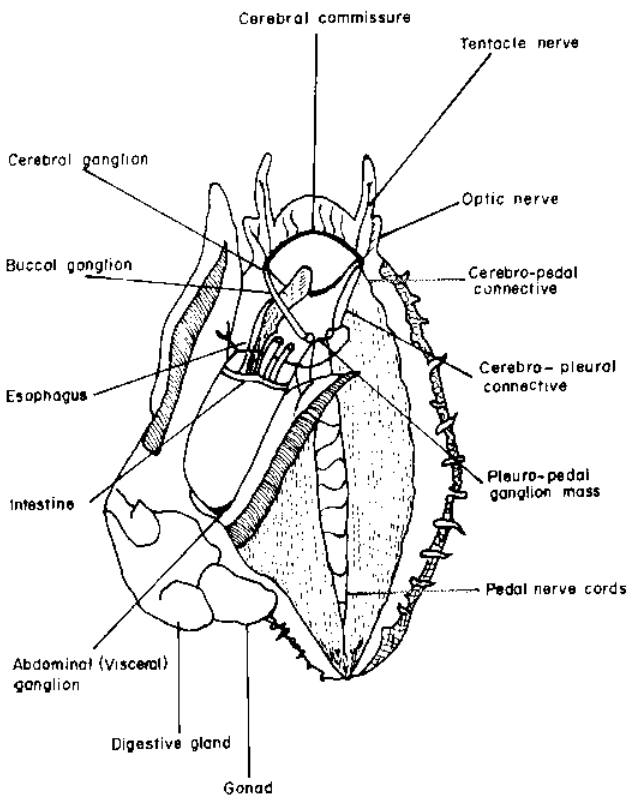

Figure 1. A diagram of the nervous system of *H. asinina*, showing various nerve ganglia linked together by nerve cords and connectives.

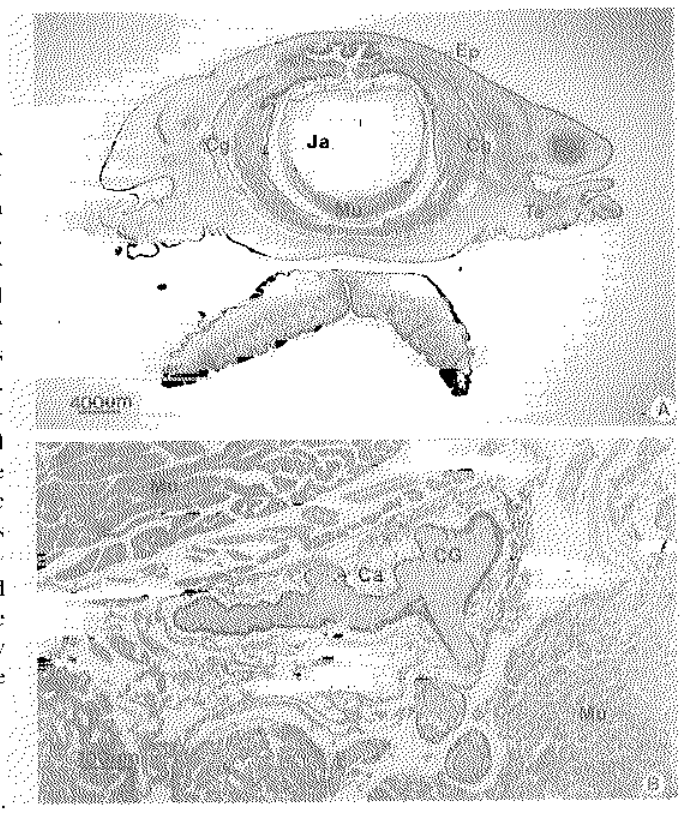

Figure 2. (A) Photomicrograph of longitudinal section showing location of cerebral ganglia (Cg) lying on both sides of jaw (Ja) and jaw muscle (Mu). Ep, epidermis; Te, tentacle. (B) A high magnification of longitudinal section of cerebral ganglion (CG). Ca, capillary.

The cells in the cerebral ganglia can be classified into eight types on the basis of their histological characteristics and stain affinity to dyes (H&E, CH-P, and PF). There are two types of neurosecretory cells (NS_1 and NS_2), three types of neurons (NR_1, NR_2, and NR_3), and three types of neuroglia (NG_1, NG_2, and NG_3).

Type 1 Neurosecretory Cell (NS_1)

These cells are very large in size (10 × 20 μm), with an oval shape. Most cells occur along the periphery of the cortex, resting on the basement membrane (Fig. 3D, see Fig. 6). The nucleus is round (8 μm in diameter) and is located toward one side of the cell. It contains mostly pale-stained euchromatin with only a thin rim of heterochromatin binding to the internal surface of the nuclear envelope (Figs. 3C and D and 4B). The nucleolus, which is round in

shape, is very distinct (Fig. 4B and C). The cytoplasm is well preserved and shows a clear boundary. It stained reddish pink with H&E and pinkish purple with CH-P. There are numerous neurosecretory granules, which stained deep violet with PF and filled the entire cytoplasm.

Type 2 Neurosecretory Cell (NS_2)

These cells are smaller than NS_1 and occur in the same layer as NS_1 and also in the inner cell layer (Fig. 3D and 4A, see Fig. 6). The cell body is round or oval and of medium size (10 × 12 μm). The nucleus is round (10 μm in diameter), with most blocks of heterochromatin attached to its periphery with some in the center. Together, they resemble a clock-face pattern (Figs. 3C and D and 4C). The nucleolus is not as prominent as in NS_1. The cytoplasm contains fewer neurosecretory granules than those of NS_1, and they stained deep violet with PF.

Type 1 Neuron (NR_1)

These cells are the largest neurons and have a pyramidal shape (15 × 30 μm) (Figs. 3D, 4D, and 5C). The nucleus is round (10 μm in diameter) and contains almost entirely euchromatin with eccentrically located nucleolus (Fig. 3D). The basal portion of the cell is flattened and lies on the basement membrane. Their slimmer cytoplasmic processes extend inward to the medulla of the ganglion (Figs. 3D, 4D, and 6). The cytoplasm stained homogeneously pink with H&E and CH-P. There are no neurosecretory granules in the cytoplasm.

Type 2 Neuron (NR_2)

These cells are the most numerous among neuronal cells. They are concentrated mostly in the middle cell layer of the cortex (Figs. 3D and 6). They have a round to oval shape (4–6 μm in diameter)

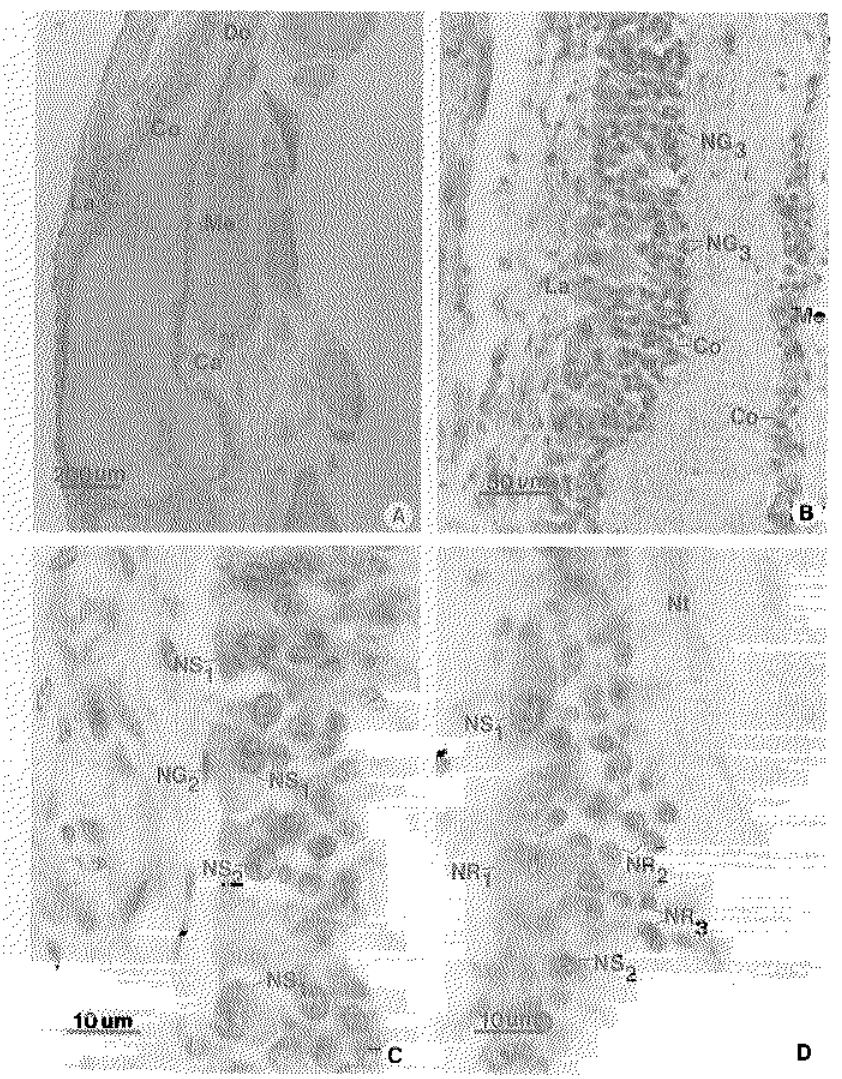

Figure 3. (A) A low-power micrograph showing longitudinal section of the upper half of a cerebral ganglion stained with H&E. Co, cortex; Ca, capillary; Do, dorsal; Me, median; La, lateral. (B) A medium-power micrograph showing longitudinal section of the cerebral ganglion stained with H&E. Notice thicker cell layers on the lateral (La) than medial (Me) sides. NG_3, Type 3 neuroglia. (C) A high-power magnification showing various types of nerve cells in the cortex stained with H&E. NS_1, Type 1 neurosecretory cell; NS_2, Type 2 neurosecretory cell; NG_2, Type 2 neuroglia. (D) A high-power magnification showing various types of nerve cells in the cortex stained with H&E. NR_1, Type 1 neuron; NR_2, Type 2 neuron; NR_3, Type 3 neuron; Nt, nerve tract.

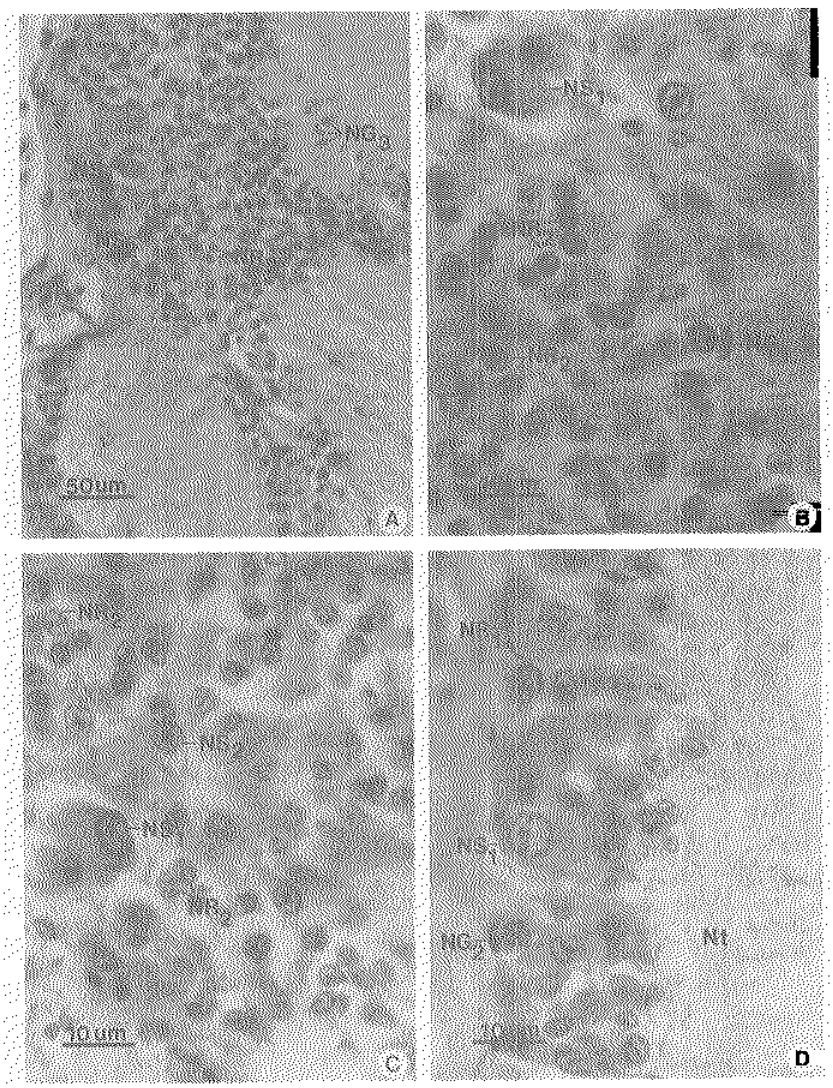

Figure 4. Medium-power (A) and high-power (B–D) micrographs showing various types of nerve cells in the cortex stained with H&E. NS_1, Type 1 neurosecretory cell; NS_2, Type 2 neurosecretory cell; NR_1, Type 1 neuron; NR_2, Type 2 neuron; NG_1, Type 1 neuroglia; NG_2, Type 2 neuroglia; NG_3, Type 3 neuroglia; Nt, nerve tract.

and contain round nuclei (4–6 µm in diameter) with patchy heterochromatin. The cytoplasm is extremely thin and does not contain neurosecretory granules (Figs. 3D and 4B and C).

Type 3 Neuron (NR_3)

These cells are a little smaller than NR_2, about 4 µm in diameter. They occur in the innermost cell layer of the cortex (Figs. 3D and 6). The nucleus is elliptical (3 µm in diameter) and contains completely dense heterochromatin (Fig. 3D). There are no neurosecretory granules in the cytoplasm.

Type 1 Neuroglia (NG_1)

These cells are scattered throughout the cortical region of the ganglion (Figs. 4B and 6). They are small (3–6 µm in diameter) and contain a spindle-shaped nucleus (Fig. 4B). The nuclear membrane is a little crenated, with a thin rim of heterochromatin attached to its inner surface, whereas most of the remaining chromatin is euchromatic (Fig. 4B).

Type 2 Neuroglia (NG_2)

The cell body and nuclear size of these cells are similar to those of NG_1, but they show completely dense chromatin (Figs. 3C and 4D). NG_2 lie in a single row on the basement membrane (Figs. 4D and 6).

Type 3 Neuroglia (NG_3)

These are small cells with spindle-shaped nuclei (2–3 µm) that contain completely dense heterochromatin. They are scattered among nerve bundles of the medulla (Figs. 3B, 4A, 5D, and 6).

DISCUSSION

The anatomy of the nervous system of the tropical abalone, *H. asinina*, is similar to those of primitive prosobranchs and other species of abalone described by Crofts (1929) and Fretter and Graham (1962). Crofts (1929) reported that in *H. tuberculata*, *H. lamellosa*, and *H. cracherodii*, the cerebral ganglia sent the nerves to supply the epipodia, tentacles, eyes, and statocysts. Through

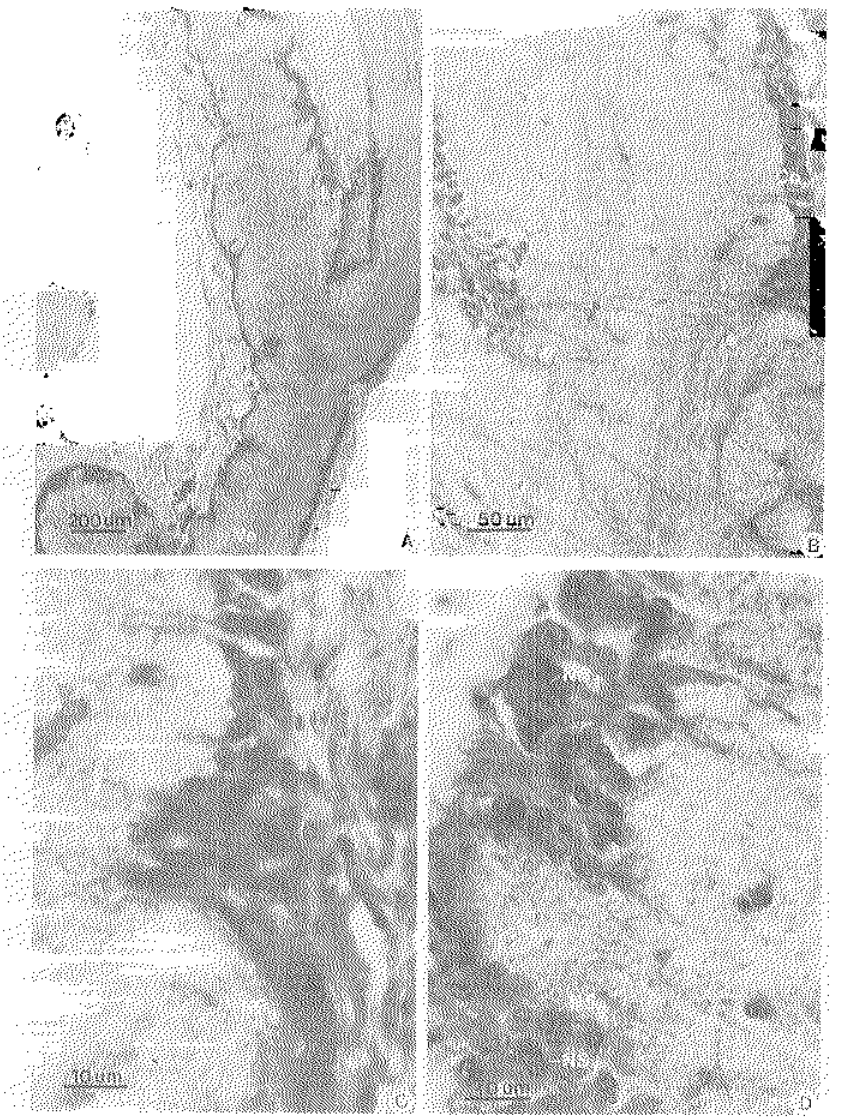

Figure 5. A low-power micrograph showing longitudinal section of cerebral ganglion stained with CH-P. Do, dorsal; La, lateral; Ve, ventral. (B–D) Medium-power (B) and high-power (C and D) micrographs showing longitudinal sections of cerebral ganglion stained with CH-P. Co, cortex; Np, neuropil; NG_3, Type 3 neuroglia; NS_1, Type 1 neurosecretory cell; NS_2, Type 2 neurosecretory cell; NR_1, Type 1 neuron; NR_2, Type 2 neuron; NG_3, Type 3 neuroglia.

these nerves, the animals receive chemosensory, mechanosensory, and visual input from their environment. Hence, the cerebral ganglia are probably the most important center for nervous integration, comparable to the brain in higher animals. The cerebral ganglia in *H. asinina* are also connected with the buccal ganglion and pleuropedal ganglion mass. It was, therefore, suggested that the cerebral ganglia could serve as a center for coordinating and modulating various functions mediated by the rest of the nervous system (Jahan-Parwar and Fredman 1976).

The cerebral ganglia of *H. asinina* are surrounded by a loose connective tissue that is rich in collagen-like fibers, threaded with capillary plexuses. This connective tissue sheath is quite different from that of *Helix aspersa* Muller, which is composed of two layers, the outer layer being packed with globuli cells and the inner being dense and lamellated (Fernandez 1966). The histological study presented here of the cerebral ganglia of *H. asinina* revealed that they contain eight cell types: two types of neurosecretory cells, three types of neurons, and three types of neuroglia. Yahata (1971) and Hahn (1994a) described four types of neurons in *N. discus* and *H. discus hannai*. They are called Type A, Type B, Type C, and Type D cells. Type A and Type B cells are neurosecretory cells. On the basis of the similarities in size and shape and their nuclear characteristics, distribution, and staining affinity, the neurosecretory cells Type 1 (NS_1) and Type 2 (NS_2) in this study should correspond to Type A and Type B cells, respectively, as reported by Yahata (1971) and Hahn (1994a). In *N. discus*, Type A cells were further divided into Type A-I cells, which contain neurosecretory granules stained with PF and CH-P, and Type A-II cells, the secretory granules of which stained with phloxine but not CH-P. Type A-II cells of *H. discus hannai* appear to be larger (20–32 μm) than the NS_1 of *H. asinina* (20 μm). However, the cell bodies of both Type A cells (Hahn 1994a) and NS_1 stained with PF.

Neurosecretory cells are found in large quantities and variety in molluscan ganglia, which are the principal source of hormones. The functions of neurosecretory cells in the cerebral ganglia are

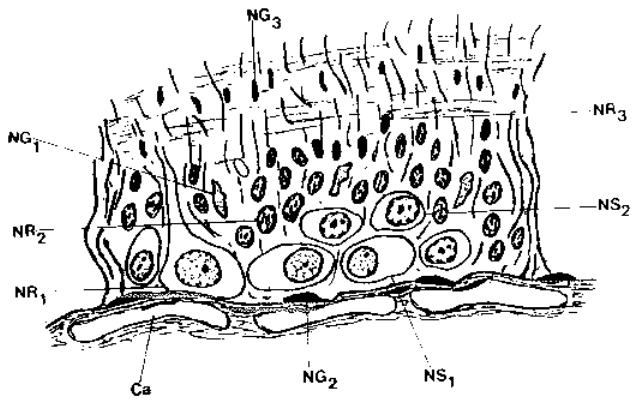

Figure 6. A diagram illustrating structure and cellular composition of the cortex and medulla of cerebral ganglion. NS_1, Type 1 neurosecretory cell; NS_2, Type 2 neurosecretory cell; NR_1, Type 1 neuron; NR_2, Type 2 neuron; NR_3, Type 3 neuron; NG_1, Type 1 neuroglia; NG_2, Type 2 neuroglia; NG_3, Type 3 neuroglia; Ca, capillary.

thought to be related to reproduction (Yahata 1971, Yahata 1973, Hahn 1994a, Hahn 1994b). Yahata (1971) found that Type A and Type B cells in the cerebral ganglia of *N. discus* showed seasonal changes in the staining intensity of PF. These cells began to accumulate PF granules in June, when the gonads started to mature, and the PF stain intensity continued to increase until it reached a maximum in September, which was the month of spawning. Hence, the rise and fall of the neurosecretory material coincided with gonadal maturation (Yahata 1971). However, the injection of crude homogenate of the cerebral ganglia into ripe females, *N. discus*, did not induce spawning, but there was a considerable gain in the mean body weight from the increase in water uptake (Yahata 1973).

Hahn (1994a) reported that the neurosecretory activities in Type A and Type B cells in the cerebral ganglia of *H. discus hannai*, as reflected by the staining intensity of cytoplasmic material, varied with the reproductive cycles. The neurosecretory activity of Type A cells was correlated with vitellogenesis in the ovaries of females, but not with gonad maturation and spermatogenesis in males. The neurosecretory activity in Type B cells in both sexes did not show any correlation with gametogenesis, vitellogenesis, or spawning. Further studies are clearly needed on the neuroendocrine activities and functions of neurosecretory cells of cerebral ganglia in abalone, including NS_1 and NS_2 cells in *H. asinina*.

There are three types of neurons in the cerebral ganglia of *H. asinina*, whereas only two types of neurons (Type C and Type D cells) were described in *N. discus* and *H. discus hannai* (Yahata 1971, Hahn 1994a). On the basis of the size and shape of cells and their nuclei, NR_2 are quite similar to Type C cells, whereas NR_3 probably correspond to Type D cells. These cells did not have any neurosecretory granules in their cytoplasm. NR_1 cells have not been reported in *N. discus* or *H. discus hannai* (Yahata 1971, Hahn 1994a). These cells are the largest neurons in the cerebral ganglia of *H. asinina*. They are pyramidal and multipolar in shape with a round nucleus, and no neurosecretory granules are observed in the cytoplasm. Compared with the classification of neurons in the nervous systems of higher vertebrates, it is possible that NR_1 may be concerned with motor activities because of their large size and multipolarity, whereas NR_2 and NR_3 are most likely to be associated neurons.

Three types of neuroglia were observed in the cerebral ganglia of *H. asinina*. To the best of our knowledge, there has not yet been any classification of neuroglia in any species of abalone. NG_1 are probably the general glia cells of the cortex because of their uniform distribution in all cell layers of the cortex. NG_2, because of their unique lining of the basement membrane, could be a part of the blood–nerve barrier that gates out the undesirable factors from the blood supplied by the capillaries. NG_3, on the other hand, are glia cells of the neuropil of the medullary region. It remains to be proved whether they are involved in the synthesis of the myelin-like structure surrounding the nerve fibers in the neuropil.

ACKNOWLEDGMENT

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RESPIRATION RATE AND THERMAL TOLERANCES OF PINTO ABALONE *HALIOTIS KAMTSCHATKANA*

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ABSTRACT Oxygen consumption rates were measured for pinto abalone, *Haliotis kamtschatkana*, held at 0.5–26.5°C. Thermal tolerances were inferred from respiration data, behavior, and survival at the test temperatures. At 0.5 and 26.5°C, pinto abalone were unable to maintain their attachment to surfaces and mortalities occurred. At 0.5°C, the first mortality occurred after 13 days, and by the end of 6 wk, 75% of the test animals had died. At 26.5°C, the first death was on Day 6, and by Day 10, all animals were dead. Between 2 and 24°C, no evidence of respiratory stress was evident and a linear relationship between oxygen consumption rate and temperature was observed.

KEY WORDS: pinto abalone, *Haliotis*, respiration, temperature

INTRODUCTION

A single species of abalone, *Haliotis kamtschatkana* Jonas 1845, occurs in Alaska, where it is harvested commercially on a small scale. Southeastern Alaska is the northern limit of their range, and it is reasonable to assume that their inability to function in the colder northern Gulf of Alaska and Bering Sea limits their northern distribution. Periodically, there is interest in culturing this cold-water species, commonly called pinto abalone, to supplement the limited natural populations. The benthic stages eat macroalgal species that are common throughout the Gulf of Alaska as well as graze on attached diatoms (Paul et al. 1977). They had also been reported to eat the planktonic diatom *Chaetoceros* (Paul et al. 1977), but that was a misidentification by one author of that article. They can be spawned in captivity, and the potential for rearing hybrids of this species in northern Japan has also been explored (Momma 1990). Thus, the species has many of the necessary attributes appropriate for artificial culturing. However, the medium-term thermal tolerances of pinto abalone are poorly understood. This information is needed to determine the suitability of different areas for the culture of this species and environmental requirements for keeping it in captivity and understanding its distribution. In this study, measurements of oxygen consumption were made to test the respiratory response of pinto abalone to thermal conditions between 0.5 and 26.5°C. The thermal range occurring in surface waters of Sitka, AK, the site where animals were captured for this study, is about 3 and 18°C (U.S. Dept. Commerce 1970), so our experiments included temperatures they would not normally encounter.

MATERIALS AND METHODS

Specimens of *Haliotis kamtschatkana* from Sitka, AK, were held captive for 3 y in the Seward IMS laboratory before these observations. During captivity, the abalone were continuously fed a variety of macroalgae with *Laminaria*, *Alaria*, *Ulva*, and *Rhodymenia* being the common genera. They also grazed on attached diatoms that grew on the sides of the tanks. They were held in 800-L tanks with seawater exchange rates of 100%/h.

Specimens were held at 0.5 (n = 12), 2 (n = 20), 4 (n = 16), 8.5 (n = 8), 12 (n = 16), 15 (n = 23), 17.5 (n = 24), 21.5 (n = 18), 24 (n = 8), and 26.5°C (n = 6) for the tests. The maximum variation in tank temperatures was ±0.5°C during any test. Oxygen consumption measurements were done at the appropriate season for the test temperature to occur in nature. Individuals were gradually acclimated to some test temperatures by the naturally occurring seasonal changes in the seawater entering the laboratory, which ranged from 3 to 11°C. When the incoming water reached 11°C, the temperature was artificially altered upward by 1°C/wk to reach the upper test temperatures in a thermally controlled room. After the incoming seawater temperature declined to 3.5°C, the temperature was artificially altered downward by 1.5°C per week to 2 and 0.5°C. The abalone were held within 1°C of the test temperature for at least 1 wk before oxygen consumption rate measurements were made.

Measurements were made to determine the role background oxygen levels had in governing respiration rates. This information was necessary to ensure that in the later, temperature-specific respiration measurements, background oxygen levels would not restrict oxygen uptake. Oxygen consumption rates at 8.5°C were determined for six individuals at the following background oxygen levels: 6.0–5.0, 5.0–4.0, 4.0–3.0, and 3.0–2.0 mL of O_2/L. Oxygen determinations were made in a 4.5-L chamber with a built-in magnetic stirrer and an Orion model 97-08 electrode that was calibrated by the Winkler technique. They were kept in these chambers for 3 days without food, and the measurement was made on the fourth day. On the fifth day, measurements were continually made as the background oxygen levels naturally declined as a result of oxygen use by the animals from a standard starting level of 6.0 to down to 2.0 mL of O_2/L. Each individual's respiration rate per unit of time was averaged for four different background oxygen levels: 6.0–5.0, 5.0–4.0, 4.0–3.0, and 3.0–2.0 mL of O_2/L. A range of animal weights were used to see if size would influence oxygen consumption relative to background oxygen levels and to help us select the size of animals that be appropriate for the temperature-specific experiments.

Abalone from 45 to 55 g live weight were used to examine the influence of temperature on oxygen consumption rates. The abalone were placed individually in 9-L open chambers immersed in a temperature-regulated seawater bath (±0.1°C). The chambers were aerated, and over them, the light level was a constant 0.3 lux.

They were kept in these chambers for 3 days without food, and the measurement was made on the fourth day. For measurements of oxygen consumption rates, chambers were sealed for 1–24 h, depending on water temperature. Salinities in all experiments ranged from 32 to 33‰. Chambers were sealed long enough so that oxygen levels decreased by at least 1 mL of O_2/L, but they were not closed long enough for oxygen background levels to drop below 3 mL of O_2/L. Initial and final oxygen determinations were made with a Monostat Digipet pipette and the Winkler titration method on BOD samples (Strickland and Parsons 1972). Afterwards, abalone were held at the test temperature and fed regularly for 6 wk to note survival after prolonged exposure.

RESULTS

Oxygen consumption rates were not modified markedly by background oxygen levels above 2 mL of O_2/L (Table 1). Because values of background oxygen levels were not allowed to fall below 3 mL of O_2/L in observations of respiration rate relative to water temperature, they did not conspicuously affect those results.

During the acclimation period, before measurement of respiration rates, behavioral anomalies were observed in abalone held at 0.5 and 26.5°C. At these temperatures, the abalone were not able to right themselves, remaining on the chamber's floor rather than attaching to a surface. Respiration rates were determined for all 12 of the 0.5°C test animals while they were in this state. They had an average oxygen consumption rate of 6.3 µL/g per h (Fig. 1A). The first death in this group occurred on Day 13 of existing at 0.5°C, and by the end of 6 wk, 75% of this test group had died. Not all of the planned measurements of oxygen consumption rates at 26.5°C were completed because there was 100% mortality in that test group (n = 6) during the study. Deaths occurred on Days 6 (n = 1) and 7 (n = 2) of existing at the test temperature. Three of six abalone in a live but distressed condition survived long enough to be tested, and they had an average oxygen consumption rate of 9.7 µL/g per h (Fig. 1A). This was a marked deviation from the previous trend of increased respiration rate with elevation of water temperature. All three of these animals died after 10 days of exposure to test temperature.

For pinto abalone 45–55 g live weight, rates of oxygen consumption generally increased as the holding temperature went from 0.5 to 24°C (Fig. 1A). No obvious behavior abnormalities were noted in the 2–24°C test groups. When the mean values for oxygen consumption rates were plotted for test animals held between 2 and 24°C, a linear relationship (df = 7, $p < 0.001$) between oxygen consumption rate and temperature was evident (Fig. 1B). There were no mortalities in the groups of animals held at other than extreme test temperatures.

TABLE 1.

Oxygen consumption (µL/g per h) by *H. kamtschatkana* at background oxygen concentrations between 6.0 and 2.0 mL of O_2/L, 8.5°C; observations were made on six individuals ranging from 22 to 111 g.

Initial Oxygen Concentration (mL of O_2/L)	Oxygen Consumption (µL/g per h)					
6.0–5.0	4	4	5	5	5	9
4.9–4.0	8	4	5	5	8	8
3.9–3.0	9	4	7	5	6	8
2.9–2.0	7	4	6	5	5	8
Mean oxygen consumption rate	7	4	6	5	6	8
SD	1	2	2	1	1	1
Abalone weight (g)	22	25	56	95	100	111

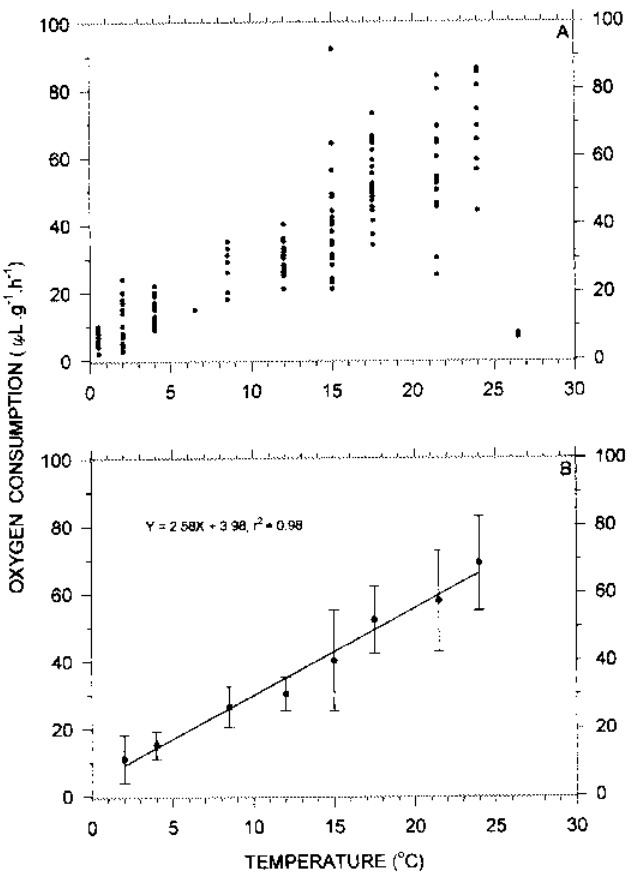

Figure 1. Oxygen consumption (µL/O_2) per gram whole weight per hour) by *H. kamtschatkana* at different temperatures. Datum points for individual animals occur in Panel A, and mean values (●) and vertical bars representing ±1 SD are shown in Panel B.

DISCUSSION

Although there are some published measurements of thermal modification of respiration rate for other species of abalone, few of them were done at the low temperatures occurring in Alaska. Also, the methods used in this study precluded a good understanding of the respiratory biology of pinto abalone. In this study, the methods did not consider several factors that might alter respiration rate. Many of our incubation times were too short to account for diurnal activity patterns, only fasting animals were used in the measurements so specific dynamic activity was not accounted for, and the specimens were all from a restricted weight range. The energetic cost of locomotion was not accounted for, and there may be a seasonal cycle of respiration rate that is not described.

The results of this study provide an indication of medium-term thermal tolerances for Alaskan pinto abalone, but the experiments are inadequate to assess the long-term survival potential of abalone at either 2 or 24°C. It is known that the shells of pinto abalone grow little when the water is <8.5°C (Paul and Paul 1981), but the optimal temperature for tissue and shell growth rates and long-term survival have yet to be established. The sea surface temperature in southeastern Alaska, where pinto abalone are abundant, is

normally between 3 and 18°C (U.S. Dept. Commerce 1970), and no indication of respiratory stress was observed at those temperatures (Fig. 1). This experiment shows that pinto abalone can survive short exposures of 0.5°C. However, prolonged exposure to ≤0.5°C causes the pinto abalone to lose its protective grip on substrate, increasing the possibility of loss to predators, and then becomes lethal if the exposure continues. North of the southeastern Alaska archipelago, in areas like Prince William Sound, as well as the Aleutian Islands, sea surface temperatures ≤0.5°C are relatively common (U.S. Dept. Commerce 1970), and this probably explains why *H. kamtschatkana* does not exist there. Our findings suggest that the southcentral Gulf of Alaska would not be good habitat in which to culture pinto abalone commercially unless seawater can be heated cheaply.

Thermally induced deaths were reported when pinto abalone were held at 17°C (Paul and Paul 1981). This study shows that with proper thermal acclimation, those temperatures are not lethal, and that the upper critical temperature falls between 24 and 26.5°C. Sea temperatures ≥24°C are not known to occur in Alaska (U.S. Dept. Commerce 1970). Tolerances of 24°C conditions may explain the species' ability to inhabit waters as far south as California. However, even in California, shallow subtidal temperatures are seldom warmer than 20–22°C (D. Leighton Marine Bioculture Inc. pers. comm.). Similar studies on more southern stocks should provide further insight into the thermal tolerance of the species and may demonstrate the existence of physiological races.

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GROWTH OF RED ABALONE, *HALIOTIS RUFESCENS* (SWAINSON), AT JOHNSONS LEE, SANTA ROSA ISLAND, CALIFORNIA

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ABSTRACT Growth of red abalone, *Haliotis rufescens*, was examined by the use of tag and recapture at Santa Rosa Island, Santa Barbara County, CA from 1978 to 1984. During five annual visits to the study site, we collected, tagged, and replaced 2,145 red abalone. Annual growth increments of individual abalones were used to estimate and compare annual Von Bertalanffy growth parameters. Single-year growth during the 1978 to 1979 period was significantly different from grouped periods 1979 to 1981 and 1981 to 1982. Red abalone grew to recreational size (178 mm) in 5.6–11.3 y and to commercial size (198 mm) from 7.6 to almost 20 y. After the 1982 to 1984 El Niño, we revisited our study site to remeasure tagged abalone. Two-year growth data comparisons (necessary because of the intervening year when no data were taken) indicated that growth of red abalone was severely depressed. This observed growth reduction could have resulted from the direct effect of elevated seawater temperature on red abalone or from the decline in food resulting from El Niño–related events, i.e., storms and kelp deterioration.

KEY WORDS: red abalone, growth, *Haliotis rufescens*, California.

INTRODUCTION

Red abalone, *Haliotis rufescens* (Swainson), is the largest of eight species of abalone in California, attaining a maximum size over 300 mm shell diameter. It occurs throughout California, in two biogeographic provinces, the Oregonian and the San Diegian, that meet at Point Conception (Fig. 1). Local distribution of red abalone is strongly influenced by seawater temperature. Thus, in cooler temperate water along northern and central California, it occurs intertidally to 15-m depth, whereas in the usually warmer water of subtropical southern California, it is subtidal to about 20-m depth. Its presence in this area often depends on local upwelling of cooler waters (Leighton 1974, Tutschulte 1976).

Abalone are slow-growing, long-lived, broadcast-spawning, benthic herbivores found on hard substrate, often in the vicinity of large brown algae, which provide their food. Partly because of their large size, red abalone have been harvested along the Pacific Coast of North America from the earliest settlers to the present. Recently, red abalone provided valuable commercial and recreational fisheries in California. During the last few decades, however, landings declined as natural and anthropogenic factors placed more pressure on abalone populations. Commercial landings declined to about 10% of historic high landings by 1996. Red abalone continue to be threatened by excessive harvest, disease, loss of habitat, and pollution.

Growth rate data are needed for effective resource management. This is especially true for species like abalone. Previous growth studies (Leighton 1974, Tegner et al. 1989, Grenier et al. 1992) concentrated on juvenile and small red abalone. Although these provided valuable insight on small abalone that are cryptic in the wild, they do not satisfy the need for information on growth throughout life. Tegner et al. (1992) reported on studies of red abalone growth in northern California. Growth there, however, may not be directly applicable to southern California abalone populations.

In 1978, we began a study of red abalone growth in southern California. We collected field data annually at Santa Rosa Island (Fig. 1) from 1978 to 1982. In 1984, we made an additional growth data collection in response to El Niño conditions, which became apparent in 1983. This location was ideal because it had a weather-sheltered cove with an abundant population of red abalone. In this article, we present red abalone growth information from a broad range of sizes at a single location and examine the study procedure, i.e., collection, tagging, measuring, and replacement of abalone, using annual growth comparisons. We also discuss potential effects of increased seawater temperature on red abalone.

METHODS

Johnsons Lee (latitude, 33°54′N; longitude, 120°06′W) is on the south side of Santa Rosa Island (Fig. 1). This site was chosen because abalone were abundant and the area is easily accessible. It was visited six times, during July of each year, from 1978 to 1982, and in 1984. The support vessel was anchored over the site with visual, shoreline cross-bearings and Loran-C coordinates. The study site, about 50 m in radius, is a rocky reef with ledges, crevices, movable rocks, and boulders. Relief varied from 0 to 2 m, and depth varied from 11 to 17 m. The site is bounded by large sand areas on the (shoreward) north and east sides and by mixed, low-relief rock reefs and sand patches on the west and south (offshore). Giant kelp, *Macrocystis pyrifera*, was consistently abundant throughout the area.

We used mark-recapture to measure growth rate. Scuba divers collected red abalone and brought them to the vessel, where they were placed in flowing seawater. Abalone were marked with numbered, stainless steel tags secured with stainless steel wire through two completely formed shell apertures (Haaker et al. 1986). Shell length (maximum shell dimension) was measured to the nearest millimeter with calipers. The foot was examined for injury, and the shell was examined for the presence of the boring sponge, *Cliona*

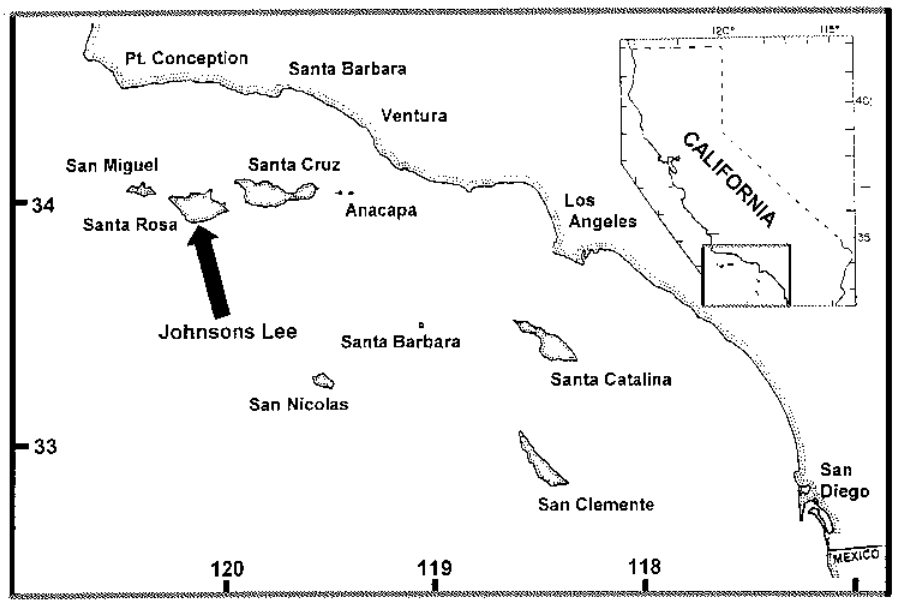

Figure 1. Southern California, showing the location of the Johnsons Lee, Santa Rosa Island, tagging site.

spp. All abalone, except for severely injured individuals, were then returned to the site. Fewer than 10 abalone collected a short distance from the study site in the course of another study were tagged and placed in the site.

On subsequent visits, we collected both tagged and additional untagged abalone. These abalone were tagged if appropriate and treated as previously described. Replaced abalone were temporarily marked with colored wire or plastic flagging to avoid repeated collection during the same visit. In 1982, no new abalone were tagged, but all previously tagged abalone that could be located were collected, measured, and replaced. No visit to the site was made in 1983. A sixth visit was made in 1984 to obtain data on red abalone growth during a prolonged period of elevated water temperature caused by warm tropical waters intruding into southern California, a phenomenon known as El Niño. Raw data collected during this study are available in tabular form (Haaker et al. 1986 Appendix A).

Because stress due to the tagging operation may affect growth rates, we investigated its possible effects on growth. A group of abalone that had been collected, measured, and replaced during three consecutive years was paired with a group only encountered during the first and last years of the same period. For example, one group consisted of abalone measured in 1978, 1979, and 1980, and its paired group consisted of abalone measured only during 1978 and 1980. A regression line was calculated for each group of abalone, with length after 2 y as the dependent variable and initial length as the predictor variable.

We used a method of comparing regression lines given in Littel et al. (1991, pp. 243–246) to compare the paired groups of abalone. Essentially, it was an analysis of covariance model: it used, in addition to the predictor variable, an indicator variable for groups, and a product of the indicator variable and the predictor variable to test for significance of the regression parameters. The hypothesis tested was that a possible handling effect would be shown by differences in the regression lines within the paired groups of abalone. Comparisons were made for three periods: 1978 to 1980, 1979 to 1981, and 1980 to 1982. A 5% significance level was used. We used the SAS general linear models procedure GLM to compare the regression lines (SAS Institute 1990).

We determined growth curves from annual increments in length of tagged individuals. We used the nonlinear methodology of Kirkwood and Somers (1984) for mark-recapture data to estimate the parameters and standard errors for the von Bertalanffy growth model. This model for mark-recapture data can be written as:

$$d = (L_\infty - r)\,(1 - \exp(-Kt))$$

where $d = c - r$, c = length at recapture, r = length at release, L_∞ = mean asymptotic length of abalone, K = Brody growth coefficient, and t = time between release and recapture; = 1 for 1-y interval, = 2 for 2-y interval.

We used the SAS procedure NLIN for nonlinear regression with the secant method to fit the von Bertalanffy model (SAS Institute 1990). The ages at recreational and commercial minimum sizes were estimated from the von Bertalanffy growth curve.

Comparisons of the growth curves for the 1-y growth periods were made using the likelihood ratio method of Kimura (1980). If two or more groups compared have the same growth curve, it would be shown by simultaneous nonsignificant differences in both the L_∞ and the K parameters. The four growth periods—1978 to 1979, 1979 to 1980, 1980 to 1981, and 1981 to 1982—were compared together to see if any significant differences occurred among them. Then, each possible pair of the growth periods was tested. When no significant differences occurred between adjacent growth periods, then the periods were combined, and a von Bertalanffy growth curve was determined for the combined period. We used the NLIN procedure from SAS to implement the likelihood ratio method of Kimura for comparisons of growth curves (SAS Institute 1990).

Because no data were collected in 1983, 2-y periods were used to test for possible effects on growth rate due to El Niño. The von Bertalanffy growth curves for the 2-y periods of 1978 to 1980, 1979 to 1981, and 1980 to 1982 were compared with the growth

TABLE 1.

Numbers of red abalone, tags, and tag recoveries collected during each annual visit to Johnsons Lee, Santa Rosa Island; total includes abalone tagged in a year (new) and abalone tagged in previous years.

Parameter	1978	1979		1980		1981		1982		1984	
	New Tags	New Tags	Total Collected	New Tags	Total Collected	New Tags	Total Collected	New Tags	Total Collected	New Tags	Total Collected
n	510	336	494	539	651	757	1032	3*	412	0	138
Mean	140.8	147.9	154.0	142.8	147.9	141.2	146.8	—	163.8	—	165.2
SD	40.6	37.6	35.5	40.1	34.4	35.2	34.4	—	23.5	—	17.9
Range	33–217	45–222	45–222	47–212	47–212	39–203	39–210	—	94–213	—	72–209
Tags from previous year	—	158		86		210		361		59	
Annual recovery (%)	—	31		17.4		32.3		35		—	

* No abalone tagged. Three abalone were tagged in previous years.

curve for the 1982 to 1984 period, using the likelihood ratio method of Kimura (1980). These comparisons determined if differences in growth might be attributable to El Niño.

RESULTS

A total of 2,145 red abalone (33–222 mm shell length) was collected, tagged, measured, and replaced during six visits to Johnsons Lee (Table 1). None of the abalone were seen again in all six sampling periods. Four (0.2%) abalone were recaptured four times, eight (0.4%) were captured three times, 137 (6.4%) were captured twice, and 632 (29.4%) were captured once. The mean recovery rate after 1 y was 28.9% and ranged per year from 17.4 to 35.0% (Table 1). Thus, tag recovery from each visit was generally high and consistent throughout the study.

The frequencies (in percentages) of initial lengths for all abalone marked were plotted with the frequencies of initial lengths for only abalone recaptured after a year (Fig. 2). The two distributions were similar, which implied that no sampling bias toward recapturing larger abalone occurred in the study.

The tags were not observed to cause either soft tissue reaction or shell damage. Although some tags were thinly encrusted with bryozoans, none had the filamentous encrusting organisms often seen on some fish tags. Some abalone (<1%) were recovered with only the attaching wire remaining, a result of twisting and abrasion against rocks by the abalone.

Stress due to handling and tagging had little effect on abalone growth. No significant difference in growth was found between abalone collected consecutively over 3 y and those undisturbed for 2 y. This was shown by nonsignificant differences between the slopes and intercepts of the paired regression lines for each of the three periods ($p \geq 0.15$; Table 2).

Significant differences in von Bertalanffy growth curves were found among the four annual growth periods ($p < 0.0001$, Kimura likelihood ratio test; Tables 3 and 4). Comparisons among the sequential growth periods showed that the 1978 to 1979 period differed from the 1979 to 1980 period; the 1980 to 1981 period differed from the 1981 to 1982 period ($p < 0.0001$). However, the 1979 to 1980 period did not differ significantly from the 1980 to 1981 period ($p = 0.13$). Thus, a single von Bertalanffy growth curve was fitted for the years 1979 to 1981 (Table 3). Separate von Bertalanffy growth curves were fitted to the other two periods (Fig. 3).

Estimated age at recreational minimum size (178 mm = 7 in) was 5.6 for the 1978 to 1979 period, 10.4 for the combined 1979 to 1981 period, and 8.3 for the 1981 to 1982 period. Estimated age at commercial minimum size (197 mm = 7.75 in) was 7.6 for the 1978 to 1979 period and 19.2 for the combined 1979 to 1981 period. For the 1981 to 1982 period, the commercial size exceeded the mean asymptotic size (Table 3).

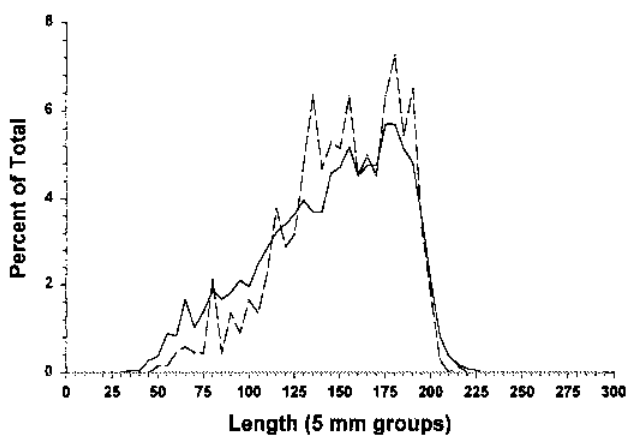

Figure 2. Length frequency distributions of initial lengths for all tagged red abalone (solid line) and initial length of red abalone recaptured after 1 y (dashed line), at Johnsons Lee, Santa Rosa Island.

TABLE 2.

Regression parameters for test of tagging effect for paired 2-y growth increments for the years 1978 to 1980, 1979 to 1981, 1980 to 1982.

Year Pair	M_n	n	r^2	Slope	P_1	Intercept	P_2
1978 to 1980	1	26	0.55	0.68	0.26	71.2	0.38
	2	27	0.67	0.51		100.23	
1979 to 1981	1	51	0.73	0.55	0.21	84.12	0.98
	2	26	0.70	0.69		61.77	
1981 to 1983	1	32	0.84	0.55	0.15	86.35	0.99
	2	77	0.80	0.63		73.39	

Each paired set is not significantly different. M_n is the number of times the abalone were handled, n is the sample size, r^2 is the squared correlation coefficient, P_1 and P_2 are the p values for the slope and intercept comparisons, respectively.

TABLE 3.

Parameters of the von Bertalanffy growth equation and years to sport and commercial size for red abalone collected at Johnsons Lee, Santa Rosa Island, CA, for each year 1978 to 1982.

Period	Size Range	n	L_∞	SE	K	SE	Years to Sport	Years to Commercial
1978 to 1979	46–204	158	223.23	6.56	0.28	0.03	5.6	7.6
1979 to 1980	80–200	86	207.09	8.49	0.21	0.04	9.4	14.5
1980 to 1981	53–212	210	196.39	4.34	0.21	0.02	11.3	*
1981 to 1982	66–210	361	194.48	2.49	0.30	0.02	8.3	*
Combined 1979 to 1981	53–212	296	200.58	4.01	0.21	0.02	10.4	19.2

n is the sample size. SE is the standard error of the corresponding parameter.

Biennial von Bertalanffy growth comparisons, which included the 1984 data, revealed that growth was significantly different among the four periods ($p < 0.0001$, Kimura likelihood ratio test: Tables 5 and 6). Growth was much lower during the 1982 to 1984 period, compared with 1978 to 1980, 1979 to 1981, or 1980 to 1982 ($p < 0.0001$). Because 1982 to 1984 was a period of elevated seawater temperatures due to a strong El Niño, these results suggested that such conditions had a negative effect on red abalone growth during that time (Fig. 4).

Growth parameters changed throughout the study period. L_∞ generally declined during each successive annual period (Table 3). Growth for the 1978 to 1979 period was relatively fast, with an average abalone attaining commercial minimum size in under 8 y. In subsequent years, 1979 to 1981, the growth to commercial size slowed to 19.2 y, over twice that of the 1978 to 1979 period. Growth rates for the 1981 to 1982 period were so low that an average abalone would not have attained commercial minimum size under those conditions (Table 3).

DISCUSSION

The mark-recapture method has been frequently used to study growth in abalone (Cox 1962, Forster 1967, Newman 1968, Keesing and Wells 1989). It has been criticized, however, because it can result in emigration (Mottet 1978), mortality (Burge et al. 1975), low recovery (typically 10–20%) (Mottet 1978), and biased growth curves (McShane et al. 1988). Shepherd and Hearn (1983) and Keesing and Wells (1989) showed that growth data collected over too short a duration and from too narrow a size range can yield distorted growth parameters. Alternatively, some abalones can be aged with annual growth rings (Sakai 1962, Shepherd et al. 1983, Prince et al. 1988, Day and Fleming 1992, Shepherd et al. 1995), but no direct aging method has been developed for red abalone. Thus, the mark-recapture method is the only one currently available for obtaining reliable growth data from natural populations.

Day and Fleming (1992) discussed assumptions inherent in using length-increment data in growth studies, including the effect of the application of the tag and its presence on growth, the importance of randomness in the recapture of tagged individuals, and the desirability that the interval between tagging and recapture should be whole years.

In this study, these assumptions were at least partly addressed. The effects of the tag application on growth were addressed by using experienced collectors, minimizing handling, and attaching temporary marks to prevent repetitive picking of recently tagged individuals. The influence of tagging-related handling on growth was addressed by tests of group pairs of differentially tagged abalone to examine growth effects. We found no differences between the groups compared.

Red abalone may move in response to various stimuli, including food supply and physical disturbance (Ault and DeMartini 1987), and there is no evidence that movements occur over extensive sandy substrate. We have not observed red abalone on sand, where it would be susceptible to being overturned and to predation. Although tagging-related handling had little effect on growth, it may induce migration of replaced abalone, thus reducing random-

TABLE 4.

Kimura's likelihood ratio test for H_0: both L_∞ and K are equal in periods compared.

Periods Compared	n	RSS(full)	RSS(red)	Test Statistic	*df*	p Value
7879 vs. 7980 vs. 8081 vs. 8182	815	43181	51906	150.0	6	0.0000
7980 vs. 8081 vs. 8182	657	32688	34206	29.8	4	0.0000
7879 vs. 7980	244	15429	17987	37.4	2	0.0000
7980 vs. 8081	296	15319	15534	4.1	2	0.13
8081 vs. 8182	571	27752	29128	27.6	2	0.0000
7879 vs. 8081	368	20875	28830	118.8	2	0.0000
7879 vs. 8182	519	27863	32681	82.8	2	0.0000
7980 vs. 8182	447	22306	22546	4.8	2	0.09

n is the sample size. RSS(full) and RSS(red) are the residual sum-of-squares for the full parameters and null hypothesis models, respectively. *df*, degrees of freedom for the test statistic.

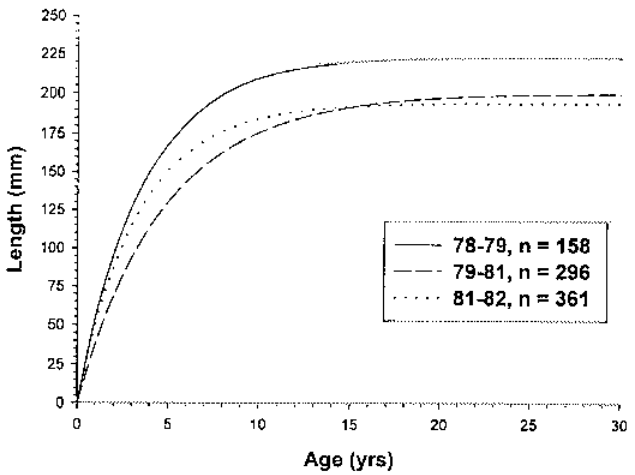

Figure 3. von Bertalanffy annual growth curves for *H. rufescens* at Johnsons Lee, Santa Rosa Island, for the years 1978 (i.e., 78 on figure) to 1982 (82). The 1979 to 1980 and 1980 to 1981 curves are not significantly different and are grouped together.

ness in subsequent collections. We believe that tagging-induced migration was minimal, because the study site was mostly surrounded by sandy substrate and included sufficient food. An abalone could not move far without encountering a broad sand expanse. Occasional searches of other reef habitats in the area surrounding the study site found few tagged abalone. Although an individual could move around the site, it would nevertheless be restricted to the general area and be available for recapture during subsequent visits. A factor not controlled was the removal of abalone by recreational and commercial harvest, but most of the tagged abalone were well below the recreational size limit and were unlikely disturbed by abalone harvesters between surveys.

We visited the sites at annual increments; thus, seasonal growth effects were minimized (Day and Fleming 1992), and we tagged abalone in a broad size range for estimation of growth curves. Annual recapture rates were generally high and consistent. The low recapture rate in 1980 may have been caused by poor weather conditions experienced in 1979, when few abalone were able to be collected, tagged, and replaced.

We believe that the stainless steel tags used here, if carefully attached with wire, are durable and will have little measurable effect on an abalone. One tagged shell from an abalone that lived 16 y after tagging was returned from a processing plant (Taniguchi and Haaker 1996). The abalone had grown from 147 mm in 1979 to 198 mm in 1995. A disadvantage of this type and size of tag is its unsuitability for abalone smaller than about 50 mm. Tags with imprinted vinyl tubing over a fine stainless steel wire and glued-on tags could be used to tag small abalone, but these are unlikely to be as durable as the washer tags that we used.

The von Bertalanffy curve is useful in describing abalone growth (Sainsbury 1982). The growth curves (Fig. 3) were based on a large number of tagged abalone with a broad range of sizes, which were measured at yearly intervals at a single location. These curves model growth within the observed size range of abalone. However, extrapolating these curves back toward the origin is questionable, because juvenile abalone may grow linearly (Yamaguchi 1975, Prince et al. 1988). Extrapolation beyond the observed maximum size is also questionable, because we need to assume, without empirical evidence, that the abalone will continue to grow at the rate as defined by the von Bertalanffy model. Thus, care should be exercised in interpreting L_∞, the asymptotic length parameter (Knight 1967).

Calculation of a growth curve for red abalone was further complicated by inability to directly establish the age of individuals and the limited availability and practicality of tagging abalone below 50 mm. Additionally, we have no information on either t_0, the age at size 0, or l_0, the length at age 0, at Johnsons Lee. These parameters were assumed to be 0 but introduce uncertainty in the interpretation of size at age.

Leighton (1968) used daily measurements to estimate annual red abalone growth of 9.8–35.0 mm/y. McBeth (1972) reported annual increases in size up to 21 mm for 5–6 mm red abalone. Tegner and Butler (1985) determined growth from a red abalone seeding experiment in 1981 at Abalone Cove, near Los Angeles. After 1 y, abalone planted at mean sizes of 71 and 45 mm had mean growth of 30 and 31.5 mm, respectively. Mean annual growth of hatchery-raised red abalone is reported to range from 20.1 to 22.4 mm (Owen et al. 1984). Hines and Pierce (1982) found that red abalone were capable of growing 50–100 mm/y in the field. Most of these estimates fall within the ranges of our growth curves, particularly when annual variation is considered.

Tegner et al. (1992) and DeMartini (pers. comm.) reported that growth of native red abalone in northern California (L_∞ = 192.4, K = 0.217) is slower than in southern California, yielding an age at recreational minimum size of 11.8 y. Here, in our study, we found that the time to recreational size ranged from 5.6 to 11.3 y Table 3). Because red abalone are adapted to cool water, slower growth would be expected in northern California, where minimum thermal tolerance may be approached and seasonal food availability varies more than in southern California. Growth varied during the study in both the 1- and 2-y increment growth curves (Figs. 3 and 4), the first period yielding the greatest growth. Whether this difference was due to natural variability in growth or attributed to diet, sea temperature, location, or seasonal factors was not investigated here.

A significant difference in growth was found between the biennial growth curve covering the 1982 to 1984 El Niño period and the other three biennial periods in the study (Fig. 4). Growth during 1982 to 1984 was reduced to such a level that most abalone would not attain sport legal size at that rate. During 1982 to 1984, a severe El Niño brought abnormally warm seawater temperatures and severe weather to southern California (Tegner and Dayton 1987). Mean sea surface water temperatures exceeded 20°C for

TABLE 5.

Parameters of the von Bertalanffy growth equation and tests for growth differences among four biennial periods, 1978 to 1980, 1979 to 1981, 1980 to 1982, and 1982 to 1984, to examine the effects of an El Niño on growth.

Perios	n	L_∞	SE	K	SE
1978 to 1980	53	212.26	13.16	0.25	0.06
1979 to 1981	77	190.59	4.19	0.27	0.03
1980 to 1982	109	194.88	3.59	0.26	0.02
1982 to 1984	59	182.05	4.92	0.12	0.02

n is the sample size. SE is the standard error of the corresponding parameter.

TABLE 6.

Kimura's likelihood ratio test for H_0: both L_∞ and K are equal in periods compared.

Periods Compared	n	RSS(full)	RSS(red)	Test Statistic	*df*	p Value
7880 vs. 7981 vs. 8082 vs. 8284	298	29035	36524	68.4	6	0.0000
7880 vs. 8284	112	13806	21086	47.4	2	0.0000
7981 vs. 8284	136	8533	11379	39.1	2	0.0000
8082 vs. 8284	168	9860	13745	55.8	2	0.0000

n is the sample size. RSS(full) and RSS(red) are the residual sum-of-squares for the full parameters and null hypothesis models, respectively. *df*, degrees of freedom for the test statistic.

93.8 days compared with 57.6 days in the prior 5 y and 50.8 in the subsequent 5-y period (Scripps Institution of Oceanography pier temperature data) (Fig. 5). Elevated seawater temperatures can affect an abalone population and its distribution in several ways. Red abalone distribution in southern California is positively influenced by the occurrence of cool seawater temperature, i.e., upwelling areas or those influenced by northern currents (Tegner et al. 1992). Leighton (1974) demonstrated a strong influence of temperature on larval and juvenile red abalone, which grew best at temperatures below 20°C. Growth variations were related to food availability in red abalone on the northern California coast (John DeMartini, Humboldt State University, pers. comm., Tegner et al. 1992). In southern California, red abalone feed primarily on giant kelp, which can be reduced in quantity and in nutrient quality during El Niño periods (Tegner et al. 1987). Strong or prolonged storm periods associated with El Niño can also physically remove kelp beds for extended periods. Giorgi and DeMartini (1977) and Ault (1985) related successful sexual maturity of northern California red abalone to food availability.

We cannot directly attribute the observed decline in growth to increased seawater temperature or reduced food availability, or both together. Both factors are known to affect reproduction in red abalone (Giorgi and DeMartini 1977) in northern California. It would not be unexpected to find decreased growth rates in red abalone populations during episodes of El Niño–related elevated seawater temperatures that approach the upper physiological temperature range or during periods of El Niño–related low food availability. If growth rates are depressed, it is likely that spawning success would also be affected, because any spawning would occur at a time of unsuitable conditions for larval and juvenile growth. These factors need to be studied further, especially in locations at the extremes of the range of red abalone. The role of such factors which can affect stock productivity need to be considered in the management of red abalone.

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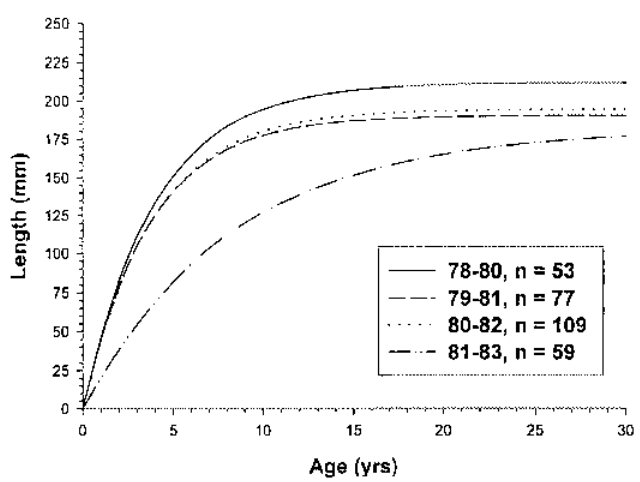

Figure 4. von Bertalanffy biennial growth curves for *H. rufescens* at Johnsons Lee, Santa Rosa Island, for the years 1978 (i.e., 78 on figure) to 1983 (83).

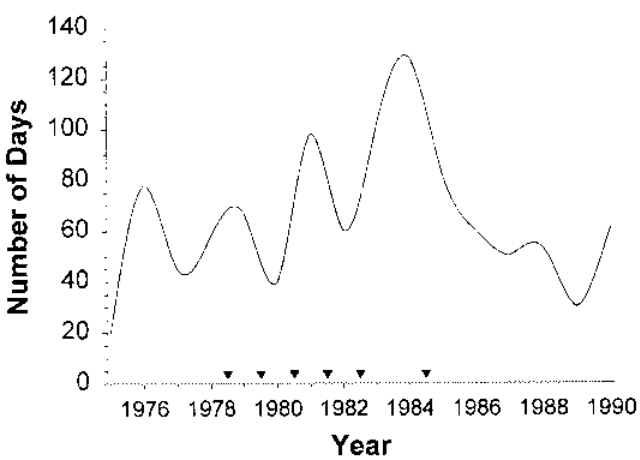

Figure 5. Number of days that mean sea surface temperature equaled or exceeded 20°C at Scripps Institution of Oceanography pier, from 1975 to 1990. Inverted triangles indicate sampling periods at Johnsons Lee.

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SCANNING ELECTRON MICROSCOPE STUDY OF RADULAE IN *HALIOTIS ASININA* LINNAEUS, 1758 AND *HALIOTIS OVINA* GMELIN, 1791 (GASTROPODA: HALIOTIDAE)

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ABSTRACT The middle parts of the radula ribbon of 10 mature snails of *Haliotis asinina* and *Haliotis ovina* were studied by scanning electron microscopy. In each transverse row, the numbers of the central and lateral teeth of *H. asinina* and *H. ovina* are 1 and 10, respectively. The number of the marginal teeth of *H. asinina* and *H. ovina* ranges from 108 to 128 and 214 to 236, respectively. The central and lateral teeth of *Haliotis* spp. are unicuspid. The marginal teeth of *Haliotis* spp. are multicuspid. In *H. asinina*, the anterior cusps are broad, short, and blunt ended in the central and first lateral teeth; long, narrow, and tapered in the second lateral tooth; spade shaped in the third to fifth lateral teeth; and with one large central and several lateral denticles on the marginal teeth. The radula morphologies of *H. asinina* and *H. ovina* are basically the same; with the differences in the middle part of the base of the central tooth, the size of the central tooth, the shape of the first and second lateral teeth, and the shape of the lateral denticles of the marginal teeth.

KEY WORDS: SEM, radula, abalone, *Haliotis asinina*, *Haliotis ovina*, Thailand

INTRODUCTION

Three species of abalone are found in Thailand: *Haliotis asinina* Linnaeus, 1758, *Haliotis ovina* Gmelin, 1791, and *Haliotis varia* Linnaeus, 1758 (Nateewathana and Hylleberge 1986, Nateewathana and Bussawarit 1988, Tantanasiriwong 1978). The average shell length and width in centimeters is 8 and 4 in *H. asinina*, 5 and 4 in *H. ovina*, and 4 and 3 in *H. varia*. *H. asinina* and *H. ovina* are cocktail-sized abalone and are potential commercial species. The prices as well as their market demand are high.

There have been several reports on the radulae of abalone, notably those on *Haliotis tuberculata* Linnaeus, 1758 (Crofts 1929), *Haliotis rufescens* Swainson, 1822 (Hickman 1984), *Haliotis rugosa* Lamarck, 1822 (Herbert 1990), and *Haliotis unilateralis* Lamarck, 1822 (Geiger 1996). In *H. tuberculata*, each transverse row of radula is characterized by a large central tooth, whereas the lateral ones are in three distinct series (Crofts 1929). The marginal teeth are too numerous to count; hence, the radula formula is $\infty \cdot (3 + 2) \cdot 1 \cdot (2 + 3) \cdot \infty$ (Croft 1929). Herbert (1990) described the radula formula *H. rugosa* to be $\infty + 5 + 1 + 5 + \infty$. The radula ribbon contains a rachidian tooth, lateral teeth with one innermost lateral, one second lateral and three outer laterals, and numerous marginal teeth (Herbert 1990). To date there has been no published work on the radula of the Thai abalone. Hence, the objective of this study is to perform a comparative study on the radulae of two potential commercial species of Thai abalone—*H. asinina* Linnaeus, 1758 and *H. ovina* Gmelin, 1791—using scanning electron microscopy (SEM).

MATERIALS AND METHODS

H. asinina and *H. ovina* were obtained form Praw Bay, Rayong Province, and from the Coastal Aquaculture Development Center, Prachuap Khiri Khan Province. Ten mature snails of each species were collected. The average shell length and width of mature snails in these samples were 66.6 and 31.6 mm for *H. asinina* and 56.4 and 43.1 mm for *H. ovina*, respectively. Each specimen was anesthesized with 5% $MgCl_2$ and three to four menthol crystals for 3–4 hours in a small, round plastic bowl (12 cm in diameter and 6.5 cm in height), covered with a piece of glass. The entire buccal mass of the well-relaxed snail was extracted and submerged in boiling 10% sodium hydroxide for 20–25 min to dissolve the tissue surrounding the radula before being washed three times in distilled water. The entire radula was mounted on a SEM stub with double-stick tape. The SEM stub was placed in a dessicator for 7–10 days and then coated with platinum and palladium for 3 min two times. Specimens were examined with the secondary electron detector and 15-KV accelerating voltage (S-2500 Hitachi). For each specimen, the central, lateral, and marginal teeth of 15 transverse rows in the middle part of the radula ribbon were examined.

RESULTS

Radula of H. asinina

The radula of *H. asinina* is a very long and ribbon-like structure. It normally extends from the anterior end of the buccal mass through about one-third of the upper esophagus. Each transverse row of the radula ribbon has a central tooth in the middle. The lateral and marginal teeth lie, respectively, on both sides of the central tooth (Fig. 1A). The central and lateral teeth are unicuspid, and the marginal teeth are multicuspid. All types of teeth lack basal denticles.

The number of teeth in each transverse row of adult *H. asinina* (average shell length and width of 66.6 and 31.6 mm., respectively) ranges from 119 to 139. The average widths of the cuspid and the base of the central tooth are approximately 196.2 and 288.5 μm, respectively. The unicuspid central tooth is large, broad, short, and blunt at the cutting edge. The base of the central tooth is strongly curved in the middle, containing thick and long pointed ridges at the lateral angles, creating a vertical groove, and is less than twice as wide as the base of the anterior cuspid (Fig. 2A and B). The first lateral tooth is moderately long and slender (its average width is 201.6 μm) (Fig. 2A and C). The anterior cuspid is

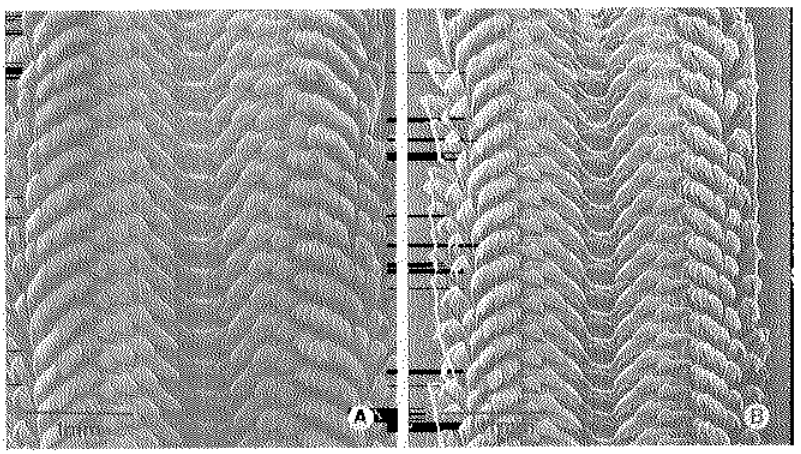

Figure 1. Scanning electron micrographs of the radula of *H. asinina* (A) and *H. ovina* (B) showing the teeth of 15 transverse rows. Each transverse row is bilaterally symmetrical, with a central tooth in the middle. The lateral teeth lie next to and on both sides of a central tooth. The inner marginal teeth are spadeshaped and lie next to the left and right lateral teeth. The outer marginal teeth are the outermost teeth on the transverse row of the radula. The sides of the outer marginal teeth are comb-like. The radula of *H. ovina* has the same arrangement of types of teeth in a transverse row.

broad, blunt, and short. The width of the base of the first lateral tooth is about the same as that of the anterior cuspid. In addition, it has thickened ridges with long pointed ends at the lateral angles, like the base of the central tooth. The second lateral tooth is equipped with a distinct shaft. The cuspid is long and narrow with a relatively rounded cutting edge (Fig. 2A and C). The third to fifth lateral teeth are similar in shape (Fig. 2E). They are broad and spadeshaped, with sharply pointed cutting edges, decreasing in size from the third to fifth (Fig. 2E). The marginal teeth are long and slender with moderately round cutting edges. They are multicuspid with several small denticles on each side (Fig. 2F). These denticles are triangular with sharply pointed ends. The base of the marginal tooth is very long and slender and has a stalk-like appearance (Fig. 2F).

Radula of H. ovina

The radula of *H. ovina* is similar to that of *H. asinina*. The number of teeth in each transverse row of adult *H. ovina* (average shell length and width of 56.4 and 43.1 mm, respectively) ranges from 225 to 247. The average widths of the cuspid and the base of the central tooth are approximately 150 and 225 μm, respectively. The central tooth is similar to that of *H. asinina* but is generally more narrow. The middle part of the base of the central tooth has a knob-like appearance (Fig. 3A and B). The lateral teeth of *H. ovina* are similar to those of *H. asinina*. However, the shape of the first lateral tooth of *H. ovina* is more broad and short (with an average width of 290.9 μm) than in *H. asinina* (Figs. 2C and 3B). The cutting edge of the second lateral tooth of *H. ovina* is more pointed than that of *H. asinina* (Figs. 2C and 3C). The shapes of the third to fifth lateral teeth and the marginal teeth of *H. ovina* are the same as those of *H. asinina* (Figs. 2D–F and 3D–F). The number of the third to the fifth lateral and the marginal teeth on each side of the central tooth are 3 and approximately 109, respectively. The lateral denticles of the marginal teeth are relatively long, narrow, and triangular, with sharply pointed ends (Fig. 3F).

Differences of radulae of H. asinina *and* H. ovina

Differences in the radular teeth of *H. asinina* and *H. ovina* are: (1) the number of the outer marginal teeth in each transverse row ranges from 108 to 128 in *H. asinina* and 214 to 236 in *H. ovina*; (2) the size of the central tooth is relatively large in *H. asinina* and is relatively small in *H. ovina*; (3) the base of the central tooth is strongly curved in *H. asinina* (Fig. 2A and B) and has a knob-like appearance in *H. ovina* (Fig. 3A and B); (4) the shape of the first lateral tooth is rather long and slender in *H. asinina* (Fig. 2C) and is broad and blunt in *H. ovina* (Fig. 3A and B); (5) the cutting edge of the second lateral tooth is tapered in *H. asinina* (Figs. 2A and C) and is strongly pointed in *H. ovina* (Fig. 3C); and (6) the shape of the lateral denticles of the outer marginal teeth is triangular and moderately long in *H. asinina* (Fig. 2F) and is triangular, but very long and narrow in *H. ovina* (Fig. 3F).

DISCUSSION

Information on the radular structure along with shell morphology is suitable to distinguish *H. asinina* from *H. ovina*. The radular structure may provide functional information related to the feeding habits and appropriate food types of *H. asinina* and *H. ovina*.

The structure of the radula has received much attention in the taxonomy of various groups of marine archaeogastropods (Thiele 1929, Wenz 1938–44, Fretter and Graham 1962, Burch 1982). The number of teeth, the number of cuspids on each type of tooth, and the shapes of these structures were observed in this study. Radular dentition patterns may provide useful information, especially if observations on the teeth are standardized. We chose to study the middle part of the radular ribbon because it is easy to locate, the teeth are fully formed, and they are not badly worn. The anterior radula is unsuitable because its teeth are worn. *Haliotis* have a rhipidoglossan type of radula (Crofts 1929, Fretter and Graham 1962, Burch 1982, Hickman 1984, Herbert 1990, Geiger 1996). Similar to other species of abalone, *H. asinina* and *H. ovina* have a typical rhipidoglossan type of radulae. Hickman (1984) studying *H. rufescens*, considered the three large teeth on either side of the central tooth to be modified marginals. In agreement with many other authors (Crofts 1929, Thiele 1929, Herbert 1990, Geiger 1996), we believe the laterals to be distinct from the marginals, common to the Vetigastropoda. In the middle part of the radular ribbon in *H. asinina* and *H. ovina*, each transverse row contains a central tooth and five lateral teeth. As stated above, the

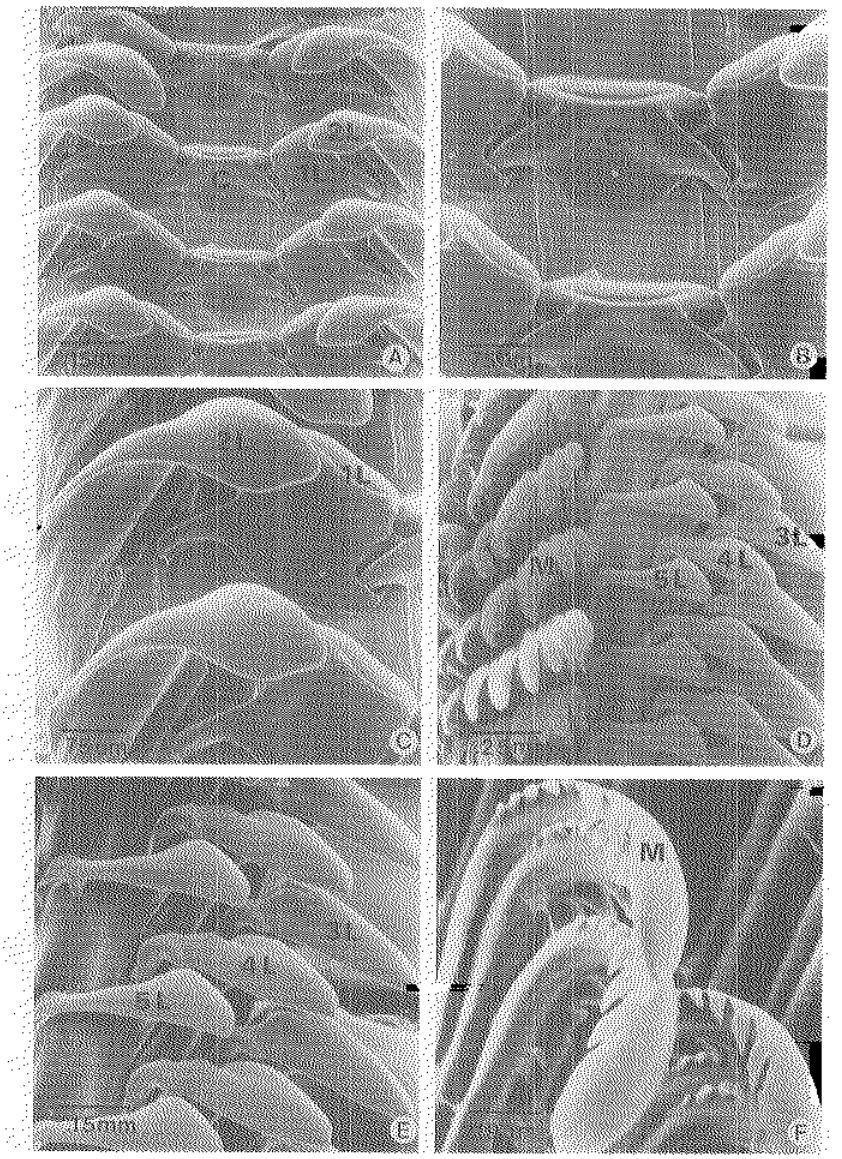

Figure 2. Higher magnifications of radular teeth of *H. asinina*. (A) Central (C), first lateral (1L), and second lateral (2L) teeth; (B) central tooth (C); (C) first lateral (1L) and second lateral (2L) teeth; (D) third lateral (3L), fourth lateral (4L), fifth lateral (5L), and marginal (M) teeth; (E) third lateral (3L), fourth lateral (4L), and fifth lateral (5L) teeth; (F) marginal teeth (M).

number of marginal teeth in *H. asinina* ranges from 108 to 128, whereas that for *H. ovina* ranges from 214 to 236. Crofts (1929) and Herbert (1990) also reported numerous marginal teeth in *H. tuberculata* and *H. rugosa*, respectively.

In general, it appears that the morphologies of radular teeth in haliotids are relatively similar with some minor differences in different species. The central or rachidian tooth was reported to be unicuspid in *H. tuberculata* (Crofts 1929), *H. rufescens* (Hickman 1984), *H. rugosa* (Herbert 1990), and *H. unilateralis* (Geiger 1996). *H. asinina* and *H. ovina* also have a unicuspid central tooth. Detailed study of the base of the central tooth revealed that *H. asinina* and *H. ovina* both contain a vertical groove similar to that found in the central teeth of *H. unilateralis* (Geiger 1996). The middle part of the base of the central tooth is strongly curved in *H. asinina* and has a knob-like appearance in *H. ovina*.

There are certain similarities in the morphology of lateral teeth among various species of *Haliotis* (Hickman 1984, Herbert 1990, Geiger 1996). Among the five laterals, the innermost or first and the second laterals are different in shape, whereas the third to the fifth laterals are similar in shape. In *H. rugosa* and *H. unilateralis*, the first lateral is broad and blunt (Herbert 1990, Geiger 1996). Geiger (1996) reported that it had a cutting edge with a distinct ridge. In *H. asinina* and *H. ovina*, the first lateral is also broad and blunt with a distinct ridge on the cutting edge. Similar to the second lateral of *H. rugosa* (Herbert 1990), *H. asinina* possesses a second lateral with a distinct shaft and relatively rounded cutting edge, whereas that of *H. ovina* has a very pointed cutting edge. The third to the fifth laterals are relatively similar in shape in *H. rufescens* (Hickman 1984), *H. rugosa* (Herbert 1990), *H. unilateralis* (Geiger 1996), *H. asinina*, and *H. ovina*. They are spadeshaped and similar to each other but decreasing in size from third to fifth.

In *H. unilateralis*, Geiger (1996) divided the marginal teeth into inner, middle, and outermost marginals. We could not distinguish these subdivisions in the marginal teeth in *H. asinina* and *H. ovina*.

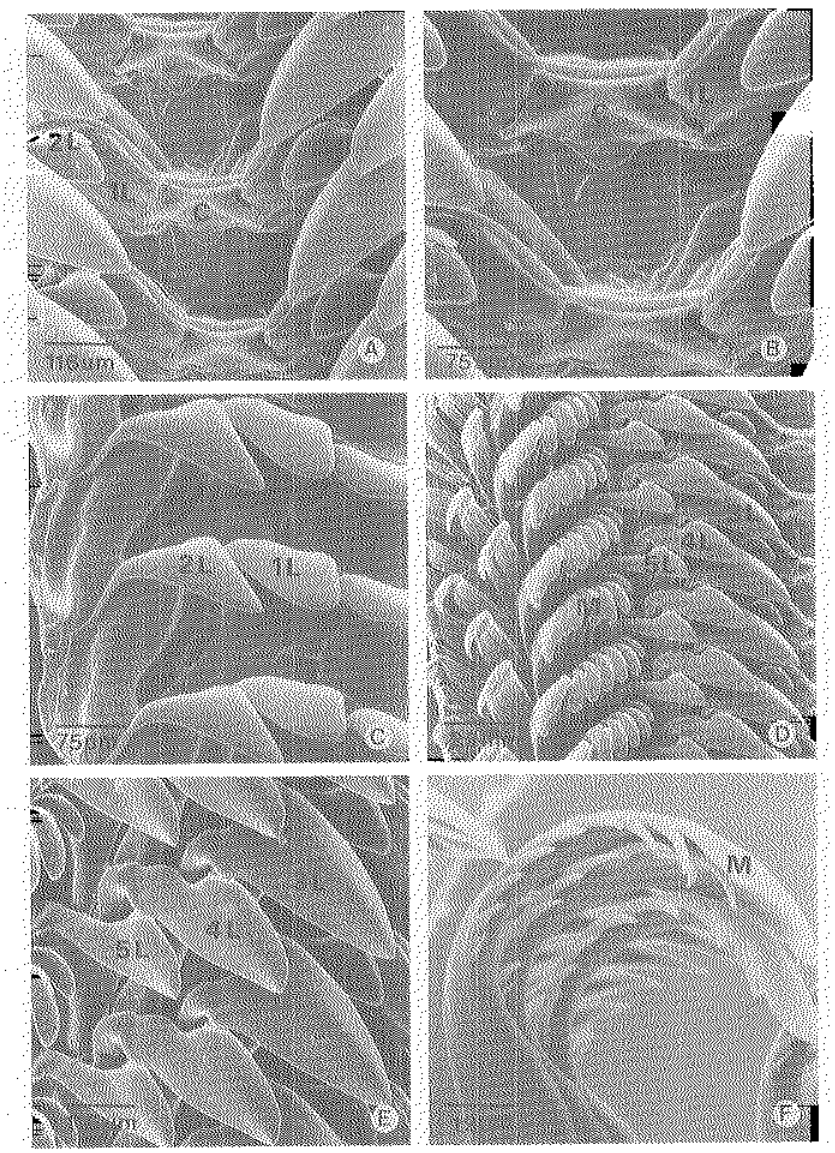

Figure 3. Higher magnifications of radular teeth of *H. ovina*. (A) Central (C), first lateral (1L), and second lateral (2L) teeth; (B) central (C) and first lateral (1L) teeth; (C) first lateral (1L) and second lateral (2L) teeth; (D) third lateral (3L), fourth lateral (4L), fifth lateral (5L), and marginal (M) teeth; (E) third lateral (3L), fourth lateral (4L), and fifth lateral (5L) teeth; (F) marginal teeth (M).

However, similar to the marginals of *H. rufescens* (Hickman 1984), *H. rugosa* (Herbert 1990), and *H. unilateralis* (Geiger 1996), those of *H. asinina* and *H. ovina* are slender in shape with rounded cutting edges and denticulated with several small projections on the sides.

Some differences in the radular structures and the number of teeth in each transverse row may indicate the capacity of radula for collecting and grinding up food. Matthews and Cook (1995) reported that abalone postlarvae (*Haliotis midae* Linnaeus, 1758) preferred to graze on prostrate diatoms. Fallu (1991) stated that young abalone use their radulae to graze on microalgae and rupture their frustules and cell walls, whereas adult abalone use the radula to shave off pieces of macroalgae. Crofts (1929) suggested that abalone use their radulae with strong lateral teeth for rasping, whereas the numerous marginal teeth with serrated edges probably serve as cogs to help food fragments move along the buccal cavity. In addition, they act as combs, working with the jaws to prevent the entrance of large fragments (Crofts 1929).

ACKNOWLEDGMENTS

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KARYOTYPES OF MARINE MOLLUSCS IN THE FAMILY HALIOTIDAE FOUND IN THAILAND

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ABSTRACT Karyotypes of gastropods in the family Haliotidae found in Thai waters, *Haliotis asinina*, *Haliotis ovina*, and *Haliotis varia*, were studied. Diploid chromosome numbers of these three abalone species were the same, 32. Two types of chromosomes (i.e., metacentric and submetacentric) were found in *H. asinina* and *H. varia*, and in *H. ovina*, additional telocentric chromosomes were found. The numbers of metacentric and submetacentric chromosomes were 20 and 12 for *H. asinina* and were both 16 for *H. varia*. The number of metacentric, submetacentric, and telocentric chromosomes for *H. ovina* were 18, 12, and 2, respectively. On the basis of the observed karyotypes, it is clear that *H. ovina* can be differentiated from *H. asinina* and *H. varia*. Because of the inconsistency of differentiation between metacentric and submetacentric chromosomes, further studies with other techniques such as fluorescence staining are required for species differentiation between *H. asinina* and *H. varia*.

KEY WORDS: chromosome number, karyotype, tropical abalone, *H. asinina*, *H. ovina*, *H. varia*

INTRODUCTION

Abalone are economically important marine gastropods that fetch moderate to high prices in the world market. In Thailand, it was reported that there were three species of abalone, i.e., *Haliotis asinina* Linnaeus, 1758, *Haliotis ovina* Gmelin, 1791, and *Haliotis varia* Linnaeus, 1758. Jarayabhand and Paphavasit (1996) reviewed the present status and the potential of commercial abalone aquaculture in Thailand. They pointed out advantages and disadvantages of culturing these species as compared with other commercial abalone species elsewhere. This article demonstrated a very high potential for commercial production of these tropical abalone species. Furthermore, many studies have been conducted to improve aquaculture technology (Jarayabhand and Paphavasit 1996). Although there is no commercial production for these marine organisms at present, it is expected to be achieved soon.

In addition to importance in taxonomic work, karyological analyses are also useful tools in providing fundamental information for animal breeding programs such as interspecific hybridization (Miyaki et al. 1997) and chromosome manipulation. Geiger (in press) reviewed studies on chromosome numbers for abalone all over the world and found that they can be divided on the basis of chromosome numbers into three groups. The first group, which has diploid chromosome numbers equal to 28, inhabits the European-Mediterranean area. The second group, which has diploid chromosome numbers equal to 36, inhabits the North Pacific region. The third group has diploid chromosome numbers equal to 32. This group is represented by abalone that inhabit the Indo-Pacific region (Table 1). This result suggests a remarkable phylogenic divergence of this character within the family Haliotidae. The aims of this article are to determine chromosome numbers and karyotypes of tropical abalone species found in Thai waters (Indopacific region) and to compare their karyotypes.

MATERIALS AND METHODS

Collection of Specimens

H. ovina samples were collected by SCUBA diving from islands in the upper Gulf of Thailand, whereas *H. asinina* and *H. varia* were collected from the East Coast of the Gulf of Thailand and from the Andaman Sea, respectively. These animals were kept alive at Angsila Marine Biological Research Station until used for chromosome preparations.

Chromosome Preparation

Live abalones were immersed individually in 0.04–0.05% colchicine seawater for 6–9 h. Animals were then rinsed thoroughly with clean seawater, and gill parts were cut out and put in 0.075% KCl for 40–60 min at room temperature. The gill parts were dried with tissue and transferred into freshly prepared carnoy's solution (glacial acetic acid:absolute methanol = 3:1 by volume) kept at 2–4°C. The solution was changed three to four times every 15 min and then kept at 4°C until time for chromosome preparation.

About 1 mm^2 of gill tissues was cut and put in 0.25 mL of 60% acetic acid. The tissue was then carefully ground with a glass rod. The solutions were added up to 2 mL with 60% acetic acid and left for 5–10 min. A fine-tipped glass dropper was used to take only the top layer of the solution consisting of cells in suspension. The solution was then dropped from 30–100 cm height onto clean glass slides preheated at 40–45°C. The slides were air dried and stained in 10% Giemsa in pH 6.8 phosphate buffer for 20–30 min. The slides were then rinsed with distilled water, dried at room temperature, and kept for further analysis.

Karyological Study

The slides were observed under a light microscope at 400 and 1,000× magnification. Diploid chromosome numbers were recorded, and at least three slides of each abalone species were

TABLE 1.

Haploid and diploid chromosome number of abalone from various geographical areas (Geiger and Groves, unpubl.), including this study.

Taxon (*Haliotis*)	Haploid No.	Diploid No.	Geographic Occurrence*	Reference
H. tuberculata	14	28	EM	Colombera and Tagliaferri 1983
H. tuberculata		28	EM	Arai and Wilkins 1986
H. lamellosa	14		EM	Colombera and Tagliaferri 1983
H. diversicolor aquatilis	16	32	IP	Nakamura 1985, Nakamura 1986
H. aquatilis	17	34	IP	Nishikawa 1962, in Nakamura 1985
H. diversicolor		32	IP	Arai et al. 1988
H. exigua		32	SJ	Aria et al. 1988
H. planata		32	IP	Arai et al. 1988
H. varia	16	32	IP	Nakamura 1986, this study
H. asinina		32	IP	This study
H. ovina		32	IP	This study
H. cracherodii		36	NP	Minkler 1977
H. discus discus		36	NP	Arai et al. 1982
H. discus hannai		36	NP	Arai et al. 1982
H. madaka		36	NP	Nakamura 1986

* EM, European-Mediterranean; IP, Indo-Pacific; SJ, South Japan; NP, North Pacific.

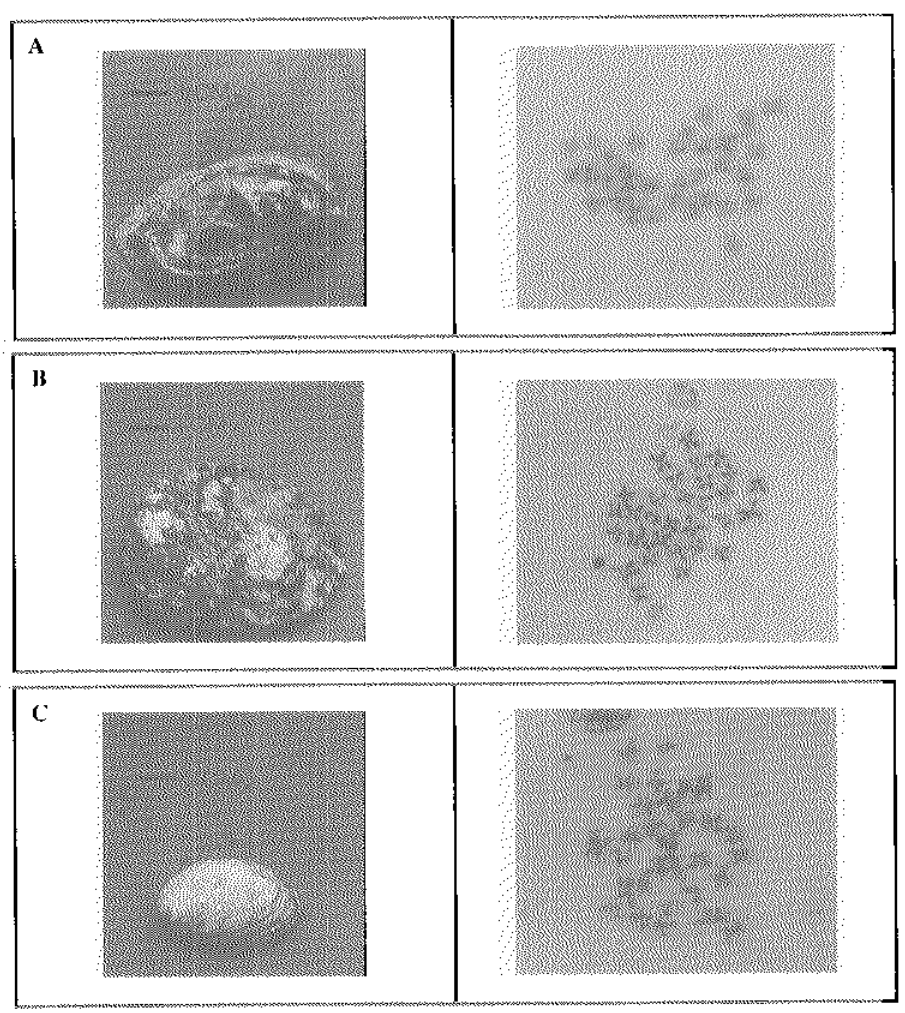

Figure 1. Morphology and mitotic metaphase chromosomes of (a) *H. asinina*, (b) *H. ovina*, and (c) *H. varia*.

selected and used for further karyological analyses. Photographs of clear mitotic metaphase chromosomes were analyzed with an image analysis program, Image Pro-Plus Version 1.0 (Media Cybernetics Inc., 1994), and centomeric indices (Sungpetch 1993) were calculated.

RESULTS

Morphology and mitotic metaphase chromosomes of *H. asinina*, *H. ovina*, and *H. varia* are shown in Figure 1. The diploid chromosome numbers of all three abalone species are 32 (Table 1). The karyotype of *H. asinina* comprises ten pairs of metacentric chromosomes and six pairs of submetacentric chromosomes (Fig. 2a). Three types of metacentric chromosomes were found in *H. ovina:* nine pairs of metacentric chromosomes, six pairs of submetacentric chromosomes, and one pair of telocentric chromosomes (Fig. 2b). The karyotype of *H. varia* comprises eight pairs of both metacentric chromosomes and submetacentric chromosomes (Fig. 2c).

DISCUSSION

Chromosome Number

The diploid chromosome number of 32 for *H. varia* (Nakamura 1986; Arai et al. 1988) is confirmed in this study (Table 1). Additionally, we demonstrate that the diploid chromosome numbers for *H. asinina* and *H. ovina* are the same at 32. This finding supports the indications of Arai et al. (1988) and Geiger and Groves (unpubl.) that abalone of the Indo-Pacific region have 32 chromosomes compared with 36 in abalone of the Pacific region and 28 in abalone of the European-Mediterranean region. It confirms a significant phylogenic divergence of this character within the family Haliotidae. However, explanations for such variation are still sought.

Karyotype

Two types of chromosomes (i.e., metacentric and submetacentric) were found in *H. asinina* and *H. varia*, whereas three types of chromosomes (i.e., metacentric, submetacentric, and telocentric) were found in *H. ovina*. This suggests that *H. asinina* and *H. varia* are more closely related than *H. ovina*. Arai et al. (1988) reported on karyotypes of abalone with 32 chromosomes: *Haliotis planata* Sowerby, 1882, *Haliotis varia*, and *Haliotis diversicolor diversicolor* Reeve 1846. He found that *H. diversicolor diversicolor* can be distinguished from the other two species by their karyotypes.

For the karyotype of *H. varia*, Arai et al. (1988) reported nine pairs of metacentric chromosomes, six pairs of submetacentric chromosomes, and one pair of submetracentric/acrocentric chromosomes. In this study, *H. varia* has eight pairs of both metacentric and submetacentric chromosomes. The different chromosome numbers obtained in these two studies may be a result of different times and temperatures used to stop metaphase.

Application

On the basis of the obtained karyotypes, *H. ovina* can be separated from *H. asinina* and *H. varia*. It is also suggested that a closer systematic relationship exists between *H. asinina* and *H. varia*

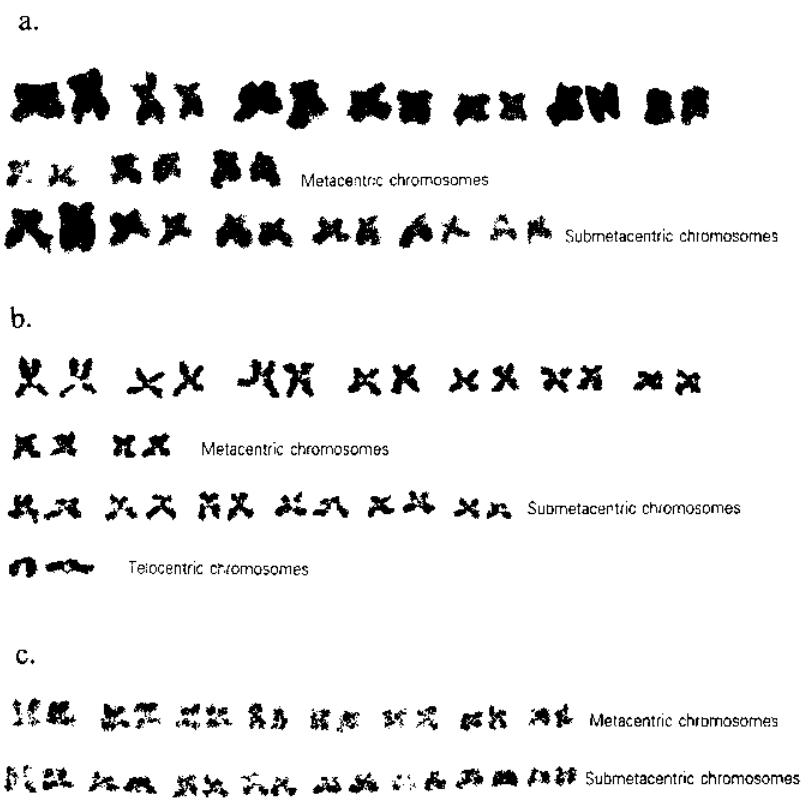

Figure 2. Karyotypes of (a) *H. asinina*, (b) *H. ovina*, and (c) *H. varia*.

than with *H. ovina*. Because of the inconsistency in differentiation between metacentric and submetacentric chromosomes, further techniques are required, such as fluorescence staining, etc., for species differentiation between *H. asinina* and *H. varia*. However, information from this study can be applied to monitoring triploid induction in this group of abalone as well as their hybridization. Under this circumstance, the chromosome preparation technique described by Okumura (1991) should be tested on these tropical abalone. Okumura (1991) used only epipodial tentacles for colchicine treatment during chromosome preparation. In this way, chromosome number can be detected without injury and the specimen can be kept alive for further investigation.

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DETERMINATION OF GUT CONTENTS OF THAI ABALONE *HALIOTIS ASININA* LINNAEUS

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ABSTRACT Samples of the abalone *Haliotis asinina* were collected from Samed Island, Raayong Province, Thailand, by SCUBA diving every 3 mo for a period of 1 y for determination of gut contents. The average shell length, shell width, and total body weight for these samples were 68.3 ± 9.1 mm, 32.1 ± 3.5 mm, and 48.2 ± 11.4 g, respectively. The percentages by weight of visceral mass, foot, and shell were 23.8, 61.6, and 14.5%, respectively. The average volume of microcontents (<120 μm) in gut contents was 80.3%, and that of macrocontents (>120 μm) was 19.7%. Most microorganisms were benthic diatoms (99.3%), and the dominant genera were *Nitzschia*, *Amphora*, *Cocconeis*, *Navicula*, and *Diploneis*. Macrocontents consisted mainly of sand granules and foraminiferans. Therefore, the main food of *H. asinina* along Samed Island appears to be composed of benthic diatoms, which were the predominant floral elements in the local environment.

KEY WORDS: abalone, *Haliotis asinina*, gut contents, benthic diatoms

INTRODUCTION

In Thailand, there are three species of abalone, *Haliotis asinina* Linnaeus, *Haliotis ovina* Gmelin, and *Haliotis varia* Linnaeus. They all inhabit coral reefs in the depth range 1–8 m (Tantanasiriwong 1978, Nateewathana and Hylleberge 1986, Tookvinart et al. 1986, Nateewathana and Bussawarit 1988, Sungthong et al. 1991). Among these three species, *H. asinina* attains the largest size. It is noted for its high percentage of edible parts; the shell makes up only about 15% of the total body weight. It is commonly distributed in Thai waters and has been the subject of an occasional fishery. Breeding of *H. asinina* has been accomplished in the laboratory (Singhagraiwan and Sasaki 1991, Singhagraiwan and Doi 1993). *H. asinina* has a high market value and is the most promising species of abalone for culture in Thailand.

H. asinina is an atypical member of the genus. It has a thin, long, and narrow shell (Hahn 1989). In its normal state, the epipodium, mantle, and foot extend well beyond the shell itself. *H. asinina* is widely distributed in the southern Pacific Ocean in subtropical and tropical regions at relatively shallow depths. For example, they are found in Thailand, Australia, Japan, and the Philippines (Hahn 1989, Nateewathana and Hylleberge 1986, Tookvinart et al. 1986, Nateewathana and Bussawarit 1988, Sungthong et al. 1991). During the day, they are usually found clinging to the undersides of rocks and dead plate corals or within crevices of the rocks or dead head corals. They are nocturnally active, as are most abalone species, and usually venture out of hiding to feed only after dark (Wood and Buxton 1996).

Among the diverse and abundant groups of invertebrates in coral reef faunas, the predatory prosobranch gastropods are especially numerous (Taylor 1986). The feeding habits of many species from hard substrate habitats of coral reefs have been investigated in detail; this is especially true for members of the families Conidae and Muricidae (Kohn 1959, Kohn 1968, Kohn and Nybakken 1975, Leviten 1978, Taylor 1978, Taylor 1984). *H. asinina* also occupies hard substrate in the coral reef environment, but its feeding habits have not been studied extensively. It has been speculated that *H. asinina* fed on the organisms that attached on hard substrate such as rocks or dead corals. Typically, feeding in abalone is by nonselective browsing or selective feeding on specific algae (Leighton and Boolootian 1963).

Abalone are herbivorous gastropods that often feed by trapping drift seaweed under their muscular foot (Wells and Keesing 1989, Fallu 1991, LaTouche et al. 1993), although they may also occasionally forage or graze on attached plants (Wood and Buxton 1996). In temperate or subtropical regions, abalone usually feed on seaweed or macroalgal species that are most abundant in their habitats (Linberg 1992, Wood and Buxton 1996). However, *H. asinina* are inhabitants of coral reef environments, where there frequently are few or no macro-algae or seaweeds. This study attempts to ascertain the constituents of the diet of *H. asinina* living in a natural habitat lacking significant macroalgae by examining gut contents.

MATERIALS AND METHODS

Specimens of *H. asinina* were collected from Ao Thein, Samed Island, Rayong Province, Thailand (Fig. 1) by SCUBA diving every 3 mo throughout a period of 1 y. For each sampling, 15 abalone were collected; 5 of them were immediately fixed in 10% formalin/seawater and brought back to the laboratory.

The total body weight, shell width, and shell length of the abalone were measured before dissection. Next, the viscera were dissected out. Gut contents were flushed from the stomach and intestine into a 5% household Clorox solution. The extract was then poured through a 120-μm-pore-size mesh sieve so that the mixture could be divided into two groups containing particles either smaller than 120 μm (microcontents) or larger than 120 μm (macrocontents). Volumes of macrocontents and microcontents were obtained by sedimentation in graduated tubes. The organisms present in the microcontents were identified as to genus or species.

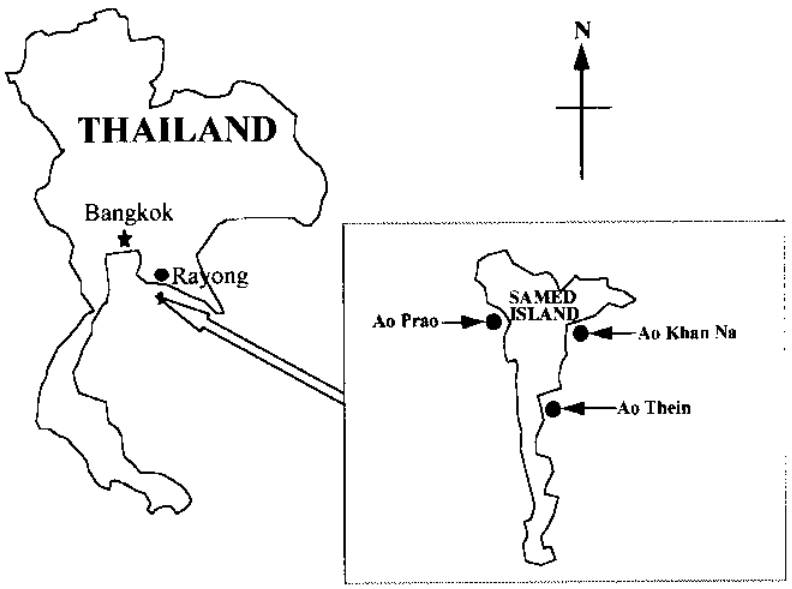

Figure 1. The sampling area for *H. asinina* at Ao Thein, Samed Island, Rayong Province, Thailand.

Their frequencies of occurrence were estimated with a Sedgwick-Rafter counting chamber under the compound light microscope. The macrocontents were examined and sorted by group or species, and total counts were made under the dissecting microscope. The volumes of sand granules, foraminiferans, algal pieces, and the remains of organisms were also measured by sedimentation in graduated tubes.

One-way analysis of variance was used to test the differences in average size of abalone and the relative frequencies of microorganisms and macroorganisms among each sampling period. The significance of differences between sampling periods were further determined by multiple comparison with Duncan's multiple range test. All statistical analyses were done with a statistical software (SPSS for Windows, Version 6.0) at $p < 0.05$.

RESULTS

Size of Samples

The average sizes (shell length, shell width, and total body weight) of *H. asinina* were similar in each period of sampling with no significant differences ($p < 0.05$). Abalone were 68.3 ± 9.1 mm in shell length, 32.1 ± 3.5 mm in shell width, and 48.2 ± 11.4 g in total body weight (Table 1). Percentages of visceral mass, foot, and shell in total body weight were 23.8, 61.6, and 14.5%, respectively (Table 1).

Gut Contents

The average volumes of microcontents and macrocontents for all samples throughout the year were 80.3 and 19.7% of the sedimented gut extract, respectively. The macrocontent portion was largely composed of sand granules (86.8%), with foraminiferans (5.6%), algal pieces (4.6%), and other organisms (3.0%) constituting the balance. The microorganisms were divided into eight groups: benthic diatoms (99.3%), crustaceans (0.2%), protozoa (0.2%), eggs of unknown organisms (0.1%), blue-green algae (0.1%), and bivalves (0.1%) (Table 2). Benthic diatoms were the dominant group in the *H. asinina* gut (Fig. 2), represented by about 36 genera (Table 3). The principal benthic diatoms were species of *Nitzschia, Amphora, Navicula, Cocconeis, Plagiogramma, Diploneis, Fragilaria, Coscinodiscus,* and *Surirella* (Table 2). The relative frequencies of these microorganisms in *H. asinina* gut content

TABLE 1.

The average sizes (mean ± SD) and percentages of visceral mass, foot, and shell of 15 *H. asinina* in each period of sampling.

Parameter	Average Size (mean ± SD)				
	October	January	April	July	Average
Shell length (mm)	63.8 ± 12.8	70.1 ± 6.6	69.1 ± 9.2	69.8 ± 6.4	68.3 ± 9.1
Total body weight (g)	48.5 ± 10.1	48.4 ± 14.3	49.5 ± 8.7	46.3 ± 12.8	48.2 ± 11.4
Visceral mass (g)	12.1 ± 2.8	11.4 ± 3.9	11.8 ± 3.9	10.7 ± 3.2	11.5 ± 3.8
	(24.9)*	(23.5)*	(23.9)*	(23.0)*	(23.8)*
Foot (g)	29.7 ± 6.5	29.8 ± 8.8	30.5 ± 5.0	28.5 ± 7.6	29.7 ± 6.8
	(61.4)*	(61.5)*	(61.6)*	(61.5)*	(61.6)*
Shell (g)	6.7 ± 1.8	7.3 ± 1.9	7.2 ± 1.4	7.2 ± 2.4	7.1 ± 1.9
	(13.8)*	(15.0)*	(14.5)*	(15.4)*	(14.7)*

* % Total weight.

TABLE 2.

The microorganism groups (<120 μm) in *H. asinina* gut.

Organism	Relative Frequency (%)*				
	October	January	April	July	Average
Benthic diatoms	99.3	98.6	99.7	99.7	99.3
Other organisms	0.7	1.4	0.3	0.3	0.7
Crustaceans	0.2	0.4	0.1	0.1	0.2
Protozoa	0.2	0.2	0.1	0.2	0.2
Unidentified eggs	0.0	0.6	0.0	0.0	0.1
Blue green algae	0.2	0.0	0.1	0.0	0.1
Bivalves	0.1	0.0	0.0	0.0	0.1
Flagellated algae	0.0	0.2	0.4	0.1	0.2
Annelids	0.0	0.1	0.0	0.0	0.0

* Relative abundance in Sedgwick-Rafter counting chamber subsample (1 mL) from the group considered in detail in Table 3.

were not significantly different ($p > 0.05$) in different sampling periods (Fig. 2; Table 3).

The macroorganisms found in samples of *H. asinina* gut content can be divided into five groups: foraminiferans (71.4%), crustaceans (10.8%), annelids (9.7%), large benthic diatoms (4.6%), and mollusks (3.5%) (Table 4). The relative frequencies for groups of macroorganisms are shown in Figure 3. They were not significantly different between sampling periods ($p > 0.05$). Most of the macrocontents in the *H. asinina* gut, as mentioned, were sand grains. Foraminiferans were the dominant macroorganisms in the gut contents.

DISCUSSION

The habitat of *H. asinina* in Thai waters and elsewhere within its range is different that of most abalone in other parts of the world. They inhabit the coral reef environment (Nateewathana and Hylleberge 1986, Nateewathana and Bussawarit 1988, Sungthong et al. 1991, Tookvinart et al. 1986). On Samed Island, there are no macroalgae in their habitat; only the small close-growing tuft algae are found. Among the tuft algae are species of *Cladophora*, *Centroceras*, and *Ectocarpus*. *H. asinina* appears to feed almost exclusively on benthic diatoms. Benthic diatoms were dominant in

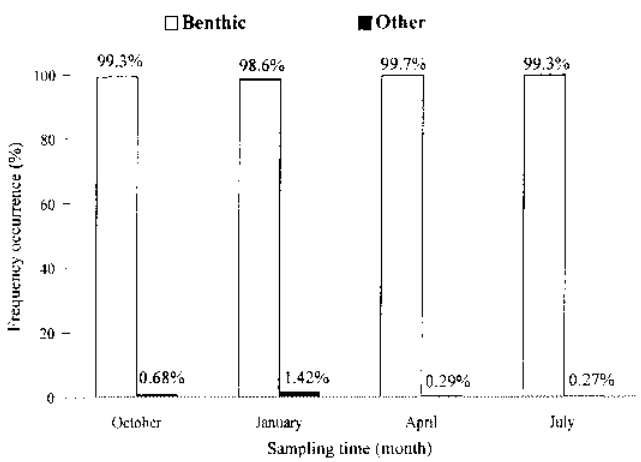

Figure 2. The microorganisms (<120 μm) in *H. asinina* gut during different sampling periods.

TABLE 3.

The genera of benthic diatoms in *H. asinina* gut.

Organism	Relative Frequency (%)*				
	October	January	April	July	Average
Benthic diatoms	99.3	98.5	99.3	99.6	99.1
Nitzschia	13.3	13.1	16.0	36.1	19.6
Amphora	22.9	11.8	16.0	18.2	17.2
Cocconeis	6.2	9.4	10.8	9.7	9.0
Fragilaria	5.6	21.0	5.2	3.2	8.7
Plagiogramma	6.7	15.7	0.5	5.0	7.0
Navicula	4.2	4.5	8.0	11.0	6.9
Surirella	4.0	1.6	10.5	1.7	4.4
Diploneis	7.5	1.5	4.5	3.2	4.2
Coscinodiscus	3.7	1.1	2.7	2.2	2.4
Licmophora	1.2	5.4	2.4	0.4	2.4
Grammatophora	3.2	2.1	2.3	0.6	2.0
Thalassionema	3.1	0.0	1.8	1.5	1.6
Melosira	3.1	1.4	0.5	0.6	1.4
Paralia	2.7	0.1	2.3	0.5	1.4
Achnanthes	0.7	2.5	1.6	0.6	1.3
Others†	6.0	3.3	8.0	4.4	5.6

* Relative abundance in Sedgwick-Rafter counting chamber subsamples (1 mL).

† Eighteen genera of benthic diatoms that have average values less than 1.0%.

the gut contents of *H. asinina* in this study. Where abundant, *H. asinina* may have a dramatic effect on benthic algal populations and their related communities that is comparable to the algal denudation commonly observed as sea urchins become dominant (Leighton et al. 1966, Paine and Vadas 1969, Sousa 1979).

The black abalone, *Haliotis cracherodii* Leach, common in the littoral of rocky California shorelines, was formerly believed to feed throughout its life principally on diatoms (Bonnot 1930, Bonnot 1940, Bonnot 1948). This concept was changed after the study of Leighton and Boolootian (1963), which concluded that *H. cracherodii* fed as adults chiefly on macroalgae such as *Macrocystis pyrifera*, *Egregia laevigata*, *Pelvetia fastigiata*, *Gigartina canaliculata*, and *Gigartina spinosa* that are often abundant in the habitat of this abalone species. In their gut contents were fragments of drift algae, filamentous algae, and diatoms. Fecal pellets often contained fragments of coralline algae, surf grass, *Phyllospadix* sp., and diatoms (Leighton and Boolootian 1963). Some diatoms in feces were observed to possess cytoplasmic and nuclear material and were therefore assumed not to have been completely digested. In contrast, the benthic diatoms in *H. asinina* gut contents in this study appeared to be completely digested except for the siliceous shells. Many researchers have reported that diatoms were the principal diet of very small abalone. Newly settled juveniles typically graze on diatoms from rock surfaces for the first few months of development (Ino 1952, Tomita and Tazawa 1971). Diatoms were secondary or supplementary to the harvesting of macroalgae by adult *H. cracherodii* (Leighton and Boolootian 1963). However, this is not the case in *H. asinina* on Samed Island. This study clearly shows that *H. asinina* on Samed Island feeds on benthic diatoms throughout its life, especially species of the genera *Nitzschia*, *Amphora*, *Navicula*, and *Cocconeis*. These diatoms were consistently found in high percentages in each period of sampling. Although the main organisms in *H. asinina* gut content were

TABLE. 4.

The macroorganisms (>120 μm) in *H. asinina* gut.

Organism	Frequency Percentage (Total Count)				
	October	January	April	July	Average
Foraminiferans	87.4	71.5	70.9	55.8	71.4
Crustaceans	6.9	14.1	12.1	9.9	10.7
Cypris	3.0	3.5	5.0	3.5	3.7
Harpacticoids	1.4	3.5	2.3	1.0	2.0
Other copepods	2.1	3.5	1.2	0.6	1.9
Crustaceans	0.0	2.6	2.0	2.3	1.7
Gnathopods	0.4	0.0	1.1	2.1	0.9
Other crustaceans*	0.0	1.0	0.5	0.4	0.5
Annelids	2.3	8.5	8.4	19.8	9.7
Mollusks	2.7	4.5	3.4	3.1	3.5
Gastropods	1.8	2.0	2.1	1.6	1.9
Bivalves	0.9	2.5	1.3	1.5	1.6
Benthic diatoms	0.7	1.4	5.2	11.4	4.7
Fragillaria	0.0	0.8	0.7	6.9	2.1
Triceratium	0.2	0.0	1.8	2.3	1.1
Campylodiscus	0.3	0.0	1.0	1.3	0.7
Rhabdonema	0.1	0.6	1.4	0.0	0.5
Coscinodiscus	0.1	0.0	0.1	0.7	0.2
Others†	0.0	0.0	0.2	0.2	0.1

* Composed of nauplei, isopods, cumacean shrimp, and amphipods.

† Composed of *Pleurosigma, Navicula,* and *Cocconeis.*

benthic diatoms, the feeding behavior involved in grazing may allow them to ingest other organisms that are also present on the substrate such as foraminiferans, pieces of tuft algae, crustaceans, annelids, and mollusks. Some abalone species, during grazing, were found to ingest hydrozoans, copepods, small gastropods, ascidians, bryozoans, foraminiferans, and even sponges (Ueda and Okada 1939, Leighton and Boolootian 1963, Shepherd 1973).

Several abalone species that frequently occur in exposed positions have been described as opportunistic graspers or trappers, e.g., *Haliotis laevigata* Donovan, *Haliotis iris* Gmelin, *Haliotis rufescens* Swainson, and *Haliotis roei* Gray (Olsen 1968, Poore 1972, Shepherd 1973, Barkai and Griffiths 1986, Wells and Keesing 1989). In some cases, abalone have been reported to starve rather than move to attached vegetation when drift components are not available, as may occur when sea urchin overgrazing removed attached seaweeds from the substrate (Leighton et al. 1966, MacGinitie and MacGinitie 1966). In common with most abalone species, *Haliotis rubra* Leach, *Haliotis cyclobates* Péron and Lesueru, *H. cracherodii, Haliotis midae* Linneaus, and *Haliotis iris* are reported to be both grazers and trappers, depending on environmental conditions (Shepherd 1973, Olsen 1968, Poore, 1972, Wood and Buxton 1996). *H. asinina* too can be both grazers and trappers. In their natural habitat at Samed Island, they must graze as juveniles and as adults on benthic diatoms exclusively. However, this species has been reared in the laboratory and fed different species of macroalgae including *Gracilaria tenuistipitata, Gracilaria fisheri, Gracilaria salicornia, Enteromorpha intestinalis,* and *Acanthophora spicifera* (Singhagraiwan and Doi 1993, Upatham et al. 1997). If macroalgae were present at Samed Island, these algae would be important to their nutrition. McNamara and Johnson (1995) studied growth of *H. asinina* on Heron reef, in tropical eastern Australia. There, the abalone fed on various species of macroalgae that occur in this habitat including *Hypnea pannosa, Laurencia* sp., and *Lobophora* sp. These researchers reported for *H. asinina* the highest growth rates recorded for any species of abalone. The coral reef environment at samed Island, devoid of tropical macroalgae, may afford an exceptional opportunity to study the growth of *H. asinina* in that context and to concentrate on the nutritional influences of the tuft algae component together with the abundant pennate diatom flora (Anunpongsuk and Sawatpeera 1991).

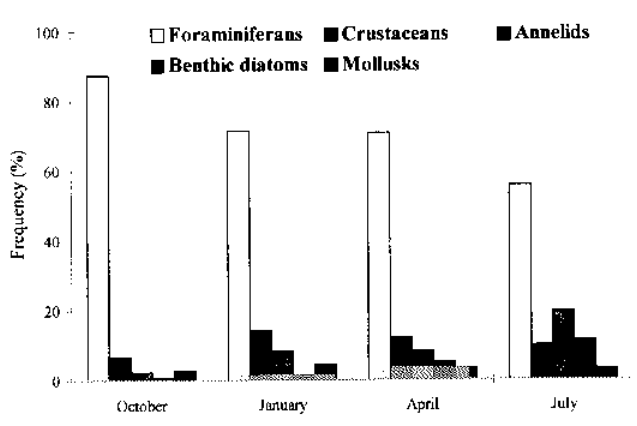

Figure 3. The macroorganisms (>120 μm) in *H. asinina* gut during different sampling periods.

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FOOD UTILIZATION BY *HALIOTIS ASININA* LINNAEUS

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ABSTRACT Experiments on food utilization by the Thai abalone, *Haliotis asinina*, were conducted over a period of 6 mo with nine species of macroalgae—*Acanthophora spicifera*, *Gracilaria fisheri*, *Gracilaria salicornia*, *Gracilaria tenuistipitata*, *Enteromorpha intestinalis*, *Caulerpa racemosa*, *Dictyota dichotoma*, *Padina minor*, and *Sargassum polycystum*. The highest growth rate (70.3 ± 3.4 μm/day in shell length; 23.6 ± 2.4 mg/day in weight), highest survival rate (95.6 ± 1.6%), and the best food conversion rate (3.3 ± 0.5) were obtained with the red alga, *G. tenuistipitata*. The brown alga, *P. minor*, gave the lowest growth (2.1 ± 3.0 μm/day in shell length; −0.3 ± 0.3 mg/day in weight) and survival (7.8 ± 1.6%) rates. The proximate composition of these nine species of algae was determined to suggest correlation with growth rates. *G. tenuistipitata* was found to have the highest content of protein and carbohydrate.

KEY WORDS: *Haliotis asinina*, macroalgal diets, relative nutritional values

INTRODUCTION

The donkey's ear abalone, *Haliotis asinina* Linnaeus, is a tropical abalone species distributed widely in coastal reef zones of southeast Asia. It is an esteemed shellfish with a high market value. Even though tropical abalone culture in Thailand is in the very early stages of development, with basic and applied research conducted only in small-scale operations, there have been several reports on culturing techniques for this species (Singhagraiwan 1989, Singhagraiwan and Doi 1993, Jarayabhand and Paphavasit 1996). Research has been done recently on growth and survival rates (Singhagraiwan 1991a, Singhagraiwan 1991b, Singhagraiwan and Sasaki 1991, Singhagraiwan 1993, Kunavongdate et al. 1995), breeding, early development and seed production (Singhagraiwan and Sasaki 1991, Singhagraiwan and Doi 1993), spawning pattern and fecundity (Singhagraiwan and Doi 1992), and salinity tolerance of *H. asinina* (Singhagraiwan et al. 1992).

Growth rates of *H. asinina* on natural algal diets are quite variable. Consequently, various diets of single algal species have been tested in an attempt to identify those diets that maximize the growth rate of *H. asinina* in culture (Singhagraiwan 1991a, Singhagraiwan 1991b, Singhagraiwan and Sasaki 1991, Kunavongdate et al. 1995). However, these reports were limited to only a few species of algae. Accordingly, in this study of food utilization by juvenile *H. asinina*, nine species of macroalgae common to the natural environment of this species were tested in order to determine which algae supported the best growth and survival rates. The proximate composition of these nine species of algae was determined.

MATERIALS AND METHODS

Juvenile *H. asinina* (age, 5 mo) were obtained from the Coastal Aquaculture Development Center, Prachuap Khiri Khan province, Thailand. They were fed *Gracilaria salicornia* (C. Agardh) Dawson before the experiment and acclimated to experimental conditions for 2 wk before collection of initial data.

The feeding experiments with monospecific algal diets were conducted in plastic aquaria (40 cm long, 25 cm wide, and 30 cm high). The animals were confined in a basket that was placed in plastic aquaria. Flow-through seawater was supplied at ambient temperature that ranged from 20°C in winter to 30°C in summer. Water flowed in at approximately 200–250 mL/min. Each aquarium was continually aerated.

Nine species of macroalgae were selected consisting of the red algae *Acanthophora spicifera* (Vahl) Børgesen, *Gracilaria fisheri* (Xia et Abbott) Abbott, Zhang et Xia, *G. salicornia*, and *Gracilaria tenuistipitata* (Chang et Xia); the green algae *Enteromorpha intestinalis* (Linnaeus) Nees and *Caulerpa racemosa* (Forsskål) J. Agardh; and the brown algae *Dictyota dichotoma* (Hudson) Lamouroux, *Padina minor* (Yamada), and *Sargassum polycystum* C. Agardh. Algae were weighed and provided in excess every 3 days. On the third day, the remaining fragments of algae were collected and weighed, and then dry matter content was determined by drying in a hot-air oven at 80°C until a constant weight was reached.

Juvenile abalone (13.29 ± 1.36 mm in shell length) were used in the experiments. Treatments were performed in triplicate. Thirty abalone were used for each replicate. Shell length, wet weight of each abalone, and mortality were recorded every 2 wk throughout

the course of 6 mo (184 days). Increases in shell length and body weight of abalone fed various species of algae were determined for each 2-wk period. Growth of abalone was expressed in terms of shell length (μm/day) and body weight (mg/day). The food conversion rates (FCR) for *H. asinina* feeding on the nine tested algae were calculated on the basis of the total dry weight gain of abalone and the dry weight of algae consumed as follows (algal value quotient; Leighton and Boolootian 1963):

$$\text{FCR} = \frac{\text{Dry weight algae consumed (g)}}{\text{Dry weight gain (g)}}$$

At the beginning and the end of experiments (184 days), the total dry weight of abalone of each tested group was determined. The animals were weighed and dried at 85°C until a constant weight was reached.

Samples of the nine species of algae tested were analyzed for crude protein (Horwitz 1980), crude fat (Pearson 1976), crude carbohydrate, ash, and moisture (Kangsadalampai and Sungpuag 1984). Each analysis was performed in triplicate.

Growth and survival rates, FCR of abalone, and nutritional values of algae were determined by one-way analysis of variance. The multiple comparison, Duncan's multiple range test was performed to determine the significant differences between each treatment. SPSS for Windows, Version 6.0, was the statistical software for all statistical analysis, at $p < 0.05$.

RESULTS

Growth Rates

The results obtained for each algal diet are shown in Table 1 and Figures 1 and 2. Growth rates (shell length and body weight increases per day) of *H. asinina* were significantly different ($p < 0.05$) among five groups of feed treatments, i.e., (1) *G. tenuistipitata*, (2) *A. spicifera*, (3) *G. salicornia*, (4) *G. fisheri* and *E. intestinalis*, and (5) *P. minor*. No data were obtained for *H. asinina* fed the remaining algal species (*C. racemosa*, *D. dichotoma*, and *S. polycystum*) because all died before the end of the experiment (Figs. 1 and 2), presumably the result of starvation.

Shell Length

The maximum cumulative increase in shell length was 97.4 ± 4.2% for abalone fed the red alga, *G. tenuistipitata* (Fig. 1). This equaled a growth rate of 70.3 ± 3.4 μm/day (Table 1). The intermediate cumulative increases of shell length were obtained with abalone fed *A. spicifera* (86.2 ± 1.8%), *G. salicornia* (82.2 ± 4.6%), *E. intestinalis* (75.1 ± 5.4%), and *G. fisheri* (66.0 ± 0.9%) (Fig. 1). These gains corresponded to growth rates of 62.2 ± 1.4, 59.4 ± 3.2, 54.3 ± 3.8, and 47.6 ± 0.6 μm/day, respectively (Table 1). The lowest cumulative increase in shell length was 2.1 ± 0.8% for abalone fed *P. minor*, with a corresponding growth rate of 2.1 ± 3.0 μm/day (Fig. 1, Table 1).

Body Weight

The maximum rate of cumulative weight increase was found in abalone fed *G. tenuistipitata* (1056 ± 118%), which resulted in a growth rate of 23.6 ± 2.4 mg/day (Fig. 2, Table 1). Abalone fed *A. spicifera*, *G. salicornia*, *G. fisheri*, and *E. intestinalis* resulted in intermediate cumulative increases in weight (726 ± 5, 610 ± 94, 508 ± 43, and 478 ± 68%, respectively) (Fig. 2). These were related to growth rates of 16.6 ± 0.6 (*A. spicifera*), 13.8 ± 2 (*G. salicornia*), 11.0 ± 0.5 (*G. fisheri*), and 10.9 ± 1.7 (*E. intestinalis*) mg/day (Table 1). The smallest increase in weight was found in abalone fed *P. minor* (−16.4 ± 0.1% weight increase), which was −0.3 ± 0.3 mg/day in growth rate (Fig. 2, Table 1).

Survival Rate

The survival rates of *H. asinina* fed the various species of algae were significantly different ($p < 0.05$) among four groups of feed treatments (Fig. 3), i.e., (1) *G. tenuistipitata* (95.6 ± 1.6%), *A. spicifera* (94.4 ± 4.2%), and *E. intestinalis*, (87.8 ± 9.6%); (2) *G. fisheri* (76.7 ± 7.2%); (3) *G. salicornia* (55.6 ± 8.3%); and (4) *P. minor* (7.8 ± 1.6%). Abalone fed *C. racemosa*, *D. dichotoma*, and *S. polycystum* all had died by the 93rd, 150th, and 162nd days of the experiment, respectively (Fig. 3).

FCR

The dry weight FCR were significantly different ($p < 0.05$) among three groups of feed treatments (Table 2), i.e., (1) *G. tenuis-*

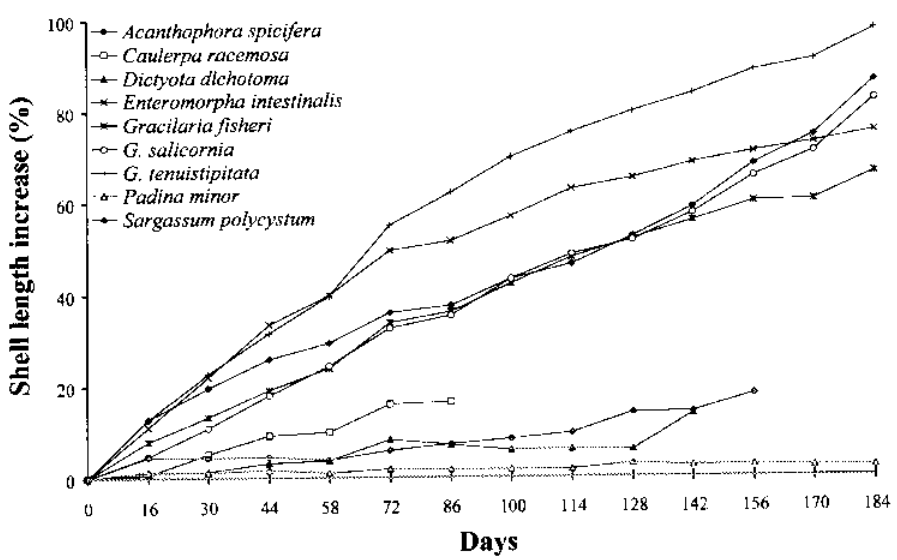

Figure 1. Percent shell length increase of *H. asinina* fed nine species of macroalgae.

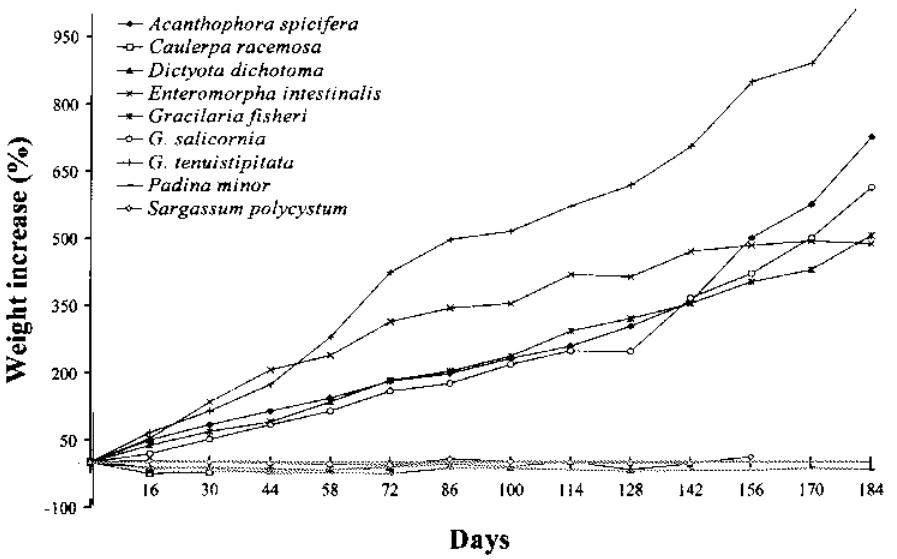

Figure 2. Percent weight increase of *H. asinina* fed nine species of macroalgae.

tipitata (3.3 ± 0.6); (2) *A. spicifera* (5.7 ± 0.2). *G. fisheri* (7.4 ± 1.0), and *E. intestinalis* (9.8 ± 2.7), and (3) *G. salicornia* (15.5 ± 4.0). The lowest or best FCR was obtained on a diet of *G. tenuistipitata*, whereas *G. salicornia* produced the highest FCR. The FCR for *P. minor* could not be calculated because of dry weight loss.

Proximate Compositions

Table 3 shows the analysis of proximate composition of the nine species of algae with respect to protein, fat, carbohydrate, ash, and moisture. *G. tenuistipitata* showed a significantly higher ($p < 0.05$) content of protein (24.7 ± 2.1%) than did species of the remaining algae.

DISCUSSION

The results of this study of food utilization by the Thai abalone, *H. asinina*, when provided nine species of algae, showed that the red alga, *G. tenuistipitata*, was the most readily consumed species. It yielded the greatest growth and survival rates. The other three species of red algae (*A. spicifera*, *G. salicornia*, and *G. fisheri*) also showed high utilization and provided relatively high growth rates. In contrast, the brown alga *P. minor* gave the lowest growth and survival rates, whereas the green alga, *C. racemosa*, was the least effective species; the abalone did not survive beyond the 93rd day of the experiment.

There are numerous reports on algal foods of abalone species from different parts of the world (see Shepherd and Steinberg 1992). Except for abalone species in the Southwest Pacific and Australasia, most abalone species prefer brown algae, especially phenolic-poor species (Shepherd and Steinberg 1992). In Australasia, the abalone species *Haliotis roei* Gray, *Haliotis laevigata* Donovan, *Haliotis rubra* Leach, and *Haliotis iris* Gmelin seemed to prefer red algae such as *Gracilaria*, *Jeannerettia*, *Pterocladia*, and *Asparagopsis* (Shepherd 1973, Shepherd 1975, Shepherd and

TABLE 1.

Growth and survival rates of *H. asinina* provided nine species of macroalgae over a period of 184 days.

Species of Algae	Survival Rate (%)	Shell Length (mm)		Body Weight (g)		% Cumulative Increase		Growth rate	
		Initial	Final	Initial	Final	Length	Weight	Length (μm/day)	Weight (mg/day)
Red algae									
A. spicifera	94.4 ± 4.2^{a}	13.3 ± 1.4	24.8 ± 3.4^{b}	0.4 ± 0.1	3.5 ± 1.6^{b}	86.2 ± 1.8^{b}	726 ± 5^{b}	62.2 ± 1.4^{b}	16.6 ± 0.6^{b}
G. fisheri	76.7 ± 7.2^{b}	13.3 ± 1.4	22.0 ± 3.2^{d}	0.4 ± 0.1	2.4 ± 1.2^{d}	66.0 ± 0.9^{d}	$508 \pm 43^{c,d}$	47.6 ± 0.6^{d}	11.0 ± 0.5^{d}
G. salicornia	55.6 ± 8.3^{c}	13.3 ± 1.4	24.3 ± 2.8^{b}	0.4 ± 0.1	3.0 ± 1.3^{c}	82.2 ± 4.6^{b}	$610 \pm 94^{b,c}$	59.4 ± 3.2^{b}	13.8 ± 2.0^{c}
G. tenuistipitata	95.6 ± 1.6^{a}	13.3 ± 1.4	26.2 ± 3.6^{a}	0.4 ± 0.1	4.8 ± 2.0^{a}	97.4 ± 4.2^{a}	1056 ± 118^{a}	70.3 ± 3.4^{a}	23.6 ± 2.4^{a}
Green algae									
E. intestinalis	87.8 ± 9.6^{a}	13.3 ± 1.4	23.4 ± 2.9^{c}	0.4 ± 0.1	2.5 ± 1.0^{d}	75.1 ± 5.4^{c}	478 ± 68^{d}	54.3 ± 3.8^{c}	10.9 ± 1.7^{d}
C. racemosa	—	13.3 ± 1.3	—	0.4 ± 0.1	—	—	—	—	—
Brown algae									
D. dichotoma	—	13.3 ± 1.2	–	0.4 ± 0.1	—	—	—	—	—
P. minor	7.8 ± 1.6^{d}	13.3 ± 1.4	13.6 ± 1.4^{e}	0.4 ± 0.1	0.5 ± 0.1^{e}	2.1 ± 0.8^{e}	-16.4 ± 0.1^{e}	2.1 ± 3.0^{e}	-0.3 ± 0.3^{e}
S. polycystum	—	13.3 ± 1.4	—	0.4 ± 0.1	—	—	—	—	

Means and standard errors are presented. All values are expressed on a wet weight basis. The results of Duncan's multiple range test performed on the means of length and weight increases and growth rate (shell length and body weight) are shown besides the means; the same letter identifies algae that are not significantly different ($p < 0.05$). The experiments were performed in triplicate ($n = 30$).

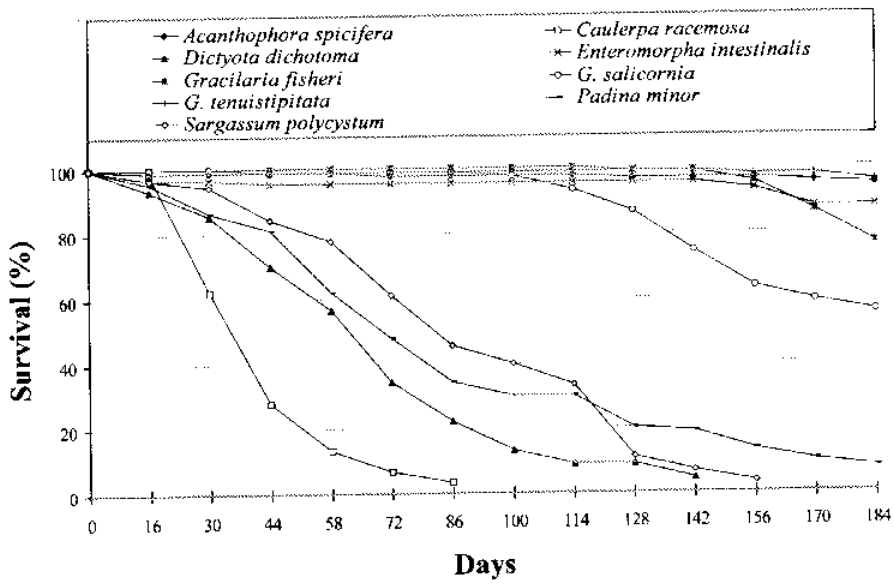

Figure 3. Percent survival of *H. asinina* fed nine species of macroalgae.

Steinberg 1992, Wells and Keesing 1989, Prince 1989, Stuart and Brown 1994, Fleming 1995). Reports on food preferences of *H. asinina* have indicated that red algae such as *Gracilaria* and *Laurencia* are preferred (Singhagraiwan 1991b, Singhagraiwan and Sasaki 1991, Kunavongdate et al. 1995). Hence, this study is in good agreement with earlier studies on *H. asinina* and several other *Haliotis* species of Australasia. In the tropics, red algae may predominate by number of species in some areas, and in terms of biomass, red algae may be more abundant than brown and green algae.

Food preferences appear to be correlated with nutritive value and growth. Generally, *Haliotis* species in temperate zones have shown better growth rates when fed brown algae than when fed red algae (Table 4). However, the Australasian species showed better growth rates when fed red algae (Table 4). In New Zealand, Stuart and Brown (1994) reported the best growth (11.6 mg/day in weight, 57.2 μm/day in shell length) in *H. iris* fed mixed algae (*Gracilaria chilensis, Ulva lactuca*, and *Macrocystis pyrifera*). In Australia, *H. rubra* fed the red alga *Jeannerettia lobata* exhibited a very high growth rate (51.0 mg/day) (Fleming 1995).

In Thailand, *H. asinina* fed red algae also showed better growth than did those fed either green or brown algae. Singhagraiwan (1991b) reported very high growth rates for body weight (51.08 mg/day) and shell length (51.6 μm/day) when *H. asinina* was fed *G. salicornia*. Kunavongdate et al. (1995) reported that *H. asinina* fed *Laurencia* sp. showed very high growth rates (24 mg/day in weight, 98 μm/day in shell length). In this study, *H. asinina* fed *G.*

TABLE 2.

FCR of *H. asinina* fed nine species of macroalgae over a period of 184 days.

	Number and Total Weight of Abalone							
	Initial		Final		Total Wet Weight Gain (g)	Total Dry Weight Gain (g)	Dry Weight Intake of Algae (g)	FCR*
Species of Algae	Number	Weight (g)	Number	Weight (g)				
Red algae								
A. spicifera	90	37.8	85	294.3	85.5 ± 2.2^{b}	32.5 ± 0.8^{b}	186.5 ± 0.3	5.7 ± 0.2^{b}
G. fisheri	90	36.1	69	203.3	43.9 ± 5.4^{c}	16.7 ± 2.1^{c}	122.0 ± 1.7	7.4 ± 1.0^{b}
G. salicornia	90	37.5	50	148.4	36.9 ± 10.7^{c}	14.0 ± 4.1^{c}	201.2 ± 1.4	15.5 ± 4.0^{c}
G. tenuistipitata	90	36.6	86	410.3	124.4 ± 14.8^{a}	47.3 ± 5.6^{a}	151.6 ± 7.1	3.3 ± 0.6^{a}
Green algae								
C. racemosa	90	37.3	0	0			25.9 ± 0.4	—
E. intestinalis	90	37.7	79	194.1	52.1 ± 15.5^{c}	19.8 ± 5.9^{c}	178.3 ± 1.0	9.8 ± 2.7^{b}
Brown algae								
D. dichotoma	90	36.7	0	0	—	—	21.2 ± 1.8	—
P. minor	90	38.2	1	2.0	—	-4.5 ± 0.06^{d}	36.8 ± 7.2	—
S. polycystum	90	37.1	0	0	—	—	34.5 ± 4.6	—

Means and standard errors are presented. The results of Duncan's multiple range test performed on the means of total wet weight gain, total dry weight gain, dry weight intake of algae, and FCR are shown beside the means; the same letter identifies algae that are not significantly different ($p < 0.05$). The experiments were performed in triplicate.

* FCR = g. algae to produce 1.0 g. abalone wt. gain

TABLE 3.

Proximate composition of the nine species of macroalgae.

Species of Algae	Composition (% Dry Weight)				Moisture (%)
	Protein	Fat	Carbohydrate	Ash	
Red algae					
A. spicifera	$15.4 \pm 2.0^{c,d}$	$1.3 \pm 1.0^{d,e}$	$34.6 \pm 4.2^{d,e,f}$	$48.7 \pm 6.8^{a,b,c}$	93.2 ± 12.9^{a}
G. fisheri	$16.8 \pm 2.5^{b,c}$	$1.1 \pm 0.1^{e,f}$	57.2 ± 7.0^{a}	24.9 ± 2.5^{d}	81.2 ± 6.7^{a}
G. salicornia	$16.9 \pm 1.8^{b,c}$	$1.3 \pm 0.1^{d,e}$	$31.1 \pm 4.6^{e,f}$	$50.7 \pm 3.3^{a,b}$	90.3 ± 13.4^{a}
G. tenuistipitata	24.7 ± 2.1^{a}	0.7 ± 0.0^{g}	$52.0 \pm 6.7^{a,b}$	22.7 ± 1.5^{d}	84.9 ± 5.0^{a}
Green algae					
C. racemosa	19.4 ± 1.5^{b}	3.2 ± 0.2^{b}	$27.4 \pm 3.9^{f,g}$	$50.0 \pm 6.2^{a,b}$	93.8 ± 13.2^{a}
E. intestinalis	18.9 ± 2.5^{b}	3.8 ± 0.4^{a}	20.8 ± 2.0^{g}	56.6 ± 3.5^{a}	94.9 ± 6.7^{a}
Brown algae					
D. dichotoma	$14.0 \pm 0.7^{c,d}$	2.8 ± 0.3^{c}	$38.3 \pm 3.9^{c,d,e}$	$44.9 \pm 5.5^{b,c}$	89.6 ± 10.0^{a}
P. minor	12.3 ± 1.4^{d}	$0.8 \pm 0.0^{f,g}$	$46.2 \pm 2.4^{b,c}$	40.8 ± 4.7^{c}	87.0 ± 5.0^{a}
S. polycystum	9.2 ± 1.1^{e}	1.5 ± 0.2^{d}	$41.2 \pm 3.2^{c,d}$	$48.1 \pm 6.7^{a,b,c}$	87.4 ± 12.9^{a}

Means and standard errors are presented. The results of Duncan's multiple range test performed on the means of protein, fat, carbohydrate, ash (% dry weight), and moisture (%) are shown beside the means; the same letter identifies algae that are not significantly different ($p < 0.05$). The experiments were performed in triplicate.

tenuistipitata showed the highest growth rates (23.6 mg/day in weight, 70.3 μm/day in shell length). These results on growth rates are similar to those from the study of Kunavongdate et al. (1995). In addition, the survival rates of *H. asinina* fed *G. salicornia* (100%), *Laurencia* sp. (100%), *G. fisheri* (100%), and *G. tenuistipitata* (95.6%), over a period of 184 days, were not significantly different (Singhagraiwan 1991b, Kunavongdate et al. 1995). Their reported FCR were 20.4 in *H. asinina* fed *G. salicornia* (Singhagraiwan 1991b), 29.49 in those fed *G. fisheri* (Kunavongdate et al. 1995), and 17.9 in those fed *Laurencia* sp. (Kunavongdate et al. 1995). In this study, the diet of *G. tenuistipitata* resulted in an FCR of 3.3.

Shepherd and Steinberg (1992) proposed three factors that may affect the acceptability of food algae in nature. These include (a) the presence of chemical metabolites in algae, (b) algal morphology (toughness), and (c) nutritional value. Among the three species of *Gracilaria* tested (*G. salicornia*, *G. fisheri*, and *G. tenuistipitata*), the *G. tenuistipitata* thallus is soft and palatable. That alga has a high protein content compared with the other algae, which may have contributed to the relatively high growth rates obtained on this seaweed.

ACKNOWLEDGMENT

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TABLE 4.

Growth rates reported for juveniles of various *Haliotis* species fed different species of algae.

Species of Abalone	Observation Temperature (°C)	Initial Size		Species of Algae	Growth Rate		References
		Weight (g)	Length (mm)		Weight (mg/day)	Length (μm/day)	
Haliotis discus hannai	18.9–20.6	1.9	24.0	*Laminaria japonica*	19.0	108.0	Uki 1981
		1.8	24.0	*Laminaria religosa*	18.0	101.0	
		1.9	24.0	*E. bicyclis*	16.3	48.0	
		1.9	24.0	*U. pinnatifida*	25.3	121.0	
Haliotis laevigata	18	0.4	15.0	*Gracilaria conifervoides*	6.7	—	Morrison & Whittington 1991
Haliotis rubra	18	7.0	—	*Jeanneretia lobata*	19.0		Gorfine & King 1991
					51.0	—	Fleming 1995
	10–20	7.0	—	*Phyllospora comosa*	8.0	—	Gorfine & King 1991
Haliotis refescens	10.6–14.2	8.3	38.3	*Macrocystis* sp.	80.3	69.0	Ebert & Houk 1984
Haliotis tuberculata	20 ± 0.5	0.2	10.0	*Palmaria palmata*	2.9		Kolke et al. 1979
		0.1	10.0	*Ulva lactuca*	2.1	—	
		0.1	9.0	*Laminaria digitata*	1.7	—	
Haliotis iris	15 ± 1	1.2	21.6	*Gracilaria chilensis*	9.7	45.5	Stuart & Brown 1994
		1.1	21.0	*Macrocystis pyrifera*	5.9	34.4	
Haliotis asinina	25	4.8	27.8	*Gracilaria salicornia*	51.1	51.2	Singhagraiwan 1991b
	25–28	9.7	31.1	*Laurencia* sp.	24.0	98.1	Kunavongdate et al. 1995
		10.9	31.3	*Gracilaria fisheri*	3.0	21.0	
		10.4	31.4	*Eucheuma* sp.	2.0	14.0	

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THE NATURAL DIET OF THE GREEN ABALONE (*HALIOTIS FULGENS* PHILIPPI) IN THE SOUTHERN PART OF ITS RANGE, BAJA CALIFORNIA SUR, MEXICO, ASSESSED BY AN ANALYSIS OF GUT CONTENTS

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ABSTRACT An attempt was made to assess the natural diet of the green abalone from the southern extreme of its range by examination of gut contents. Crop and stomach contents were identified in samples from study sites at Bahía Asunción, La Bocana, Punta Abreojos, and Las Barrancas along the Pacific Coast of Baja California Sur. Shell lengths of abalone from banks at the northern sites, Bahía Asunción and La Bocana, were greater than for abalone found at the southern sites, Punta Abreojos and Las Barrancas. The consistency and weight of gut content among individuals varied according to the extent of digestion in each. Differences were found between the weight of the particulate contents at the different sites. The smallest number of plant species in the gut was one, and the largest was six. The average number of plant species per individual gut was similar at three sites. The highest average was at Las Barrancas. Seven Rhodophytes, four Phaeophytes, and one seagrass were recorded. The number of species in samples from Las Barrancas was nine, followed by Bahía Asunción (n = 8), La Bocana (n = 7), and Punta Abreojos (n = 5). The most common food items in gut content were the seagrass *Phyllospadix torreyi* and the macroalgae *Sargassum* sp., *Eisenia arborea*, *Cryptopleura crispa*, and *Rhodymenia* sp. The other species found in the abalone diets could be considered species that are consumed incidentally with the main food according to the local flora. The brown algae *Sargassum* and *Eisenia* and the red algae *Cryptopleura* and *Rhodymenia* made up about 50% (relative frequency) of the recovered food items at Bahía Asunción and Las Barrancas and about 80% at La Bocana and Punta Abreojos. *P. torreyi* formed 32% of the recovered food items at Punta Prieta and about 15% at the other sites. No other algae formed more than 12% of the gut content items in these green abalone.

KEY WORDS: natural diet, gut content, green abalone, *Haliotis*

INTRODUCTION

Seaweed plays an important role in nearshore marine ecosystems as a source of food, habitat, or refuge for many species of fauna, mollusks, crustaceans, and fish. In the wild, the common food of the adult abalone consists of macroalgae. Abalone food preferences have been studied from both analyses of gut contents and feeding experiments. Shepherd and Steinberg (1992) reviewed the literature on the feeding biology of abalone and found that although most populations have a wide range of acceptable algae for food, each species is fairly selective and usually has a preferred food item. Abalone have available to them different types of food in different parts of the world. In the northern hemisphere, there is a general preponderance of brown algae in the abalone diet. In the southern hemisphere, abalone feed mostly on the abundant red algae.

The benthic environments inhabited by abalone off southern California are characterized chiefly by Phaeophyta (brown algae). Dominant species include *Macrocystis pyrifera* (L.), C. Ag., *Pelagophycus porra* (Lem.) Setch., *Laminaria farlowii* Setch., *Pterygophora californica* Rupr., *Egregia menziesii* (Turn.) Aresh., *Eisenia arborea* Aresh., and *Cystoseira osmundacea* (Turn.) C. Ag. Rhodophyta (red algae) present generally include articulated and encrusting coralline algae, *Rhodymenia* spp., *Gelidium* spp., *Gigartina* spp., and *Plocamium* spp. (Dawson et al. 1960). Chlorophyta (green algae) do not form a conspicuous part of the algal flora of near-shore areas south of Point Conception (Leighton 1968). The production of *Macrocystis*, understory kelps, and bottom-cover algae, together with energy imported from the plankton, supports consumers in giant kelp forests as well as in nearby communities receiving drift from these beds or forests (Foster and Schiel 1985).

In California, the bull kelp, *Nereocystis leutkeana* (Mert.) Post. & Rupr.; giant kelp, *Macrocystis*; elk kelp, *Pelagophycus*; feather boa kelp, *Egregia*; belt kelp, *Laminaria*; and palm kelp, *Eisenia*; are the main and preferred foods of red abalone, *Haliotis rufescens* Swainson; pink abalone, *Haliotis corrugata* Wood; white abalone, *Haliotis sorenseni* Bartsch; and green abalone, *Haliotis fulgens* Philippi (Cox 1962, Leighton 1966, Leighton 1968, Tutschulte and Connell 1988). *Haliotis cracherodii* Leach showed a preference for brown algae, *M. pyrifera*, *E. menziesii*, and *Pelvetia fastigiata* (J. Ag.) De Toni (Leighton and Boolootian 1963). Kelps commonly used as food for abalone in California mariculture are *M. pyrifera* and *E. menziesii*. The former is a valuable food for young red abalone, but a relatively poor diet for green abalone (Leighton 1989). *Egregia* is effectively used by both species and is the diet of choice for culture of green abalone (Leighton et al. 1981).

In contrast, red algae were found to be the main food items of two New Zealand species, *Haliotis iris* Gmelin and *Haliotis australis* Gmelin (Poore 1972). Australian abalone also apparently prefer to eat red algae but will consume some species of brown algae, generally when preferred species are uncommon (Shepherd and Steinberg 1992). For example, *Haliotis laevigata* Donovan rejects nearly all species of brown and green algae and prefers red algae and seagrass blades. *H. rubra* Leach feeds extensively on the fronds of the large "kelp," *Phyllospora*, on drifting blades of the giant kelp, *M. pyrifera*, and red algae, depending on the site. *Haliotis roei* Gray feeds preferentially on red algae, such as the short red algal "turf" seaweed, *Pterocladia* (Shepherd 1975).

Along the western coast of Baja California, Dawson et al. (1960), Guzmán del Próo et al. (1972, 1991), and Mateo-Cid and Mendoza-González (1994) have studied the associated flora in the natural habitats of the different abalone species in southern Baja California and found that the principal components of the abalone

habitat are brown algae, *M. pyrifera*, *E. arborea*, and other Laminariales; red algae, *Gelidium*, *Acrosorium*, and *Plocamium*; the articulated coralline algae, *Bossiella* and *Corallina* spp.; the crustose coralline algae, *Lithothamnium* and *Lithophyllum*; and the seagrass, *Phyllospadix torreyi* S. Watson. These species support the main gastropod grazers in the community including *Tegula eiseni* Jordan, *Tegula aureotincta* Forbes, *Ocenebra foveolata* Hinds, *Haliotis* spp., and *Astraea undosa* Wood (Guzmán del Próo et al. 1991).

Although it has sometimes been assumed by fishermen that Mexican abalone species feed extensively on *Macrocystis*, there have been no local studies on the natural diet or feeding habits of these species. Moreover, *Macrocystis* is not present in abalone banks around Punta San Roque (27°N lat) to Magdalena bay (24°N lat), the southern limit of the commercial abalone range, although as drift, *M. pyrifera* does occur as far south as Punta Abreojos (26°N lat). In Baja California, there are seven abalone species, but only five have commercial interest: *H. fulgens*, *H. corrugata*, *H. cracherodii*, *H. rufescens*, and *H. sorenseni*. Green abalone are the main resource of the Mexican abalone fishery.

The purpose of this article is to identify the food plants consumed by adult *H. fulgens* through examination of gut contents of specimens collected along the southern part of its range, Baja California Sur, México, at sites largely beyond the current distribution range of *M. pyrifera*. We sought to determine the relative importance of brown and red algae as food for abalone living in that area. Studies on natural diets, growth on specific algal diets, digestive processes, digestibility, and conversion efficiency for algae by marine herbivores of commercial interest are important. A better understanding of these subjects will help suggest optimal diets for abalone culture.

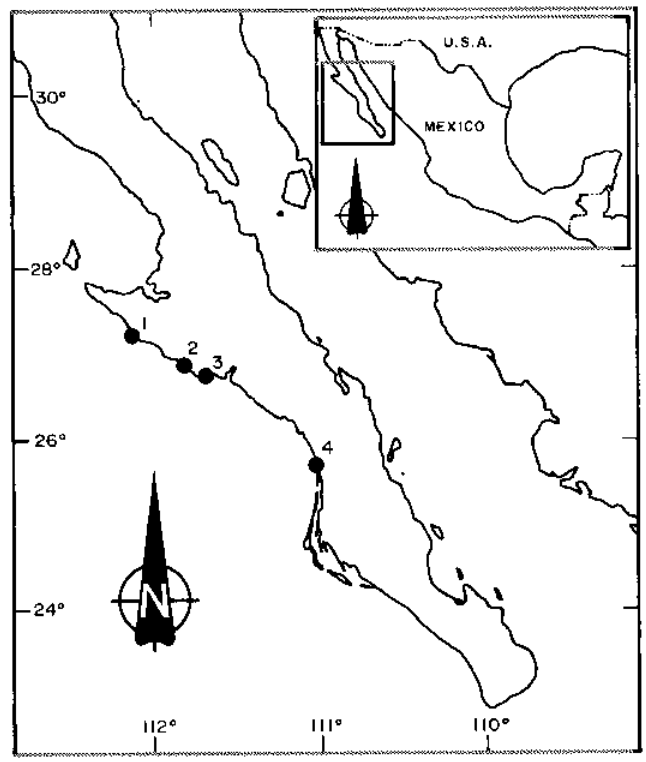

Figure 1. The study sites: 1, "Punta Prieta," 40 km south of Bahía Asunción; 2, "La Piedra de la Orilla," La Bocana; 3, "Piedra de Afuera," Punta Abreojos; 4, "La Punta, El Cora y Amarillo," Las Barrancas.

MATERIALS AND METHODS

Green abalone were collected from four study sites along the coast of Baja California Sur—"Punta Prieta," 40 km south of Bahía Asunción; "La Piedra de la Orilla" at La Bocana; "Piedra de Afuera" at Punta Abreojos; and "La Punta, El Cora y Amarillo" at Las Barrancas—in August and September 1995 and March 1996 by personnel of Centro Regional de Investigaciones Pesqueras, CRIP-La Paz (Abalone project) and of the fishermen's cooperatives Leyes de Reforma, Progreso, Punta Abreojos, and Puerto Chale (Fig. 1). These sites are beyond the present distribution range of the brown alga *M. pyrifera*. This species of kelp is found from Alaska to Baja California, México (Abbott and Hollenberg 1976). Stands can occur as far south as Punta Asunción-Punta San Hipólito in Baja California, México (27°N lat; Dawson 1951), but this southern limit varies. At the time of sampling, the most southerly plants were around Punta San Roque, over 15 km northwest of Bahía Asunción (Serviere pers. obs.).

The specimens were collected by Hookah divers. All diving was done in the early morning. Four or 5 h after collection, the specimens were delivered to the shore, and the sex and shell length of each abalone sampled were recorded. The digestive tracts of 30 specimens from each site were donated by the cooperatives for the study (Table 1). Guts were labeled and preserved in 10% formalin in seawater neutralized with sodium borate.

In the laboratory, each digestive tract was dissected and the crop and stomach contents were washed with distilled water, recovered on a 1-mm-pore-size mesh sieve, and weighed while wet (McLean 1970). The different groups present were separated by viewing with a stereoscopic microscope and identified to the lowest taxonomic level permitted by the degree of digestion of the contents. It was necessary to compare algal fragments in detail (external, internal, and reproductive morphology) with macroalgae collected from the benthic community at nearby sites or with herbarium specimens. The keys and species descriptions of Abbott and Hollenberg (1976), Dawson (1953, 1954, 1963), Hollenberg and Dawson (1961), Phillips and Meñez (1988), and Taylor (1960) were used to guide our identifications.

The frequency for each species was calculated from the number of guts in which the species occurred among the total number of guts sampled, and the relative frequency was calculated from

$$\text{Relative frequency} = \frac{\text{number of occurrences}}{\text{number of guts}} \times 100$$

Relative frequency was obtained for each site and also for the total guts analyzed.

Differences in mean shell length, weight of gut contents, and number of species per gut between study sites were analyzed with the nonparametric Kruskal-Wallis test (Sokal and Rohlf 1995). Multiple comparison procedures (Kolmogorov-Smirnov test) were used to test for significant differences between means. All statistical procedures were done with the program STATISTICA 5.0 for PC.

RESULTS

The range in shell length for the abalone studied was 71.0–182.0 mm, and the overall mean ± SD was 134.04 ± 22.09 mm. The mean size was significantly different between sites (Kruskal-

TABLE 1.

Location, shell length, and sex of the green abalone analyzed.

Sites	Geographic Location	Date	n*	Shell Length (mm)†	Sex‡
Bahía Asunción, "Punta Prieta"	114°17'40" W 27 07'00" N	Sept. 4, 1995	30	141.04 ± 15.08	11♂, 11♀ 8?
La Bocana, "La Piedra de la Orilla"	113°41'35" W 26°46'30" N	Sept. 2, 1995	29	143.96 ± 28.26	13♂, 10♀ 6?
Punta Abreojos, "Piedra de Afuera"	113°34'05" W 26°41'50" N	Aug. 31, 1995	31	129.30 ± 21.58	15♂, 12♀ 4?
Las Barrancas, "La Punta, El Cora y Amarillo"	112°27'00" W 26°14'00" N	March 8, 1996	30	120.40 ± 10.01	9♂, 6♀ 15?

* n, Sample size of abalone examined.

† Mean ± SD.

‡ ♂, male; ♀, female; ?, not identified.

Wallis test, $H = 25.54$; $p < 0.0001$). The mean size of abalone from banks at the northern sites, Bahía Asunción (141.04 ± 15.08, mean ± SD) and La Bocana (143.96 ± 28.26), was greater than the size of abalone found at the southern sites. Punta Abreojos (129.30 ± 21.58) and Las Barrancas (120.40 ± 10.01) (Kolmogorov-Smirnov test, $p < 0.05$).

The consistency and wet weight of gut contents among individuals were different, depending in part on the degree of digestion and the time since last feeding in each (Fig. 2). In general, the contents were fragments of macroalgae and seagrass larger than 1 mm. However, in some individuals, the stomach contents were more liquid, probably because digestion was more advanced, and in these, there were few fragments of macroalgae and seagrass larger than 1 mm. The average wet weight (grams ± SD) was 1.05 ± 0.73 at Bahía Asunción, 3.57 ± 2.23 at La Bocana, 1.99 ± 1.57 at Punta Abreojos, and 3.59 ± 2.6 at Las Barrancas. Differences between the content weights for abalone from the different sites were tested (Kruskall Wallis test, $H = 21.85$; $p < 0.0001$).

The smallest number of species per individual gut was one, and the largest was six (Fig. 3). The average number of plant species per gut was similar at three sites: Bahía Asunción (3 ± 1, mean ± SD), La Bocana (2 ± 1), and Punta Abreojos (2 ± 1). The highest average was at Las Barrancas (4 ± 1) and was significantly different (Kolmogorov-Smirnov test, $p < 0.01$).

The components found in the crop and stomach contents of 121 green abalone adults were 12 species, of which 4 were Phaeophyta, 7 were Rhodophyta, and 1 species was seagrass (Table 2). The number of species in all guts at Las Barrancas was nine, followed by Bahía Asunción (n = 8), La Bocana (n = 7), and Punta Abreojos (n = 5). In general, the species diversity of red algae predominated over brown algae. In Punta Abreojos specimens, however, red and brown algae were equally represented.

Guzmán del Próo et al. (1991) studied the associated flora of the abalone species in Bahía Tortugas (27°N lat) and reported 4 species of Chlorophyta, 11 species of Phaeophyta, 29 species of Rhodophyta, and 1 spermatophyta. The number of species in summer was 22, and in winter, it was 27. Mateo-Cid and Mendoza-González (1994) found 113 species of benthic marine algae at Bahía Asunción (27°N lat): 3 species of Cyanophyta, 13 species of Chlorophyta, 19 species of Phaeophyta, and 78 species of Rhodophyta. The greatest diversity occurred in summer with 110 taxa; 40 species were found in winter. At Bahía Magdalena (24°N lat), a total of 132 species were identified—22 species of Chlorophyta, 22 species of Phaeophyta, and 88 species of Rhodophyta. The greatest diversity occurred in winter, with 77 species; 66 species were found in summer (Sánchez-Rodríguez et al. 1989). *M. pyrifera*, *E. arborea*, *Corallina officinalis* L., *Corallina pinnatifolia* (Manza) Daws., and *P. torreyi* were the principal components of the benthic vegetation of Bahía Tortugas and Bahía Asunción. *Codium magnum* Daws., *Caulerpa sertularioides* (S.G. Gmel.) Howe, *Colpomenia tuberculata* Saund., *E. arborea*, *Sargassum sinicola* S. & G., *Padina durvillaei* Bory, *Hydroclathrus clathratus*

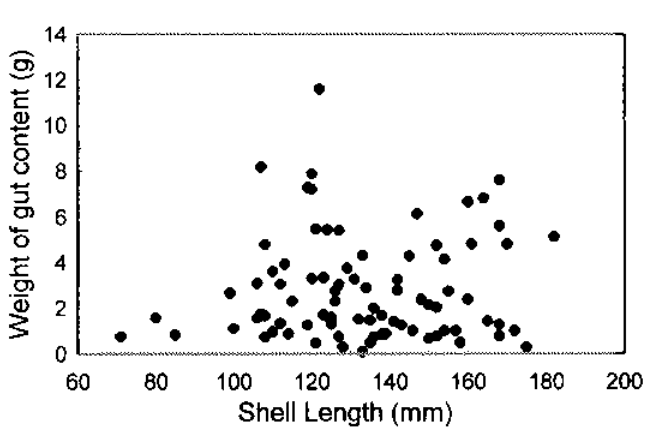

Figure 2. Shell length and weight of gut contents of green abalone.

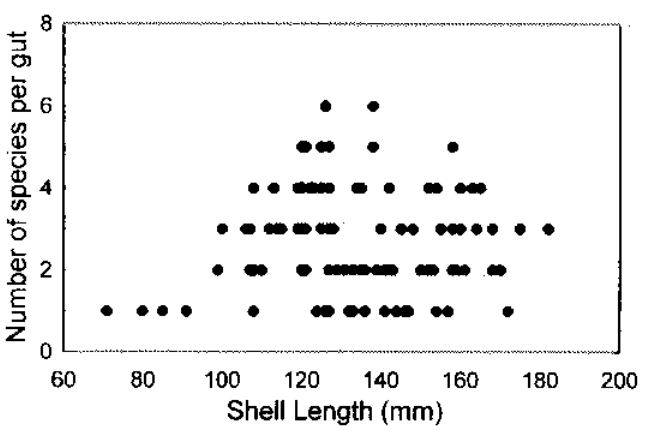

Figure 3. Shell length and number of species per gut of green abalone.

TABLE 2.

List and relative frequency of species identified in the crop and stomach contents of green abalone.

Species	Sites*				
	Bahía Asunción	La Bocana	Punta Abreojos	Las Barrancas	All Sites*
Phaeophyta					
Eisenia arborea Aresh.	14	22		22	16
Padina sp.	1			1	1
Sargassum sp.	23	19	52		19
Spatoglossum sp.			2		1
Rhodophyta					
Cryptopleura crispa Kylin	13	26	12	16	16
Gelidium robustum (Gardn.) Hollenb. & Abb.	10	5			4
Erythrotrichia sp.				4	1
Polysiphonia sp.				11	4
Prionitis sp.	1	1		1	1
Rhodymenia sp.	4	17	18	16	13
Red NI†				12	4
Seagrass					
Phyllospadix torreyi S. Watson	32	10	16	17	19
Total species	8	7	5	9	12

* Relative frequency.

† Red NI, red algae not identified.

(C. Ag.) Howe, *Corallina* spp., *Amphiroa magdalenensis* Daws., *Amphiroa zonata* Yendo, *Laurencia pacifica* Kylin, *Pterocladiella capillacea* (S.G. Gmel.) Santelices and Hommersand, and *Spyridia filamentosa* (Wulf.) Harv. were most common at Bahía Magdalena.

Five species had the greatest relative frequency and made up most of abalone diets at the sites studied along Baja California Sur. *P. torreyi* had the highest value, followed by the brown algae *Sargassum* sp. and *E. arborea* and the red algae *Cryptopleura crispa* and *Rhodymenia* sp. The value of each was different at different sites (Table 2). The brown algae *Sargassum* and *Eisenia* and the red algae *Cryptopleura* and *Rhodymenia* made up about 50% of the recovered food items at Bahía Asunción (brown, 37%; red, 13%) and Las Barrancas (brown, 22%; red, 32%) and about 80% at La Bocana (brown, 41%; red, 43%) and Punta Abreojos (brown, 52%; red, 30%) and averaged about 60% over all sites (brown, 35%; red, 29%). *P. torreyi* was present in 32% of guts at Bahía Asunción and about 15% in the other localities and averaged about 15% over all sites. No other plant occurred in more than 12% of the guts of the green abalone examined.

DISCUSSION

The analysis of shell length of abalone from Bahía Asunción, La Bocana, Punta Abreojos, and Las Barrancas suggests that the mean size of green abalone decreases from north to south. The shell length (in millimeters) of abalone at Bahía Asunción (141.04 ± 15.08, mean ± SD) was larger than that at Las Barrancas (120.40 ± 10.01). Guzmán del Próo et al. (1976) observed the size and weight means for *Haliotis* spp. to decrease from north to south, following a gradient along the peninsula. Data for samples from the commercial catch showed the same trend for the maximum length of *H. corrugata* and *H. fulgens* (Vega-Velazquez et al. 1994).

The range of gut content weights for individuals of the same size was wide. For example, at the shell length of 130 mm, values from 0.12 to 4.33 g were measured. The differences between individuals of the same or different populations appear related more to the progression of digestion in each individual rather than its size, because the weight of gut content was from the fragments of macroalgae and seagrass larger than 1 mm, and not for the liquid stomach content. The progress of digestion in the organisms sampled is relative to the time that the abalone began or stopped feeding and with the time of collection of abalone. The digestive tracts were preserved 4 or 5 h after the time of collection, and the content weight then consists of fragments not immediately digested. Nevertheless, no abalone with an entirely empty crop and stomach were found in the samples, and in general, in the contents, we always found fragments of macroalgae and seagrass larger than 1 mm. Faole and Day (1992), in laboratory assays, found that when abalone are well fed, they eat only the preferred species and process these rapidly, but when food is scarce, they are less selective and process the food slowly. Therefore, one must be cautious in assessing diet based only on gut contents.

The average number of species per individual gut was 2 ± 1 (mean ± SD), except at Las Barrancas (4 ± 1). Abalone from three similar sites were collected during summer and, at Las Barrancas, during winter. The season of the sampling is important because it has been suggested that the diets reflect the species most abundant in the habitat at the time of sampling, and the relative abundance of food item in the diet may be related to its availability in the environment. Southern sites on the peninsula of Baja California have the greatest floral diversity in winter. Sánchez-Rodrígez et al. (1989) identified 77 species in winter and 66 species in summer. Poore (1972) found seasonable variation in the diet composition of a New Zealand species, *H. iris*, and Barkai and Griffiths (1986) describe the diet of a South African abalone *Haliotis midae* at two sites being more diverse in winter than in summer. However, site

appears to be a more important variable than season. It is necessary to extend this preliminary survey with detailed field studies on the natural diets of Mexican abalone species with quantitative analysis of local flora to determine the relation between availability of food and principal food to abalone. Feeding experiments are needed to determine preference and utilization.

Although along the Pacific Coast of Baja California Sur, the benthic environments inhabited by abalone are characterized by a flora with more than 50 species (Guzmán del Próo et al. 1991, Mateo-Cid and Mendoza-Gonzalez 1994, Sánchez-Rodrígez et al. 1989), only seven red algae, four brown algae, and one seagrass were identified from gut content analysis of green abalone. Of these, *Macrocystis*, and *Eisenia* have been reported as algal foods of *H. fulgens* in southern California (Leighton 1966, Tutschulte and Connell 1988). *Gelidium robustum, E. arborea,* and *P. torreyi* have been concluded to be important species in abalone communities and potential abalone food along Baja California (Guzmán del Próo et al. 1972, Guzmán del Próo et al. 1991). The red algae *C. crispa, Erythrotrichia* sp., *Polysiphonia* sp., and *Prionitis* sp. and the brown algae *Padina* sp., *Sargassum* sp., and *Spatoglossum* sp. had not been previously reported as algal food of green abalone.

Guzmán del Próo et al. (1972, 1991) suggested that species such as articulated corallines *Bossiella* and *Corallina* spp. and crustose corallines *Lithothamnium* and *Lithophyllum* are important elements that form part of the habitat of abalone species in Baja California, as well as all over the world, and are related to postlarval diet. Although corallines and crustose algae are found in the local flora of the sites studied (Abbott and Hollenberg 1976, Dawson 1953), we did not observe them in the gut contents. These species and their epiphytes are used by small abalone.

The relative frequency of species at the four sites suggests that although green abalone in Baja California Sur accept a wide range of macrophytes, the principal conspicuous elements in the ingesta were the seagrass *P. torreyi* and the macroalgae *Sargassum* sp. (Fucales), *E. arborea* (Laminariales), *C. crispa* (Ceramiales), and *Rhodymenia* sp. (Rhodymeniales). The other species found in abalone guts could be considered species that are eaten incidentally with the main food according to the local flora. Poore (1972) studied the diets of a single species (*H. iris*) from two widely differing locations and concluded that the floral composition has a major effect on the abalone diet, which varies markedly from place to place, even within a small geographical area. Similarly, Leighton and Boolootian (1963), from study of gut contents of *H. cracherodii*, a common littoral inhabitant of rocky southern California and Baja California shore lines, found that the contents largely reflected the flora of the local intertidal zones.

Our results show that for green abalone along Baja California Sur, both brown algae (*Sargassum* and *Eisenia*) and red algae (*Cryptopleura* and *Rhodymenia*) have importance as food plants. These species occurred in about 50% of the content samples from Punta Prieta and Las Barrancas and about 80% at La Bocana and Punta Abreojos and averaged 60% at all sites. Cox (1962) and Shepherd and Steinberg (1992) mentioned that brown algae predominate in the diet of green abalone. Leighton (1966) conducted feeding experiments that demonstrated that green abalone had a distinct preference for brown algae, in particular, *Macrocystis* and *Egregia*. The second species is the diet of choice for culture of green abalone (Leighton et al. 1981, Leighton 1989). The only red alga tested in Leighton's 1966 study, *Gigartina armata*, had a relatively low preference value.

The diet an abalone consumes in the field must be seen as a compromise between the need to consume a balanced diet and such factors as food availability, avoidance of algae with chemical deterrents, and an inability to consume tough food (Fleming 1995). Where feeding studies have been done with captive green abalone, algae like *Padina* and *Sargassum* were little ingested and had relatively limited food value. The same is true of the red alga *Pterocladia* and the surf grasses *Phyllospadix* spp. (Leighton pers. comm.) This study suggests that controlled feeding and growth experiments and *in vitro* digestibility tests should be conducted wherein the algae found in abalone gut content samples may be appropriately evaluated for their nutritive value. It may be that *H. fulgens* in the southern part of its range has adapted enzymatically and may use some of these algae more effectively than in northern populations (Serviere-Zaragoza et al. 1997). *Eisenia* is a fairly good food, and where it predominates in drift or among rocks and is available to the abalone, it will support growth.

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TRIPLOID INDUCTION IN PACIFIC ABALONE *HALIOTIS DISCUS HANNAI* INO BY 6-DIMETHYLAMINOPURINE AND THE PERFORMANCE OF TRIPLOID JUVENILES

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ABSTRACT Pressure shock, thermal shock, and cytochalasin B treatment have been the main treatments used in inducing triploid gastropod. In this study 6-dimethylaminopurine (6-DMAP) was attempted to induce triploid in Pacific abalone, *Haliotis discus hannai* Ino, by inhibiting formations of either polar body 1 (pb1) or polar body 2 (pb2). Although the highest triploid (73.5%) was obtained by blocking pb2 for 15 min with a 6-DMAP concentration as high as 300 μM (the recommended dose for triploid induction in bivalve), no veligers could survive. At the ranges of 75–150 μM 6-DMAP, blocking pb2 for 20 min resulted in higher abnormalities than blocking pb2 for 15 min. The relative survival rate was higher and the abnormality was lower in the pb2 group than those in the pb1 group. No significant difference in triploid induction ($p > 0.05$) was found both between 15- and 20-min treatments and between treatments targeting pb1 and pb2 at 75–150 μM 6-DMAP. At 6-DMAP concentrations of 75, 100, 125, and 150 μM, in groups that blocked the pb 2 formation, the triploid yields (number of triploid larvae/fertilized eggs) were 30.0, 46.0, 47.0, and 54.0% and the relative survivorships at the trochophore stage were 96.8, 95.3, 94.8, and 90.5% for 15-min treatments, respectively. The triploid yields were 30.1, 49.0, 51.2, and 56.0% and the relative survivorships at the trochophore stage were 95.8, 90.2, 88.5, and 82.6% for 20-min treatments, respectively; in groups that blocked pb1 for 15 min, the triploid yields were 30.4, 43.0, 46.6, and 51.0% and the relative survivorships at the trochophore stage were 70.6, 68.4, 68.0, and 61.8%, respectively. The shell dimensions of triploids and controls were measured at 4 mo postfertilization. In the large-size group (1.2–1.3 cm), differences between 3n and 2n groups in both dimensions and weight were significant ($p < 0.01$), whereas in the small-size group (0.7–0.8 cm), no significant differences were found in dimensions ($p > 0.05$), but they were found in total weight ($p < 0.01$). Overall, the optimal treatment criteria for triploid production in Pacific abalone with 6-DMAP appear to be 125–150 μM for 15–20 min at 500–600/mL zygote density and 23.0°C.

KEY WORDS: abalone, triploid, 6-dimethylaminopurine (6-DMAP)

INTRODUCTION

Pacific abalone, *Haliotis discus hannai* Ino, is one of the most valuable seafoods in the Asian market and has been farmed in northern China for many years. Abalone are now the major marine products in this area. However, in recent years, abalone farming has dropped tremendously in terms of scale and productivity. Deterioration of environments and disease contamination were found to be the main reasons. For example, 80–90% of land-based abalone hatcheries in Dalian, China, were contaminated by disease in 1996, which caused up to 85–90% mortalities.

The large-scale abalone mortality happening in Dalian might also result from, in addition to the above-mentioned reasons, decreases in heterozygosity, so animals became more sensitive to environmental changes and more easily attacked by the pathogens. Triploid animals, especially polar body 1 (pb1) triploids should, theoretically, have higher heterozygosity than their diploid siblings; therefore, triploid chromosome manipulation techniques would provide a novel approach in protection against disease. Chromosome engineering such as polyploidy induction has also been considered one of the genetic tools that could improve shellfish production. Chemical (cytochalasin B) and physical (thermal and pressure shocks) treatments were applied shortly after fertilization for triploid induction in shellfish (Stanley 1981, Stanley 1984, Allen 1987). However, the methods usually result in low survival rates and high levels of aneuploid.

Thermal shock, cytochalasin B, and pressure shock were applied, respectively, by Arai et al. (1986), Wang et al. (1990), Rong et al. (1990), Sun et al. (1993), and Stepto and Cook (1998) to induce triploid in *Haliotis discus hannai* Ino, *Haliotis diversicolor*, and *Haliotis midae*. Some of those treatments could result in high percentages of triploids, but because of the very high mortalities in treated animals, none of those methods could be commercially used in triploid abalone hatcheries. Recent work of Desrosiers et al. (1993) and Gerard et al. (1994) showed that treatment with 6-dimethylaminopurine (6-DMAP) was far more effective than other methods in generating viable triploid oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis*). The effectiveness of this chemical in triploid induction in abalone, *H. discus hannai* Ino, is reported in this article.

MATERIALS AND METHODS

Broodstock

Pacific abalone, *H. discus hannai* Ino, about 6.5–8.0 cm in shell length, were collected in the vicinity of coastal Dalian. The animals were conditioned for 80–100 days at 20°C, during which they were fed *Laminaria japonica*, and the effective accumulation temperature reached 1000–1200°D. The gonad of a well, mature individual is gray-blue in color in females and milk-white in males, with a distinct incise between gonad and alimentary glands.

Gamete Collection and Insemination

In each experiment, gametes from at least two females and several males were collected by drying the broodstock in darkness

with 75–80% moisture first, and then spawning them in ultraviolet- (400–600 mw/h per L) treated seawater and raising the water temperature to 3–4°C above the conditioning temperature (20°C). High-quality oocytes are round and even blue in color, with a well-distributed yolk and clear membrane. The average egg diameter is 220 μm; the average yolk diameter is 180 μm. Successful fertilization could be obtained by using fresh sperm, which could stand erectly on the egg surface when checked under the microscope. Sometimes, a preexperiment fertilization was used to determine the vigor of sperm. Eggs and sperm were pooled within 30 min after being released at 23°C.

6-DMAP Solution Preparation

The commercial product of 6-DMAP is white powder. The stock solution used in this study was prepared in 1/100 (wt/vol) sterilized filtered seawater 1–2 h before the commencement of experiments and was stored at 4°C in a refrigerator.

Determination of Commencement Times and Treatment Duration

Although the first polar body could be found as early as 8 min postfertilization at 22.8–23.0°C, 45–50% zygotes released their pb1 within 22 min. Therefore, 6–7 and 20–22 min postfertilization were, respectively, chosen as the timings of the commencements of chemical treatments for inducing pb1 or pb2 triploids. On the basis of the observations and the previously published data, 15–20 min exposure durations were determined.

The time when sperm was added to the suspension of eggs was considered as the start of the experiment. Eggs and sperm were mixed gently. The fertilized eggs were equally divided into beakers according to replicates needed in each experiment.

Triploid Induction

In the first experiment, eggs were subjected to 6-DMAP at concentrations of 150 mm, 300 μM, and 450 μM. Chemicals were added either 6–7 min postfertilization to inhibit pb1 formation or 20–22 min postfertilization to retain pb2. Water volume was individually adjusted before 6-DMAP solution was added; the beakers were then stirred gently. The first experiment revealed that Pacific abalone appeared to be more sensitive to 6-DMAP than bivalves, so 6-DMAP concentrations were reduced to 75, 100, 125, and 150 μM in the later experiments. A 15- or 20-min treatment was applied in all experiments; eggs were washed with clean seawater and hatched under the same conditions as controls. During treatments, the zygote density remained at 200–600/mL, and the temperature was 22.6–23.0°C.

Rearing of Larvae and Juveniles

After treatment, each replicate was stocked at a density of 30–50/mL for embryos, 5–8/mL for trochophore larvae, and 4–5/mL for veliger larvae. At about 65 h postfertilization at 20°C, the eyed larvae were settled on the transparent waved polyethylene plates (42 × 33 cm), which were preplanted with benthic diatoms and turned to brown-yellow in color. The settlement plate density was kept consistently at 80/m^2, and 4.0–4.8 ($\times 10^4$) healthy eyed larvae were used for settlement in each replicate.

Juvenile abalone were kept on the transparent plates for 2 mo, until they reached a size of 4–5 mm in shell length; they were then transferred to the black waved plates for growout until the animals were about 1.5–2.0 cm in shell length. Juveniles from treated and control groups were maintained under the same conditions.

Ploidy Verification

Twelve to 15 h postfertilization, trochophore larvae swimming in water were selected and placed in a 0.01% colchicine solution for 0.5–1.0 h and then subjected to a hypotonic treatment for 1 h with 0.075M KCl and fixed in four changes of Carnoy solution (3:1, methanol:acetic acid) at 15-min intervals. The samples were stored at 4°C overnight in a refrigerator. Cell suspension was made by replacing Carnoy suspension was made by replacing Carnoy solution with 50% acetic acid and shaking heavily. The suspension was then dropped onto a warmed (48–52°C) microscope slide, air dried, and stained in 4% Giemsa solution (pH 6.8) for 30–60 min. According to Arai et al. (1986), diploid chromosome number of *H. discus hannai* Ino is 36. The following ploidy classification was applied in this study: metaphase spreads with about 36 chromosomes were classified as diploid; those with 52–54 chromosomes were classified as triploid (Fig. 1). A total of 80–100 metaphase spreads were counted in each replicate.

Formulations

Fertilization rates and survivorship at trochophore and veliger stages were calculated. Deformed trochophores or trochophores with irregularly divided cells were counted as abnormal.

Fertilization rate (FR) = fertilized eggs/total eggs (100%).

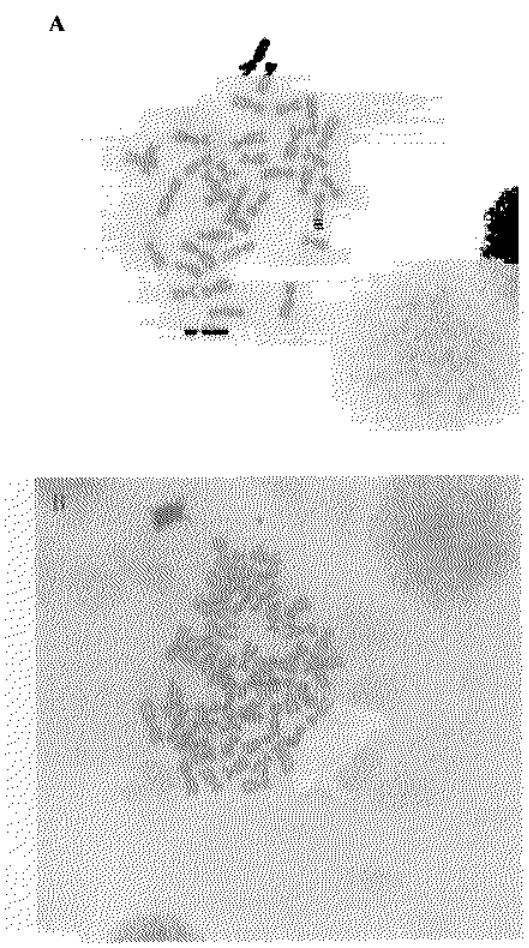

Figure 1. (A) A metaphase of commercial (2N = 36). (B) A metaphase of chromosome (3N = 54).

TABLE 1.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis II lasting 15 min (fertilized rate is 52.1%, treated density is 140-250/mL, and hatchery density of zygote is 30–55/mL).

6-DMAP (μM)	Trochophore Larvae					E. Veliger		P. Veliger
	SR	RSR	AR	RAR	TY	SR	RSR	RSR
150	49.2 ± 1.5	74.4	52.5 ± 5.9	106.9	55.9	22.5 ± 2.1	66.9	69.6
300	42.1 ± 0.4	63.7	93.2 ± 1.3	189.8	73.5	—	—	
450	2.0 ± 2.8	3.0	100.0 ± 0.0	203.7	-	—	—	
Control	66.1 ± 3.5	100.0	49.1 ± 3.5	100.0	0.0	33.6 ± 5.1	100.0	100.0

Survival rate (SR) = trochophore or veliger numbers/fertilized eggs (100%).

Relative survival rate (RSR) = survival rate of each replicate/survival rate of control (100%).

Veliger survivorship = veliger number/trochophore number (100%).

Abnormal trochophore rate (AR) = abnormal trochophore larvae/(normal trochophore + abnormal trochophore) (100%).

Relative abnormal rate (RAR) = abnormal rate of treated group/abnormal rate of control (100%).

Triploid yield (TY) = $N_{3n}/N_{3n} + N2_n$ (100%).

N_{3n} is the number of metaphase chromosomes of triploids, and N_{2n} is the number of chromosomes of diploids.

RESULTS

Effect of 6-DMAP Concentration on Triploid Induction by Blocking Meiosis II Division for 15 min

At concentrations of 150, 300, and 450 μM, the normal veliger larvae were found in the 150 μM group and their relative survival rate was 66.9%. In the other two groups, only 2.9% of fertilized eggs reached the trochophore stage in the 300 μM group and no normal trochophore larvae were found in the 450 μM group. The triploid yields were 55.9 and 73.5% in the 150 and 300 μM 6-DMAP groups, respectively (Table 1).

With increases of concentrations form 75 to 150 μM, the triploid yields increased from 30.0 to 55.1%, whereas the relative survival rates of veligers remained more than 77.2% (Table 2). The increases of triploid yields were 16.0% from 75 to 100 μM treatments, only 1.0% from 100 to 125 μM treatments, and 7.0% from 125 to 150 μM treatments.

Effect of 6-DMAP Concentration on Triploid Induction by Blocking Meiosis II Division for 20 min

At a given 6-DMAP concentration, treatments lasting for 20 min resulted in higher triploid yields but lower survival rates than did treatments lasted for 15 min. The number of abnormal larvae in the 20-min group was 10.0% higher than that in the 15-min group (Table 3). As in the 15-min groups, triploid yields also depended on concentrations of 6-DMAP. From 75 to 100 μM, the increment of triploid yield was 18.9%, which was the biggest increase between groups treated with chemicals at the other concentrations. Comparison of triploid yields and relative survival rates of postveligers revealed significant differences ($p < 0.01$) between treatments with different 6-DMAP concentrations, but no significant differences (*Argopecten purpuratus* > 0.05) between treatments with the same 6-DMAP concentration for different durations (15 or 20 min). The relative survival rate decreased 4.8% from the 15- to the 20-min treatments, whereas relative abnormality increased 37.0%. The relative survivorships were almost the same at early veliger stage in both 15- and 20-min treatments at the given concentration, but 5.1% higher in 15-min treatments than that in 20-min treatments at the postveliger stage.

Effect of 6-DMAP Concentration on Triploid Induction by Blocking Meiosis I Division for 15 min

Except at 75 μM 6-DMAP, at other given concentrations, the triploid percentages resulted from blocking meiosis I division was lower than that from blocking meiosis II division. The relative survival rate of the pb1 triploid group was 26.9% lower and the abnormal rate was 8.18 times higher than those of pb2 triploid group. The relative survival rates at early and postveliger stages were only 2.5 and 27.2% of those from meiosis II treatments (Table 4).

Comparison of the Effects of Different Egg Densities on Triploid Yields

No positive correlation was found between triploid percentages produced and different egg densities (150–500/mL) at the given chemical concentrations. Differences in survivorship, relative sur-

TABLE 2.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis II lasting 15 min (fertilized rate is 71.2%).

6-DMAP (μM)	Trochophore Larvae					E. Veliger		P. Veliger
	SR	RSR	AR	RAR	TY	SR	RSR	RSR
75	65.5 ± 4.8	96.9	6.6 ± 6.5	103.1	30.0	76.8 ± 10.3	89.3	96.8
100	64.1 ± 4.6	94.3	7.4 ± 7.8	115.6	46.0	74.1 ± 8.4	86.1	95.3
125	64.1 ± 5.2	94.8	9.3 ± 5.6	145.3	47.0	73.3 ± 8.3	85.2	94.8
150	61.2 ± 6.4	90.5	7.1 ± 6.3	110.9	54.0	66.4 ± 7.3	77.2	90.5
Control	67.6 ± 4.7	100.0	6.4 ± 5.1	100.0	0.0	86.0 ± 3.4	100.0	100.0

TABLE 3.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis II lasting 20 min

6-DMAP (μM)	Trochophore larvae					E. veliger		P. veliger
	SR	RSR	AR	RAR	TY	SR	RSR	RSR
75	79.4 ± 4.1	95.9	14.2 ± 7.9	124.6	30.1	82.7 ± 10.3	89.4	95.8
100	74.7 ± 4.8	90.2	18.1 ± 6.1	158.8	49.0	81.4 ± 3.7	88.0	90.2
125	73.3 ± 7.4	88.5	19.4 ± 5.1	170.2	51.2	77.5 ± 6.0	83.3	88.5
150	68.4 ± 7.2	82.6	19.3 ± 5.0	169.3	56.0	75.2 ± 11.2	81.3	82.6
Control	82.8 ± 5.2	100.0	11.4 ± 5.5	100.0	0.0	92.5 ± 7.3	100.0	100.0

vivorship, and abnormality seemed dependent on the fertilization rates only (Table 5).

Comparison of the Developmental Speeds Between Treated and Control Groups

The development of treated embryos was slower than that of control embryos (Table 6).

Effects of 6-DMAP Treatments on Growth of Juvenile Abalone

In the first after treatments, the growth rate (54.1 μm/day) in shell length of treated group was still slower than that in control (59.1 μm/day) and showed a significant difference ($p < 0.01$). The growth rate in shell width of the treated group was similar to that of the shell length (Table 7).

Four months postfertilization, the growth rates in both treated and control groups were calculated again. In large-size groups (1.2–1.3 cm), triploid and diploid abalone showed a significant difference ($p < 0.01$) in all dimensions and weights. In small-size groups (0.7–0.8 cm), however, no significant difference ($p < 0.01$) was found between triploid and diploid groups in their dimensions, but differences were found in total weights (Table 8). Comparisons between Tables 6, 7, and 8 revealed that the growth rates in triploid groups were slower than those in controls at the early stage, whereas at the later stage, the mean shell dimension(s) of triploid groups were significant longer than those of diploid siblings in large-size groups.

DISCUSSION

Cytochalasin B is one of the most important chemicals in oyster polyploidy induction and has been extensively used in shellfish chromosome manipulations. Although cytochalasin B could induce as high as 100% triploids in some experiments (Allen et al. 1982, Downing and Allen 1987, Wang et al. 1990, Nell et al. 1996, Stepto et al. 1998), its high toxic nature and expense impede its use in commercial hatcheries. High mortality of larvae (about 85–90%) after treatment is another consideration. Therefore, new approaches that should be inexpensive, nontoxic, and consistent with high percentage of triploid yields are needed, especially new chemicals and comprehensive techniques. *C. gigas* may produce more than 40–50 million eggs by strip spawning a 2-y-old oyster. Therefore, 10% survivorship after cytochalasin B treatment, the common cases in most experiments, could be tolerated in hatcheries. Pacific abalone, on the other hand, could not be strip spawned. A 7–8 cm female abalone could produce only 0.8–1.2 million eggs in a spawning season, even after 70–80 days conditioning in the hatchery. Without any treatments, the survival rate of abalone from zygotes to juveniles about 1.0 cm in shell length is usually less than 10.0%. If eggs are treated with cytochalasin B, the survivorship will drop to 1–5% (or less) at as early as veliger stages (Wang et al. 1990). Therefore, techniques of including triploid abalone by the use of cytochalasin B could hardly be applied in commercial scales. The results from this study suggested that the 6-DMAP technique could overcome the above-mentioned disadvantages in triploid induction and thus meet the large-scale requirement in abalone industries.

pb1 Triploids should, theoretically, have higher heterozygosity than their pb2 triploid and diploid siblings; therefore, they should be very useful in aquaculture (Stanley et al. 1984). In this study, however, no high performances were found in pb1 triploids. In addition, at optimal 6-DMAP concentrations (125–150 mM), the relative abnormalities of pb1 triploids increased to 1,317–1,625%, which were much higher than those of pb2 triploids. Therefore, as in oysters, pb2 triploid techniques would be the potential methods that could be used in large-scale triploid productions in abalone.

As in a previous report on bivalves (Desrosiers et al. 1993), increasing periods of incubation with 6-DMAP also improved the

TABLE 4.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis I lasting 15 min

6-DMAP (μM)	Trochophore larvae					E. veliger		P. veliger
	SR	RSR	AR	RAR	TY	SR	RSR	RSR
75	56.6 ± 16.6	70.6	11.0 ± 0.2	282.1	30.4	85.8 ± 6.6	92.7	70.6
100	54.8 ± 16.8	68.4	25.8 ± 14.2	661.5	43.0	78.9 ± 1.8	85.2	68.4
125	54.5 ± 12.5	68.0	51.4 ± 19.8	1317.9	46.6	77.8 ± 10.9	84.0	68.0
150	49.5 ± 13.7	61.8	63.4 ± 15.9	1625.6	51.0	72.4 ± 8.4	75.7	61.8
Control	80.1 ± 8.7	100.0	3.9 ± 4.4	100.0	0.0	92.6 ± 2.6	100.0	100.0

TABLE 5.

The induction of triploid in *H. discus hannai* Ino by 6-DMAP in meiosis II lasting 15 min in different treated densities.

6-DMAP (μM)	DT* (egg/mL)	FR	Trochophore Larvae SR	Trochophore Larvae AR	Trochophore Larvae TY	E. Veliger SR	P. Veliger RSR
150	150	52.1	48.5	48.3	45.2	65.1	53.9
150	250	52.1	50.4	56.7	66.7	71.3	69.2
150	500	71.2	51.2	7.1	54.0	77.2	90.5

* DT, density of treatment.

production of triploid eggs in Pacific abalone. However, when the eggs were exposed to 6-DMAP for periods of time covering all of the meiosis II and overlapping the first mitotic division in the control, the proportion showing abnormal chromosome behavior increased and survival decreased sharply. These results suggest that a high percentage of triploids and good yield of normal larvae may be obtained after exposure to 6-DMAP for a period of incubation that is shorter than the duration of meiosis II.

The purine analog 6-DMAP was initially used as a cleavage inhibitor in sea urchin eggs (Rebhun et al. 1973). Recent studies on 6-DMAP have demonstrated that the drug exerts its actions on protein phosphorylation, microtubule organization, metaphase spindle formation, and cortical filament organization in different animals (Dufresne et al. 1991, Rime et al. 1989, Szollosi et al. 1991). These cellular effects can explain how the triploidy state can be induced by blocking chromosome movement and extrusion of polar bodies by 6-DMAP.

Comparison between methods used in previous studies and methods used in this study clearly reveals that treatment with 6-DMAP is the most simple, inexpensive, and efficient technique to induce triploidy in abalone. 6-DMAP treatment does not require expensive or specialized equipment. 6-DMAP is water soluble and can be easily washed out in seawater after the treatment. 6-DMAP is safer than cytochalasin B, which is known to be a carcinogenic product. Treatment with 6-DMAP in abalone was as effective as in bivalves (Desrosiers et al. 1993, Gerard et al. 1994). Therefore, 6-DMAP should be an efficient and safe alternative method to induce triploidy with a high potential in commercial aquaculture in abalone.

The best yield of 6-DMAP to produce triploid abalone was 73.5%, which is slightly lower than that to produce triploid bivalves (Desrosiers et al. 1993, Gerard et al. 1994). The main reason for lower triploid yields in this research could result from the eggs, which were not well synchronized, because the efficiency to induce triploid depends on the synchrony of meiosis in the eggs (Downing and Allen 1987), the time of initiation, and the duration of treatments (Desrosiers et al. 1993).

In Pacific abalone, the optimal 6-DMAP concentrations for triploid induction are much lower than those recommended for bivalves. For example, the relative abnormalities at trochophore stages were as high as 93.2% at 300 mM 6-DMAP and 100.0% at 450 mM, although the doses were within the optimal ranges suggested for oyster ploidy manipulations. At a concentration of 150 mM the performance of abalone larvae improved tremendously, the survival and relative survival at trochophore stage increased to 49.2 and 74.4%, respectively, and the relative postveliger survival remained at 69.6%. The abalone industries in China have already shown much interest in the methods.

The high survivorship of juveniles expressed in treated groups remained to at least 4-mo-old abalone. At 125–150 mM 6-DMAP, the survival rate from shell sizes of 3–4 mm in length to 10–12 mm was about 1.5% in controls, whereas it was 30.0–35.0% in treated groups.

Unlike oysters, in which the growth rate of triploid and diploid larvae is generally similar (Downing and Allen 1987), the growth rates of abalone larvae and early juveniles in treated groups were lower than those in controls. At 20.0°C, 47.2% of the embryos in control groups reached archenteron 8.25 h postfertilization, whereas only 43.5% in treated groups turned up at this stage at the same time. The slight difference might result from the prohibition of development in treated groups. However, this situation changed 4 mo later: triploid groups showed faster increases in both shell dimensions and total weight. The bigger the size of the groups, the faster the triploids grew.

Much of the utility of triploid shellfish to aquaculturists or to fisheries' managers is a consequence of their sterility or semisterility, which precludes the possibility of unwanted reproduction.

TABLE 6.

The developmental comparison between the treated and control groups of *H. discus hannai* Ino (22 ± 0.5).

D	T (h)	Group	Developing Rate (%)
2 Cells	1.05	Treated	45.4
		Control	64.3
4 Cells	1.35	Treated	40.3
		Control	49.0
Archenteron	8.15	Treated	43.5
		Control	47.2
Trochophore	12.30	Treated	43.0
		Control	47.9

Note: D, developmental stage; T, time of postfertilization.

TABLE 7.

The comparison of early growth between the treated for triploid and control groups of *H. discus hannai* Ino that settled on May 13 (n = 20).

Group	Item	2/6	5/6	13/6
Treatment	SL (μm)	785.4 ± 53.9	1,035.4 ± 92.0	1,358.0 ± 108.1
	SW (μm)	710.8 ± 64.3	956.9 ± 81.1	—
Control	SL (μm)	902.9 ± 64.5	1,154.6 ± 117.2	1,552.7 ± 173.0
	SW (μm)	824.0 ± 71.5	1,056.4 ± 125.7	—

Note: SL, shell length; SW, shell width.

TABLE 8.

The comparison of growth in triploid treated and control groups of *H. discus hannai* Ino rearing for about 4 mo (n = 30 for each group).

Group		Shell Length (cm)	Shell Width (cm)	Shell Thickness (cm)	Total Weight (g)
3N	Group 1	1.37 ± 0.12	0.95 ± 0.08	0.23 ± 0.04	0.38 ± 0.14
	Group 2	0.82 ± 0.22	0.58 ± 0.13	0.15 ± 0.04	0.09 ± 0.06
2N	Group 1	1.27 ± 0.12	0.89 ± 0.09	0.21 ± 0.03	0.29 ± 0.10
	Group 2	0.78 ± 0.15	0.55 ± 0.11	0.14 ± 0.04	0.08 ± 0.03
(3N-2N)/2N	Group 1	7.87%	6.74%	9.52%	34.48%
	Group 2	5.13%	5.45%	7.14%	12.50%

because they produce, theoretically, aneuploid gametes. The mature individuals show fast growth and high quality of meat in the reproductive season. However, juvenile triploid abalone produced in this study began to show better performance in growth than did diploid controls at about 1 cm in shell length. The advantages of triploid groups could result from the relatively high heterozygosity of the triploid itself or from the selection effects of a chemical on individuals or both. Zygotes sensitive to chemical treatments stopped development or became abnormal, whereas the remaining were triploids and/or selected zygotes. Animals developed from those zygotes might, therefore, have greater vigor and thus the ability to withstand environmental variability at juvenile stage.

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ISOLATION, CLONING, AND EXPRESSION OF A DNA SEQUENCE ENCODING AN EGG-LAYING HORMONE OF THE BLACKLIP ABALONE (*HALIOTIS RUBRA* LEACH)

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ABSTRACT Egg laying in the two gastropods, *Aplysia californica* and *Lymnaea stagnalis*, has been shown to be a physiological hormone response to expression of genes located in neural ganglia. These hormone-producing genes had been previously cloned and provided for DNA probes and polymerase chain reaction (PCR) primers to be made for the identification and amplification of an abalone DNA encoding an egg-laying hormone (aELH). PCR amplification of genomic blacklip abalone DNA with *Pfu* polymerase, and subsequent DNA sequencing, showed a sequence relating to an aELH. PCR was then performed to amplify this sequence and add 5′*Bam*HI and 3′*Eco*RI sites. A double-digested PCR product was cloned into a pGEX-2T expression vector for high-level expression of aELH, as a fusion protein with glutathione-*S*-transferase. This fusion product was purified and cleaved to release aELH, which is being tested in bioassays on various abalone species, as well as being used to produce antibodies for physiological studies. The aELH gene had 5 base-pair differences (95.4% homologous) with an analogous gene in *L. stagnalis*, and in regard to the deduced amino acid sequence, the abalone had only 1 of 36 different (97.2% homologous). Homology to the equivalent sequences in *A. californica* was 51.9 and 47.2%, respectively. These data infer that the genes relating to egg laying in some gastropods are highly conserved, and further abalone species are being investigated.

KEY WORDS: abalone, *Haliotis rubra*, egg-laying hormone, aELH, PCR, expression protein

INTRODUCTION

Hormonal control of egg-laying behavior in the gastropod molluscs, *Aplysia californica* and *Lymnaea stagnalis*, has been well documented (Joosse and Geraerts 1983, Joosse 1988). In particular, the control of egg laying in both species is known to be controlled by related super-gene families that encode prohormones that are cleaved proteolytically into a number of peptides. Of these, peptides of 36 amino acids have been implicated as the main egg-laying hormones.

In *A. californica*, the peptides associated with egg-laying behavior are released from bag cells (BCs) of the abdominal ganglia (Scheller et al. 1982, Nagle et al. 1988a, Nagle et al. 1988b). Although the egg-laying hormone (ELH) gene is primarily expressed in BCs, where it constitutes approximately 50% of the total cellular protein, it is also expressed in small populations of neurones situated in the cerebral and pleural ganglia. Other members of the ELH family are expressed in a tissue-specific manner (Mahon and Scheller 1983, Mahon et al. 1985, Nagle et al. 1994). The 36-amino-acid ELH is produced from cleavage of a 271-amino-acid precursor protein and is released into the circulation, where it can travel to distant target sites (Chiu et al. 1979, Newcomb et al. 1988, Newcomb and Scheller 1990).

In *L. stagnalis*, egg laying is initiated and coordinated by release of peptides from caudodorsal cells (CDCs) of the cerebral ganglia (Geraerts et al. 1988). One of these peptides is an ovulation and egg-laying-inducing hormone composed of 36 amino acids and has been termed the caudodorsal hormone (CDCH) (Ebberink et al. 1985).

Molecular studies have shown strong homologies in DNA sequences of the ELH and CDCH super-family genes, as well as some of their expressed peptides (Geraerts et al. 1988). In particular, there is a 51% homology between the primary structure of ELH and CDCH, and both peptides are amidated. However, there are notable differences. In the first and last series of regions (sequences 1–12 and 29–36), a homology of about 60% exists, and in the middle region (sequence 13–28), there is only a homology of 25%. It has been suggested that the binding site for the ELH receptor involves a positive charge, which is in the middle region (sequence 19–23), whereas this region for CDCH has a net negative charge (Vreugdenhil et al. 1988).

Relatively little is known about the presence of egg-laying genes, and hormones, in abalone. The cerebral ganglia of abalone are connected by a long commissure and have connectives to the buccal, pleural, and pedal ganglia (Dorsett 1986, Hahn 1992). Injection of homogenates of pleuropedal and visceral ganglia have resulted in increased body weight and subsequent spawning of large numbers of fertilizable eggs (Yahata 1973). Studies of the cerebral ganglia of the Japanese abalone, *Haliotis discus hannai*, have shown the presence of four types of nerve cells (A, B, C, and D), of which, neurosecretory activity associated with egg production was only found in Cell Types A and B (Yahata 1971, Hahn 1994).

This report describes the identification and characterization of genomic DNA relating to an abalone ELH (aELH) in *Haliotis rubra*, the Australian blacklip abalone. As well, it gives the results of the subsequent cloning of an aELH gene and *in vitro* production of an aELH with a bacterial expression system.

MATERIALS AND METHODS

Abalone Genomic DNA Extraction

Approximately 1 g of fresh female abalone foot muscle was used for each extraction. After adding 10 mL of lysis buffer containing 10 mM Tris-HCl, 0.1 M EDTA, 0.5% sodium dodecyl sulfate (SDS), and 0.1 mg of RNase A, the mixture was incubated at 37°C for about 1 h; then, 1 mg of proteinase K (Boehringer Mannheim) was added and the mixture was incubated at 55°C for several hours, until the tissue dissolved. The DNA was then extracted by the standard phenol/chloroform method, concluding with precipitation and washings with ethanol. The DNA was finally pelleted by centrifugation, dried under vacuum, dissolved in TE buffer overnight at 4°C, and then stored at −20°C.

Polymerase Chain Reaction Conditions

The amplification of abalone genomic DNA was performed by polymerase chain reaction (PCR) in a DNA thermal cycler (Perkin Elmer Cetus), for 30 cycles of 94°C, 45–60°C (depending on primers), and then 72°. An additional extension at 72°C for 3 min was performed after the last cycle to ensure complete synthesis. The proofreading and thermostable DNA polymerase, *Pfu*, was used throughout.

Three degenerate primers, designed from relatively conserved regions of known ELH gene precursors of both *Lymnaea* and *Aplysia*, were used for amplification of abalone genomic DNA fragments. The sequences of the three primers were as follows:

CDCH-1, sense, 33-mer, T_m = 94–102°C

5′-CCGAGACT(A/G)(C/A)G(C/G)TTCTATTCAC(T/G)T-(G/C)-G(C/A)AAAGGA-3′,

CDCH-2, sense, 26-mer, T_m = 72–78°C

5′-CAAACGA(A/C)TTTC(C/G)ATCA(A/C)CCATGA(C/TC-3′

CDCH-3, antisense, 21 mer, T_m = 62–68°C

3′-CC(G/T)TTATC(C/A)(C/T)CAAGA(C/G)CGCTC-5′

In relation to the CDCH cDNA sequence, the positions of these primers were at 621–654, 775–801, and 908–929 base pairs (bp), respectively, charge (Vreugdenhil et al. 1988). Therefore, it was anticipated that these primers would produce two abalone genomic DNA PCR products of 308 bp from CDCH-1 and CDCH-3 (designated PAF-1) and 154 bp from CDCH-2 and CDCH-3 (designated PAF-2), both of which should contain a sequence encoding an aELH. Primers used for amplifying inserts and sequencing were standard vector primers.

Hybridization Probe for an ELH

A cDNA clone of the CDCH preprohormone mRNA, constructed in the plasmid vector pBluescript II SK (+/−), was kindly provided by Dr. Guus Smit (Biological Lab. Vrije University, The Netherlands). The CDCH cDNA was amplified by the PCR method with T3 and T7 vector primers, and the PCR products were radiolabeled with ^{32}P by a random priming system, Prime-a-Gene (Promega), according to the manufacturer's instructions. The radioisotope [α-^{32}P]dATP (3,000 Ci/mmol) was supplied by Bresatec.

Dot blots of genomic DNA samples (5–50 μg), or Southern-transferred PCR products, on Hybond N$^+$ nylon membrane, were first bound in an oven and then hybridized with a ^{32}P radiolabeled CDCH cDNA probe at a washing stringency of 1× SSC for 30 min at 60°C and 0.5× SSC for 10 min at 65°C. Autoradiographs were then made after washing and drying membranes.

DNA Sequencing and Sequence Analysis

Clones of abalone DNA were sequenced in both directions, by the dideoxy chain-termination method, by Westmead Hospital (Sydney) and Australian Animal Health Laboratory (Geelong). GenBank and EMBL databases were searched for similar matches to abalone DNA sequences; predicted peptide sequences were obtained with the BLAST network service.

Cloning

Genomic abalone DNA amplified by PCR were initially cloned into the pCR-Script AMP SK (Strategene) according to the suppliers' protocols. Once inserts had been sequenced, new PCR primers were designed to amplify the aELH and to simultaneously add *Bam*HI and *Eco*RI restriction sites to the ends of the products, for directional cloning into the expression vector, pGEX-2T (Pharmacia). The inserts were sequences again. Transformation of competent *Escherichia coli* DH5α was performed by the $CaCl_2$/heat-shock method, or electroporation with a Pulser Transformation apparatus (Bio-Rad), and the cells were plated out on selective LB media.

Preparation of Fusion Protein and Cleavage To Release an aELH

Expression of the fusion protein of aELH with glutathione-*S*-transferase (GST) from pGEX-2T was performed in accordance with standard protocols (Pharmacia Biotech). Transformed *E. coli* cells were grown aerobically at 37°C in LB medium containing ampicilin, and expression of fusion protein was induced by the addition of IPTG. After an incubation for 4 h at 37°C, the cells were harvested by centrifugation and the pellet was resuspended in ice-cold phosphate-buffered saline. The cells were lysed by adding 10% Triton X-100 and then lysozyme solution and finally were sonicated in a Branson Sonifier (Model B-12) sonicator on ice for two 10 sec. The cellular lysis was immediately centrifuged, and the supernatant (with soluble proteins) and the resuspended pellet (with insoluble proteins) were stored at −20°C. Glutathione Sepharose 4B beads were mixed with supernatant to bind the GST fusion protein and were then washed three times with repeated centrifugations. The GST fusion protein was then removed by elution buffer, or the aELH was cleaved from the GST fusion protein by the site-specific protease, thrombin, before collection in supernatants after centrifugation. Purified protein supernatants were then stored at −20°C.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblots

Protein samples were first analyzed by discontinuous sodium dodecyl sulfate–polyacrylamide electrophoresis (SDS-PAGE) in a Mini-PROTEIN II apparatus (Bio-Rad). Prestained low-range molecular-weight markers (Bio-Rad) were run with the protein samples, and protein bands were visualized by Coomassie Blue staining. A further analysis of proteins involved separation by SDS-PAGE and electrotransfer to a nitrocellulose membranes with a Mini Trans-Blot apparatus (Bio-Rad), before immunological detection of the GST and GST fusion proteins with a rabbit anti-GST (kindly produced and provided by Dr. L. F. Wang at CSIRO, AAHL), diluted 1:1,000. An alkaline phosphatase–conjugated sheep anti-rabbit (Promega), diluted 1:2,000, was used in the detection of GST.

RESULTS

Detection of aELH Sequence in Abalone DNA

The CDCH cDNA probe hybridized strongly with dot blots of abalone genomic DNA (Fig. 1A), indicating that the abalone DNA had good sequence homology with the egg-laying gene of *L. stagnalis*. This was strengthened further by the PCR amplification of a single-band PAF-1 product of ~308 bp from abalone genomic DNA (Fig. 1B), which also hybridized strongly with the probe (Fig. 1C). Acting as a positive control, the labeled probe also

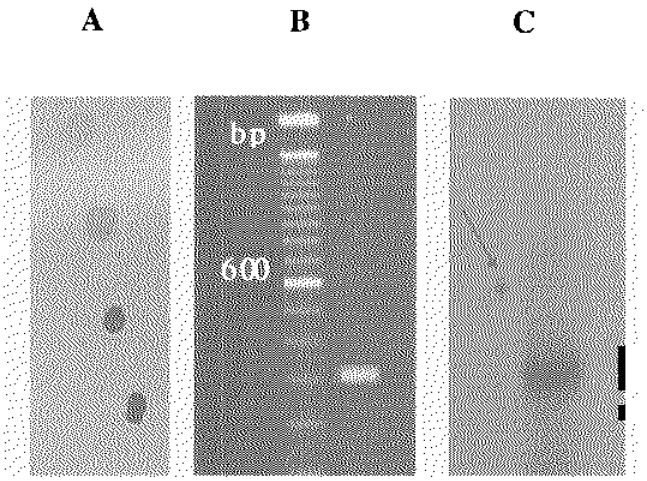

Figure 1. Detection of *H. rubra* aELH with a CDCH cDNA probe. (A) Autoradiograph of a dot-blot analysis of genomic DNA hybridized with a ^{32}P-labeled CDCH cDNA probe. Spots (from top to bottom) are 5, 10 and 50 μg of abalone DNA and 10 μg of CDCH cDNA (positive control). (B) Agarose gel electrophoresis showing a PCR-amplified product (designated PAF-1) of genomic abalone DNA. Lanes (left to right): molecular markers (100-bp ladder, Gibco); PCR product of an expected ~308-bp size. (C) Positive hybridizations of the labeled CDCH cDNA probe with PAF-1, as well as a dot of unlabeled CDCH cDNA as a positive control (arrow).

hybridized with unlabeled CDCH cDNA. PCR amplification of abalone genomic DNA also produced a single PAF-2 product of ~154 bp, and it, too, hybridized strongly with the probe (data not shown). The larger PAF-1 PCR product was successfully cloned into pCR-Script for sequence analysis.

Sequence Analysis of aELH

The nucleotide sequence encoding aELH was derived from sequencing a clone containing PAF-1 and is shown in Fig. 2A, together with analogous nucleotide sequences encoding *A. californica* ELH and *L. stagnalis* CDCH (Scheller et al. 1983, Vreugdenhil et al. 1988). There was a very high nucleotide homology (95.4%) between aELH and *L. stagnalis* CDCH, with only 5 bp different in 108. However, homology to the equivalent sequence in *A. californica* was 51.9%. In addition, six nucleotides at the 5′ end of the aELH sequence, and nine at the 3′ end, were identical in all three species.

An amino acid sequence of aELH was derived from the nucleotide sequence (Fig. 2A), by BLAST, and is shown in Fig. 2B, together with known sequences of the *A. californica* ELH and *L. stagnalis* CDCH. This sequence also had a high homology (97.2%) with CDCH, with only 1 of 36 amino acids different. However, there were 19 differences with the amino acid sequences of *Aplysia* ELH (47.2% homologous).

The conserved genomic nucleotides, at the 5′ and 3′ ends of the aELH sequence, encode Lys-Arg and Gly-Arg-Lys, respectively. These indicate proteolytic cleavage sites of an aELH from a prohormone, as well as amidation of the C-terminus, both of which are known to occur with *Lymnaea* CDCH and *Aplysia* ELH (Vreugdenhil et al. 1988, Nambu and Scheller 1986).

Cloning and Expression of aELH

The PAF-1 sequence was used to design two primers for the PCR amplification of the aELH sequence from genomic DNA and contains 5′ *Bam*HI and 3′ *Eco*RI restriction enzyme sites for the directional cloning, in phase, into the expression vector pGEX-2T. Figure 3 shows an expected band size of ~150 bp, after double digestion of amplified PCR products. After cloning of a product into pGEX-2T, and transformation of *E. coli*, two positive recombinants clones were selected. One of these was sequenced to confirm the previous results of sequencing PAF-1 and to check that the insert was in phase for correct expression of an aELH. The sequence was the same as before and in a correct reading frame for suitable expression.

E. coli cells containing the correct plasmid constructs plasmids were cultured and induced with IPTG to produce the desired GST fusion protein. This was shown as a protein corresponding to a predicted size of ~32.5 kD by SDS-PAGE (Fig. 4A). Western blotting confirmed the presence of GST within the expressed fusion protein (data not shown). Thrombin cleavage of the aELH from fusion protein, bound to Sepharose 4B beads, was detected

Figure 2. Comparisons of nucleotide and deduced amino acid sequences of *H. rubra* aELH with *L. stagnalis* CDCH (Vreugdenhil et al. 1988) and *A. californica* ELH (Scheller et al. 1983). (A) Nucleotide sequences with numbers indicated above. Asterisk indicates same sequence compared with aELH, and the hyphens indicate gaps within the nucleotide sequence to obtain maximum alignment. (B) Amino acid sequences with numbers indicated above. Asterisk indicates same sequence compared with aELH. Underlined sites are potential polypeptide cleavage sites.

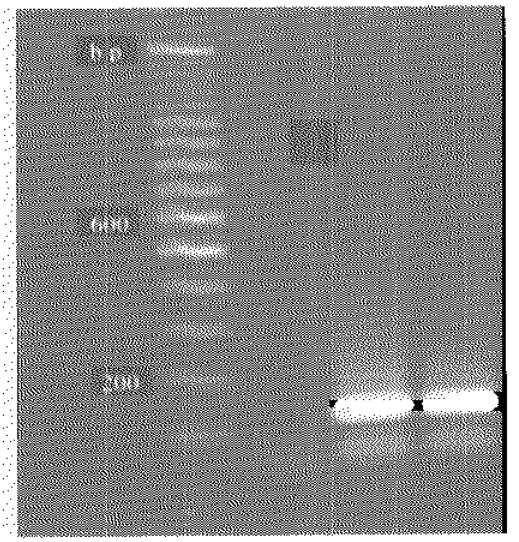

Figure 3. Agarose gel electrophoresis showing PCR amplification products of the aELH sequence, from *H. rubra* genomic DNA, and containing 5′ *Bam*HI and 3′ *Eco*RI sites for cloning into pGEX-2T. Lanes (left to right): molecular markers (100-bp ladder, Gibco); no DNA template (negative control); PCR product of aELH with an ~150-bp size; PCR product of CDCH cDNA (positive control).

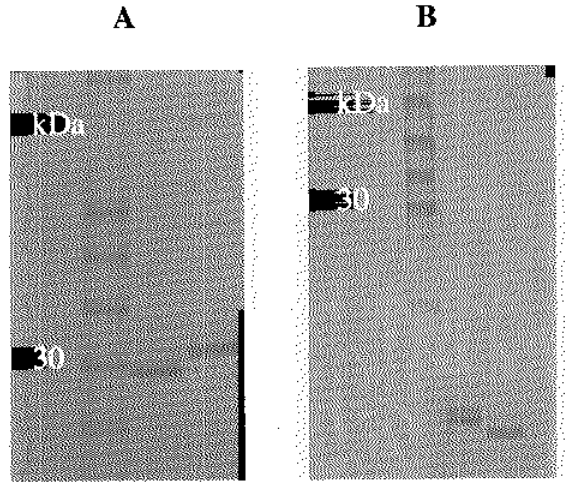

Figure 4. Detection of expressed aELH. (A) SDS-PAGE showing a ~32.2 kD expressed fusion protein. Lanes (left to right): low-range molecular-weight protein markers (Bio-Rad); GST protein expressed from pGEX-2T without an insert; expressed GST-aELH fusion protein of ~32.2 kD. (B) SDS-PAGE of aELH cleaved from the fusion protein by thrombin. Lanes (left to right): positive control of a 5 kD expressed protein of Capripoxvirus PLm2 (supplied by the CSIRO Australian Animal Health Laboratory, Geelong); aELH of ~4.3 kD.

by separate SDS-PAGE, which showed the aELH to be ~4.3 kD (Fig. 4B).

DISCUSSION

Genes encoding ELH of *Aplysia* and *Lymnaea* were cloned in 1983 (Scheller et al. 1983) and 1988 (Vreugdenhil et al. 1988), respectively. However, the aELH gene had not been studies, so we obtained and used a cDNA clone, encoding *Lymnaea* CDCH, for use in identifying an ELH in abalone genomic DNA. Dot blotting with a labeled CDCH cDNA probe indicated that the abalone genomic contained a complementary sequence (Fig. 1). This was confirmed by two PCR amplifications of abalone genomic DNA, with primers designed from nucleotide sequences of *Aplysia* ELH DNA and *Lymnaea* CDCH DNA, and hybridization of products with the labeled probe.

The nucleotide sequence of cloned abalone PCR product, PAF-1, amplified with primers derived from the CDCH sequence of *Lymnaea*, was found to have high homology with the corresponding sequence in the *L. stagnalis* CDCH super-gene family. In particular, the region encoding an aELH was found to have a 95.4% homology with that of *L. stagnalis* CDCH sequence, but only 51.9% with that of *A. californica* ELH sequence. This is almost the same as the 51% nucleotide homology observed between *L. stagnalis* CDCH and *A. californica* ELH. Total aELH superfamily sequences of *H. rubra* and several other species are currently being analyzed.

Subsequent cloning of genomic aELH into pGEX-2T, and re-sequencing, confirmed the previous sequence. Comparisons of amino acid sequences mimicked the homologies found in nucleotide sequences; aELH and CDCH were 97.2% homologous, but aELH and ELH were only 44.5% homologous.

The pGEX-2T fusion protein expression vector was chosen for use in this project for the induction of a high-level intracellular expression of an aELH as a fusion protein with bacterial GST (Ausubel, 1994). The resulting fusion proteins within bacterial lysates were subsequently purified with ease under nondenaturing conditions and with the absence of isoforms of GST. Expression as fusion proteins is known to increase the stability and solubility of the protein, compared with when the protein is expressed alone, although sometimes the fusion protein is even less soluble (Frangioni and Neel 1993). In the expression of aELH, a fusion protein was successfully obtained and then cleaved to produce an aELH with an M_r of ~4.3 kD. Bioassays of the effects of this aELH in the egg-laying process are in progress. As well, polyclonal antibodies have been produced against the aELH and are now being used in physiological studies to determine seasonal changes in and tissue-specific production of the aELH peptide.

Egg laying is a vital component of the reproductive capacity of abalone, and the finding of an aELH gene should intimately lead to a better understanding of the biological process. A gene product that is involved in the regulation of reproduction could also have been involved in reproductive isolation events that led to speciation, and to examine this point, the aELH of several abalone species have been cloned and are currently being sequenced. The findings reported here provide the basis for further studies leading to a better understanding of egg laying in abalone.

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IDENTIFICATION OF THREE POLYMORPHIC MICROSATELLITE LOCI IN BLACKLIP ABALONE, *HALIOTIS RUBRA* (LEACH), AND DETECTION IN OTHER ABALONE SPECIES

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ABSTRACT Three microsatellite were obtained from the screening of random amplified polymorphic DNA products and a genomic DNA library of blacklip abalone (*Haliotis rubra* Leach). They were assigned RUBGT1 (for *H. rubra* GT1) containing (GT)n repeats, RUBCA1 containing (CA)n repeats, and RUBGACA1 containing (GACA)n repeats. All were polymorphic in 100 blacklip abalone samples collected from the Victorian coast and Eden, New South Wales. There were 41 alleles identified at the RUBGT1 locus, 30 at the RUBCA1 locus, and 8 at the RUBGACA1 locus. Polymerase chain reaction primers of the three microsatellites, together with primers of two minisatellites, a growth hormone gene repeat (GHR) and a molluscan insulin-related peptide gene repeat (MIPR), were used in cross-species amplification of 14 abalone species from the United States, South Africa, South Korea, and Australia. Most amplifications occurred in the Australian species, *Haliotis conicopora*.

KEY WORDS: microsatellite sequences, polymorphism, abalone, *Haliotis*

INTRODUCTION

Satellite DNA, a type of tandemly arranged highly repetitive sequence, has been found to exist widely in animals and plants (Beridze 1986). By definition, the repeat unit size within the range 7–70 base pairs (bp) is known as a minisatellite or variable number of tandem repeat (VNTR), whereas the repeat unit size between 1–6 bp is known as a microsatellite or short tandem repeat (STR) (Budowle et al. 1991, Kimpton et al. 1993). Minisatellites are usually located in introns (Griffiths et al. 1993), or 3′ end nontranslated regions (Budowle et al. 1991, Huang et al. 1997), and a considerable number have been identified in animals and plants (Jeffreys et al. 1991, Rafalski et al. 1996). There are abundant microsatellites located in both genic and extragenic regions of the eukaryotic cell genome (Kimpton et al. 1993). For example, in the snail, *Bulinus truncatus*, it is estimated that on average there is a dinucleotide microsatellite locus every 40,000 bp and a tetranucleotide microsatellite locus every 60,000 bp (Jarne et al. 1994).

The abundance of VNTRs and STRs, their polymorphic nature, and amenability to amplification by polymerase chain reaction (PCR) have made them ideal markers for genetic studies, including genetic linkage analysis, antenatal diagnosis, forensic medicine, population genetic structure, and evolutionary studies (Bosch et al. 1993, Jeffreys et al. 1991, Budowle et al. 1991, Deka et al. 1995, Primmer et al. 1996). There is only one report concerning two minisatellites in blacklip abalone (*Haliotis rubra* Leach), *viz.* a growth hormone gene repeat (GHR) and a molluscan insulin-related peptide gene repeat (MIPR) (Huang et al. 1997). There are no reports concerning microsatellites in abalone, and there is a need for them, particularly for use in population studies and aquaculture.

We describe in this report three microsatellites obtained form screening a blacklip abalone genomic DNA library and random amplified polymorphic DNA (RAPD) products of blacklip abalone. As well, we report on three microsatellite alleles identified in blacklip abalone samples from Victorian and New South Wales waters, together with cross-species amplification of DNA from additional abalone species with three microsatellite primer sets and two minisatellite primer sets.

MATERIALS AND METHODS

Abalone Samples

Fourteen species of abalone were collected from Australia, Korea, the United States, and South Africa (see Table 2). In addition, 100 blacklip abalone collected for a population genetic structure study were from nine sites along the Victorian coast and one site from New South Wales. The site details have been described by Huang et al. (1997), and the abalone were provided frozen at −20°C or preserved in 75% ethanol. Each abalone was dissected with a new set of latex gloves and surgical blades in order to prevent cross-contamination. After removing the shell, 1.5 g of soft inner muscle or gonad was taken and stored in sterile 1.5-mL Eppendorf tubes at −70°C.

DNA Extraction and Purification

Abalone DNA was extracted by a method of microwaving tissue in lysis buffer followed by phenol:chloroform (1:1) extraction (Goodwin and Lee 1993). DNA to be cloned and sequenced was purified with a QIAquick™ Gel Extraction kit (QIAGEN), according to the manufacturer's instructions.

Detection of Microsatellites in Blacklip Abalone RAPD Products and a Genomic Library

RAPD products were generated from abalone genomic DNA with primers UBC 101 (5′-GCGGCTGGAG-3′), UBC135 (5′-AAGCTGCGAG-3′), and M13 repeat (5′-GAGGGTGGCGGTTCT-3′), in 50-μL volumes according to Williams et al. (1990). RAPD products were transferred to membranes by Southern transfer described in Sambrook et al. (1989) and were subsequently hybridized with labeled probes to detect bands containing microsatellite sequences. The procedures for hybridization and detection of microsatellites used 3′ end fluorescein-labeled oligonucleotide probes produced with a RENAISSANCE™ kit (Du Pont). Labeled probes used in this study included: $(CA)_{10}$, $(GA)_{10}$, $(CT)_{10}$, $(GT)_{10}$, $(GGT)_7$, $(GATA)_5$, $(GACA)_5$, $(GGGT)_5$, and $(AACT)_5$. Positive bands were excised from additional agarose gels; DNA fragments were extracted and cloned into a pCR-Script Vector

(Stratagene) for sequencing, following the manufacturer's instructions. The inserts were sequenced with T3 and T7 primers.

A blacklip abalone genomic library was obtained from Dr. Z. Chai (Medical School, Monash University). It was constructed by *Hae*III digestion of whole juvenile blacklip abalone genomic DNA and subsequent ligation of 1-3 kilobase fragments into λ *gt*10 phage. Double plaque lifts were prepared, and membranes were hybridized with labeled probes. Positive plaques were subcultured, and inserts were amplified by PCR with λ primers. PCR products were purified from gels and sequenced in both directions with λ sequencing primers.

Cloned RAPD products, or purified PCR products, were sequenced with a Perkin Elmer ABI PRISM™ Dye terminator cycle sequencing kit and autosequencer (Model 377). Alleles of microsatellites were designated by the number of repeat units within sequences, for example, allele RUBGACA1-5 contained 5 units of a GACA repeat.

Electrophoresis and Staining of Gels

Minisatellites (GHR and MIPR) were analyzed with a 0.4-mm ultrathin 6% polyacrylamide gel, following the method of Budowle et al. (1991). Microsatellites (RUBGT1, RUBCA1, and RUBGACA1) were analyzed with standard 6% denaturing polyacrylamide sequencing gels. Gel preparation and electrophoresis conditions followed the manufacturer's instructions (Bio-Rad). A nonmutagenic silver stain method, described by Budowle et al. (1991), was used to visualize separated PCR fragments in both types of gels.

PCR Conditions

Protocols for PCR amplification of MIPR and GHR minisatellites followed Huang et al. (1997). PCR conditions for analyzing variation among individuals were performed with 30× three-step cycling reactions, consisting of a hot start at 94°C for 5 min, followed by 30 cycles of denaturing at 94°C for 30 sec, an annealing step for 1 min, and extension at 72°C for 15 sec. The primer sequences and annealing temperatures are listed in Table 1.

RESULTS

Identification of a (GT)n Microsatellite in Blacklip Abalone

Abalone RAPD fragments, amplified with the M13 primer, are shown in Figure 1A. Subsequent Southern transfer of the RAPD products and hybridization with a 3′ end fluorescein-labeled $(GT)_{10}$ probe resulted in hybridization-positive bands of 900-1,000 bp (Fig. 1B). A hybridization-positive band from A7 (corresponding to Lane 7 in Fig. 1A) was found to contain 38 GT repeats. The

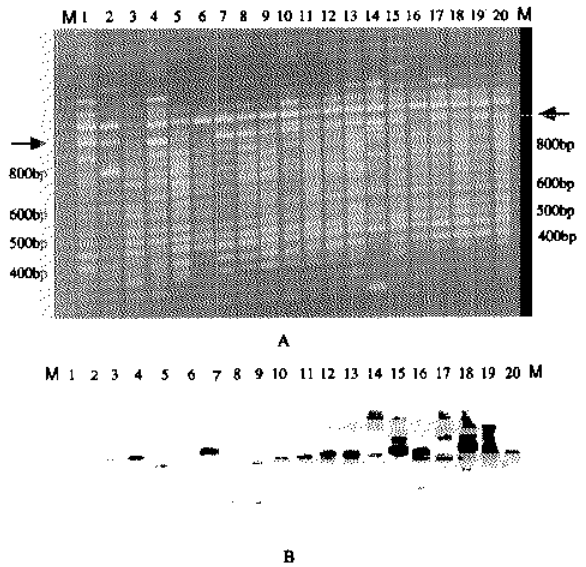

Figure 1. Identification of a (GT)n repeat in blacklip abalone RAPD products. (A) RAPD products of blacklip abalone DNA amplified with a M13 random primer (5′-GAGGGTGGCGGTTCT-3′). Arrows indicate location of hybridizing bands in Panel B. Lanes: 1–10, animals from Tullaburga Island; 11–20, animals from Eden; M, 100-bp DNA ladder (Promega). (B) Hybridization of a $(GT)_{10}$ probe to RAPD products shown in Panel A.

sequence was submitted to GenBank (Accession Number AF027572). This microsatellite locus was designated RUBGT1 (for the first GT microsatellite identified in *H. rubra*).

Identification of a (CA)n Microsatellite in Blacklip Abalone

Abaolone RAPD products, amplified with primers UBC 101 and UBC 135, are displayed in Figure 2A. Hybridization of Southern-transferred RAPD products with a 3′ end fluorescein-labeled $(CA)_{10}$ probe resulted in positive bands of 800–900 bp (Fig. 2B; Lanes 1–16). Positive bands were present in the RAPD products amplified with primer UBC 101, but not in RAPD products amplified with primer UBC 135. Sequencing of a hybridization-positive band from Petrel Point (corresponding to Lane 1 in Fig. 2A) showed the presence of a 30-repeat microsatellite, composed of $(CA/G)_{18}(CA)_{12}$. The sequence was submitted to GenBank (Accession Number AF027573). This microsatellite locus was designated RUBCA1.

Identification of a (GACA)n Microsatellite in Blacklip Abalone

Screening of a λ *gt*10 blacklip abalone genomic DNA library produced 1 hybridization-positive plaque in 2,500 plaques

TABLE 1.

Primer sequences and annealing temperatures used in the analysis of microsatellite loci.

Locus	Temperature (°C)	Primer Designation	Primer Sequence
RUBGACA1	50	RUBGACA1F (forward)	5′-CGCCGTTTTATTCGTCACCAATC-3′
		RUBGACA1R (reverse)	5′-CCACATATACAAATAAATATATC-3′
RUBCA1	61	RUBCA1F (forward)	5′-CCAATTTTACTTGAAGACTTGTGATGC-3′
		RUBCA1R (reverse)	5′-ATGTGTACGCGTTGGTGGATGG-3′
RUBGT1	61	RUBGT1F (forward)	5′-AGGGTGGCGGTTCTGGTCCTAAATC-3′
		RUBGT1R (reverse)	5′-GGCAGTGATGATATAGCGTTGTTCG-3′

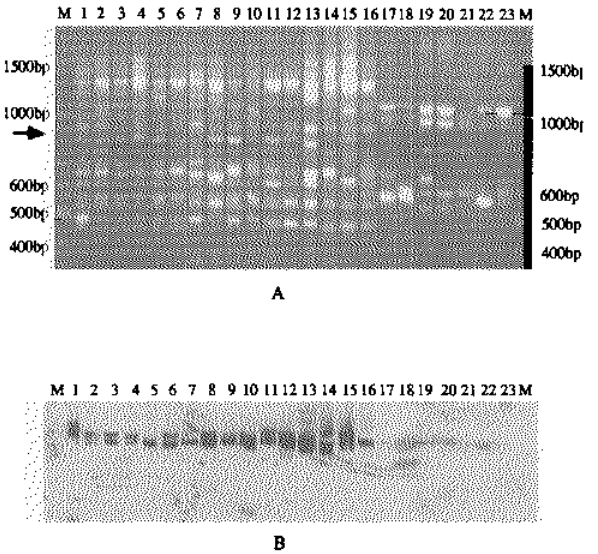

Figure 2. Identification of a (CA)n repeat in blacklip abalone RAPD products. (A) RAPD products, with arrows indicating the location of hybridizing bands in Panel B. Lanes: 1–16, RAPD products amplified with UBC-101 (5′-GCGGCTGGAG-3′), in which 1–7 are RAPD products of animals from Sandpatch Point, and 8–16 are from Petrel Point; 17–23, RAPD products from Sandpatch point amplified with UBC-135 (5′-AAGCTGCGAG-3′); M, 100-bp DNA ladder (Promega). (B) Hybridization of a $(CA)_{10}$ probe to RAPD products of Panel A.

screened. Subsequent sequence analysis of the insert showed that there were six repeats of GACA tetranucleotides. The sequence was submitted to GenBank (Accession Number AF027574). This microsatellite locus was designated RUBGACA1. Figure 3 shows amplified blacklip abalone microsatellite alleles containing known GACA repeats, with the exception of the RUBGACA1-13 allele.

Allelic Frequencies of Three Microsatellites in Blacklip Abalone

Amplification of DNA extracted from 100 blacklip abalone collected along the Victorian coastline and Eden, New South Wales, with the three microsatellite primer sets, showed that all three microsatellite loci were polymorphic. There were 41 alleles found at the RUBGT1 locus, which were defined as RUBGT1-1 to -51. Alleles RUBGT1-2, -4, -6, -7, -8, -9, -10, -16, -28 and -50 were not observed. There were 30 alleles identified at the RUBCA1 locus. These alleles ranged from RUBCA1-13 to -47, with the exception of alleles RUBCA1-14, -16, -17, -19 and -46, which were not observed. There were eight alleles identified at the RUBGACA locus, ranging from RUBGACA1-1 to -13, except alleles RUBGACA1-2, -8, -9, -10, and -12, which were not observed. A summary of the allele frequencies of the three microsatellite loci for the 100 abalone are displayed in Figure 4. The allele frequencies of all three loci were found to depart significantly from the Hardy-Weinberg equilibrium ($p < 0.001$).

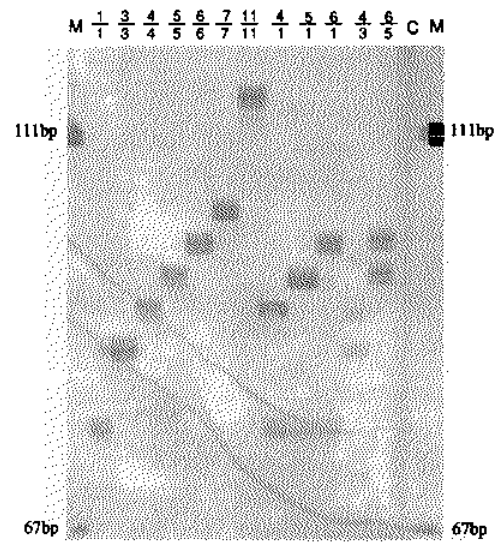

Figure 3. Alleles of the RUBGACA1 locus identified in blacklip abalone. Alleles are designated by number of repeat units. Genotypes of animals are given at the top of each lane and indicate the alleles present.

The Application of the Five Sets of Mini-/Microsatellite Primers to Other Abalone Species

Fourteen abalone species collected from four countries (United States, South Africa, South Korea, and Australia) were used in a study with all five primer sets developed for minisatellite and microsatellite studies of blacklip abalone populations. The results listed in Table 2 show that there were no amplifications of DNA from four species, *Haliotis rufescens, Haliotis cracharodii, Haliotis pervum,* and *Haliotis midae,* from the United States and South Africa. However, primers for amplifying the RUBGT1 locus amplified the DNA of the two South Korean species: *Haliotis gigantea* and *Haliotis sieboldi.* Of the other four Australian abalone species, DNAs from two species, *Haliotis roei* and *Haliotis scalaris,* were amplified with two sets of primers. DNA from *Haliotis conicopora* was amplified with four sets of primers, but was not amplified with the MIPR locus primers. None of the five primer sets could amplify DNA from *Haliotis laevigata.*

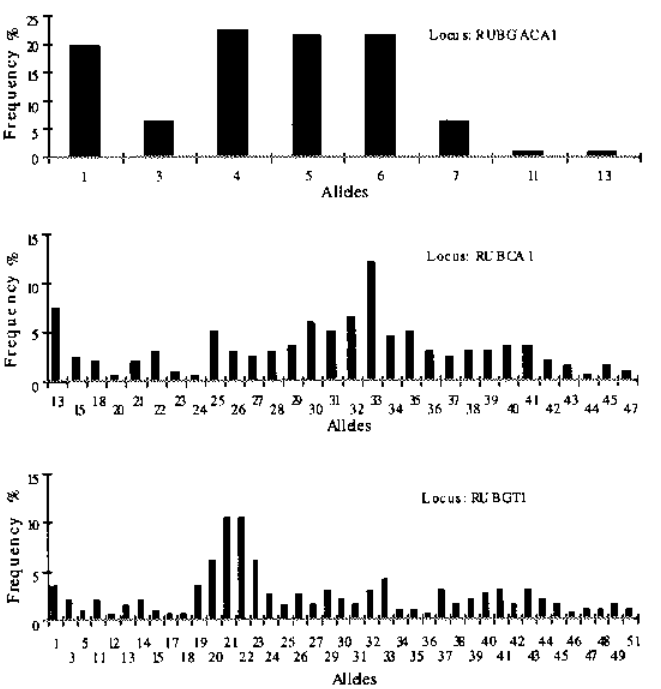

Figure 4. Allelic frequenices of the three microsatellites observed in 100 blacklip abalone of the Victorian coast and Eden, New South Wales.

TABLE 2.

The use of mini- and microsatellite primer sets on DNA of different abalone species.

Country	Species	Sample No.	Microsatellites A	B	C	Minisatellites D	E
United States	*H. rufescens*	1	—	—	—	—	—
		2	—	—		—	—
	H. cracherodii	1	—	—	—	—	—
		2	—	—	—	—	—
South Africa	*H. pervum*	1	—	—	—	—	—
	H. midae	1	—	—	—	—	—
		2	—	—	—	—	—
		3	—		—	—	—
		4	—	—	—	—	—
South Korea	*H. discus**	1		—	—	—	—
		2	—	—	—	—	
	H. discus†	1	—		—	—	—
		2	—	—	—	—	—
	H. gigantea	1		—	28/28		—
	H. sieboldi	1	—	—	27/27	—	—
		2	—		27/27	—	—
	H. discus hannai	1	—	—	—	—	—
		2	—	—			—
	H. diversicolor	1	—	—	—	—	—
		2	—	—	—	—	—
Australia‡	*H. rubra*	1	4/5§	37/37	13/13	5/5	14/18
		2	1/4	31/31	9/19	5/6	14/20
	H. laevigata	1	—	—	—	—	—
		2	—	—	—	—	—
	H. roei	1	3/3	—	29/29	—	
		2	3/3	—	19/19	—	—
	H. scalaris	1	—	7/7	11/28	—	—
		2	—	11/11	11/11	—	—
	H. conicopora	1	1/1	36/39	20/24	–	16/16
		2	1/1	40/43	9/11	—	16/17

Note: A, RUBGACA1; B, RUBCA1; C, RUBGT1; D, MIPR; E, GHR; —, no amplification.

* *H. discus* was sampled from a hatchery.

† *H. discus* was sampled from the sea.

‡ Samples of *H. rubra*, *H. laevigata*, *H. roei*, and *H. scalaris* were from South Australia, and samples of *H. conicopora* were from Western Australia.

§ Alleles present in a diploid individual are expressed in the number of repeats (e.g., 4 and 5).

DISCUSSION

Microsatellite sequences are normally obtained by screening genomic DNA libraries (Jarne et al. 1994). However, it is a tedious process to construct and screen genomic DNA libraries and to purify and sequence the clones containing STRs. Recently, it has been found that the bands amplified with a single arbitrary primer (i.e., RAPDs) often contain microsatellite DNA sequences (Richardson et al. 1995). Because the RAPD method does not require any DNA sequence knowledge of the organisms and is relatively easy to perform, the products provide a good source and starting point to search for microsatellites. This approach circumvents the construction of genomic DNA libraries but has limitations. First, a number of RAPD primers need to be tested in order to determine those showing reliable band variations among population samples. Second, there is also some difficulty extrapolating from hybridization-positive bands back to the complementary band in agarose gels, so that cloning and sequencing of DNA can be achieved.

In this study, three microsatellites were successfully cloned and sequenced. Of these, two dinucleotide microsatellites, RUBCA1 and RUBGT1, were identified and cloned from two RAPD products of blacklip abalone. The microsatellite motif (CA)n/(GT)n may be the most frequent microsatellite in the blacklip abalone genome, because hybridizations of other microsatellite probes [i.e., $(GA)_{10}$, $(CT)_{10}$, $(GGT)_5$, $(GATA)_5$, $(GGGT)_5$, and $(AACT)_5$], with RAPD products and a λ *gt*10 library, were all negative. Interestingly, the (CA)n/(GT)n microsatellites are reported to be the most frequently occurring dinucleotide microsatellites in mammalian genomes (Rafalski et al. 1996). The polymorphism and codominant features of the three microsatellites have made them useful in population differentiation and genetic structure studies of blacklip abalone (Huang 1997). In particular, they have revealed significant molecular variation in a study of 10 blacklip abalone populations ($\phi_{PT} = 0.067$, $p < 0.001$).

Minisatellite and microsatellite primers developed for blacklip abalone were applied to some of the abalone species in the world to determine whether microsatellite marker systems established here could be transferable to other abalone species. It has been reported that the detection of polymorphic STR loci in birds, using the primers developed for swallow, is related to evolutionary distance (Primmer et al. 1996). Therefore, the evolutionary relatedness of abalone species could possibly be assessed with the five mini- and microsatellites markers developed for blacklip abalone. Of the 14 species tested, no amplifications occurred for the overseas abalone species, with the exception of the South Korean species *H. gigantea* and *H. sieboldi* at the RUBGT1 locus. The minisatellite MIPR was a species-specific locus for blacklip abalone, because there was no amplification detected in other species. Amplifications occurred mainly in the Australian sympatric species, *H. roei*, *H. scalaris*, and *H. conicopora*. It is expected that abalone species in Australia. *H. conicopora* displayed amplification at four out of five loci, and *H. roei* and *H. scalaris* did so at two out of five loci. Therefore, *H. conicopora*, also named brownlip abalone, appears to be more closely related to blacklip abalone than the other three species. Similar results were observed in allozyme analysis by Brown (1991), in which the genetic distance of blacklip abalone to other species, from closest to least related, were *H. conicopora*, *H. roei*, *H. scalaris*, and then *H. laevigata*. It was unexpected that there were no amplifications at the five mini- and microsatellite loci for greenlip abalone, because the observed incidences of hybridization between blacklip and greenlip abalone in nature indicate that a close evolutionary correlation exists between these two species (Brown 1991). Additional microsatellites need to be characterized to assess more fully the evolutionary distances among abalone species.

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IDENTIFICATION OF MICROSATELLITES IN THE CALIFORNIA RED ABALONE, *HALIOTIS RUFESCENS*

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ABSTRACT We report on the progress of our genetic studies on red abalone (*Haliotis rufescens*). The first microsatellite locus in red abalone was obtained by creating size-selected genomic libraries and screening for all combinations of dinucleotide and trinucleotide repeats. Genomic libraries were created for samples from northern California (Punta Gorda Reserve), central California (Morro Bay), and southern California (Santa Barbara). Initial sequencing of positive clones identified 21 microsatellites. The most common repeats were GT/TG and AC/CA. Primers were designed for the polymorphic microsatellite locus Hruf200, which had 21 alleles ranging from 97 to 149 base pairs in size. Some rare alleles were observed in only northern or southern California collection sites. Various tissue types were evaluated for abalone genomic library construction, and gonad tissues produced the highest yield of clean DNA. Mantle tissues were tested for field treatments such as alcohol, freezing, and air drying amplified Hruf200. A DNA archive was established for the red abalone.

KEY WORDS:

INTRODUCTION

The commercial fishery for the California red abalone (*Haliotis rufescens* Swainson) has undergone a major decline since the 1960s (Tegner et al. 1989). Although restocking programs have not been notably successful, management of the fishery will need to involve the eventual translocation of adult broodstock from northern California to depleted southern fishing grounds as well as continued hatchery seeding programs. With the closure of the commercial red abalone fishery, it is crucial to the development of a management plan that there be a tool to identify populations. However, to date, there have been no known genetic markers for population structure in the red abalone. Traditional allozyme studies have not detected any significant population differences. In many other marine species where allozyme studies have not detected population differences, microsatellites and mitochondrial markers have been useful in detecting such differences (Garcia et al. 1994, Geller et al. 1993, Palumbi and Wright 1995, Wright and Bentzen 1994).

Microsatellites are a class of nuclear DNA markers that are abundant in all eukaryotic genomes (Tautz 1989) and consist of series of 1–5 bases in repeating arrays that exhibit high levels of codominant allelic variation in repeat number (Wright and Bentzen 1994, O'Reilly and Wright 1995). Polymorphisms exhibited by specific microsatellites are readily detected by polymerase chain reaction (PCR) amplification with oligonucleotide primers specific to the nonrepetitive regions that flank the repeat array. Microsatellite analysis has the advantage of using small amounts of tissue so that nonlethal sampling is possible. However, the use of microsatellites as DNA indicators of genetic diversity has slowly been applied to marine species because it is entirely dependent on the availability of species-specific primers for the PCR. In order to adapt this technology for use in the red abalone, species-specific primers have to be developed because there are virtually no reports on microsatellite DNA in any abalone species.

We report here on the development of microsatellite analysis in the red abalone and the first microsatellite locus, named Hruf200. Because our goal was also to establish a DNA archive for red abalone and create a DNA database, our project also included the following studies: (1) determine what and how tissues would be collected in the field, (2) optimize protocols for DNA extraction for genomic library construction and microsatellite analysis, (3) construct genomic libraries for three geographical regions of California, (4) initiate the development of a DNA archive of abalone samples, (5) identify microsatellites in red abalone, and (6) identify polymorphic loci for population analysis.

METHODS

Sample Collections

Adult red abalone, *H. rufescens*, were collected from five sites in California for construction of genomic libraries. In northern California, 12 adults were collected from Punta Gorda Reserve (CDFG, Kon Karpov). In central California, two adult abalone from Morro Bay were provided by Mr. Frank Oakes (The Ab Farm, Cayucos, CA). In southern California, five adults were from the Santa Barbara region (CDFG). Tissues collected from the adult abalone were held at −70°C until further analysis.

Mantle tissues were collected, extracted, and archived for DNA analysis from 400 abalone from a total of nine coastal or island sites and two abalone aquaculture facilities. These sites (Fig. 1) were divided into northern and southern California regions for analysis. Samples were either directly frozen on dry ice in the field or were kept on wet ice in the field and frozen within 12 h of collection. The original tissues were archived for storage at −70°C, whereas the DNA extractions were archived at −20°C. For the initial microsatellite analysis, a total of 170 subsamples were randomly selected from northern California ($n = 85$) and from southern California ($n = 85$). For microsatellite analysis, a small sliver of the tissue was clipped from the frozen field sample and put into 200 μL of 5% Chelex-100 (BioRad) in distilled water (wt/vol). These tubes were then (1) incubated for 20 min at 65°C, (2) vortexed for 3 sec, (3) incubated for 10 min at 95°C, (4) vortexed for 10 sec, and then (5) centrifuged at 10,000 *g* for 3 min. Chelexed samples were then used in a polymerase chain reaction (PCR) analysis or frozen at −20°C until PCR analysis.

For determination of acceptable types of tissues for microsat-

Figure 1. Collection sites for red abalone, *H. rufescens*, in California. PG, Punta Gorda; SC, Shelter Cove; GC, Gerstle Cove; PA, Point Arena; SP, Salt Point; BB, Bodega Bay; FI, Farallon Islands; PP, Pigeon Point; MB, Morro Bay; SB, Santa Barbara; SM, San Miguel Island.

ellite analysis, we collected matched tissue samples from two abalone from Punta Gorda Reserve and treated these samples by (1) freezing directly, (2) holding on ice for 24 h and then freezing, (3) air drying, or (4) fixing in 80% ethanol (Table 1). The tissue samples were from the foot muscle, epipodium, mantle, and gonad biopsy. In our abalone genetic enhancement research, we routinely collect nonlethal tissue biopsies from gonad, foot, mantle, or tentacles. Samples as small as 1–5 mg (a very small tentacle or sliver of tissue) provide ample amount of DNA for individual typing.

Nonlethal gonad biopsies were collected to confirm sex as well as provide gonad tissue for comparison of extraction protocols from the abalone collected at Pigeon Point. Gonad tissue was aspirated into a 1-mL syringe with a 25-gauge needle held at an angle of approximately 15–30°, depending on the size of the gonad. By passing the needle just under the surface of the connective tissue covering the gonad, one can observe the needle's path, which must be essentially parallel to the gonad but just under the protective connective tissue. Slight back pressure is exerted on the syringe plunger to collect tissue into the needle.

TABLE 1.

Four tissue types were treated by four methods of preservation and then tested for amplification of Hruf200 microsatellite.

	Tissue Type			
Sample Treatments	Foot Muscle	Epipodium +/-Tentacle	Mantle at Leading Edge	Gonad Biopsy
Freeze within 12 h	+	+	+	+
On ice 24 h, then freeze	+	+	+	+
Allow to air dry	+	+	+	+
80% alcohol	+	+	+	+

* + indicates microsatellite DNA recovered from that tissue or that treatment.

Constructing Size-Selected Genomic Libraries

Size-selected genomic DNA libraries were constructed for red abalone from three geographical regions of California. The northern California library was represented by abalone from Punta Gorda; central California was represented by Morro Bay; and southern California was represented by Santa Barbara abalone. One library was used to optimize and validate the following procedures. Tissues from foot, hypobranchial-gill glands, digestive gland, and ovary were homogenized in liquid nitrogen with a mortar and pestle, digested with proteinase K at 65°C for 2 h, and phenol:chloroform extracted (Ausubel et al. 1995). DNA was ethanol precipitated, and its purity was spectrophotometrically measured. It was determined that ovary produced the highest yield and cleanest DNA. All other sample types had high levels of RNA and mucopolysaccharides. The other libraries were then extracted from ovary tissue and stored at −70°C. The genomic DNA was digested with *Sau*3A restriction enzyme and separated on an agarose gel. The 200–700 base pair (bp) bands were excised and purified by Centricon spin columns. Bands of this size were selected in order to allow for efficient sequencing of cloned fragments. DNA concentration was determined by spectrophotometry before storing at −20°C.

Cloning and Hybridization

The resulting size-selected genomic DNA was ligated to KS-pUC18 vector (Stratagene) at various DNA:vector ratios. The constructs were then inserted into competent Bluescript *Escherichia coli* bacteria via heat shock (Sambrook et al. 1989). After incubation at 37°C overnight, colonies were lifted onto nylon membranes for hybridization. Dinucleotide and trinucleotide repeats (Gibco-BRL) were end-labeled with P32 and used to probe the ultraviolet-cross-linked nylon membranes (Sambrook et al. 1989). Positives were cultured in 5 mL of DYT media, and plasmid DNA was recovered by alkaline mini-preps. This DNA was then sequenced via the Sequenase 2.0 kit (US Biochemical) with S35-dATP. Sequences were read directly off of the autoradiographs for primer design.

Identification of Microsatellites

Those microsatellite sequences having sufficient flanking DNA at both the 5′ and 3′ ends were chosen for primer design. Although a total of seven primer pairs were tested for PCR product, only Hruf200 produced nonambiguous microsatellite alleles that could be consistently scored. Microsatellite primers were tested for band amplification with hot start PCR as described by Nielsen et al. (1994). One microliter of chelexed sample was added to a PCR mixture of 1.25 μL of 10× PCR buffer, 1.25 μL of 10 mM each dNTP, 1.25 μL of 10 mM primer A, 0.63 μl of primer B, 0.63 μL of primer B end-labeled with γ-^{32}P-ATP, 0.06 μL of Taq polymerase (Promega), and 3.5 μL of ddH_2O. Primer sequences for the Hruf200 locus were (from the 5′ to 3′): Hruf200A: GAGATAGT-

TCGATTCAAGAT; Hruf200B: CCATTATAAAGGGCCGGACTA.

After denaturing for 5 min at 94°C, the samples were cycled for 30 cycles of 40 sec at 94°C, for 60 sec at 50°C, and for 120 sec at 72°C. Initial PCR cycles used touch down cycling to locate the appropriate annealing temperatures. After PCR amplification, the samples were mixed with loading dye and then denatured at 95°C for 5 min. The samples were then electrophoresed on standard 8% polyacrylamide: 7.2 M urea denaturing gels (40 × 30 cm) at 60-W power, mounted on Whatman paper, dried, and exposed to x-ray film (Kodak XAR-5) for 24–72 h at room temperature. Hruf200 alleles were scored with a M13-labeled sequenced ladder (Sequenase V.2.0; US Biochemical) as a size standard (Yanisch-Perron et al. 1985). Only unambiguous microsatellite bands were scored for this report. If the bands were too faint to positively identify a microsatellite as homozygous or heterozygous, that sample was not scored and the sample was tagged for reanalysis. For shadow bands, the darkest band was scored as the allele. If bands were of equal darkness, they were not scored for that sample.

Statistical Analysis

Frequency distributions of allele sizes for Hruf200 were analyzed. Heterozygosities and genotype frequencies were obtained for all samples combined and separately for northern California and southern California with an unbiased estimate of the Fisher's exact test (GENEPOP; Raymond and Rousset 1994). Combined and regional groups were tested for Hardy-Weinburg equilibrium with the χ^2 test.

RESULTS

Sample Collections

We treated the mantle tissue in a variety of ways to simulate field conditions such as: (1) immediately frozen, (2) kept on ice for 24 h and then frozen, (3) air dried, and (4) stored in 80% ethanol (Table 1). Mantle tissue kept under these conditions amplified Hruf200. Epipodium, foot muscle, and gonad tissues also amplified Hruf200. Although this analysis was only done on four tissues from one abalone, it suggested that any of these tissues collected in the above manner may be used for Hruf200 microsatellite analysis. However, this may not be true for other microsatellite loci; therefore, we have kept all samples frozen for the study until we can repeat these preliminary experiments with more replicates and, more important, use these in PCR for different microsatellite loci.

The gonad biopsy, which can be used in the field, is more definitive for determining the sex of animals than is the standard visual examination. We noted that in the field, most individuals ($n = 16$) that appeared to be female by visual examination were actually immature males on the basis of biopsy. At the same time of year, California Department of Fish and Game field researchers had observed that there seemed to be mostly females in the field at one of the Channel Islands. Because some abalone have a fair amount of dark pigment in the connective tissue covering the gonad, a male with poor gonad development could be visually mistaken for a female. Also, in the case of a male with a completely refractive gonad, the hepatopancreatic organ shows through the connective tissue, giving a dark appearance. It may be that in some areas, the males are not developing their gonads at the same time as the females. We have observed this in abalone aquaculture with abalone 5 inches or less in size for the last 3 y. Animals of the same age did not have sexually mature gonads during the same season.

We also note that of the 205 biopsies performed at our laboratory, only one abalone died, and it was one of the very first animals to be tested by our biopsy protocol. If care is taken to avoid puncturing the digestive gland by inserting the needle at a shallow angle to the surface of the gonad, the biopsy is nonlethal and can be done several times on the same abalone.

Abalone Microsatellites

A total of 26 microsatellites were isolated from 31 positive clones from the partial genomic library. Perfect, imperfect, and compound repeat core sequences (as defined in Weber 1990) were observed. $(GT/TG)_n$ microsatellite repeats together with the $(AC/CA)_n$ repeats represented 83% of the abalone microsatellites. Dinucleotides were the most common type of microsatellite (75% of the total).

The initial population analysis for this report used 170 samples randomly selected from northern and southern California, including Punta Gorda, which is the most northern of all of the collection sites. Of these samples, 74 amplified a microsatellite band that could be unambiguously scored for variation in allelic size. Most of the 96 samples that could not be scored unambiguously had amplified microsatellite bands, but they were either too faint to determine whether they were homozygous or heterozygous or stutter bands were too dark to determine which allelic band was the actual correct bp size. For the samples that produced nonambiguous microsatellite bands, it was determined that there were 21 alleles at the Hruf200 locus (a GT repeat), which ranged in size from 97 to 149 bp.

A frequency distribution analysis (Fig. 2) indicated that the most common alleles were 97–103 bp for the northern populations and 101–103 and 121 for the southern populations. These appeared to be a bimodal distribution of alleles with a cluster corresponding to 99–105 bp and a second cluster between 115–121 bp. A more complex pattern may emerge as more samples are evaluated. Punta Gorda abalone, the most northern population, were not distinguishable from other northern California red abalone, but they did share alleles that were only observed in the northern populations. North-

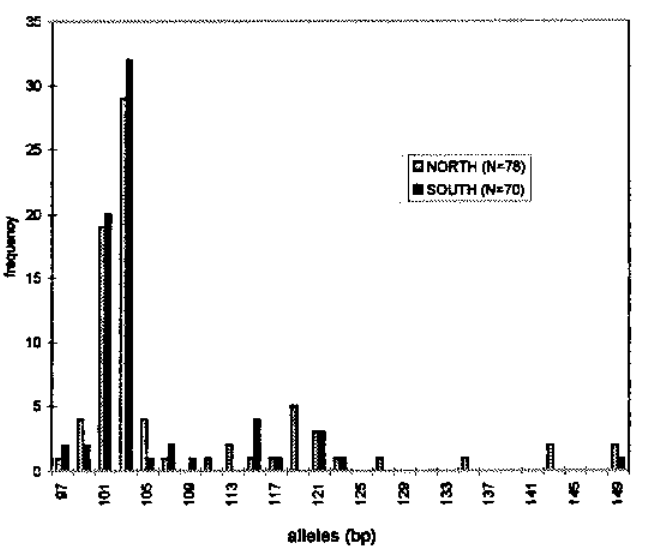

Figure 2. Frequency distribution of Hruf200 alleles by region.

TABLE 2.

Genetic variance at Hruf200 locus in total sample population (all of California) and separately as northern and southern California regions.

Region	n	Heterozygosity: Observed (Direct Count)	Heterozygosity: Expected (Hardy-Weinberg)	No. of Alleles
All California	74	35	56	28
Northern CA	39	23	31.1	17
Southern CA	35	12	24.9	12

ern California red abalone had unique alleles of 111, 113, 127, 135, and 143 bp. In contrast, southern California abalone had the one unique allele of 109 bp. However, more samples will have to be analyzed to determine if any of these alleles can be used as population markers.

The observed heterozygosity (Table 2) indicated that there was a relatively high level of variability of Hruf200. The genotype frequencies for all regions combined conformed to Hardy-Weinberg, as do those for southern California animals. However, the northern California data did not, indicating that further samples are needed before further statistical analysis.

DISCUSSION

In this report, we have shown that microsatellites can be found in red abalone and we have reported on the first microsatellite marker, designated as Hruf200. GT/CA repeats, such as Hruf200, represented 83% of the microsatellites observed. This is consistent with the literature, where GT/CA repeats predominate (Stallings et al. 1991). Hruf200 was polymorphic, with 21 alleles detected in 74 abalone. Almost all of the original 170 samples amplified microsatellite alleles with Hruf200, but they could not be unambiguously scored. Preliminary reanalysis of some of these samples suggests that too much DNA was used in the PCR, and many of these samples were the first samples that we chelexed. We later determined that a much smaller quantity of DNA was adequate for chelex extraction. Several alleles were observed in only either the northern or the southern samples. These alleles were generally rare and may or may not prove to be unique to the respective populations. However, there are over 400 more samples in our DNA archive to be analyzed with Hruf200 to determine if allelic variation at this locus can be used as a population marker.

Our project objectives included the evaluation of microsatellite analysis for red abalone. When we initiated this project, there was no literature available on technique for abalone microsatellite analysis. Because this was also the first attempt on red abalone to develop microsatellite primers, we concluded that our first investigations should include building genomic libraries for microsatellite screening, determining the type of tissues and their condition necessary for microsatellite analysis, and then initiating screening of genomic libraries. Although only one library is necessary for screening, we have taken the long-term approach by building libraries for different geographical regions of California to eventually screen for site-specific loci.

We have successfully completed the first stages and determined that either mantle, epipodium, foot muscle, or gonad biopsy can be used for Hruf200 microsatellite analysis. These tissues can be either fresh, frozen, alcohol preserved, or dried. As we continue to sequence the number of clones, we expect to find more polymorphic loci for the analysis of population structure in red abalone. We will continue to screen more individuals as well as search for other microsatellite loci for statistical analysis of population differences. Ultimately, we hope that DNA fingerprinting technology that uses the analysis of microsatellites will provide information to allow us to address questions related to: (1) distinguishing between abalone populations, (2) monitoring the success or failure of abalone restocking and conservation efforts, and (3) providing forensic markers for law enforcement of protected stocks.

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SHELL LESIONS IN NEW ZEALAND *HALIOTIS* SPP. (MOLLUSCA, GASTROPODA)

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ABSTRACT Shell lesions are reported in the three New Zealand species of *Haliotis* (*Haliotis iris* Martyn, 1784, *Haliotis australis* Gmelin, 1791, and *Haliotis virginea virginea* Gmelin, 1791). The lesions are described as blisters of conchiolin, and occasionally nacreous material, forming on the inside of the abalone shell near the apex. Twelve (16%) of 76 *H. iris*, 21 (38%) of 56 *H. australis*, and 5 (100%) of 5 *H. virginea virginea*, had lesions, some of which disrupted the adductor muscle scar. Specific histological stains confirmed the presence of fungal hyphae within lesions and the shell matrix of affected *H. iris* and *H. australis*. No pathological changes or evidence of fungal infection were observed in any of the soft tissues of the lesion-bearing animals examined. Lesions were most prevalent in populations in the Catlins region of South Otago and off the northeast coast of Stewart Island, although they were found throughout the southern New Zealand range of *H. iris*, including offshore islands. The mean shell length of lesion-bearing *H. iris* was significantly ($p < 0.05$) less than that of nonlesion-bearing *H. iris* at 4 of 11 locations sampled.

KEY WORDS: abalone, paua, *H. iris*, *H. australis*, *H. virginea virginea*, New Zealand, shell lesion, fungus

INTRODUCTION

The marine genus *Haliotis* (Mollusca: Gastropoda) is distributed through the temperate and tropical regions of the world, including New Zealand, where it is represented by three endemic species: *Haliotis iris* Martyn, 1784, *Haliotis australis* Gmelin, 1791, and *Haliotis virginea virginea* Gmelin, 1791 (Powell, 1979). Commonly called abalone, or paua, the largest and most abundant species, *H. iris*, is the basis of a substantial inshore fishery. Harvested abalone are often exported as a highly prized food, whereas the shells are used for jewelry and in the inlaying of furniture. They are also increasingly important as an aquaculture species, for both meat and pearl production.

Recently, it was observed that some individuals of *H. iris*, in the vicinity of Stewart Island (47°S and 168°E), had lesions on the inside of the shell. The lesions generally appeared as brown, jelly-like material resembling conchiolin. The lesions severely damaged the shell of affected individuals, with a resulting loss of earnings for both fishers and exporters. Furthermore, aquaculturalists reported that this condition could be fatal to animals kept in captivity (D. Langdon pers. comm.). The effects of this condition, and its prevalence and distribution in wild abalone populations, were unknown. A search of English language journals revealed no reports of similar shell lesions among the many other species of *Haliotis* found outside New Zealand. There are several reports of parasites that invade the shells of abalone. Crofts (1929) reported that *Haliotis tuberculata* Linnaeus shells were frequently damaged by boring bivalves such as *Lithodomus* sp. and *Pholadidea* sp. Further reports include a boring barnacle (Batham and Tomlinson 1965), a sponge (Clavier 1992), and parasitic worms (Cox 1962, Oakes and Fields 1994). In New Zealand, Sinclair (1963) and Sainsbury (1977) described various shell conditions in *H. iris* from the Wellington area and from Banks Peninsula and Peraki Bay areas, respectively. Both authors describe shell pathologies that appeared to share some characteristics with those described here.

This study provides a detailed description of the macroscopic and microscopic structure of the shells and soft tissues of affected and unaffected specimens of the three abalone species native to New Zealand. In addition, the geographic distribution and information on the prevalence of these lesions in wild populations of abalone in New Zealand are provided.

MATERIALS AND METHODS

Description of Lesions and Histology

A total of 76 *H. iris* (50.8–151.7 mm shell length), 56 *H. australis* (26.5–86.0 mm shell length), and 5 *H. virginea virginea* (47.2–63.8 mm shell length) were collected by divers (using SCUBA) from shallow subtidal populations at Little River, Stewart Island (46°53′S, 168°06′E), in February 1995 and from Jacks Bay in South Otago (46°30′S, 169°44′E), in December 1995. The shell was detached from the soft tissue of the animal, and the inside was examined for the presence of lesions. The size, location, and description of shell lesions were characterized from these lesion-bearing animals. Selected individuals were sampled for microscopic analysis, as given below. To determine the extent to which the adductor muscle attachment site was affected by the lesion, a random selection of 63 affected *H. iris* shells and 24 affected *H. australis* shells were set aside from the commercial catches sampled as described below.

Square pieces of shell measuring approximately 1 cm^2 were excised from affected and unaffected animals with a small hand-held electric grinder. These samples were placed in Bouins solution (Humason 1979) in seawater for at least 14 days, embedded in Paraplast™, and sectioned transversely at a thickness of 7 μm by the techniques described by Humason (1979). Shell tissue sections were stained with periodic acid Schiff's reaction (PAS), Hotchkiss-McManus method (McManus 1948), and Grocott's adaptation of Gomori's methenamine-silver nitrate method (SS) for fungi

(Grocott 1955). Samples of shell were taken from 11 lesion-bearing and 11 nonlesion-bearing *H. iris* and 10 lesion-bearing and 14 nonlesion-bearing *H. australis*.

In order to examine the effect of lesions on soft tissues, we examined all major tissues including foot, digestive gland, kidney, gill, and mantle from 10 *H. iris* and 10 *H. australis* with and without lesions. For animals with lesions, a sample of tissue adjacent to lesions was also examined. Cross-sections (3–4 μm thick) of selected tissues were placed in Davidson's invertebrate fixative (Shaw and Battle 1957) for 24–48 h and processed for routine paraffin histology (Humason 1979). Deparaffinized sections were stained with Lillie-Mayer haematoxylin and eosin (Lillie 1965) and PAS and viewed by bright field microscopy.

Distribution

Samples of commercially caught *H. iris* (i.e., animals larger than 125 mm shell length) from 21 locations around the South Island and Stewart Island of New Zealand were sampled at a processing factory in Dunedin. One to three groups of 250 abalone were sampled from each geographic location, and the presence or absence of the condition was noted for each animal (7,500 *H. iris* were examined in total). Twelve affected *H. iris* shells were also collected from a commercial abalone processor on the Chatham Islands (44°53′S, 176°30′W). Fifteen specimens of *H. australis* were collected from the Snares Islands (48°00′S, 166°35′E), and five specimens of *H. virginea virginea* were collected from Campbell Island (52°30′S, 169°10′E).

Size

To ascertain if an association exists between the size of commercially caught abalone and the presence of shell lesions, we examined *H. iris* from selected locations throughout the South Island of New Zealand. At least 107 *H. iris* were randomly selected from each of the commercial catches, and the shell lengths of affected and unaffected animals were measured to the nearest 0.1 mm with calipers. Catches in which more than 10% of the measured abalone were affected with shell lesions were selected for analysis, resulting in a total of 1,679 abalone in 11 catches from 9 locations. Single-factor analyses of variance were used to compare the shell lengths of affected and unaffected *H. iris* from each catch.

To assess the size range of animals exhibiting lesions, 56 specimens of *H. iris* and 36 specimens of *H. australis*, ranging above and below the minimum legal size (125 and 80 mm, respectively), were collected from Little River, Stewart Island. The shell lengths were measured to the nearest 0.1 mm, and the shell was dissected from the soft tissues, as before, to examine for lesions. A Kolmogorov-Smirnov two-sample test was used to assess the relationship between animal size and presence of shell lesions.

RESULTS

Description of Lesions

Lesion prevalence in populations of *H. iris* at the Little River and Jacks Bay sites was 54 (n = 56) and 45% (n = 20), respectively, whereas in *H. australis*, the estimates were 47 (n = 36) and 50% (n = 20), respectively (Table 1). All *H. virginea virginea* sampled from Little River (n = 2) and from Jacks Bay (n = 3) were affected. In over 95% of all affected specimens examined, we observed no indication of the presence of lesions on the exterior of the shell. Although *Polydora* tubes were evident on the exterior of the shells, irrespective of the presence of lesions, none penetrated through the innermost layers of the shell. A few *H. iris* did have lesions that were evident externally, and these were characterized by the collapse and destruction of the shell around the apex region (Fig. 1). Usually, a shell lesion appeared as a golden-brown growth on the inside of the shell. The condition was always evident at the apical region and, in large lesions, extended from the apex to include the site where the adductor muscle attaches to the shell. Small lesions were not usually beneath the attachment site of the adductor muscle (Table 2). In 21 (16%) of *H. iris* and 15 (38%) of *H. australis* shells examined, the adductor muscle scar was disrupted by an extensive lesion (Table 3). Lesions ranged in size from 0.01 to 40 cm^2, were soft with a jelly-like matrix, often contained a gritty component, and had a pungent odour (Fig. 2).

Sometimes, a spherical nacreous blister encompassed the soft jelly-like lesion (Fig. 3). In a few cases where the exterior of the shell had collapsed, the nacreous layer completely covered the lesion and isolated it from the mantle cavity. The nacreous blisters ranged in size from 9 to 30 cm^2. Shell lesions, similar to those described above for *H. iris*, were also observed in *H. australis* and *H. virginea virginea* (Fig. 2).

TABLE 1.

Number of specimens of *H. iris*, *H. australis*, and *H. virginea virginea* examined from each of two locations (Little River and Jacks Bay) and the number (and percent) affected by shell lesions.

Species	Location	No. of Specimens	Number Affected (%)
H. iris	Little River	56	30 (54%)
H. iris	Jacks Bay	20	9 (45%)
H. australis	Little River	36	17 (47%)
H. australis	Jacks Bay	20	10 (50%)
H. virginea virginea	Little River	2	2 (100%)
H. virginea virginea	Jacks Bay	3	3 (100%)

Histology of Shell and Soft Tissues

All unaffected *H. iris* and *H. australis* shells examined by light microscopy (n = 11 and n = 14, respectively) showed an organized, layered structure that consisted of alternating layers of cal-

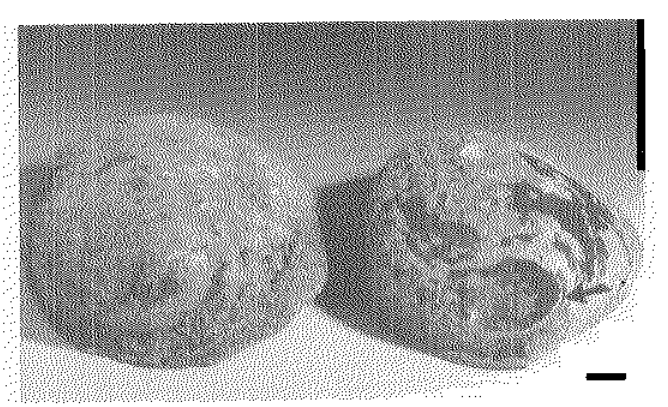

Figure 1. An affected *H. iris* (on the right) showing the external crumbling and caving-in of the apex of the shell (arrow). This occurs in some extreme cases of the condition. The *H. iris* on the left is unaffected by the condition. Scale bar, 1.5 cm.

TABLE 2.

The number of affected individuals of *H. iris* and *H. australis* and the relationship between the area of their shells with lesions and the area of their adductor muscle site exhibiting lesions.

Area of Shell Covered by Shell Lesion (%) in *H. iris* and *H. australis*	No. of Animals and Area of Adductor Muscle Scar Covered by Lesion (%)					
	None	+−10%	11–15%	15–20%	21–25%	26–50%
H. iris +−10%	16	—	—	—	—	—
11–25%	21	7	1	—	1	
26–50%	4	4	2	2	1	1
51–75%	—	—	—		—	1
H. australis +−10%	4	1	—	—	—	—
11–25%	3	3	1	—	—	
26–50%	1	4	1	1	1	—
51–75%	—	—	1		—	3

cium carbonate and protein (Fig. 4). In all affected *H. iris* and *H. australis* shells (n = 11 and n = 10, respectively), normal shell organization was disrupted and damaged. Gaps and necrotic tissue or conchiolin were frequently observed between shell layers, and the shell was thickened adjacent to the lesion (Fig. 5). The shells of all lesion-bearing animals contained hyphae-like structures that were generally straight, sparsely branched, and septate (Fig. 6). These structures were generally thin (1.20–2.38 μm in diameter), and no prochlamydospores were observed. Hyphae-like structures with apical swellings (4.8–7.2 × 7.2–15.0 μm) were observed in three specimens examined. These hyphal structures reacted positively to specific staining (PAS and SS) for fungi. Although similar hyphal structures were present in all of the affected shells of *H. iris* and *H. australis* that were examined, they were also sometimes found in nonlesion-bearing shells, either on the outside of the shell or, in a few instances, as a shallow penetration of the shell (Table 4).

Macroscopic and microscopic examination of the auricles, ventricles, right and left kidneys, hypobranchial glands, gonads, gills, adductor muscles, and mantle revealed no detectable differences in tissue architecture between animals with and those without lesions. Tissue sections taken from sites on the conical appendage directly adjacent to severe lesions revealed no pathological changes (e.g., no metaplasia or infiltration of hemocytes). No fungal hyphae were found in any of the soft tissues examined.

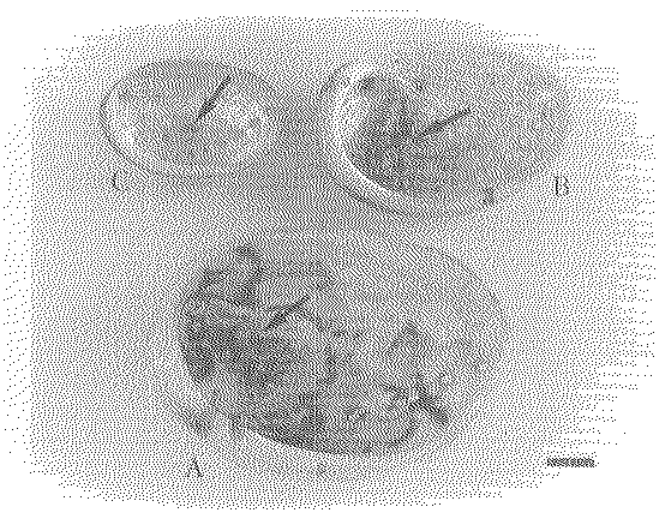

Figure 2. The interior shell surface of lesion-bearing *H. iris* (A), *H. australis* (B), and *H. virginea virginea* (C) shells. Lesions (arrows) are present on the inner surface. Scale bar, 1.5 cm.

Geographic Distribution

The 21 locations surveyed around the South Island and Stewart Island showed that shell lesions were found in *H. iris* throughout

TABLE 3.

Number of *H. iris* and *H. australis* that had a portion of the adductor muscle site disrupted and those that did not.

Species	Affected or Unaffected	Total Number	Attachment Site Not Affected	Attachment Site Affected
H. iris	Unaffected	67		
(n = 130)	Affected	63	42 (32%)	21 (16%)
H. australis	Unaffected	16		
(n = 40)	Affected	24	9 (23%)	15 (38%)

Figure 3. Transverse section of an affected *H. iris* shell. This shell has a lesion (L) completely covered by a nacreous layer (N). Note the thickening of the shell in the region of the lesion. Scale bar, 0.75 cm.

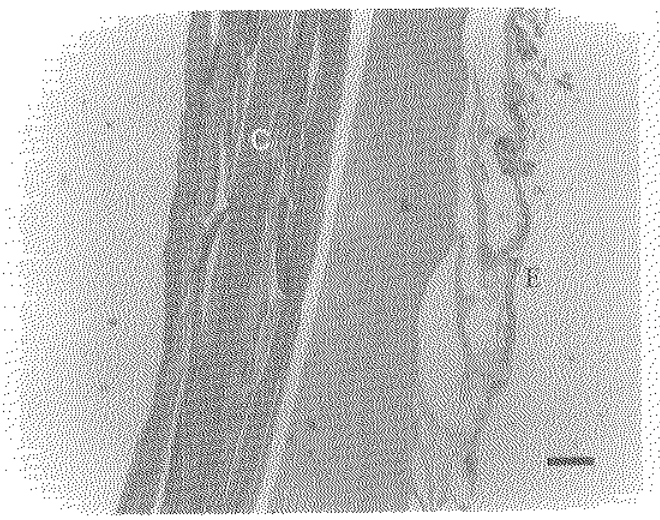

Figure 4. Transverse section of an unaffected *H. iris* shell stained with PAS. The exterior (E) of the shell and the alternating calcium carbonate (C) and protein layers are highlighted. Scale bar, 25 μm.

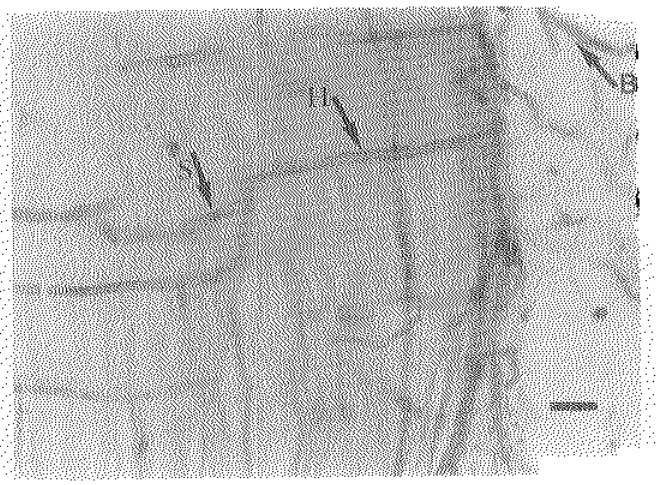

Figure 6. Transverse section of an affected *H. iris* shell stained with PAS. Fungal hyphae (H) may be seen throughout the shell matrix. The hyphae are sparsely branched (B) and separate (S). Scale bar, 10 μm.

the area sampled (Fig. 7). The prevalence of affected *H. iris* ranged from 0% at several sites, particularly in the Marlborough Sounds region, to a maximum of 70% at one location in the Catlins region. The two catches from the Breaksea/Dusky Sound location had lesion prevalences of 0.4 and 42.4%. Lesions were present in animals at all three of the off-shore islands sampled. Eight of the 15 *H. australis* from the Snares Islands were affected, and 1 of the 5 *H. virginea virginea* from Campbell Island was affected. Twelve affected shells of *H. iris* were collected from a Chatham Island fish processor.

Although the mean shell length of affected *H. iris* was less than the mean shell length of unaffected *H. iris* from all 19 catches sampled, differences were significant at only four locations: Cape Jackson, the northeast coast of Stewart Island, Port Adventure, and Coal River (Table 5). Animals sampled from Little River showed that lesion-bearing *H. iris* ranged in shell length from 64.3 to 147.0 mm, whereas lesion-bearing *H. australis* ranged in shell length from 44.8 to 86.0 mm (Figs. 8 and 9). No significant correlation was observed between shell length and the presence of shell lesions in *H. iris* ($p > 0.05$, $D_{max} = 0.3127$, $n = 56$) sampled from Little River (Fig. 8). However, we did observe a significant trend of increasing prevalence of shell mycosis to increasing shell length in *H. australis* (Fig. 9: $p < 0.05$, $D_{max} = 0.5202$, $n = 36$).

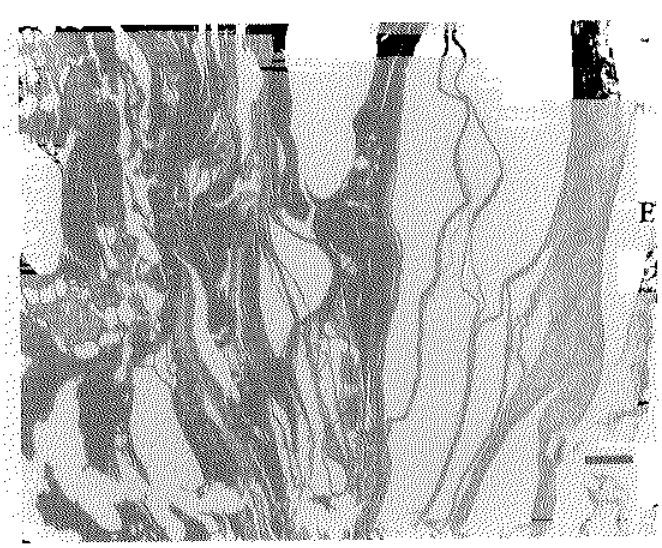

Figure 5. Transverse section of an affected *H. iris* shell stained with PAS. The shell layers are disrupted, broken, and considerably thickened. The magnification and orientation are as for Figure 8, with the exterior (E) of the shell being marked. Scale bar, 25 μm.

DISCUSSION

The lesions described in this study of the New Zealand species of *Haliotis* were rarely visible on the exterior of the shell (Fig. 1) but were always evident on the inside of the shell. Unlike many damaged mollusc shells (Vermeij 1993), lesion-bearing *H. iris* and *H. australis* collected from the wild always exhibited damage around the apex, the oldest part of the shell. In specimens with extensive damage, lesions appeared to initiate at the apex and from there expand toward, and eventually under, the site of adductor muscle attachment. Small lesions (*circa* 0.01 cm^2) were jelly like in consistency, whereas larger lesions, which often covered considerable proportions of the inner shell (*circa* 40 cm^2 in area; Figs. 2 and 3), including the adductor muscle scar (Table 2), were hard in consistency, caused shell thickening (Fig. 5), and may have calcified.

The presence of fungal hyphae associated with all of the affected shells was confirmed with PAS and SS for fungi (Grocott

TABLE 4.

Number of lesion-bearing and nonlesion-bearing *H. iris* and *H. australis* in which hyphae were either absent, present on the shell surface only, or embedded in the shell or lesion.

	No. of Animals			
	No Visible Lesions			Lesions
Species	No Hyphae	Surface Hyphae Only	Hyphae Within Shell	Hyphae Within Lesion
H. iris	4	3	4	11
H. australis	8	4	2	10

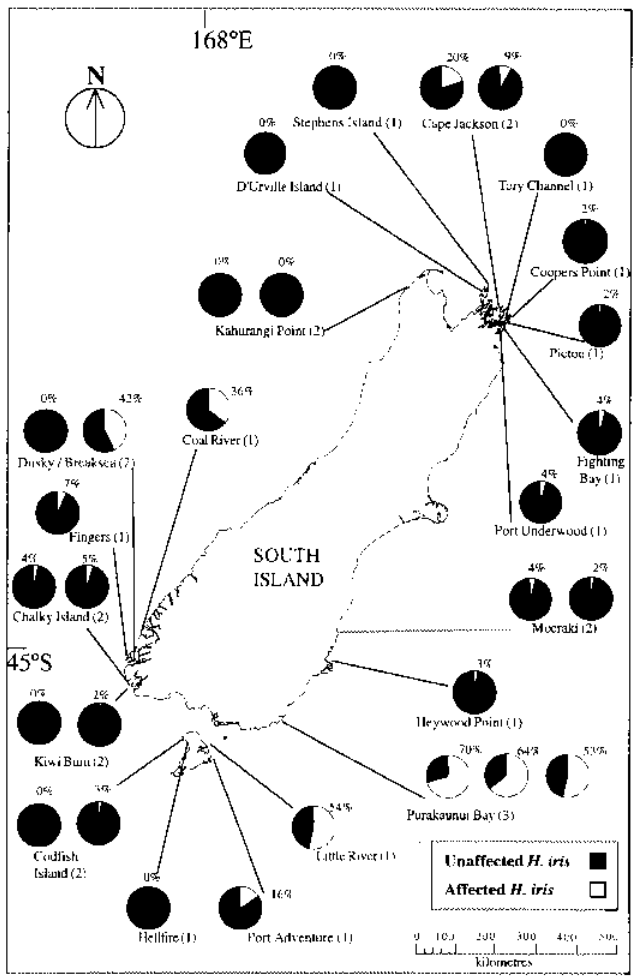

Figure 7. The South Island of New Zealand showing the locations of commercial catches of *H. iris* sampled for the presence of shell lesions. The percentage of affected *H. iris* from each catch is given and the number of catches sampled at each location is indicated in parentheses after the location name.

1955; Fig. 6). Although fungal hyphae were also found in a few shell samples taken from nonlesion-bearing shells (Table 4), they were only found in the surface layers of the shell, which suggests that they may represent an early stage in the development of lesions. Microscopic examination of stained tissue sections from visceral, pedal, mantle, and adductor muscle tissues of several lesion-bearing animals appeared normal (Bevelander 1988) and did not contain fungal hyphae. These lesions were thus confined to the shell of the animal.

Sinclair (1963) described a number of irregularities occurring in shells of *H. iris* from the Wellington district that share some macroscopic characteristics with the lesions we describe. Although the site of some lesions described by Sinclair (1963) is similar to those described here (i.e., posterior to the adductor muscle scar), the tubercules were described as similar in structure to nacreous pearls. No mention was made of a brown-colored, soft, jelly-like substance or of the potential for the adductor muscle to become detached from the shell. Sainsbury (1977) also briefly described blisters in *Haliotis* from Banks Peninsula, which in some cases resulted in the animal losing its shell. These blisters were attributed

TABLE 5.

Location, mean shell length, and number of unaffected and affected *H. iris* sampled, together with estimates of significant differences (bold indicates $p < 0.01$) between the shell length of unaffected and affected animals at each site.

Location	Mean Length (Unaffected)	Mean Length (Affected)	p Value
Cape Jackson (Marlborough)	130.2 (n = 100)	129.4 (n = 18)	0.5396
Cape Jackson (Marlborough)	129.7 (n = 104)	127.5 (n = 57)	**0.0004**
Fighting Bay (Marlborough)	131.1 (n = 95)	130.7 (n = 12)	0.9568
Northeast Stewart Island	152.5 (n = 105)	147.5 (n = 82)	**0.0029**
Port Adventure (Stewart Island)	145.1 (n = 128)	137.9 (n = 40)	**0.0008**
Dusky/Breaksea (Fiordland)	132.4 (n = 91)	132.0 (n = 54)	0.6858
Coal River (Fiordland)	143.8 (n = 101)	138.7 (n = 89)	**0.0004**
Fingers Peninsula (Fiordland)	133.2 (n = 134)	133.1 (n = 17)	0.9251
Purakaunui Bay (South East Otago)	134.9 (n = 70)	134.0 (n = 82)	0.3637
Purakaunui Bay (South East Otago)	133.1 (n = 64)	132.1 (n = 88)	0.2350
Catlins (South East Otago)	134.4 (n = 45)	133.2 (n = 103)	0.1900

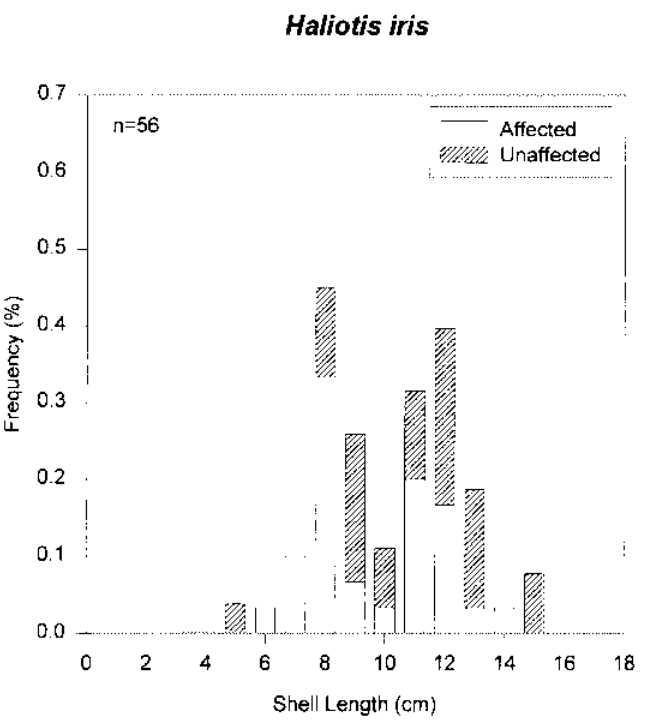

Figure 8. Histogram showing the percent frequency of affected and unaffected *H. iris* in different size classes collected from Little River.

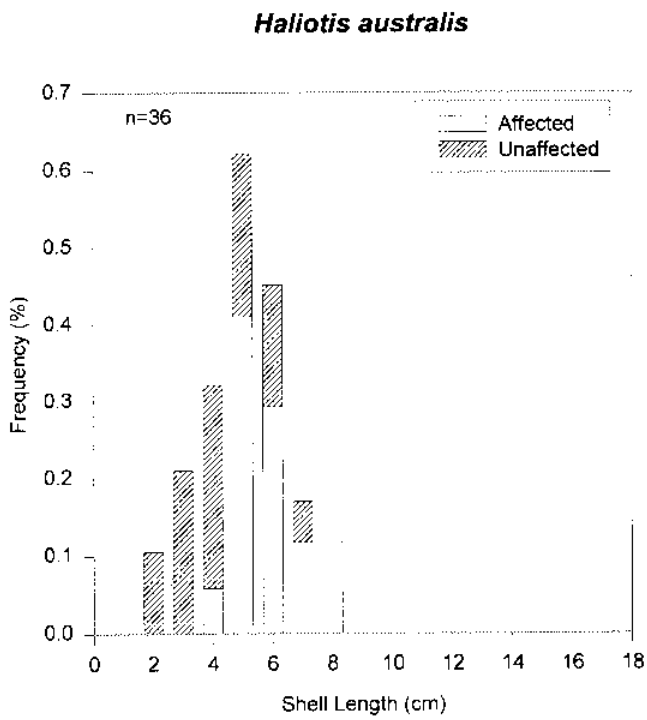

Figure 9. Histogram showing the percent frequency of affected and unaffected *H. australis* in different size classes collected from Little River.

to irritation by boring organisms and/or sand grains lodged in the mantle cavity. Although many of the abalone in our study were infested with *Polydora* sp., no penetration into the nacreous layer was observed. In addition, we did not observe any evidence of sand within the shell cavities of affected or unaffected animals. The lesions we describe appear to be composed of conchiolin and fungal hyphae. In an earlier study, we isolated pure cultures of a fungus from shell lesions of New Zealand abalone (Friedman et al. 1997). On the basis of the limited information provided by Sinclair (1963) and Sainsbury (1977), the lesions we describe differ from those they report.

Records of fungus-like structures invading the calcareous parts of marine animals, particularly molluscs (e.g., Stirrup 1872), date back more than 100 y (Kohlmeyer 1969). Johnson and Anderson (1962) reported hyphae and chlamydospores of a fungus that showed affinities to *Endogone* (Phycomycetes) in a shell of *Anomia*, and Najdenova and Zakhaleva (1992) described fungal shell diseases in oysters (*Ostrea edulis* Linne) and mussels (*Mytilus galloprovincialis* Lamarck) from the Black Sea. Korringa (1951) reported a shell disease that resulted in conchiolin warts on the inner surfaces of the shells of *O. edulis* and that was later attributed to the fungus *Ostracoblabe implexa*.

The possible role of the fungus reported here in the development of shell lesions in New Zealand *Haliotis* awaits further elucidation as does the extent, if any, to which the presence of the fungus is facilitated by the presence of worms and/or other irritants, such as sand grains. Although it is clear that these lesions are restricted to the shell, as was also apparently the case with those described by both Sinclair (1963) and Sainsbury (1977), it is possible that the presence of the lesion and associated fungus may affect these molluscs in ways not yet documented. Friedman et al. (1997) did not observe an association between size of lesion and serum protein levels, sex, or condition index. However, a significant relationship was observed between number of circulating hemocytes and degree of shell mycosis in *H. australis* and was thought to represent a sign of stress or response to the shell disease. Thus, further examination of physiological parameters and reproductive development of abalone with and without shell lesions is warranted.

Some lesion-bearing *H. iris*, collected from the wild and held in captivity, have been reported to have very loosely attached shells (Langdon pers. comm.). The existence of shells in which the adductor muscle attachment site was disrupted by a lesion (Table 3) led us to conclude that, in such extreme cases, the abalone may lose its shell, as has been indicated by Sainsbury (1977). Thus, populations with affected animals may experience elevated mortality relative to those that are free of the shell lesions described in this study. Other shells exhibiting similar lesions showed evidence of having isolated the lesion beneath nacreous blisters (Fig. 3). This suggests that some animals have the potential to deposit shell layers over a lesion, thereby possibly containing it and preventing the shell from being lost.

Lesion-bearing *H. iris* were widespread along the South and Stewart Island coasts, with prevalences in individual populations that ranged from 0 (e.g., Stephens Island) to 70% (e.g., Purakanui Bay) (Fig. 7). Samples from adjacent populations (e.g., Dusky/Breaksea) also showed remarkably different levels of incidence of shell lesions (Fig. 7). The presence of shell lesions in all species examined, from several of the off-shore islands where human habitation is low, suggests that they occur naturally. Furthermore, abalone divers assert that this condition has existed for at least 25 y (Grindley 1997), supporting the contention that lesions are a well-established feature of New Zealand *Haliotis*. Until more is known about how such lesions are caused, it will remain problematic to explain the extent of these variations.

Regardless of the ultimate cause, the available evidence does not support the contention that the three lesion-bearing species of *Haliotis* in New Zealand have different etiological agents. In the two instances where specimens of *H. iris*, *H. australis*, and *H. virginea virginea* from the same site were examined, all three species were found to bear lesions (Table 1). In addition, morphologically indistinguishable fungi were isolated from early shell lesions of both *H. iris* and *H. australis* (Friedman et al. 1997). The existence of shell lesions in *H. iris* and *H. australis* confirms that they can occur in two (*Paua* and *Padollus*) of the three subgenera within *Haliotis* (Lee and Vacquier 1995). Without a more detailed examination of other marine invertebrate species, both in New Zealand and elsewhere, it is premature to comment on the host specificity of the lesions that we describe.

The mean shell length of affected abalone, from all sites combined, was significantly ($p = 0.006$) smaller than that of unaffected animals (Table 5). More detailed analysis (Table 5) suggested that at only 4 of the 11 sites examined were shell lengths of affected and unaffected animals significantly different. Although there are many possible reasons for these differences, it is of note that in all four cases, the shell lengths of affected animals were less than the shell lengths of unaffected animals (Table 5). This suggests that either growth and/or survival may be impaired by this condition, at least at some locations. Although the age of affected animals has not been estimated in this study, the large size range of *H. iris* and *H. australis* exhibiting advanced lesions (Figs. 8 and 9) suggests that lesions may be acquired early in life. This hypothesis is supported by Figure 9, in which the frequency of shell

lesions increased with increasing shell length. Determining the effect of shell lesions on growth, reproduction, and survival of affected individuals relative to those without lesions and clarifying the possible role of epizooties in the development of these lesions will be important areas for future research.

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STUDIES ON SOUTHERN AUSTRALIAN ABALONE (GENUS *HALIOTIS*) XIX: LONG-TERM JUVENILE MORTALITY DYNAMICS

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ABSTRACT The survival of newly settled cohorts from an age of c. 2 months to an age of 2½ to 4 years of the abalone species *Haliotis laevigata* and *Haliotis scalaris* was measured in an under-boulder habitat at West Island, South Australia from 1983 to 1997 by sequential sampling of the habitat to estimate changes in density of cohorts over time. Estimates were compared with those resulting from two Beinssen–Powell-type mark-recapture experiments each conducted over 1 year. Mortality (M) of juveniles in the initial phase (2–8 months) was density dependent, with possible inverse density dependence at higher densities. Instantaneous coefficients of M ranged from 0.05 to 0.4 mth^{-1} during this period. At age 8 months to 3 or 4 years, according to species, M of cohorts was independent of density and ranged from 0.2 to 3.2 yr^{-1}. M was consistently higher for *H. laevigata* than for *H. scalaris*, possibly because of differences in shell sculpture, but M rates between the two species were correlated. During the latter phase, collections of dead shells from the habitat showed that invertebrate predators (mostly crabs) and unknown causes accounted for a mean M rate of 0.12 to 0.13 yr^{-1} on the two abalone species and vertebrate predators (wrasses) the remainder. Hence, M attributed to wrasses was relatively more severe on *H. laevigata* than on *H. scalaris*. The mortality rate of small abalone may be controlled by the density of their major predator, the blue-throated wrasse, *Notolabrus tetricus*.

KEY WORDS: *Haliotis laevigata*, *Haliotis scalaris*, juvenile mortality, density dependence, postsettlement mortality, settlement, recruitment, Beinssen–Powell experiment, dead shell assemblage, crab predation, fish predation

INTRODUCTION

An understanding of recruitment variability and its causes is a central problem in the dynamics of exploited abalone stocks (McShane 1995). There is much uncertainty over the relative importance of three key elements affecting recruitment: the size of the parent stock; oceanographic factors influences on larval dispersal; and postsettlement mortality. This uncertainty has practical import when a population is in decline, and it is unclear whether the decline is attributable to natural recruitment failure (Sainsbury 1982, McShane 1992, 1995) or to recruitment overfishing (Shepherd and Brown 1993, Shepherd and Partington 1995, Shepherd and Baker 1997). Evidence of a strong effect of spawning stock size (Prince et al. 1988, McGarvey et al. 1993, Shepherd and Brown 1993, Shepherd and Partington 1995) and, alternatively, oceanographic factors (Shepherd et al. 1998) illustrate the first two elements, but the role of postsettlement mortality on cryptic juvenile abalone remains almost wholly unknown (Sasaki and Shepherd 1995, Seki and Taniguchi 1996, McShane 1996).

This paper examines the juvenile mortality of *Haliotis laevigata* Donovan and *Haliotis scalaris* Leach in a boulder habitat at West Island, South Australia (35°37′ S, 138°35′ E). The larvae of both species settle on crustose coralline algae in this boulder habitat between about November and May each year (Shepherd and Daume 1996). Some of the major competitors and predators of small abalone in this under-boulder habitat are described by Kangas and Shepherd 1984, Clarkson and Shepherd (1984), Mower and Shepherd (1988) and Shepherd and Gray (1986). Annual recruitment strength from about 2 mm shell length (SL) was measured for both species, and the survival of annual cohorts was estimated from 1983 to 1997 from periodic census data. Survival was independently estimated by using the experimental design of Beinssen and Powell (1979) to measure the movement and mortality of marked abalone at the study site in two experiments. The results of one of the experiments was published by Shepherd and Godoy (1989) and are re-analyzed in this paper together with an analysis of the second experiment. Last, collections of empty (i.e., dead) abalone shells taken from the study site periodically were analyzed, and natural mortality (M) was partitioned between the different classes of agents of mortality.

MATERIALS AND METHODS

The study site on the north shore of West Island is a slope of rounded boulders 30–40-cm diameter, and extends for ~80 m along the shore at a depth of 4–5 m. The field of boulders is ~5 m wide at this depth range and is bounded at each end by granitic blocks and by sand on the seaward side and unsuitable shallower boulder habitat on the shoreward side. *H. laevigata* and *H. scalaris* settle in this habitat (Shepherd and Turner 1985) and remain cryptic under boulders until ~2½ years of age in the case of *H. laevigata* and ~3½ years of age in the case of *H. scalaris*. At these ages, the two species emerge into a more exposed crevice habitat (Shepherd 1990, Shepherd and Cannon 1988).

Surveys

The abalone populations under boulders were censused 4 to 9 times a year by overturning upper boulders and searching their lower sides and then searching the boulders below. Crustose coralline algae were searched with an underwater magnifier (× 3–4 magnification) (Mladenov and Powell 1986). In each census, a 0.25 m^2 quadrat was used to take 25 to 40 samples according to the conditions and diving time available. The quadrat was placed haphazardly on the boulders in a sampling procedure stratified by depth and horizontally to cover the whole of the study site. All small abalone >1 mm SL found within the quadrat area were measured *in situ*, and the boulder was replaced with minimum disturbance to the habitat. Empty shells found within the quadrat were measured, after noting damage to the shell, and removed. In 1983 and 1984, however, numbers of abalone were recorded per boulder per unit time and then converted to density with the relationship: searching time per quadrat = 20.4 minutes (SE = 1.4 min).

Length–frequency data for each species from each census were

decomposed into age classes either by modal analysis (see Shepherd 1988, Shepherd et al. 1988) or, where numbers were not high enough, by knife edge separation based on known growth rates. The numbers in each cohort per 0.25 m^2 were then determined. A plot of the natural logarithm of density of a cohort (weighted for diver efficiency—see below) versus time, fitted by a least-squares linear regression, gives a straight line of slope -M, the instantaneous coefficient of natural mortality (Gulland 1983).

Diver Efficiency

Because a diver's efficiency at finding small abalone changes over the size range examined, it is necessary to make a correction for this change. An experiment was carried out at the study site, in which 130 small abalone, 8 to 50 mm SL were marked with plastic numbered tags (Shepherd and Godoy 1989) and placed in the central columns of a grid 30 m × 4 m described below. The grid was then searched thoroughly by divers on three occasions at 7-day intervals commencing the day after tagging, and the marked individuals found were recorded. It was assumed that, over this short time interval, no marked abalone died or moved out of the grid. The probability (p) of sighting a marked abalone is given by:

$$p = N_m/N_o$$

where N_o is the total number of marked abalone, and N_m is the number seen in an exhaustive search in which every boulder was overturned.

A second assumption that no migration from the study site occurs was shown to be satisfied by Shepherd and Godoy (1989). For this study, the mean birthdates of *H. scalaris* and *H. laevigata* were taken to be 1 January (Shepherd et al. 1985, Shepherd 1988).

Beinssen–Powell (B–P) Experiment

A chain grid measuring 30 m × 4 m divided internally into 1-m columns (Fig. 1) was laid out at the same site as an earlier experiment of similar design (Shepherd and Godoy, 1989). The second experiment was modified to incorporate: (1) a much longer grid because of the known extent of movement of small abalone; and (2) columns in place of squares, because only longitudinal movement was of interest. On four occasions at 2 to 3 month intervals, the six central columns of the grid were searched and small *H. scalaris* 8–50 mm SL were taken to the surface and marked with numbered tags glued to the spire of the shell with superglue. Marked abalone were held overnight in containers on the seabed to detect morbidity, and then replaced in the grid in one of the three categories of replacement column (Fig. 1). On the 29 and 30 March 1989, 1 year after the first marking episode, the grid was searched exhaustively three times for marked individuals, and in addition, two areas each 10 × 4 m beyond each end of the grid were searched for any that may have moved farther.

The survival of marked abalone was calculated with the method of Beinssen and Powell (1979) (and see also Shepherd and Godoy 1989, where more details are given). The movement of abalone recorded within the grid during the study is used to estimate the proportion of marked abalone that do not move beyond the boundaries of the grid. In this experiment, there were 18 possible types of movement, k, of abalone (up to 17 columns east or west) within the grid from the three kinds of release column that did not take an abalone out of the grid. The probability, p, of recapture of a marked abalone in the grid is given by the formula:

$$p = \sum_{k=1}^{18} \frac{C_k N_k}{p_k} \qquad (1)$$

where N_k is the number of movements of type k, p_k is the probability of a movement of type k and

$$C_k = 1/\sum_{j=1}^{3} n_j \cdot A_{j,k} \qquad (2)$$

where n_j is the number of marked abalone released in the jth column, and A_{jk} is the fraction of movements not taking the abalone out of the grid. In this experiment, movements of abalone were all less than 18 m horizontally so $\Sigma\, p_k = 1$; that is, there were no types of movement other than those observed in the grid. The assumption that abalone do not move laterally out of the grid, as in the earlier experiment, held true.

Where there are several releases Beinssen and Powell (1979) showed that:

$$\ln p = \ln \alpha - Xt \qquad (3)$$

where t is the period at liberty. A regression of ln p versus t gives a straight line of slope -X, which is the coefficient of reduction of marked abalone attributable to natural mortality and loss of marks. Ln α is the y-intercept, which is not of interest in this paper.

Estimates of M for *H. scalaris* obtained in the two B–P experiments were compared with those obtained by the change-in-density method after weighting the M rates obtained for the 1+, 2+, and 3+ cohorts with the latter method according to the proportions of each cohort marked in the B–P experiments.

To estimate M for annual periods for combined year-classes of *H. laevigata* and *H. scalaris* in the under-boulder habitat I weighted the M value for each cohort by its initial density for each 6-month period and by addition obtained a mean value of M for all cohorts combined for that time period. The annual value of M was the sum of the two 6-month values.

Agents of Mortality

The predators of small abalone are mainly: wrasses, which eat the soft parts and crush the shell (Shepherd and Turner 1985), crabs, which leave the shell intact except for chipped edges (Mower and Shepherd 1988), and predatory mollusks, which leave the shell intact.

Data on the abundance, size, and type (intact or chipped) of empty shells of both species were collected from the study site during the under-boulder censuses. These data were then used to

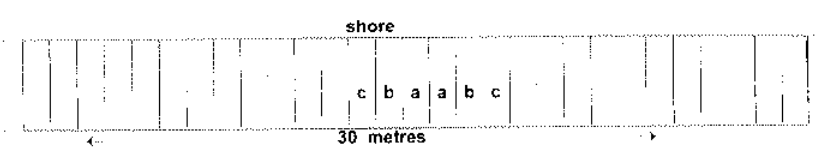

Figure 1. Grid design of Beinssen–Powell experiment. Columns are 1-m wide. The three types of replacement column are numbered a to c where abalone were placed after tagging.

partition M into two components, M_w, the natural mortality attributable to wrasse predation, and M_c, the natural mortality attributable to predation by crabs, mollusks and unknown causes. Hence

$$M = M_w + M_c \quad (4)$$

M_c was calculated for the years 1984 to 1996 as follows. Consider a cohort of an abalone species in the under-boulder habitat. If wrasse predators leave no trace of the shells of abalone eaten by them, then the annual production, P_s, of empty shells resulting from M_c of all cohorts of a species in the habitat is:

$$P_s = \sum_{i=1}^{n} N_i(1 - e^{-Mc}) \quad (5)$$

where N_i is the density of the ith cohort at the beginning of the year, and n is the number of cohorts, two in the case of *H. laevigata* and three in the case of *H. scalaris*. However, empty shells comprising P_s gradually disintegrate or disappear by dissolution, comminution, and bioturbation. If X_s is the rate of disappearance of empty shells, then it can be shown, following Ricker (1975), that the mean density of empty shells, D, is:

$$D = P_s (1 - e^{-Xs})/X_s \quad (6)$$

A mean annual value of ΣN_i was derived by summing the estimated densities of the 1+ and 2+ cohorts of *H. laevigata* and the 1+, 2+, and 3+ cohorts of *H. scalaris* at the beginning of each year, obtained from the regression analyses in Figures 2 and 3. A mean annual value of D for both species was derived from the census data on abundance of empty shells 10–50 mm SL.

The rate of disappearance of empty shells was obtained from three replicate experiments; in each experiment 20 to 25 shells, evenly distributed over the size range 15–45 mm SL, were marked with numbered tags glued to the *inside* of the shell and then placed among and under boulders in a marked out area of 1 m^2. Careful searches for the shells were made at intervals of 1 to 3 months for a year, and the number still present was recorded. A regression of the natural logarithm of the number of surviving shells over time gives a straight line whose slope is $-X_s$. By substituting the values of D and X_s in Equation (6), annual estimates of P_s are derived, and then by substituting these in Equation (5) annual estimates of M_c are obtained. Finally, M_w is obtained from Equation (4).

RESULTS

Diver Efficiency

The sighting probabilities of four size classes of small *H. scalaris* in three censuses, obtained in conditions that were subjectively assessed as ranging from poor to good according to swell height and underwater visibility, are given in Table 1. The data were analyzed by Friedman's two-way analysis of variance (ANOVA) which showed a significant effect of conditions (χ^2 = 42.5; $p < .001$). Sighting probabilities increase with increasing size class and from poor conditions to good. Because it is difficult to correct census data objectively for different diving conditions, the mean sighting probabilities for each size class were used to weight cohort densities (× 1/p) according to their size range. For the size range 1–8 mm SL, the same weighting (× 1/0.36) was used as for the 8–19 mm SL size class for the purpose of comparing survival in the first 6 months and subsequently. This was because Shepherd et al. (1992) found no difference in detectability of small abalone between 1 and 15 mm SL by divers (see Discussion section).

Natural Mortality

Plots of log-transformed density of annual cohorts versus time are shown in Figure 2 for the two species from 1983 to 1995 for *H. laevigata* and to 1996 for *H. scalaris*. For many of the years, there was a distinct change in slope of the regression of ln density versus time when the cohort was about 6–8 months old. So M was examined separately for the two successive periods. The first (called the initial phase) commenced from about 2 months after settlement and continued for up to about 6 months; this coincides with the period during which juveniles live on crustose coralline algae. The second period was from about 6–8 months of age to about 2½ to 3 years of age (for *H. laevigata*) and about 3 to 4 years of age (for *H. scalaris*).

Initial Phase

Settlement of *H. laevigata* was high in 1983, but then declined, and, except for a slight recovery in 1988 and 1989, remained very low after the population collapse (Shepherd and Brown 1993). In every year, there was one episode of settlement a year, except in 1983, when there were two (Shepherd and Daume 1996). Settlement of *H. scalaris* occurred in 1 to 3 episodes, which sometimes made estimation of M difficult in the first 6 months (here called the initial phase). When settlement was low, or there were multiple episodes of settlement, mortality rates could not be estimated unless one episode was strong and the others were well separated in time, which allowed them to be distinguished by size. Mortality rates with standard errors for the initial phase are given by Shepherd and Daume and here only the mean monthly M rates for annual settlements from 1983 to 1997 versus initial density are plotted in Figure 3. M rates vary from about 0.05 to 0.4 mth^{-1} but do not differ significantly between species (see Table 7).

Second Phase

The data for this phase did not warrant fitting more than one linear regression per cohort (except for 1988 for *H. laevigata*, when a broken stick model was clearly better). After weighting for changes in diver efficiency according to Table 1, the calculated rates of M for individual cohorts are given in Table 2 and plotted as log-transformed cohort densities versus time (Fig. 2). Mean cohort M for *H. laevigata* was 0.90 (SE 0.15) and for *H. scalaris* was 0.51 (SE 0.09). The difference was significant ($t = 2.13$; $p < .05$).

Changes in density and annual rates of M of combined cohorts within each species from 1984 to 1996 are shown in Figure 4. Annual M rates are given in Tables 5 and 6. Mean annual M was 0.98 (SE 0.15) for *H. laevigata* and 0.43 (SE 0.06) for *H. scalaris*. The difference was significant ($t = 2.78$; $p < .02$).

Next I examined the data for evidence of density dependence of M by testing for correlations, first between the M rates of cohorts and their respective initial densities, and then between the annual M rates of the combined cohorts for each species and their respective densities at the beginning of the year, for annual periods in the second phase. Neither the M rates of cohorts of each species nor the annual M rates for combined cohorts of each species (Table 2) were correlated with initial density (for *H. laevigata*, $r = 0.33$ and 0.04, respectively; NS; for *H. scalaris* $r = 0.24$ and 0.07, respectively; NS). I conclude that mortality was independent of density in the second phase.

Except for the 1988 to 1990 spike, the density of *H. laevigata* has declined to low levels since the 1984 collapse of the population

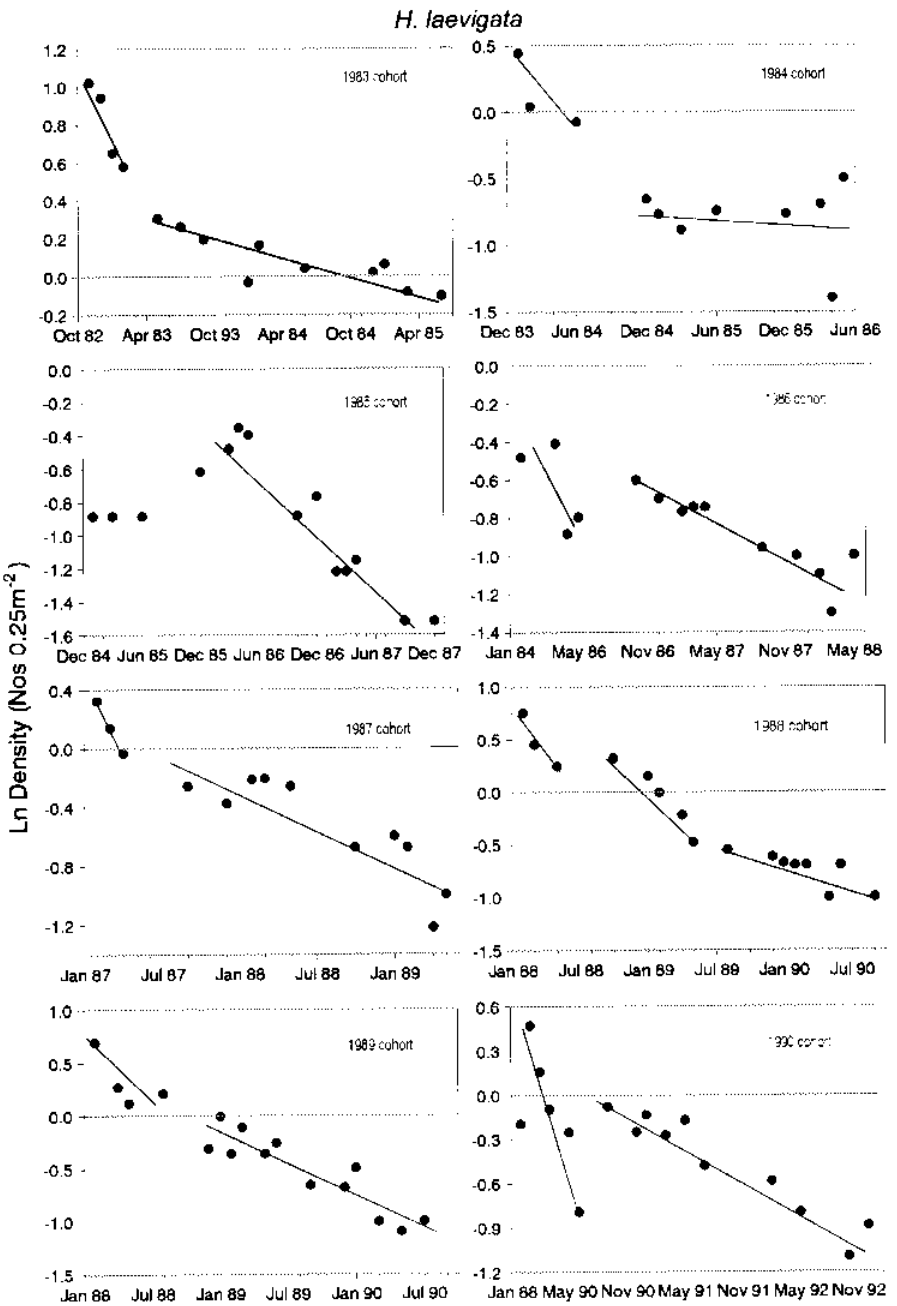

Figure 2. Plots of the logarithm of density of cohorts of *H. laevigata* and *H. scalaris* for years 1983 to 1997 for the period from about 2 months of age to 2½–4 years of age according to species. Fitted regression lines are shown. Regressions were not fitted to datapoints in the initial phase in those years where several episodes of settlement or very low settlement occurred; i.e., in 1985, 1991, and 1992 for *H. laevigata,* and in 1984 to 1987, 1990 and 1992 to 1996 for *H. scalaris*.

(Shepherd and Brown 1993). In contrast, the density of *H. scalaris* has fluctuated between 7 and 14 m^{-2} until 1991 and then increased to almost 20 m^{-2} by 1996. Natural mortality of both abalone species was highly correlated ($r = 0.78$; $p < .01$) and pursued the same cycle upward from 1984 to a peak in 1989 declining to a low in 1992 to 1993, and then slowly increasing again until 1995 (Fig. 4).

Beinssen–Powell (B–P) Experiments

The second experiment, conducted on a grid more than three times the size of that of the first experiment, showed greater movement of abalone than previously thought. The movement data, to be presented elsewhere, enabled a recalculation of the values for Σp_k (the probability that an abalone stays within the grid) used in the first experiment and so permitted a recalculation of X (see Shepherd and Godoy 1989). In the second experiment 223 *H. scalaris* were released, and 82 were recovered after periods at liberty from 57 to 358 days. Because no individuals in this experiment moved >17 m outside the grid, $\Sigma p_k = 1$. Release–recapture data for the two experiments are given in Table 3; X values calculated from Equation (3) represent the natural mortality rate plus the rate of disappearance of tags. Shepherd and Godoy found no

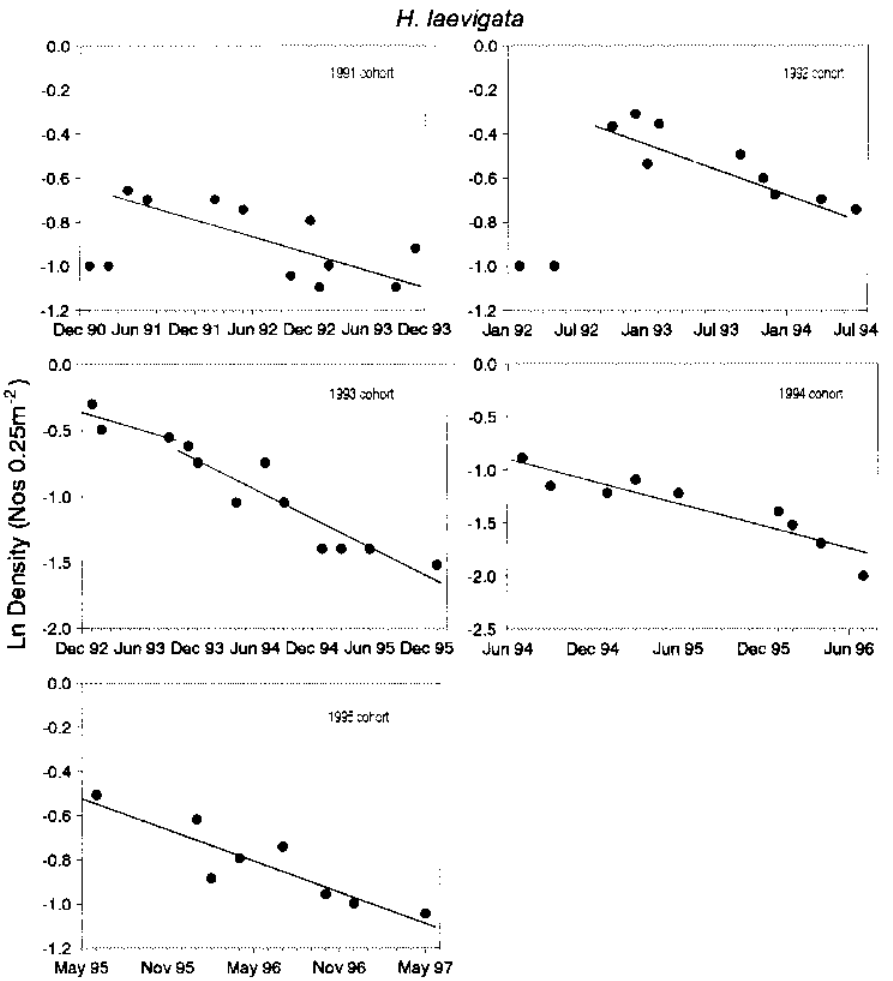

Figure 2. Continued.

tag loss but suggested that tags could become overgrown with bryozoans. In the second experiment, the dorsal surface of all abalone shells found was examined for overgrown tags. The rate of disappearance of tags from overgrowth was estimated from 74 marked abalone at liberty for 1 year and then recovered, and was found to be 0.027. This value was used to adjust the M rates in the first experiment, but not in the second experiment, where care was taken to search for overgrowth.

Annual M values calculated from the two B–P experiments are compared with the equivalent values of M derived from the change-in-density method for the same periods (Table 4). Mortality estimates are higher in the B–P experiments, but the standard errors are large in the first experiment and overlap in the second, suggesting that the differences are not significant.

Agents of Mortality

Size frequency distributions of damaged (mostly with chipped edges) and undamaged shells of the two abalone species, representing accumulated data for 13 years (Fig. 5) did not differ significantly between species ($\chi^2 = 4.9$; NS). This suggests that the causes of mortality that leave intact or damaged shells (mainly crabs—see Discussion section) act similarly on each species.

The mean proportion of damaged shells was 51% for *H. scalaris* and 55% for *H. laevigata*. The proportion of damaged shells in the samples increased significantly with size for *H. laevigata* ($\chi^2 = 25.0$; $p < .001$) but not for *H. scalaris* ($\chi^2 = 0.6$; NS).

The three experiments to measure the disappearance rate of empty shells showed that breakdown was rapid, with a mean half life of 48 days, although one was seen 916 days after marking. The disappearance rates, X, in the three experiments were 5.85 y^{-1} (SE 0.37), 5.17 (SE 0.26) and 4.85 y^{-1} (SE 0.94). So a mean value of X = 5.29 y^{-1} was used in Equation (6) to calculate p, and then from Equation (5) M_c was calculated for each year of the study. The mean annual density of empty shells and calculated annual values of p, M, M_0 and |from Equation (1)| M_w are given in Tables 5 and 6.

Mean M attributable to crabs was 0.12 (SE 0.02) for *H. laevigata* (after excluding zero values for 1992 to 1996, when densities were too low to give detectable values) and 0.13 (SE 0.01) for *H. scalaris;* the difference was not significant (t = 0.8; NS). In contrast, mean M attributable to wrasses was 0.92 (SE 0.15) for *H. laevigata* and 0.30 (SE 0.05) for *H. scalaris:* this difference was significant. Thus, wrasse predation was relatively greater and crab predation relatively lower on *H. laevigata* than on *H. scalaris*.

DISCUSSION

Postsettlement and juvenile mortality of abalone, as for marine invertebrates generally (reviewed by Hunt and Scheibling 1997), is

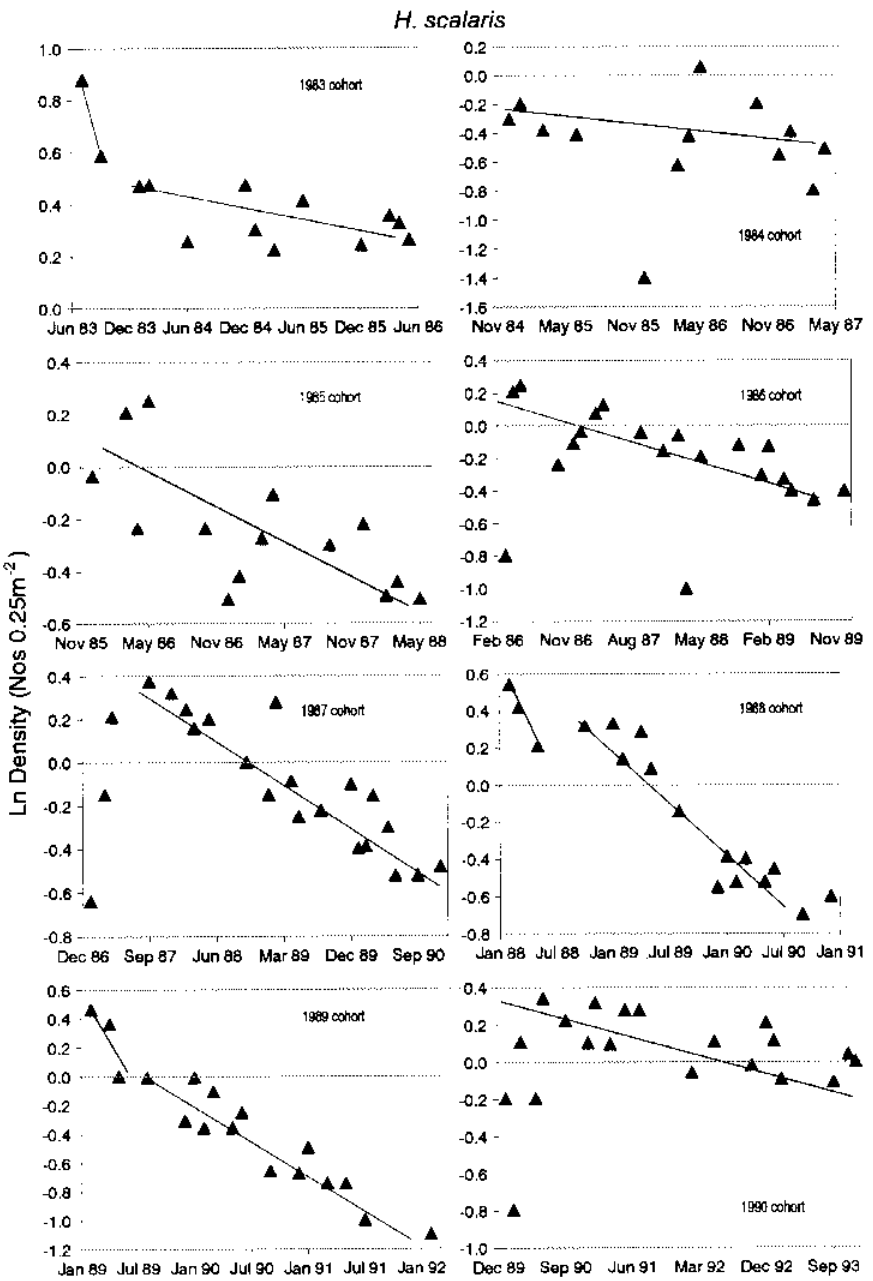

Figure 2. Continued.

poorly known. Most data for the initial phase come from studies of larval seeding and for the second phase, from transplants of hatchery-reared juveniles (reviewed by Tegner and Butler 1989, Shepherd and Breen 1992, McCormick et al. 1994). This study has shown that M is much higher in the initial phase than in the second phase and that there are large interannual differences in M in both phases.

Initial Phase

Our estimates of M (Fig. 2) are almost certainly low for two reasons. First, diver efficiency at detecting small abalone over the size range 2–8 mm SL must increase, despite the inability of Shepherd et al. (1992) to detect any change in efficiency (see Castell et al. 1996). Second, it is possible that a trickle of settlers in the few weeks following a major settlement could occur. These would have be too small to be registered in the initial census but would be included in later censuses and so tend to bias M downward. Settlement episodes separated by a month or more could, if strong enough, be recognized by their size difference.

The rates of M given here (Fig. 2) are lower than most previously reported values (see Table 7) but are strictly comparable only with the study of Preece et al. (1997) because only that study covers the same period from settlement. The value given by Preece et al. (1997) from 7 wk to 5 mo (0.7 mo^{-1}) and from 7 weeks to 11 months (0.24 mo^{-1}) are not much more than the 14-year mean M (0.22 mo^{-1}) for *H. laevigata* and *H. scalaris*. However, initial densities in the study of Preece et al. were over three times the average initial densities in this study (see Table 7), so, although M

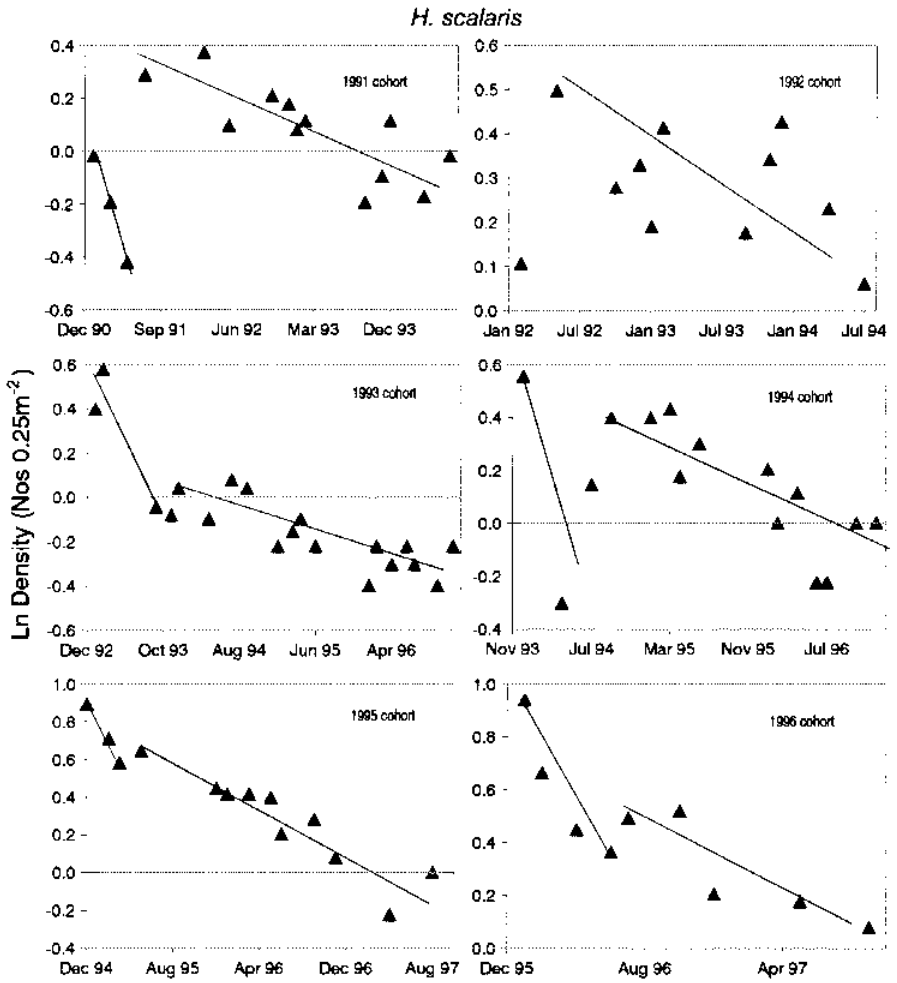

Figure 2. Continued.

values in this study are undoubtedly low; those of Preece et al. may be exaggerated by density-dependent effects.

Over the range of densities recorded in the 14-year period, M seems to be density dependent at low to moderate densities and inversely so at higher densities (Fig. 3). Density dependence in the initial phase has also been found by McShane (1991) and Preece et al. (1997). In the absence of any strong evidence of density dependence in other stages of the life history, this phase may be the only period in which density-dependent M operates or is important (see McGarvey 1996). The possible causes of density-dependent M in this study are discussed by Shepherd and Daume (1996).

Another noteworthy feature is that the timing of settlement of *H. scalaris* has apparently advanced from February to May in the

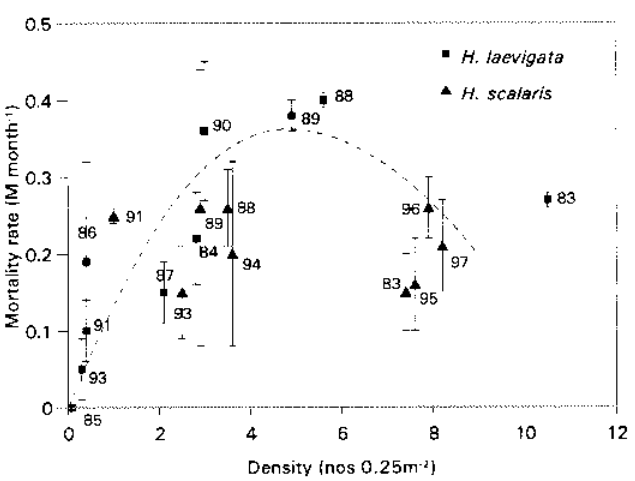

Figure 3. Plots of monthly mortality rates of *H. laevigata* and *H. scalaris* vs. initial density for cohorts of *H. laevigata* for the years 1983 to 1996 for the period from about 2 months of age to 6 to 8 months of age. Vertical bars are standard errors. The dashed line was fitted by eye.

TABLE 1.

Sighting probabilities, p, for four size classes of *H. scalaris* in three censuses taken in different conditions of underwater visibility and swell; N_o is the number of individuals marked in each size class, and the number in parentheses is the number of dead marked shells found during the searches.

Size Class, mm	N_o	Conditions			$\bar{p}$
		Poor	Average	Good	
8–19	33 (2)	0.27	0.23	0.58	0.36
20–29	30 (1)	0.37	0.48	0.50	0.45
30–39	41	0.44	0.41	0.49	0.45
40–50	29 (1)	0.48	0.45	0.55	0.49

$\bar{p}$ = mean.

TABLE 2.

Natural mortality rates (M) for cohorts of *H. laevigata* and *H. scalaris* in the second phase estimated from changes in density over time from the data shown in Figure 2. Estimates are mean values for individual cohorts (identified by year of birth) from an age of 6 (–11) months to 2.5–3 years; n is the number of censuses, and the period given is the time span to which the mortality estimate relates; a broken stick regression model was fitted to the 1988 cohort of *H. laevigata;* data for 1995 and 1996 are provisional.

	H. laevigata			*H. scalaris*		
Cohort	n	Period	M y^{-1} (SE)	n	Period	M y^{-1} (SE)
1983	9	July 83–June 85	0.35 (0.09)	11	Jan. 84–June 86	0.13 (0.08)
1984	8	Dec. 84–June 86	0.17 (0.46)	13	Dec. 84–Apr. 87	0.22 (0.17)
1985	11	Dec. 85–Dec. 87	1.23 (0.29)	14	Dec. 85–May 88	0.50 (0.15)
1986	10	Oct. 86–May 88	0.89 (0.16)	16	Oct. 86–Aug. 89	0.28 (0.08)
1987	10	Sept. 87–May 89	1.21 (0.22)	19	Sept. 87–Dec. 90	0.62 (0.06)
1988	5	Oct. 88–May 89	3.16 (0.38)	14	Oct. 88–Dec. 90	1.24 (0.12)
1988	9	May 89–Sept. 90	0.85 (0.20)			
1989	13	Aug. 89–July 91	1.49 (0.17)	14	Aug. 89–Feb. 92	0.85 (0.14)
1990	10	Sept. 90–Dec. 92	0.96 (0.10)	16	June 90–Dec. 93	0.21 (0.05)
1991	8	July 91–Nov. 93	0.38 (0.12)	12	July 91–Jan. 95	0.33 (0.07)
1992	10	Oct. 92–Oct. 94	0.54 (0.12)	16	June 92–Jan. 96	0.31 (0.05)
1993	10	Sept. 93–Jan. 96	1.06 (0.14)	17	Sept. 93–Dec. 96	0.28 (0.04)
1994	9	July 94–July 96	1.11 (0.12)	14	Sept. 94–Sept. 97	0.46 (0.07)
1995	8	June 95–Sept. 97	0.28 (0.14)	10	June 95–Sept. 97	0.76 (0.13)
1996		Not detected		5	July 96–Sept. 97	0.89 (0.28)

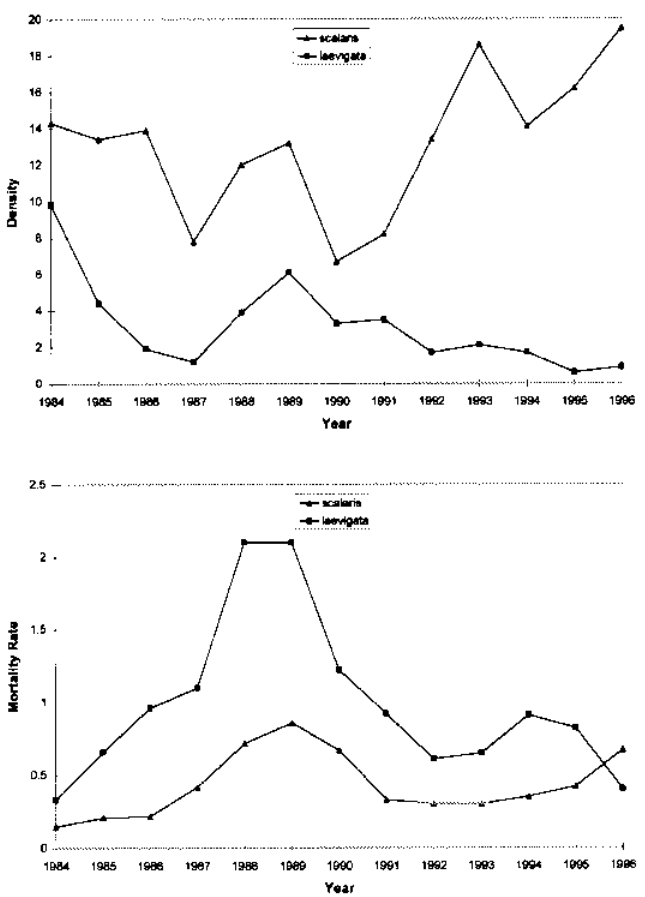

Figure 4. (upper) Plot of density (nos m^{-2}) of combined cohorts of 1 to 3-year-old *H. laevigata* and *H. scalaris* vs. time from 1984 to 1996; (lower) plot of M y^{-1} of combined cohorts of 1 to 3 year olds of *H. laevigata* and *H. scalaris* vs. time for 1984 to 1996.

1980s to October to December in the 1990s (see dates of settlement given in Shepherd and Daume 1996) as *H. laevigata* has declined. There is the intriguing possibility that resource competition between postlarvae of the two species in the crustose coralline habitat kept timing of settlement apart when both species were abundant, but the decline of one has permitted the other to expand or advance its period of settlement. A possible mechanism for this would be the enhanced survival of early settlers that mature into early spawners.

Second Phase

Estimation of M by diver census seems more robust during the second phase than during the initial one, because the changing efficiency of the diver is more easily measurable, and M can be validated by independent experiments. The two methods used gave

TABLE 3.

Release–recapture data for two Beinssen–Powell experiments at the study site, (a) gives the data for the first experiment published by Shepherd and Godoy (1989) with recalculated p_k and p values; (b) gives data for the second experiment in the larger grid; n is the number of *H. scalaris* marked and released; R is the number recaptured; "Days" is the number of days at liberty; Σp_k is the probability that a marked abalone will not move out of the grid; and p is the probability of recapture.

	Release	Date	n	R	Days	Σp_k	p (SE)
(a)	1	5.xi.86	15	2	375	0.61	0.29 (0.11)
	2	5.ii.87	28	7	283	0.79	0.39 (0.04)
	3	2.vi.87	25	8	166	0.81	0.43 (0.03)
	4	4.viii.87	42	14	103	0.85	0.60 (0.03)
(b)	1	6.iv.88	53	16	358	1	0.31 (0.04)
	2	30.vi.88	59	20	274	1	0.34 (0.03)
	3	21.ix.88	54	19	190	1	0.35 (0.03)
	4	15.1.89	57	27	74	1	0.49 (0.02)

TABLE 4.

A comparison of annual mortality rates estimated from the two Beinssen–Powell (B–P) experiments and the change-in-density data.

Period	B–P Experiment (SE)	Density Data (SE)
5.xi.86–15.xi.87	0.86 (0.18)	0.44 (0.11)
6.iv.88–30.iii.89	0.56 (0.14)	0.43 (0.06)

reasonably consistent results and increased confidence in their general accuracy. It is possible that the B–P method tends to overestimate M, because it is sensitive to differences in the probability of movement outside the grid. Shepherd and Godoy (1989) overestimated M, because they underestimated Σp_k. Because a much larger grid was used in the second B–P experiment, and extensive searching was done outside it, serious overestimation of M seems unlikely in that experiment. The change-in-density method of estimating M is less demanding in terms of diving time, but the variability of diver efficiency according to underwater conditions introduces another source of variation. However, a regression analysis of census data collected over 2 to 3 years during the whole under-boulder life of a cohort should smooth this variability and give an unbiased estimate of M for each cohort.

The method is also subject to other assumptions (e.g., the random movement of abalone in the habitat and absence of migration) that must be validated experimentally. The B–P method does this. Subject to these assumptions, the change-in-density method is an efficient way to estimate M. At this study site, the method may tend to underestimate M for *H. scalaris* because of the possible settlement of larvae of this species at shallower depths (around 3 m) and their subsequent migration into the study site at about 3.5–5 m depth. Shallower depths are occupied by *H. rubra*, and *H. scalaris* is rare here (Shepherd 1973, Shepherd and Cannon 1988), so this bias is likely to be small.

TABLE 5.

***H. laevigata*; mean annual density of empty shells (D), annual empty shell production (p_s), summed cohort densities (ΣN_i for $i = 1,2$), and the mean annual mortality rate of combined cohorts (M), partitioned between mortality attributable to crabs (M_c) and mortality attributable to wrasses (M_w); values of D, P_s, and ΣN_i are in numbers m^{-2}.**

Year	D (SE)	P_s	ΣN_i	M (SE)	M_c	M_w
1984	0.08 (0.02)	0.43	9.8	0.33 (0.11)	0.04	0.28
1985	0.13 (0.03)	0.69	4.3	0.66 (0.18)	0.17	0.49
1986	0.06 (0.02)	0.32	1.9	0.96 (0.25)	0.18	0.78
1987	0.03 (0.02)	0.16	1.2	1.10 (0.14)	0.14	0.96
1988	0.07 (0.03)	0.37	3.8	2.10 (0.21)	0.10	2.00
1989	0.11 (0.04)	0.57	6.1	2.12 (0.20)	0.10	2.02
1990	0.07 (0.02)	0.38	3.3	1.22 (0.11)	0.12	1.10
1991	0.05 (0.02)	0.27	3.5	0.92 (0.08)	0.08	0.84
1992	0	0	1.7	0.61 (0.08)	0	0.61
1993	0	0	2.1	0.65 (0.10)	0	0.65
1994	0	0	1.7	0.91 (0.10)	0	0.91
1995	0	0	0.6	0.82 (0.09)	0	0.82
1996	0	0	0.2	0.40 (0.06)	0	0.40

Studies of the mortality of juvenile abalone during this phase in the wild are rare (reviewed by Shepherd and Breen 1992). Published annual values of M are: 0.7–0.9 for *H. rubra* (Prince et al. 1988, Day and Leorke 1986), and 1.7 for *H. tuberculata* (Clavier and Richard 1985), both in the same range as those in Tables 5 and 6. There are many studies of the mortality of hatchery seed transplants in the wild (see Table 8 and see also Tegner and Butler 1989, McCormick et al. 1994). However, these studies must be interpreted cautiously, because cultured seed and wild abalone behave differently in the presence of predators, with initial mortality rates about 25% higher in seed abalone (Schiel and Weldon 1987). Table 8 lists only those studies that extended for over 1 year

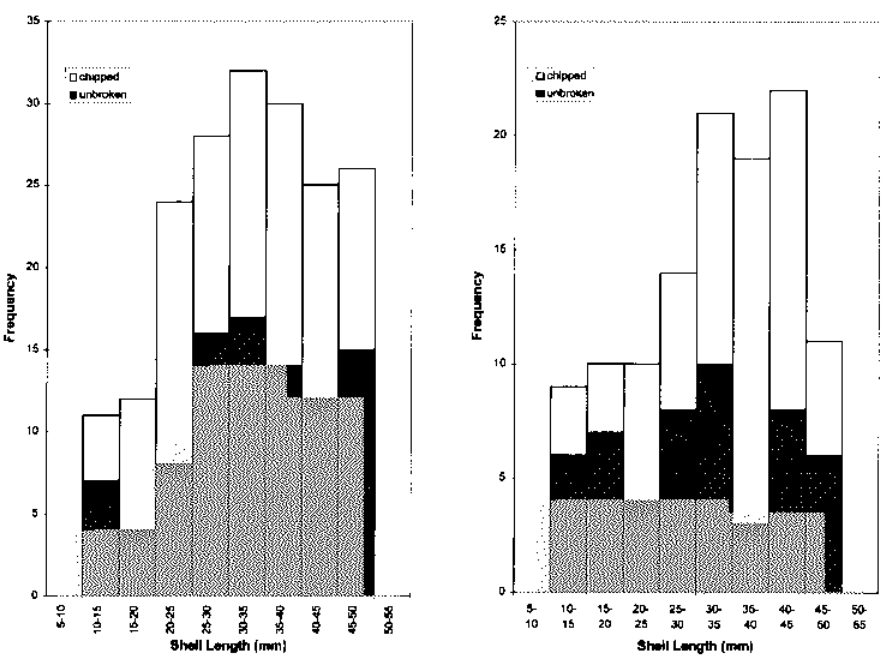

Figure 5. Length frequency distributions of *H. laevigata* (left) and *H. scalaris* (right) showing proportions of damaged and undamaged shells taken from under boulders at West Island.

TABLE 6.

***H. scalaris*. Mean annual density of empty shells (D), annual shell production (P_s), summed cohort densities (ΣN_i for i = 1, 2, 3), and mean annual natural mortality rates (M), partitioned between mortality attributable to crabs (M_c) and mortality attributable to wrasses (M_w); values of D, P_s, and ΣN_i are in numbers m^{-2}.**

Year	D (SE)	P_s	ΣN_i	M (SE)	M_c	M_w
1984	0.18 (0.08)	0.96	14.3	0.15 (0.07)	0.07	0.08
1985	0.24 (0.05)	1.28	13.5	0.21 (0.07)	0.10	0.11
1986	0.22 (0.06)	1.16	13.9	0.22 (0.07)	0.09	0.13
1987	0.30 (0.03)	1.60	7.9	0.42 (0.06)	0.23	0.19
1988	0.45 (0.09)	2.39	12.0	0.73 (0.07)	0.22	0.51
1989	0.34 (0.19)	1.81	13.2	0.86 (0.08)	0.15	0.71
1990	0.21 (0.07)	1.12	6.7	0.67 (0.06)	0.18	0.49
1991	0.17 (0.08)	0.90	8.2	0.33 (0.06)	0.12	0.21
1992	0.26 (0.10)	1.38	13.4	0.31 (0.04)	0.11	0.20
1993	0.30 (0.07)	1.60	18.6	0.30 (0.05)	0.09	0.21
1994	0.33 (0.03)	1.76	14.1	0.35 (0.04)	0.13	0.22
1995	0.26 (0.06)	1.38	16.3	0.42 (0.06)	0.12	0.30
1996	0.44 (0.15)	2.34	19.2	0.66 (0.08)	0.13	0.53

and notes those in which the seed received protection from predators (i.e., in cribs or with some kind of netting) to show the effect of reduced predation. McCormick et al. showed that protecting seed against predators, if only for the first few days, substantially reduced M. These studies (Table 8) give a wide range of values as expected in different latitudes, habitats, and conditions, but two generalizations can be made from them (see McCormick et al.). Smaller abalone (1< y old) suffer higher M than larger seed (>1 y old), and seed protected from predators have lower M rates than unprotected ones. Thus, critical determinants of M seem to be predator abundance and abalone size. Of these, the former may be the more important, because the only study that compared mortality over a range of sizes at replicate sites found no significant difference in M values between size classes except for the smallest one (Zhao et al. 1991). This is consistent with the results of this paper, which found no evidence of a change in M during the second phase.

Predator Abundance

Predation by crabs on abalone at West Island leaves empty shells sometimes chipped at the edges (Mower and Shepherd 1988). Predation by the whelks *Pleuroploca australasia* and *Cominella lineolata* and the seastar *Coscinasterias muricata* leaves undamaged shells (unpublished data). However, the densities of these whelks and this seastar are low (<0.1 m^{-2} for *P. australasia* and *C. lineolata* combined, and <0.001 m^{-2} for *C. muricata*) at the study site. Hence, undamaged empty shells are most likely to result from predation by omnivorous crabs with small chelipeds, such as *Paguristes sulcatus, P. brevirostrus,* and *Lomis hirta* (Mower and Shepherd) and rarely by whelks or seastars, from which abalone have an escape response anyway (Day et al. 1995). Damaged shells, on the other hand, are most likely the remains of predation by crabs with larger chelipeds (*Megametope carinatus, Nectocarcinus tuberculosus,* and *Paguristes frontalis*). The density of crabs with small chelipeds is ~10 m^{-2}, and of the latter group ~2–3 m^{-2}, so the proportions of damaged and undamaged shells in the collections may rather reflect the relative intensity of predation by the two groups of crabs. The under-representation of the 1+ age class in the empty shell samples (Fig. 5) implies some size-selective predation by crabs, or more likely, that very small shells are less easily visible to divers. The greater proportion of damaged shells with size of *H. laevigata* as compared with *H. scalaris* may be caused by: (1) the different sizes at which the two species emerge from the under-boulder habitat into more open crevices; and (2) size-selective predation by the large crab *N. tuberculosus* and the large hermit crab *P. frontalis* on emergent individuals. The size of emergence is 40–45 mm SL in the case of *H. laevigata* and >50 mm SL in the case of *H. scalaris*.

The dominant predators of small abalone >5–10 mm SL (i.e., >~6 months old) are the wrasses *Notolabrus tetricus* and *Pictilabrus laticlavius* (Shepherd and Turner 1985). Both species are voracious predators than forage under boulders within home

TABLE 7.

Monthly natural mortality rates during the initial phase for various species of abalone; densities given are initial densities of postlarvae after settlement.

Species and Reference	Period	M mo^{-1}	Comments
H. rubra McShane (1991)	Settlement—5 mo	0.6–0.8	Exposed vertical surfaces. Density: 10–1000 m^{-2}
H. discus hannai Sasaki and Shepherd (1995)	Settlement—2(–3) mo	1.4–2.7 mean 2.1	Boulder habitat. Initial density: 200 m^{-2}
H. iris Schiel (1993)	Settlement—5 mo	1.0	Larval seeding in boulder habitat. Density: 200 m^{-2}
McShane and Naylor (1995)	Settlement—4 mo	0.6	Larval seeding in boulder habitat. Density at 2 days: 120 m^{-2}
H. laevigata Preece et al. (1997)	6 day–7 wk	2.6	Larval seeding in boulders. Density at 6 days: 50 m^{-2}
	7 wk–5 mo	0.7	
	7 wk–11 mo	0.24	
H. laevigata	2–6 mo	0.24	Diver census with underwater magnifier. Mean values over 15 yr. Average initial densities 13–18 m^{-2}
H. scalaris Shepherd and Daume (1996)	2–6 mo	0.21	

TABLE 8.

A summary of studies of the mortality of seed transplants of juvenile abalone (6 mo old–2 or 3 y); length is mean shell length at time of seeding; mean age is estimated where not given in the paper.

Species and Reference	Length (mm) (age, y)	M y^{-1}	Comments
H. gigantea	10 mm (6 mo)	2.3	On artificial reefs. M was lower in concrete cribs and higher on natural reefs
Inoue (1976)	30 mm (1 y)	0.36	
Haliotis discus hannai	10–20 mm	0.6–0.7	On natural and artificial reefs.
Saito (1979)	(1 y)		
Momma et al. (1980)	15 mm (1 y)	1.8	In concrete cribs filled with boulders
	21 mm (1.5 y)	1.1	and protected from fish predators
Miyamoto et al. (1982)	15–22 (1 y)	1.7	In natural habitat, for 1.3 y
	>22 (1 + y)	0.9–1.1	
Saito (1984)	20 mm (1 + y)	0.8–1.0	On natural reefs, averaged over 3 y
	31 mm (2 + y)	0.8–0.9	
Asano et al. (1989)	<30 mm (2 y)	0.22	Released into artificial reefs in *Eisenia bicyclis* forest
Zhao et al. (1991)	8 mm (6 mo)	2.14	Each value is the mean for four sites.
	13 mm (9 mo)	1.22	Released on natural reefs, for
	18 mm (1 y)	1.24	12–18 mo
	23 mm (1+ y)	1.10	
	29 mm (2 y)	1.25	
Haliotis d. discus	45–71 mm	1.0	On concrete artificial reefs,
Sakamoto et al. (1982)	(2–3 y)		averaged over 2 y
Kojima (1995)	20–35 mm	0.25	Data from 1980 to 1985 on natural reefs.
	(1 y)		Based on recoveries over 4 y
Tanaka et al. (1991)	10–24 mm (1 y)	11.9	Experiment on natural reef to
	22–30 mm (2 y)	2.3	measure sighting rates, etc.
H. rufescens	45–71 mm	0.6–4.6	
Tegner and Butler (1985)	(2–3 y)		In natural habitat (some predators removed). Lower estimate from dead shell collections; upper estimate from live recoveries
H. iris	5–20 mm	1.2	In natural boulder habitat. Mean for six
Schiel (1993)	(0.5 y)		sites (omitting two sandy sites).

ranges. Abalone are a preferred prey of *N. tetricus* whose food, feeding preferences, and behavior are described by Shepherd and Turner and Shepherd and Clarkson (in prep.). These fishes pick small abalone off the rock, or, in the case of larger ones, ram the shell and in the same action pull them off the rock. In either case, the fish ingests the shell or breaks it into small pieces. Densities of *N. tetricus* are 0.05–0.2 m^{-2} at the study site, and those of *P. laticlavius* are ~0.005 m^{-2} so the latter is of little significance as compared with *N. tetricus*, which may be a key predator in this habitat.

What was the cause of the interannual variability of M at West Island? Abalone density that might affect predator behavior (see Shepherd and Daume 1996) was not a factor, because M was independent of density. But the synchronous change in M for both species over the 14-year period (Fig. 4) implies that some external factor, such as predator abundance, controls the mortality of both abalone species. The variability of M attributed to wrasses was much greater than that to crabs, which inflicted a more or less constant M. Furthermore, the consistently higher M of *H. laevigata* as compared with *H. scalaris* may reflect the greater vulnerability of *H. laevigata* to wrasse predators. The shell of *H. laevigata* is rather flat, smooth, and elongate and more easily crushed than that of *H. scalaris*, which is rounded and sculptured strongly with corrugations and "keels," an architectural adaptation to shell-crushing fish predators (Vermeij 1978). The mortality rate attributable to wrasses was correlated over the 14-year study with the density of these wrasses, and this will be described in a later paper.

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ALTERNATIVE APPROACH FOR ESTABLISHING LEGAL MINIMUM LENGTHS FOR ABALONE BASED ON STOCHASTIC GROWTH MODELS FOR LENGTH INCREMENT DATA

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ABSTRACT Stochastic parametrization of length-increment data was used to estimate the distribution of maximum length of abalone populations. The distribution of maximum length provides important information for establishing a legal minimum length (LML). Legal minimum length is commonly used to optimize yield and/or reproductive potential in abalone populations. Although choice of LML is generally based on deterministic estimates of average values of growth parameters, there is strong evidence in abalone growth data that distributions of maximum length in abalone populations have large variances and proportions of abalone that cannot attain the LML. However, deterministic estimates give no indication of the proportion of individuals that may or may not attain a specific LML. Consequently, deterministic estimates of growth can be misleading and may result in setting inappropriate LMLs. Length-increment data from tagged individuals in three *H. rubra* populations in Victoria, Australia were analyzed using new stochastic versions of the Gompertz model reparameterized for tagging data. Outputs from the stochastic Gompertz model for tagging data include distributions of L_∞ that show the proportion of abalone in each studied population that were unlikely to attain the LML and enter the stock. It is presently unknown whether this level of protection will ensure long-term sustainability. The proportion conserved may also be viewed as lost yield to the fishery. If the proportion of abalone required to provide sufficient egg production for sustaining a particular population is known, then the stochastic Gompertz model for tagging data provides additional information to select an appropriate LML that balances reproductive output against yield to the fishery.

KEY WORDS: Length-increment data, legal minimum length, stochastic Gompertz model.

INTRODUCTION

The objective of employing legal minimum lengths in management regimes applied to abalone fisheries is to conserve sufficient egg production for sustainable exploitation and/or optimization of yield. Legal minimum lengths (LML) must be set at sizes that allow enough abalone to reach reproductive maturity and spawn prior to recruitment to the stock. Eggs-per-recruit analyses (EPR) have typically been used to arrive at these determinations of LML. The value of the LML for each particular abalone population is strongly dependent on the size distribution of the population. However EPR analysis based on deterministic estimates does not allow for heterogeneity of growth within a population. Abalone are characteristically variable with respect to many biological parameters including those that describe growth (Day and Fleming 1992).

Although the von Bertalanffy model adequately describes the growth of the more mature part of the population whose growth is slowing, extrapolation through the juvenile phases is less reliable. This is because the von Bertalanffy model assumption that length increment decreases with increasing length at release inadequately represents juvenile growth data. There is evidence that the Gompertz model is more suitable, because this model can accommodate both the exponential increase in growth expected during the early phases of life history (e.g., Nash 1992), and the asymptotic phase that occurs as growth slows with onset of maturity, and energy is channelled into reproduction. For example, the data of Poore (1972), Shepherd et al. (1985), and Prince et al. (1988) show that the mean growth rate of juvenile abalone tends to increase when they are very small and is almost linear for larger juveniles, before the growth rate decreases as they mature.

The Gompertz growth function has been used in population studies and to represent the average course of animal growth since 1940 (Medawer 1940). Yamaguchi (1975) has pointed out that this curve provides a better fit than the von Bertalanffy curve for many species when the growth of small juveniles as well as subadults is considered. The original Gompertz growth model has been applied to size-at-age growth data. However, for animals that cannot be aged with reasonable resolution (such as abalone), growth data are almost always produced from tagging studies. Thus, it is important to use a version of the Gompertz model that utilizes length-increment data and accommodates the heterogeneity in these growth data. If residuals from an average growth function are larger than the measurement error, then models with distributed parameters can be applied to describe effectively the data. One such unified method of stochastic paramterization of data by models with one or more random parameters, and the distinctive properties of the models in the form of probability density is described in Troynikov (1998). For analysis of abalone growth, we use stochastic versions of the Gompertz growth model for length-increment data with distributed parameter L_∞ that represents asymptotic size. We selected the Gompertz model because of its potential for expansion to data that incorporate juvenile growth.

In this study, we examined existing length increment data from tagging studies previously published by McShane and Smith (1988). Some of these data have been applied in eggs-per-recruit analyses (McShane 1992). The new stochastic model for tagging data was applied to length increments for three Victorian blacklip (*H. rubra*) abalone populations. A stochastic approach allows us to estimate the portion of a population that is unlikely to attain the LML; in other words, the percentage of the population whose reproductive output is unaffected by fishing.

Estimated percentiles of the maximum length for each population were output from the model and compared with the respective LMLs. This comparison showed the proportion of abalone conserved by current LMLs. Fecundity and mortality rates will influence whether the conserved abalone provide sufficient egg production for sustainable exploitation. Additionally, environmental factors will cause interannual variation in recruitment to abalone populations for any given proportion that is conserved by an LML.

With further information about the egg production required for sustainability, it may be possible to increase yield to the fishery by reduction of LMLs in some instances.

MATERIALS AND METHOD

Data Collection

Growth increment data from tagging studies of blacklip abalone were collected at Boulder Point, Seal Rocks, and Tullaberga Island in Victoria. The study locations (Fig. 1) were separated by several hundred kilometers and were all harvested regularly by commercial abalone divers. Only data from abalone where the tags were glued to shells were included in the analysis; the tags attached by wire through tremata were rejected, because they have been shown to retard growth (McShane and Smith 1988). Growth increments were the differences between maximum shell diameters of each tagged abalone at times of release and recapture. Shell diameters were measured to the nearest millimeter using Vernier calipers. Length-frequency distributions at time of release for each location show that most tagged abalone were >60 mm (Fig. 2).

Data Analysis

The original deterministic Gompertz growth model for length-at-age data was reparameterized to a model for length-increment data (Troynikov et al. this volume).

$$\Delta l(\Delta t) = L_\infty (l_1/L_\infty)^{\exp(-g\Delta t)} - l_1 \qquad (1)$$

Model (1) has two Gompertz growth parameters L_∞ and g, one parameter less than in the Gompertz model for length-at-age data. Here l_1 is length at release, Δt is time interval at liberty, and Δl is length increment. From formula (1) follows that $\Delta l(0) = 0$ and $\Delta l(\infty) = L_\infty - l_1$. There are several examples of using a Gompertz growth model for length-increment data in the literature. Kurogane et al. (1974) and Kaufmann (1981) have proposed special techniques for approximate estimation of the Gompertz growth parameters using restrictive additional assumptions about length-increment data. Equation 1 is the direct reparameterization of the Gompertz model without any additional assumptions.

Three stochastic growth models in the form of conditional (on the length at release l_1) probability density of length-increments Δl for time at liberty Δt that incorporate the distribution of L_∞ (Weibull, gamma, and log-normal) and parameter g of the Gompertz growth model were used for parameterization of growth heterogeneity in the abalone populations. Weibull, gamma, and log-normal distributions are the most commonly used distributions for the description of positively distributed continuous variables. We used the positive distributions for L_∞ to avoid negative tails that

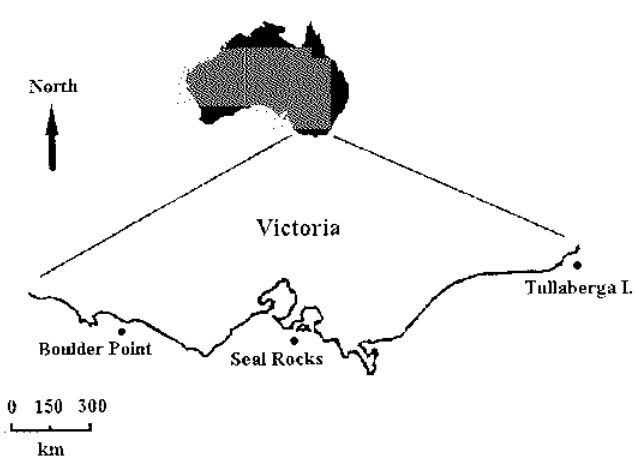

Figure 1. Locations of abalone populations where growth data were sampled.

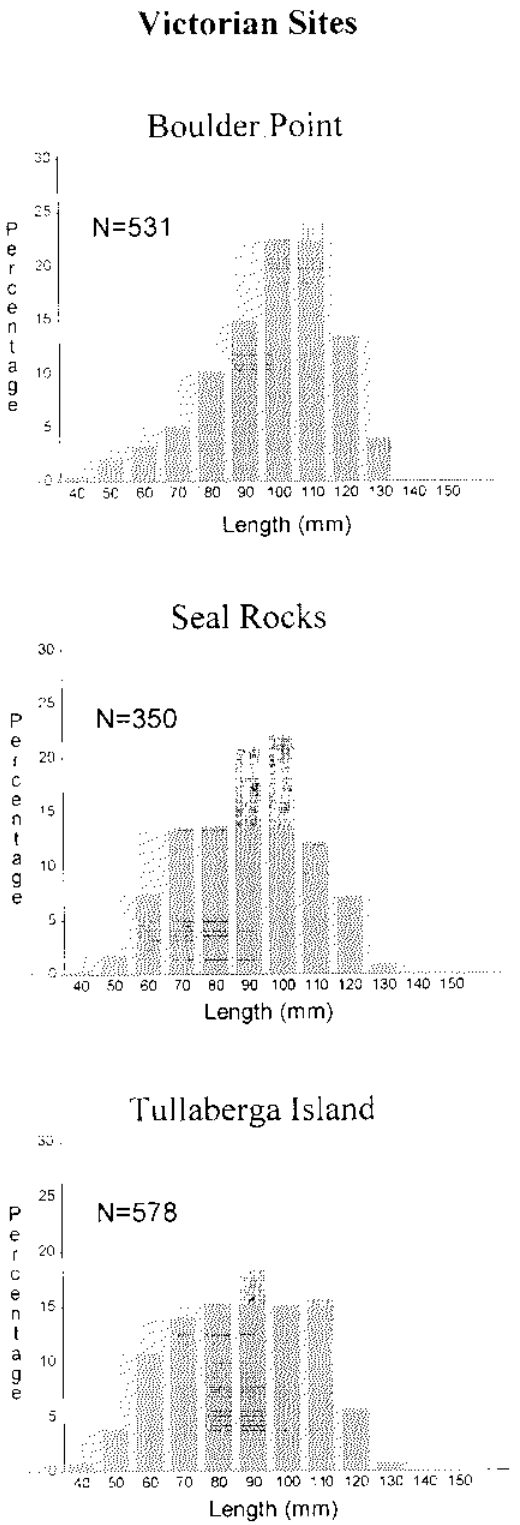

Figure 2. Length frequency at release of tagged abalone for three abalone (*H. rubra*) populations.

are inconsistent with the biological meaning of size of animal (maximum shell diameter, in this instance). When the variance of L_∞ is large, then the negative tail of the distribution can be large. In this situation, assumption about normal distribution of L_∞ is inappropriate for describing the data.

The following expressions are stochastic versions of the deterministic growth Model (1) (Troynikov et al. this volume).

$$WG_{\Delta l}(x,\Delta t;g,\eta,\alpha|l_1) = \{\eta(L_\infty(x)/\alpha)^{\eta}{}_{\exp}[-L_\infty(x)/\alpha)^{\eta}]/L_\infty(x)\}\frac{dL_\infty(x)}{dx} \quad (2)$$

here η and α are parameters of the Weibull distribution. Model (2) is the conditional on size-at-release l_1 probability density function of length increment Δl, and Δt is a parameter of the distribution;

$$GG_{\Delta l}(x,\Delta t;g,\lambda,\rho|l_1) = \{[\lambda L_\infty(x)]^{\rho}{}_{\exp}[-\lambda L_\infty(x)]/[\Gamma(\rho)L_\infty(x)]\}\frac{dL_\infty(x)}{dx} \quad (3)$$

here λ and ρ are parameters of the gamma distribution, $\Gamma(x)$ is the gamma function;

$$LNG_{\Delta l}(x,\Delta t;g,\mu,\sigma|l_1) = [\exp\{-[\log(L_\infty(x) - \mu]^2/(2\sigma^2)\}/(\sigma\sqrt{2\pi}\,L_\infty(x)]\frac{dL_\infty(x)}{dx} \quad (4)$$

here μ and σ are parameters of the log-normal distribution. Where formulae (2), (3), and (4) we have (Troynikov et al. this volume)

$$L_\infty(x) = [(l_1 + x)l_1^{-\exp(-g\Delta t)}]\frac{1}{1-\exp(-g\Delta t)}$$

WG stands for Weibull–Gompertz, GG for Gamma–Gompertz, and LNG for Log-normal–Gompertz. Semicolons are used to separate the parameters that should be estimated from data. Equations 2, 3, and 4 have three parameters for estimation, only one more then in the deterministic Eq. 1.

We use the Kullback's informative mean to discriminate between Eqs. 2, 3, and 4 with respect to the fit to data (Wilks 1962). Although this discrimination between models is not a statistical test of hypotheses, it allows us to avoid large errors in assumptions when selecting a particular model. More formal tests of separate families of hypotheses for some families of distributions were developed by Cox (1962).

NUMERICAL RESULTS

In this section, we demonstrate the ability of the new stochastic growth models to extract the information about distribution of asymptotic size from size-increment data with high heterogeneity.

The program is written in FORTRAN 77 for UNIX with a simple, convenient shell used for estimating parameters and calculating different growth characteristics. The Nelder–Mead simplex method is used for numerical approximation of the maximum log-likelihood function.

For Seal Rocks and Boulder Point, the lognormal distribution of L_∞ provided the best fit to the data and Weibull the worst (Table 1). For Tullaberga I., the gamma distribution seemed to fit better; however because Kullback's informative mean Δ(LNG,GG) = −0.003, the difference is relatively small. Note that the values Δ(LNG,WG) and Δ(GG,WG) are significantly bigger than Δ(GG,LNG), and the distinction between values g and $E(L_\infty)$ for "WG" model from those for "LNG" and "GG" is bigger than between "LNG" and "GG" models. These numerical results show that Kullback's informative mean provides reliable discrimination between models with respect to fit to empirical data distribution.

TABLE 1.

Kullback's informative mean Δ(M1,M2) (if Δ(M1,M2) > 0 then model M1 fit the data better; if < 0 then M2 fit better respectively).

Location	Δ(GG,WG)	Δ(LNG,WG)	Δ(LNG,GG)
Seal Rocks	2.00×10^{-1}	2.24×10^{-1}	2.42×10^{-2}
Tullaberga I.	2.37×10^{-2}	2.08×10^{-2}	-2.86×10^{-3}
Boulder Point	2.88×10^{-1}	3.48×10^{-1}	5.96×10^{-2}

WG = Weibull–Gompertz, GG = Gamma–Gompertz, and LNG = log-normal–Gompertz.

We use numerical integration to calculate the quantiles of a distribution and "the proportion less than the Legal Minimum Length" (see Table 3). Derivation of the statistical error for these integrals is unknown. If the subject of the analysis is the growth of a specific population, then known bootstrap techniques can be used to generate confidence limits for the numerical results. Because this study focuses on the presentation of a new approach, rather than the specific numerical results, the computer time-consuming task of generating confidence limits (which, in our case, also, required numerical integration) was not undertaken.

The values of the mathematical expectation $E(L_\infty)$ were close to the medians of the lognormal distributions of each population (Tables 2 and 3). Note that in Table 2, the standard errors are expressed in percentages to enable comparison of errors in estimates of different parameters. The estimates of L_∞ and g for the deterministic version of the Gompertz growth model for length-increment data were close to $E(L_\infty)$ and g values for the stochastic version of the Gompertz growth model with a lognormal distribution of L_∞ for Seal Rocks and Tullaberga I., but different for Boulder Point. This difference could be attributable to a greater departure from the distribution of L_∞ from normality. Despite the biggest LML for Boulder Point the percentage of abalone that are unlikely to attain LML is smallest (Table 3). This highlights the utility of our approach in estimating the asymptotic size distribution rather than arbitrarily setting what may be perceived as relatively large size limits to conserve a population.

DISCUSSION

Identification of the proportion of abalone unlikely to attain legal size provides important quantitative information for determining if LMLs are sufficiently conservative to ensure reproductive sustainability, or overly conservative and wasteful of yield. This information is also important, because quantitative assessments of mortality, catchability, and fecundity, as well as other population parameters, are limited for wild abalone populations.

Through application of the stochastic growth model for length increment data we can estimate the lower bound of this proportion. In this study 26–39% of the tagged abalone would have remained smaller than the LML. The extent to which these proportions could be expected to conserve the stock will depend not only on their egg production, but also upon the contributions made by the remaining

TABLE 2.

Likelihood estimates of parameters for the stochastic Gompertz growth model for length-increment data from three *H. rubra* populations in Victoria; E and SD are the mathematical expectation and standard deviation of L_∞; here η and α are the parameters of the Weibull distribution, ρ and α are the parameters of the gamma distribution; and μ and σ are the parameters of the log-normal distribution.

Model	Parameter	Location: Seal Rocks	Tullaberga I.	Boulder Point
WG	η	7.00 (5%)	8.28 (7%)	5.21 (6%)
	α	116 (1%)	120 (1%)	132 (1%)
	g	0.52 (4%)	0.47 (5%)	0.49 (6%)
	$E(L_\infty)$	109	113	121
	$SD(L_\infty)$	17%	14%	22%
GG	ρ	43.92 (11%)	37.84 (13%)	26.77 (9%)
	λ	0.38 (11%)	0.31 (14%)	0.20 (10%)
	g	0.40 (4%)	0.37 (6%)	0.33 (4%)
	$E(L_\infty)$	115	121	134
	$SD(L_\infty)$	15%	14%	19%
LNG	μ	4.75 (1%)	4.79 (1%)	4.92 (1%)
	σ	0.16 (6%)	0.17 (7%)	0.21 (5%)
	g	0.36 (5%)	0.34 (6%)	0.28 (5%)
	$E(L_\infty)$	117	123	140
	$SD(L_\infty)$	16%	14%	22%
Gompertz deterministic	L_∞	115 (1%)	120 (<1%)	131 (<1%)
	g	0.36 (1%)	0.36 (1%)	0.33 (1%)
Sample size		350	578	531

WG = Weibull–Gompertz, GG = Gamma–Gompertz, and LNG = log-normal–Gompertz. (*%) is relative statistical error.

61–74% of abalone that will be recruited into the stock. This partly depends upon growth rate, with those growing slowly having more time to reproduce before becoming available to the fishery. However, fecundity has been shown to be proportional to length for reproductively mature individuals, implying that faster growing individuals have greater reproductive potential per time interval (Nash et al. 1994). The contribution of legal-size abalone to egg production and subsequent recruitment depends upon mortality and catchability. Catchability, in turn, depends upon the extent of available cryptic habitat, numbers of abalone that are not in aggregations and diver's expectations of acceptable catch rates. For example, McShane and Naylor (1995) postulated that those abalone that are disaggregated may contribute sufficient egg production to account for all the recruitment necessary for sustainability. The basis for his assertion is that abalone divers target larger aggregations of abalone to the exclusion of scattered individuals. However, in instances where bottom topography is such that the spatial distribution of abalone exposes most of the legal-sized abalone to fishing, then the LML becomes a critical determinant of stock replenishment. The closeness of the $E(L_\infty)$ and median values (Tables 2 and 3) shows that if $E(L_\infty)$ values (or deterministic estimates of L_∞) were chosen as LMLs, then about 50% of the abalone would remain unavailable to the fishery. This may be overly conservative and result in substantial loss of potential production.

The estimates of distributions of asymptotic length parameter L_∞ in this study have large variances. This property of the L_∞ distributions is consistent with information about growth in abalone populations. Tarr (1995) and Shepherd et al. (1995) estimated L_∞ for several populations of *H. midae* and *H. mariae*, respectively. Approximate estimates of minimum standard deviations of L_∞ from these studies are 37 mm for *H. midae* and 75 mm *H. mariae*, indicative of the large variability within populations that characterizes abalone generally. When McNamara and Johnson (1995) plotted growth rate against shell length for *H. asinina* up to 100 mm they showed that for many abalone larger than 60 mm, annual increments were close to zero. A corollary of this is that maximum lengths attained by individual abalone in the study population fell within a wide range that can start from 60 mm. This is consistent with our results (Fig. 3), which show a wide range of asymptotic lengths distributed above 70 mm.

The stochastic version of the Gompertz growth model for length-increment data with a lognormal distribution L_∞ was the most appropriate given the dataset (Table 1). The sigmoid shape of the Gompertz curve is consistent with the growth trajectory during the prematurity phase of abalone life history. However, previous studies have mostly suggested that growth is linear among juvenile abalone. Although it is clear from this study that, for our data, the Gompertz curve fits well, a more convincing argument could be made where a larger proportion of juveniles were included in the samples. Under these circumstances, a wider range of such growth functions as the logistic and Richards (Schnute 1981) could be tested. Model (1) as well as other growth functions, can be used for development of stochastic growth models in the form of probability density function (Troynikov 1998) with one or two random growth parameters.

This study and previous work has highlighted the lack of length increment data for juvenile abalone. Satisfying this need presents substantial problems however, because the preference of juvenile abalone for cryptic habitat results in their under-representation in population samples. Most abalone tagging data relates to adult abalone whose growth is generally much slower than for juveniles. Whilst juvenile growth is important when attempting to estimate time to recruitment, the growth data from adult abalone are more

TABLE 3.

Quantiles of the log-normal distribution of the asymptotic length L_∞ (mm) for *H. rubra* from three locations in Victoria; shaded cells represent the part (P%) of the abalone population that will not reach legal minimum length (LML) for fishing.

Location	LML	P%	Percentile: 10%	20%	30%	40%	50%	60%	70%	80%	90%
Seal Rocks	110	39	94	101	106	111	116	120	126	133	142
Tullaberga I.	110	30	97	105	110	116	121	126	132	140	151
Boulder Point	120	26	104	114	122	130	137	144	153	163	180

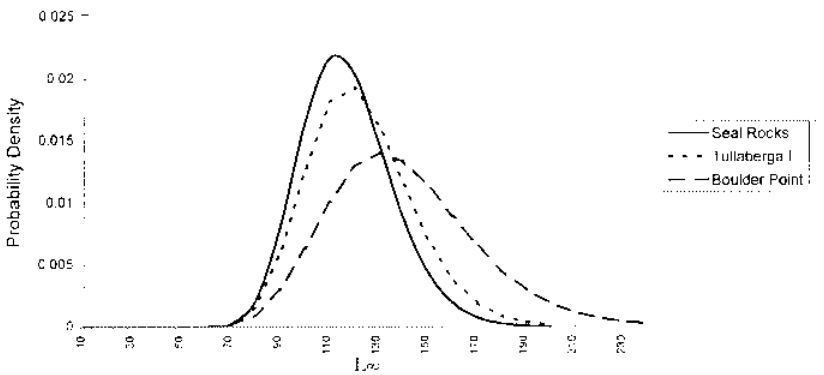

Figure 3. Distribution of asymptotic length for three abalone (*H. rubra*) populations in Victoria.

informative for assessment of the proportion of a population excluded from the stock by the LML.

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ESTIMATION OF SEASONAL GROWTH PARAMETERS USING A STOCHASTIC GOMPERTZ MODEL FOR TAGGING DATA

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ABSTRACT This paper addresses the problem of growth estimation from tagging data that exhibit large heterogeneity in size-increments. Precision in growth estimation is essential for stock assessment, especially for abalone fisheries, because they are managed in part by size limits. However, abalone growth is notoriously variable, changing dramatically between seasons and sites. It is also known that juvenile growth does not fit the commonly used von Bertalanffy model. We present a modified deterministic Gompertz model for tagging data and three stochastic versions in which asymptotic length is a random parameter. The Kullback's informative mean was used to discriminate between models with respect to the fit to data.

Every 3 months over an 18-month period, abalone were collected from within an area of approximately 200 m × 200 m at Point Cook reef, in Port Phillip Bay. They were brought to shore, tagged, measured, and returned within 1–2 hours. Previously tagged abalone were found and measured *in situ* with vernier calipers to minimize disturbance. Thus, the data provide information on the growth of abalone for six consecutive 3-month periods (seasons). Sizes ranged from 15 to 125 mm maximum shell length at release. The distribution of asymptotic length was estimated from all the length increment data for large size classes. The parameter g of the Gompertz model, and distributions of length-increments for various initial sizes, were obtained for each season. The numerical results show that juvenile growth varies more markedly with season than with initial size; whereas, in larger abalone, growth increments vary with initial length and are less dependent on season.

KEY WORDS: growth, stochastic, Gompertz model, length-increment data

INTRODUCTION

Predictive models of stock dynamics are the basis for all stock assessment and, thus, fishery management (Hilborn and Walters 1992). Good estimates of growth are essential to predict the dynamics of the stock, including yield, reproductive output, and its response to fishing (Beamish 1990, Day and Fleming 1992). This is especially true for abalone fisheries, because in most cases, the abalone cannot be aged with any certainty, and growth is highly variable (Day et al. 1995, Shepherd et al. 1995). Minimum legal lengths are used worldwide to protect reproductive capacity of stocks (Prince and Shepherd 1992), and adequate models of growth are needed to determine minimum length for fishing.

Usually the abalone growth data available are produced by tagging experiments. Here, initial size and the growth increment over a specified time are known, but not the ages of the animals. In this case, size cannot be directly related to age. Abalone growth, as well as growth of other marine species, has traditionally been fitted to the von Bertalanffy growth curve using the method of Fabens (1965). However the data of Poore (1972), Shepherd et al. (1985), Shepherd (1988), and Prince et al. (1988) show that the mean growth rate of juvenile abalone tends to increase when they are very small and is almost linear for larger juveniles, before the growth rate decreases as they become mature. Such growth is best fitted by a Gompertz growth curve (e.g., Nash 1992). Yamaguchi (1975) has pointed out that this curve is a better fit than the von Bertalanffy curve for many species when considering the growth of small juveniles as well as subadults.

Large variation in growth is characteristic of abalone populations, and this has important consequences for management. In particular, *Haliotis rubra* in Australia display extremely variable growth, both between local areas and within reefs (Day and Fleming 1992, Nash 1992). Sainsbury (1982) showed that to represent populations with high growth heterogeneity, models with distributed growth parameters should be applied. In this case, the "average growth parameters" do not represent the growth of a population.

In this paper, we present a deterministic Gompertz growth model for length-increment data, which is an exact reparametrization of the classical Gompertz growth model for length-at-age data, and three stochastic Gompertz models that are developed on the basis of the technique for stochastic parametrization proposed by Troynikov (1998). In these models, the parameter of asymptotic length is positively distributed, with Weibull, gamma, or log-normal probability density functions. The length-increment in these models has a probability density conditional on length-at-release. We demonstrate the use of these models in describing the growth of abalone and seasonal changes in the growth of juveniles.

It is important to obtain data and estimates of growth parameters that provide information on the rapid growth phase of juveniles, because data on the slow growth of larger individuals reveal very little about the rate of recruitment of abalone to adult stocks and the fishery, and, thus, about the dynamics of the stocks under fishing. Shepherd and Hearn (1983) have also shown that growth of abalone changes seasonally, with the growth of juveniles being most affected. In this study, we investigate the seasonal patterns of growth of a wide size range of blacklip abalone (*Haliotis rubra*) by tagging individuals and measuring them at 3-month intervals over six seasons. We use a two-step procedure wherein parameteres are estimated independently from subsets of the data. Data for all seasons are first pooled, and a distribution of asymptotic length is fitted. This distribution is then fixed, and we estimate the Gompertz growth parameter *g*, which indexes the expected rate at which abalone grow to reach their final size, from data for each season.

SAMPLING METHOD

Abalone were tagged to estimate growth on Point Cook reef, in Port Phillip Bay, Australia (37°55′S, 144°48′E) (Fig. 1). This is a slowly sloping reef of basalt boulders and smaller rocks interspersed with sand, which extends 300–400 m from shore and to about 5 m depth. The reef has supported a large harvest of blacklip abalone over many years. The area within 100 m of shore is a marine reserve, and the study was conducted at the north end of the reserve, by SCUBA diving from shore, at a depth of up to 4 m.

Larger abalone were removed from rocks using a commercial "abalone iron," and juveniles were collected by overturning boulders and removing them by hand once they started to move. They were carried to shore and placed in trays with 1–2 cm of seawater while they were tagged, and the maximum shell length was measured using plastic vernier callipers. Two kinds of tags were used: for larger abalone tinted epoxy glue was painted onto the shell to form numbers, and for juveniles; small numbered polyethylene tags (Hallprint, South Australia) were fixed to the shell with Superglue. After tagging, abalone were placed on rocks in plastic mesh cages on the reef and left overnight to recover, before being returned to the reef. This procedure was designed to minimize stress, which an earlier study (Leorke unpubl.) had shown induced increased movement.

At intervals of about 3 months, divers searched for marked abalone and remeasured them *in situ* on the reef using the plastic callipers to minimize disturbance. Further abalone were then tagged in an attempt to ensure about 100 recaptures could be recorded each season. Varying numbers of abalone were found and measured in each of six seasons, from February to May 1983 (Autumn) to May to August 1984. Most recapture times were 90 days ± 10 days. To check on the precision of the underwater length measurements, cases where the same abalone had been measured twice on the same day, either by the same diver, or by two different divers, were collated.

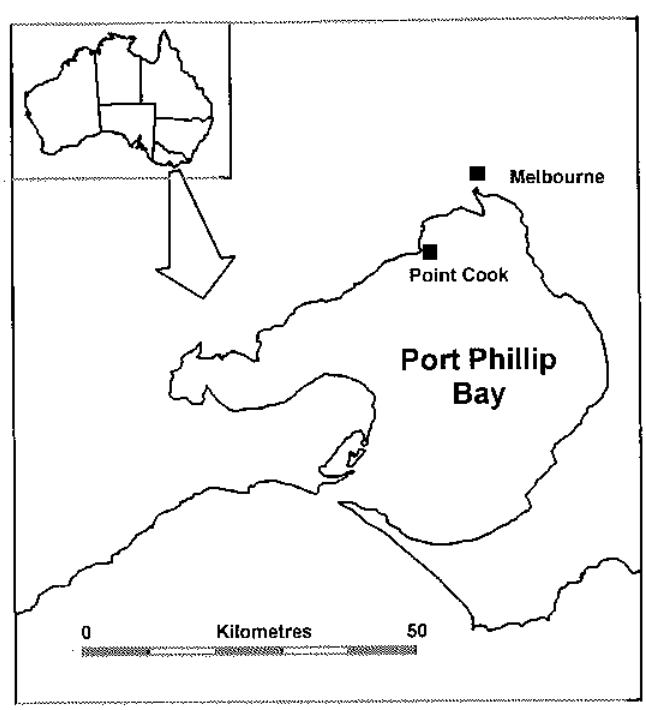

Figure 1. Map of Australia showing the location of Port Phillip Bay and map of Port Phillip Bay showing the location of the reef off Point Cook.

GOMPERTZ MODELS FOR LENGTH-INCREMENT DATA

The deterministic Gompertz growth model for length-increment data was obtained as a direct reparametrization of the original Gompertz growth function into a model with parameter Δt as the time interval between observations. The Gompertz model for aging data is:

$$l(t) = L_0\exp[G\,(1 - \exp(-g\ t))] - L_\infty \exp[-G \exp(-g\ t)] \quad (1)$$

Here $L_\infty = l(\infty) - L_0\exp(G)$ is asymptotic length, $L_0 = l(0)$ and $g > 0$, $G > 0$. Let $l_1 = l(t_1)$ and $l_2 = l(t_2)$ be size-at-release and size-at-recapture, $t_2 = t_1 + \Delta t$, $\Delta l(\Delta t) = l_2 - l_1$. Then, using simple algebra, we have a model for size-increment data. From Model 1, we have

$$\begin{aligned} l_2 &= L_\infty\exp[-G \exp(-g\,(t_1 + \Delta t))] \\ &= L_\infty\exp[-G \exp(-g\ t_1)\exp(-g\ \Delta t)] \end{aligned} \quad (2)$$

and

$$l_1/l_\infty = \exp[-G \exp(-g\ t_1)] \quad (3)$$

Then using Eqs. 2 and 3 with a simple transformation we have

$$\Delta l(\Delta t) = L_\infty(l_1/L_\infty)^{\exp(-g\ \Delta t)} - l_1 \quad (4)$$

Here $\Delta l(0) = 0$, $\Delta l(\infty) = L_\infty - l_1$. Model 4 is a direct reparametrization from the model (1) and has two growth parameters, L_∞ and g. This reparametrization is analogous to the reparametrization, without additional assumptions, of the von Bertalanffy (1938) growth model for length-at-age data to the Fabens (1965) model for length increment data. In both cases, the model for length increment data has one parameter less then the model for length-at-age data. Note that Kurogane et al. (1974) and Kaufmann (1981) proposed special techniques to estimate Gompertz growth parameters using length-increment data. However, the extra assumptions they used produced approximate estimates of Gompertz parameters. Model 4 can be applied to length-increment data without any additional assumptions.

To study seasonality in abalone growth we used stochastic versions of the Gompertz growth model for size-increment data with a random parameter L_∞ and a constant parameter g. The models have the form of probability density functions. We used the positive distributions for L_∞ to avoid large negative tails when variances of L_∞ are large. Large variances of L_∞ distributions are consistent with information about growth in abalone populations (Day and Fleming 1992, McNamara and Johnson 1995).

Consider Δl as a function of L_∞, then from Model 4 the inverse function is

$$L_\infty(\Delta l) = [(l_1 + \Delta l)l_1^{-\exp(-g\Delta t)}]^{\frac{1}{1-\exp(-g\Delta t)}} \quad \text{and}$$

$$\frac{dL_\infty(\Delta l)}{d\Delta l} = \frac{1}{1-\exp(-g\Delta t)}\left(\frac{l_1 - \Delta l}{l_1}\right)^{\frac{1}{\exp(g\Delta t)-1}}$$

Let L_∞ be a positively distributed random variable with a Weibull, gamma, or log-normal distribution. Using the rule for changing the variable of integration in a probability density function we have:

$$WG_{\Delta l}(x,\Delta t;g,\eta,\alpha|l_1) = \{\eta|L_\infty(x)/\alpha]^\eta \exp[-L_\infty(x)/\alpha]^\eta/L_\infty(x)\}\frac{dL_\infty(x)}{dx} \quad (5)$$

Here η and α are parameters of the Weibull distribution. Model 5 is a probability density function of length increment Δl, which is conditional on size-at-release l_1, and Δt is a parameter of the distribution.

$$GG_{\Delta l}(x,\Delta t;g,\lambda,\rho|l_1) = \{[\lambda L_\infty(x)]^\rho \exp[-\lambda L_\infty(x)]/[\Gamma(\rho)L_\infty(x)]\}\frac{dL_\infty(x)}{dx} \quad (6)$$

Here λ and ρ are parameters of the gamma distribution, $\Gamma(x)$ is the gamma function.

$$LNG_{\Delta l}(x,\Delta t;g,\mu,\sigma|l_1) = \{\exp[-\log(L_\infty(x) - \mu)^2/(2\sigma^2)]/[\sigma\sqrt{2\pi}\,L_\infty(x)]\}\frac{dL_\infty(x)}{dx} \quad (7)$$

Here μ and σ are parameters of the log-normal distribution. WG stands for Weibull–Gompertz, GG for Gamma–Gompertz, and LNG for log-normal–Gompertz. We use a semicolon to separate those parameters that should be estimated from the data. Models 5, 6, and 7 have three parameters that should be estimated from data. Therefore, there is only one more parameter than in deterministic models for size-increment data.

The Gompertz growth models where the second parameter is random can be obtained using the technique described in Troynikov (1992). Note that, in these models, the growth parameter L_∞ of an individual is not assumed to be constant during the life history of that individual, and the individual's growth trajectory is considered to be the realization of a random process.

RESULTS

The average difference between measurements on the same day was 0.95 mm ($n = 81$). Analysis of variance (ANOVA) showed no significant differences in the measurements between divers ($F = 3.017$, $df = 1,77$). However, there was a significant trend for measurement differences to increase with abalone size ($F = 5.023$, $df = 1,79$), as might be expected.

Growth increments were initially adjusted linearly to a 90-day period, to facilitate comparison and examination of the data only (because this process introduces bias). In each season, growth increments were quite variable: for example, adjusted increments are plotted against size for summer 1983/1994 and winter 1984 (Fig. 2A,B). For each season, initial sizes were grouped into six 20-mm categories, and the mean adjusted increment for each size class was plotted against initial size (Fig. 3). In all seasons, the increments for smaller size classes do not decrease with size, which suggests a Gompertz growth model. In contrast, a von Bertalanffy growth curve requires that increments decrease with initial size in a linear fashion. Figure 3 also shows that the mean increments differed substantially between seasons for abalone smaller than 60 mm length, but not for large abalone. The negative growth data shown for larger abalone may be a result of the measurement error described above, but our observations indicate it may also represent erosion of the edges of abalone shells.

Increments for small abalone were unlikely to provide reliable information on asymptotic length: essentially the smaller abalone do not "know" what length they will reach. The distribution of asymptotic length should be independent from seasonality in growth. Therefore, we pooled the data for all seasons and used the raw increments and times at liberty for abalone over 60 mm long to estimate the distribution of asymptotic length (Fig. 4). We fitted models with Weibull, gamma, and log-normal distributions of asymptotic length to the data. The parameters of the distributions of asymptotic length are shown in Table 1. The relatively large standard deviations indicate the substantial variability of abalone growth. Kullback's informative mean (Wilks 1962) indicated that the stochastic Gompertz model with a log-normal distribution of asymptotic length provided the best fit to the data for abalone >60 mm.

The raw data for smaller abalone (<60 mm) were then used to estimate the Gompertz growth parameter g for each season (Table 2), by fixing the log-normal distribution of asymptotic length as a constraint in the model when running the fitting routine. In this way, we avoided correlation between the estimates of seasonal growth parameters and the parameters of the asymptotic length distribution. The standard errors measure the precision of the estimated parameters. They are relatively small as compared to both the means and the differences between seasons, which indicates that it is important to account for differences between seasons in juvenile growth when modelling abalone growth. The Gompertz parameter g is large in spring and summer and lowest in winter. The values of g are similar for the winters in each year.

Figure 5 shows the estimated distributions of growth increments for various initial sizes. This figure shows more graphically that the differences between mean increments across seasons are substantial. The periodicity of the seasonal changes in growth is

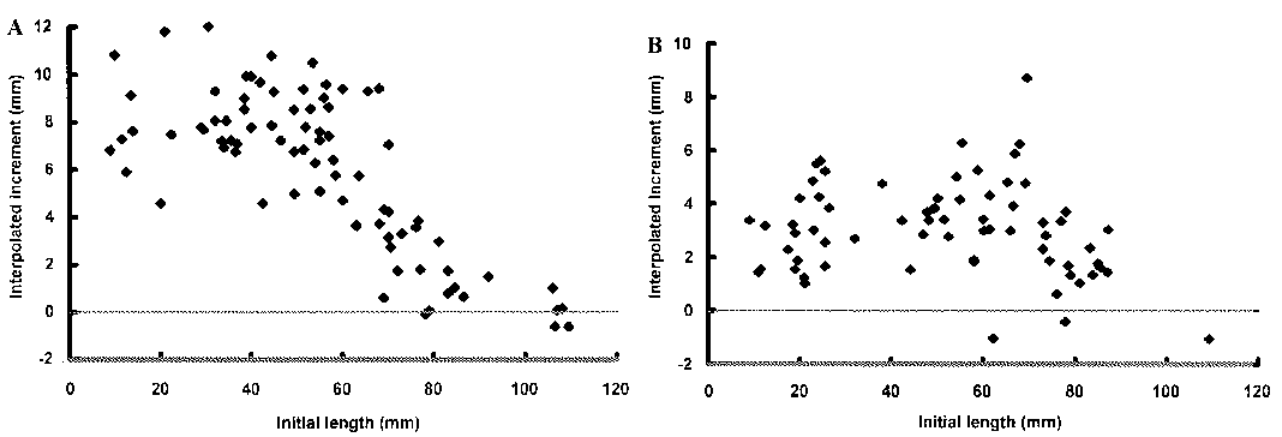

Figure 2. Growth increments of abalone, adjusted linearly to a 90-day period, plotted against initial length; A. data for summer 1983/4; B. data for winter 1984.

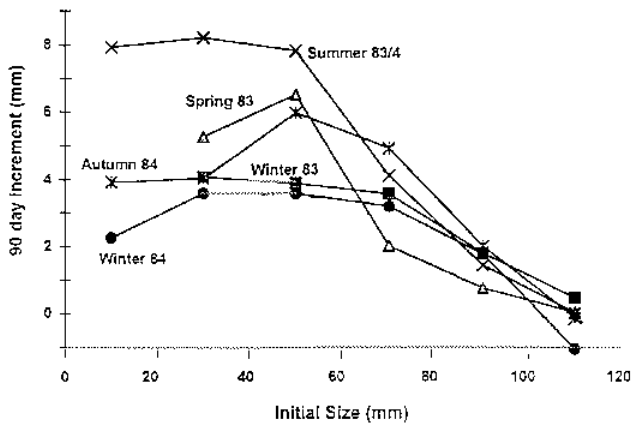

Figure 3. Mean growth increments, in each 3-month period, of abalone in 20 mm length classes, plotted against initial length. The increments have been adjusted linearly to a 90-day period. Squares: winter 1983 (June to August); triangles: spring 1983 (September to November); crosses: summer 1983/4 (December to February); stars: autumn 1984; circles: winter 1984.

evident, and note that in seasons when growth is faster, there is also more variable growth. The length increments of individuals 10, 20, and 40 mm long are always greater than zero for all seasons, but in every season, some of the 60 mm abalone have length increments close to zero. The variance of the length increments increases with size, but the mode of the length increment distribution for 60 mm abalone is smaller than for 40 and 20 mm abalone. There is a large overlap in the ranges of the length increments of different size classes. For example, the range of length increments of the 20 mm size class is included in the range of length increments of the 40 mm size class in all seasons, and the length increment range of the 60 mm size class includes that of all other size classes.

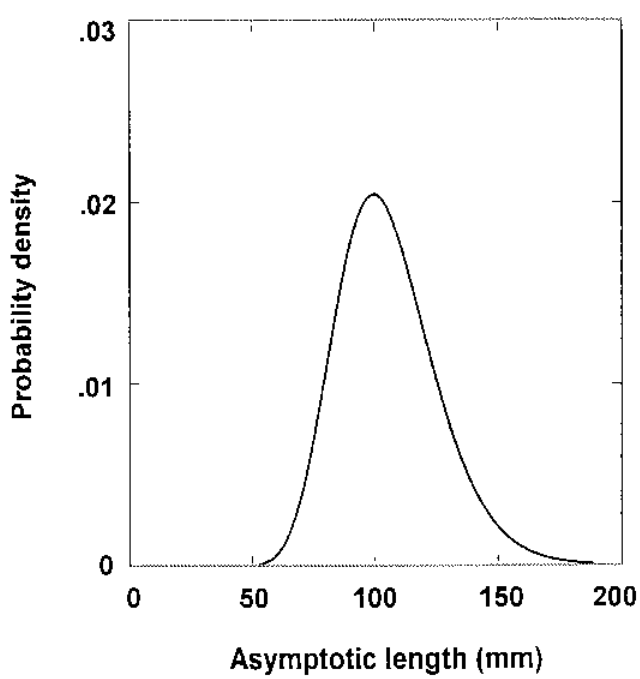

Figure 4. The log-normal probability density of asymptotic length fitted to growth increment data for abalone >60 mm in length; data from all seasons were pooled.

TABLE 1.

The mean $E[l_\infty]$ in mm and standard deviation $SD[l_\infty]$ of asymptotic length distributions fitted to abalone >60 mm, sample size n = 153 and maximum length ML = 118 of recaptured abalone.

Moment	Log-normal	Gamma	Weibull
$E[l_\infty]$	105.5	102.6	95.9
$SD[l_\infty]$	20.5	18.4	19.2

To estimate the parameter g over 1 year, the model was fitted to data pooled from the last four seasons: spring 1983 to winter 1984, with the same log-normal distribution of asymptotic length as above. The estimated g was 0.74, with a standard error of 0.011. Note that the number of abalone <60 mm in the samples from spring 1983 and summer 1983/1984 is larger than in autumn 1984 and winter 1984, so the value of g over one year is likely to be overestimated. The mean value of g over 1 year is 0.67.

DISCUSSION

The use of tagging data involves the assumptions that the tag does not affect growth, and the animals tagged and recaptured are a random subset of the population (Day and Fleming 1992). We attempted to minimize any effect of tagging on growth by minimizing the handling time of abalone out of the water, by using numbers painted or glued onto the shell, and by allowing abalone to recover in cages immediately after tagging. In addition, in each dataset, some of the animals had been tagged previously, and the increments are differences between two measurements of the animals *in situ* with minimal disturbance. Boulders were overturned to ensure both cryptic and exposed individuals were tagged and recaptured, and the short tagging periods minimize any effect of differential mortality of fast and slow growing abalone (Francis 1988).

Our model produces a distribution of asymptotic lengths, rather than a single parameter. This accords well with the experience of abalone divers and biologists, which is: abalone that seem to be very old vary widely in size. This variation means that not all of the abalone in a population will reach the size limit and recruit to the fishery; the proportion that recruit will depend upon the relation between the distribution of asymptotic length at each site and the size limit.

Abalone divers classify some areas as "stunted stocks," because few of the abalone grow past the size limit. Wells and Mulvay (1995) compared populations of *Haliotis laevigata* in such areas with those in "good growth" areas. They were unable to

TABLE 2.

The estimated Gompertz parameter g for each season, obtained by fitting the model to juveniles (<60 mm) with fixed log-normal distributions of asymptotic size (parameters in Table 1) and standard error and sample-size (n).

Season	g	SE	n
Winter 1983	0.45	0.012	93
Spring 1983	0.66	0.020	88
Summer 1983/4	1.01	0.026	80
Autumn 1984	0.60	0.023	66
Winter 1984	0.41	0.010	70

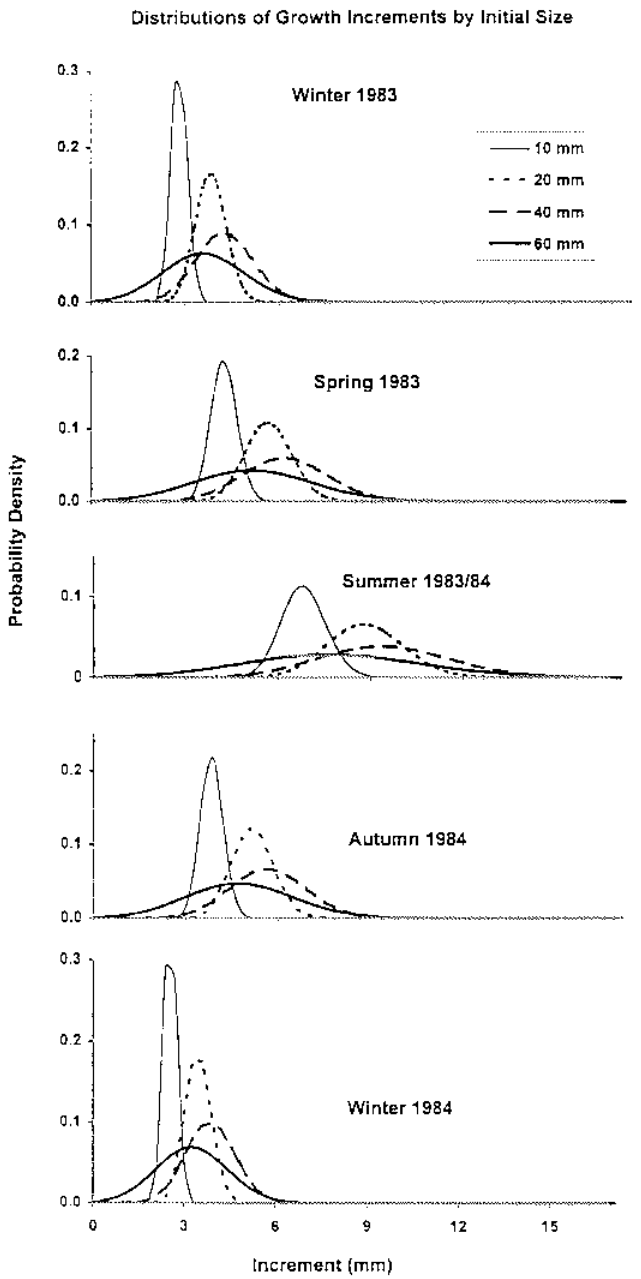

Figure 5. Probability density of estimated growth increments of abalone of 10 to 60 mm initial size, in each season.

demonstrate growth differences when fitting tag data to a deterministic von Bertalanffy model. We suggest this is, at least in part, a consequence of the variable growth at each site that is evident in their figures, so that the fitting of a single asymptotic length at each site is inappropriate.

The procedure of splitting the data at 60 mm initial length allowed us to produce an estimate of the distribution of asymptotic lengths based on a large dataset and independently estimate the Gompertz parameter g for each season, based on the smaller abalone that provide "seasonal" information on this parameter. Note that 60 mm was chosen as an approximate size for subdivision of the data on the basis of Figure 2, but Figure 5 shows that 60 mm juveniles differ in growth between seasons. We have not yet explored the consequences of varying the 60 mm dividing line between the larger animals used to estimate the asymptotic length distribution and the smaller animals used to estimate the values of g. For any application of the model, this decision would be based on the relative quantities of data available in each size range.

Growth was most rapid in summer and slowest in winter. Previous studies differ about which season produces the most rapid growth in abalone (Sakai 1962, Newman 1968, Keesing and Wells 1989, Shepherd and Hearn 1983), and the variation has been related to temperature, spawning cycle, and most commonly, to variations in food supply (Day and Fleming 1992). At Point Cook, the more rapid growth in summer may be related to the availability of more nutritious ephemeral red algae (Fleming 1995), which seem to be more abundant at this site in the summer.

The growth of smaller abalone varied most between seasons. In large, mature abalone, it seems likely that in seasons when food resources are more abundant the extra resources fuel gonad development and gametogenesis, rather than growth. Smaller, immature individuals would devote all of their available resources to growth. Cubberley (1990) has shown that at Point Cook, the gonad first becomes visible in abalone at about 60 mm shell length, which corresponds neatly with the pattern shown in our data. In the 60 mm size class, individuals that have not begun to develop the gonad may grow faster. In the 60 mm size class, the variance of the growth increment may be larger than in the smaller size classes, because at this size, there is a mixture of individuals with different growth activity.

This paper is the first study of seasonality in growth using stochastic Gompertz growth models for tagging data. We focused on the differences between seasons in juvenile growth, and thus, a fixed parameter g seems appropriate. Although the general method of stochastic parameterization (Troynikov 1998) provides a variety of stochastic growth models with different distributions of the growth parameters, the models with two or more random parameters have the form of an integral, so consumption of computer time to estimate parameters increases rapidly.

As the discussion above shows, our stochastic model captures far more features of the data than previous models, and thus, brings up more issues that require a biological explanation. We believe that one of the major strengths of these models is the process whereby the model suggests new hypotheses for testing. This will lead us to an enhanced understanding of the factors affecting abalone growth. Applying these models to other abalone species, or other organisms that display very heterogeneous growth, should lead to further advances in our understanding.

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DECLINE OF THE ABALONE FISHERY AT LA NATIVIDAD, MÉXICO: OVERFISHING OR CLIMATE CHANGE?

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ABSTRACT Abalone have been exploited commercially at La Natividad Island, Baja California since about 1956. The fishery for *Haliotis sorenseni* collapsed after about 7 years, and those for *Haliotis corrugata* and *Haliotis fulgens* in 1984. Subsequently, the fishery recovered somewhat before the recent decline in 1994 to 1997. Egg-per-recruit (EPR) analyses for the two major species were carried out with information on growth rate, fishing mortality rate, and size at sexual maturity and other data obtained mostly during the 1990s. Egg production conserved before the 1984 collapse was probably somewhat low for *H. corrugata* at ~30–40% of the maximum possible in unfished conditions, and certainly low for *H. fulgens* at ~20%. After the collapse with better control of the fishery, the egg production improved slightly for *H. corrugata* to ~30–50%, and for *H. fulgens* to ~25–40%, but from 1995 has declined again as fishing mortality increased. The periodic El Niños cause elevated sea temperatures and loss of *Macrocystis* in the region. The total abalone catch from 1965 to 1996 was correlated with mean sea surface temperature anomalies with a lag of 8 years, which is the average period from larval settlement until recruitment into the fishery. This implies that sea temperature anomalies have a positive effect on recruitment. On the other hand, there is also slight evidence of recruitment failure during severe El Niños. Although environmental variables and recruitment overfishing can each cause reductions in catch, the presence of both makes a decline practically inevitable. Quota managed fisheries must take into account environmental effects on recruitment if they wish to avert declines.

KEY WORDS: *Haliotis corrugata*, *Haliotis fulgens*, abalone fishery, population decline, egg-per-recruit analysis, El Niño, sea surface temperatures, recruitment overfishing, environmental variables.

INTRODUCTION

The Mexican abalone fishery of central Baja California, México has a troubled history. From the mid-1950s, the fishery flourished for two decades, then declined, and finally collapsed in 1984, subsequently recovering for some years before going into decline again. Data on the population biology of the species fished are few and recent; hence, knowledge of the status of the fishery "is slender but the tradition fabulous."

Analyses of the fishery in specific coastal areas in the region have been done by Doi et al. (1977), Marín (1981), Rocha and Arreguín-Sánchez (1987) and Prince and Guzmán el Próo (1993), but they have depended on some dubious parameter estimations, and the conclusions are in some respects implausible. There are no analyses of the fishery for the Island of La Natividad. Vega et al. (1997) have recently reviewed the fishery for the whole of Baja California and concluded that environmental factors, as well as overfishing, have been causally implicated in the recent declines. Current studies providing the basis for aging the shell (Shepherd et al. 1995, Shepherd and Avalos-Borja 1997, Shepherd and Turrubiates-Morales 1997) have enabled a more accurate estimation of such population parameters as growth and mortality rates, and hence allowed us to undertake this egg-per-recruit (EPR) analysis together with a more reliable assessment of the fishery and its decline. Our approach is to use this model to assess the role of recruitment overfishing at the critical periods prior to the declines of the abalone fishery at La Natividad, and then to consider an alternative explanation for the declines; namely, the effect of El Niño oceanographic events and sea surface temperature (SST) anomalies on the fishery.

We have chosen the fishery at La Natividad Island for this study, because we have the data for an EPR analysis, and the abalone populations can be considered as ecologically independent of other abalone populations in the region. La Natividad is about 20 km from Cedros to the north and about 9 km from Punta Eugenia to the southeast. Although tidal excursions are several km, abalone larvae are unlikely to be transported between La Natividad populations and those of the neighboring mainland or Cedros because tidal currents flow transversely into and from the Bay of Vizcaíno (Fig. 1). Therefore, our analysis is based on the assumption that the La Natividad populations are self-recruiting. Recent genetic studies by Rodríguez et al. (1997) support this assumption.

History of the Fishery

The account given here draws partly on the more general ones given by Guzmán del Próo (1992), Prince and Guzmán del Próo (1993), and Vega et al. (1997) and their sources, and partly on our own data and those of the La Natividad Cooperative. The abalone fishery at La Natividad probably started in about 1956 (Guzmán del Próo 1975). The mean annual catch was initially 980 t (in shell weight) declining to around 500 t after 3 years. Catches are presented from 1960 in Figure 3 and remained stable until about 1975, when a decline set in. Four species were exploited, *Haliotis fulgens* Philippi, *Haliotis corrugata* Wood, *Haliotis sorenseni* Bartsch, and *Haliotis cracherodii* Leach. Until about 1970, *H. corrugata* comprised 70–80% of the catch, but with the decline of that species, *H. fulgens* and *H. sorenseni* were exploited more intensively. By 1976, the fishery for *H. sorenseni* had collapsed, and later catches were small and incidental; the species was formally protected in 1995. *H. cracherodii* was apparently exploited only sporadically and in small amounts between 1974 and 1984. The quantities taken are not known, but in the period 1981 to 1988, were only about 1.5% of the total catch (Lelevier-Grijalva et al. 1989).

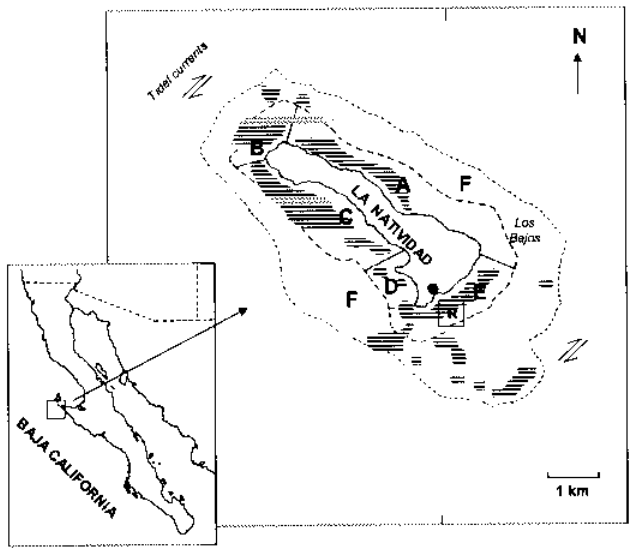

Figure 1. Map of the island of La Natividad. The location of the fishery reserve is shown as R. The directions of tidal currents are indicated with arrows. The zones of the fishery are indicated by the letters A–F. Shaded areas around the Island are *Macrocystis* beds. The isobaths are 10 and 20 m, respectively.

Management

From 1956 to 1984, the fishery was managed by size limits (which were rarely observed) and seasonal closures. After the 1984 collapse, stricter controls were gradually introduced and more rigorous stock assessments carried out. Legal minimum lengths (LML) were enforced for the first time. These were: 145 mm shell length (SL) for *H. fulgens* and 135 mm SL for *H. corrugata*.

Quotas were introduced in 1981. After preliminary surveys in 1985, annual surveys were done from 1988 with divers from the cooperative supervised by research personnel. The methods are given in detail by Reinecke-Reyes (1997) and summarized below.

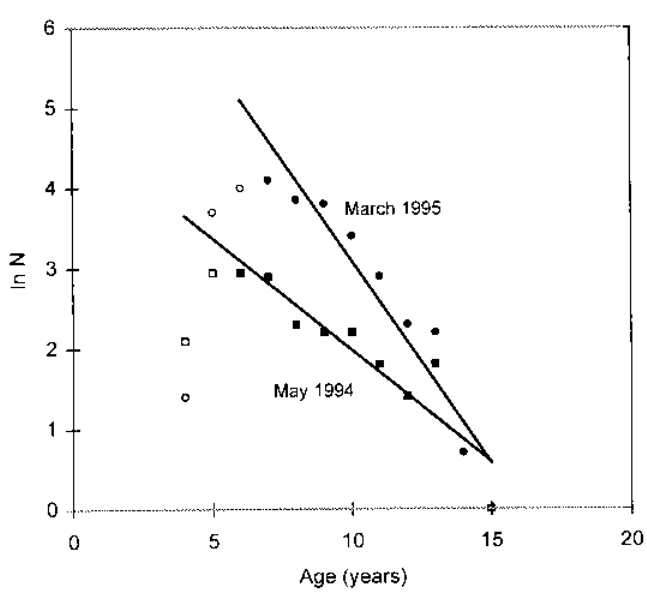

Figure 2. Catch-curves i.e. age frequency plots from which Z is calculated as the negative slope of the regression fitted to the solid data points (those age groups fully recruited to the fishery) for *H. fulgens* in May 1994 and March 1995.

The areas of abalone-bearing reefs were estimated by using buoys to delimit their boundaries and global positioning system (GPS) fixes on the buoys to map their extent. Abalone reefs to 20 m depth around the Island (Fig. 1) were divided into a 500-m grid to give blocks of 500 × 500 m, with minor adjustments for smaller areas according to the geography. In each block 8–10 samples, each a 5 × 2 m transect, were taken by a random sampling strategy, stratified by depth. All abalone in the samples were taken to the surface and measured in the boat and a subsample retained for determination of total weight, meat weight, and reproductive state.

The survey data were analyzed to give estimates (and standard errors) of: (1) total density; (2) density of those below; and (3) of those above LML. From the length frequency data, the mean weight of abalone in each 5 mm SL category was estimated.

From 1990 to 1995 quotas for each block were fixed at 30% (in the last year between 15 and 30%) of the legal-sized abalone in each block, and the annual quota was the sum of the quotas for each block. In 1996, quotas were based on the estimated biomass of prerecruits predicted to recruit into the fishery during the year. In 1997, a more elaborate method was used (see FAO 1982). The Hilborn and Walters (1992) dynamic version of the Schaefer surplus production model, modified to include the environmental effect of mean sea temperature anomalies (see Sierra et al. 1997), was used to estimate the surplus available for capture. Bayesian techniques were used to revise the estimates based on the comparison of catches of previous years and the calculated surpluses estimated from the survey data. A decision table was drawn up for different levels of quota and the associated risk of decline. Quotas were fixed by negotiation between the Instituto de Pesca (CRIP) and the cooperative. In addition, by internal regulation of the divers by the cooperative, size limits of the major two species were increased by 5 mm SL from 1985, and by a further 5 mm in 1997. A small area of about 15 ha at the southern end of the island, was also designated a fishery reserve in 1985 (Fig. 1).

Oceanography and Subtidal Ecology

The coast of La Natividad is influenced by the California current, which causes strong upwellings from February to June each year that lower sea temperatures to about 12–13°C. From October to January, the current is displaced seaward, and the Davison countercurrent brings tropical waters northward and elevates temperatures to about 19–20°C (Arntz and Fahrbach 1996). During El Niño years, when the upwellings are weaker, both maximum and minimum temperatures may be increased by 3–4°, and *Macrocystis* may disappear for some months or even a year (see Shepherd and Avalos-Borja 1997 for temperature data).

Forests of *Macrocystis pyrifera* dominate inshore habitats with extensive reef of high relief to about 20–25 m depth (Zones B–E) around the island, except for Zone A on the northeastern coast, which is largely sandy bottom with only a few low density stands of *Macrocystis*. Other common subdominants are *Eisenia arborea*, *Pelagophycus porra*, and the seagrass *Phyllospadix torreyi*. Deeper bottom, called Los Bajos, much of it rubbly or with boulders and some outcropping reef, from 12–30 m deep (Zone F) has a red algal flora of *Corallina* spp. *Bossiella*, *Gelidium* spp., *Rhodymenia* spp., and *Plocamium coccineum*, which also extends up into the *Macrocystis* forest (see Guzmán del Próo et al. 1972 and Serviere-Zaragoza this volume).

For *H. fulgens*, the most productive zones are Zones B, D, and E at the northwest and southeast sides of the island (1994 mean

legal densities 0.25–0.3 m^{-2}); Zones A and C are moderately productive (1994 legal densities ~0.15 m^{-2}), and Zone F the least productive with low legal densities in 1994 of <0.05 m^{-2}. For *H. corrugata* the deeper Zone F, and those at the extremities, B and E, are more productive (1994 legal densities 0.1–0.15 m^{-2}) than Zones A, C, and D (1994 legal densities 0.05–0.1 m^{-2}). The proportional production from the six zones for 1994 is given in Table 1.

MATERIALS AND METHODS

Catch and effort data are published by Anuarios Estadisticas de Pesca, and more detailed data are archived in the La Paz office of CRIP. Catch data for individual species are available only from 1986, so for the earlier years, we used the proportions derived in market measuring programs and summarized from various sources by Guzmán del Próo (1992). For missing years, we interpolated and smoothed proportions.

Catch per unit effort (CPUE) data in kg/diving team/day were considered by Prince and Guzmán del Próo (1993) to be a crude index of abundance, in contrast to most other abalone fisheries Breen (1992), because divers use lead-weighted boots to walk over the bottom. Hence, searching is not dependent on the presence of aggregations. The total catch is highly correlated with CPUE ($r = 0.90$; $p < .0001$), so in this paper, we have used the total catch as a crude index of abundance.

The annual survey data were used to estimate density of recruits and total numbers. Recruitment values were: for *H. corrugata*, the density of individuals 40–110 mm SL, corresponding roughly to the 2+ and 3+ age classes combined, and for *H. fulgens*, the density of those 50–100 mm SL, corresponding to the 2+ age class. Mean SST anomalies of ocean waters adjacent to central Baja California, originally from the COADS data set (Roy and Mendelssohn 1994), were extracted from Vega et al. (1997).

EPR Analysis

An analytical model was used to examine the effect of size at first capture and exploitation rate on the egg production of each species. Modeling was done with the equations given in Nash (1992), which are age-structured dynamic pool models based on Baranov's (1918) classic catch equation. The spawning potential of each age class is summed from the age of reproductive maturity to maximum age (20 y) for an unfished stock to produce the total egg production per recruit. This is the well-known measure of population finess, R_0 (Stearns 1992). The spawning potential of the fished stock, under chosen levels of natural mortality, M, is expressed for a range of levels of fishing mortality, F, and sizes at first capture, as a percentage of R_0 to produce the percentage egg-per-recruit (% EPR) values plotted as isopleths.

The parameters given in Table 2 were used as inputs to the model. The von Bertalanffy growth parameters for *H. corrugata* are the means of the two sets of values given by Shepherd and Avalos-Borja (1997), and for *H. fulgens*, are those in Shepherd and Turrubiates-Morales (1997). The length–weight equations are unpublished data of one of us (J. T-M), and the fecundity equations, for the nearest known populations in the absence of data from La Natividad, are from Shepherd et al. (1991) for *H. fulgens* and from Ortiz-Quintanilla and Gonzalez-Aviles (1986) for *H. corrugata*.

Natural mortality rates, M, summarized by Shepherd and Breen (1992), are poorly known. Published values of 0.35–0.43 by Doi et al. (1977) and Marín (1981) for *H. corrugata* were based on unsubstantiated estimates of longevity of 15–20 years. They also overestimated growth rates that would have contributed to the overestimation of M (see Prince and Guzman de Próo 1993). The value of 0.2 for *H. corrugata* by Tutschulte (1976) and 0.07 for *H. fulgens* by Olsen (see Shepherd and Breen 1992) may be more realistic than other published values. We have recorded longevities of 25 years for both species at La Natividad, which would suggest a value of about 0.18 using Hoenig's (1983) formula (see Shepherd and Breen 1992).

The method of fixing quotas used until 1996 should also give a crude estimate of F. The capture of 30% of the legal-sized stock gives an estimate of $F = 0.36$. However, the mean estimate of Z for the years 1992 to 1995 for both species was only 0.38, suggesting that estimates of abundance and/or of M are very low. Taking these considerations into account, we have used two values of M, 0.15 and 0.25, which should encompass the range of likely values.

Total mortality rates, Z, were obtained by catch-curve analyses, using the shell-aging techniques of Shepherd and Avalos-Borja (1997) and Shepherd and Turrubiates-Morales (1997). Fishing mortality rates can be derived from the equation $Z = F + M$.

TABLE 1.

Proportional production of *H. fulgens* and *H. corrugata* for each area shown in Figure 1.

	A	B	C	D	E	F
H. fulgens	0.15	0.18	0.33	0.03	0.08	0.24
H. corrugata	0.20	0.09	0.25	0.04	0.03	0.39

TABLE 2.

Parameters used in the EPR analysis. Standard errors are in parentheses.

Parameter	*H. corrugata*	*H. fulgens*
Growth		
K	0.29	0.32
L_∞	165.0	183.3
T_0	0.6	0.8
Length–weight		
$A \times 10^{-5}$	1.38	2.24
b	3.50	3.36
r^2	0.89	0.94
N	418	314
Fecundity		
c	——	−0.61
d	——	0.0026
e	0.037	——
x	2.19	——
Size at sexual maturity (mm)	103.5	121.5
Total mortality (Z)		
c. 1980	0.28 (0.02)	——
1992	0.38 (0.02)	0.43 (0.03)
1993	0.38 (0.02)	0.34 (0.04)
1994	0.40 (0.04)	0.29 (0.06)
1995	0.66 (0.05)	0.51 (0.06)
1996	0.27 (0.13)	0.39 (0.07)

Equation for length–weight is: $W = a L^b$, for fecundity is $F = c + d W$ or $F = e L^x$.
W in g; L in mm; F in millions of eggs.

RESULTS

The catches of *H. corrugata, H. fulgens,* and *H. sorenseni* (the proportions were estimated in some early years, see Methods) and a mean annual catch rate (where available) from 1960 to 1996 are shown in Figure 3, together with the survey data since 1985. *H. sorenseni* suffered an early demise after about 7 years fishing and after that was taken in very small numbers (a few kg day^{-1}) until 1995, when it was protected. Catches and catch rates of the remaining two species declined to a low level in 1980 reaching a minimum in 1984, thereafter catches increased somewhat before the last decline in 1992 to 1996. Density data for the annual surveys show a continuing decline in abundance of *H. fulgens* since 1985, but some increase in abundance for *H. corrugata* since 1985.

We examined correlations between total catch and SST anomalies separately for the "stable" period of the fishery (1965 to 1980) and the period from collapse to the present (1980 to 1996) for lag times of 7–9 years, which covers the period from larval settlement to entry to the fishery. We excluded data from 1960 to 1964, because animals caught in these years would have been spawned by virgin or near-virgin stocks. For the period 1965 to 1980, the total catch was significantly correlated with SST anomalies when the catch was lagged 8 years ($r = 0.50$; $p < .05$). In the second period, correlations were significant for 7- and 9-year lags in the catch ($r = 0.6$–0.8; $p < .01$), but highest for an 8-year lag time ($r = 0.86$; $p < .001$).

Catch-curves published by Shepherd and Avalos-Borja (1997) show that *H. corrugata* is partially recruited to the fishery at age 5, and fully recruited at age 8. The principal ages exploited in 1995 were 6–10 years (87% of catch). Overall, Z has increased gradually since 1979 to the present, implying a gradually increasing F from about 0.1–0.5. Recent catch-curves for *H. fulgens* (Fig. 2) show that this species is partially recruited to the fishery at age 3 and fully recruited at age 6 or 7. The principal ages exploited are 6–10 y (71%). Z has varied during the 1990s (Table 1) averaging about 0.39, indicating a likely range of F of 0.15–0.25.

EPR values at M = 0.15 and 0.25 (Fig. 4) show that %EPR conserved for *H. corrugata* has declined from >50% in about

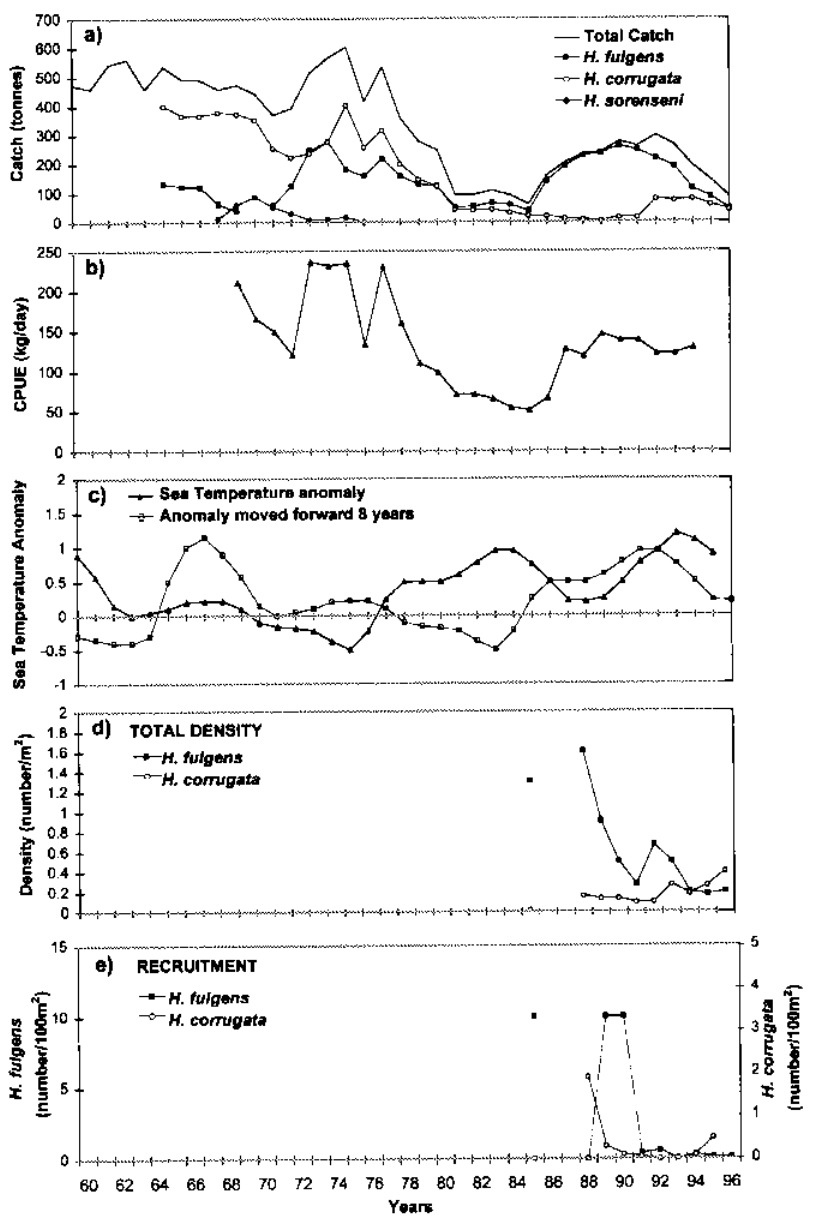

Figure 3. a. Catch in tonnes total weight of *H. corrugata, H. fulgens,* and *H. sorenseni,* individually and combined, from 1960 to 1996. b. Catch per unit effort (CPUE) in kg per diving team per day for all species combined for 1960 to 1996. c. Mean sea temperature anomalies (°C) for the central Baja California Pacific coast from 1960 to 1995, and the same anomalies moved forward 8 years. d. Survey density data (total numbers m^{-2}) for *H. corrugata* and *H. fulgens*. e. Recruitment density (numbers 100 m^{-2}) for *H. corrugata* and *H. fulgens*.

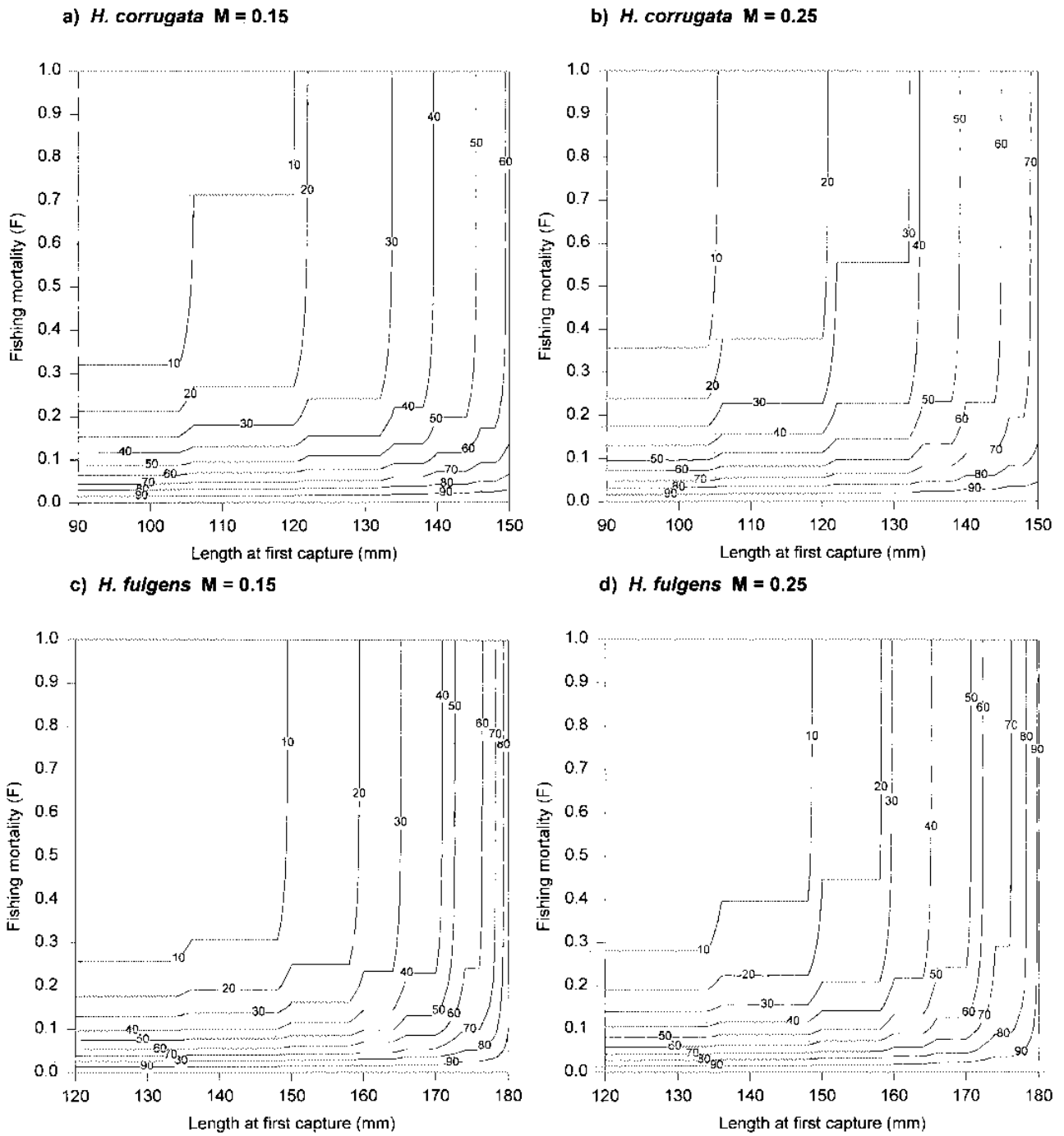

Figure 4. Egg-per-recruit isopleths for *H. corrugata* (a,b) and *H. fulgens* (c,d) at M = 0.15 and 0.25 for various fishing mortality rates (F) and lengths at first capture.

1980, to 30–50% in 1990 to 1994, to 20–40% in 1995 at the ranges of F given. Values during the 1970s when the catch declined seriously are conjectural. However, we note that at F = 0.25, which seems reasonable in the open habitat of *H. corrugata* the %EPR conserved would have been <40% at the higher M value and <30% at the lower value. The known widespread failure to observe size limits would reduce these percentages slightly. Overall, the different values of M caused differences of 10–20% in %EPR.

For *H. fulgens* different M values had even greater relative effects (Fig. 4). At M = 0.15, %EPR was about 20% from 1985 to 1994, declining to <10% in 1995. At M = 0.25, %EPR was about 40–50% until 1994, falling to <20% in 1995. These %EPR values do not take into account the additional egg production conserved in the reserve area, which contains predominantly *H. fulgens*. Assuming that F is 0.25, which is probably a conservative estimate given the high effort in the fishery, then the %EPR conserved before the 1984 collapse is <20% over the chosen range of M.

DISCUSSION

The catch history of the two major abalone species suggests an initial period of stability from 1960 to about 1975 and a second

period of instability characterized by two declines from 1975 to the present (see Vega et al. 1997). Assuming an average lag time of 6–8 years from larval settlement until entry of recruits into the fishery the data suggest that recruitment overfishing may have occurred as early as 1971 to 1973 to produce the severe declines in the catch in 1978 to 1980. During this period, mean densities of both species of abalone in neighboring cooperatives where catches followed the same downward trajectory fell from 0.18 m^{-2} to 0.03 m^{-2} (Guzmán del Próo 1989, Guzmán del Próo 1992). We assume that densities would have similarly declined in La Natividad. Such a decline in density would have had serious consequences for fertilization success as well as over-all egg production (see Shepherd and Partington 1995). The likely low %EPR values of 20% for *H. fulgens* and the margin value of ~35% for *H. corrugata* support our recruitment overfishing hypothesis whatever value of M is chosen. On the latter point, we note that our study, as with most EPR analyses (e.g. Nash 1992) are sensitive to changes in M.

Following the 1984 collapse, the densities of both species have been low, despite the increase for *H. corrugata,* and it is likely that the Allee effect is operating to reduce still further fertilization success. Shepherd and Partington (1995) found that a critical density was about 0.2 m^{-2}, below which the risk of recruitment failure was high.

What evidence is there that oceanographic events have contributed to the changes in abalone abundance since 1975? The major feature in the region is the episodic El Niño referred to above. El Niño events during the period of interest were in 1972 to 1973, 1982 to 1983 (strong), 1987, and 1991 to 1992 (strong). *Macrocystis* beds disappeared from around La Natividad in late 1983 and in late 1991 (Shepherd and Avalos-Borja 1997, Shepherd and Turrubiates-Morales 1997). The El Niño could affect abalone populations in several ways. First, the higher temperatures could reduce algal food abundance, as well as eliminating the *Macrocystis* canopy (Tegner and Dayton 1987, Dayton et al. 1992, Tegner et al. 1997). The absence of the *Macrocystis* canopy during spawning could affect recruitment, because abalone larvae, without a canopy to retain them close to the reef system are more likely to be transported away. Jackson and Winant (1983) have shown that kelp forests attenuate water movement and can retain larvae for the whole of their larval life within a forest. Similarly, McShane et al. (1988) postulated that abalone larvae were retained around natal reefs by macroalgal canopies of *Phyllospora comosa,* a much smaller species than *Macrocystis.* On this point, note that the most productive zones around La Natividad are at the northern and southern extremities, where *Macrocystis* forests are the most extensive and the most dense (Fig. 1). Second, the reduced abundance of algal food could cause reduced fecundity, as in California (Tutschulte and Connell 1981, Tegner and Dayton 1987), or could lead to increased M in the population either by starvation or poor physiological condition and greater susceptibility to predation. These possibilities are not mutually exclusive, but note that two of them act at the recruitment stage and one (increased predation) at the adult stage. Shepherd and Avalos-Borja (1997) and Shepherd and Turrubiates-Morales (1997) showed that growth checks were laid down in the shell of both species during El Niños and gave evidence that they coincided with high temperatures and spawning. However, they found no significant differences in growth rates of *H. corrugata* during the El Niño as compared with later years. This implies that low food algal abundance, if it occurs, is unlikely to cause increased M.

The strong, positive 7–9 year lag time correlations between total catch and SST anomalies; that is, the period from larval settlement to age of capture, suggest that SST anomalies have a positive effect on recruitment. This is in accord with the findings of Shepherd et al. (1985) and Forster et al. (1982) that higher sea temperatures generally were more favorable for abalone recruitment. Elsewhere, El Niño events are known to enhance recruitment of many mollusks (Tarazona et al. 1985; Wolff 1985), including *H. corrugata* (Tegner and Dayton 1987). On the other hand, there is some evidence of poorer recruitment during some El Niño years. No recruitment of the 2+ age class was recorded in the 1993 survey data for either species, nor for *H. corrugata* in 1985, both two years after strong El Niños. Although the survey data are inadequate to estimate recruitment accurately, they may be sufficient to record serious recruitment failures, so it is possible that these El Niños adversely affected abalone settlement. Upon this hypothesis we would expect to have seen downturns in the catch some 6–9 years after the El Niño when "El Niño" year classes enter the fishery.

Downturns certainly occurred in 1978 to 1981 (following the 1972 El Niño) and in 1993 to 1996 (following the weak 1987 El Niño), but not in 1988 to 1992 (following the strong 1982 to 1983 El Niño), when there was an upturn. At present, the evidence is still equivocal, but we hypothesize that SST anomalies have a positive effect on recruitment, but that very strong El Niño events; that is, those in which the *Macrocystis* disappears; may have a negative effect.

The survey and stock assessment procedures at La Natividad present a number of problems, some of them considered by Lelevier-Grijalva and Cabrera-Muro (1997). The proportion of abalone habitat sampled (~0.04%) is very low and should be increased by an order of magnitude (cf. Shepherd and Baker 1998). This would give better estimates of recruitment and reduce the sampling bias of commercial divers, who tend to set the transect line where they can see abalone. Routine age determination of catch samples from the different zones of the fishery would provide valuable information on fishing intensity and allow the use of age-structured models.

Vega et al. (1997) observed an inverse correlation between SST anomalies and the total catch of abalone from Baja California without applying a lag time. They inferred an indirect causal relationship; that is, that above average sea temperatures decreased algal food abundance, which presumably made the abalone weaker and more susceptible to predation. However, we have already shown that the strong 1991 to 1992 El Niño did not reduce the mean growth rate of *H. corrugata,* although growth was temporarily interrupted. So, on the present evidence, this hypothesis is unconvincing. Myers and Cadigan (1993) and Myers and Pepin (1994), in support of Hjort's (1926) hypothesis, argue that, although oceanographic instability does cause recruitment variability, it operates largely at the presettlement phase of the life history. Hence, we prefer to seek a mechanism operating at the recruitment phase, which, in these abalone populations, would only become evident in the adult population some 6–9 years later, rather than a direct causal relationship, as suggested by Vega and colleagues. If our hypothesis is correct, then the productivity of abalone should increase over the next few years at La Natividad following the warming trend from 1988 to 1993, except that the strong 1991 event may have caused a recruitment failure, which would become apparent in 1998 to 1999.

However, what do SST anomalies mean? As pointed out by Bakun (1996), they are the net outcome of the interaction between the California and Davison currents and include a suite of oceanographic features, such as enhanced Ekman transport and wind-

mixing index. Positive anomalies correspond to a general intensification of the over-all dynamic system. The effect of positive anomalies on abalone at La Natividad is still unclear. Observations by one of us (JRT) suggest that the spawning season is truncated into 1 or 2 months. The survey recruitment data are inadequate to support the generally positive effect of positive anomalies on settlement implied by our analysis. Therefore, our hypotheses on this are necessarily provisional, and we cannot pretend to place much confidence in the observed correlations or use them as predictive tools without much better recruitment data.

The most plausible interpretation of the fishery data is that recruitment overfishing has been the proximate cause of the declines and that climatic variability has contributed to variability in recruitment. Management of the fishery by quota without regard to such variability must almost inevitably lead to decline caused by the practical difficulties of estimating the annual replacement rate. One interpretation of the increasing statistical significance level of the lag time correlations between the two periods is that the influence of environmental effects has increased as the fishery has declined. This accords with the view of May et al. (1978) and the findings of Shepherd and Baker (1998) that, as productivity of a population declines, recruitment variability increases. At low levels of abundance, the fishery seems to be increasingly driven by climatic effects, or at any rate, climatic effects become more conspicuous.

The question arises as to the diagnostic value of EPR analyses in this abalone fishery. Shepherd and Baker (1998) reviewed the persistence of abalone fisheries worldwide and showed that only those fisheries that conserved %EPR values of 40–50% were sustained in the long term; small populations required much higher values. An implication of their model is that if large populations are fished down to low levels of productivity, the consequent increased recruitment variability will require the conservation of relatively greater egg production (see May et al. 1978) to prevent an ongoing decline. Low egg production in the 1970s sufficiently explains the failure of the *H. fulgens* fishery and is a likely explanation for the decline of the *H. corrugata* fishery. However, after the 1984 collapse, egg production has been conserved at much higher levels, but the populations still have not recovered their former productivity. Vega et al. (1997) suggested that a lower productivity state may now exist for exploited abalone in Baja California, and this is consistent with our analysis of the La Natividad fishery. Shepherd and Baker (1998) proposed a similar low density equilibrium (see Connell and Sousa 1983) for the West Island abalone population in South Australia. Therefore, we hypothesize that restoration of the fishery to its former higher level of productivity will require a much higher level of egg production for some years to come. A benefit, other than that of higher productivity, is that increased population size will help insulate the fishery against recruitment fluctuations. Greater egg production could be achieved by increasing the size limit, reducing F, setting aside more fishery reserves, or a combination of these measures. Recent quota reductions and increased size limits are a step in this direction.

The use of fishery reserves to increase egg production is novel in abalone fisheries, but is potentially a very effective and efficient way of increasing egg production (Baker et al. 1996). The reserve is well located in dense *Macrocystis* forests at the southern end of the Island, where larval retention is likely to be high. The major practical problem of reserves in abalone fisheries; that is, the difficulty of enforcement, does not exist in this fishery, because divers are directly controlled by managers who live on the Island.

The current method of setting quotas in La Natividad is sophisticated and, arguably, more advanced than anywhere else in the world. México is the only country that seriously attempts to estimate absolute abundance and to base quotas on that estimate. Unfortunately, as elsewhere, analytical capacity exceeds understanding of the key biological processes. Thus, we still know very little about stock–recruitment relations for these or any abalone or about the effect of oceanographic large-scale events on the life history. The task for the future is to measure these effects and provide a sounder basis for the models already in use.

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AN EVALUATION OF THE ANNUAL QUOTA MECHANISM AS A MANAGEMENT TOOL IN THE MEXICAN ABALONE FISHERY

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ABSTRACT Abalone constitute one of the most valuable natural resources on the west coast of the Baja California Peninsula, México. Because of its economic importance, federal agencies and fishers' organizations have devoted special attention to management of the abalone resource. Total allowable catch per cooperative (TACCs) have been in effect since 1973. However, criteria for quota assessment have changed widely, over time, toward scientifically based management. We review three quota criteria that have been considered during the history of the fishery: (1) constant exploitation rate; (2) prerecruitment index; and (3) dynamic model biomass estimations. We compare these results with a newly proposed criterion derived from the Sinclair method. It seems clear that, although TACC-based regulation may be considered the best option for management to date, great efforts should be made to reduce the discrepancy between different criteria. Moreover, it is our belief that before any further progress can be achieved regarding TACC management, a more complete understanding is required of the effects on abalone populations of factors other than the fishery, such as the warm and cold water events, El Niño and La Niña.

KEY WORDS: total allowable catch per cooperative (TACC), abalone, fishery, México, El Niño

INTRODUCTION

Traditionally, abalone and lobster fisheries have been the basis of social and economic development for most of the human settlements along the west coast of the Baja California peninsula. At present, some 30 communities depend heavily upon these resources. Abalone production is highly meaningful, not only from a social perspective, but also as an important source of foreign trade for the State of Baja California Sur (nearly $30 million US per year, Vega et al. 1997, Ponce-Díaz et al. this volume).

Because of its social importance, abalone exploitation rights were allocated exclusively to cooperative organizations by legal documents as early as 1936. These exclusive rights were in effect until 1992, when federal legislation was modified, and species allocations were removed, although geographic concessions are still in effect (Secretaría de Pesca 1992). Although the 1992 legislation permitted the participation of the private sector, this requires the agreed transference of concessions from cooperatives to the private sector, which could occur only after specific approval by authorities. For abalone, no such transfer has occurred, and harvesting is still due only by the cooperatives.

Two abalone species represent nearly 97% of the total production (León-Carballo and Muciño-Díaz 1996): green (*Haliotis fulgens*) and pink abalone (*H. corrugata*). The remaining landings include red (*H. rufescens*), black (*H. cracherodii*), and white (*H. sorenseni*) abalones.

Abalone landings peaked during the 1963 to 1964 season, at 3,500 tons of meat and declined to about 400 tons until the 1982 to 1983 season. Although some recovery was observed during the late 1980s, production has not surpassed values >1,000 tons since 1979 (Fig. 1).

At the same time, the number of people who depend upon this resource has increased because of population growth (nearly 40% during the last 7 years, INEGI 1991; Ponce-Díaz et al. in press). Although some plans and projects are under development to provide an alternative economical activity for these communities (Ortega-Rubio et al. in press), at present, the abalone fishery is still their major source of income. This has resulted in increasing concern among federal authorities and fishers' organizations.

Management is based primarily on a zonal limited entry system, with the addition of regulation strategies that have included minimum legal size, closed seasons and annual quotas, complemented by internal controls of the cooperatives (Vega et al. 1997). Biomass evaluations have been made by direct and analytical methods (Doi et al. 1977, Guzmán del Próo 1992, 1994, Prince and Guzmán del Próo 1993). Population censuses by the Instituto Nacional de la Pesca and the cooperatives have been conducted annually since 1989.

Total allowable catch per cooperative has been in effect since 1973. It may be that TACCs are among the most important management strategies for this fishery. However, criteria for TACC assessment have varied widely over time, ranging from simple negotiations between cooperatives and federal authorities during the early years, to estimations based upon the application of dynamic biomass models. The main criteria and temporal changes are summarized in Table 1.

Until 1988, TACCs were determined after simple negotiations with the participation of fishers' organizations and federal authorities. Although technical considerations were always taken into account (such as recently observed trends in total catch and catch-per-unit-effort), fishery-independent estimates on the existing potentials were unavailable. Therefore, decisions were more frequently based on social, economical, and political considerations than on scientific population estimates.

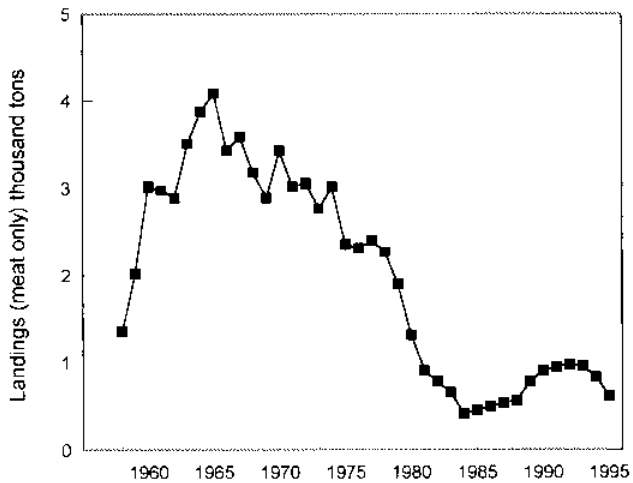

Figure 1. Abalone landings in the central zone of the Baja California Peninsula.

However, as the fishery yields declined, the need for technical information on the status of the populations became a priority. Fishers' organizations developed important technical capability and strong collaboration between organizations, and authority was established towards a scientific assessment of the abalone populations.

Since 1989, TACCs estimates have been based on annual population censuses and structure in each fishing area. Censuses are a joint effort between fishers' organizations and the Instituto Nacional de la Pesca (INP). INP is the Mexican federal agency responsible for stock assessment for fishery management. From 1989 to 1994, the main condition for TACC estimations was that quotas should not exceed 25% of the fishery biomass (i.e., organisms at or larger than the minimum legal size). The basic assumption behind this criterion was that the remaining 75% of the population was large enough to assure recruitment, at least equal to the extracted fraction, thus allowing a sustained or even an increasing biomass.

In 1989 to 1990 and 1990 to 1991 the TACCs were given directly as biomass (i.e. total weight). Fishers' organizations suggested, and obtained agreement, that quotas should be started in terms of the total numbers of abalones. These organizations believed that this would encourage the extraction of larger individuals rather than smaller ones. In fact, this resulted in an increase in the average size of abalone harvested above the minimum legal size (up to 8 mm in some fishing areas). Because this result was considered desirable from a fisheries perspective, quotas were given in numbers of organisms from 1992 to 1994.

TACC criteria were changed again in the 1995 to 1996 fishing season in response to the decreased catches since the early 1990s. In 1995, the primary goal was to maintain extraction below recruitment levels. Thus, the strategy was to keep the TACC less than the number of prerecruits. In 1995, prerecruits were animals smaller than 10 mm for green and 5 mm for pink abalone. However, recent data have demonstrated that the annual average growth of these organisms is much greater. In addition, it was agreed that, regardless of these criteria, TACC was not to exceed 25% of the number of legal size and larger individuals.

For the 1996 to 1997 fishing season, TACCs were estimated under a totally new approach. This new approach used the application of state-of-the-art fishery models as management tools, and in particular the dynamic biomass model of Hilborn and Walters (1992). To have the new approach accepted, the INP conducted a series of workshops with fishers' organizations and independent research institutions. These workshops concluded that the model, as originally formulated, was unable to describe the abalone fishery properly because of the effects of climate variability on abalone abundance (Lluch-Belda et al. in press).

A modified version of the Hilborn and Walters (1992) model was applied. This attempted to incorporate the effect of climate variation on population growth by using sea surface temperature anomalies as coefficients affecting population growth rate (r), with negative (positive) sea surface temperature (SST) anomalies increasing (decreasing) r. The model was fitted using Bayesian estimations, and different catch level scenarios were tested using risk analysis. Selected TACCs were those resulting in projected biomass equal to current levels.

TACC-based management for the Mexican abalone fishery has not been reviewed in terms of yielding the desired goal. Such reviews have been done for other abalone fisheries, results indicate that TACCs usually are not based on systematic stock assessment, mainly for reasons of cost (Shepherd 1992). In the present paper, we attempt a similar review of the advantages and disadvantages of the different criteria that have been either used or proposed for estimating TACCs within the Mexican abalone fishery.

DATA AND METHODS

We qualitatively analyzed the historic TACC criteria effects on abundance, as inferred from trends on catch and catch-per-unit-effort (CPUE) for all the central zone and for the period from 1956 through 1995. Despite limitations of considering CPUE as an index of abundance, harvesting capabilities have remained relatively unchanged during the considered period (León-Carballo and Muciño-Díaz 1996), so it is safe to consider that significant CPUE changes are caused by variations in abundance.

We quantitatively compared TACC estimates by different methods, using catch and census size composition data for the period 1995 to 1997 for one cooperative considering three fishing areas from Isla Natividad, BCS (see Fig. 2). All data were provided by the Federación de Cooperiativas de Baja California.

Estimates of TACC for the 1997 fishing season were attempted for the three selected areas under the following considerations: (1) a quota equal to 25% of the available biomass during 1996 (similar to that considered during the 1989 through 1995 period); (2) a quota given by the prerecruitment index during the previous year; and (3) an approach using the total allowable catch (TAC) for each area and species, estimated during the 1997 fishing season. These

TABLE 1.

Criteria used for TACC definition in Mexican abalone fishery.

Criteria	Period
Evaluation meetings (i.e., direct negotiations)	1973–1988
25% of available biomass quota in biomass	1989–1992
25% of available biomass quota in biomass and number	1993–1994
Prerecruitment index, less than 30% of legal size	1995
Modified H & W* model leveled biomass	1996

* H & W = Hilborn and Walters 1992.

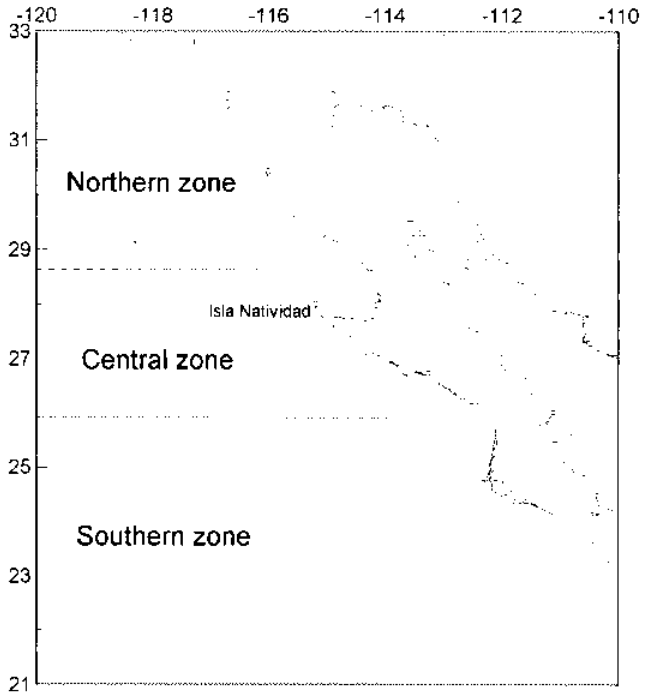

Figure 2. Study area.

estimates were based on the results from the Hilborn and Walters (1992) model needed to maintain the biomass level. Although all other TACC estimates are presented as biomass and numbers, in this analysis, only biomass TACCs are available.

Besides exploring those criteria already in use, we tested an alternative approach for TACC computation for the same areas and species using the following expression (Sinclair 1993):

$$TACC = \{[W_i * N_i * (1 - e^{-Z}) * F_i]/Z_i\}$$

where: $_i$: size; W_i: weight at size i; N_i: population number at size i; Z: instantaneous rate of natural mortality; and F_i: instantaneous rate of fishing mortality at size i.

To use this expression, we estimated the von Bertalanffy (1938) growth equation parameters for green and pink abalone. Also, length–weight relations were determined for each area and species. Total and fishing mortality rates were estimated as described by Pauly (1983). F_i values were estimated from virtual population analysis (VPA, Jones 1984).

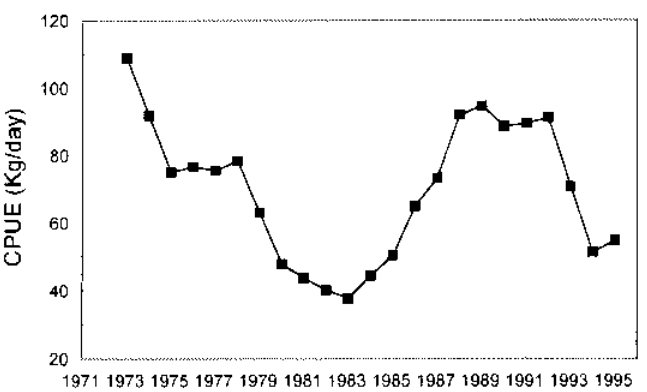

Figure 3. Catch-per-unit-effort in the central zone of the Baja California Peninsula.

TABLE 2.

Yearly proportion of available biomass allocated to total TACC in the central zone of the Baja California Peninsula.

Fishing Season	% of Available Biomass
1989–1990	25.02
1990–1991	25.09
1991–1992	24.77
1992–1993	25.11
1993–1994	25.19
1994–1995	24.76

RESULTS

Figure 3 presents the CPUE series for the central zone since 1973, when TACC management was initiated. Table 2 presents the percentages of available biomass of individuals at or above the minimum legal size set as total TACC for the entire fishery from 1989 to 1994 (25% criteria, Table 1).

The von Bertalanffy growth equation parameters and the natural and total mortality rates for each species are summarized in Table 3. Table 4 shows an example of the computations of TACCs using the Sinclair (1993) expression for only green abalone and fishing area A. Finally, Table 5 includes all TACC estimates for each area and species for the 1997 fishing season.

DISCUSSION

Figure 3 shows that the period from 1973 to 1988, when TACCs were decided during evaluation meetings, was one of high variability of fishery yields. CPUEs were stable from 1973 to 1977, after 1977, values dropped to their minimum during 1982. The lowest levels coincided with El Niño of 1982 to 1983. El Niño had a major adverse effect on giant kelp beds along the central zone; however, it is evident that CPUE was decreasing several years before that El Niño event (Fig. 3).

CPUE progressively increased during the 1980s and was at a high level when TACC criteria changed in 1989. Despite the limitations of assuming CPUE as an index of abundance, catches also increased during the same period (Fig. 1). However, with CPUE and catch trends the only available data, it is difficult to assess how the criteria for TACC determination caused the increase in yields.

Despite the high levels of CPUE and catch, the adaptative management used until 1988 was considered too empirical, so in 1989, a new technically based TACC approach was used. For this second criterion, TACCs were at equal to 25% of the fishable biomass. Table 2 presents the proportions that were actually ap-

TABLE 3.

Parameters of the von Bertalanffy growth equation, total and natural mortality estimated as described by Pauly (1983) for green and pink abalone at Isla Natividad.

Variable	Green Abalone	Pink Abalone
L inf.	241.196	196.469
K	0.227	0.303
to	0.951	0.789
Z	1.580	1.380
M	0.570	0.730

TABLE 4.

Example of TAC computation of green abalone in area "A."

Size Class mm	Wi	Ni	Fi	Zi	TAC Kg	TAC n
147	0.213	3,074	0.000	0.570	0.00	0
152	0.238	6,148	0.011	0.581	21.46	90
157	0.266	7,173	0.037	0.607	87.43	329
162	0.296	13,321	0.167	0.737	672.10	2,270
167	0.328	9,222	0.193	0.763	578.07	1,760
172	0.363	19,469	0.538	1.108	2,587.15	7,124
177	0.400	15,370	0.709	1.279	2,569.79	6,418
182	0.440	5,123	1.354	1.924	1,195.93	2,716
187	0.483	8,197	1.012	1.582	1,907.81	3,951
192	0.528	4,099	3.188	3.758	1,383.98	2,620
197	0.577	3,074	0.000	0.570	0.00	0
202	0.628	1,025	0.750	1.320	275.60	439
Total n 95,295			Total TAC 11,279.32			27,717
Percentage of total biomass 29.08						

proved by the federal authorities. The criteria were strictly enforced, and deviations from the 25% reference level were, consequently, very small. This management strategy corresponded to stable CPUEs and catch until 1992, when decreases in catch and CPUE took place.

Because the proportion of extracted biomass remained relatively constant, and this proportion initially allowed the sustained yields observed up to 1991, some factors in addition to fishing pressure must have affected abalone abundance. The 1992 to 1994 declining period corresponded to an extraordinary warm period. It was the first time every recorded that warm water conditions persisted for such a prolonged time. Therefore, it seems reasonable to suggest that under these adverse conditions, TACCs based on a fixed criteria (25% of fishable biomass) are too high to support recruitment rates sufficient to replace the extracted biomass. It is not clear if this change in quota definition from biomass to numbers in 1991 played any role in setting TACCs too high. At this point, it is very clear that further research is needed to understand and evaluate the impact of TACC and weather on the population biology.

An attempt to reverse this situation was made in 1995. That year TACCs were defined on the basis of the estimates of present-year recruitment: the number of legal-size abalones entering the fishery size class during that season. In general, these criteria resulted in lower TACCs, because estimations of recruits varied from 8 to 25% for the more important green abalone. The TACC was set at 30% of the fishable biomass for pink abalone, because recruit estimations were generally in excess of the 38% value.

The reduction in TACCs enabled some increase in CPUE during 1995, although catch still decreased. However, the criteria resulted in a significative increase in available biomass for 1996 (Fig. 3).

At present, it is difficult to make recommendations regarding the use of any particular TACC criteria. Results of the comparative analysis (Table 5), clearly show that different criteria result in different TACC estimates. Pink abalone total biomass was estimated as nearly 6,000 kg by the Hilborn and Walters (1992) model; whereas, the Sinclair (1993) equation resulted in an estimate in excess of 20,000 kg. Moreover, differences were greater when specific areas were evaluated (five times greater for green abalone in area A).

However, some general trends might be inferred from the results (Table 5). First criteria can be divided into two groups regarding TACCs estimated: the first group includes the fixed 25% and the Sinclair equation results, and the second the recruitment-based and the Hilborn and Walters (1992) model. However, the estimates from the first group are much higher than those of the second. At this point, we do not have enough evidence to decide which group drives more accurate TACCs.

CONCLUSIONS

It is clear that TACC-based regulation is far from perfect. At least regarding the Mexican abalone fishery, it is clear that quota definition is at present the most useful management tool available. It is important to improve TACC criteria to reduce the uncertainty caused by different criteria yielding different results.

In particular, useful information may come from the evaluation of which of the two groups provides TACC estimates enabling

TABLE 5.

Comparison of all criteria used in TACC definition for the 1996–1997 fishing season at Isla Natividad.

	Kilograms				Number			
	E Fixed 25%	Prerecruitment Index	H & W[a] Model	Sinclair[b] Equation	E Fixed 25%	Prerecruitment Index	H & W[a] Model	Sinclair[b] Equation
Green abalone								
Zone A	8,799	1,796	3,362	11,279	23,824	4,862		27,717
Zone B	4,662	2,105	1,789	5,597	13,310	6,011	—	13,302
Zone C	16,850	6,973	6,909	16,528	52,650	21,788	—	43,260
TACC	30,311	10,874	12,060	33,404	89,784	32,661	—	84,279
Pink abalone								
Zone A	4,412	2,681	1,364	5,363	18,751	11,393	—	20,319
Zone B	1,740	1,099	680	968	7,744	4,891	—	3,776
Zone C	12,603	8,180	3,949	13,785	56,193	34,143		52,501
TACC	18,755	11,960	5,993	20,116	82,688	50,427	—	76,596

[a] Hilborn and Walters 1992.
[b] Sinclair 1993.

high and sustainable yields. This is not an easy task, because population size may be affected greatly by climate. Conservative estimates, from recruitment-based TACCs and the Hilborn and Walters (1992) model, may prove prudent because of the effects of El Niño and associated warm water events. Higher estimates under the fixed 25% criteria and the Sinclair (1993) equation may allow more income to the fishery whenever favorable environmental conditions occur. Currently, the very strong El Niño is having wide and negative effects on Mexican abalone populations. Damage to kelp beds have probably resulted in food limitations for adults; some abalone are weak and easily removed from the bottom rocks by surf. No evaluations of the effects of the El Niño on larvae and juveniles have been conducted; however, spawning was observed 2–3 months earlier than usual. Because negative impacts are unavoidable, every effect should be made to evaluate these effects and incorporate the findings into new, more flexible criteria for TACC estimation.

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SOCIOECONOMIC CHARACTERISTICS OF THE ABALONE FISHERY ALONG THE WEST COAST OF THE BAJA CALIFORNIA PENINSULA, MÉXICO

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ABSTRACT This work describes the current characteristics of the abalone fishery along the peninsula of Baja California, México from a social and economic point of view. The species *Haliotis fulgens* and *H. corrugata* are the principal products of this fishery. During 1996, the abalone fishery had a total production of about 400 t (meat weight). The value of this production has been estimated to be 36 million dollars (US), considering the price of the end product is US $2,200.00 per box of 48 cans. This resource has been placed third in importance of export for México, because the price has increased substantially in recent years. In this paper, incomes and estimates of production costs for all aspects of the fishery within the Central zone of Baja California are given. The direct employment in the industry is about 450 employed in the catch phase and 350 at the processing plant. Along the central west coast of the Baja Peninsula, there are nine fishing cooperatives and 150 abalone fishing boats. In each are a diver, a boat captain, and an air handler. Each boat is 22-ft long, with a 55-HP motor and "Hookah" diving gear and compressor. Eight plants process the catch.

KEY WORDS: socioeconomics of abalone fishery, value of abalone

INTRODUCTION

Rarely are the economics and social aspects of fisheries considered in fishery analyses. However, such information is very important for a comprehensive evaluation of the fishery characteristics, the behavior of fishers, and insight into the effectiveness of current management.

Despite the high price and demand for abalone, there is little information relating these to the fishery. In México, a few authors have (partially) addressed these issues (Instituto Nacional de Pesca 1980, Mateus 1985, Ortíz and León 1988, Ortíz et al. 1992, Guzmán del Próo 1992, León and Muciño 1996). They point out the importance of the abalone fishery to many small towns in western Baja California and report on landing records and estimation of abalone revenues. Vega et al. (1997) describe the role of limited access in the development and management of the abalone and lobster fisheries along the Baja California peninsula, which provide the main support, origin, and development of many fishing villages.

With respect to similar fisheries, there is little information on this matter. For the California fishery, Tegner et al. (1992) analyzed the relation between abalone price and the consumer price index from 1973 to 1988. Farlinger and Cambell (1992) reported on variation of abalone price, diver's rent, and total value of landings between 1986 to 1988 for *Haliotis kamtschatkana* in Canada. Schiel (1992) analyzed the individual transferable quota systems for the abalone fishery in New Zealand and gave the total value of this fishery. Because more information is needed for the Mexican abalone fishery, in this paper we consider the social, legal, and economic issues.

APPROACH

Social aspects, including population statistics, were obtained from the National Branch of Statistics and Geography. The information on the legal framework is based on three legislative aspects of the Mexican Federal government that regulate activities on fisheries, environment, and organization of cooperatives (social organizations of fishers).

The economic information was provided by surveys of the producers, including managers and fishers. The data requested were the following: number of members per cooperative; income distribution within the crew; price trends; number of boxes of canned abalone produced; production costs of harvesting, processing, marketing; and costs of surveillance and management.

The seafood industry in the central zone of the Baja California Peninsula has eight plants that process abalone, among other kinds of catch. Two are in Bahía Tortugas, one on Isla Natividad, one on Isla de Cedros, two in Bahía Asunción, one in La Bocana, and one at Punta Abreojos. They employ a significant portion of the regional population, about 350 people. These facilities generally have many years of life left, except for a few that are now very old. However, all the plants, such as the modern plant on Isla Natividad, are adapting the Analytical Hazard Critical Control Point procedures.

PRINCIPAL FINDINGS

Social Elements

Populations and Communities Formed to Harvest and Market Abalone

The fishing activity that developed for lobster and abalone from mid-1900s on the western coast of the Baja California Peninsula was responsible for establishing villages in the region. Among the larger villages developed on this basis are Isla Cedros, Punta Eugenia, Bahía Tortugas, Isla Natividad, Bahía Asunción, Punta Pri-

eta, Punta Abreojos, and La Bocana, which in total now have a population of almost 10,000 people.

The geographical location of these fishing villages is shown in Figure 1. Those considered here are in the central zone of the Baja California Peninsula. The inhabitants of these places depend almost totally on the abalone and lobster fisheries (Table 1). Infrastructure of the villages is limited for various reasons, among them distance to other population centers, difficult access to these sites, and limited investment in this zone by state and federal governments.

Organization to Produce

The abalone resource in México has been developed by groups of fishers constituted into fishing cooperative societies to whom the Mexican government has granted the right to catch the reserved species since 1938. Since 1992, they have been governed by a revised plan of fishing concessions and permits.

Approximately 85–90% of abalone harvested in México are landed in the central region of the Baja California Peninsula. In this zone, there are nine fishing cooperatives that fish the abalone resource. These are: S.C.P.P. Pescadores Nacionales de Abulón, Buzos y Pescadores, La Purísima, Bahía Tortugas, Emancipación, California de San Ignacio, Leyes de Reforma, Progreso, and Punta Abreojos. The number of associates that participate in each of these cooperatives (Table 2) total 1,305 people. Of these associates, some work on abalone, others lobster, some on minor resources (e.g., snails) and some in the administration of the society. Some are devoted to labor directly related to the fishing, processing, and marketing of abalone.

Legal Elements

Law of Cooperative Associations

In recent years, the legal framework under which the activities of the cooperative societies in México are regulated has been modified. The object of making this change in the original law of 1938 was to modernize this framework, because the law did not reflect what, in reality, was happening. For this reason, the government wanted to modernize the constitution and administration of the cooperative societies and to help these organizations have greater entrepreneurial capacity, as occurs in other countries, without losing their identity in the collective effort.

However this modernization means losing government subsidies. This situation causes financial problems, because the cooperatives must spend their money on such social services as electricity, medical insurance, road maintenance, surveillance, and fresh water.

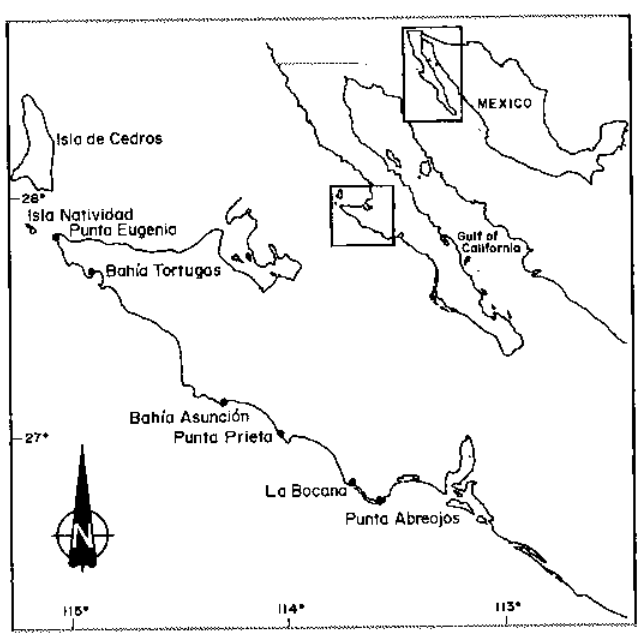

Figure 1. Geographic location of the central zone of the Baja California Peninsula, México.

TABLE 1.

Fishing villages in the central zone of the Baja California Peninsula, México

Village	Population
Isla de Cedros	2,696
Punta Eugenia	136
Bahía Tortugas	2,679
Isla Natividad	397
Bahía Asunción	1,381
Punta Prieta	189
Punta Abreojos	808
La Bocana	1,084
Malarrimo	15
Puerto Nuevo	113
San Pablo	22
San Roque	17
San Hipólito	107
Total	9,644

Federal Fishing Law

During 1992, in the wake of a process of modernizing the legal framework, the federal fishing law was modified. The goal was to strengthen the rational management of the fishing resources of the country and to promote responsible fishing. In addition, the law was designed to be simple and clear, to offer certainty to investors, and to permit the productive sectors to plan for the long term. This modified legislation had a new regulation for the cooperatives for fishing abalone and other resources. This regulation forms a concession that confers fishing rights for 20 years and is renewable.

General Law of the Environment and the Ecological Equilibrium

In relation to the environmental law, we can look again at the principal goals in terms of the concerns about the environment and its approach to work on a social basis to make decisions concerning the use, management, and conservation of natural resources. That implies encouragement of the co-management strategies for fishing purposes.

Economic Component

Abalone Production

The abalone fishery has been and continues to be one of the most important activities in the development of fishing communities in a region of huge importance for the country, which would be practically depopulated without it (Lluch-Belda et al. 1997). Figure 2 shows the historical landings for abalone in the central zone of the Baja California Peninsula, observing that the catch of

TABLE 2.

Production units within the central zone of the Baja California Peninsula, México

Cooperative Association	Year of Origin	Location	No. Members	No. Fishing Boats
Pesc. Nal. de Abulón	1942	Isla de Cderos	167	22
Buzos y Pescadores	1942	Isla Natividad	100	16
La Purísima	1944	Bahía Tortugas	139	11
Bahía Tortugas	1944	Bahía Tortugas	99	15
Emancipación	1939	Bahía Tortugas	97	22
California de San Ignacio	1936	Bahía Asunción	196	20
Leyes de Reforma	1974	Bahía Asunción	196	12
Progreso	1944	La Bocana	127	25
Punta Abreojos	1943	Punta Abreojos	184	10
Total			1,305	153

abalone reached almost 3,000 t annually at the end of the 1960s, reducing drastically by the mid-1970s to levels of only 500 t annually.

Apparently, the optimal levels of fishing (maximum sustainable yields) had been reached during the early 1960s, following expansion that supported high levels of harvest during several seasons. During the second half of the 1970s, the catch declined dramatically to historic minimums in 1983 to 1984 (Guzmán del Próo 1994) and again in 1995 to 1996. These collapses were attributed mainly to high fishing of the resource and, recently, to the combined effect of the level of effort, deficiencies in management, and, in a very important way, to broad-scale environmental changes, according to Lluch-Belda et al., (1997).

Currently the fishery remains a highly profitable activity, because of the high value of the product, but the catch levels are very low as compared to the levels of the 1960s and first half of the 1970s. The deficiencies that the combination of management measures traditionally have demonstrated call for adoption of multidisciplinary approaches (Vega et al. 1997) to permit the administration of the fishery concerning such matters as adjustable fishing and timely knowledge of changing environmental factors (Lluch Belda et al. 1997).

Value of Production

Income from Fishing

Use of the abalone resource involves several stages. The first is harvesting, in which a diver, a boat captain, and an air handler participate. These workers have become specialists. According to the internal organization developed by the fishing cooperative societies, the diver receives 50% of the value of abalone captured, the boat captain 25%, and the airhandler 25%, on average.

Price of the Final Product

Abalone are shucked, trimmed, and canned. Canning is the standard final product form of abalone. The market destination is export. The cans are 450 g total weight and 254 g drained weight. Estimates of the price of a box of 48 cans of abalone during different fishing seasons have been made. From these, we see an accelerated increase in recent years in the price of the canned abalone, up to $2,200 US per box of 48 cans (Fig. 3).

Total Income of the Fishery

The revenue for the fishery has been high, because of a substantial increase in price paid for abalone in the last 10 years. From the yield (cans) obtained from a ton of abalone and the selling price of a case of 48 cans in the 1966 season, it has been estimated that the total revenue of the Mexican abalone fishery is approximately, in current prices, $36,000,000 US (Fig. 4).

Costs

Production Costs (Catch, Processing, and Marketing)

For determination of the different production costs of cooperatives (direct costs), a survey among the producers showed these as average percentages of the main costs (Fig. 5). Production costs are composed of the following.

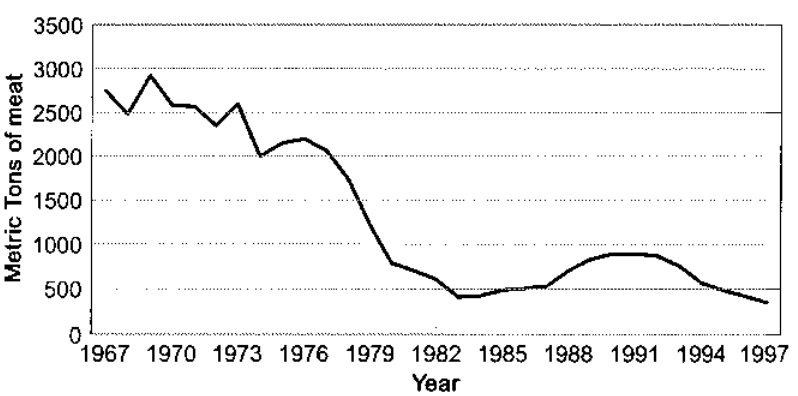

Figure 2. Abalone landings in the central zone of Baja California Peninsula, México.

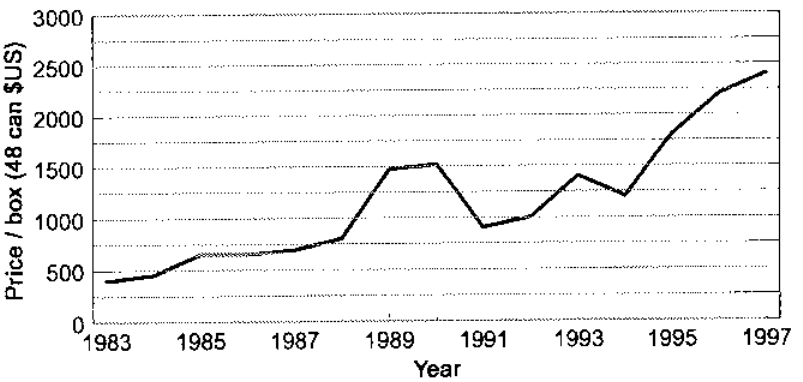

Figure 3. Abalone prices in the central zone of Baja California Peninsula, México

(1) *Harvesting.* These costs are those of the fishing phase of the abalone. On average, this cost is 67.96% of total production costs in the central zone of the Baja California Peninsula.
(2) *Processing.*—costs associated with the transformation phase of the product. Canning is done exclusively, because this is the basic form for the export market. It was 23.00%.
(3) *Research and managing costs (stock assessment, technician staff, etc.).* Because in recent years, this fishery has been administered under a quota system, it has needed an annual evaluation of the wild stock to be captured from the abalone banks in the future. Abalone beds are assigned to specific cooperative societies. Organizations making these surveys generally rely on technical personnel who make the evaluations and other fishing analyses together with the staff of the National Fishing Institute, whom provide technical advice to the Office of Fisheries Management.

Technical participation of the producers has a determined cost and that, according to a survey made among the cooperatives of the central zone of the Baja California Peninsula, is of the order of 3.87% of the total cost, considering the major elements; catch, processing, research, managing, and surveillance.

Surveillance Costs

The costs incurred in surveillance (5.17%) are those economic resources do this work by the social organizations. These tasks are to protect all the natural resources under concession. It is well known that abalone and the lobster are fisheries that experience major problems with furtive fishing activities. Poaching of abalone occurs in other abalone fisheries, however in Mexico fishing rights obligated the Cooperatives to spend more money on surveillance activities.

DISCUSSION

The abalone fishery in Mexico has been faced with problems of resource exploitation, broad-scale environmental change, new regulations, and increasing independence from governmental subsidy.

Environmental factors has been recently proposed (Lluch-Belda et al. 1997) as elements that have a significant impact on dynamics of abalone populations in Mexico. The impact associated with fishing effort has been proposed as a cause of recent declines in the Mexican abalone fishery. This has gradually limited the income of the people that depend for their livelihoods on this resource throughout much of Baja California.

Illegal fishing, which in the opinion of the fishers, has grown significantly, requires that each year more must be spent to curtail it. The abalone population biomass is lowered by poaching, and if this is not recognized and stopped, a drastic decrease in legal catch may ensue.

Along with the catch of abalone and lobster along the coast of the Baja California Peninsula, is the need to adapt alternative plans for fishing for finfish, snails, and macroalgae.

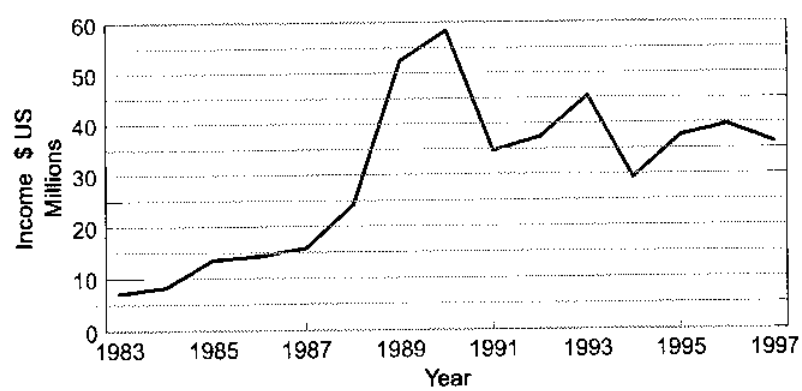

Fig. 4. Abalone income in the central zone of Baja California Peninsula, México.

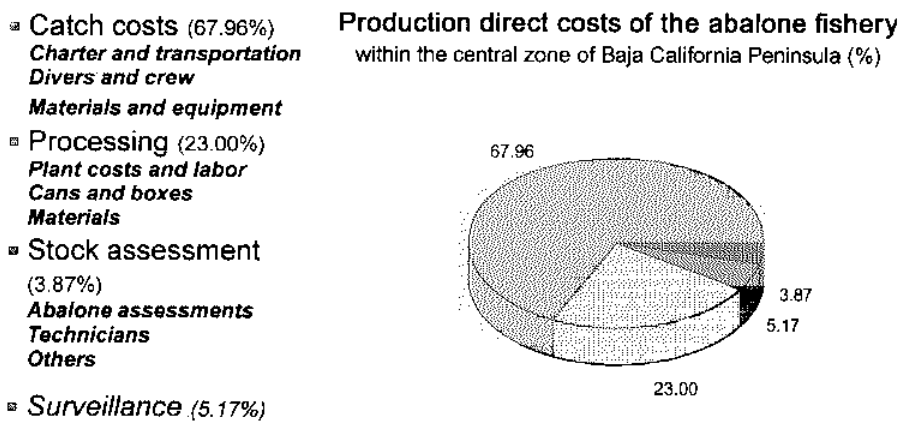

Figure 5. Production costs of the abalone fishery in the central zone of Baja California Peninsula, México.

Because the abalone fishery is in difficulties, it will be necessary to apply, quickly, programs based on aquaculture. The central zone of the Baja California Peninsula offers substantial opportunities for aquaculture development. These efforts should contribute to diversification of production and an increase in overall income.

Restocking is now in progress to enhance the abalone fishery. Its usefulness must be re-evaluated and, if necessary, should be increased.

The abalone fishery in México presently has low landings. This situation does not imply a drop in income, because prices have been increasing rapidly in recent years. In other places, abalone fisheries also have low landings (e.g., California and South Africa), but circumstances are very different in Mexico in terms of fishery, environmental, social, and economical conditions to generalize a single reason for the decline. Thus, management must be different and must respond to the specific problems of specific areas.

ACKNOWLEDGMENT

This work is dedicated in memory to G. León Carballo.

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POACHING PRESSURES ON NORTHERN CALIFORNIA'S ABALONE FISHERY

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ABSTRACT An examination of poaching pressure on red abalone (*Haliotis rufescens*) in Northern California, based on anecdotal accounts, courtroom testimony, newspaper reports, and situation assessments by resource professionals revealed a major threat to abalone resources and demonstrated the effectiveness of community involvement in resource management.

KEY WORDS: abalone, poaching, Northern California, HoJo

INTRODUCTION

The abalone fishery off Central and Southern California is in a state of collapse. A harvesting moratorium has been in effect for black abalone (*Haliotis cracherodii*) since August 1993. Harvesting of green (*H. fugens*), pink (*H. corregata*), and white (*H. sorenseni*) abalone ceased in January of 1996. The red abalone (*H. rufescens*) fishery was placed under an emergency closure in May of 1997 following a full season when less than 87 metric tons were landed commercially (K. Karpov, Associate Marine Biologist, CA Dept. of Fish and Game, Fort Bragg, 9/28/97 pers. tele. comm.). The situation is entirely different in Northern California, where less than 14% of the state's potential abalone habitat yields annual recreational fishery landings of red abalone estimated to exceed 900 metric tons (Karpov and Haaker 1997). The northern fishery flourishes and, assuming no major environmental changes, California resource managers expect that current harvest levels can be sustained indefinitely.

The stage was set for this study to contrast two adjacent fisheries of the same state almost 50 years ago by regulations that split California at San Francisco. Commercial abalone harvesting in Northern California was banned in 1949, and, in 1952, use of SCUBA gear for recreational harvesting north of San Francisco was also prohibited (California Department of Fish and Game 1995). Those regulations established *de facto* reserves in waters too deep or too remote for most recreational breathhold divers to reach. Estimated to encompass as much as 40% of total stocks (Karpov and Haaker 1997), the resulting refuge population may well be responsible for replenishing abalone taken from more accessible waters.

From about 1971, the economic value of abalone landings approximately doubled every 6 years (Lundy 1997); whereas, the commercially exploitable Central and Southern California fisheries steadily declined. Before the May 1997 closure, red abalone were worth as much as $50 apiece at the dock (California Department of Fish and Game 1995a). In Northern California, where it is easy to take 100 abalone per SCUBA tank, the 1-day, tax-free earning potential of an unrestrained poacher easily exceeds $3,000. One result of these financial incentives is that abalone poaching in Northern California is common enough to be seen by any interested observer. Law enforcement officials close to the situation estimate 4,800 abalone per diveable day are being poached from Northern California waters (*Sacramento Bee* 12/22–26/97).

SPORT VIOLATIONS

The most obvious poaching in Northern California consists of recreational harvesters committing violations of fish and game regulations involving undersized, over limit, or illegal possession of abalone out-of-shell. The California Department of Fish and Game periodically sets up roadside checkpoints. United States constitutional law requires that the location and times of these checkpoints be advertised in advance and that a randomizing method for vehicle selection be determined in advance. The occupants of each stopped vehicle are asked whether they have taken any abalone. If the answer is no and there is no evidence to the contrary, the vehicle is sent on its way. These checkpoints are neither intended nor expected to detect an alert or wary poacher. But they do provide some insight into the extent of sport violations. For example:

- A 1990 Sonoma County checkpoint inspected approximately 400 vehicles resulting in 36 citations and 120 warnings plus seizure of 175 abalone (*Santa Rosa Press Democrat* 6/25/90).
- The June 1997 Sonoma County roadside checkpoint inspected 200 vehicles, issued 28 citations, and confiscated 73 abalone (*Santa Rosa Press Democrat* 6/23/97).

The over-all impact of sport violations is generally limited to an increase in pressure on stocks already subject to recreational harvesting. When there is no financial incentive involved, petty poaching would be a minor concern except for the apparently large numbers of sport harvesters involved.

COMMERCIAL-TO-COMMERCIAL POACHING

At the other extreme of visible poaching activity is a small and elusive segment of the commercial diving industry that takes or creates opportunities to harvest abalone illegally in Northern California. Though well out-of-sight, the existence of this type of poaching is known and easily illustrated. Beginning in 1985, Northern California was the focus of a boom–bust cycle in commercial sea urchin fishing. Most of the abalone permittees from Southern California also have commercial sea urchin permits that enable them to move north and work Central California for abalone and Northern California for sea urchin. Landing records from the mid- to late-1980s document that some dual-permit divers were making landings of up to 564 abalone per vessel day and averaging three times the catch per unit effort (CPUE) as compared to Central California operators (California Department of Fish and Game 1/95). Suspicious boats were targeted for surveillance, which resulted in the 1990 seizure of two commercial boats for north coast poaching violations. Landing data available from as late as 1993 suggests that other operators continued with similar illegal activities (K. Karpov 9/28/97 pers. tele. comm.). Recently publicized cases further bolster assertions that commercially licensed divers continue to be involved in abalone poaching. For example:

- Fish and game wardens arrested a Half Moon Bay commercial abalone diver in January of 1996 following observations that he was diving in Fitzgerald Marine Reserve, a no-take preserve, during the closed month of January. Agents monitoring his movements became suspicious when one SCUBA tank stayed in the back of this truck while others were being refilled in a local dive shop. When the agents moved in, they found a SCUBA tank with a false bottom--perfect for hiding the seven abalone found inside.
- Two Santa Barbara commercial divers were arrested in August of 1996 for landing 46 abalone at Sonoma County's Fort Ross (*Santa Rosa Press Democrat* 11/6/96). Using radios to maintain contact with a third accomplice, these poachers used SCUBA gear for their illegal activities (Sonoma County Superior Court, testimony before Judge Frank Passalacqua 8/22/96). An earlier encounter with Northern California law enforcement officers indicates the August foray was not their first. For one of the commercial divers, the Sonoma County arrest and conviction landed him in state prison for 2 years when his probation was revoked by a Southern California court that had sentenced him earlier the same month for fish and game violations (B. Halsey, Jr. Dep. D.A., Sonoma County 9/30/97 pers. tele. comm.).
- Investigation of a Point Area bed-and-breakfast lodging that seemed to cater only to commercial sea urchin divers and tenders led to prison for those involved in what turned out to be an abalone for heroin operation. (M. Hee, Patrol Captain, Abalone Enforcement Coordinator, CA Dept. of Fish and Game, Sacramento, CA 9/23/97 pers. tele. comm.).

With the May 1997 closure shutting down the commercial abalone fishery throughout the State of California, concern over poaching by commercially licensed divers has been substantially reduced. The presence of more than sport quantities of abalone aboard any boat now constitutes *prima facie* evidence of commercial poaching that can lead to loss of boat and equipment; abalone poaching in Northern California has been too easy to risk valuable assets unnecessarily. Even so, sufficient evidence continues to accumulate to justify ongoing, close scrutiny of the commercial diving fleet working Northern California waters.

SPORT-TO-COMMERCIAL POACHING

Of much greater current concern to resource professionals and legitimate stake holders is the black market in poached abalone and those occasions where it has led to large-scale commercial operations. Historically, there has been an underground trade involving local restaurants and seafood markets willing to purchase or barter for abalone landed by individuals having nothing more than a sport fishing license. For example:

- The owner of a San Francisco seafood market was arrested on July 14, 1996, when she bought 20 kilograms of abalone from an undercover game warden after driving 100 kilometers to take delivery in a parking lot meeting place (*Santa Rosa Press Democrat* 7/15/96).
- A San Francisco Chinatown fish market owner was arrested on May 29, 1997 in possession of 75 kilograms of abalone on his business premises. The only available records to indicate where the abalone had been purchased were bank drafts made out to "cash" (*San Francisco Chronicle* 10/4/97).
- Two Fort Bragg restaurants in business for decades, one under the same owner, were successfully prosecuted based on information obtained from a poacher. When that same poacher was released from prison months later, he was again caught poaching abalone, and he revealed that one of the two restaurants was back in the business of buying poached abalone. The follow-up investigation led to a second round of arrests and the discovery of more abalone than could be accounted for by undercover transactions preceding the arrests (Hee 9/23/97 pers. tele. comm.).
- In 1996, an East Bay resident came under suspicion when it became obvious he was diving for abalone on a daily basis. A background check revealed that he was residing more than 150 kilometers from the diving locations, driving a new vehicle, and had no obvious source of income. The investigation that led to this arrest revealed he was taking his four abalone per day, processing his landings to maximize the value, and making daily deliveries to local sushi restaurants. A search of the individual's home turned up an additional 129 abalone (Hee 9/23/97 pers. tele. comm.).

More than 24 seafood markets and restaurants have been successfully prosecuted for Northern California abalone violations during the past 18 months, with more cases pending (Hee 9/23/97 pers. tele. comm.). Some of these relate to the traditional black market, and others are associated with a new class of poachers. These new participants make almost daily appearances on the coast, take their sport limits, and return to homes 150 to 300 kilometers away. Although more wary poachers tend to work alone, some of the newcomers have been observed traveling together in caravans with up to six people per vehicle.

- Thirty-five suspects were arrested August 9, 1995, when approximately 70 individuals were detected illegally working Fort Ross Terrace in the middle of the night (*Santa Rosa Press Democrat* 9/23/95). Having similar San Francisco home addresses plus common employers in the garment and hotel industries suggest the defendants were part of an organized group (Halsey 9/30/97 pers. tele. comm.). Five of the defendants had a total of 23 prior citations that included commercial poaching (more than seven times sport limit), undersized, and out-of-shell violations (*Santa Rose Press Democrat* 1/18/96; Sonoma County Superior Court, courtroom testimony before Judge Rex Sater 12/10/96).
- Four individuals were arrested north of Mendocino County's Navarro Beach on July 11, 1995, in possession of 309 abalone. About 90% were under legal size including some that were less than half the legal size minimum (*Santa Rosa Press Democrat* 7/14/95).
- Eight people were arrested June 5, 1996, in possession of 70 abalone taken from Fisk Mill Cove. They used SCUBA gear, camouflaged equipment, lookouts, and worked in relay teams to land the abalone (*San Francisco Examiner* 6/9/96). This group had one dive shop refuse to rent them dive gear when it became clear they intended to use it for north coast poaching activities; they went forward with their plans despite being told by dive shop employees the consequences of getting caught (Sonoma County Superior Court, testimony before Judge Mark Tansil 11/6/96).
- Surveillance of one Mendocino location during a middle-of-the-night low tide in August 1997 resulted in the arrest of 24 poachers. In the middle-of-the-night 2 weeks later, nine more individuals were arrested at a different site (Lt. S. Morse, Wild Life Protection, CA Dept. of Fish and Game, Fort Bragg, CA 10/8/97 pers. tele. comm.).

In some cases, small scale operations have tied in with international businesses involving millions of dollars in abalone poached from Northern California waters.

- The HoJo poaching ring began in the late summer of 1993 with two individuals selling part of their sport landings to local restaurants. Within a few months' time, more than a dozen individuals were involved, and a San Diego commercial fisherman was offering to buy as much abalone as the group could land. By the end of 1993, the San Diego contact had the poaching ring set up with a safe house near the coast, bought the equipment needed to properly pack and freeze the product, and established air freight and money exchange procedures to efficiently ship the product south. On receipt in San Diego, the abalone would be immediately transferred to another receiver in a chain that led to the Orient or the U.S. eastern seaboard. When free diving methods proved too inefficient to meet demand, he convinced the group to switch to the illegal use of SCUBA equipment that made it possible to remove and shuck 80 to 100 abalone per tank (Sonoma County Superior Court, courtroom testimony before Judge Bryan Jamar 7/24/96). This group of poachers ended up removing at least 20 metric tons of red abalone from five locations along a 10 kilometer stretch of coastline during the less than 1 year they were in operation.
- During pretrial interviews in the Van Howard Johnson case, statements were made about a larger operation that included the involvement of 12 commercial sea urchin boats based in Sonoma and Mendocino Counties (Halsey 9/30/97 pers. tele. comm.). Those statements in 1994 eventually led to the May 1997 arrest of a Los Angeles seafood processor and two of his associates following buys of abalone from a fish and game agent posing as a north coast sport diver (*Santa Rosa Press Democrat* 5/30/97, 6/3/97, San Francisco Examiner 6/8/97, *San Francisco Chronicle* 5/31/97). One of the participants in that illegal Southern California seafood processing business made statements to an undercover agent that they were paying commercial sea urchin boats in Bodega Bay as much as $45,000 per load for Northern California abalone (*Sacramento Bee* 6/9/97). More than 3,000 abalone, almost one metric ton of sea cucumber, and $20,000 cash were confiscated during the raid of their illegal processing plant (Hee 9/23/97 pers. tele. comm.). Bookkeeping records seized during the arrests document traceable business transactions totaling millions of dollars since 1994 (Halsey 9/30/97 pers. tele. comm.).

COUNTERMEASURES

Building concern over the blatant rise in poaching led to direct public involvement, which, in turn, spurred increased attention on the problem by government agencies at the local, state, and federal levels. The results have been impressive, although not necessarily publicized. Quietly processing cases through the legal system lessens the attention drawn to focused law enforcement efforts and potentially makes defendants more useful during follow-up investigations.

- The commercial boat seizures in 1990 led to the first use of felony conspiracy charges in cases involving misdemeanor fish and game violations. Convictions for felony conspiracy carry maximum sentences of up to 3 years in state prison, as compared to 1 year in county jail for a misdemeanor. In 1996, 16 felony convictions were obtained in Sonoma County abalone cases resulting in two 3-year and one 2-year state prison sentences, as well as numerous county jail sentences.
- The 1990 seizures also resulted in legislation that increased abalone poaching fines to $30,000. With penalty assessments tacked on, financial exposure for commercial poaching convictions now exceeds $80,000.
- Groups of as many as 45 recreational harvesters have demonstrated the importance of the poaching issue by sitting through courtroom hearings involving abalone poaching cases.
- Fines for petty sport violations in Sonoma County have been standardized at $500 for a violation involving one abalone, with $250 added for each additional abalone; for example, four undersized abalone equates to a $1,250 fine (B. Halsey, Jr. 7/24/97 presentation at Sonoma Co. Abalone Network gen. mtg.). If violations are associated with sport-to-commercial poaching, punishment for first time offenders has resulted in jail time with fines starting at $3,000.
- Virtually nonexistent during 8 of the last 10 years, state funding has been made available for law enforcement efforts that focus on abalone poaching. More than 200 court cases have been filed during the past 18 months involving the illegal commercialization of Northern California abalone (Hee 9/23/97 pers. tele. comm.).
- Businesses found to be purchasing poached abalone are now beginning to face civil suits for engaging in unfair business practices. Compared to criminal prosecutions, civil actions require a much lower standard of proof and the "fines" have no ceiling.
- Successful prosecution at the local level draws stepped up scrutiny by federal tax, customs, and marine enforcement agencies.
- Grassroots stewardship efforts have sprung up to educate recreational harvesters and build on peer pressure against petty poaching.
- Legislation that includes an annual fee on recreational harvesters as a means of funding abalone research and law enforcement projects received widespread and active support from the scientific and recreational communities and was signed into law October 8, 1997.

CONCLUSION

Abalone poaching in Northern California has been a serious concern for decades, and the escalating value of abalone provides good reason to expect it will continue. The public and private sectors have responded with a number of deterrents and have had some limited success. The question for the future is whether the resolve needed to counter the plundering of public resources can be maintained.

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THE RED ABALONE, *HALIOTIS RUFESCENS*, IN CALIFORNIA: IMPORTANCE OF DEPTH REFUGE TO ABALONE MANAGEMENT

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ABSTRACT Two different approaches have been used for management of red abalone, *Haliotis rufescens*, in California. In central and southern California, use of compressed air has been allowed for both commercial and sport fisheries, permitting take of red abalone throughout its depth range. In northern California, commercial fishing has not been allowed, nor has use of compressed air by the sport fishery, resulting in refuge from take at depths below 8.4 m. The central and southern California fisheries were recently closed because of stock declines, while the northern California fishery continues to provide high yields. Commercial catch and stock density showed parallel trends of significant decline at two of three southern California islands (Santa Rosa Island and Santa Cruz Island, but not San Miguel Island) between 1983 and 1996. At Van Damme State Park, a highly fished northern California site, stock density increased threefold between 1986 and 1992. Dive survey data indicate major recruitment events are sporadic in northern California. Density of young-of-the-year (YOY) recruits at Van Damme in 1990 to 1992 averaged three times that at Santa Rosa Island in 1978 to 1982. The proportion of red abalone larger than the sport size limit was 43% at Van Damme in depths >8.4 m, but was only 16% at depths <8.4 m, and 15% at Santa Rosa Island at all depths. Refuge by depth at Van Damme apparently protected sufficient adults from harvest during protracted periods of low recruitment to allow sustainable harvest.

KEY WORDS: *Haliotis rufescens*, density, abundance, refuge, young-of-the-year, recruitment

INTRODUCTION

The red abalone, *Haliotis rufescens* (Swainson), is found on rocky substrate in kelp forests along the entire California coast. In northern California, they occur most abundantly in subtidal areas in 7–8 m depth, and out to 25 m. In southern California, they occur deeper at depths of 12 to 25 m (Tegner et al. 1992).

Recreational and commercial red abalone fisheries declined in southern and central California since the 1970s. Commercial landings declined from an average 1,300 MT in 1955 to 1977 to 105 MT in 1982 (92% decline) (California Department of Fish and Game Landing Statistics). Continued decline led to closure of all abalone take in central and southern California in 1997. In marked contrast, a red abalone fishery continues to flourish in northern California (Tegner et al. 1992).

Tegner et al. (1992) contrasted the outcomes of two very different management approaches for red abalone in northern California versus central and southern California. In central and southern California, commercial take was allowed, and sport divers could harvest using SCUBA. In northern California, commercial take was prohibited and SCUBA is not allowed for sport take. The northern sport fishery take, estimated to range between 552 to 1,550 MT in 1985 to 1989 comparable to southern California commercial landings that averaged 900 MT from 1931 to 1968 (Karpov and Tegner 1992, Tegner et al. 1992).

Parker et al. (1988) found high densities of red abalone in underwater surveys of heavily fished areas in northern California in 1986 and suggested that refuge by depth may protect these stocks from overutilization. Here, we compared the outcome of two different management approaches for California red abalone. We address the question of whether refuge by depth provided protection for northern California red abalone.

METHODS

Our analysis focuses on fisheries-independent dive survey data, primarily in areas with time series. We also examine commercial landings at the southern California dive survey areas and sport fishermen intercepts (creel surveys) for Van Damme State Park (VDSP) in northern California.

Fishery-independent data were mostly in the form of two kinds of underwater surveys: "emergent" and "invasive." "Emergent" surveys are designed to enumerate and measure noncryptic (=emergent) juvenile and adult abalone that can be seen without the use of flashlight or moving the substrate (Tegner et al. 1989, Karpov et al. 1997, Kalvass et al. 1991, Davis 1985). "Invasive" surveys are designed to enumerate and measure both cryptic and exposed abalone using flashlights and overturning rocks (Tegner et al. 1989, Karpov et al. 1997).

Southern California

In southern California, three of the Channel Islands, San Miguel (SMI), Santa Rosa (SRI), and Santa Cruz (SCI) had time series dive survey data that provided abundance trends (Fig. 1). These included a dive survey by Tegner et al. (1989) from 1978 to 1982 at Johnsons Lee, SRI and Channel Islands National Park (CINP) surveys from 1983 to 1997 at SMI, SRI, and SCI (Davis 1985, Kushner et al. 1997). CINP surveys included 10 to 12 (10 to 1984 and 12 thereafter) annual 60 m^2 emergent transects at 16 stations (Davis 1985). Transects were randomly placed off a permanent 0.1-km leadline. Five of the 16 CINP locations sampled contained enough red abalone to be included in our analysis. These included Hare Rock and Wyckoff Ledge at SMI, Johnsons Lee, SRI, and Gull Island, SCI. These locations were selected by CINP as index stations to monitor change in macro-invertebrate assem-

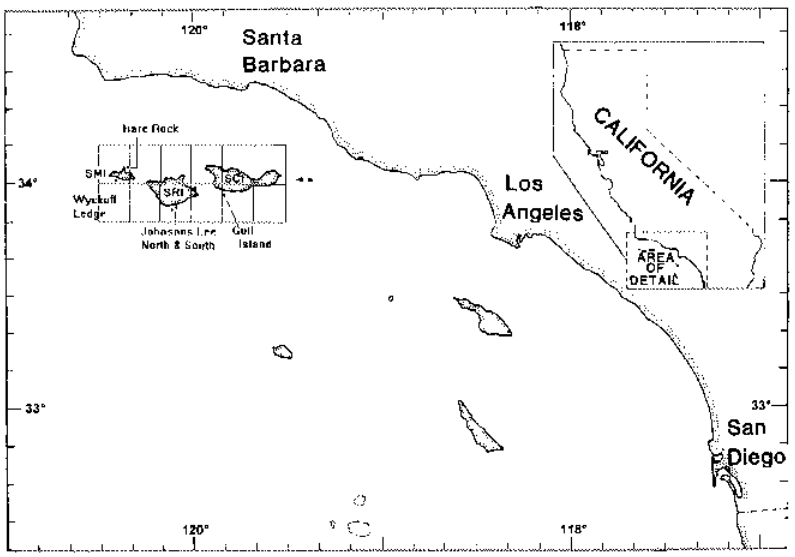

Figure 1. Southern California vicinity with San Miguel Island (SMI), Santa Rosa Island (SRI), and Santa Cruz Island (SCI). SMI, SRI, and SCI show commercial fishery sampling blocks and dive survey locations.

blages (Davis 1985). We pooled transects by island: SMI and SRI each included 24 transects annually and SCI 12 (Table 1). We also used emergent and invasive red abalone density and size data from Tegner et al. (1989) collected at Johnsons Lee from 1978 to 1982 as a basis for examining relative recruitment at VDSP northern California.

Commercial landing trends from 1981 to 1996 were also compared to red abalone densities at SMI, SRI, and SCI to validate independently if locations sampled by CINP were representative of red abalone abundance. Location of commercial abalone take is recorded on landing receipts by origin of catch. Catch is assigned by the processor to California Fish and Game blocks, each of which spans 10 minutes of latitude and longitude (Fig. 1). Two of the 12 blocks surrounding the islands, were shared by adjacent islands and catch data were allocated evenly between the ambiguous blocks.

Northern California

In northern California, our primary focus is at VDSP, where we examine red abalone abundance in a time series spanning 1986 to 1992, comparing fished to "refuge" (unfished) depths (Fig. 2, Table 1). We also examined red abalone abundance from other recent emergent dive surveys for a broad-based comparison between areas with refuge in northern California to areas without refuge in southern California. Parker et al. (1988) completed 114 emergent transects in 1986 at four high sport use sites [VDSP (25), Point Arena (16), Salt Point Park (31), and Fort Ross State

TABLE 1.

Red abalone dive locations, length of coastline surveyed, type (invasive or emergent), year(s), number of transects, depth range in northern and southern California.

Area of California Location (Source)	Coastline (km)	Survey Type	Survey Years	No. of Transects	Depth Range (m)
Northern	1.8	Invasive	1990 to 1992	60	2–18
Van Damme State Park	1.8	Emergent	1986, 1989 to 1992	120[a]	2–18
Cabrillo Reserve	1.3	Emergent	1986	30	5–17
Salt Point, Fort Ross, and Pt. Arena (Parker et al. 1988)	11.0	Emergent	1986	89	5–17
Sonoma and Mendocino (Kalvass et al. 1991 and Kalvass and Taniguchi 1993)	143	Emergent	1989 and 1991	66	5–17
Southern	0.2	Emergent	1983 to 1997	352	6–16
Hare Rock and Wyckoff ledge, San Miguel Island					
Gull Island off Santa Cruz Island	0.1	Emergent	1983 to 1997	176	15–18
J. Lee, Santa Rosa Island (CINPS)	0.2	Emergent	1983 to 1997	352	8–18
J. Lee, Santa Rosa Island	1.2	Invasive	1978 to 1982	166	7–16
(Tegner et al. 1989)	1.2	Emergent	1978 to 1982	83	7–16

[a] Includes 25 emergent stations surveyed by Parker et al. (1988) in 1986.

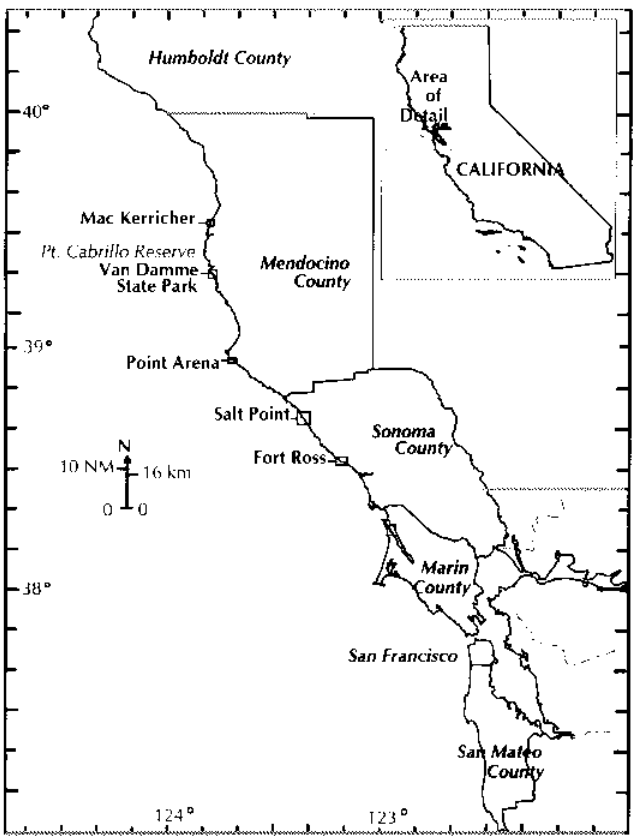

Figure 2. Northern California dive survey locations.

Park (42)] and also a no-take reserve off Point Cabrillo. Kalvass et al. (1991), and Kalvass and Taniguchi (1993) conducted separate broad-scale surveys in 1989 an 1991 in which 66 emergent transects were surveyed from Mac Kerricher to Fort Ross. We combined the two surveys for our comparison.

Free divers in northern California seldom dive deeper than 8.4 m to collect abalone. We segregated dive station results at VDSP by two depth strata "Shallow" and "Deep" using 8.4 meters as the dividing depth. An effort was made, each year, to randomly distribute dive station locations at VDSP throughout the study area by depth strata. In 1989 to 1992, an additional 95–30 m^2 emergent transects were added to the 25 completed in 1986 by Parker et al. (1988). Sixty invasive 10 m^2 transects were added in 1990 to 1992. Rough seas often forced our sampling to alternate reef sites in the more sheltered areas of Van Damme Bay. As a result of this procedure, most of our sampling locations were in close proximity to the areas of fishing concentration, because the fishery is also constrained by weather to targeting available reefs (Fig. 3a–c).

Creel surveys were conducted during spring minus-tides from 1990 to 1994 at VDSP. The location of fishing was recorded to the nearest 0.2 min of latitude and longitude, which allowed mapping fishing concentration (Fig. 3a). Transect locations were also mapped in a similar fashion, allowing direct visual comparison (Figures 3b,c).

Statistical Comparison

In southern California red abalone density differences between years were tested using one-way analysis of variance (ANOVA), followed by Scheffe multiple-comparison test to identify years that were the most significantly different. At VDSP, both density and sizes were tested for significance between year and depth strata using the two-way ANOVA. The model used was density or size = year|depth; where year and depth were class variables. Only emergent densities and sizes were tested at VDSP for difference over time. The invasive survey period was too short to evaluate time trends. Before applying one- or two-way ANOVA comparisons, densities were transformed using the method of Pearce and Hines (1987) (transformed density = Ln (density + 1)). Significance for all tests was at $\alpha = 0.05$. To examine size by density at VDSP, red abalone sizes were weighted by density at each transect. The weighted means were then combined for each depth strata and year to produce density–size frequency histograms.

RESULTS AND DISCUSSION

Southern California—Stock Abundance SMI, SRI, and SCI

Visual comparison of catch and emergent dive survey data show parallel trends at SRI and SCI, but not SMI (Fig. 4). Density

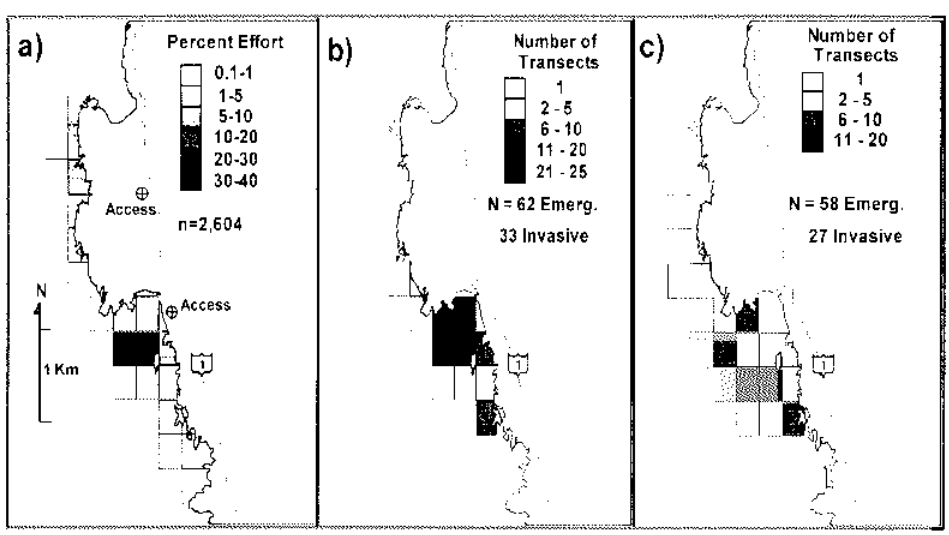

Figure 3. a) Distribution of abalone fishing effort from creel census interviews at Van Damme State Park access point, Mendocino County, California, 1990 to 1992; b) distribution of dive sampling at shallow (1.8–7.6 m); and c) deep (8.4–17.6 m) sampling stations from 1986 to 1992.

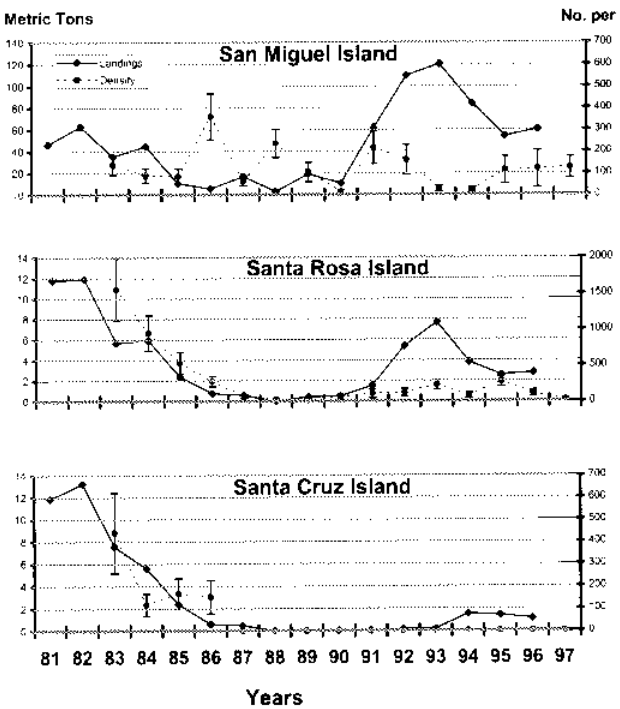

Figure 4. Red abalone commercial landings reported from fishery blocks adjacent to SMI, SRI, and SCI, and red abalone emergent dive survey densities and standard errors from those same islands.

differences were significant over time (ANOVA test, Table 2). SMI densities showed no definitive trend during this period, and catch increased to a peak 120 MT in 1993, a year of relatively low density (28 abalone-per-ha).

In 1997, an emergent dive survey was conducted by one of us (Haaker) at SMI. Stations targeted in the survey were selected as areas having high red abalone abundance, based on information offered by commercial fishermen. Density results ranged from 0 to 2,900 abalone-per-ha. Size frequency distributions from this survey showed a robust distribution of juveniles, and were similar to distributions found by Tegner et al. (1989) for SRI during 1978 to 1982. These results indicate that the CINP stations at Hare Rock and Wyckoff Ledge do not represent indices of peak red abalone abundance at SMI.

SRI showed a close correlation between catch and density for most years (Fig. 4). Density (abalone-per-ha) at Johnsons Lee declined from a maximum of 1,563 in 1983 to a low of 21 in 1997. Although abalone density did not change in 1993, commercial catch increased to 8 MT. Density differences between years were significant, with 1983 and 1984 as significantly greater than all other years (ANOVA test $p = .0001$ and Scheffe test Table 2). Tegner (1989) found emergent red abalone abundance showed no significant change from 1978 to 1982 at Johnsons Lee; density averaged 632 abalone-per-ha. In 1993, two of us (Haaker and Karpov) conducted 17 random emergent transects at Johnsons Lee to corroborate independently our findings on the CINP index stations. We found 60 abalone-per-ha (SE = 60), as compared to 223 abalone-per-ha (SE = 67) on the CINP stations. Collectively, the fishery-dependent and fishery-independent results provide convincing evidence of significant stock decline at SRI over the last 15 years.

SCI showed the most profound declines in both commercial catch and density (Fig. 4). From 1983 to 1996 commercial catch declined from 7.5 MT to 1.1 MT, and density declined from 440 to 0 abalone-per-ha. Density differences between years were significant, with 1983 greater than all other years (ANOVA test $p = .0001$ and Scheffe test Table 2).

Red abalone abundance differences at SMI, SRI, and SCI reflect differences in temperature regime and fishing pressure, with the only refuge provided by difficulty of access: that is, cold SMI, warm SCI, and intermediate SRI (Engle 1994). SRI and SCI are more readily accessible to sport and commercial fishers than SMI. Red abalone at SMI varied from year to year and showed no consistent trend. SRI and SCI underwent severe fluctuations and decline in abundance, probably because of combined effects of the 1982 to 84 El Niño and fishing pressure. Although our results were inconclusive, SMI would be expected to fare better because of its geographic position in the direct flow of the southward moving California current (Engle 1994).

Northern California—Van Damme State Park

VDSP (Fig. 2) is among the highest used area in the northern California abalone fishery. Fishing at VDSP is focused on a relatively small area: between 1990 to 1994, 57% of the fishing effort was concentrated in two adjacent grids of 21 hectares (Fig. 3a). Distances traveled from the parking areas to take abalone for divers swimming from shore averaged only 0.63 km (SE = 0.02), boat divers 0.83 km (SE = 0.02), and shore pickers 0.50 km (SE = 0.02). Despite the concentration of fishing effort, abalone density at VDSP increased over time (Fig. 5). Differences were significant by time and by depth (Table 3).

The increase in red abalone abundance from 1986 was most pronounced at shallow depths, reaching a plateau in 1989, and deep transects continued to increase through 1992 (Fig. 5). The 1986 deep water densities must be interpreted cautiously given the small number of deep stations sampled that year (six) relative to shallow stations (19). Our results suggest that one or more major cohorts, first seen as juveniles in 1989, replenished first shallow and then deep water stocks. One effect of refuge by depth was the

TABLE 2.

Red abalone one-way ANOVA probability values and Scheffe multiple comparison (years paired as significant) for transformed density by year (1983 to 1997) from emergent dive transects at San Miguel, Santa Rosa, and Santa Cruz Island.

Location	Years	ANOVA Prob. Values	Scheffe Year(s) Paired as Significant
San Miguel Island	1983–1997	0.001	None
Gull Island, Santa Cruz Island	1983–1997	0.0001	1983 vs. 1984–1997
Johnsons Lee, Santa Rosa Island	1983–1997	0.0001	1983, 1984 vs. 1985–1997

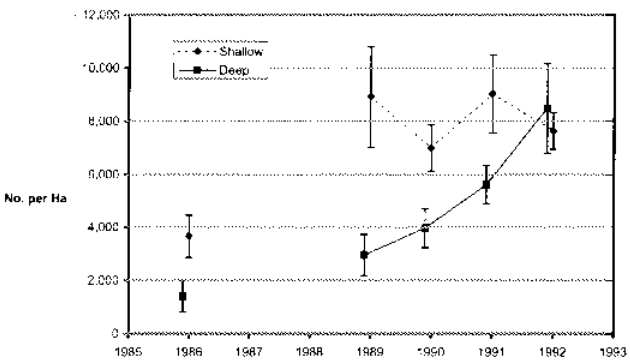

Figure 5. Densities of red abalone from emergent dive transects at Van Damme State Park in 1986 and 1989 to 1992.

increase in density of sport-legal abalone (>178 mm) at the deep but not shallow stations from 1991 to 1992 (Fig. 6).

The dynamics of the changes in emergent red abalone density become more understandable when changes in size and density are examined together over time (Fig. 6). Size–density distributions of red abalone showed no evidence of juvenile recruitment at either shallow or deep stations in 1986. In 1989, the shallow distribution reflected emergence of new cohorts. Juvenile recruits became a significant component of the deeper transect distributions in 1990 and later. Two alternate explanations are possible for these observations: (1) that YOY (size <31 mm) recruited in previous years at shallow depths only and were dispersed through movement to deep depths in subsequent years; or (2) separate recruitment pulses occurred first in shallow depths followed by a separate event deeper. Ault and DeMartini (1987) found onshore and offshore movement during winter and summer months, respectively, during an 11-year mark and recapture study off Point Cabrillo (Fig. 2). Tegner et al. (1992) reported that intertidal red abalone picked in northern California by shore pickers are replaced by shoreward movement.

Our results clearly show that major recruitment events are sporadic in northern California. Recruitment potential can be underestimated if only a single year with a low number of juveniles is examined. Tegner et al. (1992) compared emergent densities from Johnsons Lee to 1986 density distributions at four high-use northern California sites from Parker et al. (1988). All four northern California locations in 1986 showed similar narrow distributions with a high density of adults and few juveniles (Parker et al. 1988). Tegner et al. (1992) concluded that recruitment levels were lower in northern California relative to Johnsons Lee. When our 1990 to 1992 VDSP densities are added to 1986 the juvenile abundance was clearly greater than the Johnsons Lee densities from 1978 to 1982 (Tegner et al. 1989) (Fig. 7). Tegner et al. (1989) also found evidence for periodicity of recruitment success at Johnson's Lee. During their 5-year study they found YOY recruits ranged from 50 (SE = 50) to 360 (SE = 190), averaging 176 (SE < 190) abalone-per-ha (Table 4).

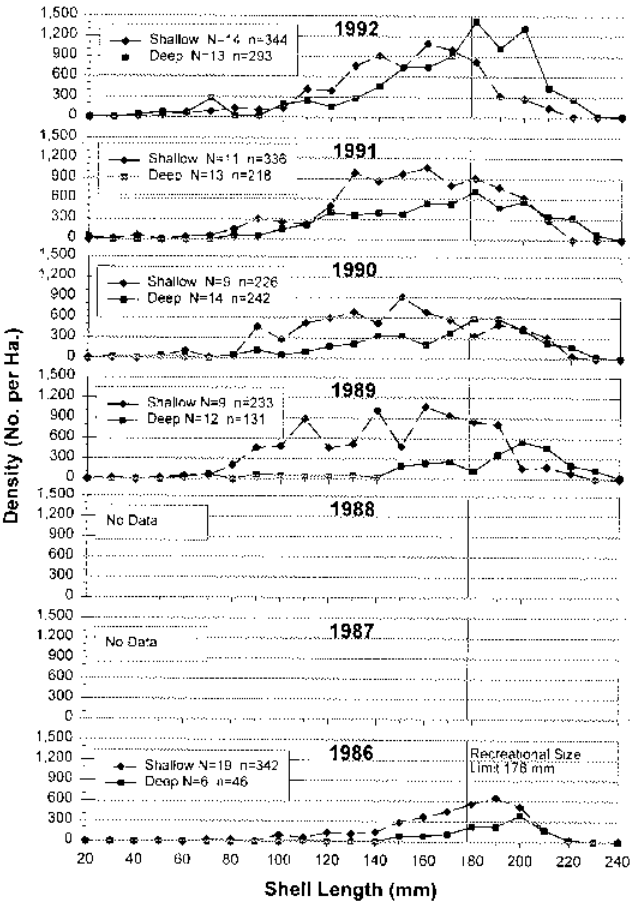

Figure 6. Density by length class for emergent red abalone sampled in dive surveys at Van Damme State Park. N = numbers of transects; n = number of abalone measured.

At VDSP the cryptic density of the population was about half the cryptic density observed at SRI. VDSP invasive shallow transects averaged 11,788 abalone-per-ha (cryptic plus emergent) (Table 4); whereas, emergent shallow transects (emergent only) averaged 7,765 (Table 5); thus the portion of cryptic animals is estimated at 34%. Deep transect densities were similar, with the portion of cryptic abalone estimated at 42% (Tables 4 and 5). The combined depths density of cryptic abalone is estimated at 39% (Tables 4 and 5). In comparison, red abalone at Johnsons Lee in 1978 to 1982 were 65% cryptic (1,790 invasive and 632 emergent abalone-per-ha, Tegner et al. 1989). The larger density of cryptic abalone in areas without refuge may result from fishing down of exposed emergent animals. An alternate explanation for these differences could be the result of differences in rugosity providing more cryptic habitat.

TABLE 3.

Red abalone two-way ANOVA probability values for size and log transformed density comparison by year, depth, and year/depth interaction for emergent stations at Van Damme State Park in northern California.

Comparison	Class Variable	Red Abalone
Density	Year	0.004
	Depth	0.005
	Year/depth	NS
Size	Year	0.0001
	Depth	0.0001
	Year/depth	0.0001

Invasive Survey Density and Size Comparisons—Northern California versus Southern California

Invasive size–density distributions allow direct comparison of YOY recruitment. Invasive surveys at VDSP showed similar levels

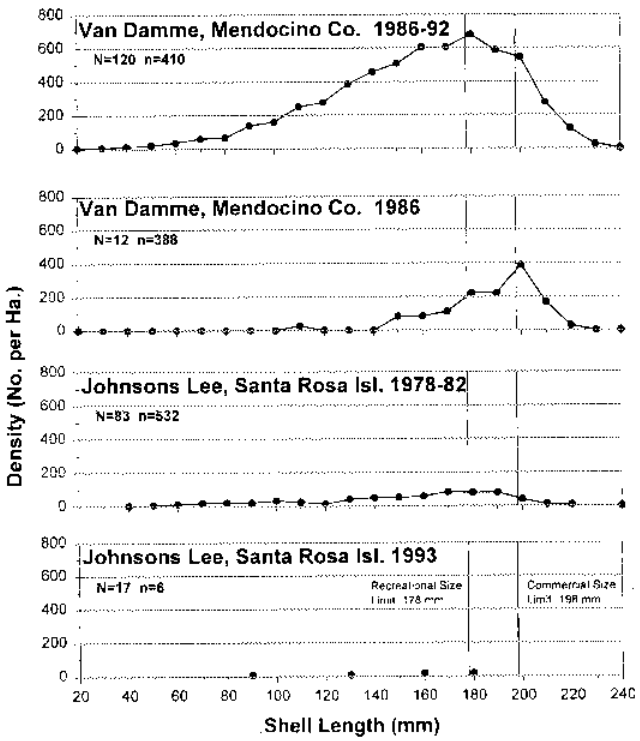

Figure 7. Density by size of emergent 1982 red abalone from Van Damme State Park and Johnsons Lee. Johnsons Lee 1978–1982 (Tegner et al. 1989) and Johnsons Lee 1993 (California Department of Fish and Game data). N = number of transects; n = number of abalone measured.

of YOY recruitment at shallow transects (666 abalone-per-ha, or 6% of total density) and at deep transects (446 abalone-per-ha, or 4% of total density) (Table 4). In contrast, YOY at Johnson's Lee in 1978 to 1982 averaged 176 (SE = 190) abalone-per-ha, comprising 10% of total density (Tegner et al. 1989). Thus, the percentage of recruits at VDSP was smaller than at Johnson's Lee, but the actual density of recruits was over three times greater (Fig. 8).

Invasive survey size–density distributions also allow comparison of the densities of sport legal (>178 mm) and commercial legal (>197 mm) abalone. At the VDSP shallow invasive stations, 16% were sport legal and 3% were commercial legal (Table 4). At the VDSP deep stations, 43% were sport legal and 18% were commercial legal (Table 4). These results demonstrate that refuge by depth combined with a period of good recruitment in northern California protects large adults from harvest at three times the density found at fished depths. At Johnsons Lee in 1978 to 1982, 15% (270, SE = 130 abalone-per-ha) were sport legal and 3% (50, SE = 35 abalone-per-ha) were commercial legal (Tegner et al. 1989).

Depth Refuge Effectiveness in Stock Abundance and Sustainability

Indirect proof of the large-scale positive effects of refuge can be seen in abundance differences and current status of the fisheries in the areas of California beyond those we examined for time series comparisons. Point Cabrillo Reserve is the largest no-take refuge in northern California (Fig. 2). In 1986, emergent red abalone abundance at this location was 12,139 (SE = 1,615) abalone-per-ha (Fig. 9). This was more than twice the emergent density at four popular sport use areas that included VDSP surveyed in 1986 (Parker et al. 1988). When we examine areas without refuge, such as SCI and SRI in southern California, their densities are now at critically low levels that can no longer support fisheries.

CONCLUSIONS

Refuge by depth protected red abalone stocks from overharvest at VDSP, an area where red abalone densities increased despite intense fishing pressure. In contrast, stocks off SRI, where no refuge existed, ultimately collapsed under intense fishing and environmental events. At VDSP, the high densities of deep water sport legal abalone, which are protected from fishing, may represent the type of refuge population needed to survive sporadic re-

TABLE 4.

Red abalone densities and sampling statistics for invasive dive transects at Van Damme State Park (1990 to 1992) and Johnsons Lee, Santa Rosa Island (1978 to 1982 from Tegner et al. 1989). YOY and harvested sizes as percent of total counts.

Location (Depth)	Species (Size Categories)	No. of Trans.	Meas. n	Counts n	Density No./ha	SE	% Total
Van Damme State Park							
(Shallow: 1.8–7.6 m)	All sizes	33	409	389	11,788	1,476	
	(YOY < 31 mm)		27	22	666	330	6
	(>177 mm)		51	64	1,939	356	16
	(>198 mm)		12	13	393	134	3
(Deep: 8.5–17.7 m)	All sizes	27	282	299	11,111	2,480	
	(YOY < 31 mm)		15	12	446	418	4
	(>177 mm)		116	130	4,830	540	43
	(>198 mm)		62	55	2,043	483	18
(Combined: 1.8–17.7 m)	All sizes	60	691	688	11,483	1,368	
	(YOY < 31 mm)		42	31	571	257	5
	(>177 mm)		167	194	3,261	305	28
	(>198 mm)		74	68	1,496	226	13
J. Lee SRI (Tegner et al. 1989)	All sizes	83	1392	--	1,790	<666	
	(YOY < 31 mm)		——	——	176	190	10
	(>177 mm)		——	— —	270	130	15
	(>198 mm)		——	....	50	35	3

TABLE 5.

Comparison of emergent to invasive density for red abalone at Van Damme State Park, during 1990 to 1992 when both emergent and invasive stations were conducted.

Location (Depth and Source)	Number of Transects	Density	SE	% Cryptic
Van Damme State Park				
(Shallow)	34	7,765	856	34
(Deep)	40	6,425	1,051	42
(Combined)	74	7,041	691	39
J. Lee SRI (Tegner et al. 1989)	83	632	—	65

cruitment and periods of environmental stress. In 1986, stocks at VDSP, and at other high use areas of northern California examined by Parker et al. (1988), showed lower juvenile and adult abundance relative to recovered levels observed at VDSP by 1992. The fact that even the reduced 1986 levels were five times the abundance observed at the Johnsons Lee study area in 1978 to 1982, a population that failed to sustain fisheries, supports the value of refuge for recruitment and environmental uncertainty.

Our study examined refuge by depth. How other forms of refuge may protect stocks is unresolved. Habitat refuge, in the form of crevice or relative rugosity, and refuge by access must be considered in future studies.

Minimum Viable Populations

Maintenance of genetically effective population size is complicated by decreased spawning aggregations (Frankel and Soule 1981, Shepherd and Brown 1996). A study on southern Australia abalone *H. laevigata* suggested adults aggregated to improve spawning success (Shepherd 1986). There is evidence that sea urchin, a broadcast spawner, must be in within a meter of one another (Pennington 1985) for successful fertilization. Shepherd and Brown (1993), working with *H. laevigata*, defined aggregations as three or more large individuals (>110 mm shell length) less than 1 m apart. They found that aggregations declined over 7 years from 67 to 16% when density declined from 1,800 to 700 abalone-per-ha. They explained that effective population size declined more rapidly than true population as density declined. When densities fell below about 1,000 abalone per-ha spawning aggregations disappeared and recruitment failure occurred.

Refugia and Stock Recovery in Southern California

Shepherd and Brown (1993) discussed the possible benefits of completely closed areas with "source populations" of abalone that could serve to replenish adjacent areas subject to fishing. Refuge by depth seems to work for red abalone in northern California. In southern California, refuge by depth could be applied to pink abalone. Pink abalone may be the best southern California candidate, because it closely resembles the depth range of red abalone in northern California. Pink abalone occur in the lower intertidal zone to 60 m in depth; most are found subtidally at depths of 6 to 24 m (Cox 1960, 1962, Tutschulte 1976). Green abalone, the other once major species in southern California, may exist at depths that are too shallow to be well protected from free divers able to reach depths of 8.5 m. Green abalone are found from low intertidal to subtidal depth zones to about 9 m, and occasionally to 15 to 18 m, but most are found in 3 to 6 m of water (Cox 1960, 1962, Tutschulte 1976).

Marine Protected Areas (MPA)

Single species refuges may be a poor alternative used to promote recovery of red abalone stocks in southern California. Initially protected areas must contain or be close to sources of genetically effective populations to allow recovery. The presence of

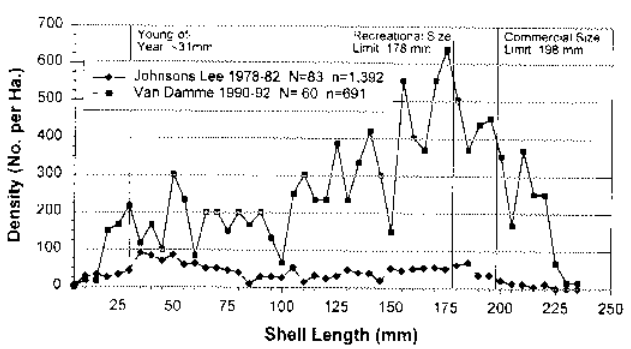

Figure 8. Red abalone density by size from invasive transects at Johnsons Lee (Tegner et al. 1989) and Van Damme State Park. N = number of transects; n = number of abalone measured.

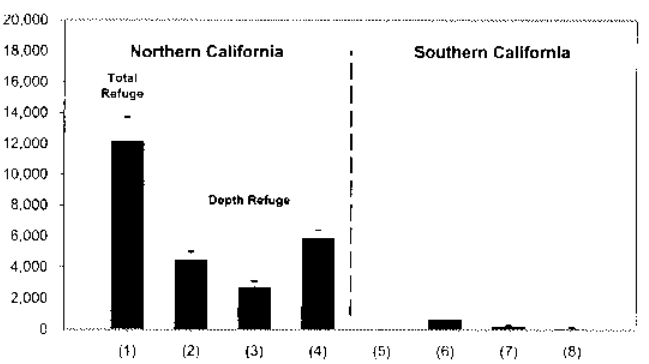

Figure 9. Emergent red abalone density at:
(1) Point Cabrillo Reserve, Mendocino County in 1986 (N = 30) (Parker et al. 1988);
(2) four high use sites in Sonoma and Mendocino Counties, including Van Damme State Park (VDSP) (Parker et al. 1988);
(3) Sonoma and Mendocino Counties sampled in 1989 (Kalvass et al. 1991) and 1991 (Kalvass and Taniguchi 1993) (N = 66);
(4) VDSP, Mendocino County, in 1986 and 1990 to 1992 (N = 120);
(5) Gull Island on Santa Cruz Island in 1997 (N = 12) (Channel Island National Parks [CINP] unpublished data);
(6) Johnsons Lee, Santa Rosa Island (JL, SRI) in 1978 to 1992 (N = 83) (Tegner et al. 1989);
(7) JL, SRI in 1993 (N = 24) (CINP Unpublished data);
(8) JL, SRI in 1993 (N = 17) (California Department of Fish and Game unpublished data).

such competitors and predators as sea urchin, fishes, lobsters, octopus, and crabs are vital to the health of the kelp community supporting abalone populations (Dayton et al. 1998). Sea urchin barrens, areas where large numbers of sea urchin congregate, have a serious derogatory effect on abalone populations. Barrens develop where the marine community structure has been degraded by urchin predator removal (Dayton et al. 1998, Tegner 1989). Where food of sea urchins; for example, kelps and other vegetation, are low, sea urchins will compete with one anther (Tegner 1989, Wilson and North 1983), and, less mobile abalone may be out competed. During prolonged episodes of starvation, sea urchins are able to survive on minimal water-borne nutrients; whereas, abalone cannot. Even if food is not limiting, it may be unavailable because the more mobile sea urchins will obtain the food first. Once this situation occurs, reestablishment of abalone is difficult, because sea urchins out compete other herbivores indefinitely (Tegner 1989).

To reestablish and maintain the natural order, reestablishment of the whole marine community must occur, allowing natural processes to control predator prey relationships. This concept is inherent in the establishment of marine protected areas. Such areas, once established in areas with source populations, could enhance surrounding areas open to harvest. Properly planned and protected, these areas could provide insurance against virtual total annihilation of California's marine community.

ACKNOWLEDGMENTS

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THE PERILOUS CONDITION OF WHITE ABALONE *HALIOTIS SORENSENI*, BARTSCH, 1940

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ABSTRACT The white abalone, *Haliotis sorenseni*, seems to be on the brink of extinction. We searched 107,650 m^2 of white abalone habitat at 39 locations around the California Channel Islands, the species' historical center of abundance. At SCUBA depths, 25–42 m, where mean densities in the 1970s were 2,000 to 10,000 white abalone per hectare, we found a mean density of 1.6 ± 0.5 ha^{-1} in the early 1990s. Surveys conducted by submersible in 1996 and 1997 at depths of 27–67 m revealed the same extremely low population densities (1.0 ± 0.4 ha^{-1}), in a remarkably narrow band of suitable habitat on deep reefs, and demonstrated the suitability of the research submersible DELTA for abalone surveys. Following a 270 metric ton commercial harvest in the 1970s, landings of white abalone virtually ceased. No fishery-independent population assessment was made until 1992 to 1993, and the fishery remained open until 1996. The management scheme, based on a minimum harvest size of 153 mm and a closed season during spawning, apparently failed to protect adequate spawning stock density. The population has not recovered from the harvest, and the survivors are currently dying of old age. Spontaneous recovery is highly unlikely, even in the absence of continued harvest. Active management intervention will be required to prevent extinction and to restore the species to a viable status. We identify a program of capture rearing and refugia-based management, with a public education component and using existing governmental processes, to restore the white abalone population.

KEY WORDS: extinction, restoration, fishery, California Channel Islands

INTRODUCTION

Only in the last few years has the scientific community considered human-mediated extinction possible for invertebrates (Malakoff 1997). People believed that their large geographic ranges, high fecundity, and planktonic dispersal made marine invertebrates essentially "extinction-proof." Fishery management strategies based on these beliefs have treated the reproductive capacities of old, large, and extremely fecund individuals as surplus and nonessential for sustaining populations of long-lived species. Evidence of fished populations collapsing worldwide now challenges those beliefs (Parfit 1995, Botsford et al. 1997).

Following a 270 metric ton harvest in the 1970s, white abalone, *Haliotis sorenseni*, dropped to a critically low population density (1.6 ha^{-1}) on reefs near its historic center of distribution (Davis et al. 1996). White abalone are the deepest-dwelling of eight species on the Pacific Coast of North America. They inhabit low relief rocky reefs mainly at depths of 25–65 m around offshore islands between Point Conception, California to Punta Eugenia, Baja California Sur (Cox 1962). Because much of the white abalone habitat lies below normal scientific SCUBA diving depth (32 m) and commercial harvests in the 1970s were reportedly taken from depths of 60 m, we hoped to find remnant groups of white abalone at sustainable population densities (>5,000 ha^{-1}) in depths of 30–65 m. This report describes the results of deep reef surveys for white abalone using the research submersible DELTA, identifies actions needed to restore viable populations and prevent white abalone extinction, and describes a novel effort to initiate these actions.

METHODS

In November 1996 and October 1997 we searched white abalone habitat on low relief rocky reefs near Santa Barbara, Anacapa, and Santa Cruz Islands and on Osborn Bank from the research submersible DELTA (Delta Oceanographics, Channel Islands Harbor, CA). Each dive provided 60 to 120 minutes of observation that were continuously recorded on video, indicating depth and ambient water temperature. The position of the R/S DELTA was tracked continuously using a differential global positioning system with a sonar link to a surface support vessel to determine where and how much habitat was searched. We recorded the number and size of live white abalone and estimated the number of white abalone shells found, because they were frequently broken and partially covered by sand.

RESULTS

We conducted a total of 24 dives over 6 days, searched 77,050 m^2 of rocky reef believed to be white abalone habitat between 30 and 67 m depth, and found nine live white abalone (Table 1). We found live white abalone between depths of 38 and 51 m (mean depth 45 m). They were all larger than 150 mm. We also observed more than 300 empty white abalone shells throughout the depth range searched, indicating that the survey sites were previously inhabited by white abalone. Suitable abalone habitat; that is, rocky reef, was restricted to a relatively narrow band (<400 m wide), within the reported depth limits of white abalone. At Santa Cruz and Anacapa Islands, most areas below 50 m depth appeared unsuitable for abalone, virtually devoid of macro algae, and dominated by suspension-feeding organisms, primarily brittle stars (ophiuroids) and sponges. At Osborn Bank and Santa Barbara Island, apparently suitable habitat extended down to 67 m.

DISCUSSION

The rapid spread of modern diving technology in the midtwentieth century greatly facilitated abalone exploitation (Cicin-Sain et al. 1977). Just 25 years ago, California divers and shore pickers gathered 4,000 to 5,000 metric tons of abalone every year, creating recreational and commercial fisheries worth more than $20 million annually. In Southern California, as supplies of popular pink (*H. corrugata*) and red (*H. rufescens*) abalone began to wane in the early 1970s, divers explored deeper reefs for the even more valu-

TABLE 1.

Summary of white abalone habitat surveys in R/S DELTA.

DELTA Dive Number	Date	Location Latitude/Longitude	Depth (m)	Dive Duration (min)	Habitat Surveyed (m^2)	White Abalone Found
3945	3 Nov 96	34°00.383′N/119°26.439′W	46–61	121	5,200	0
3946	3 Nov 96	33°59.926′N/119°22.150′W	31–61	84	2,850	0
3947	3 Nov 96	33°59.994′N/119°23.241′W	43	97	4,300	2
3948	3 Nov 96	33°59.826′N/119°25.113′W	39–43	113	5,300	0
3949	4 Nov 96	34°00.281′N/119°26.408′W	43–47	112	4,800	1
3950	4 Nov 96	34°01.954′N/119°30.558′W	43–46	76	2,550	0
3951	4 Nov 96	33°59.229′N/119°31.316′W	44–56	92	4,100	0
3952	4 Nov 96	33°59.029′N/119°33.781′W	37–66	116	2,150	0
3953	5 Nov 96	33°56.681′N/119°49.736′W	33–61	97	3,000	0
3954	5 Nov 96	33°56.810′N/119°46.057′W	31–61	65	700	0
3955	5 Nov 96	33°57.037′N/119°43.021′W	49	119	4,000	0
3956	5 Nov 96	34°00.135′N/119°30.556′W	49	73	3,650	2
3957	6 Nov 96	34°00.615′N/119°24.462′W	31–67	122	2,800	0
3958	6 Nov 96	34°00.885′N/119°26.954′W	37–49	90	2,750	0
3959	6 Nov 96	34°00.383′N/119°26.439′W	31–48	97	2,200	0
4250	19 Oct 97	33°21.562′N/119°02.458′W	47–67	89	4,050	0
4251	19 Oct 97	33°27.906′N/119°03.127W	35–44	83	1,450	0
4252	19 Oct 97	33°29.594′N/119°04.057W	35–47	93	3,675	1
4253	19 Oct 97	33°30.459′N/119°02.998W	44–60	91	3,000	0
4254	19 Oct 97	33°29.982′N/119°00.034W	45–56	82	350	0
4255	20 Oct 97	33°27.575′N/119°02.167W	37–60	101	4,150	0
4256	20 Oct 97	33°27.408′N/119°02.146W	31–50	65	2,400	1
4257	20 Oct 97	33°29.562′N/119°00.942W	32–52	103	4,875	2
4258	20 Oct 97	33°29.901′N/119°03.754W	51–62	60	2,750	0
Total				2,241	77,050	9

able white abalone. In 1972, commercial divers alone landed more than 60 metric tons of white abalone (Davis et al. 1996). After just 7 years, the original adult populations were exhausted, and white abalone catches dropped to near zero.

No one worried, because it was believed there must be even deeper, more isolated reefs where divers did not go, so some white abalone would escape fishing, and eventually their reproduction would replace those taken by divers. People also believed that because white abalone are so fecund, with individual females producing 3.6 to 6.5 million eggs a year (Tutschulte and Connell 1981), only a few survivors would be needed to restore depleted populations. California's fishing regulations were believed to ensure this survival by prohibiting harvest of white abalone under six inches (152 mm) in length and during only 3 months per year. These beliefs now seem false.

California's abalone management strategy disregarded the Allee Effect, reproductive failure that occurs below some threshold of population density. The surviving white abalone, although protected by size and season limits, were so few and so scattered they could not find mates, and now they are dying of old age, isolated and alone on small deep reefs. This incredibly fecund, long-lived species is now facing extinction. California's annual economy has lost millions of dollars, and the people of California have lost the equivalent of a multimillion dollar capital asset that once produced the annual landings.

The white abalone's decline has been exceptionally rapid and severe. When Tutschulte (1976) began studying white abalone populations in the early 1970s, he found mean densities of 2,000 to 10,000 ha^{-1} on reefs at depths of 26 m to 42 m around the California Channel Islands. In the early 1980s, National Park Service and California Department of Fish and Game scientists surveying the new Channel Islands National Park found white abalone population density had shrunk to only 21 ha^{-1}. Ten years later, white abalone teetered on the brink of extinction: with an average of only 1.6 ha^{-1} of rocky reef (Davis et al. 1996). The mean density of one white abalone per hectare reported here for reefs below 31 m was slightly lower than that observed at SCUBA depths in 1992 to 1993. These submersible surveys confirmed the critically low population density, and demonstrated the absence of de facto refugia beyond normal SCUBA depths. White abalone populations have decreased 99.99% in the last 25 years. If current population densities at the California Channel Islands, the historic population center, are typical of the entire range, a crude calculation of suitable habitat within the species range reveals that fewer than 1,000 white abalone may exist today, scattered sparsely along nearly 800 km of narrow coastal shelf from Point Conception to Punta Eugenia (Table 2).

As do many sessile or slow-moving marine bottom-dwellers, abalones have external fertilization and spawn synchronously en masse. Females must be within a few meters of males when they both spawn to get their eggs fertilized, or the sperm will be too diluted by diffusion (Davis et al. 1996). Because adult abalone move very little and because the average distance between solitary adults is currently more than 50 meters, the last remaining survivors have virtually no chance of getting critical masses of adults close enough to spawn successfully and regularly produce viable cohorts of juveniles. Even if rules prohibiting further fishing for abalone were completely effective (i.e., no poaching), white abalone will not likely produce another viable cohort without human intervention. The last large cohort of white abalone was apparently

TABLE 2.

Estimated total area of white abalone habitat (rocky reef 25–65 m depth) within the species historic range from Point Conception to Punta Eugenia.

Location	Shelf Length at 25–65 m Depth Contour (km)	Mean Shelf Width (km)	Total Shelf Area (ha)	Potential White Abalone Habitat (ha)[a]
Pt. Conception to Santa Barbara	75	0.5	3,750	112
San Miguel Island	34	0.3	1,020	31
Santa Rosa Island	61	0.4	2,440	73
Santa Cruz Island	40	0.3	1,200	36
Anacapa Island	34	0.2	680	20
San Nicolas Island	42	0.3	1,260	38
Santa Barbara Island	34	0.3	1,020	31
Osborn Bank	23	0.3	690	21
Santa Catalina and Farnsworth Bank	90	0.4	3,600	108
Bishop Rock	53	0.5	2,650	80
Tanner Bank	41	0.5	2,050	62
Cortez Bank	83	0.4	3,320	100
San Clemente Island	33	0.4	1,320	40
Islas Los Coronados	17	0.2	340	10
Islas de Todos Santos	21	0.4	840	25
San Martin	19	0.4	760	23
Cedros	70	0.4	2,800	84
Punta Eugenia	60	0.4	2,400	72
Total	830		32,140	966

[a] Potential white abalone habitat estimated as the rocky substratum between 25 and 65 depth contours, reported as 3% of total shelf by Thompson et al. for the Southern California Bight (1993).

spawned in the late 1960s (Davis et al. 1996). Given their 35-year life span (Tutschulte 1976, Tutschulte and Connell 1988), survivors of the 1970s fishing cannot live much longer.

ACTION PLAN TO SAVE AND RESTORE WHITE ABALONE

The Mexican white abalone fishery apparently collapsed in 1995. The following year, the California Fish and Game Commission also recognized the plight of white abalone and closed the California fishery to protect the survivors. Those were essential first steps, but alone are insufficient to save and restore white abalone, because the population is so severely depleted. Even after 20 years of passive protection, abalone populations along a large section of the Orange County coast of California closed to all abalone harvest in 1977 have not yet shown any sign of spontaneous recovery (Tegner 1993). Clearly, more proactive actions must be taken to prevent white abalone extinction and to rebuild populations to the point they can once again support fisheries. Given the perilous current population densities, white abalone biology, and that spontaneous recovery is virtually impossible, an *ad hoc* group of scientists, attorneys, and fishermen from the United States and Mexico, representing fishing cooperatives, conservation organizations, universities, and federal and state agencies, and mariculturists in private enterprise, has joined forces as the White Abalone Working Group to develop and execute a plan to restore white abalone populations.

They have developed a four-step strategy based on public education, existing governmental processes, and research. The four steps are: (1) locate survivors by surveying historic habitat; (2) collect brood stock from the survivors; (3) breed and rear a new generation of brood stock; and (4) re-establish refugia of self-sustaining brood stocks in the wild. Laying the foundation for white abalone restoration will take 10 to 15 years. Actual population recovery will take an additional decade or more. All four steps must be taken to ensure success. Without these actions, white abalone will probably be extinct by 2004, less than 10 years from now, only 64 years after its discovery by science.

Educational Outreach

Ignorance has been the worst enemy of the white abalone so far; what scientists, decision makers, and the public don't know has almost sealed the species' fate. White abalone survival is doubtful unless public interest increases immediately. An outreach program must reach a broad audience of legislators, marine resource managers, potential funders, and the general public, including divers, school-age children, and senior citizens. An education program must describe the plight of white abalone, explain why restoration is needed (e.g., to get past denial that fishing caused the population collapse), and raise public awareness of the resources at risk. This education program is especially important, because the public has been far more responsive to calls for conserving large, charismatic mammals and birds (such as whales and eagles) than invertebrates. Fortunately, abalones are exceptionally charismatic invertebrates. They are large, have beautiful iridescent shells that have long been used for ritual and decorative purposes, have almost mythic status as food in California and Asia, and are consequently better known than most invertebrates. White abalone can also be portrayed accurately as leading indicators, or symptoms, of broader environmental issues. To reach a broad audience, the program should employ a variety of communication media, including video, radio, museum exhibits, and the press.

Governmental Processes

Governmental institutions are overwhelmed with critical environmental, social, and economic issues. Even the process of iden-

tifying endangered species is backlogged. Current discussions of reauthorization of the Federal Endangered Species Act further delay recovery actions and reduce the probability of adequate public funding for restoration. Because white abalone are publicly owned resources, governmental agencies should lead the restoration effort, but the proposed restoration of a marine invertebrate is essentially unprecedented. The National Marine Fisheries Service of the Commerce Department's National Oceanic and Atmospheric Administration is responsible for listing and recovering listed species under the Endangered Species Act of 1973, but the National Marine Fisheries Service has yet to list a marine invertebrate under the act, and has rejected all requests to do so. Therefore, the working group believes it is not prudent to rely solely on government to rescue white abalone and proposes a collaboration between government and private interests.

Three appropriate levels of government need to be involved in white abalone restoration activities: state, federal, and international. Each level has existing administrative mechanisms for addressing threats to species, such as listing procedures for threatened and endangered species, and planning processes for restoring depleted populations. Legislation and regulation at all levels are also options for white abalone protection and restoration, because it occurs in and beyond California's territorial sea in federal waters and in México. At the state level, people are working actively to secure passage of legislation that would protect all of California's marine resources, including abalone, by establishing a network of no-take marine reserves, by requiring the collection of fundamental data on population dynamics prior to the prosecution of a fishery, and by labeling certain fishing practices (e.g., shrimp trawling) as "sea life destructive" in the marketplace (Keeley 1997). In federal waters, others are working to establish no-take marine reserves and to designate essential fish habitat that would trigger consultation with agencies contemplating projects that have the potential to harm it, leading to recommendations to protect habitat. On the international level, diplomatic discussions with México must begin. Surviving white abalone must be protected from fishing there as well as in the United States. Mexican scientists must be encouraged to participate in habitat surveys and other restoration activities.

The *ad hoc* group is exploring new territory with this effort by combining public and private interests in the restoration process. Rules of engagement to establish limits of authority, responsibility, liability, and benefits must be clarified and reviewed to ensure that all interests are appropriately served and all interested parties are allowed to participate. For example: is new, critical information on breeding biology of white abalone made possible from publicly provided brood stock and developed by private interests proprietary? As brood stock are collected from the wild, how will private facilities qualify to receive them? For example, many California abalone culture facilities are currently plagued by alien sabellid worms that would preclude using brood stock raised in these facilities from being released in the wild. How many facilities are needed to spread the risk of extinction in captivity?

Research and Restoration

White abalone restoration depends on acquiring crucial new information about the species' reproductive biology and ecology and then applying that information to rebuild depleted populations. Building on a firm foundation of public knowledge and support, restoration requires that all four steps of the strategy be executed. Although these four steps must take place sequentially, they should be linked to reduce costs and the uncertainty of success.

The first step in restoration is to find the survivors. The White Abalone Working Group weighed the options of moving individuals to facilitate successful reproduction in the field and of spawning and rearing individuals to captive breeding facilities. They decided that the former was far too likely to fail, given the high likelihood that these animals will not produce juvenile recruits before they die, even if they were to spawn successfully. Many long-lived organisms do not reproduce each year, and even those that do fail in most years to produce young that will enter the breeding population. The absence of any white abalone produced after the late 1960s suggests that recruitment in this species is too unreliable to opt for *in situ* breeding at this time. Captive breeding—even with the different set of risks it entails—is the preferred option for now.

Soon after the population is surveyed, brood stock need to be collected for captive breeding, ideally a few hundred individuals, and held until synchronous spawning in captivity can produce the next generation (Leighton 1972, Leighton and Lewis 1982). If more than a few hundred survivors are found, the additional individuals may be translocated to a few refugia reefs where they can be protected, creating denser populations and thus facilitating *in situ* reproduction. Adult survivors collected from the wild will be used to produce the next generation of brood stock, either from captive breeding with progeny returned to the wild as adults, or from natural reproduction by aggregations on refugia reefs created by translocation of wild stock.

Locate Surviving White Abalone

The area to be surveyed for survivors is large, ranging 1,000 km from Point Conception, near Santa Barbara, California, to Punta Eugenia, halfway down the Pacific coast of Baja California, México. A first step is to obtain high resolution side-scan sonar maps of low-relief rocky reefs from which to select appropriate dive targets and design a stratified random sampling system to maximize survey efforts. These maps may be available without additional field surveys for some areas, but a preliminary search of public and proprietary archives indicated few data are available. The depths at which white abalone occurred, 25–65 m, will require submersible and support ship time, in addition to time and support for SCUBA surveys.

On known historic white abalone reefs, we were able to survey 10–15% of the suitable habitat (15,400 m^2) along 10 km of coast per day with the research submersible DELTA. At that rate, 100 days of submersible time would be required to survey statistically the historic white abalone range for breed stock. Analyzing bathymetric maps with early survey data in a geographic information system may help narrow subsequent searches to more productive reefs to reduce survey and brood stock collection costs.

Collect Wild Brood Stock

When survivors are located during the historic habitat survey, adult brood stock could be collected immediately or the site precisely marked for capture teams to revisit shortly thereafter. Diving technology for short dives by collectors on known white abalone locations is readily available, but requires extreme exposures for scientific divers. Abalone locations could be marked with transponders during surveys to facilitate subsequent collection, if they were not collected simultaneously during the survey. Holding facilities and funds to maintain the brood stock must be available when collecting wild brood stock begins. Current costs are approximately $2/abalone/day to hold live abalone in commercial

facilities (T. McCormick, pers. comm.). To minimize the kind of mishap that almost spelled extinction for captive endangered blackfooted ferret populations—a distemper epidemic that killed off all the captive breeders—collected brood stock should be held and maintained in several different facilities.

Captive Breeding and Rearing

Captive breeding has proved to be an essential method of recovering species in danger of extinction, but captive breeding does not always succeed, even where general aspects of a species' breeding biology are known. Successful captive breeding requires some understanding of a species' behaviors, reproductive physiology, and genetics. Fortunately, captive breeding of white abalone is not without precedent (Leighton 1972, Leighton and Lewis, 1982), and several decades of breeding red abalone and other species provide a firm foundation (Shepherd et al. 1992). The breeding biology of white abalone is not known as well as other abalone species, and each species studied thus far has distinct aspects of reproductive biology. Enough is known to begin spawning large numbers of white abalone in the laboratory and rear them to maturity (Leighton and Lewis 1982). Most outplanting of marine species from mariculture operations has been done to augment depleted but, nonetheless, substantial populations in the field (Tegner and Butler 1989, Tegner 1992); there are very few precedents for recovering marine species nearing extinction with captive breeding. The only precedents are those established from long-standing experience in captive breeding of terrestrial species developed by the World Conservation Union (IUCN) Species Survival Commission's Conservation Breeding Specialist Group.

In California, 25 years of experimental outplanting of juvenile and younger abalone has failed to demonstrate effective population restoration or to prevent population collapses (Tegner and Butler 1989), although a current red abalone larval seeding experiment seems promising (P. Raimondi, University of California, Santa Cruz, pers. comm.). Because the surviving white abalone are nearing the end of their life span, and few opportunities exist to produce succeeding generations, we believe progeny should be held in captivity until they are mature and large enough to escape high mortality in the wild. That probably means 4 years growth and releasing them at >100 mm (Tutschulte 1976, Tutschulte and Connell 1988). Annual mortality rates for somewhat smaller (40 mm) hatchery-raised red abalone released at the California Channel Islands in 1989 were 38–68% (Davis 1995); whereas, annual natural mortality of fishery-vulnerable sized abalone is only 10–15% (Tegner and Butler 1989). To overcome these high mortality rates, we propose outplanting 1,000 large individuals at each of 10 sites every year for 10 consecutive years. This would establish a large, young, reproductively active population on refugia reefs and provide several opportunities to initiate juvenile recruitment on site and on adjacent reefs. The genetics of the parental stocks and their progeny should be assessed to evaluate the effectiveness of captive breeding, to avoid inbreeding depression, and thereby maximize long-term survival prospects for recovered populations of white abalone.

Create White Abalone Refugia

The final step in restoring white abalone is to establish self-replacing populations on a variety of reefs through its historic range that are protected from depletion. Half of this action requires outplanting the brood stock produced by the captive breeding and rearing program described above, and half requires political establishment of protected zones to serve as refugia (Dugan and Davis 1993, Keeley 1997).

CONCLUSIONS

White abalone status is perilous, but not hopeless. Rapid political and scientific actions could prevent extinction and probably restore the species' former productivity. If we lose this species, we cannot claim we were not warned.

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EXPERIMENTAL SEEDING OF HATCHERY-REARED JUVENILE RED ABALONE IN NORTHERN CALIFORNIA

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ABSTRACT The feasibility of enhancing populations of red abalone, *Haliotis rufescens*, by seeding juveniles was evaluated in Northern California. We seeded 50,000 hatchery-reared juvenile red abalone averaging 8 mm in length in October 1995 into six sites: Caspar, Van Damme, Salt Point, Bodega Marine Life Refuge north, BMLR south, and Half Moon Bay. Recovery of juveniles at 6 months, 1 year, and 2 years totaled less than 1% of the number seeded, however seed accounted for one-third of the 1995 cohort. Recovered juveniles with obvious hatchery markings grew 15 mm per year. Recapture rates were higher at three sites with urchin spine canopy microhabitat as compared to three sites without sea urchins. Although juvenile recoveries may not be good indicators of survival, poor recapture rates highlight the present importance of conservative fishery management strategies.

KEY WORDS: enhancement, *Haliotis rufescens*, stocking, seed survival, Northern California

INTRODUCTION

The red abalone, *Haliotis rufescens* (Swainson), is the largest abalone in the world and is harvested by both commercial and recreational divers in California. Commercial harvest rates have declined more than 80% from an average of 2,000 metric tons in the 1950s and 1960s to 330 mt in 1989 (California Dept. Fish and Game statistics, Karpov and Tegner 1992). Commercial abalone fisheries are in decline worldwide. In 1997 the commercial abalone fishery in California was closed. The north coast of California supports a large recreational free-diving fishery, with daily limits of four abalone, which seems to have stable landings, ranging from 1–3 million pounds per year in the late 1980s (Karpov 1991, Karpov and Tegner 1992). This region, however, has sporadic natural recruitment. Because of the economic importance of abalone coupled with irregular natural recruitment, there has been considerable interest in abalone enhancement.

Abalone enhancement by seeding hatchery-reared juveniles has been practiced in many parts of the world. Success rates however, have been mixed (Tegner and Butler 1989, McCormick et al. 1994). In Japan, a large-scale seeding effort has been underway with more than 10 million seed produced and distributed by fishing cooperatives in 1979 alone (Tegner and Butler 1989). Nevertheless, reports of juvenile survival vary widely, ranging from 1–80% (Saito 1984, Tegner and Butler 1989, Kojima 1995). Similarly, in New Zealand survival of juveniles varies spatially ranging from 1–72% (Schiel 1993). In Southern California, juvenile recapture rates have been low, averaging less than 1% for seeded red abalone (Tegner and Butler 1985, 1989); however, similar studies have not been conducted in Northern California. Despite low recapture rates in Southern California, recent genetic work indicates that adult abalone at previous seeding sites are genetically similar to hatchery abalone, suggesting high juvenile seed survival (Gaffney et al. 1996).

In this paper, we examine the results of a large-scale seeding experiment using juvenile red abalone in Northern California. In October 1995, 50,000 juvenile abalone averaging 8 mm in shell length were seeded in six sites ranging from Half Moon Bay to Fort Bragg. Because juvenile abalone have been observed under the spine canopy of adult urchins (Tegner and Dayton 1977, Kojima 1981, Tarr et al. 1996, Rogers-Bennett pers. comm.), three sites were selected with and three without red sea urchins, *Strongylocentrotus franciscanus* (Agassiz). We report on the number of seed recovered and their growth after 6 months, 1 year, and 2 years.

MATERIALS AND METHODS

Study Sites

Six sites were selected for the seeding experiment: Caspar Reserve, Van Damme, Salt Point, Bodega Marine Life Refuge (BMLR) north, BMLR south, and Half Moon Bay (Mavericks). Caspar, Salt Point, and BMLR north have red sea urchins at 2–3/m^2, and urchins are absent from the remaining three sites. The sites were all in shallow water (5–8 m) and were selected to maximize cobble and movable substrate while minimizing sand scour and silt. Potential abalone predators at these sites include Cabezon fish, *Scorpaenichthys marmoratus* (Ayers), red and rock crabs, *Cancer* spp., and the sea stars, *Pycnopodia helianthoides* (Brandt), *Dermasterias imbricata* (Grube), and *Pisaster* spp.

Experimental Seeding

Juvenile red abalone were cultured at Bodega Farms, Bodega, California. Seed from three spawnings in spring of 1995 were randomly mixed and used. Larvae settled 7–8 days after fertilization. Animals were given a complete health examination including inspection for the presence of polychaetes by Dr. C. Friedman (California Dept. Fish and Game) and assessed as healthy. Delivery of juveniles occurred in September when the animals were weaned from microalgae and feeding on macroalgae averaging 8.22 mm in length (SD 1.08, n = 100). Their shells were turquoise or pale green, indicative of their hatchery diet of diatoms and *Macrocystis pyrifera* (Linnaeus) (Tegner and Butler 1989). Juvenile abalone were placed in mesh bags (1-mm mesh) in groups of 500. One group of 500 was counted and weighed (45.8 g), and subsequent bags were stocked by weight. Fleshy red algae, *Cryptopleura* spp., were added to the bags to acclimate the juveniles to a wild diet. Animals seemed to clump actively onto the algae and feed, and no transfer mortalities were observed.

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Juveniles were seeded into the six sites during an 11-day period in October, 1995. Two seeding methods were used: hand planting and release from abalone modules. Red algae were used as seed substrate and divers hand placed the algae with attached juveniles into cryptic microhabitats, including rock crevices, under moveable cobble, and under adult red urchins (where available). Hand-planting juveniles on the algal substrate took approximately 6 hours per site. Abalone were also placed in seeding modules (see Ebert and Ebert 1988 for module design) for release. The modules are large cement boxes with doors closed by rubber straps (bungee) attached to magnesium links that dissolve after 18 hours, freeing the juveniles at midnight the following night.

At Caspar, Van Damme, and Half Moon Bay the two seeding methods were used. Half the abalone were hand planted and the other half were placed in three abalone modules at a density of approximately 1,700/module. At the other three sites, all juveniles were hand seeded. The seeding area encompassed roughly a 4 × 15 m core area within the larger 24 × 30 m site. Juvenile density after seeding was approximately 150–170 seed/m^2 within the smaller core area. Sea star predators were removed from the sites at seeding.

Surveys

At 6 months, 1 year, and 2 years, sites were searched invasively by lifting movable cobble, removing adult urchins, searching in coralline and other red algae, rock crevices, and under ledges. The 6-month survey was a 3-hour invasive search, but no identification of hatchery seed was attempted, because juveniles were not collected. At the 1 and 2 year surveys, all juvenile abalone found were collected and measured. These surveys were conducted along six parallel 4 × 30 m transects for a total area searched of 720 m^2 per site. This extensive search took approximately 8–10 hours depending on the habitat type and abalone density. We compared the number of seed recaptured from sites with and without urchin spine microhabitat in 1996 and 1997 using a chi-square test.

Growth

All juvenile abalone collected from the sites with obvious hatchery shells were measured in the laboratory to assess original and final size. Growth is reported as the difference between original size determined from the green shell, and final size (See Table 1). In addition, juvenile seed were maintained in the laboratory and their final size was assessed after 2 years.

RESULTS

Surveys

At 6 months, fewer than 20 juveniles between 6–16 mm were found at each site in February and March 1996 (Table 1). No dives were made at 6 months at Half Moon Bay because of rough ocean conditions. No shells indicating mortality were found at any of the sites.

One year after release, shells with obvious hatchery coloration (turquoise and pale green) ranged in size from 18.4–28.4 mm. Hatchery-reared seed accounted for one-third of all the juveniles found between 16–30 mm (Table 1). Seed recoveries ranged from 0–0.17% for the six sites. Juvenile density in the study sites (each 24 × 30 m) ranged from 0–0.07 juveniles/m^2 at one year. Caspar had the most seed in the target size range and the highest seed recovery of 17 animals; whereas, at Half Moon Bay, neither juvenile nor adult abalone were found. At 1 year, we recovered significantly more seed from the sites with urchins, as compared to the sites without urchins ($\chi^2 = 18.78$, df = 1, $p < .001$).

Two years after release, shells with obvious hatchery-reared colors ranged in size from 28.4–51.0 mm. Again, hatchery-reared seed made up approximately one-third of all the juveniles found between 26–52 mm (Table 1). Recoveries from the sites ranged from 0–0.21% of the total number of juveniles seeded. Juvenile density in the study sites ranged from 0–0.08 juveniles/m^2 at 1 year. Again, Caspar had the most seed in the target size range and the highest seed recovery of 21 animals. In Half Moon Bay, no juvenile or adult abalone were found in the survey and none were found in a broad scale emergent survey conducted over an area 10 times the size of the study site. Again, we recovered significantly more seed from sites with urchin spine canopy microhabitat ($\chi^2 = 9.77$, df = 1, $p < .01$).

Using the most liberal interpretation of the recovery data, so that all juveniles between 16–30 mm in the 1st year and all juveniles between 26–52 mm in the 2nd year are counted as seed, still yields less than 1% of the total 50,000 recovered after 2 years. Here, we use the more conservative estimate of seed recovered including only those seed with obvious hatchery coloration. Although seed recovery was exceedingly low (<1%), one-third of the 1995 cohort was hatchery-reared seed (Table 1).

TABLE 1.

Number of juvenile red abalone seeded (size 8.22 mm), number of juveniles recovered of potential recapture size, number of definite recaptures, and mean increase in shell size of the recaptured juveniles. Recaptures are from six field sites (each 720 m^2) in Northern California where sites 1–3 have red sea urchins and sites 4–6 do not have urchins.

	1995	1996 (6 mo)	1996 (1 Year)			1997 (2 Years)		
Site	# Seeded	# (6–16 mm)	# (16–30 mm)	# Seed	x Growth (SD)	# (26–52 mm)	# Seed	x Growth (SD)
With urchins								
1. Caspar	10,000	11	47	17	15.75 (SD 3.7)	59	21	28.49 (SD 6.0)
2. Salt Point	10,000	16	35	10	17.07 (SD 3.1)	11	5	30.64 (SD 9.0)
3. BML north	5,000	5	13	4	13.5 (SD 1.2)	4	2	27.9 (SD 3.7)
No urchins								
4. Van Damme	10,000	8	5	5	15.03 (SD 3.3)	23	9	30.57 (SD 7.6)
5. BML south	5,000	2	5	0	No data	2	0	No data
6. HMB	10,000	No data	0	0	No data	0	0	No data
Total	50,000	42	105	36		89	37	

Growth

Juveniles averaging 8 mm at the time of seeding grew a mean of 15 mm during the 1st year and 15 mm more in the 2nd year (Table 1). There were no significant differences in growth between sites or between years. Seed reared in the laboratory grew 19.48 mm (SD 1.63, n = 9) over 2 years.

DISCUSSION

The feasibility of seeding red abalone populations with small hatchery-reared juveniles was explored. We recovered fewer than 1% of 50,000 juvenile abalone (8 mm) after 2 years from six sites in Northern California. Recoveries seemed unrelated to local adult abalone density, and the recreational abalone fishery in Northern California did not seem to have an impact on the recovery of seeded juveniles. These recoveries contrast sharply with results from Japan and New Zealand, where some sites had greater than 50% juvenile survival (Saito 1984, Schiel 1993, Kojima 1995). Our results are more consistent with recoveries from the Palos Verdes Peninsula in Southern California, where fewer than 1% of the juveniles (45 and 71 mm) were recovered after 1 year (Tegner and Butler 1985). Growth of seeded juveniles was lower in Northern California, with juveniles growing 15 mm per year than in the Palos Verdes study, where juveniles grew 30 mm per year (Tegner and Butler 1985). Elsewhere in Southern California, recovery of seed abalone (41 mm) from inside artificial habitats in the Channel Islands was also low, ranging from 0–6% after the first year and 0–2% after the second year (Davis 1995).

Sites with adult red sea urchins had significantly higher juvenile abalone recoveries, as compared to sites without urchins. Many of the hatchery-reared juveniles were found under the spine canopy of red sea urchins. Juvenile abalone in Southern California (Tegner and Dayton 1977, Tegner and Butler 1989), Japan (Kojima 1981), and South Africa (Tarr et al. 1996) have been found in association with the spine canopy of sea urchins. Sites designed as commercial urchin harvest refugia enhanced the recovery of hatchery-reared juvenile abalone. This result supports an ecosystem-based fishery enhancement strategy.

Poor recoveries have been attributed to high predation mortality. There is evidence for differential mortality of hatchery-reared as compared with wild juveniles, because laboratory experiments indicate that wild juveniles are better able to find cryptic shelter and avoid predation (Schiel and Weldon 1987, Tegner and Butler 1989, but see Tegner and Butler 1985). In this study, we found few shells resulting from predation or other causes of mortality; however, wave action in this area may hinder shell recovery.

An alternative explanation is that juveniles not recovered survived but evaded recapture and that recoveries are a poor indicator of juvenile survival. In this study, recoveries did not decrease over time (Table 1). Surprisingly, the poorest recoveries occurred at the earliest time point (6 mo). This suggests juveniles were cryptic and difficult to relocate in the complex habitat. In addition, juvenile abalone are known to be highly mobile. For example, Tegner and Butler (1989) showed that 38% of the small abalone (13 mm) moved out of a study area in 1–2 days. Therefore, juvenile abalone in our study could have easily moved outside of the study areas between survey periods.

Genetic evidence also suggests that seed survival rates may be greater than recoveries indicate. Resident adult abalone from Tyler Bight on the south side of San Miguel Island in the Channel Islands, a site that was seeded repeatedly in the 1970s and 1980s, currently seem to have genotypic and allelic frequencies similar to hatchery animals (Gaffney et al. 1996). This is further evidence that, despite poor recoveries, many juveniles could have survived to become adults. Genetic markers such as these, although raising concerns about the genetic diversity of seeded juveniles, may prove useful in tracking their fate.

It seems clear that although juvenile recoveries in this study were low (<1%) more work is needed to determine long-term survival. With one-third of the juveniles in the 1995 cohort originating as hatchery seed (Table 1), it will be important to follow their survival over time. If the survival trend observed in 1996 and 1997 continues, hatchery seed have the potential to contribute to the local population, despite poor recoveries. At present, the future reproductive contribution of these hatchery juveniles is unknown. From an economic perspective, this approach does not seem cost effective; however, better information regarding survival to reproductive size and to minimum legal size (177 mm) is required for economic analyses. In the meantime, our poor recovery results (<1%) support careful conservation of red abalone resources and conservative stock management policies, because northern populations are the only healthy abalone stocks remaining in California today.

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DISPERSAL OF TAGGED BLACKLIP ABALONE, *HALIOTIS RUBRA*: IMPLICATIONS FOR STOCK ASSESSMENT

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ABSTRACT Dispersal will cause tag disappearance during abalone tagging studies when the area in which abalone were tagged is the same as the area subsequently searched. The degree of dispersal will depend upon the magnitude of the movements of tagged individuals. Despite the potential for dispersal to bias estimates of natural mortality it is mostly ignored. We describe a method we developed to estimate dispersal from movements of tagged blacklip abalone, *Haliotis rubra*, from fished and unfished experimental plots. Although the movements of tagged abalone were small, dispersal contributed 40–60% of total tag disappearance. Indeed, dispersal had at least as much effect on over-all estimates of tag disappearance as did the combination of tag loss and observer error. Substantial contributions from dispersal may explain why estimates of natural mortality from tagging studies often seem larger than anticipated. The magnitude of movements varied with habitat quality and was affected by fishing. Preferred habitat occurred on medium-to-high relief reef that accounted for only 37% of the study site but contained 60% of the total population. Prefishing dispersal rates were smaller in areas with more preferred habitat, and the removal of 40% of the population during experimental fishing caused a decrease in the proportion of abalone dispersing from the fished plots. This study demonstrates that substantial dispersal will occur despite only small movements resulting from heavy fishing pressure within complex reef structure.

KEY WORDS: *Haliotis rubra*, abalone, dispersal, movement, natural mortality, tag–loss, tag–recapture

INTRODUCTION

Abalone species are generally long-lived; consequently, it is reasonable to expect that they have low natural mortality rates (Beinssen and Powell 1979, Shepherd et al. 1982). However, published rates of instantaneous natural mortality (M) are often higher than expected (Shepherd and Breen 1992). Shepherd and Breen (1992) suggested that of all methods for estimation of M, a well designed tag–recapture model has potential for the most accurate results for a given effort. Unfortunately, poorly designed models that do not consider all components of tag disappearance are likely to overestimate M. Illustrating this overestimation, tagging studies of *H. kamschatkana* have provided M values ranging from as low as 0.15 to as high as 2.51 (Shepherd and Breen 1992).

Although complex models have been successfully applied to estimate natural mortality from mark–recapture studies of birds (Peach 1993), most abalone tag–recapture studies have ignored tag disappearance caused by dispersal or emigration of tagged abalone beyond the survey area. Beinssen and Powell (1979) provided a method for estimation of dispersal and obtained an M value of 0.2 for *H. rubra*. Although their study violated the main assumption of localized movement, because movements up to 200 m were observed after just 1 month, ignoring these large movements would only result in an increase in dispersal and a further reduction in M. In contrast a tag–recapture study by Nash (1995), which did not include quantification of dispersal, obtained an M value of 1.56 for *H. rubra*. Nash (1995) identified dispersal as the likely cause of his high estimate.

Any movements of tagged abalone can result in losses caused by dispersal if the area searched is the same as the area in which they were tagged. Whether a movement does result in dispersal depends upon the initial position of the tagged individual relative to the boundary of the tagging/searching area and the direction and magnitude of the movement. Because dispersed movements will not be detected, it is necessary to predict the expected number of tagged individuals to disperse by accounting for all magnitudes and directions of possible movements in relation to initial tagging positions.

Our study estimated dispersal rates from the magnitude and direction of movements of tagged abalone. We attempted to satisfy the assumption of random movement direction by locating our experimental plots at a site of uniform depth devoid of swell or currents. Also, movements were calculated to an estimated error of less than half a meter; whereas, Beinssen and Powell (1979) observed movements between 10 × 10 meter blocks. An *in situ* tagging technique was used to reduce tag-induced disturbance.

Estimates of the number of tagged abalone expected to remain and the actual number resighted within a plot were used to obtain annual rates of dispersal and tag disappearance. We compared the initial magnitude of movements for the *in situ* tagging method with the method applied by Beinssen and Powell (1979). To determine whether movement varied with habitat, as reported by Shepherd (1986), we then compared available preferred habitat with changes in dispersal.

METHOD

Study Site and Experimental Design

The study site was at Point Cook (37°55.893′S, 144°47.104′E), on the northern end of Port Phillip Bay, Victoria, Australia, an area supporting a dense stock of blacklip abalone (*H. rubra*). Water depths were similar across the site (3–4 m), and the reef consisted of basalt boulders, mostly less than 1-m in height, on a sandy substrate. The study site covered an area of 58 × 58 m that we subdivided into four plots each of 24 × 24 m separated by corridors 10-m wide (Fig. 1). Each plot was further divided into 1 m^2 cells using a grid system. Two diagonally opposed plots were randomly

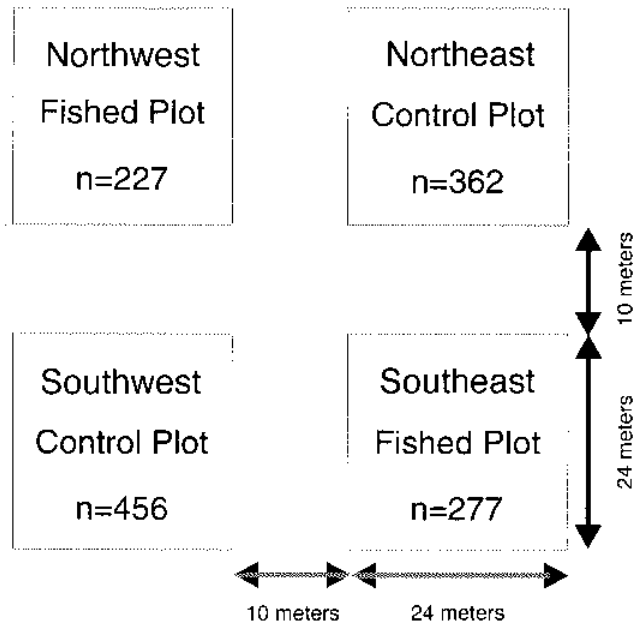

Figure 1. Diagram showing the numbers tagged (n) and plot dimensions at Point Cook.

selected for fishing to test whether fishing affected dispersal rates, and the remaining pair of plots were designated as controls. In the fished plots, 40% of the untagged abalone population were removed during experimental fishing.

Two prefishing and two postfishing dive surveys were completed, with approximately 7 months between the first and fourth surveys for each plot. During the initial prefishing survey, each cell in the grid was systematically searched and the number of abalone in the cell recorded. Every fifth abalone encountered was tagged *in situ* with a rivet tag inserted through a respiratory pore using a method similar to that used by Prince (1991). The position of each tagged abalone was recorded to the nearest 0.1 m within the cell. The relief of each cell was subjectively characterized as being either flat, low, medium, or high. During subsequent surveys, each of the plots was systematically searched as before, and the position of every tagged abalone resighted was recorded. The second prefishing survey was conducted 3–4 months after the initial survey and immediately prior to experimental fishing. The third survey occurred 3–4 weeks after fishing and the fourth and final survey 10–11 weeks after fishing. Searches up to approximately 30 m beyond the plot boundaries were conducted to detect large movements of tagged abalone.

Calculation of Dispersal Rate

To estimate the number of abalone expected to disperse from a plot, we first calculated distances moved by resighted tagged abalone. The frequency distribution of distances moved was then adjusted by predicting movements of individuals emigrating from the plot. We applied all predicted movements, in all possible directions of travel, to each initial tag position to estimate the probability of each individual moving outside the plot. The sum of each individual probability was the number expected to be lost through dispersal.

Predicting Unobserved Movements

The position of each tagged abalone within the plot was recorded as an x and y coordinate. The distance traveled d_k for abalone k, from its initial position (i) to its finishing position (f) was calculated using the following equation.

$$d_k = \sqrt{[(x_{fk} - x_{ik})^2 + (y_{fk} - y_{ik})^2]}, \quad \text{where i, f, k} \in [1, n_{res}] \quad (1)$$

and n_{res} is the total number of re-sighted tags.

A distribution of distances d_k for resighted tagged abalone was obtained from all movements within an experimental plot. From an initial tag position $x_{ik}y_{ik}$, a distance moved d_k in direction θ will result in the hypothetical finishing position $x_{hk}y_{hk}$. Distances d_k are biased against larger movements, because the larger the movement d_k, the greater the probability that $x_{hk}y_{hk}$ will be outside of the plot. Therefore, a predicted distribution of distances was calculated using the resighted distribution by obtaining the proportion of occasions $x_{hk}y_{hk}$ was outside the plot for all possible hypothetical movements ($1° \leq \theta \leq 360°$), assuming random direction of movement (Fig. 2).

The displacement (x_{disp}, y_{disp}) of each hypothetical movement ($1° \leq \theta \leq 360°$) was obtained by assigning all absolute angles of θ from $x_{ik}y_{ik}$ and its actual distance moved d_k, (Eq. 2–3).

$$x_{disp} = d_k \times \sin\theta \text{ and } y_{disp} = d_k \times \cos\theta \quad (2)$$

for $\theta \in (0°, 90°)$ or $\in (180°, 270°)$

$$x_{disp} = d_k \times \cos\theta \text{ and } y_{disp} = d_k \times \sin\theta, \quad (3)$$

for $\theta \in (90°, 180°)$ or $\in (270°, 360°)$.

The hypothetical finishing position of $x_{hk}y_{hk}$ was compared with each of the four plots' boundaries to determine if it was inside the plot (Eq. 4).

Let $x_{hk} = x_{ik} \pm x_{disp}$ and $y_{hk} = y_{ik} \pm y_{disp}$.

Define x_{min}, y_{min} = minimum x/y ordinate of the plot.
and x_{max}, y_{max} = maximum x/y ordinate of the plot.

If $(x_{min} \leq x_{hk} \leq x_{max})$ and $(y_{min} \leq y_{hk} \leq y_{max})$
then the tagged abalone at $x_{hk}y_{hk}$ remained within the plot. (4)

The probability P_{dk} of abalone k remaining within the plot having moved distance d was calculated to be

$$P_{dk} = \sum_{\theta=1}^{360} \text{count}/360, \text{ where count} = 1, \text{ if } x_{hk}y_{hk} \text{ is inside the plot.} \quad (5)$$

The predicted frequency of the distance d_k was defined by

$$\text{freq}_{dk} = 1/P_{dk}. \quad (6)$$

The predicted and actual frequencies were summed across abalone k for each distance d to produce a frequency distribution function for all possible distances. Calculations from Eqs. 1–6 were repeated for all abalone resighted for each plot and survey. A frequency distribution function was created from all plots and surveys combined (see Fig. 4).

Estimation of Number Dispersed

For each distance d_k and all possible directions, Eqs. 2–4 were repeated for all initial tagged positions, $x_{ia}y_{ia}$ (instead of $x_{ik}y_{ik}$),

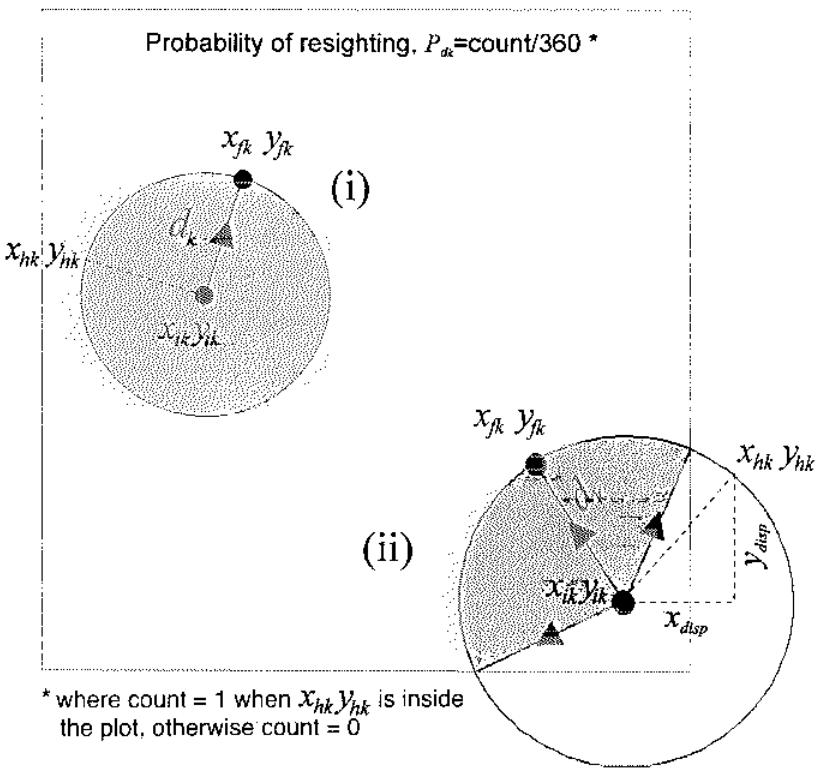

Figure 2. The probability of P_{dk} of a tagged abalone being resighted within a plot given its initial position $x_{ik}y_{ik}$, distance traveled d_k (assuming random direction of movement), and final position $x_{fk}y_{fk}$. Hypothetical finishing positions $x_{hk}y_{hk}$ that remain within the plot are shaded. Examples are shown for an abalone: (1) that would remain within the plot, regardless of the direction of movement; and (2) when some directions of movement resulted in $x_{hk}y_{hk}$ being outside plot boundaries, determined by adding x_{disp} and y_{disp} to its initial position $x_{ik}y_{ik}$.

even if not re-sighted, for abalone a where i.a $\in [1, n_{tot}]$ and n_{tot} is the total number tagged in a plot. The number of times the individual was found inside and outside a plot (Eq. 4) was multiplied by the predicted frequency at distance d_k (Eqs. 7 and 8).

$$\text{freq}_{in\_a,dk} = \text{freq}_{d_k} \times \text{count where count} = \begin{cases} 1 \text{ if } x_{ha}y_{ha} \text{ is inside the plot} \\ 0 \text{ otherwise} \end{cases} \quad (7)$$

$$\text{freq}_{out\_a,dk} = \text{freq}_{d_k} \times \text{count where count} = \begin{cases} 1 \text{ if } x_{ha}y_{ha} \text{ is outside the plot} \\ 0 \text{ otherwise} \end{cases} \quad (8)$$

The probability P_a of the theoretical finishing position for each initially tagged abalone, $x_{ha}y_{ha}$, being outside a plot for all possible movements was given by

$$P_a = \Sigma \text{ freq}_{out\_a}/\Sigma (\text{freq}_{in\_a} + \text{freq}_{out\_a}). \quad (9)$$

The expected dispersal (E_d) outside a plot and the expected remaining (E_r) within a plot for each survey were given by

$$E_d = \sum_{a=1}^{n_{tot}} P_a \quad \text{and} \quad E_r = n_{tot} - E_d \quad (10)$$

The number of tagged individuals expected and the number resighted were regressed against the median of the number of days at liberty for resighted individuals for each plot and survey. Their slopes were determined as the instantaneous rate of tag dispersal and disappearance, respectively. Fishing mortality was assumed as zero, because all legal fishing within 100 m of the site ceased 6 months before and throughout the project. Cryptic loss was also assumed as zero, because there was minimal cryptic habitat inaccessible to divers. Therefore, the difference between the slopes represented disappearance caused by M, tag loss, and observer error.

Differences in dispersal rates were compared with the type of relief in each plot by expressing the number of tagged abalone expected to remain E_r as a percentage of the number tagged n_{tot}. The effect of experimental fishing was determined as the change in percentages between the second and third surveys.

RESULTS

Annual rates of dispersal ranged from 0.49 in the northwest fished plot, to 0.78 in the northeast control plot (Fig. 3), contributing 40–60% of total tagged abalone disappearance for all plots. The remaining three components of tag disappearance (natural mortality, tag loss, and observer error) ranged from 0.49 in the northwest fished plot to 0.82 in the southeast fished plot (Table 1).

The majority of abalone movements were less than 10 m (Fig. 4). The largest movement detected between the first and second surveys (4 months) was less than 50 m. The maximum distance traveled by any tagged individual over the course of the study (7 months) was 60 m (Fig. 4). Searches beyond plot boundaries failed to detect any movements greater than those measured within the plots.

The number of resighted tagged abalone, n_{res}, decreased between 50–55% from initial tagging (first survey) to the second

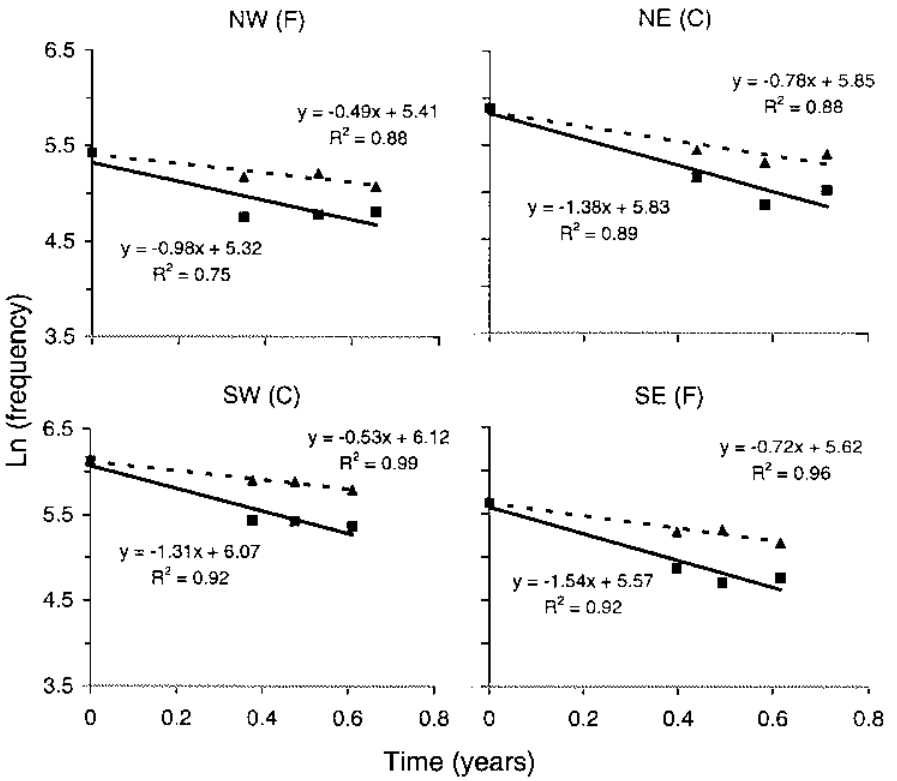

Figure 3. Regressions of the natural logarithm of the number of tags observed (■) and the number of tags expected (▲) after dispersal against time for each plot.

survey (Table 2). Numbers resighted remained fairly consistent or decreased only slightly after the second survey, decreasing by 5% from the second to fourth survey in the northeast and southwest plots, decreasing by 4% in the southeast plot and an increase of 2% in the northwest plot. The expected number of tagged abalone remaining, E_t, varied between plots after the second survey (Table 2). In the northeast plot, only 64% of tagged abalone were expected to remain after the second survey, and in the southwest plot, 80% were expected to remain. From the second to fourth surveys, only 3 to 8% more were expected to disperse outside each plot (Table 2).

Prefishing dispersal varied depending on the amount of relief of the reef (Table 3). The southwest control plot contained the highest proportion of medium to high relief (56%) and showed the lowest initial dispersal (20%) of the tagged population. In contrast, the northeast control plot had the lowest amount of high relief (14%) and showed high initial dispersal (36%). Dispersal decreased in fished plots following fishing, by 2 and 3% in the southeast and northwest plots, respectively (Table 3). The southwest control plot showed a small increase of 1%; whereas, in the northeast control plot, dispersal increased by 8% after fishing.

TABLE 1.

The components of tag disappearance for all plots expressed as annual rates and dispersal expressed as a percentage of tag disappearance.

	Fished Plots		Control Plots	
Annual Rates of:	**NW**	**SE**	**NE**	**SW**
Disappearance (observed)	0.98	1.54	1.38	1.31
Dispersal (expected)	0.49	0.72	0.78	0.53
Dispersal as a percentage of disappearance	50%	47%	57%	40%
M+tag loss+observer error	0.49	0.82	0.60	0.78

DISCUSSION

Dispersal rates contributed between 40 to 60% of total tag disappearance in the four plots at Point Cook (Table 1). In this study, dispersal had greater potential to bias estimates of natural

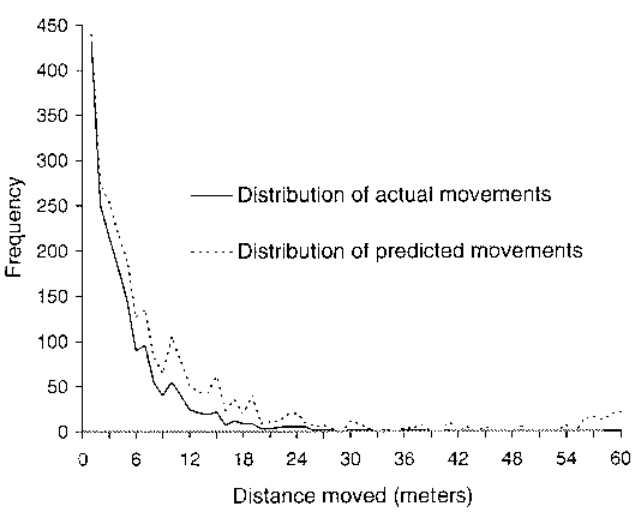

Figure 4. Actual and predicted movement frequency distribution functions of tagged abalone at Point Cook.

TABLE 2.

The number and percentage of tagged abalone expected to remain within each plot after dispersal, E_r, and number and percentage of tagged abalone resighted, n_{res}.

Plot	Plot Type	Number Tagged n_{tot} 1st Survey	Expected Remaining E_r (%) 2nd Survey	3rd Survey	4th Survey	Number Resighted n_{res} (%) 2nd Survey	3rd Survey	4th Survey
NW	Fished	227	176(78)	183(81)	160(70)	103(45)	104(46)	106(47)
SE	Fished	277	198(71)	203(73)	174(63)	137(49)	117(42)	124(45)
NE	Control	362	232(64)	203(56)	222(61)	177(49)	136(38)	158(44)
SW	Control	456	366(80)	360(79)	328(72)	228(50)	224(49)	207(45)

mortality than did tag loss and observer error combined. Ignoring dispersal would have resulted in overestimation of natural mortality by more than 200%. These high dispersal rates existed despite the fact that the majority of movements were under 10 meters. Some studies have shown that abalone populations can be highly mobile, moving hundreds of meters within months and kilometers in their lifetime (Newman 1966, Ault and DeMartini 1987). In such circumstances, the contribution of dispersal to over-all tag disappearance will increase, causing further bias to natural mortality estimates.

There seems to be two different rates of tag disappearance between initial tagging and the second survey, as compared to the second to fourth surveys (Table 2). Although the number of tags resighted, n_{res}, should have reduced at a similar rate after each survey, this was not the case. Two factors may have increased initial tag disappearance.

Higher initial dispersal rates may have been caused by disturbance caused by tagging. While *in situ* tagging may reduce initial tagging-induced dispersal, it is unlikely to eliminate the problem. Results from this study have shown that even small movements will considerably affect dispersal. Alternatively, initial tag loss may have been higher than tag loss from the second to fourth surveys. Treble and Day (1993) showed that the rate of tag loss for glue tags applied to the shell of limpets changed over time. The initial rate of tag loss was low while the glue maintained full adhesion; however, as the glue reached a critical age its adhesive ability reduced rapidly, and the rate of tag loss increased. In our opinion, the opposite occurs with *in situ* rivet tags, because initial rates of tag loss may be relatively high. Poorly inserted tags or weak shells have a high rate of shedding, leaving tags only in those animals tagged securely.

Beinssen and Powell (1979) modeled abalone dispersal by observing tag movements between 10 × 10 m blocks. Their technique was repeated by Shepherd (1986) for the greenlip abalone *H. laevigata*, but was unsuccessful, because the assumption of random movement was violated. Shepherd (1986) found that *H. laevigata* migrated toward the direction of swell or main currents, using a technique described by Clavier and Richard (1984) for *H. tuberculata*. No such patterns were observed for *H. rubra* during this study, probably because Point Cook is not subject to any swell or currents. It could be then argued that our assumption of random movement is reasonable.

Beinssen and Powell (1979) alluded to a violation of their main assumption of localized movements. Within a month, movements of up to 200 m were observed, three times greater than the maximum distance detectable within their resurveyed area. The maximum distance detectable within the Point Cook plots was 70 m, and searches made beyond the plot boundaries roughly doubled this distance. Because the largest detected distance moved was under 60 m over 7 months, the assumption of localized movement seems valid. Another assumption of our dispersal estimation method was that plotting disappearance and dispersal against the median number of days caused minimal bias to the estimates. This was necessary, because each survey took several days to complete. It was considered a reasonable assumption, because the majority of movements were small, and the variation in the days at liberty was minimal relative to the time between surveys for each plot.

Tagging-induced disturbance seemed to increase the initial movements of tagged abalone during Beinssen and Powell's (1979) study. Approximately one-third of movements greater than 50 meters occurred within 1 month of tagging during their 3-year study. Detected movements for our study did not exceed 50 m after the second survey, approximately 4 months after initial tagging.

The tagging method adopted by Beinssen and Powell (1979) required removal of the abalone from the substrate. Several studies have shown that disturbance of abalone by tagging induces abnormal movement (Forster 1967, Fournier and Breen 1983, Ault and DeMartini 1987). The tagging technique used in this experiment has the advantage of being an *in situ* method. Animals were never

TABLE 3.

Effect of available preferred habitat on estimates of dispersal for fished and control plots.

Plot	Plot Type	% Medium and High Relief	Prefishing Dispersal (1st–2nd Survey)	Postfishing Dispersal (1st–3rd Survey)	Change after Fishing (2nd–3rd Survey)
NW	Fished	38%	22%	19%	Decrease 3%
SE	Fished	41%	29%	27%	Decrease 2%
NE	Control	14%	36%	44%	Increase 8%
SW	Control	56%	20%	21%	Increase 1%

removed from the substrate, and the tag was inserted into a pore in an operation taking less than 1 minute. This technique provided less stimulus for rapid initial movement, because the strong foothold was never disrupted. It was observed that a low percentage of animals underwent rapid initial movement after *in situ* tagging, as compared to those that were removed and returned to the substrate in other studies (C. Dixon, pers. comms.).

Tagging methods may also influence over-all rates of tag disappearance in three ways: tag loss, observer error, and tag-induced mortality. A poor tagging method resulting in high tag loss will obviously result in high rates of tag disappearance. Perhaps just as important is the ease with which remaining tags are found. Inconspicuous tags may be missed, resulting in higher rates of observer error. Third, tagging methods that require removal of individuals from the substrate often cause mortality by injuring the foot. This mortality is difficult to differentiate from natural mortality. In this study, all of these factors were minimized. The rivet tag reduced rates of tag loss (Prince 1991), the use of brightly colored large plastic tags ensured that tags remained conspicuous throughout the period of the study, and *in situ* tagging removed the possibility of damaging the foot.

The position an abalone occupies on a reef is often referred to as a home-site (Momma and Sato 1969, Shepherd 1973, Tarr 1995). Home-sites such as crevices or gutters may be chosen, because they provide protection from predators, or they are sites that are likely to act as areas for entrapment of drift algae. Shepherd (1986) found that the magnitude of movements of greenlip abalone *H. laevigata* in Waterloo Bay, South Australia, were larger in areas with little cryptic habitat. In Shepherd's (1986) study, cryptic habitat provided the greatest number of preferred home-sties, but at Point Cook, cryptic habitat was scarce. Assuming that each abalone occupied a unique home-site, then the spatial density distribution of the prefished population should have reflected preferred habitat. The grid system used in our experiment allowed categorizing of cells by relief (height of the reef). Preferred habitat seemed to be of medium-to-high relief reef. Such reef accounted for only 37% of the total planar area but contained 60% of the total abalone population in this study.

Our results were consistent with Shepherd's (1986) findings, that movement, reflected by estimated dispersal, decreases as the area of preferred habitat increases (Table 3). The southwest control plot contained the highest amount of medium and high relief and had the lowest percentage of initial dispersal. In contrast, the northeast control plot had by far the lowest amount of high relief and had a higher initial dispersal. Initial dispersal was calculated from movements prior to any removal of abalone from the population.

The removal of 33% of the population during experimental fishing increased the amount of available home-sites/preferred habitat within the two fished plots. If dispersal increased over time, a reduced rate of increase would be expected. There was an estimated reduction in the percentage of dispersal at the two fished plots of both 2 and 3%, respectively (Table 3). In the unfished control plots, a continued increase in dispersal was expected. The southwest plot showed only a 1% increase; whereas, in the northeast plot, dispersal continued to increase by 8%. This discrepancy seems to be attributable to the difference in the number of home-sites/preferred habitat available.

Although this study required substantially more searching effort than that available to most tag–recapture studies, carefully planned experiments that estimate dispersal and observer error can overcome the need for thorough searching of large areas. Searching small areas thoroughly will reduce observer error, but if tagged abalone movements are larger than the maximum dimension of the search area, then the assumption of localized movements will be violated. Random sampling from a larger area would be preferable, because it will ensure that the largest magnitudes of movement could be detected. If double counting exercises are used, estimates of observer error as well as dispersal may be achieved.

This study could only estimate dispersal attributable to emigration outside the survey area, although further work is planned to estimate tag loss and observer error. At Point Cook, the lack of cryptic habitat meant that disappearance of tagged abalone into crypsis was minimal. However, at sites with more cryptic habitat, the capacity for such disappearance will be greater. Therefore, site selection should consider the complexity of the reef for potential losses attributable to crypsis as well as the number of available home-sites in relation to magnitude of potential movement. The tagging method used in future studies should consider how tagging affects movement and tag induced-mortality. We believe that an *in situ* tagging method is preferable, because this study has shown that this approach reduces initial magnitudes of movement.

Past estimates of natural mortality could have benefited from a quantitative estimate of dispersal. We have shown that with a defined area of tag release and recapture and a distribution of population movements, estimation of dispersal can be achieved. Future tagging studies that aim to estimate natural mortality will have to consider all components of tag disappearance. Aside from natural mortality, these include dispersal, cryptic loss, fishing mortality, tag loss, tagging-induced mortality, and observer error. Failure to account for any one of these components will overestimate natural mortality.

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A PCR TECHNIQUE FOR FORENSIC, SPECIES-LEVEL IDENTIFICATION OF ABALONE TISSUE

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ABSTRACT Although the incidence of abalone poaching is increasing in South Africa, several alleged poachers have been acquitted in cases where the state has been unable to prove that the confiscated meat is of the local abalone, *Haliotis midae*. This species is illegally exported to the Far East by poaching syndicates; a practice that is undermining the legitimate industry. To solve this, a polymerase chain reaction (PCR) technique that targets a portion of the lysin gene of several abalone species and unequivocally distinguishes between *H. midae* and *H. spadicea* (a sympatric congeneric) has been developed. The PCR primers specifically amplify approximately 1,300 bp of genomic DNA from dried, cooked, and fresh abalone tissue. A smaller fragment of 146 bp is used for canned abalone. Restriction fragment length polymorphism (RFLP) exploit interspecific polymorphisms that discriminate between these two species. The method can also be used to identify *H. rubra* and can easily be adapted to other abalone species under the same threat of overexploitation.

KEY WORDS: polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), forensic, abalone, poaching, lysin

INTRODUCTION

The abalone *Haliotis midae* (Linnaeus 1758) is the only commercially exploited abalone of the six species that occur on the South African coast. *H. spadicea* (Donovan 1808) is exploited by recreational divers but is too rare and small to be commercially viable. For *H. midae*, the annual commercial quota stands at 550 metric tons (Stuttaford 1997). It is estimated that the amount exploited by recreational divers approximates the commercial quota (Payne and Mathews 1995, Tarr 1997). A third category of exploitation, abalone poaching is difficult to quantify. However, estimates in just one area in the Western Cape province show that at least 40 tons of *H. midae*, valued at approximately US $1 million to local producers, is poached annually (Tarr 1997).

The incidence of abalone poaching in South Africa has increased in recent years following the establishment of sophisticated syndicates who illicitly procure, process (dry and canned abalone), and export the abalone to the Far East (E. Pieters, pers. comm.). The effects of these activities are beginning to affect the legitimate industry, which employs several hundred people and also provides a significant source of foreign currency. Lower demand on the oversupplied international market and depreciation of the value because of the poor quality of the illicit abalone reaching the Far East have resulted in losses to legitimate exporters. Reduced returns, resulting from these effects, threaten the development of abalone farming, in which there is currently a substantial capital investment. Abalone poaching and illegal trade in abalone products are international problems and occur for most major abalone fisheries, mainly because of the attractive prices obtained (up to US $75 per animal, Daniels and Floren, this volume). The overexploitation of abalone (attributable to the combined effects of both commercial and illegal fishing) in California has caused a sharp decline in stocks and the possible extinction of at least one species, *H. sorenseni* (Tegner et al. 1996, Davis et al. this volume).

In several trials of alleged poachers in South Africa, the accused have either claimed that the abalone in question are not *H. midae*, claiming instead that they are *H. spadicea* (a congener for which less stringent regulations exist) or that the product in question is not abalone at all. Consequent to such claims, several alleged poachers have been acquitted because the State could not show that the confiscated material (especially in the case of processed abalone) was, in fact, *H. midae*. In one case, canned abalone labeled as "Product of Australia" was suspected to contain poached South African abalone, but the authorities had no means of determining this from visual inspections. For this reason, a method is required that would unequivocally identify fresh or processed abalone to species level, and would conform to the legal requirements for forensic purposes.

A polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) technique has been developed, based on the lysin sperm protein cDNA sequences previously used in a systematic study of the *Haliotis* genus (Youn-Ho and Vacquier 1995). That study included 23 of the 55 described extant species (Geiger, 1998) and several of the approximately 25 commercially exploited abalone species (Cox 1962, Shepherd et al. 1992). During fertilization, this protein creates a hole in the vitteline membrane in the ovum (Vacquier et al. 1990), and given a proposed example of positive Darwinian selection, is unusually divergent between species; however, highly conserved within species, thereby conferring a high degree of species specificity (Vacquier et al. 1990, Shaw et al. 1994, Vacquier et al. 1997). These features make the lysin gene an informative tool for the characterization of abalone species, and the methodology developed in the present

TABLE 1.

Details and sequences of PCR primers. Y = C + T, W = T + A, in degenerate positions. HGEN = generic primers, HMID = *H. midae*-specific primers, HSPAD = *H. spadicea*-specific primer. Positions (numbered from the 3′-end) and direction (F = forward and R = reverse) are according to the *H. midae* lysin cDNA sequence of Youn-Ho and Vacquier (1995; see Fig. 4). Partially complementary restriction site sequences (underlined) were incorporate into the 5′-end of some of the primers to facilitate cloning of products, in the case of HMID-A, noncomplementary bases were incorporated in the 5′ region to increase the TM of the primer.

PCR Primer	Sequence (5′ to 3′)	Position
HGEN-1	GGTCAGGAGAAACTTGATCCC	424-F
HGEN-2	ACTATYGWACAATGTTTACGAGTTAAATAGA	620-R
HMID-A	TCTATCTACAACCGTGACACGACC	524-F
HMID-UP2	CGTTCTAGAGAAACTTGATCCCTTCC (*Xba*I)	428-F
HMID-LP1	GCGCTGCAGCAAAAATATTTATTTAC (*Pst*I)	649-R
HSPAD	GGTTCCACGTGCAATTATGC	483-R

study for identification of *H. midae* products can be readily adapted to other *Haliotis* species for which similar problems of illegal exploitation exist.

MATERIALS AND METHODS

Specimen Collection

Fresh *H. midae* and *H. spadicea* specimens were obtained from False Bay near Cape Town, South Africa. *H. rubra* tissue from two individuals was obtained from Dr. B. Degnan of the Department of Zoology at the University of Queensland, Australia and was transported to the University of Cape Town in a frozen state. Dried and canned abalone, for control experiments or as test cases, were either supplied by the South African Police Services or from a commercial company. Abalone mucous was obtained by placing fresh shucked and cleaned *H. midae* specimens in unused plastic bags. After 1 hour, the secreted mucous was collected and frozen until DNA extraction.

DNA Extraction

DNA was extracted from fresh abalone tissue using a CTAB extraction method. Briefly, 0.5 g adductor muscle (or 0.5 ml mucous) was digested in extraction buffer (100 mM Tris, 50 mM EDTA, 400 mM NaCl, 1% SDS, pH 8) with fresh proteinase K (0.5 mg/ml) at 55°C overnight. To extract mucopolysaccharides differentially, (that otherwise coprecipitate with the DNA) NaCl (0.75 M) and CTAB (1%, Sigma, USA) were added, samples were incubated at 65°C for 1 h, and then extracted once with chloroform/isoamyl alcohol (24:1). DNA was precipitated by addition of ethanol, washed, and dissolved in 2 ml TE buffer. For the dried and processed tissue, DNA from approximately 25 mg of tissue was extracted using a commercially available DNA extraction kit (Quiamp Tissue Kit, Quiagen Inc., Germany).

PCR Reactions

Each 100 µl PCR reaction contained approximately 500 ng abalone DNA (diluted from stock extractions in TE buffer), 200 µm of each dATP, dCTP, dGTP, dTTP, 50 pmoles of each primer (Table 1), 0.04 mM $MgCl_2$, 2 U of *Taq* polymerase (Boehringer Mannhein, Germany) in a buffer supplied by the manufacturer. A PCR blank, containing no template, was used for each series of reactions. PCR reactions were overlaid with 100 µl of mineral oil (Sigma, USA) prior to 35 cycles of amplification on a Techne PHC-2 thermal cycler. Each cycle consisted of: denaturing at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 45 s with a final 10 min extension at 72°C. PCRs were performed using DNA templates extracted from fresh/frozen, dried, or canned tissues and from mucous. In cases where poor quality DNA was recovered, further purification of the DNA or reamplification of the PCR products was conducted. PCR blanks (no template) and, in test cases, extraction blanks (run in parallel with DNA extractions from disputed abalone samples to exclude contamination during the extraction procedure) were run where appropriate. In cases where no amplification was obtained or anticipated (e.g., negative controls), the reactions were tested for PCR inhibitors by spiking the refractory DNA template with an appropriate freshly prepared DNA template. All PCR reagents were stored, and reaction preparations were conducted in a laboratory separate from where the PCR reactions were run or the templates stored.

DNA Sequencing

Where appropriate, PCR products were directly sequenced using a USB Sequenase Version 2.0 DNA sequencing kit modified according to the methods of Casanova et al. (1990) and Bachmann et al. (1990).

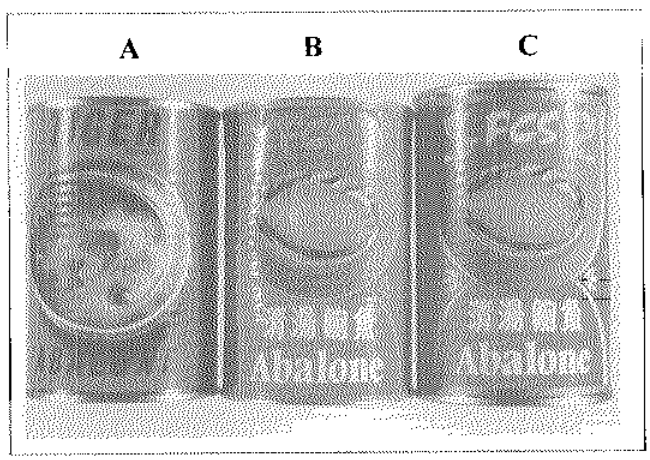

Figure 1. Confiscated abalone cans. Can A is a can of South African abalone, and can C contains Australian product. Can B is the suspect can, which is shaped like the South African can (has the same color and size), but has a copy of the Australian label (i.e., labeled "Product of Australia") and lacked the required embossing. The can was claimed to contain *H. rubra* and alleged to contain *H. midae*. The contents of can B were shown to be *H. midae* (see text and Fig. 5).

TABLE 2.

Predicted and estimated PCR fragment sizes from various primer combinations. D = difference between predicted and detected band sizes in base pairs. M = *H. midae*, S = *H. spadicea*, R = *H. rubra*.

Primer Combination	Predicted	Estimated	D *H. midae*	D *H. spadicea*	D *H. rubra*
HMID-UP2 + LP1	272	1,300	1,028	—	—
HMID-A + HGEN-2	146	146	0	—	—
HGEN-1 + HSPAD	105	1,000	—	895	—
HGEN-1 + HGEN-2	M S R 250, 255, 250	M S R 1,000, 1,300, 700	1,050	947	450

Strategies for the Identification of H. midae *Amplification Products*

Species-Specific Approach

H. midae-specific PCR primer combinations were designed by comparison with all the published abalone lysin cDNA sequences (Youn-Ho and Vacquier 1995) and positioning of one of the primer pairs 3′-ends at sites unique to *H. midae* (see Fig. 4). Degenerate generic primers were designed to amplify DNA from *H. midae*, *H. spadicea*, and *H. rubra* and sequence data derived from these PCR products were then used to design *H. spadicea*-specific primers. The sequences of the six PCR primers designed for this study are presented in Table 1. HMID-A, HMID-UP2, and HMID-LP1 are *H. midae*-specific primers, HSPAD is *H. spadicea*-specific, and HGEN-1 and HGEN-2 are generic primers.

Primers HGEN-2, HMID-A, HMID-LP1, and HSPAD were located in the less conserved 3′ untranslated region of the cDNA, and HGEN-1 and HMID-UP2 were located in the conserved coding region of the cDNA. The combination of primers HMID-UP2 (F) + HMID-LP1(R) was predicted to amplify a fragment of 272 bp in length, and HMID-A(F) + HGEN-2(R) targeted a smaller fragment of 146 bp from *H. midae*. The combination of primers HGEN-1(F) + HSPAD(R) targeted a fragment of 105 bp in *H. spadicea*. The generic primers HGEN-1(F) + HGEN-2(R) targeted a fragment of approximately 250 bp in length. The primers were synthesized by the Department of Biochemistry at the University of Cape Town, South Africa.

To test the specificity of the species–specific primers, PCRs were performed on a *H. midae* DNA template with *H. spadicea*-specific primers and vice versa. *H. midae* primers and the generic primers were also tested on a *H. rubra* DNA template.

For canned abalone, the combination of primers HMID-A + HGEN-2 was used. A test case concerning a consignment of abalone of disputed origin (see Fig. 1) was used to test this primer combination, as well as to establish a forensic protocol.

PCR–RFLP Approach

Informative interspecific polymorphisms that consistently differentiate between *H. midae* and *H. spadicea* were detected by restriction endonuclease digestion of PCR products amplified by the generic primers (HGEN-1 + HGEN-2). The restriction digests were either performed directly on PCR products or on gel-purified PCR products (Quiex gel extraction kit, Quiagen, Germany).

The 20 µl digestion reaction consisted of 5–10 µl of the PCR reaction, and 5 U of the appropriate restriction endonuclease in a buffer supplied by the manufacturer. Digestion was allowed to occur overnight at 37°C. PCR products and digested fragments were visualized by UV illumination after electrophoresis on 2% agarose gels containing ethidium bromide. Restriction fragment lengths were estimated by comparison to a molecular weight marker (100 bp ladder) using a UVP gel image analyzer. A panel four, five, and six-base cutters were tested, from which the restriction endonucleases *Cfo* I, *Dra* I, *Hin*fI, and *Taq*I were selected for analysis.

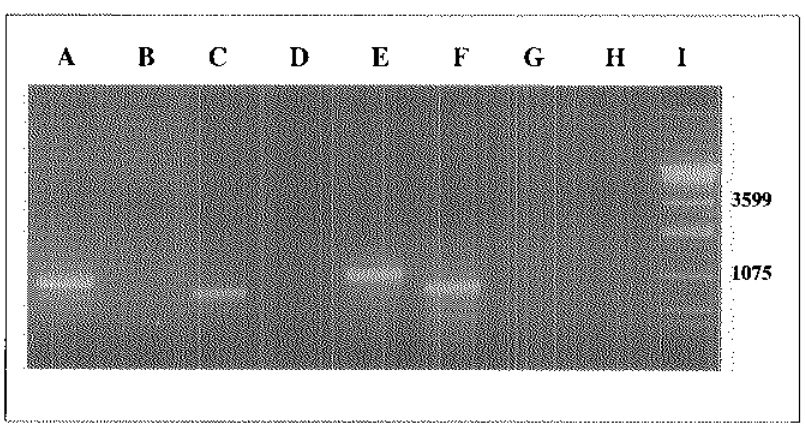

Figure 2. Species-specific PCR products on a 1% agarose gel. Lane A—*H. midae*-specific PCR primers (HMID-UP2 + HMID-LP1) on *H. midae* DNA template. Lane B—*H. midae*-specific primers on *H. spadicea* DNA template. Lane C—*H. spadicea*-specific primers (HGEN-1 + HSPAD) on *H. spadicea* DNA template. Lane D—*H. spadicea*-specific primers on *H. midae* DNA template. Lanes E and F—*H. midae*- and *H. spadicea*-specific primers, respectively, on mixed DNA (*H. midae* and *H. spadicea*) as positive controls to show absence of inhibition of *Taq* polymerase. Lanes G and H—PCR-blank with respective templates. Lane I—fragment size marker: *Dra*I digested 1 DNA, sizes in base pairs.

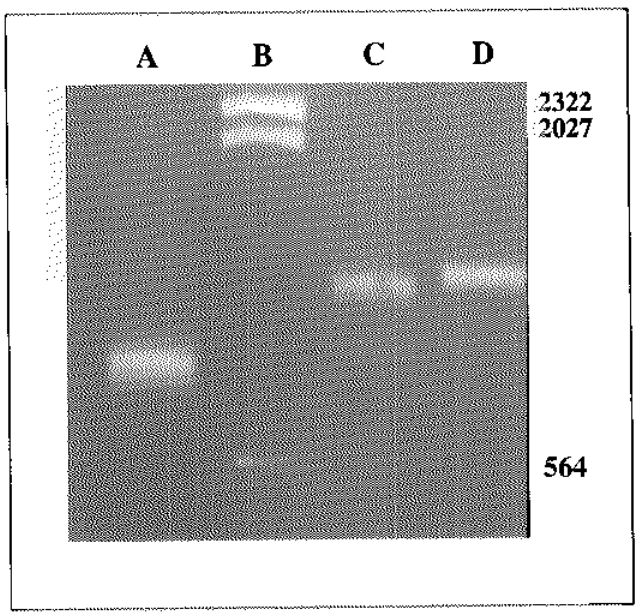

Figure 3. PCR amplification products of DNA templates with generic primers on a 1% agarose gel. Lane A—*H. rubra* DNA template. Lane B—fragment size marker: *Hind*III digested 1 DNA, sizes in base pairs. Lane C—*H. spadicea* DNA. Lane D—*H. midae* DNA.

RESULTS

High molecular weight DNA was recovered from all tissue types, except in the case of dried and canned abalone, when relatively low molecular weight DNA was recovered probably attributable to degradation caused by the processing of the abalone.

The fragment size for all primer combinations, except HMID-A + HGEN-2, differed from the size predicted from the cDNA sequences (Table 2, Figs. 2 and 3). DNA sequencing of the larger than predicted PCR products revealed the presence of an intron of between 895 and 1,100 bp at position 459 on the cDNA sequence (Fig. 4) in *H. midae*, *H. spadicea*, and *H. rubra*. The same intron (of varying size) has been found in *H. rufescens*, *H. fulgens*, and *H. corrugata* (E. Metz pers. comm.).

The fragment size difference between *H. midae* and *H. spadicea* (seen in Fig. 2) is attributable not only to the relatively different positions of the specific primers but also to length polymorphisms in the intron. Generic primers HGEN-1 and HGEN-2 amplified products of varying size from *H. midae* (1,300 bp), *H. spadicea* (1,200 bp), and *H. rubra* (700 bp. Table 2, Fig. 3), providing further evidence of interspecific length polymorphisms in the intron, which are in themselves informative characters.

Using the species-specific PCR primers HMID-UP2 and HSPAD, amplification did not occur on nontargeted species template (Fig. 2). Decreasing the annealing temperature from 55 to 45°C, to relax the stringency of the PCR reactions, resulted in a number of nonspecific amplified products, although no fragments of a similar size to the product in *H. midae* were amplified in *H. spadicea* and vice versa.

Amplification (primers HMID-UP2 + HMID-LP1) of large DNA fragments (1,300 bp) from all *H. midae* tissue types was efficient when DNA was prepared from fresh material, but not from canned abalone samples (Fig. 5). In some instances, the efficiency of the PCR reaction was considerably reduced when DNA extracted from dried tissue was used as template. To obviate the lack of amplification from canned tissue, the HMID-A + HGEN-2 combination was used to avoid the intron (Fig. 4), and the resultant fragment of 146 bp amplified efficiently from DNA template extracted all tissue types, including canned (Fig. 5: Lanes H–L). Thus, this fragment is useful where DNA quality is poor and where only low molecular weight DNA template is available. This primer combination was used successfully in identify-

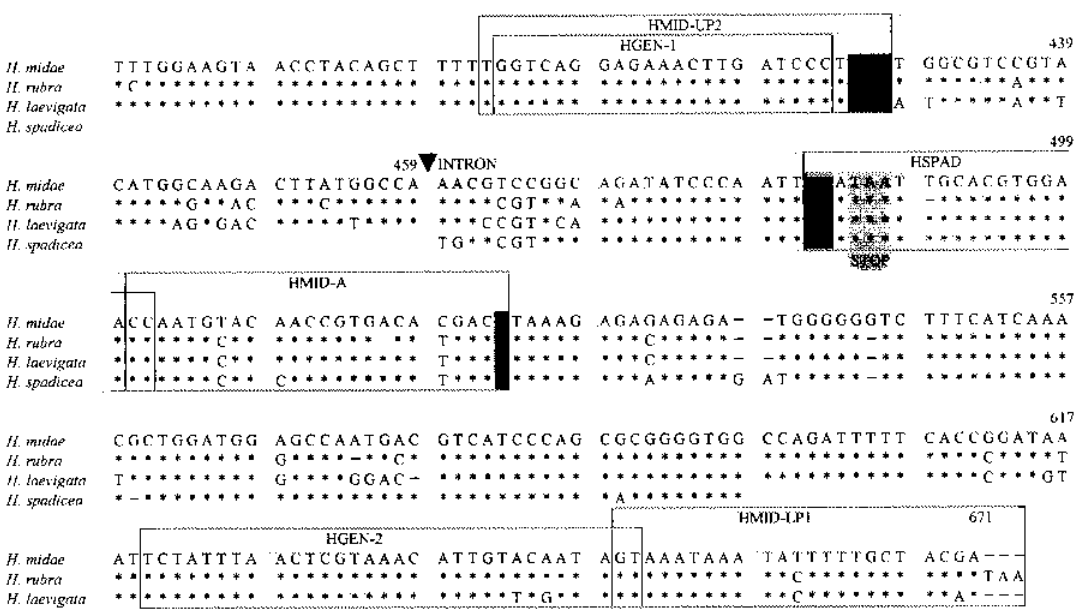

Figure 4. Alignment of lysin cDNA (Youn-Ho and Vacquier 1995) sequences showing relative position of PCR primers (these do not accurately reflect the sequences of the primers as certain changes were made for technical reasons, see Table 1). *H. spadicea* sequence is included where obtained. The positions of the introns and stop codon are also indicated where determined for *H. midae* and *H. spadicea*. Stars indicate identical nucleotide bases to *H. midae*, dashes indicate insertions for alignment.

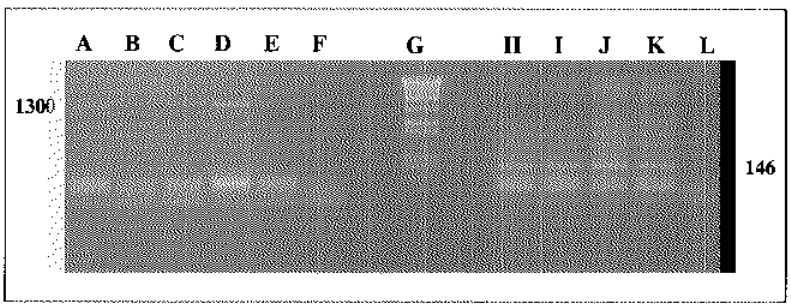

Figure 5. PCR amplification products from DNA extracted from several *H. midae* tissue types on a 2% agarose gel. Lanes A–F—primer combination HMID-UP2 and HMID-LP1. Lanes H–L—primer combination HMID-A and HGEN-2. Lanes A and H—fresh/frozen tissue DNA extract. Lanes B and I—canned tissue. Lanes C and J—dried tissue. Lanes D and K—abalone mucous. Lane E—canned abalone DNA with fresh tissue DNA added to test for inhibition. Lanes F and L—PCR blanks. Lane G—fragment size marker: *Dra*I digested 1 DNA, size in base pairs. Note lack of amplification in lane B and successful amplification of the smaller fragment from the same tissue in Lane I. Size estimates of PCR products are in base pairs.

ing the origin of samples of confiscated canned abalone (Figs. 1 and 6).

RFLPs revealed both site presence/absence and length characters that differentiated *H. midae* from *H. spadicea* (Fig. 7, Table 3). The digests were repeated on 40 individuals (from geographically separate populations) and revealed no intraspecific polymorphisms with the enzymes tested. Digests carried out directly on PCR products were as efficient as those performed on purified PCR product.

DISCUSSION

Molecular and biochemical methods for the identification of species exist for several purposes and utilize species-specific protein and DNA polymorphisms detected by a variety of systems. For medical and agricultural purposes, diagnostic methods that identify parasites or infection to species level have been applied (e.g., Armstrong et al. 1997, Crabtree et al. 1997, Kageyama et al. 1997, Lampo et al. 1997, Roehrdanz 1997). Food quality control and authentication of processed food contents rely on such techniques (Hunt et al. 1997, Ruggeberg et al. 1997), especially in the case of fish and other marine products (e.g., Ward et al. 1995, Meyer and Candrian 1996, Partis and Wells 1996, Ram et al. 1996, McKay et al. 1997, Rehbein et al. 1997). Such methods have been applied to crustaceans (Gangar et al. 1996, Powell et al. 1995) and mollusks where mtDNA has been used to identify which species of land-snail were contained in disputed canned products (Borgo et al. 1996) and to identify sibling species of invading bivalve species (Geller et al. 1994, Geller and Powers 1994). Molecular identification of species for forensic purposes has been used in plants (e.g., Gigliano et al. 1997) and mammals (e.g., Miller et al. 1995, Murray et al. 1995) where DNA polymorphisms, amplified from DNA templates extracted from bloodspots, have been used to determine whether animals have been poached.

The features of the lysin gene, species-specificity, the high degree of divergence, and the presence of the intron are advantageous for the identification of abalone species for forensic purposes. It may be especially useful where discrimination between closely related species or subspecies such as *H. discus discus* and *H. discus hannai* is required.

Although the structure of the lysin protein has been well studied (Shaw et al. 1994), the genomic structure remains unresolved. Although positive Darwinian selection on the lysin protein has resulted in a remarkable degree of divergence at the protein level between species (Vacquier et al. 1990, Shaw et al. 1994, Vacquier et al. 1997), the introns are more conserved (Vacquier, unpublished data). Nevertheless, there is variability with respect to the length of the intron examined in this study. It was fortuitous that the PCR primers designed here were located in adjacent exons, and that the defined genomic fragment was small enough to be easily amplified under the reaction conditions used.

The species-specific primers used in this study demonstrate

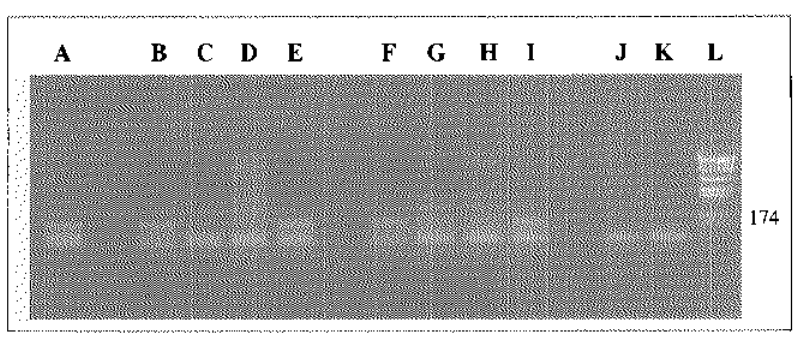

Figure 6. Test case of disputed origin of canned abalone using, primer combination HMID-A and HGEN-2 for the establishment of a protocol on a 2% agarose gel. Lane A—positive control with fresh *H. midae* DNA template. Lane B—positive control with canned *H. midae* (Can A, Fig. 1) DNA. Lane C—negative control with fresh *H. rubra* DNA. Lane D—DNA extracted from contents of a can of disputed origin (not shown in Fig. 1). Lane E—DNA extracted from contents of disputed can B (Fig. 1). Lanes F–I—positive controls to test for inhibition of *Taq* polymerase—50% mixtures of *H. midae* DNA with original templates as in lanes B–E, respectively. Lane J—PCR blank. Lane K—extraction blank. Lane L—fragment size marker *Dra*I digested 1 DNA (lower fragment of doublet = 174 bp).

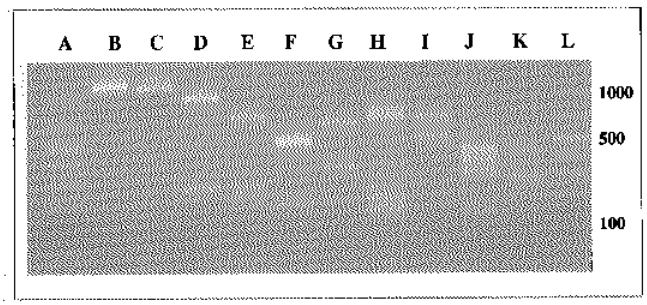

Figure 7. RFLPs of generic PCR (HGEN-1 + HGEN-2) products from *H. midae* and *H. spadicea* on a 2% agarose gel. Lanes A and L—100 bp DNA ladder (fragment size marker, sizes in base pairs). Lanes B, D, F, H, J—*H. midae*. Lanes C, E, G, I, K—*H. Spadicea*. Lanes B, C—undigested PCR product. Lanes D, E—*Dra*I. Lanes F, G—*Cfo*I. Lanes H, I—*Taq*I. Lanes J, K—*Hinf*I.

TABLE 3.

Summary of restriction fragments differentiating between *H. midae* and *H. spadicea* after digestion of PCR products using generic primers. Sites = number of recognition sites. Sizes are length in base pairs. Sizes were estimated from a standard curve calculated from a 100 bp DNA ladder (Fig. 7). Note that the 508 bp band for *Cfo*I and the 171 bp and 180 bp for *Taq*I appear as one band in Figure 7 because 2% agarose has a resolving power of 30 bp.

	H. midae		*H. spadicea*	
R.E.	Sites	Sizes	Sites	Sizes
*Dra*I	1	1032, 268	2	768, 40, 192
*Cfo*I	3	508, 508, 207, 76	2	654, 350, 204
*Taq*I	3	725, 217, 180, 171	1	685, 515
*Hinf*I	3	455, 390, 312, 142	3	410, 331 263, 196

complete specificity. At an annealing temperature of 55°C, *H. midae*-specific primers did not amplify any DNA tested, other than the targeted fragment in *H. midae*. Notwithstanding this, the species-specific approach relies on a negative result (nonamplification) to prove that a sample is not of a particular species. Even with the appropriate controls, this might present legal problems, and for this reason, the species-specific amplification approach should be used as a complement to the RFLP approach.

Use of the generic primers to amplify across the intron, has the advantage of differentiating between the abalone species because of the size difference of the PCR product, even before digestion by restriction endonucleases. The size of the intron varies between species substantially, ranging between approximately 500–1,100 bp. Preliminary data suggest that the size of the intron might be substantially larger (in the region of 4,500 bp) in *H. laevigata*, making this a robust differentiating character in general. The RFLP pattern, however, provides incontrovertible evidence as to the origin of the PCR product. Although all four restriction endonucleases can differentiate between *H. midae* and *H. spadicea*, not all four would need to be used in one instance.

In other examples, where PCR, RFLPs have been used to identify species, the target fragments have been between 100 and 100–500 bp, with most less than 500 bp in length (Borgo et al. 1996, Ram et al. 1996, Rehbein et al. 1997). In the present study, the fragments were originally designed to be less than 300 bp in length, but because of the presence of the intron, they were found to be at least three times this size. This has proved to be problematic in cases where canned abalone has been analyzed, because DNA can become degraded by the canning process (Ram et al. 1996). This was partly true for dried abalone, where the PCRs that targeted the larger fragments worked inefficiently. Most illegally exported abalone is likely to be shipped either canned or dried (morphologically distinguishing features are destroyed in the process), and therefore, it is important that smaller PCR products can be targeted to obviate this potential problem. Where sequences are too short to find differentiating loci, site-directed mutagenesis exploiting interspecific polymorphisms to generate species-specific restriction sites have been employed (Geller and Powers 1994).

Provided that the appropriate precautions and controls are used to exclude the possibility of carryover or contamination from other sources (Knight 1991) and that disputed tissue is collected and stored correctly, this technique will help to minimize the number of erroneous acquittals in abalone poaching cases, in South Africa in particular. For this to occur, a process of accreditation of this technique is to be completed by the South African Police Services' forensic laboratories, where legal standards of control are maintained.

The efficient amplification of *H. rubra* DNA with the generic primers shows that the technique can be easily adapted to other *Haliotis* fisheries where similar problems exist or where quality control requires species-level identification. Currently, such investigations are underway for Californian and Australian abalone species. Ultimately, the success of this technique will depend upon proper legal implementation and application. To deal with poaching, however, this contribution is but one of several approaches to attempt to police and control this complex threat to the stability and sustainability of the South African and other abalone fisheries.

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EXPERIMENTAL ABALONE (*HALIOTIS MIDAE*) SEEDING IN SOUTH AFRICA

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ABSTRACT The experimental release of juvenile abalone (*Haliotis midae*) in an area beyond the natural range of the species, on the northwest coast of South Africa was evaluated. A novel release mechanism was designed and tested for these experiments. Mean minimum survival rates at three experimental sites, at 6 months postrelease, were about 30%, and growth rates were within the range of those reported for naturally occurring populations farther south. Seasonal growth effects as well as between release site variability were observed. These factors as well as age at release and some economic considerations (recapture rate and associated profit for one ton of market size abalone) were considered. Based on these results, it is concluded that seeded abalone will reach market size between 3.5 and 6 years postsettlement, which we suggest offers good prospects for commercial-scale abalone ranching on this coast.

KEY WORDS: abalone ranching, *H. midae*, South Africa, reseeding

INTRODUCTION

Consequent to the development of several abalone culture facilities in South Africa (Cook 1990), the feasibility and potential of abalone seeding, for both commercial (ranching) and stock enhancement purposes, requires appraisal. Currently in South Africa, the commercial abalone quota stands at 550 mt/y (Stuttaford 1997). This represents a reduction of some 65 tons from the 1995 to 1996 season, resulting from a decision taken because of a decline in the southwestern Cape stocks where overexploitation (by fishing, poaching, and recreational utilization) has occurred (SANCOR 1996). The decline has resulted in a lowering of abalone density to below what is considered high enough to facilitate reproduction (Tegner 1993, SANCOR 1996, Tarr et al. 1996) with many sublegal-sized abalone targeted by poachers. This factor, together with a marked increase in predation of sea urchins (on which abalone recruits depend for protection, Day 1998) and juvenile abalone by crayfish has led to a recruitment failure in the area, which forms part of the region of most concentrated fishing effort for abalone (Tarr et al. 1996). In light of this decline and the need for the stimulation of economically depressed coastal regions such as the northern west coast of South Africa (Solomons 1996), the prospects of abalone seeding are particularly relevant.

Abalone ranching is the basis for a significant proportion of the abalone fishery in Japan (Saito 1979, Saito 1984, Kojima 1995), and experimental seeding and the transplant of both juveniles and broodstock has been attempted with various species, with a range in estimates of success (reviewed by McCormick et al. 1994).

Although several devices for abalone seeding have been tested, ranging from sophisticated timed-release systems to disposable PVC containers (Ebert and Ebert 1986, McCormick et al. 1994), other factors, such as size at release, stocking density, predation, microhabitat, and economics have all been identified as important contributors to the success or failure of abalone seeding. Economic viability is a factor that has generally been neglected, with the notable exceptions of Emmet and Jamieson 1989, Schiel 1992, Schiel 1993, and Kojima 1995, where it is demonstrated that economic success is not always attainable, even where biological viability of seeding was achieved.

Where hatchery-produced abalone seed are easily distinguished from naturally occurring seed (e.g. *Haliotis discus discus*, Kojima 1995), the difficulty with assessment of success, in terms of survival and growth rate, is easier to overcome than where they are indistinguishable, as is the case with *Haliotis midae*. More recently, an assessment of the success of abalone seeding has been achieved using genetic markers (Gaffney et al. 1996).

Here, we report on the results of seeding experiments at Port Nolloth, on the northwest coast of South Africa. Port Nolloth lies approximately 400 km north of the range of the abalone *Haliotis midae* (Branch et al. 1994, Fig. 1). Although the subtidal environment is typical West Coast kelp bed (Eekhout et al. 1992, Emanual et al. 1992), and superficially resembles that of abalone habitat farther south, only fossil evidence of an extinct species, *Haliotis saldanhae* occurs on the coast in 2-million-year-old diamond rich sediments (Kensley and Pether 1988). This renders the environment ideal for experimental abalone seeding by obviating the need to distinguish the seed from naturally occurring recruits. Seeding into environments previously uninhabited by abalone has precedent in Japan. Saito (1979) reports on a fishery of 150 mt from Funka Bay, which was devoid of abalone prior to seeding. The efficacy of a novel release device was tested, and some economic factors of abalone ranching were modeled.

MATERIALS AND METHODS

Abalone Seed

Abalone seed were provided by Port Nolloth Sea Farms. The first cohort to be released (CHT1) was obtained from abalone larvae that were settled in the hatchery at Port Nolloth Sea Farms in April 1995 and grown for 6 months prior to release in September 1995. A second cohort (CHT2), released in April 1996 comprised 4-month-old individuals obtained from a separate settlement at the hatchery. The seed were fed on varied diet of diatoms and macroalgae (*Ecklonia maxima*) before release. Shell lengths of a random sample of 130 seed form each cohort were measured at the time of release and assumed to be representative of the size at release of the seed.

Site Selection

The releases took place in September 1995 and April 1996 at Stilbaai in Port Nolloth (29°14'S, 16°53'E, Fig. 1), a small sheltered bay, with a southern rocky beachhead that supported an extensive kelp bed. Before release, three sites were carefully selected on the basis of protection from direct wave action and the presence of sea urchins (*Parechinus angulosus*), because juvenile abalone are generally found sheltering under sea urchins (Day 1997) in the near subtidal shallow water (Tarr 1989).

The first site (A) endured more seawater wash than the second (B). Although site A, which was located at about mean low water springs and was protected from wave action by rocks, it was shallower than the site B and had no kelp canopy. Site B was located in a relatively deeper depression (1.5 m at low tide) and was protected by a subtidal rocky ridge and a relatively high density of kelp. For the second release experiment, a third site was employed (C), which was chosen on the basis of its close resemblance to site B.

Release Method

The method employed for release of the seed used a specially designed release module (a series of welded PVC gutter-pipes, Fig. 2A,B), which was mounted on a concrete platform (Fig. 2C). This design was based on the criteria that handling of abalone should be minimized, and that the release device should be cheap, stable, durable, retrievable, and non-polluting. Basal dimensions of the release module were 30 × 16 cm, and the total internal surface area of the module, including base and half-pipes, was 9268 cm^2.

The selected release position at sites A, B, and C were cleared of urchins and other organisms (to prevent crushing them and attracting predators) and the concrete platforms (after leaching for several days in sea water) were set in place at least 1 day before release. On the day of release, abalone seed (4 to 6 months) were enumerated on kelp fronds to which they adhere in the hatchery.

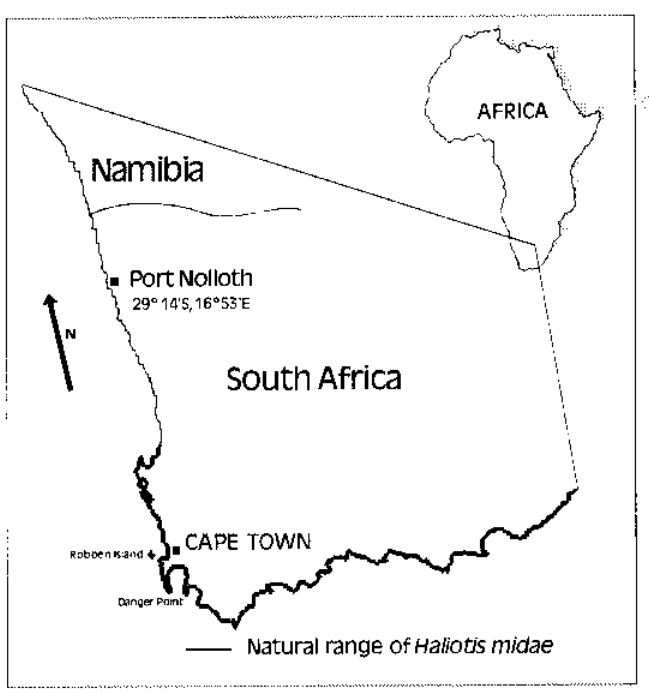

Figure 1. Experimental area.

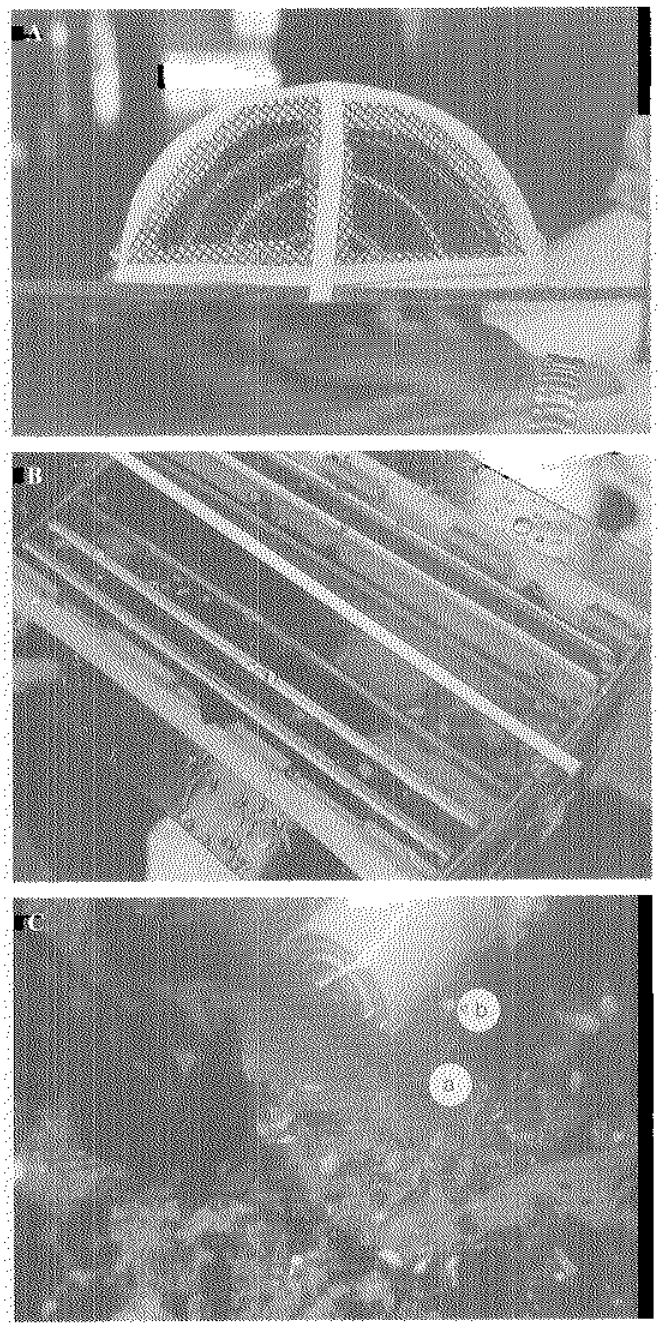

Figure 2A. Sealed release module (side view) consisting of PVC half gutter-pipes. See text for details.
Figure 2B. Release module containing 800 hatchery-reared abalone seed with transparent PVC base. (bottom view).
Figure 2C. A concrete ingot-shaped block measuring 0.5 × 0.4 × 0.2 m at the base, impregnated with metal offcuts (a) for weight and with nylon bolts (b) embedded to fit the release modules is placed at the preselected release point.

The abalone were either transferred directly into the release modules along with the kelp fronds or the fronds were quickly transferred to glass tanks filled with sea water, which contained the release module. In the latter case, the seed were induced to move into the module by exposure to ambient light, in the absence of alternative shelter. Once the seed had entered the module it was sealed with nylon mesh and a plastic-heat gun, to keep the seed

inside and predators out, without impeding the flow of water through the release module (see Fig. 2A). The stocking density was approximately 500 seed per module (i.e.,500 seed per site) at sites A and B (CHT1) in September 1995 and 800 seed/site at sites B and C in April 1996 (CHT2).

After placement of the modules by a diver, a number of sea urchins were collected and placed in close proximity to the platform. Twenty-four hours after the release modules were placed on the platforms (allowing time for the seed to acclimatize and mucous to wash away), the modules were opened, and the seed were allowed to move out spontaneously. The opening of the traps was timed to coincide with early evening, because this is when abalone are most active (Hayashi 1988, Tarr 1989). Twenty-four hours after release, the modules were inspected for the presence of seed, and those without seed were recovered.

Recapture

In April 1996 (6 months after release), samples of seed from the first cohort were recaptured from sites A and B (first growth period, summer). In September 1996, a second sample of CHT1 was recaptured (12 months) from sites A and B (second growth period, winter). In March 1997, an additional sample from Site B (18 months) was obtained for CHT1 (third growth period, summer), but by this time, no further seed could be found at site A. Samples of the second cohort (sites B and C) were concurrently sampled (6 months after release, September 1996, winter), and March 1997 (12 months after release, summer). Therefore seasonal samples for two summer periods and one winter growth period were obtained for CHT1 and a summer and a winter growth period for CHT2. Sampling was conducted by random searches, radiating from the point of release and covering a circular area with a radius of about 15 m. To locate the seed, sea urchins were removed, and small boulders were turned. To allow for repeated sampling, nonexhaustive sampling of the sites was conducted. Once approximately 100 individuals were collected (where possible), sampling was terminated. Thus, estimates of maximum survival were not obtained from these experiments. However, estimates of minimum survival after 6 months could be made by summing the total number of seed collected at each site during the duration of the experiment and expressing this as a proportion of the number seeded.

Data Analysis

Mean shell lengths were compared after growth periods of 6 and 12 months (sites A and B) postrelease for CHT1 and after 6 and 12 months postrelease (sites B and C) for CHT2 with Mann–Whitney U tests. Because CHT2 were younger than CHT1 at release and were released in a different season, a comparison of mean shell length was not attempted between the cohorts.

Growth rate analysis for the 18 months postrelease was performed for CHT1 at site B for which most data was available. The nonlinear iterative facility in the Statistica Ver. 5 (Statsoft 1992) software package was used to perform regressions on the age–length data. Two growth models were evaluated: the Von Bertalanffy growth function, and a power curve (see Kerstan 1995). Size at age 0 was assumed to be 0.

The size-at-age data were compared with those calculated from Von Bertalanffy growth equations in published data from *H. midae* populations within its natural range (Tarr 1995). The instantaneous linear growth increments were also determined to compare the results for growth periods monitored.

Minimum survival at 6 months postrelease was estimated from the cumulative proportion of recaptured juveniles. Because the first collection date was 6 months after seeding, all those collected in subsequent samples had also survived up until this point. Using this method of estimation, the more a site is sampled, the better the estimation of minimum survival. Because the release sites were not destructively sampled, estimates of minimum mortality were not obtained. The release sites were searched for the presence of dead abalone shells.

Economic Modeling

A simple 1-ton model was developed to investigate some of the variables associated with the production of 1 ton (1,000 kg) seeded abalone. Recapture rate (defined as the proportion of a seeded cohort recaptured at market size) was plotted against the number of seed that would have to be seeded to produce 1 ton of market size animals (100-mm length/100-g whole weight). Cost of the seed was estimated at R1.00 and R1.50/animal (+10% for handling and marketing costs). In the model, the value of abalone was estimated at US $35 per kg (R157500.00 per ton).

RESULTS

Release Modules

Juvenile abalone (4–6 months) moved spontaneously into the release modules when transferred on kelp fronds. In all cases, approximately 30 minutes was required to fully stock the release modules. Bunching of seed did occur, but they were evenly spread throughout the module within 2 hours (Fig. 2B). Mortality at this stage was below 1%, based on retrieval of dead abalone from the glass transfer tanks.

Twenty-four hours after release, approximately 95% of the seed had left the module and were located under adjacent sea urchins and under the release platforms. In all cases, all the seed had left the module within 48 hours of release. No dead abalone shells were located on or near the release sites. No noticeable increase in predator density was observed, although data were not collected in this regard.

TABLE 1.

Numbers of abalone seed released and recovered from samples at Port Nolloth. CHT1 released in 9/95, CHT2 released in 4/96.

	Released	April 1996	Sept. 1996	March 1997	Minimum Survival after 6 Months
Site A-CHT1	500	97	40	Not sampled	27.4%
Site B-CHT1	500	107	70	19	39.2%
Site B-CHT2	800	——	124	99	27.8%
Site C-CHT2	800	——	145	60	25.6%

Survival

Table 1 shows the number of individuals recaptured from each of the release sites and the minimum survival estimates at 6 months.

A number of predatory species were observed at the release sites, although it is not known whether there was an associated increase in density of these species at the sites. At site B, a single octopus (*Octopus vulgaris*) was located beneath the release platform, and at site C, two rocksucker fish (*Chorisochismus dentex*) were located beneath the release platform. Both of these species are known predate on abalone (Scott 1997). Only one dead abalone shell was recovered during sampling.

Growth

Estimates of mean size-at-age for 6, 12, 18, and 24-month-old abalone, from two sites within the natural range of *H. midae* (representing the lowest and highest growth rates), are shown in Table 2a. The empirical mean shell lengths (±1 SD) for Port Nolloth CHT1 and CHT2 at sites A, B, and C for the various sample dates are presented in Table 2b. Length increments for the growth periods by season are indicated in Table 2c. Although the data fall within the range of growth rate estimates for the natural populations, length increments after the two growth periods were generally less than those estimated for the natural populations after an equivalent period of growth. The data for CHT1, sites A and B, are represented graphically in Figure 3 and demonstrate the marked difference in growth for these two sites. The seasonal effect on growth increment is also clear from the mean growth increments (Table 2c, Fig. 4). Summer growth increments were generally 20–50% greater than those for winter growth.

In the first summer growth period for cohort1 at site B, the mean growth increment was almost double that at site A, resulting in a statistically significant difference between mean lengths of abalone at sites A and B ($p < .0001$, Fig. 3). Although the mean shell lengths between sites remained significantly different after the second growth period ($p < .001$) the growth increment was similar in both sites, indicating that growth at site B had slowed to a similar rate to that at site A. The growth increments at sites B and C were similar for both growth periods monitored after release for CHT2, however the difference in mean shell lengths was statistically significant ($p < .002$).

Growth Rate Estimates

A power curve (Fig. 5) best fitted the data ($r = 0.88$) for CHT1, site B. Although statistically significant parameters for the Von Bertalanffy growth equation were attained, L_∞ was unreasonably low ($r = 0.88$, Fig. 5). This has occurred as a result of the attempt to estimate the parameters on only part of the growth curve rather than a complete growth profile (Kerstan 1995). Both equations were used to plot growth curves, along with the maximum and minimum growth curves (Von Bertalanffy) for the first 2 years in natural populations (Fig. 5). Although the estimates indicate a relatively slow rate, they fall within the natural range reported (Tarr 1995).

TABLE 2a.

Size at age calculated for natural populations.[a]

Site/Age Natural Stocks	6 Months	12 Months	18 Months	24 Months
Robben Island	19.1	36.0	50.9	64.3
Danger Point	9.5	18.5	27.1	35.2
Mean	15.5	29.5	42.1	49.3

[a] Calculated from growth curves after Tarr 1995.

TABLE 2b.

Mean shell lengths at Port Nolloth.

Port Nolloth	Sept. 95[b]	April 96	Sept. 96	March 97
CHT1				
PN: A-CHT1 (± SD)[a]	14.8 (3.0)	22.2 (3.8)	28.0 (4.7)	
PN: B-CHT1 (± SD)[a]	14.8 (3.0)	26.6 (4.6)	32.2 (5.8)	41.6 (4.0)
CHT2		**April 96[b]**	**Sept. 96**	**March 97**
PN: B-CHT2 (± SD)[a]	——	8.2 (1.7)	14.6 (2.5)	22.7 (3.9)
PN: C-CHT2 (± SD)[a]	——	8.2 (1.7)	13.7 (2.1)	21.2 (2.9)

[a] Mean shell lengths for Port Nolloth sites A, B, or C.
[b] Size at release.

Economic Model

The results from the economic model are plotted in Figure 6. This shows that seeding is not profitable at return rates below about 10% and that a return rate of about 20% or above would be desirable to ensure a profitable operation. The position of the curves and resulting profit margin depend upon several factors not included in this model. These include volume (tonnage), internal rate of return of investment, production cost, price and exchange rate.

DISCUSSION

Handling of abalone in hatcheries inevitably leads to mortality (McCormick et al. 1994). The system designed and tested here successfully obviates the need to handle the abalone seed at any stage of the release procedure. Although the experiments were conducted on a relatively small scale, adaptation to a commercial scale should present no insurmountable obstacles. Another detri-

TABLE 2c.

Average growth increments by season for natural stocks and Port Nolloth.

Site/Period	$\Delta 6^a$	$\Delta 6^b$	$\Delta 6^c$	$\Delta 12$	$\Delta 18$
Robben Island*	16.9	14.9	13.4	31.8	45.2
Danger Point*	9.0	8.6	8.1	17.7	25.7
Mean*	14.0	12.6	7.2	26.6	33.3
Port Nolloth	Summer	Winter	Summer		
PN: A-CHT1	7.4	5.8	——	13.2	— —
PN: B-CHT1	11.7	5.7	9.2	17.4	29.9
Port Nolloth	Winter	Summer			
PN: B-CHT2	6.4	8.1		14.5	
PN: C-CHT2	5.5	7.5		13.0	

* Calculated from growth curves after Tarr 1995; $\Delta 6^a$ = increment after first 6 months postrelease/settlement; $\Delta 6^b$ = increment after second 6 months postrelease/settlement; $\Delta 6^c$ = increment after third 6 months postrelease/settlement; $\Delta 12$ = increment after 12 months postrelease/settlement; $\Delta 18$ = increment after 18 months postrelease/settlement (settlement in the case of natural stocks).

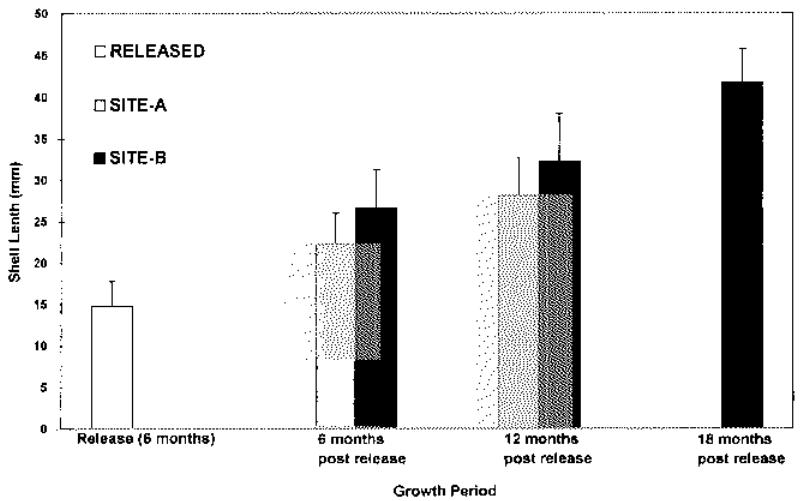

Figure 3. Mean shell length (mm) for cohort 1 (CHT1) at sites A and B. No seed were found at site A 18 months after release. Means were significantly different after 6 and 12 months postrelease (see text).

mental factor identified by McCormick et al. (1994) is the distance from the hatchery to the point of release. Several options do exist to control this factor, such as holding facilities nearer the point of release.

By keeping the release module sealed for 24 hours after placement, the accumulated mucous secretions (which attract such predators as whelks) are allowed to wash away while the abalone acclimatize to the subtidal conditions. Using a diver to complete the operation is complicated by unpredictable diving conditions.

Estimates of survival and minimum mortality are notoriously difficult to achieve for seeded abalone (McCormick et al. 1994). Therefore, results reported for other abalone species vary significantly. Many considerations, including age/size at release, time elapsed before estimation, and several physical factors seem to influence these estimates (McCormick et al. 1994). Although almost no dead abalone shells were recovered in these experiments, it would be presumptuous to assume that this represents low mortality, especially because some predators crush shells (Scott 1997). A more meaningful estimate is that of survival, because this is what will matter in a commercial scale operation.

Survival at three sites at Port Nolloth was around 30% 6 months postseeding. This high rate of return occurred despite the fact that the region experienced one of the biggest storms on record during the experimental period. Although the estimates are of minimal survival, they are likely to be underestimates, because the sampling regime targeted the point of release, and therefore, those individuals that had moved away from the point of release would not have been included in the sample. Abalone are known to move substantially in their life span (Newman 1966), with younger abalone occurring more in the shallow subtidal than larger/older conspecifics; whereas, adults seem to be sedentary in deeper water (Tarr 1995).

Another factor that may contribute to this effect would be the microhabitat and topography of the environment into which abalone are released. *H. midae* occur in fairly patchy distributions and aggregations (Newman 1966, Newman 1969, Tarr 1995), which is

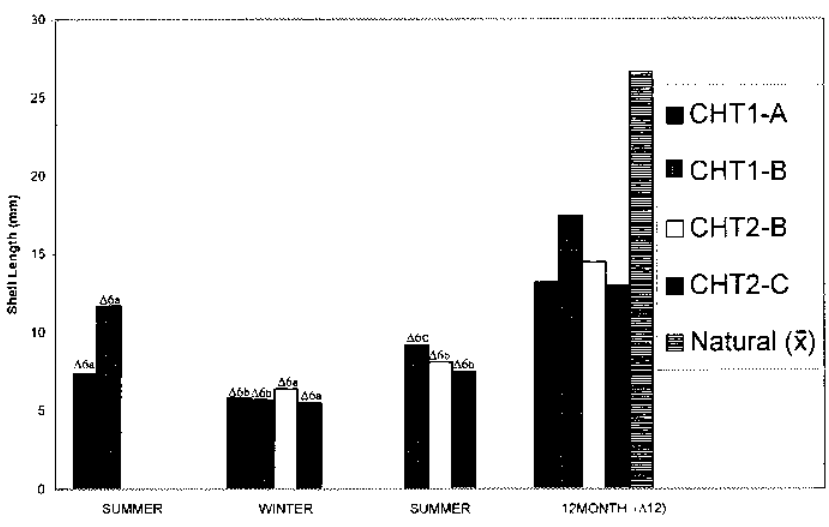

Figure 4. Average shell length increments for cohort 1 and cohort 2 at sites A, B, and C by season. Δ6a = from release to 6 months postrelease. Δ6b = from first sample to second sample (6 months). Δ6c = from second sample to third sample (6 months). Δ12 = increment from release to 12 months postrelease. Natural = calculated for natural populations, from Tarr 1995.

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