REFINEMENTS IN CULTURE TECHNOLOGY FOR THE PURPLE-HINGE ROCK SCALLOP

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INTRODUCTION

Preliminary biological studies of the purple-hinge rock scallop, Hinnites multirugosus, with focus on its aquaculture potential, were begun in 1972. The project first received support from the UC Sea Grant Program in 1975. Thenceforth, a three-year research effort yielded much information of value toward the eventual commercial development of aquaculture for this bivalve (Leighton and Phleger, Technical Report 1980). Resulting literature has appeared largely in the Proceedings of the World Mariculture Society (1976, 1977, 1979), Marine Biology and Sea Frontiers (1979), and the Journal of Food Science (1978). Three Master's theses (Jacobsen 1978, Foe 1979 and Monical 1980) have been completed by Sea Grant trainees and one is in late stages of preparation (Cary 1981).

The present research was undertaken to allow improvement in the "state of the art" of hatchery culture and field rearing approaches. Results reported herein show enhanced yields of spat through improved hatchery procedures and simplified effective extensive culture of market size adults by development of a patent-protected rearing panel for use in protected and/or deep offshore waters. Other observations and findings have related to control of gametogenesis (and thereby spawning) and the utilization of dissolved and particulate (detritus) organic matter by juveniles.

The major portion of the laboratory work has been done at the Southwest Fisheries Center (NMFS), La Jolla, and at the San Diego State University Marine Laboratory, Sea World, San Diego. Field experiments were performed largely in Quivira Basin, Mission Bay, San Diego, wherein a floating laboratory is maintained*.

MAJOR STUDY AREAS

1. A first laboratory culture of larvae to yield substantial numbers of juveniles. These juveniles have since been reared to sexual maturity. Their larvae are currently in culture.

2. Diet/growth studies with early and advanced larvae, and with metamorphosed postlarvae. A large portion of these studies was conducted by Craig Cary, Sea Grant trainee and Master's degree candidate. Principal findings include demonstration of changes in dietary requirements with age.

3. The development of a reusable plastic panel for extensive culture of Hinnites to market size. Patent protection was applied for, November 1979, through the University of California Board of Patents.

4. Experiments comparing exogenous factors (photoperiod and temperature) in regulation of gametogenesis in young adult Hinnites.

5. An attempt to extend open sea observations on growth/depth in young Hinnites on shelfland five miles off San Diego. Buoys and lines holding caged scallops were lost, possibly due to entanglement with deep-draft military vessels in the area. Scarcity of early juveniles in 1980 precluded a second attempt.

6. Studies of uptake of both dissolved and particulate non-living organic matter by juvenile Hinnites using C^{14} labelled kelp exhudate and pulverized fecal material from abalones fed the kelp. Positive uptake was demonstrated in

*Space donated by Ocean Studies Division, World Research, Inc.

each case. Further study may suggest the extent to which dissolved organic matter and detritus complement the algal nutrition of the rock scallop.

RESULTS

Improvements in Hatchery Methods

Survival of larvae through the 4-5 week period of intensive culture to metamorphosis has been generally very low. Most attempts to rear larvae have yielded less than 1% through premetamorphic stages. Frequently, mortality of the greater portion of larvae in a batch has occurred during the second and third weeks when shell lengths range from 110 to 140 μ. A variety of experiments was conducted to reveal causes of this observed mortality and indicate optimal conditions for larvae in mass culture. Observations have concerned nutrition, temperature, light, salinity, water motion, aeration, and frequency of water changes. Also examined were antibiotic therapy, level of feeding and culture container size and form. Some of these studies are reported in the 1978 Technical Report (Leighton and Phleger 1980). Recent findings are presented here.

Earlier study had shown food algae, Isochrysis galbana and Monochrysis lutheri, species common in cultured oyster diets, supported good growth and survival of Hinnites larvae (Leighton and Phleger 1977). The flagellate Rhodomonas sp. proved especially valuable to advanced larvae entering metamorphosis (Leighton and Phleger 1980). Tetraselmis and Gymnodinium, species too large to be utilized by early larvae, were valuable to advanced larvae and postlarvae (Table 1).

In an experiment described elsewhere (Leighton and Phleger 1980), the advantage of mixed diets to larval growth and survival was clearly demonstrated. Similar observations have been made recently. Results of a feeding run in

Table I: Growth of early juvenile Hinnites multirugosus provided nine different algal diets during a 24-day period.

Diet	Mean Size at Start (mm)	Mean Size at End (mm)	Mean Increase (mm)	Std. Dev. (mm)
Tetraselmis suecica	4.89	5.95	1.06	0.48
Rhodomonas sp.	5.38	5.88	0.50	0.21
Gymnodinium splendens	5.36	5.78	0.42	0.24
Isochrysis galbana	4.95	5.28	0.33	0.36
Monochrysis lutheri	5.60	5.90	0.30	0.24
Phaeodactylum tricornutum	5.19	5.36	0.17	0.13
Dunaliella salina	5.09	5.20	0.11	0.08
Chaetoceras affinis	5.01	5.09	0.08	0.29
Skeletonema costatum	5.44	5.48	0.04	0.12
Starved group	5.70	5.74	0.04	0.19

Each group was comprised of eight individuals. Water in containers was changed (UV sterilized and 5 μ filtered) and groups freshly fed three times each week. Temperature was maintained between 16 and 18°C.

which early juvenile H. multirugosus were fed Rhodomonas, Monochrysis, Isochrysis and Phaedodactylum singly and in combination show significantly (p .05, t test) greater growth rates when the mixture was used (Table II).

Feeding observations with both larvae and juveniles to determine optimal concentrations of food algae in cultures show that feeding levels must be maintained within limits and that excessive inoculation may be detrimental and extravagent. Six algal diets were fed to Hinnites larvae at four concentrations to test growth and survival. Isochrysis galbana and Tahitian Isochrysis were the most successful diets. A concentration of 10^4 cells/ml was found to be best. Eighty percent survival through metamorphosis was supported by the Tahitian Isochrysis diet. This is higher than we have ever observed in experiments of this type.

A nutrition study was designed to examine effects of five algal diets on growth and survival of Hinnites postlarvae. The diets included I. galbana, Monochrysis lutheri, Thalassiosira pseudonana (3H), Tahitian Isochrysis sp., and Phaeodactylum sp. As in the previous study Tahitian Isochrysis and I. galbana provided best growth with least mortality at a concentration of 10^5 cells/ml. The remaining diets supported little or no growth.

An early feeding study was initiated to determine if the feeding of Hinnites larvae prior to the straight-hinge (D) veliger stage enhances survival and growth. We usually do not commence feeding until onset of the D stage. Growth of these young larvae was enhanced by Tahitian Isochrysis; considerably more so than other diets tested. Thus, we recommend Tahitian Isochrysis for best survival and growth of pre-veliger larvae since in this case, also, this alga has proved to be an excellent food.

Groups of larvae invariably have shown best survival through the premetamorphosis culture period when held in darkened containers and given frequent changes of water (by "meshing out" each 3-5 days), as discussed earlier (Leighton and

Table II: Growth of early juvenile Hinnites multirugosus provided single and combined algal diets for a period of 30 days.

Diet	Mean Size at End of Period (mm)	Standard Deviation (mm)	Number in Sample
Rhodomonas sp.	2.91	0.79	12
Monochrysis lutheri	2.85	0.39	10
Isochrysis galbana	1.94	0.44	10
Phaeodactylum tricornutum	1.86	0.24	13
All four algae	3.48	0.59	17
Starved group	1.95	0.30	12

All individuals were 1.3-2.3 mm at the start (mean 1.90 mm, std. dev. 0.27). Liter polypropylene beakers held each group; temperature range 14-16°C.

Phleger 1980). Antibiotic therapy was routinely used during the first week of larval culture. It remained to be ascertained whether increased survival rates would result if antibiotic treatments were given periodically throughout the four-week pre-metamorphosis interval of intensive care. An experiment was designed to examine the influence of both light and antibiotics on success of larvae. Growth rate was enhanced initially in containers under full period illumination, but overgrowth by algae soon occurred with deleterious effects on larval survival. Survival was superior under conditions of darkness together with periodic antibiotic treatment.

Best survival and growth of larvae through metamorphosis and into early juvenile stages was achieved using darkened containers with nylon mesh bottoms. Following a laboratory-induced spawning October 26, 1978 which yielded an especially hardy group of larvae, tests were made of a black-surfaced fiber-glass tank with mesh bottom (86 µm) to which approximately 10,000 late-stage larvae were introduced. Food algae included Isochrysis, Monochrysis and Rhodomonas. Approximately 3,000 postlarvae continued to early juvenile stages from this batch. The culture apparatus is described elsewhere (Leighton and Phleger 1980).

Routine use is now made of black plastic (ABS) cylindrical containers with mesh bottoms for culture of advanced larvae (150 µ) through metamorphosis. Sections of 20 cm diameter ABS pipe cut at lengths of 30 cm are modified with leg pieces after gluing nylon mesh in place. These containers fit within larger plastic containers holding seawater and algal food (Figure 1). Gentle aeration is supplied by airstones both within and outside the cylinders.

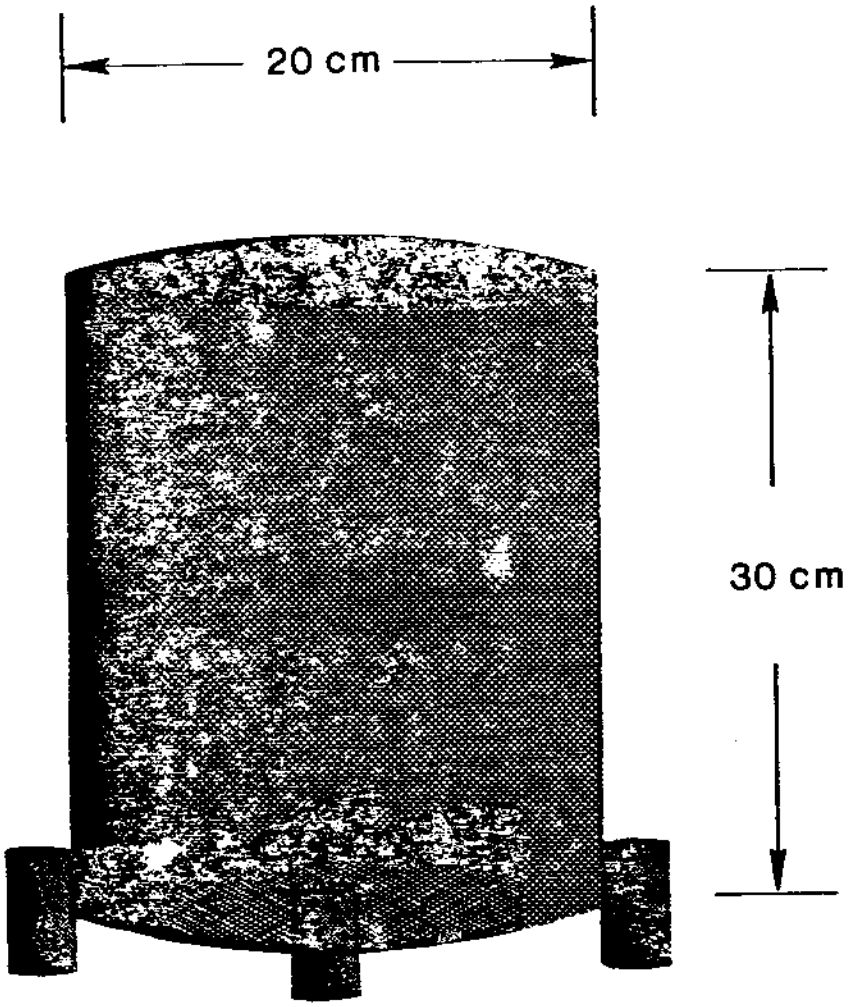

Figure 1. Mesh bottom cylindrical rearing vessel (ABS) for Hinnites larvae.

Growth Rate and Age at First Sexual Maturity in Progeny from the October 1978 Spawning

Juvenile scallops completing metamorphosis and progressing into postlarval stages were transferred to a 100-liter acrylic plastic tank (cauldron style) and reared for two months. At the age of three months, scallops were 3 ± 2 mm and large enough to be held in mesh sided pens in natural waters of Mission Bay. Growth over the succeeding year (Figure 2) was comparable to that observed in earlier studies (Leighton and Phleger 1980). At an age of 18 months, these scallops became gravid and were induced to spawn. Survival throughout the cauldron laboratory culture period and the following year in Mission Bay remained amazingly good, in most subgroups better than 95%. These results confirm our observations on growth, survival and age of first sexual maturity (see Leighton and Phleger 1980) in progeny from an April 6, 1976 spawning. In that case, scallops at 18 months spawned (October 2, 1977). Their size averaged 53.7 mm (S.D. 9.18, sample n=20), the range, 39.1-69.0 mm. Survival during 14 months in Mission Bay was 100%.

On several occasions, larvae from spawned laboratory-reared scallops have been reared to maturity. It has not, however, been the focus of this research to select strains and conduct rigorous inbreeding programs. Should this work be undertaken, a minimum generation time of 18 months may be expected.

An Effective Structure Facilitating Containment, Growth and Harvest of Scallops in Extensive Culture

The following concerns the conception, development, testing and patent procurement for a reusable plastic rearing panel applicable to culture of H. multirugosus (and other similar species) from juvenile to market adult size in natural waters. The subject is thoroughly described in patent literature

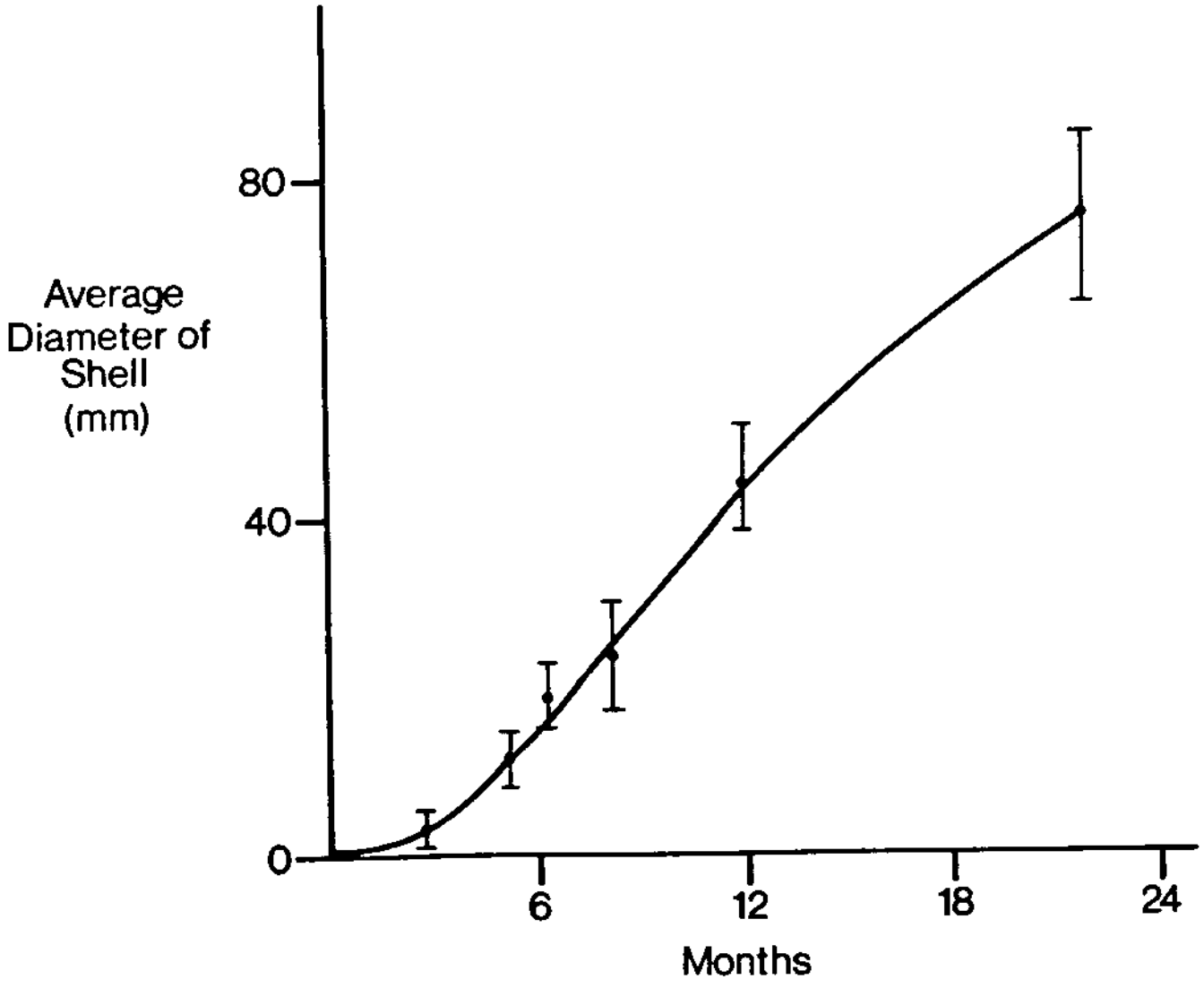

Figure 2. Shell growth for Hinnites multirugosus produced October 26, 1978, cultured for three months to early juvenile stages in the laboratory and subsequently reared to young adulthood in cages in Quivira Basin, Mission Bay. First sexual maturity and spawning occurred in May 1980 (18 mo). Vertical bars indicate standard deviations.

(Leighton 1979).

Earlier study (Leighton 1979b, Leighton and Phleger 1980, Monical 1980) had shown scallops exhibit maximum growth when distributed on rearing surfaces at densitites of $1/dm^2$ of less (ca. $6/ft^2$) to an age of approximately 2.5 years. Scallops were either secured as pre-cementing juveniles to flat durable sheet (asbestos board to thinly cast concrete) with underwater setting epoxy glue, or confined until naturally cemented by small cup-shaped mesh cagelets affixed to the surface in appropriate spatial distribution (Phleger and Leighton 1978, Leighton and Phleger 1980). More recently, concepts circumventing the labor-intensive gluing operation and obviating the requirement for use of heavy or hazardous materials (asbestos sheet) were explored.

A plastic panel with attachment sites for scallops at $1/dm^2$ was designed. A plywood form with taper-finished dowel sections spaced appropriately was taken to a plastics manufacturer (vacuum forming process, DECO Products, Santee, California) and a series of panels cast in polyethylene, styrene, and ABS (Acrilonitrile-butadiene-styrene) sheet. Each panel was 40 x 60 cm in stock 60 mil (1.5 mm) in thickness and contained 12 circular depressions (Figure 3). Several holes (1/4") were drilled in each well, and a temporary retaining mesh glued or taped over each. Scallops 8-15 mm were admitted singly to each site through a slit cut into the mesh.

Most tests of the performance of the plastic panels and growth of contained scallops were done in water beneath a floating laboratory in Mission Bay. Generally, scallops cemented to the well bottoms with shell material deposited into the 1/4" holes in a three-month period and the temporary retaining mesh could be removed. Continued growth yielded market size scallops within the following 1.5 year interval as expected (Figure 4).

Refinements in the rearing panels have followed tests of other mesh

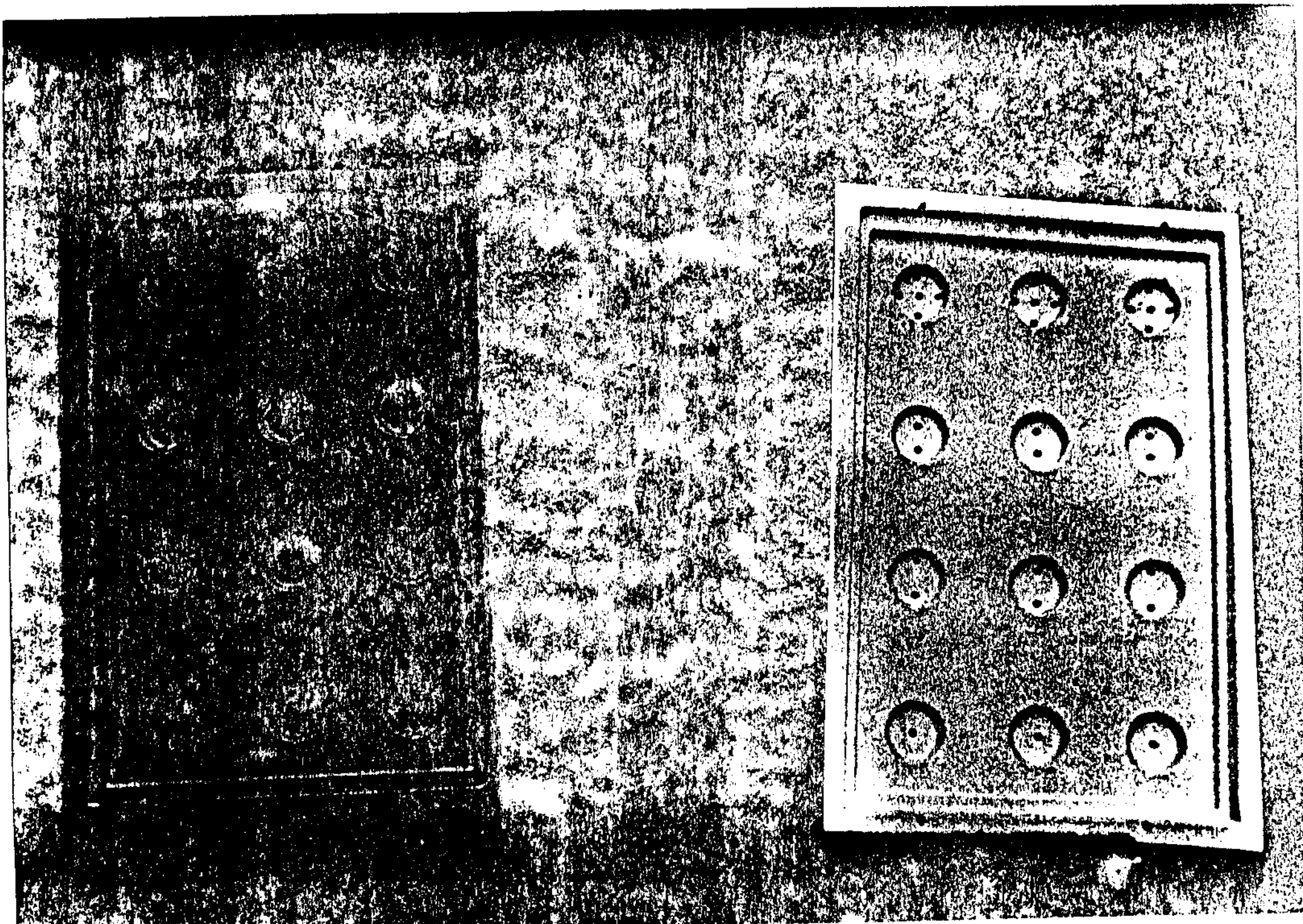

Figure 3. Photograph of plastic panels designed to rear the purple-hinge rock scallop.

Figure 4. Market size purple-hinge rock scallops reared for 1.5 years on the plastic rearing panels.

materials which either were short-lived in seawater (cellulosic fiber) or anti-fouling (copper and aluminum screen). It was found that gauze and cheesecloth disintegrated too rapidly. Nylon (veil) mesh stood up well in seawater, but the fine mesh (2-3 mm) became occluded too rapidly by fouling growths. Copper screen failed since it proved highly toxic to the contained scallops. Aluminum screen remained intact through the required 2-3 month cementing period, but has been abandoned in favor of the polyethylene 1/4" mesh for its larger mesh size and lower cost.

An additional refinement has been made in panel preparation to assure secure attachment of scallops. A loop of nylon monofilament line is tied through two or three holes in each well. Shell material is deposited by the scallops to include the monofilament. At harvest, the line is simply cut from behind the panel. This final improvement is included in the patent disclosure and description by addendum.

The styrene sheet panels have proved highly durable in seawater. Following harvest, these panels are readied for reuse by cleaning (soaking in fresh water, followed by brine and light scrubbing).

We see wide application of the rearing panels to scallop culture. Other molluscs (e.g. oysters, other species of scallops, mussels) may also be reared from juvenile to adult stages using this development.

A full description of the "Reusable Plastic Rearing Panel Applicacable to Aquaculture of the Purple-Hinge Rock Scallop" was presented to the University of California Board of Patents and official application for patent protection was made to the United States Patent and Trademark Office, November 30, 1979.

Offshore Studies

Preliminary results of observations on growth of juvenile and adult rock

scallops beneath the NOSC (U.S. Navy) Oceanographic Platform off the San Diego coast were reported in Leighton and Phleger (1980). A detailed analysis of these studies is now available (Monical 1980). During a year-long observation period, scallops held in cages at 3, 9 and 18 m grew in shell diameter 5-6 cm; closely comparable to growth of scallops held in Mission Bay during the same interval. Maximum growth rates occurred at ocean and bay stations at mid-depths. Slow growth was observed in shallow water, due in part to fouling competition. In the bay, salinity reduction during winter storm periods caused extensive mortality in shallow waters. Scallops held within 1 m of the bottom also grew slowly, possibly due to the presence of nutritionally poor organic and inorganic particulates. Temperatures near the bottom averaged about 5°C colder than surface waters.

To find a maximum effective depth for scallop growth, another study (Leighton 1979) was conducted over a three-month period at a station 5 km off La Jolla. Two buoyed lines held pairs of cages at six depths from 8 to 130 m. Growth of scallops in the cages was comparable to best bay locations between 8 and 30 m, but declined at greater depths. The proximity of the La Jolla Submarine Canyon with its deep cold and phytoplankton-poor water might have contributed to that decline in growth.

Accordingly, another location was sought over more level sea bottom to study growth of scallops in the depth range 15 to 70 m. Possibly the vast areas of inshore shelfland off the southern California coast could support commercially attractive growth of rock scallops held in appropriate rearing structures. A station 8 km off the coast near Mission Bay was established which could be relocated precisely by three pairs of landmarks (allowing intersection of three bearing coordinates). The depth was 65 m (210'). Two lines, each with a set of paired cages at depths of 16, 31, 46 and 62 m, were installed May 19, 1979

(Figure 5). Each cage contained eight juvenile scallops cemented to asbestos board strips, half were laboratory-reared scallops, the others were natural recruits collected near the mouth of Mission Bay. Two submerged 60 cm diameter polyurethane-filled fiberglass buoys supported the lines. A 30 m horizontal line interconnected the two floats to allow simple recovery by grapple when advancing the boat along the primary bearing line.

This experiment was unfortunately not completed since the entire structure could not be relocated. Several trips were made to the area throughout the summer. On one occasion, divers descended with propulsion devices, but no trace of the buoys and lines was found. Deep-draft vessels used on Navy maneuvers in the area may have entangled the lines. We have not yet had the opportunity to attempt a repeat of this experiment since natural recruited scallop juveniles have not returned in numbers to the jetty habitat. Winter rain dilution in the bay exterminated all scallops at depths 0-4 m during 1979 and 1980.

An Algal Culture System to Support Young Adults in Preliminary Studies of Gametogenesis

An algal culture system was developed at the San Diego State University Marine Laboratory at Sea World, San Diego to support growth of post-larval and juvenile rock scallops. Use was made of the laboratory also to maintain young adult scallops held under different light regimes and temperatures to make preliminary observations on the influence of these exogenous factors on gonadal ripening.

Algae (Tetraselmis suecica and a Tahitian strain of Isochrysis) were inoculated into four 10-liter carboys held in a transparent water bath and illuminated by four fluorescent bulbs. Three 100-liter cauldrons received a heavy

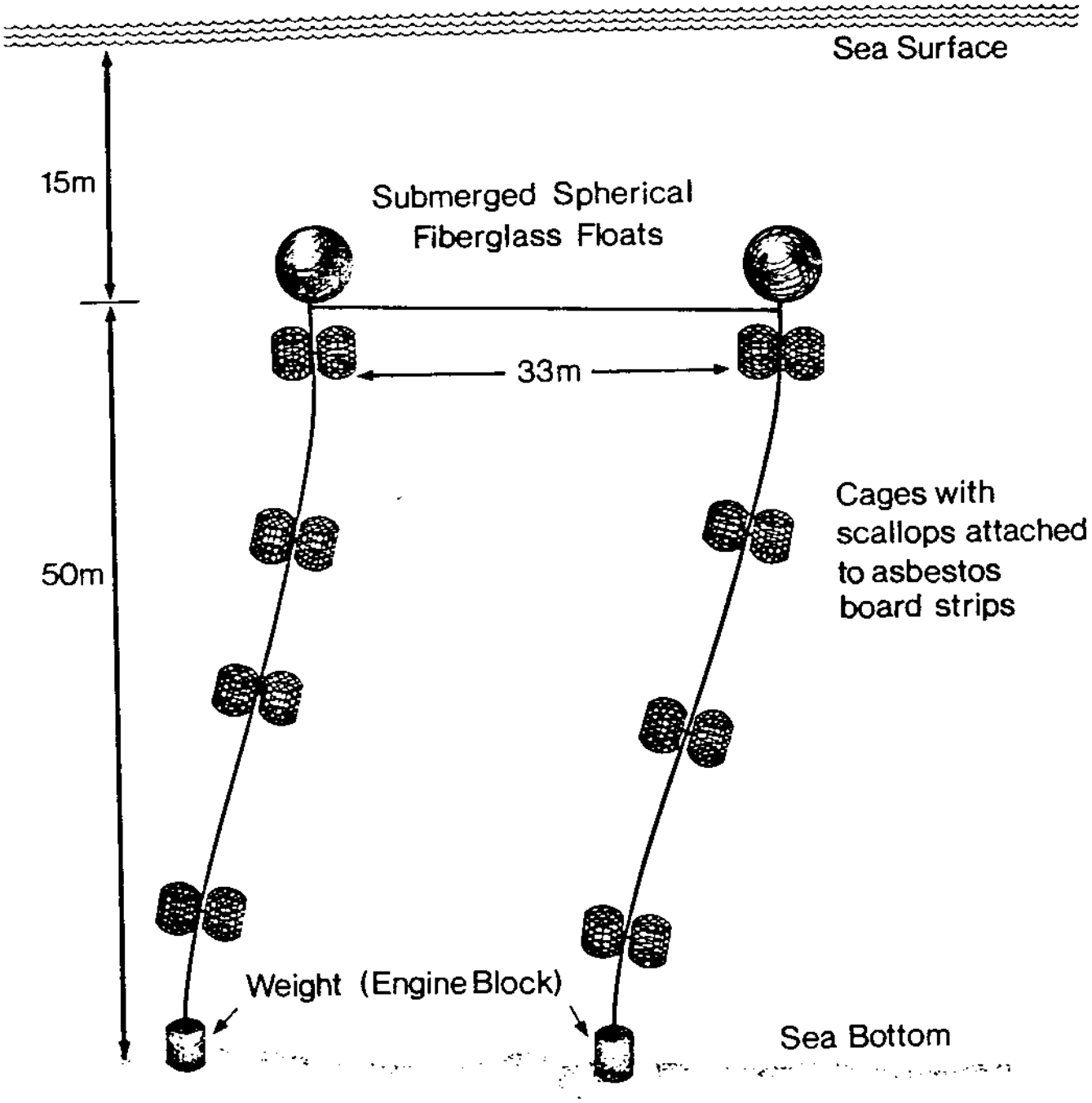

Figure 5. Lines supported caged juvenile scallops buoyed by submerged floats.

inoculum from the carboys once algae were in log phase growth. This arrangement was sufficient to provide food for both postlarvae and the young adults in the ripening experiments, but during summer months water quality declined and algal growth was hindered. Preparations are now being made to translocate the culture system in experiments to the Scripps Institution of Oceanography aquaculture laboratory where both filtered and unfiltered high quality coastal seawater will be supplied.

Dissolved and Particulate Organic Matter as Supplements to Scallop Nutrition

A study was conducted during summer and fall 1980 to examine uptake of dissolved and detrital organic matter by juvenile H. multirugosus.

Dissolved organic matter was obtained from giant kelp, Macrocystis pyrifera, by radiolabel in situ with two mC $NaHC^{14}O_3$ in a polyethylene bag containing three liters of seawater. The apical scimitar and about eight healthy and clean terminal blades were enclosed in the bag for 24 hours spanning two sunny days in June 1980. The bag was closed on the intact kelp stipe by wiring and taping it to two rubber gaskets surrounding the stipe. A second outer bag containing seawater protected the inner sample bag. This was sampled after the experiment for C^{14} to check for leaks. There were no leaks. Control experiments done with sodium fluorescein dye also did not leak. Following techniques reported by Fankboner and de Burgh (1978) the seawater was filtered through 0.22 μ willepore filters to remove particulate organic matter. The pH was lowered with HCL to remove excess $NaHC^{14}O_3$. Thus, the remaining 4.4×10^7 cpm in the 3 l seawater were attributable to dissolved organic matter only.

Juvenile rock scallops for uptake experiments were collected beneath Mission Bay jetty rocks by scuba diving. Scallops were maintained in a 2:1 mixture of

seawater (filtered through 0.22 μ millepore filters) and the C^{14} dissolved organic matter from kelp for 24 hours in the laboratory of A. A. Benson at Scripps Institution. Samples of living juvenile scallops (in triplicate) were sacrificed at appropriate time intervals (see Figure 6). Steamed juvenile scallops were used as controls at each time interval. Soft body tissue was homogenized on a Polytron tissue hemogenizer prior to measuring total radioactivity on a Beckman LS-8000 scintillation counter with 2% error. Scintillation fluid used included toluene (600 ml), Triton-X-100 (257 ml), ethanol (106 ml), ethylene glycol (37 ml), and PPO (3 g). Dissolved organic matter uptake into biochemical fractions of scallops was also measured utilizing microassay techniques of Holland and Gabbot (1971).

The results of total uptake of dissolved organic matter into scallops are summarized in Figure 6. Carbon-14 activity increased from 200 dpm/mg dry weight of tissue after ten minutes to 2300 dpm/mg dry weight of tissue after 24 hours. These results are significantly different. There was a general increase of carbon-14 into living scallops over the 24-hour period (Figure 6). Preliminary data on uptake of C^{14} dissolved organic matter into the biochemical fractions of scallops revealed significant incorporation into carbohydrate (5%), free-reducing substances (5%) and RNA (3%). These results demonstrate dissolved organic matter uptake from kelp by juvenile rock scallops for the first time. We are currently examining uptake into biochemical fractions (lipids, protein, carbohydrate, RNA, DNA and free-reducing substances) over the 24-hour time period to see if there is an increase and/or shift in uptake into different fractions. Chemical composition of the kelp exhudate is not known. P. V. Fankboner (pers. comm.) reports exhudate from Macrocystis integrefolia to consist almost entirely of amino acids, similar to those that occur naturally in seawater of kelp beds. Rock scallop populations are abundant in kelp beds off

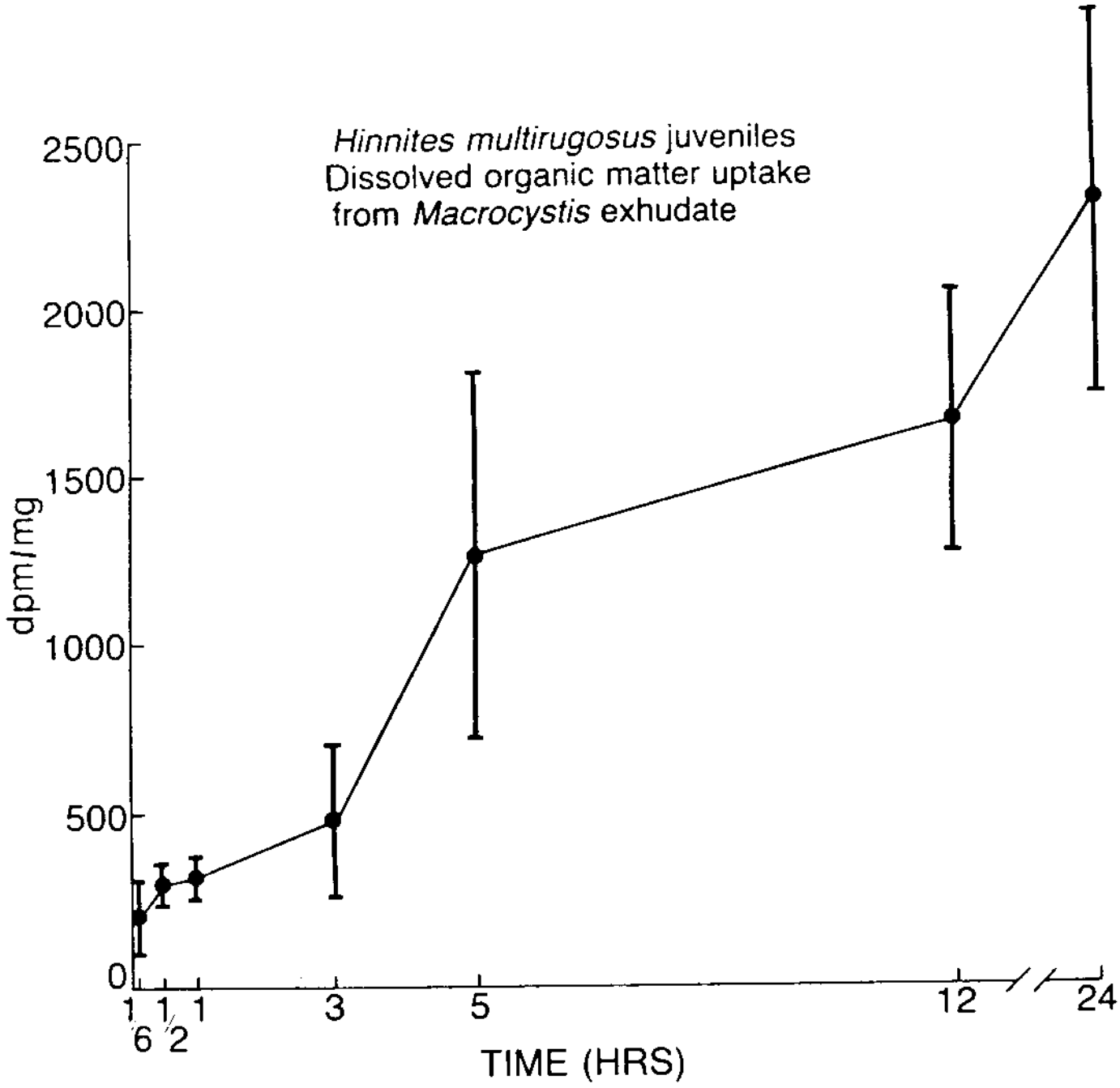

Figure 6. Total uptake of dissolved organics during one day.

southern California. Dissolved organic matter, as kelp exhudate, could provide an important supplementary food source for developing Hinnites larvae and juveniles in the kelp beds and associated areas. The aquaculturist should be aware of this, as dissolved chemicals, particularly amino acids, might improve growth and survival in the larval growth stages of rock scallops. This work is being presented at the Western Society of Naturalists Meeting in Seattle, Washington, 1980 (C. F. Phleger and S. S. Rossi, Dissolved organic carbon accumulation by juvenile rock scallops).

The kelp that had been radiolabelled in the polyethylene bag with $NaHC^{14}O_3$ was fed to three young adult red abalone, Haliotis rufescens. It was recovered within two days as fecal material and fed in suspension to small 1-2 cm rock scallops as a source of particulate organic matter. Specific activity of fecal matter was 1614 cpm/mg dry weight. After 48 hours, a single juvenile scallop had accumulated 821 cpm/mg dry weight. Duplicate scallops, sacrificed after one week exposure to abalone feces, had 423 cpm/mg dry weight and 475 cpm/mg dry weight. These data indicate uptake of particulate organic matter and possible turnover. Biochemical fractions are currently being assayed for radiolabel. Particulate organic material of fecal origin from such sources as abalone, copepods and other invertebrates in the natural environment may thus be an important food source for scallops.

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