# Nutrition and Technical Development of Aquaculture

## PROCEEDINGS OF THE TWENTY-SIXTH U.S.-JAPAN AQUACULTURE SYMPOSIUM

Edited by W.H. Howell, B.J. Keller, P.K. Park,
J.P. McVey, K. Takayanagi and Y. Uekita

UJNR Technical Report No. 26

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Durham, New Hampshire, U.S.A.
September 16-18, 1997



Edited by W. Huntting Howell, B. Jane Keller,
Paul Kilho Park, James P. McVey, Kazufumi Takayanagi,
and Yukio Uekita

Panel Chairmen: Yukio Uekita, Japan
James P. McVey, United States

Under the U.S. - Japan Cooperative Program in Natural Resources (UJNR)

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# PREFACE

The United States and Japanese counterpart panels on aquaculture were formed in 1969 under the United States-Japan Cooperative Program in Natural Resources (UJNR). The panels currently include specialists drawn from the government and academic departments most concerned with aquaculture. Charged with exploring and developing bilateral cooperation, the panels have focused their efforts on exchanging information related to aquaculture that could be of benefit to both countries.

The UJNR was begun during the Third Cabinet Level Meeting of the Joint United States-Japan Committee on Trade and Economic Affairs in January 1964. In addition to aquaculture, current subjects in the program include toxic microorganisms, air pollution, energy, forage crops, national park management, mycoplasmosis, wind and seismic effects, protein resources, forestry, and several joint panels and committees in marine resource research, development, and utilization.

Accomplishments include: increased communication and cooperation among technical specialists; exchanges of information, data, and research findings; annual meetings of the panel, a policy-coordinating body; administrative staff meetings; exchanges of equipment, materials, and samples; several major technical conferences; and beneficial effects of international relations.

The 26th U.S.-Japan Aquaculture Panel Symposium was held in Durham, New Hampshire, from 16-18 September 1997. Following the symposium, field trips during a seven-day period included the areas of Portsmouth, New Hampshire; and Bar Harbor, Eastport, Camden, and Boothbay Harbor, Maine. The symposium was organized by program chair Anne Bucklin, Sea Grant Director; Hunt Howell, Professor of Zoology; and Rollie Barnaby, Sea Grant Extension Officer, at the University of New Hampshire.

Panel Chairmen:

James P. McVey, United States

Yukio Uekita, Japan

Participants in the 26th UJNR Aquaculture Panel Symposium, held in Durham, New Hampshire, U.S.A., September 16-18, 1997.

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# RESEARCH IN FLATFISH CULTURE IN THE NORTHEASTERN UNITED STATES

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## ABSTRACT

Although flatfish have been commercially cultured for over a decade in Europe and Asia, their culture in North America has only recently been commercialized. The commercialization of Atlantic halibut *Hippoglossus hippoglossus* and summer flounder *Paralichthys dentatus* culture followed years of collaborative effort between industry and university researchers. Other important flatfish species are being evaluated for commercial culture throughout the region, including winter flounder *Pleuronectes americanus*, witch flounder *Glyptocephalus cynoglossus*, and yellowtail flounder *Pleuronectes ferruginea*. Juvenile production continues to be an impediment to commercialization. Commercial on-growing strategies include both net pen and land-based tank culture systems.

## INTRODUCTION

### Summer flounder *Paralichthys dentatus*

Research is underway in New Hampshire, Massachusetts, Rhode Island, Connecticut, and New York. Commercial production was begun in New Hampshire in 1995, Massachusetts and New York in 1997, and is slated for 1998 in Rhode Island. Much of the research in New Hampshire has taken place at the University of New Hampshire's (UNH) Coastal Marine Laboratory and at GreatBay Aquafarms, Inc. (GBA), a commercial hatchery. UNH research has been supported by both the U.S. Department of Commerce's National Oceanic and Atmospheric Administration (NOAA)/National Marine Fisheries Service (NMFS) Saltonstall-Kennedy Industry Grants Program (S-K) and the U.S. Department of Agriculture Northeast Regional Aquaculture Center (NRAC). The S- K work investigated substrate color preferences and effect on pigmentation, juvenile stocking densities as a percentage of bottom coverage (100, 150, and 200%), feed preferences, growth performance, and use of a recirculating seawater system. The NRAC research investigated the efficacy of natural spawning vs. hormonal inducement, larval stocking densities at 10, 20, 30, and 60 larvae/L, and weaning diet protein contents of 45, 50, and 55%. Much of this NRAC-sponsored research is being undertaken at GBA.

GreatBay Aquafarms, the New Hampshire Industrial Research Center (NHIRC), Sea Grant, NRAC, and the Electric Power Research Institute (EPRI) have cooperatively funded a number of research projects. The NHIRC research includes microbiology and veterinary diagnostics, wastewater characterization and effluent treatment design, and thermal engineering to capture the waste heat of a utility for heating the seawater and air. UNH Sea Grant and GBA are working to identify and develop a probiotic approach for early larval rearing in order to increase survival and limit pathogen habitation of the culture environment or larval gut. In addition, UNH Sea Grant researchers are working with GBA to identify genetically superior broodstock by tracking the performance of individual families raised in a common environment. The EPRI funding assisted in the development of a commercial scale grow-out demonstration system where research is being undertaken to evaluate the performance of alternate recirculating life support systems, specifically the biofilters. One biofilter is a fluidized sand bed, the other is a

plastic media submerged in an aerated tank. This research is also assisting in the engineering of a land-based tank farm being planned for GBA. This demonstration farm will allow GBA to compare variables such as stocking density on biofilter performance. The operating costs of the two systems will also be compared.

In Massachusetts, Aqua Future, Inc., a hybrid striped bass grow-out operation, is building a hatchery for the production of summer flounder juveniles and, as the recipient of a NOAA Fishing Industry Grant (FIG), is providing oversight and design input for two grow-out demonstration sites, one in New Bedford, Massachusetts, and one in Quonset, Rhode Island. The objectives of this work are to demonstrate the commercial culture of summer flounder, the utilization of underutilized seafood processing space as culture sites, and the viability of aquaculture as an alternative means of employment for displaced fishermen and plant workers. Trio Algarvio Seafoods, Inc. harvests and processes groundfish in New Bedford. The company owns a processing facility that is currently underutilized because of government-mandated reduced fishing effort which has, in turn, made it difficult for the company to supply local fish. Trio has installed into its facility the first phase of a grow-out farm utilizing raceways and a recirculating seawater system. Trio is stocking this system starting in late 1997 with juvenile summer flounder.

The University of Rhode Island (URI) has been involved with flatfish culture for many years, most recently investigating commercial culture for stock enhancement and grow-out. URI's flatfish aquaculture research has been funded in part through S-K, Sea Grant, and NRAC awards. The S-K research was done in collaboration with the Universities of Massachusetts and New Hampshire, the New England Fisheries Development Association (NEFDA), Northeast Organics, Inc., and GBA. The S-K research supported efforts for taking the culture of summer flounder to commercial scale by demonstrating the ability to repeatedly spawn summer flounder in captivity, to improve survival rates through larval and weaning stages, and to further elucidate larval nutritional requirements. Sea Grant research has investigated causes of larval mortality, influence of thyroid hormone on larval development and survival, hormonal influences on developing embryos, optimal culture environment conditions, marketing, economics, and outreach. URI is also the lead institution in a multi-institution effort currently funded by NRAC. This research is investigating larval stocking densities in greenwater, natural vs. hormonal induction of broodstock, comparison of survival, growth, health, and behavior of fish reared in recirculation systems vs. open ocean net pens. V&G Seafarms, in Quonset, Rhode Island, has constructed a commercial scale demonstration grow-out site as part of the FIG program administered by NMFS. This facility utilizes recirculating technology with minimal new water addition and a shallow raceway tank system. The tank system is stacked two layers deep. Commercial production is planned for early 1998.

The University of Connecticut is conducting research as part of the NRAC project being coordinated by URI. The objectives of this research are to identify protein and lipid levels in grow-out diets and an optimum calorie to protein ratio.

Mariculture Technologies, Inc. in New York is a private commercial production farm which is presently culturing summer flounder in open ocean net pens. The company supports its net pen operations from land-based nursery operations. Juveniles in excess of 100 g are stocked in the spring and fed through November, when they are typically harvested. As this is the first season summer flounder have ever been stocked in these net pens, it is unknown if the fish will be able to successfully overwinter in this location. In an effort to determine their thermal tolerance, some fish will be held in the net pens throughout the winter of 1997-98. Under a NOAA/NMFS FIG, Mariculture is testing a number of net pen systems, including ones manufactured by Bridgestone, Northern Plastics, Ocean Spar Technologies, and Atlantic Aqua Cage. Finally, as a commercial participant on the NRAC project coordinated by URI, Mariculture is monitoring fish growth, survival, health and behavior. These are being compared to fish raised in land-based systems.

### Winter flounder *Pleuronectes americanus*

Winter flounder supports a strong recre-

ational and commercial fishery from the Atlantic Maritimes through the mid-Atlantic states. Like summer flounder, commercial landings for winter flounder are at historic lows. There is strong interest in aquaculture research for purposes of both stock enhancement and commercial culture. Research in New Brunswick, Maine, New Hampshire and Rhode Island, and a commercial operation in Nova Scotia, support the development of this promising culture candidate.

In Maine, the Department of Marine Resources (DMR) has devoted considerable time and resources to evaluating the potential of stock enhancement for groundfish, particularly cod. Recently, DMR has given consideration to winter flounder for stock enhancement. DMR personnel are rearing small numbers of winter flounder juveniles from captive broodstock.

In New Hampshire, researchers at the UNH Coastal Marine Laboratory are also investigating the potential of stock enhancement as well as the commercial culture of winter flounder. They have been successful in batch rearing juvenile flounder to over 500 g. Graduate students are working on methods to improve larval survival through metamorphosis, and are comparing hatchery-reared and wild-caught juveniles as part of a program to determine the efficacy of stock enhancement. The stock enhancement research is being done in collaboration with the personnel at the Maine DMR, the Massachusetts Division of Marine Fisheries, and URI.

Winter flounder research is also underway in the maritime provinces of Canada.
The University of New Brunswick is working on developing commercial culture techniques and the optimization of feeding. In Nova Scotia, research and development are being undertaken by Sambro Fisheries Limited, which is a commercial processor, wholesaler, and distributor of groundfish species. Recognizing that its supply was being limited by a combination of overfishing and government regulation, Sambro has begun to investigate the commercial culture of winter flounder. It began on a pilot scale in 1994-96, maintaining a population of captive broodstock and rearing several batches of juveniles. Based on this experience, Sambro is planning large scale production systems starting in 1997.

**Atlantic halibut *Hippoglossus hippoglossus***

Atlantic halibut, the largest of our flatfish with individuals exceeding 200 kg not unheard of in the commercial fishery, are being commercially cultured in Europe to between 5 and 10 kg. At present the harvest is small, about 50 tons; however, with capacity expanding this number is sure to increase, particularly as North America starts to bring both academic and industrial resources into the development of a halibut culture industry. These fish are active swimmers and could be cultured in both tanks and net pens. Research programs have been developed at a number of North American locations. At the Memorial University of Newfoundland, investigators are seeking to develop broodstock management protocols, improve live-feed enrichment diets, refine start-feeding protocols, and develop juvenile-formulated diets. Also in Newfoundland, the privately owned company Maritime Mariculture is working closely with government and academic researchers to develop a commercial hatchery and grow-out farm for Atlantic halibut. Maritime has incorporated proven Norwegian culture technology into its rearing strategy. In New Brunswick, the Canadian Department of Fisheries and Oceans in St. Andrews is working on broodstock management, juvenile production, and the improvement of culture systems for rearing halibut. Researchers at the University of Maine are working on methods to refine spawning, fertilization, and larval rearing. They are also working on sex determination methodology and larval development issues. Future work at the University will seek to optimize culture techniques, and to improve larval and juvenile nutrition.

**Yellowtail flounder *Pleuronectes ferruginea***

For decades, yellowtail flounder has been a major component of the commercial fishery landings for groundfish from New Bedford, Massachusetts, north through Atlantic Canada. Landings have declined dramatically, and if this flatfish can be shown to be a viable cold water culture candidate it will enjoy a ready market throughout the Northeast.

Research on this species is occurring at the Memorial University of Newfoundland, where investigators are looking closely at the potential of yellowtail as a commercial culture candidate. Their

initial efforts are focused on broodstock management, improving juvenile on-growing protocols, determining an optimal culture environment, and needs for diet formulation.

**Witch flounder *Glyptocephalus cynoglossus***

The witch flounder is considered by many the premier flounder in the Northwest Atlantic. This statement is borne out in the prices paid for this fish by commercial dealers, with boat prices often in excess of $8/kg. Because it is such a prized fish, there is interest in its commercial culture, but the interest is tempered somewhat by the fact that witch flounder appear to be a relatively slow growing, difficult-to-rear species. The only research being conducted on witch flounder is through a joint project between the Memorial University of Newfoundland and UNH. At these two institutions, researchers are investigating protocols for broodstock development and management, as well as larval rearing protocols. The larval research seeks to determine the optimal incubation temperature for eggs and early larvae, to ascertain the necessity of greenwater for larval culture, and to compare growth rates of first-feeding larvae fed wild, cultured, and enriched cultured diets. They have been successful in rearing small numbers of fish through the juveniles stage.

## ACKNOWLEDGMENTS

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# JAPANESE FLOUNDER SEED PRODUCTION FROM QUANTITY TO QUALITY

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## ABSTRACT

Japanese flounder *Paralichthys olivaceus*, distributed widely from the coast of Hokkaido to Kyushu, is one of the most important target species for stock enhancement trials and aquaculture in Japan. More than 20 million juveniles were released on the coast of Japan in 1994. Studies of seed production in this species are divided into three phases: the challenge of seed production, the quantitative expansion, and the qualitative improvement. The first phase, started at the end of the 19th century, involved egg collections from wild-caught fish, artificial fertilization, and hatching experiments. During the period of the 1950s to 1960s, suitable food organisms were found for the feeding of marine fish larvae. Then, seed production of Japanese flounder became successful following the success of red sea bream *Pagrus major* seed production. During this period, matured eggs were taken from wild-caught fish in the spawning season and fertilized artificially. Fish larvae and juveniles were fed rotifers, *Artemia*, minced meat, and then chopped sand eels as they grew. During this phase, the quality and the quantity of fertilized eggs fluctuated, because stripping and artificial fertilization were conducted from the wild-caught fish. Several years after the first success, fertilized eggs were taken from the spawners which grew in captivity from newly hatched larvae. We then could enter the second phase when brood stocks kept in land-based tanks matured and continuously spawned large numbers of eggs with high quality. Tank spawning expanded the quantities of the Japanese flounder seed production very quickly, for we could transfer the basic technologies of red sea bream seed production. Moreover, according to the maturation control, we could acquire fertilized eggs almost year round. Since the larvae of this species could accept formula feed more readily than other species, formula diets were actively introduced to reduce costs together with the nutritional improvement in produced fish. In the third phase, we have been trying to improve the quality of produced juveniles. We have succeeded in several aspects: dramatic reduction in the percentage occurrence of albinism by nutritional improvement during the larval stage, behavioral improvement by training in semi-natural conditions before releasing, and labor savings and automization in the seed production process. We still face many problems that include egg quality, abnormal blind side pigmentation, vertebral abnormality, disease control, and genetic diversity. In addition, hormonal control during larval metamorphosis, biotechnological trials, and mechanism of sex determination should be elucidated in relation to the seed production of this species.

## INTRODUCTION

Japanese flounder, distributed from Hokkaido to Kyusyu, is one of the most important fish for Japanese cooking, along with red sea bream. Annual production from aquaculture is more than 6000 tons, exceeding landings from boat fishing in 1994. Moreover, Japanese flounder is one of the most important target species for the stock enhancement project. Thirty-four prefectures released more than 20 million juveniles in coastal areas as part of governmental activities in 1994 (Furusawa 1997). This became possible based on the mass seed production that integrates various technologies from biology to engineering. Japanese flounder seed production has expanded quickly within the past 20 yr (Fig. 1). As for the quantitative expansion of seed production, related technologies need to develop concurrently and harmoniously. We Japanese are now facing new situations in the transition from the pioneer trials to the enterprise of stock enhancement to produce higher quality juveniles.

I present a historical review of studies in Japanese flounder seed production, several trials for the transition from quantity to quality, and the future issues and remaining problems.

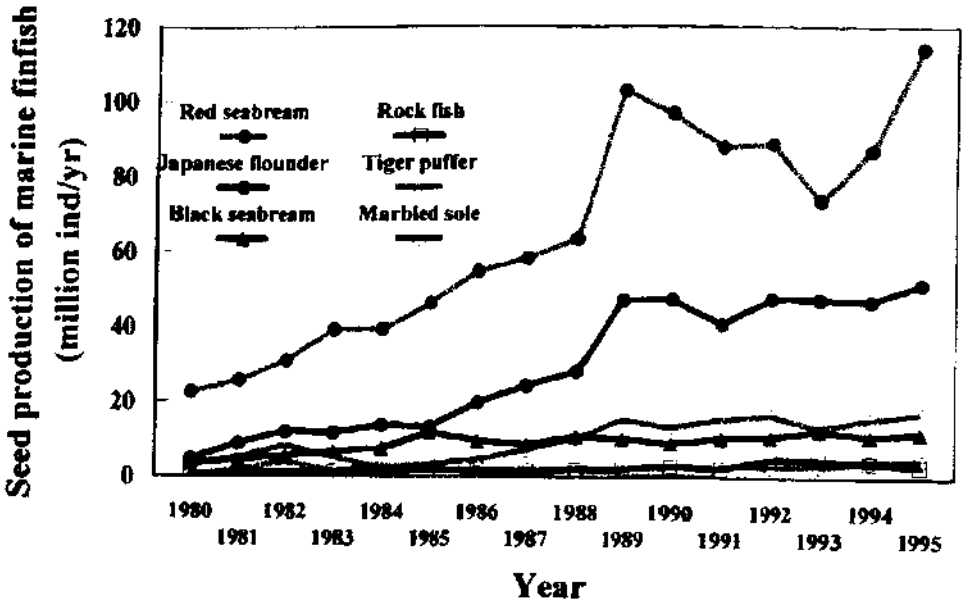


**Figure 1.** Seed production of marine finifsh in Japan, 1980-1995.

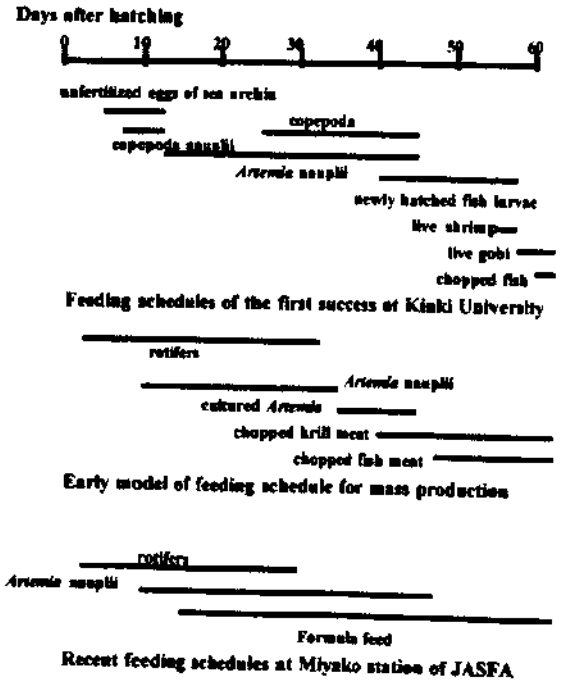


**Figure 2.** Comparison of feeding schedule in Japanese flounder seed production from the first success at Kinki University to the recent data at Miyako station of the Japan Sea-Farming Association.

## Challenge of larviculture and seed produ

In Japanese flounder, artificial fertili and hatching trials using wild fish began at t of the 19th century (Fujita 1903). Howe trials ended at the completion of yolk abso because of the lack of initial food organism

From the 1950s to the first half 1960s, the feeding scheme for marine finfish was nearly developed, and larval reari Japanese flounder from hatching to juvenil successful in 1965 (Harada et al. 1966). A time, eggs were taken from wild fish caught spawning season, and were fertilized artifi Unfertilized eggs of sea urchins, copepods, *A* nauplii, newly hatched fish larvae, live shrir gobi, and chopped fish meat were fed succes as the flounder grew (Fig. 2). Survival rat 0.7% during the first 63 days from hatchi juveniles at 3.03 cm TL (Fig. 3). From that serious cannibalism and abnormal pigmentat the ocular side were noticed as characte phenomena of this species.

## Establishment of basic technologies quantitative expansion

In 1969, cultured flounder raised hatching matured in captivity, and fertilized

were taken from these fish (Harada 1980). During the 1970s, the Japan Fisheries Agency (JFA) supported the basic studies for the mass production of Japanese flounder in several prefectural experimental stations.

During the first half of the 1970s, the quantity and the quality of eggs were unstable, as eggs were stripped and fertilized artificially from wild fish. Then, cultured fish started to spawn large amounts of high quality eggs in land-based tanks. Since the basic technologies of seed production in red sea bream could be transferred to Japanese flounder, spawning became readily possible and the seed production expanded very rapidly (Takahashi et al. 1980, Hiramoto et al. 1981a). Moreover, the conditioning of maturation by the control of photoperiods and temperature permitted fertilized eggs to be taken year round (Ijima et al. 1986) (Fig. 4). General procedures at the present time in Japan are as follows: spawners are kept in captivity throughout the year; maturation is controlled by photoperiods and temperature; fertilized eggs are taken from natural spawnings in land-based tanks; newly hatched larvae are stocked in several 10-$m^3$ concrete tanks at a density of 10,000-50,000/$m^3$; they are raised in several tanks until juveniles become 3 cm TL (Fig. 5), and fed on rotifers, *Artemia* nauplii, and a formula diet as they grow (Fig. 2). Food organisms are enriched with special oils containing highly unsaturated fatty acids and fat-soluble vitamins (Torii et al. 1994).

In the first phase of seed production, minced or chopped fish meat was generally used as juvenile feed (Hiramoto et al. 1981b) (Fig. 2). Because these diets lose their nutrients to water and deteriorate the water quality, they were replaced with a formula diet. Since the larvae can feed on a formula diet with relative ease (Tange and Nagahama 1986), and the formula diet could reduce the appearance of albinism in the juveniles, many hatcheries are active in the use of formula feed which reduce labor and cost in the seed production (Takahashi 1990) (Fig. 2).

Serious cannibalism should be mentioned as one of the main factors of mortality during the seed production of this species (Harada et al. 1966), especially when juveniles have a large size variance of 1.5 to 2.0. Biting and cannibalism frequently occurred in the rearing tanks (Torii et al. 1994).

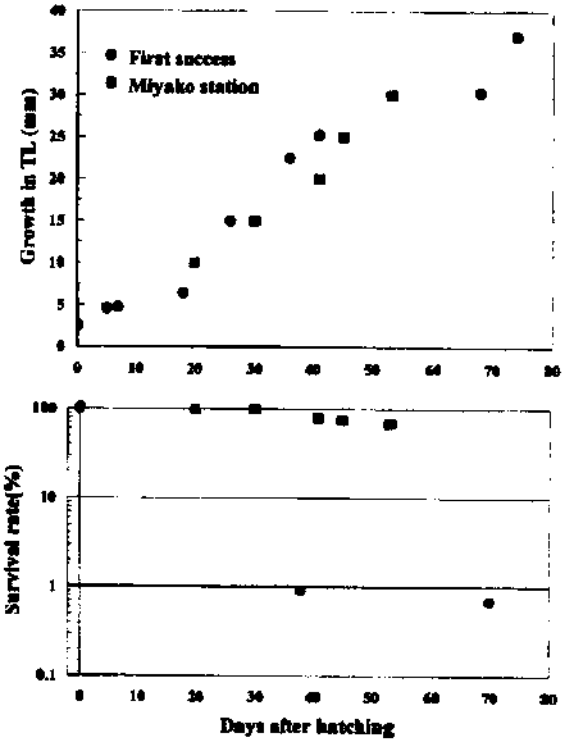


**Figure 3.** Comparison of growth and survival between the first success and the recent results at Miyako station of the Japan Sea-Farming Association corresponding to the development of technology.

To reduce the mortality by cannibalism, it is necessary to maintain reasonable stocking density and feeding to reduce size divergency, and to do size selection thoroughly during the seed production process.

## Trials for qualitative improvement

### *Egg quality improvement and genetic diversity*

Japanese flounder spawn frequently during a long spawning season (Hirano and Yamamoto 1992). Even in the land-based tanks, the egg quality from natural spawning is not always high. However, the most important factors affecting egg quality might be spawner's feed and the mating environment, but these factors have not been analyzed thoroughly in many species (Morimoto 1994). At the present time, frozen fish supplemented with several nutrients, such as vitamins, are used for spawners (Torii et al. 1994). Special formula diets for spawners were examined

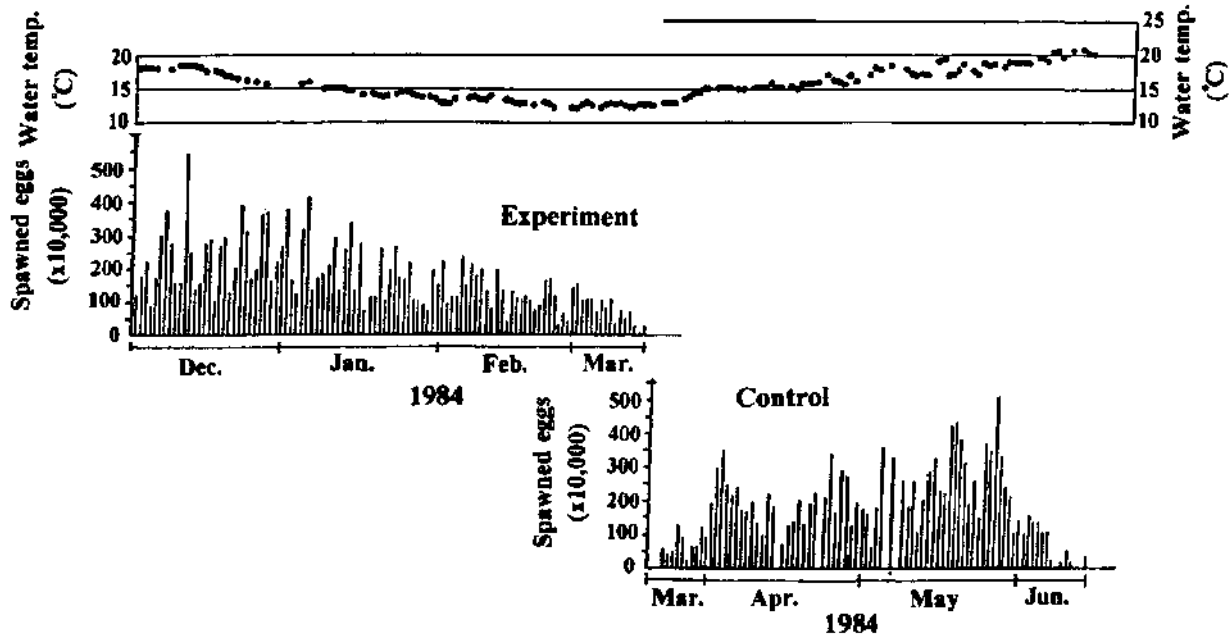


**Figure 4.** Enhanced maturation and spawning of Japanese flounder with photoperiod control. Photoperiod control was started at 42 days before first spawning in experimental tank. (modified from Iijima et al., 1986)

to obtain higher quality eggs, but the feed composition is still under investigation (Table 1).

As to the mating environment, stocking ratios between females and males, and stocking densities in spawning tanks are maintained at 1:1-3, and 3 kg/m$^3$, respectively, to improve the fertilization rate and fecundity (Y. Hondo, Miyazu Station, JASFA, personal communication).

A recent concern in seed production is the genetic problem. Continuous use of limited spawners from the hatchery-reared fish results in the mass release of juveniles with simplified genetic information. Therefore, it has become popular to replace the spawners from artificially produced fish to locally caught wild fish to keep genetic diversity intact with local characters in produced juveniles (Tanaka et al. 1997).

*Nutritional study and development of formula diet*

A large number of nutritional studies have been conducted from the viewpoints of growth, survival, vitality, pigmentation, and vertebral deformation (Kanazawa 1990, Takeuchi 1997). These studies have helped to improve the composition and processing of formula diets and enrichment procedures of live food organisms. Since commercially available formula diets and enrichment materials contain many industrial secrets, hatcheries are choosing brands according to their experiences. Formula diets have better and more stable nutritional qualities than live food or chopped meat. Therefore, formula diets can improve the tolerance ability to stress and handling during the seed production. For example, the Miyako Station of JASFA began to produce more than 2 million juveniles (3 cm TL) at a survival rate higher than 80% from hatching with the effective use of formula diets (Torii et al. 1994).

*Studies on abnormal pigmentation and practical prevention*

In association with the development of seed production in this species, typical abnormal pigmentation has been studied seriously. There are two types of abnormal pigmentation in flatfish—albinism (hypomelanosis on the ocular side) and ambicoloration (hypermelanosis on the blind side) (Seikai 1985, Seikai et al. 1987). However, both albinism and ambicoloration appeared in hatchery-reared flounder at extremely high percentages, and the former phenomenon attracted biologists from the outset of the seed production.

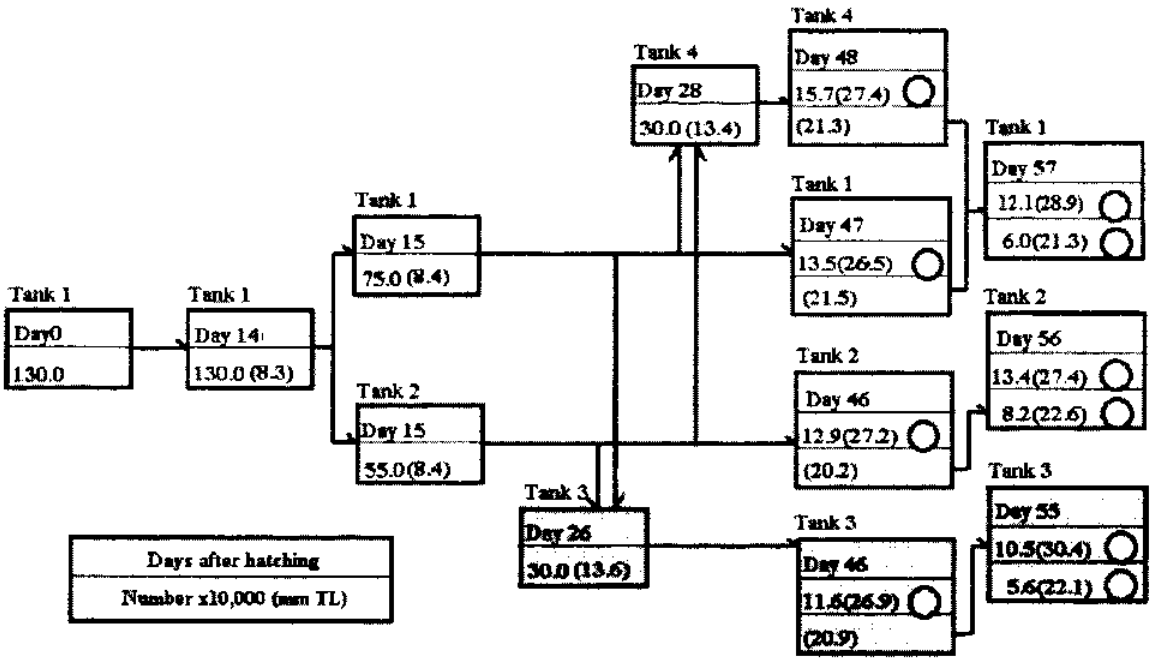


**Figure 5.** Sequence of separation and harvest during Japanese flounder seed production at Miyako station of the Japan Sea-Farming Association (Modified from Torii et al. 1994).

The factors were analyzed mainly from the nutritional viewpoint during the larval stage. It was revealed that the percentage in appearance of albinism was highly dependent on the feed during the larval stage (Seikai 1985, Seikai et al. 1987, Kanazawa 1993). In the practical seed production, enrichment of live food organisms with vitamin A and docosahexaenoic acid (DHA) (Miki et al. 1988, 1989), and early feeding of formula diets for larvae (Kitajima et al. 1985) could reduce the percentage in occurrences to negligible levels. However, the percentage appearance of albinism increased because of the outbreak of larval diseases (Takahashi 1992) or genetic background of spawners (Tabata 1991). There are still unresolved factors in relation to this abnormality.

On the other hand, nearly all cultured fish show hypermelanosis on the blind side. Stocking density and the type of feeds during larval stage, light irradiation during juvenile stage, effects of ocular side pigmentation abnormality, and source of examined fish (wild or hatchery-reared) were suggested as the factors for this abnormality (Seikai 1991, Takahashi 1992). Recently, it was revealed that the effect of substratum was the most important factor for hypermelanosis (staining type) during juvenile and young periods (Iwata and Kikuchi, 1998). Many unresolved factors remain in relation to other types of hypermelanosis (true and spotting types). At present, hypermelanosis is used as the effective marker, which nearly all artificially produced fish have without any treatments, to distinguish released fish from wild fish (Furusawa 1997). Since heavy pigmentation on the blind side decreases the price of recaptured flounder at the fish market, this is becoming a problem for the cost performance of the stock enhancement program (Furusawa 1997).

*Morphological abnormalities and prevention*

Japanese flounder never show lordosis like red sea bream because this species does not have an air bladder. Artificially produced juvenile flounder show a high percentage in occurrence of vertebral abnormalities such as fusion, deformation, and compression (Iseda et al. 1978, Seikai 1979). Recently, the percentage of occurrence has increased from the past. One reason for this was suggested to be the effect of enrichment to live food organisms with fat-soluble vitamins to reduce albinism. It was revealed that the excessive

| Exp. lots | 1 | 2 | 3 |
|---|---|---|---|
| Feed | Control | +Lecithin 1.5% | +VE 0.1% |
| Female | | | |
| Mean BW[1] (no.) | 1,388(40) | 1,389(40) | 1,391(40) |
| Male | | | |
| Mean BW (no.) | 807(80) | 814(80) | 813(80) |
| Spawning (1990) | | | |
| Duration | 13 Mar-8 Aug | 13 Mar-2Aug | 20 Mar-2Aug |
| Frequency | 93 | 90 | 88 |
| Total eggs (x1,000) | 35,721 | 69,620 | 59,038 |
| Egg quality | | | |
| Floating rate (%) | 46.7 | 49.8 | 48.7 |
| Fertilization rate (%) | 54.0 | 48.0 | 38.0 |
| Egg diameter (mm) | 0.88 | 0.88 | 0.88 |
| Hatching rate (%) | 81.4 | 88.3 | 86.7 |
| Deformation rate (%) | 41.4 | 42.9 | 50.3 |
| SAI[2] | 50.6 | 35.1 | 35.3 |

[1]Body weight (g)
[2]SAI (survival activity index) = $\Sigma$ (N-hi)i/N (i=1 to k);
N = initial number of larvae, hi = accumulated mortality at day i,
k = day of no survivor
(modified from Wakui and Otaki 1991).

**Table 1.** Experiment of formula feed for spawners

administration of vitamin A through enriched live food during the special period when ossification vertebrae advances induced the high percentage of occurrence of this abnormality (Dedi et al. 1995, Takeuchi et al. 1995, Dedi et al. 1997). Other factors such as genetic effects in relation to this abnormality are expected (Y. Hondo Miyazu Station, JASFA, personal communication).

*Labor and cost savings*

Simplification of operation and labor savings are essential factors for the expansion of quantities in production. In many cases, simplification of the feeding schedule, active use of formula feed, introduction of automatic feeders, and cleaning robots could bring success to some extent. Labor savings with automization sometimes backfire to increase the running cost and the risk which produce poor quality juveniles because of disregard for species and developmental stage-specific characters. There is a concept that large rearing tanks are considered as small ecosystems, where self-discipline of organisms in rearing tanks is attained and reduction in daily work is possible, called "Hottoke-Siiku" (Hottoke means 'let' and Shiiku 'grow') (Takahashi 1990).

*Quality improvement for releasing*

Most of the juveniles produced in public hatcheries are released in the coastal areas of Japan. The effects which are extremely different between areas are dependent on timing and location of releasing, and the quality of juveniles. Different behavioral patterns and chemical compositions in the artificially produced juveniles from wild fish are expected as the reasons for higher predation mortality (Furuta 1996). It was revealed that released sizes larger than 8 cm TL could promise good survival and recapture because flounder juveniles have a relatively higher hierarchy in the nursery ground ecosystem (Yamashita et al. 1994). As the cost for the production increases logarithmically as fish grow larger in land-based tanks, it is desirable to produce juveniles with stronger potential for survival at smaller sizes than 8 cm. The concept of the quality of juveniles for releasing is well accepted (Tsukamoto 1993), but the standard for this concept is now under investigation using feeding behavior, and burrowing abilities. Earlier seed production which can realize earlier releasing to avoid predator and crush of mysids in the nursery ground is conducted. At the same time, hatcheries are trying to produce juveniles which have a competitive edge over wild fish. For this purpose, juveniles were raised to the releasing size to acclimate to the wild environment in the enclosures (Furuta 1993). Under such environment, juvenile flounder approximate their behavior and chemical compositions closely to that of wild fish.

*Outbreak of diseases and countermeasures (Table 2)*

Diseases of larvae, juveniles, and spawners occur during the seed production process (Muroga 1992). Bacterial diseases for juveniles include infections with *Flexibacter maritimus*, *Vibrio anguillarum*, and *Edwardsiella tarda*. The latter two can infect the young and spawners. Protozoan diseases are caused by infections with *Ichthyobodo* sp., *Cryptocaryon irritans*, and *Scuticociliatida* gen. sp. Bacterial diseases of larvae and juveniles are bacterial enteritis infected by *Vibrio ichthyoenteri*. Viral diseases are epidermal hyperplasia and viral nervous necrosis (VNN). These diseases during the larval stage cause extremely high mortality of over 90%. The safeguards against diseases are: (1) no introduction of pathogens, (2) maintenance clean environments, (3) enhancement of live food activities, (4) sterilization of tanks and tools, and (5) utilization of UV-treated seawater for rearing. Frequent outbreaks of viral diseases during seed production caused serious problems for the stock enhancement project. As viral-infected fish (e.g., VNN) are impossible to treat, we must destroy all juveniles if the produced fish have had an attack of viral disease.

*Biotechnology in seed production*

Japanese flounder is one of the most advanced species in relation to biotechnological studies (Tabata 1991, Yamamoto 1995). As females can grow faster than males in this species, chromosome manipulation was attempted to produce all females by gynogenetic diploid. During such attempts, phenotypic expression of sex in this species was found to be determined by genetic and environmental factors (e.g., temperature, diet, and

| Disease | | Pathogen | Note |
|---|---|---|---|
| Viral | Epidermal hyperplasia | Flounder herpesvirus (FHV) | 10-25 days after hatching high mortality |
| | Viral nervous necrosis | Striped jack nervous necrosis virus (SJNNV) | larvae and juveniles, high mortality |
| | Birnaviral disease | Hirame birnavirus | 1-2.4 g juveniles |
| | Hirame rhabdoviral disease | Hirame rhabdovirus (HIRRV) | adult |
| Bacterial | Streptococcicosis | *Streptococcus iniae* | juveniles, adult |
| | Vibriosis | *Vibrio anguillarum* | juveniles |
| | Bacterial enteritis | *Vibrio ichthyoenteri* | larvae |
| | Gliding bacterial disease | *Flexibacter maritimus* | juveniles |
| | Edwardsiellosis | *Edwardsiella tarda* | juveniles, adult |
| Protozoan | Ichthyobodosis | *Ichthyobodo* sp. | juveniles |
| | White spot disease | *Cryptocaryon irritans* | juveniles |
| | Scuticociliatidosis | *Scuticociliatida* gen. sp. | juveniles |
| Parasitic | Skin fluke disease | *Neobenedenia girellae* | juveniles |

**Table 2.** Diseases of Japanese flounder during seed production and culture

stocking density). Several cloned progeny which could be produced by using these technologies have strong possibilities to produce effective strains for aquaculture purposes for shorter than usual breeding procedures (Yamamoto 1997). Biotechologically treated fish are impossible to use for releasing; however, such strains with high growth potential or resistance ability to diseases are useful for aquaculture (Yamamoto 1997).

## CONCLUSION AND REMAINING PROBLEMS

Juveniles of Japanese flounder are now produced in mass scale at many public hatcheries. Such rapid expansion in quantity presents many related problems, and if we cannot resolve these problems together, it will have a negative impact on the trials of the Japanese flounder stock enhancement project.

First of all, it is necessary to stabilize further the mass seed production (Furusawa 1997). If the object of production is not achieved, problem situations could occur frequently, such as the introduction of fertilized eggs or produced juveniles from other areas. These bring more serious concerns to fish disease and genetic problems. The concerns include: (1) egg quality improvement, (2) clarification of nutritional demand, (3) development of formula feed, (4) automization and labor savings, (5) reconsideration of biological function including food organisms, and (6) prevention of fish disease. Regarding disease, the amounts of restocking to the coastal area have already attained the levels of natural resources in Japan, and we should establish a better system to protect the diversity of carrier fish to the wild environment.

The next step is to improve the quality of juveniles which balance with the stock enhancement releases economically (Tsukamoto 1993). For this, the most important is the feedback from the ecological and fisheries resource surveys after releasing. In addition, produced juveniles for stock enhancement should maintain the local genetic information and their diversity (Nishida et al. 1997). Recent mt-DNA analyses revealed that unexpectedly few males and females compare with the number of stocked spawners in the spawning tank to the mating behavior of 1-day egg batches (Fujii and Nishida 1996). To resolve this problem, we should reconsider egg-taking methods, for instance stripping and artificial fertilization of males and females. Recently, the role of hormones in eggs has attracted attention, and the effects of maternal hormones on the survival after hatching have been revealed in different species. The relationship between egg qualities including maternal hormones and survival and development should be one of the topics to concentrate on in future efforts (Tagawa 1997).

In the case of Japanese flounder, relatively large amounts of biological information have been accumulated. Recently, a well organized review on biology and stock enhancement of Japanese flounder was published from the viewpoints of ecological aspects (Minami 1997, Nishida et al. 1997, Noichi 1997, Tanaka et al. 1997), ecology and physiology of metamorphosis (Seikai 1997, Tanaka 1997, Yamano 1997), and stock enhancement (Takeuchi 1997, Yamamoto 1997, Yamashita 1997). Recent topics on the biology of Japanese flounder include the role of thyroid hormones regulating metamorphosis (Yamano 1997), and sex determination mechanisms depending on genetics and environments (Tabata 1991, Yamamoto 1995). Basic information on the biology can solidify the foundation of the seed production technology and stock enhancement.

## LITERATURE CITED

Dedi, J., T. Takeuchi, T. Seikai, and T. Watanabe. 1995. Hypervitaminosis and safe levels of vitamin A for larval flounder (*Paralichthys olivaceus*) fed *Artemia* nauplii. Aquaculture 133: 135-146.

Dedi, J., T. Takeuchi, T. Seikai, T. Watanabe, and K. Hosoya. 1997. Hypervitaminosis a during vertebral morphogenesis in larval Japanese flounder. Fish. Sci. 63: 466-473.

Fujii, T. and M. Nishida. 1996. Heriditical diversity in hatchery-reared juvenile flounder analyzed from the basic sequence in D-roop region of mt-DNA. Abstract. Autumn meet., Jpn. Fish. Sci. Soc. 92 p. [In Japanese].

Fujita, T. 1903. Artificial fertilization and hatching of Japanese flounder. Zool. Mag. 15(179):

316-322. [In Japanese].

Furusawa, T. 1997. Key problems of sea-farming associated with its perspective, pp. 117-126. *In:* T. Minami, and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Furuta, S. 1993. Releasing techniques and fry quality, pp. 94-101. *In:* C. Kitajima (ed.), Healthy Fry for Release, and Their Production Techniques. Koseishakoseikaku, Tokyo. [In Japanese].

Furuta S. 1996. Predation on juvenile Japanese flounder (*Paralichthys olivaceus*) by diurnal piscivorous fish: field observations and laboratory experiments, pp. 285-294. *In:* Y. Watanabe, Y. Yamashita, and Y. Ozeki (eds.), Survival Strategies in Early Life Stages of Marine Resources. A.A. Balkema, Rotterdam.

Harada, T. 1980. Present state and problems in Japanese flounder culture. Yoshoku 17(4): 48-53. [In Japanese].

Harada, T., S. Umeda, O. Murata, H. Kumai, and K. Mizuno. 1966. Rearing and growth of Japanese flounder larvae from artificially fertilized eggs. Rep. Fish. Res. Sta., Kinki Univ. (1) 28: 9-303. [In Japanese].

Hiramoto, Y., N. Miki, and K. Kobayashi. 1981a. Studies on the Japanese flounder seed production-III. Rearing on fish meat during juvenile period (TL 15-50mm). Saibai Giken 10(2): 89-103. [In Japanese].

Hiramoto, Y., N. Miki, and K. Kobayashi. 1981b. Egg collection from natural spawning and larval hatching in Japanese flounder. Rep. Tottori Pref. Fish. Sta. 23: 7-12 . [In Japanese].

Hirano, R. and E. Yanamoto. 1992. Spawning rhythm and egg number at the individual female spawning experiment in the hirame flounder, *Paralichthys olivaceus*. Rep. Tottori Pref. Sta. Fish. 33: 18-28. [In Japanese with English summary].

Ijima, T., T. Abe, R. Hirakawa, and Y. Torishima. 1986. Induced spawning with long day treatment in Japanese flounder. Saibai Giken 15(1): 57-62. [In Japanese).

Iseda, H., S. Sumida, M. Ishihara, M. Owaki, and S. Tabata. 1978. Experiment of Japanese flounder seed production. II. The relation between feed and albinism, and ossification stage and the deformation. Annu. Rep. Showa 51st Kumamoto Pref. Fish. Exp. Sta., pp. 257-261. [In Japanese].

Iwata, N., and K. Kikuchi, 1998. Effects of sandy substrate and light on hypermelanosis of the blind side in cultured Japanese flounder *Paralichthys olivaceus*. Environmental Biology of Fishes. 52: 291-297.

Kanazawa, A. 1990. Nutrition and feed in Hirame culture. Suisannzousyoku 38: 398-399. [In Japanese]

Kanazawa, A. 1993. Nutritional mechanism involved in the occurrence of abnormal pigmentation in hatchery-reared flatfish. J. World Aquacult. Soc. 24: 162-166.

Kitajima, C., G. Hayashida, M. Shimozaki, and T. Watanabe. 1985. Reductive effect of micro-binded diet on occurrence of color anomaly in hatchery-reared flounder, *Paralichthys olivaceus*. Bull. Nagasaki Pref. Inst. Fish. 11: 29-35. [In Japanese with English summary].

Miki, N., T. Taniguchi, and H. Hamakawa. 1988. Effect of rotifer enriched with fat-soluble vitamins on occurrence of albinism in hatchery-reared "Hirame" *Paralichchys olivaceus* (preliminary report). Suisan Zoshoku 36: 91-96. [In Japanese].

Miki, N., T. Taniguchi, and H. Hamakawa. 1989. Adequate vitamin level for reduction of albinism in hatchery-reared Hirame *Paralichthys olivaceus* fed on rotifer enriched with fat-soluble vitamins. Suisan Zoshoku 37: 109-114. [In Japanese].

Minami, T. 1997. Life history, pp. 9-24. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Morimoto, H. 1994. Egg quality, pp. 83-96. *In:* M .Tanaka and Y. Watanabe (eds.), Studies on Early Life Mortality of Fishes. Koseishakoseikaku, Tokyo. [In Japanese].

Muroga, K. 1992. Hatchery diseases of marine fish in Japan, pp. 215-222. *In:* I. M. Shariff, R. P. Subasinghe, and J. R. Arthur (eds.), Diseases in Asian Aquaculture.

Fish Health Section, Asian Fisheries Society, Manila, Philippines.

Nishida, M., T. Ohkawa, and T. Fujii. 1997. Population structure, pp. 41-51. *In:* T. Minami and M.Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Noichi, T. 1997. Early life history, pp 25-40. *In:* T. Minami and M.Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Seikai, T. 1979. Studies on the abnormality of vertebrae and scales in company with the occurrence of abnormal coloration in the juvenile and young of hatchery-reared flounder, *Paralichthys olivaceus*. Bull. Nagasaki Pref. Inst. Fish. (5): 19-25. [In Japanese with English summary].

Seikai, T. 1985. Influence of feeding periods of Brazilian *Artemia* during larval development of hatchery-reared flounder *Paralichthys olivaceus* on the appearance of albinism. Nippon Suisan Gakkaishi 51: 521-527.

Seikai, T. 1991. Influence of fluorescent light irradiation, ocular side pigmentation, and source of fishes on the blind side pigmentation in the young Japanese flounder, *Paralichthys olivaceus*. Suisanzoshoku 39(2): 173-180. [In Japanese with English summary].

Seikai, T. 1997. Mechanism of abnormal pigmentation, pp. 63-73. *In:* T. Minami and M.Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Seikai, T., T. Watanabe, and M. Shimozaki. 1987. Influence of three geographically different strains of *Artemia* nauplii on occurrence of albinism in hatchery-reared flounder *Paralichthys olivaceus*. Nippon Suisan Gakkaishi 53: 195-200.

Tabata, K. 1991. Application of chromosomal manipulation in aquaculture of hirame *Paralichthys olivaceus*. Bull. Hyogo Pref. Fish. Exp. Sta. 28: 1-134. [In Japanese with English summary].

Tagawa, M. 1997. Involvement of thyroid hormones in early development of teleosts. Nippon Suisan Gakkaishi 63(4): 498-501. [In Japanese].

Takahashi, Y. 1990. Reduction of the amount of food organisms supplied and simplification of rearing operations in the seedling of the Japanese flounder. Suisan Zoshooku 38(1): 23-33. [In Japanese with English summary].

Takahashi, Y. 1992. Appearance and prevention of abnormally pigmented fish in Japanese flounder seed production. Japan Sea-Farming Assoc. Spec. Rep. 3: 1-58. [In Japanese].

Takahashi, K., Y. Hayakawa, D. Ogura, and H. Nakanishi. 1980. Egg collection from natural tank spawning in Japanese flounder. Saibai Giken 9: 41-46. [In Japanese].

Takeuchi, T. 1997. Nutritional requirement for improvement of rearing seed production, pp. 96-106. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Takeuchi, T., J. Dedi, C. Ebisawa, T. Watanabe, T. Seikai, K.Hosoya, and J. Nakazoe. 1995. The effect of ß-carotene and vitamin A enriched *Artemia* nauplii on the malformation and color abnormality of larval Japanese flounder. Fish. Sci. 61(1): 141-148.

Tanaka, M. 1997. Ecological significance of metamorphosis, pp. 52-62. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Tanaka, M., T. Minami, and Y. Koshiisi. 1997. Research direction, pp. 127-130. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Tange, K. and K. Nagahama. 1986. Secondary phase of flounder juvenile rearing fed on formula feed. Saibai Giken 15(1): 63-71. [In Japanese].

Torii, S., Y. Shiokawa, Y. Hayakawa, S. Siota, K. Igarashi, Y. Uji, K. Sato, A.Ito, H. Wakui, M. Kawanobe, A. Iwamoto, and T.

Fukunaga. 1994. Corpus of hirame seed production technologies in Notth Pacific Area. Japan Sea-Farming Assoc., Tokyo, pp. 87. [In Japanese].

Tsukamoto, K. 1993. Fry quality, pp. 102-113. *In:* C. Kitajima (ed.), Healthy Fry for Release, and Their Production Techniques. Koseishakoseikaku, Tokyo. [In Japanese].

Wakui, H. and K. Otaki. 1991. Results of the experiment for the development of formula diet for Hirame brood stock. Heisei 3rd Annu. Rep. Seedling Inst. Fukushima Pref., pp. 57-79. [In Japanese].

Yamamoto, E. 1995. Studies on sex-manipulation and production of cloned populations in Hirame flounder, *Paralichthys olivaceus* (Temminck and Schlegel). Bull. Tottori Pref. Fish. Exp. Sta. (34): 1-145. [In Japanese with English abstract].

Yamamoto, E. 1997. Biotechnology, pp. 83-95. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Yamano, K. 1997. Mechanism of metamorphosis, pp. 74-82. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Yamashita, Y. 1997. Ecology and releasing techniques, pp. 107-116. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseishakoseikaku, Tokyo. [In Japanese].

Yamashita, Y., S. Nagahora, H. Yamada, and D. Kitagawa. 1994. Effects of release size on survival and growth of Japanese flounder *Paralichthys olivaceus* in coastal waters off Iwate Prefecture, northeastern Japan. Mar. Ecol. Prog. Ser. 105: 269-276.

# SUSTAINABLE FLOUNDER CULTURE AND FISHERIES: A REGIONAL APPROACH INVOLVING RHODE ISLAND, NEW HAMPSHIRE, VIRGINIA, NORTH CAROLINA, AND SOUTH CAROLINA

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## ABSTRACT

Interest in culturing flounders along the east coast of the United States has increased greatly due to reduced fishery landings and increased consumer demand. Three flounder species are being evaluated for aquaculture development by research teams from several states. Through region-wide meetings, these researchers have shared information regarding progress and provided input to a regional plan containing future research needs and priorities related to flounder culture and stock enhancement. The resulting strategy will continue to serve as blueprint for an integrated approach to research and outreach activities supported by Sea Grant in the coming years.

## INTRODUCTION

This paper presents a brief overview of recent planning and research developments concerning flounders on the Atlantic coast of the United States. The author is not directly involved in any of the research described; rather, he is one of the Sea Grant program administrators who has facilitated the interactions and collaborations that are being developed. The following, then, is a description of the emergence and content of a multi-state complex of Sea Grant-supported research into various aspects of flounder aquaculture focusing on three different species.

### Sea Grant and the national network

Within the structure of the National Sea Grant College Program, there are state-based program administrative units that are usually located at universities. Together, these so-called local Sea Grant programs form the national Sea Grant network, and there is a local program in each coastal and Great Lakes state, including Hawaii and Puerto Rico. The local Sea Grant programs provide the focal points for developing partnerships between the local universities, state agencies and federal government, primarily through the National Oceanic and Atmospheric Administration (NOAA) within the U.S. Department of Commerce. Within each local Sea Grant program are research, education, and outreach/extension projects related to various aspects of marine and coastal resources, including aquaculture, that are important to that state.

Of particular relevance to the topic of this paper are the flounder aquaculture projects supported through the local Sea Grant programs in New Hampshire, Rhode Island, Virginia, North Carolina, and South Carolina.

### The regional plan

In mid-1996, a group of flounder culture researchers, extension leaders, industry representatives, and Sea Grant program administrators met to discuss the status of flounder culture activities in the states noted above and to produce a document setting forth a strategy for research and extension activities that will provide the technical foundation upon which commercial flounder culture will be able to further develop and prosper along the U.S. east coast. The resulting strategic plan for "Sustainable Flounder Culture and Fisheries" (Wa-

ters 1996) provides an agenda for focused, integrated public and private investment in research, facility development, and education by recommending priority actions in six key areas:

1. Hatchery and reproduction technology
2. Production and culture systems
3. Stock enhancement
4. Economic feasibility and marketing
5. Policy and regulation
6. Education and outreach

Space does not permit going into the specific priorities identified and discussed in the document, but a copy can be obtained by writing to North Carolina Sea Grant, Box 8605, North Carolina State University, Raleigh, NC 27695-8605, USA.

This strategy is now serving as the basic justification for researchers from the states noted, as well as prospective researchers from other Sea Grant programs, to develop project proposals for support within their respective states and for such emerging projects to be linked and integrated with other flounder culture research within the overall Sea Grant network. It is the intention of the Sea Grant network to develop an inter-related sequence of research and education projects that will lead to commercial flounder culture.

**Current flounder culture within Sea Grant**

Several flounder culture studies are ongoing in the New England states, but these will only be mentioned here because they were presented in detail in other parts of the UJNR meeting agenda.

In the meeting's first presentation, George Nardi described the commercial success that his company, GreatBay Aquafarms, is having with the production of juvenile summer flounder *Paralichthys dentatus*. Much of the technology used has been derived from New Hampshire Sea Grant studies conducted several years ago.

In a later session of this meeting, speakers described recent studies at the University of New Hampshire on the effects of stocking density on larval and juvenile growth and survival in summer flounder.

In addition, researchers at the University of Rhode Island presented production economics resulting from their studies of summer flounder grow-out. The same research team is also conducting studies on larval survival and growth in the winter flounder *Pleuronectes americanus* with the ultimate goal of assisting efforts in wild stock enhancement.

Proceeding southward along the coast, the next state which is participating in this regional activity is Virginia. Discussion of flounder culture activities in Virginia is presented in a later section of this paper.

In both North Carolina and South Carolina, the species of primary interest is the southern flounder *Paralichthys lethostigma*. Later sessions of the meeting included a presentation on South Carolina studies of pond production, including controlled reproduction, tank, and pond nursery systems as well as grow-out to market size in this species. The tri-state research team for these studies includes collaborators from Rhode Island and North Carolina.

Studies in North Carolina are focusing on southern flounder reproduction, in collaboration with investigators from South Carolina, upon nursery rearing of larvae and fingerlings, and upon pond production of food-sized fish. In addition, research is underway to determine the ecological dynamics of stock enhancement using southern flounder through assessment of food habits, survival rates, release timing optimization, and habitat availability. The next generation of Sea Grant projects in North Carolina will examine the possibility of creating an all female population of fingerlings, controlling egg quality in broodstock, rearing conditions for larvae, pond production of fingerlings, and optimized grow-out conditions for producing food fish. Several of the studies noted thus far are supported with funding from sources in addition to Sea Grant, and there are non-Sea Grant flounder culture projects that are not included in this summary because their justification for funding is not tied to the Sea Grant strategic plan noted previously. However, the results of all studies, regardless of funding support, will be integrated through outreach/extension activities to assist with the commercialization of flounder culture.

**Flounder culture in Virginia**

Virginia is a relatively recent entrant to the field of flounder culture, but the reduced landings of wild-harvest flounder, increasing market demand,

and recognition of the potential to apply recirculating culture system technology to flounder has generated considerable interest in our academic and business communities.

In 1996, researchers at the Virginia Institute of Marine Science constructed recirculating systems and purchased summer flounder juveniles from GreatBay Aquafarms. Initial activity focused on system optimization and preliminary grow-out, feeding, survival, and water quality maintenance in the recirculating systems. In addition, these studies encountered significant disease outbreaks that resulted in considerable mortality among the juveniles in culture.

In 1997, the development of grow-out protocols for use in recirculating systems has continued; and, as a result of the 1996 work, an inter-institutional and inter-disciplinary team has been formed to investigate diseases that occur in cultured summer flounder. This group includes expertise in parasitic and non-infectious disease pathology, bacteriology, virology, immunology and therapeutant application, and water quality monitoring. Through Sea Grant project seed money in 1997, this group is developing a fundamental understanding of the water quality needs of summer flounder, the variety of pathogenic conditions that affect the species in culture, and the information needed for rapid and accurate diagnosis of disease conditions.

Our flounder disease group is also functioning as a focal point for disease issues that arise at other flounder culture projects within the Sea Grant network. In other words, this is emerging as a cross-cutting project not only within Virginia's flounder research, but it also provides the same function in concert with other flounder research within the Sea Grant network.

This flounder disease research and diagnostics activity will continue as a seed effort through 1999 and is expected to develop into a sequence of fully-supported Sea Grant projects as of 2000. In the meantime, others encountering flounder disease problems are invited to establish collaboration and cooperation with the Virginia Sea Grant research team. Names and addresses of the team members are readily available through either the Virginia Sea Grant College Program or the National Sea Grant College Program, NOAA, 1315 East West Highway, Silver Spring, MD 20910-3282, USA (e-mail: Jim.McVey@noaa.gov).

## LITERATURE CITED

Waters, E.B. 1996. Sustainable flounder culture and fisheries. North Carolina Sea Grant Publ. UNC-SG-96-14. 12 pp. (North Carolina State Univ., Raleigh, NC.)

# TANK AND POND NURSERY PRODUCTION OF JUVENILE SOUTHERN FLOUNDER (*PARALICHTHYS LETHOSTIGMA*)

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## ABSTRACT

In South Carolina, studies have been conducted to develop nursery techniques for southern flounder *Paralichthys lethostigma* which could be used for aquaculture development and for stock enhancement programs. Wild caught adults were tank-conditioned and spawned using hormone implants. Larvae were stocked in tanks at 6 days of age and used in a two-part study. Three diet treatments were tested which included feeding rotifers and *Artemia* nauplii with and without a commercial larval diet supplement. The second part of the study examined the effect of rearing larvae under two light intensities (low - 457 lux, high - 1362 lux). At 24°C, metamorphosis began on day 23 and was completed by day 30. Analysis of survival, size, and pigmentation data at completion of metamorphosis indicated there were no significant differences among feed treatments or light treatments. Overall survival was 33.8% and mean length was 11.5 mm TL . However, only 30% of the larvae were normally pigmented.

Development of pond nursery systems was examined during 1995-1997. Three 0.1-ha ponds at the Waddell Mariculture Center were stocked with 3 to 5-day-old larvae. Stocking densities ranged from to 284,760 to 740,000 fry/ha and fish were harvested after 2½ to 7 months. Survival was low and ranged from 3.5 to 6.1% (mean 4.5%). However, approximately 99% of the fish had normal pigmentation. Results indicated that southern flounder are tolerant of a range of environmental conditions and that pond systems may be useful in seasonal production of juveniles. Two short-term salinity tolerance tests were conducted. In a 72-h study, fish which had recently metamorphosed (13.7 mm TL, 50 days old) exhibited low survival at 0 g/L salinity (16-20%) while those exposed to 5-30 g/L had a mean survival of 99.1%. A 2-wk study using older juveniles (95.2 mm TL, 220 days old) showed that they could tolerate salinities of 0-10 g/L (100% survival). Thus, salinity tolerance increases with age. Two weaning studies were conducted with pond-produced juveniles. Younger juveniles (47 mm TL, 77 days old) were transferred to dry diets over a 2-wk period with over 80% survival while older juveniles (95 mm TL, 220 days old) required 106 days to wean and exhibited a 58% survival. Results of the various nursery trials and related studies suggest that mass production of southern flounder juveniles should be possible with refinements to current techniques.

## INTRODUCTION

Along the east and gulf coasts of the United States, there is growing interest in producing flatfishes for food and for stock enhancement (Smigielski 1975, Arnold et al. 1977, Brisbal and Bengston 1993, Daniels et al. 1996). In 1996, the National Sea Grant College Program convened a 'Task Force on Flounder Culture and Stock Enhancement.' The resulting report, which

included inputs from Sea Grant directors, scientists, extension specialists, and industry representatives, summarized the research, outreach and policy needs for successful flounder culture and possible stock enhancement of U.S. flounder stocks (Waters 1996).

The South Carolina Department of Natural Resources (SCDNR) initiated culture research on the locally occurring southern flounder *Paralichthys lethostigma* in 1994. This is a euryhaline species well known in seafood markets and in anglers' creels (Wenner et al. 1990). The southern flounder inhabits coastal waters from Albemarle Sound, North Carolina, through the south Atlantic states to Corpus Christi Pass, Texas (Ginsburg 1952). From spring through fall, southern flounder typically inhabit coastal bays, sounds, and river systems (Ginsburg 1952, Gutherz 1967) and are most abundant in the mid- to upper estuarine areas with occasional movement into freshwater (Dahlberg 1972). They prefer silt and organic mud substrates (Powell and Schwartz 1977). In South Carolina, southern flounder are commonly located in shallow tidal flats, shellbanks, and around pilings (Bearden 1961). Although some adults may remain within the estuary all year, most adults migrate offshore to spawn in late fall and winter (Ginsberg 1952). Additional information on the life history and ecology of southern flounder throughout its range is provided by Powell and Schwartz (1977, 1979), Stokes (1977), Music and Pafford (1984), and Wenner et al. (1990). The southern flounder can attain a size of 9 kg, making it the largest bothid inhabiting inshore waters along the south Atlantic and Gulf of Mexico. As such, it has substantial commercial and recreational importance. Landings data for the southern flounder are difficult to obtain as this species is commonly landed and reported with the sympatric summer flounder *P. dentatus*, and the gulf flounder *P. albigutta*. In South Carolina, there are no focused flounder fisheries. Instead, landings occur primarily associated with shrimp trawlers as bycatch with most of the fish provided to the vessel's crew as part of their wages. These fish are then sold or consumed by the crew. In South Carolina, flatfish landings are relatively low, but are almost exclusively southern flounder (J. Moran, SCDNR Fisheries Statistics Section, personal communication). During 1991-1996, landings

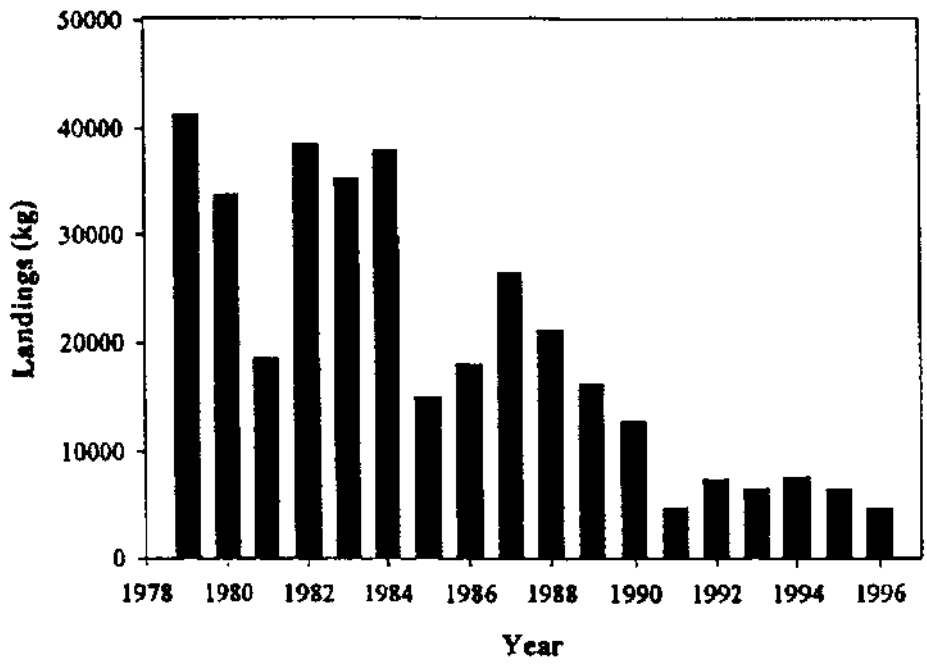


**Figure 1.** Reported landings of flounder (primarily southern flounder) in South Carolina during 1978-1996.

averaged 6,238 kg which is substantially lower than in previous years (Fig. 1). This may be due to several factors including data collection difficulties, increase in the legal minimum size (30 cm TL), and a decline in abundance.

Market prices for east coast flounders reflect seasonal abundance, with wholesale prices in the range of $3.85-5.50/kg (NMFS 1995). Retail prices in the order of $22.00/kg are received for filleted products. Due to their benthic and gregarious behavior, and resistance to handling stress, flounders appear to be excellent candidates for live sales. In fact, live east coast flounders are regularly sold to upper-scale Japanese restaurants in the Northeast where they command a premium price (~$15/kg) and are also shipped to Tokyo where they are valued at $45-60/kg (Ackerman 1997). In addition to the wild fisheries studies, there has been some culture research. Much of this research has been focused on identifying spawning techniques. Arnold et al. (1977) successfully tank-spawned 3 of 6 females using photothermal conditioning and produced 120,000 eggs with fertilization ranging from 30-50%. In another trial using carp pituitary extract, only 25,000 eggs with 80% fertility were produced from 14 females (Lasswell et al. 1978). Ten years later, a similar attempt using luteinizing hormone-releasing hormone analog (LHRHa) implants was unsuccessful, apparently due to lack of male participation (Henderson-Arzapalo et al. 1988). However, in 1995 success was obtained using gonadotropic hormone-releasing hormone analog (GnRHa implants to induce final maturation in 12 photothermally conditioned females with oocytes ≤500 µm (Berlinsky et al. 1996). These fish were manually stripped and a total of 1.6 x $10^6$ eggs (batch fertility 7-95%) was obtained. During 1997, repetitive tank-spawnings were accomplished over a 3-month period (Smith et al. unpublished). Thus, the spawning technology for wild broodstock appears to be reasonably developed and fry may be available throughout the year using photothermal manipulation of broodstock.

The next step in the culture process is development of suitable nursery techniques. Daniels et al. (1996) provided some basic information on the effects of density, light intensity, and salinity on larval growth and survival. However, overall survival was low and there was a high percentage of larvae that did not complete metamorphosis. Thus, additional research is needed to develop the culture technology necessary for mass production of juveniles. Similarly, information is lacking on weaning techniques to transfer juveniles to dry rations for rearing to market size.

Based in part on the above information, the southern flounder appears to have desirable aquaculture characteristics. Reported growth rates of wild fish in South Carolina indicate that females attain a mean size of about 0.9 kg at 2 yr of age (Wenner et al. 1990). However, small juveniles recruited to coastal impoundments containing abundant food may substantially exceed this growth rate. Fisheries data suggest that this species is regularly captured in freshwater and nearly freshwater environments and, thus, there may be opportunity to grow this species in inland and coastal sites as is done with the euryhaline hybrid striped bass (*Morone saxatilis* x *M. chrysops*) (Smith and Jenkins 1996, Smith et al. 1996). An overview of research is provided focused on the development of suitable nursery systems; results to date on tank studies and pond trials as well as information on techniques for weaning fish to formulated rations. In addition, results of short-term studies focused on elucidating the salinity tolerances of small juveniles are included.

## MATERIALS AND METHODS

### General

Larvae used during the various nursery trials were obtained from captive wild adults which had been photothermally conditioned for at least 6 months prior to spawning. Spawning was delayed until March by holding broodstock at a constant temperature of 17°C, and photoperiod of 12 h light. This allowed zooplankton blooms consisting primarily of rotifers (~2,200/L) to be developed in the outdoor ponds. Females were induced to spawn using GnRHa implants. For each study, eggs were obtained from a minimum of two females and fertilized with milt from several males. Eggs were obtained by either stripping or tank-spawning and incubated in 30-34 g/L salinity and at a temperature of 16-20°C. Hatching occurred in 36-40 h and the

fry were held for 3-6 days, until the eyes were pigmented and the mouthparts fully formed, before stocking in the nursery trials. At this time, larvae were a mean size of 2-3 mm TL.

As appropriate, data were statistically analyzed using parametric (ANOVA and T-Test) and non-parametric tests (Kruskall-Wallis One Way Analysis of Ranks, Mann-Whitney Rank Sum Test). Dunn's method was used to identify specific differences among more than two groups. Percent data were normalized using the arcsin transformation before analysis. Significance was accepted at $P \leq 0.05$.

**Pond Nursery**

During 1995-1997, three 0.1-ha ponds at the SCDNR's Waddell Mariculture Center (WMC) were stocked with fry. The ponds were lined with 30 mil high-density polyethylene and the bottoms covered with 20 cm of native soils. Ponds were sloped from a minimum depth of 1 m to a maximum depth of 2 m. The ponds contained a 4.9-m-long x 1.5-m-wide sloped harvest basin to assist in harvesting fish. Water, either saline or brackish, was supplied at the shallow end of the pond with an additional water valve located above the harvest basin. During the studies, ponds were filled with filtered (500 µm) saltwater from the adjacent Colleton River. At harvest, water of low oxygen content and containing a high silt load occurred in the catch basin near the end of pond drainage. To reduce stress and mortalities, clean and highly oxygenated water was added to the basin during harvesting. Fish were stocked near the end of March during each trial and harvested 2½ and 7 months later. Stocking density ranged from 284,760-740,000 larvae/ha based on volumetric estimation. Water quality was measured regularly during trial 1 and less frequently during the other trials. To reduce risk of low oxygen concentrations and temperature stratification, a 0.75-kw paddlewheel aerator was run continuously. After approximately 1 month, supplemental feed consisting of sinking salmon starter (48% protein) was added 6 days/wk during trial 1. Feed size was gradually increased, and beginning on 5 July a 2.4-mm pellet (38% protein) was fed (Jenkins et al. 1997). In trials 2 and 3, no supplemental feed was provided. Fish were first sampled using a plankton sled and then with a seine as they grew larger. At harvest, fish were dip-netted from the harvest basin and placed into tanks containing clean seawater. Survival was based on an individual count of the fish harvested while a subsample was used to determine fish size.

**Tank Nursery**

A tank nursery study was conducted during 1996 to evaluate the effect of three larval diets and two light intensities on growth, survival, and pigmentation (Denson and Smith 1997). Cylindrical 70-L black fiberglass tanks containing center standpipes and filled with 1-µm filtered water at 23°C and 34-35 g/L salinity (combination of Charleston Harbor water and evaporated sea salts) served as the experimental culture units. Tanks were filled with 16 L of water initially, with the volume increased to 40 L when *Artemia* nauplii were added as food. The diet treatments consisted of: treatment 1 - rotifers (*Brachionus plicatilis*) fed at a concentration of 10/ml during days 1-9, and *Artemia* (3/ml) fed days 7 through metamorphosis; treatment 2 - rotifers fed days 1 through metamorphosis with *Artemia* added beginning day 7; treatment 3 - rotifers fed days 1-9, and *Artemia* and an artificial larval feed (Larva "Z" Plus, Zeigler Brothers Inc., Gardeners, PA) fed days 7 through metamorphosis. Rotifers were fed a diet of algae (*Isochrysis tahiti*) and a commercial highly unsaturated fatty acid (HUFA) enriched feeding product (Culture Selco, Artemia Systems Inc., Baasrode, Belgium) before being placed in the larval rearing tanks. Lighting was continuous using overhead fluorescent lights (215 W cool white high output bulbs). Light intensity was measured at the surface of each tank and the lowest and highest light readings were averaged to provide a combined tank value. Two treatments were examined: high light - 1362 lux, and low light - 457 lux. At initiation of the study, each tank received 200 individually counted larvae, and four replicates per treatment were utilized in the experimental design. The study was completed on day 30 and all post-metamorphic fish were hand-counted, measured, and pigmentation characteristics categorized based on a subjective key modified from Seikai (1985a) (Denson and Smith 1997). Water quality was monitored every two days in each tank

and adjustments made as necessary. In-tank sponge filters were used to provide biological and particulate filtration.

**Salinity Tolerance**

Two short-term salinity tolerance studies were conducted. The first study utilized recently metamorphosed juveniles produced from the tank study while the second study examined the tolerance of larger, 6-month-old juveniles. These larger fish were obtained from the 1995 pond nursery trial. During both studies, fish were inspected several times daily and dead fish were removed when observed. Lighting was continuous using overhead fluorescent lights. Water quality was monitored at the beginning and end of study 1, and daily during study 2. Dechlorinated tap water (0 g/L salinity; mean alkalinity 45 mg/L; hardness, 66 mg/L ($CaCO_3$ equivalent); pH 7.5) was added several times during the studies to adjust for evaporative loss and to maintain salinities at appropriate treatment levels.

In the first study, the recently metamorphosed juveniles (13.7 ± 2.6 mm TL, 50 days old) were stocked into 1-L glass bowls filled with 30 g/L seawater. Each treatment was replicated in three bowls each containing 10-14 juveniles which were acclimated to seven different salinity levels: 0, 5, 10, 15, 20, 25, and 30 g/L (control). Fish were acclimated to treatment salinities by diluting the Charleston Harbor seawater with dechlorinated tap water at a rate of 5 g/L/h. In the 0 g/L treatment, water was completely replaced with dechlorinated tap water after acclimation to near 0 g/L using dilutions of seawater. Total acclimation time for the 30 to 0 g/L treatment was 6 h. Additionally, as a separate treatment, flounder in three bowls were converted to 0 g/L without acclimation to examine the influence of acclimation time. Juveniles in all treatments were fed newly hatched to 24-h-old *Artemia* nauplii (400 µm) stocked at a density of 5/ml. The study was conducted for 72 h after acclimation was completed.

Study 2 evaluated the effects of four salinity levels on survival and feeding behavior over a 2-wk period. Five juveniles, mean size 95.2 ± 14.0 mm TL, 7.4 ± 3.6 g, 220 days old, were stocked into three replicate 70-L black cylindrical fiberglass tanks (45 cm diameter x 45 cm deep with rounded bottom) fitted with center standpipes. All three replicates of each treatment were connected to a recirculating system which continuously recirculated filtered water to the tanks. Tanks were filled with 28 g/L Charleston Harbor seawater and the juveniles acclimated to one of four salinity treatments (0, 1, 5, and 10 g/L) using dechlorinated tap water. Juveniles were fed live mosquitofish *Gambusia sp.* to satiation daily.

**Transition Diets**

Two studies were conducted to wean fish from live feeds to commercially available dry rations. Fish used in the trials were obtained from the pond nursery studies. After harvesting, fish were placed in clean water and transported to the Marine Resources Research Institute. Fish were measured, counted, and placed in 1.8-m-diameter x 0.8-m-deep cylindrical tanks fitted with center drains and equipped with screened standpipes which drew water from throughout the water column. Water was recirculated through a biological filter and supplied to the tanks at a rate of 20 tank volumes/day. Water was aerated using four air stones spaced around the perimeter of the tank. Based on data collected from the salinity tolerance studies and the desire to reduce the potential for infestation with the dinoflagellate *Amyloodinium sp.*, fish were reared at 3-10 g/L salinity using a combination of dechlorinated tap water (freshwater), low salinity well water, and settled seawater from the Charleston Harbor estuary.

Water temperature was recorded daily while other water quality parameters were measured weekly. Fish were weighed several times during trial 1 (study duration 103 days) and at beginning and end of trial 2 (27 days duration). Fish were fed dry feeds (salmon starter crumbles #2-#3, Zeigler Brothers, Gardeners, PA) at 1 1/2-h intervals 24 h/day using overhead automatic feeders (Sweeney Enterprises Inc., Boerne, TX). The live feeds and previously frozen feeds were provided 2-3 times/day during normal working hours (0830 - 1700 h). When feeding a mixture containing dry feeds, the amount of natural feed was gradually reduced during each feeding period, while the amount of dry feed was increased. Once fish had been conditioned to exclusively accept dry feeds,

the weaning experiments were terminated.

In trial 1 (1995), 200 fish (mean size 8.3 g and 96 mm TL, 220 days old) were stocked in each of two replicate tanks at a density of 77 fish/m$^2$ of tank bottom. Fish were fed as follows: live and dead grass shrimp *Palaemonetes* sp. during days 0-15; chopped mullet *Mugil cephalus* and spot *Leiostomus xanthurus* during days 16-52; #2 salmon starter (50% protein) mixed with refined menhaden *Brevoortia tyrannus* oil and chopped fish during days 53-78; #3 salmon starter and refined menhaden oil during days 79-105; and dry feed only beginning day 106.

In trial 2 (1997), 400 fish (mean size 1.2 g, 46.7 mm TL, 77 days old) were stocked in each of the two replicate tanks at a density of 154 fish/m$^2$. Fish were fed as follows: ground Atlantic mackerel *Scomber scombrus*, frozen blood worms *Tubifex* sp., and frozen adult *Artemia*, days 0-8; #1 and #2 salmon starter and emulsified menhaden, days 9-13; and dry feed only, day 14.

## RESULTS

### Pond Nursery

During harvesting of trial 1, the pond was drained rapidly as is done with other fish species. Unfortunately, most of the juvenile flounder became stranded, burrowed into the soft pond bottom, and had to be individually harvested by hand. During trials 2 and 3, ponds were rapidly drained to near exposure of the pond bottom. Then, during the night, the remaining water was very slowly drained which allowed the small flounders to move with the water and become concentrated in the harvest basins. Nighttime draining eliminated bird predation problems and water temperature increases due to solar heating. Collection of the fish in the basin made harvesting more efficient although removing fish from the bottom of the basin was still difficult.

Water quality recorded during the trials appeared satisfactory although temperatures on some days in July and August during trial 1 exceeded 31°C (Table 1). Fish reared for 2½ months (trials 2 and 3) were about half the length of those reared for 7 months (Table 2). Survival was low (mean 4.5, range 3.5-6.1%) during each trial (Table 2). Examination of pigmentation pattern indicated that about 99% of the harvested fish were normally pigmented.

### Tank Nursery

No significant differences in water quality were detected among tanks. Mean water quality values were as follows: temperature 22.9°C; salinity 35.7 g/L; dissolved oxygen 6.6 mg/L; pH 7.6; and total ammonia nitrogen 0.8 mg/L. Larvae began actively feeding on rotifers at age 6 days post-hatch (dph) and on *Artemia* nauplii at age 14 dph. On study day 17 (23 dph), mean size was 8.2 ± 0.6 mm TL. At that time, complete metamorphosis and a variety of transition stages were observed among fish in all treatments. By day 30, 98% of the animals had completed metamorphosis. There were no significant differences detected among survival levels between feed treatments (mean 37.5 ± 15.5%) or light treatments (mean 26.4 ± 12.8%) (Denson and Smith 1997). Similarly, there were no differences in total length (overall mean 11.5 ± 1.3 mm) among fish in any treatments (Denson and Smith 1997).

Body pigmentation was highly variable. On day 30, no significant differences in pigmentation were detected among the three feed treatments, but on average only 29.7% of the population had normal pigmentation. Rechecking pigmentation patterns a week later indicated that there had not been any significant change. On day 30, there was no difference in the degree of albinism among fish reared in high and low light conditions, but again there was only a low percentage of normally pigmented fish. Subsequent to the study, the light intensity in the low light treatment was increased to high light conditions on day 30. On day 37, there was statistically more normally pigmented (34.5% vs. 13.3%) animals than on day 30. No change in pigmentation was observed among fish in the high light treatments which were held at high light intensity for an additional week (24.5% on day 30 vs 23.0% on day 37) (Denson and Smith 1997).

### Salinity Tolerance

In study 1, no differences were observed in water quality between treatments. Mean water temperature was 25.2°C, pH was 7.5, and total ammonia nitrogen was within acceptable limits (≤ 2 mg/L). Some fish in each of the treatments, including the non-acclimated 0 g/L

| Month | Temp. (C) Mean | Temp. (C) Range | Oxygen (mg/L) Mean | Oxygen (mg/L) Range | pH Mean | pH Range | Salinity (g/L) Mean | Salinity (g/L) Range |
|---|---|---|---|---|---|---|---|---|
| | | | | TRIAL 1 | | | | |
| March 1995 | 19.0 | 17.4-20.2 | 7.9 | 7.2-9.0 | 8.7 | 8.6-8.8 | 22.0 | 20.0-24.0 |
| April 1995 | 20.8 | 15.8-25.0 | 6.8 | 5.6-8.6 | 8.5 | 8.2-9.0 | 24.7 | 23.0-26.0 |
| May 1995 | 24.8 | 21.4-27.4 | 5.3 | 4.2-6.7 | 8.5 | 8.2-8.8 | 27.7 | 26.0-30.0 |
| June 1995 | 26.8 | 24.5-29.9 | 4.9 | 3.4-6.2 | 8.2 | 8.1-8.3 | 25.0 | 24.0-26.0 |
| July 1995 | 29.7 | 27.2-31.3 | 4.4 | 3.4-5.8 | 8.4 | 8.2-8.5 | 25.0 | |
| August 1995 | 28.8 | 22.7-31.9 | 4.4 | 3.5-6.0 | | | | |
| September 1995 | 25.4 | 30.9-28.2 | 5.1 | 6.2-6.2 | | | | |
| October 1995 | 24.7 | 23.8-25.7 | 6.2 | 5.6-6.7 | | | | |
| | | | | TRIAL 2 | | | | |
| March 1997 | 21.0 | | 7.2 | | | | 30.0 | |
| April 1997 | 20.5 | 18.4-22.5 | 7.3 | 6.2-9.9 | 8.7 | | 33.0 | |
| May 1997 | 23.8 | 22.6-26.0 | 6.2 | 5.7-7.1 | | | 26.0 | |
| June 1997 | 21.0 | | 7.1 | | | | 28.0 | |
| | | | | TRIAL 3 | | | | |
| March 1997 | 20.9 | | 7.0 | | | | 30.0 | |
| April 1997 | 20.4 | 18.2-22.3 | 6.9 | 5.6-9.1 | 8.5 | | 33.0 | |
| May 1997 | 23.9 | 22.5-26.0 | 6.1 | 5.5-6.9 | | | 26.0 | |
| June 1997 | 21.0 | | 7.1 | | | | 28.0 | |

**Table 1.** Water quality parameters monitored during pond nursery trails with southern flounder. Data without ranges indicate only one observation was made.

| Trial | STOCKING Date (d/m/y) | STOCKING Density (no./ha) | STOCKING Fish size (TL mm) | Duration (d) | PRODUCTION Fish size (TL mm) | PRODUCTION Fish size (wt. g) | PRODUCTION Survival (no./ha) | PRODUCTION Survival (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 22/03/95 | 284,760 | 2.0±0.1 | 217 | 96.3±13.5 | 8.3±4.4 | 17,460 | 6.1 |
| 2 | 28/03/97 | 740,000 | 2.0±0.1 | 74 | 55.5±8.0 | 1.9±0.9 | 30,020 | 4.0 |
| 3 | 28/03/97 | 740,000 | 2.0±0.1 | 74 | 36.6±6.8 | 0.5±0.2 | 25,850 | 3.5 |

**Table 2.** Stocking and production data for pond nursery trials with southern flounder.

treatment, were observed feeding on *Artemia* immediately after they were added to the culture containers. Within 24 h, some juvenile flounder began to die (25%) in the non-acclimated 0 g/L salinity treatment. At this time, only one fish in one replicate had died in the acclimated 0 g/L treatment. At the conclusion of the 72-h study, there was significantly higher mortality in the 0 g/L acclimated (80%) and non-acclimated (84%) treatments ($p=0.007$) than in the higher salinities (Table 3). Mean overall survival in 5-30 g/L salinity treatments was 99.1% and no differences were detected (Table 3).

In study 2, no mortalities were recorded during the 2-wk study period. No significant differences were detected between fish total length (mean 102.5 ± 14.6 mm TL) (Table 4).

## Transition Diets

In trial 1, fish would not consume pelleted feeds initially even though large amounts of feed had been provided to the nursery pond for 6 months

| Treatment salinity (g/L) | Survival (%) |
|---|---|
| 0[1] | 16.7 A |
| 0 | 20.0 A |
| 5 | 97.0 B |
| 10 | 100.0 B |
| 15 | 100.0 B |
| 20 | 97.3 B |
| 25 | 100.0 B |
| 30 (control) | 100.0 B |

[1]Fish were not acclimated but placed directly from 30 g/L water into 0 g/L dechlorinated tap water

**Table 3.** Mean survival of recently metamorphosed southern flounder (13.7 ± 2.6 mm TL) in a 72-h salinity tolerance test. Values followed by the same letter are not significantly different.

prior to harvest. Population sampling on day 15 indicated that very little growth had occurred while fish were being fed grass shrimp (Table 5). During the next period, fish readily consumed chopped fish and increased to a mean weight of 11.9 g by day 36. This trend continued during the period with fish attaining a mean size of 15 g and 116 mm TL on day 53 (Table 5). Chopped fish was completely removed from the diet on day 79. The fish were not weaned from the menhaden oil and salmon starter combination until day 106 at a size of 28.6 g and 140 mm TL. Survival during this 106-day weaning period averaged 58.2%. As in the previous trial, fish would not consume dry feeds initially in trial 2. However, no live feeds were provided in trial 2. Instead, fish were provided a combination diet of ground mackerel, adult *Artemia*, and blood worms which they readily consumed. On day 9, this combination diet was replaced with a mixture of pelleted feed and emulsified menhaden. Fish ate this diet and were weaned to the dry salmon starter diet by day 14. At sampling on day 27, survival averaged 80.4% with fish more than doubling in size to 2.6 g and 64 mm TL (Table 5).

## DISCUSSION

Intensively managed tank nursery systems are typically used to produce flatfishes in Japan and Europe (Fukusho et al. 1985, Holmefjord et al. 1993, Minkhoff and Broadhurst 1994). Such systems have a number of advantages including the ability to strictly manage water quality and feeding regimes and to produce juveniles year around (Table 6). However, they are more costly to construct and require more skilled labor. Also, fish produced in such systems may have varying

| Salinity treatment (g/L) | Fish size (mm TL) Initial | Final | Survival (%) |
|---|---|---|---|
| 0 | 91.1 ± 12.4A | 96.5 ± 13.1A | 100 |
| 1 | 96.5 ± 15.8A | 103.4 ± 17.5A | 100 |
| 5 | 96.4 ± 13.8A | 104.0 ± 14.7A | 100 |
| 10 | 96.6 ± 14.6A | 106.1 ± 12.3A | 100 |

**Table 4.** Mean total length (± SD) of southern flounder juveniles in 14-day salinity study. Values in columns followed by the same letter are not significantly different.

| Day # | Mean size Wt. (g) | Mean size TL (mm) | Diet | Survival (%) |
|---|---|---|---|---|
| | | | TRIAL 1 | |
| 0 | 8.3 | 96 | live and dead grass shrimp | 100.0 |
| 16 | 8.7 | 99 | chopped mullet + spot | |
| 52 | 15.0 | 116 | chopped mullet + spot + menhaden oil + #2 salmon starter | |
| 79 | 20.6 | 133 | menhaden oil + #3 salmon starter | |
| 106 | 28.6 | 140 | #4 salmon starter | 58.2 |
| | | | TRIAL 2 | |
| 0 | 1.2 | 47 | dead bloodworms + adult *Artemia* + ground mackerel | 100.0 |
| 9 | | | emulsified menhaden + #1 and #2 salmon starter | |
| 14 | | | #2 salmon starter | |
| 27 | 2.6 | 64 | #2 salmon starter | 80.4 |

**Table 5.** Data for weaning trials with juvenile southern flounder.

| | Advantage | Disadvantage |
|---|---|---|
| Tank culture | High control of rearing environment<br>Rapid assessment of growth, survival and health status<br>Ability to provide specific feeds of known nutrient level<br>Production independent of external ambient conditions<br>More predictive production levels<br>Can produce all year | Requires daily maintenance and monitoring<br>Requires intensive culture of food items<br>Requires more costly facilities and labor |
| Pond Culture | Lower initial investment<br>Utilizes natural foods<br>Requires low daily maintenance<br>Reduced skilled labor requirements<br>Reduced susceptibility to mechanical failures | Requires coastal sites<br>Production subject to variations in zooplankton<br>Production subject to ambient conditions<br>Production seasonably limited |

**Table 6.** Characteristics of intensive tank and extensive pond nursery systems for production of juvenile southern flounder.

degrees of pigmentation abnormalities which make them less desirable for sale as a live product. In contrast, pond nursery systems are common in the southern United States for a number of species including channel catfish *Ictalurus punctatus* (Tucker and Robinson 1990), striped bass and its hybrids (Harrell et al. 1990), and red drum *Sciaenops ocellatus* (McCarty et al. 1986). Pond systems allow less control of the rearing environment but are cheaper to develop and manage. However, production is seasonally limited and fish must be converted to dry feeds after harvest (Table 6).

Development of nursery systems for southern flounder is still in the early stages but results to date indicate that juveniles can be produced in tank and pond systems. The tank study showed that they can be reared on a rotifer/*Artemia* diet with a survival level of about 37%. This is an improvement over that reported by Daniels et al. (1996). However, a high incidence of pigmentation abnormalities occurred in our study (~70%) as well as among juveniles (30-40%) produced by Daniels et al. (1996). In contrast to our tank culture results, production of juveniles in ponds was low (mean survival 4.5%) but so was the incidence of pigmentation abnormalities (~1%). Albinism in intensively reared flatfishes is a common problem. The cause appears to be complex and may be related to environmental and nutritional factors (Seikai 1985 a,b, Støttrup and Attramadal 1992, Seikai and Matsumoto 1994, Spedicato et al. 1994).

Refinements to pond and tank nursery systems over the next several years should result in improved production levels and production of more normally pigmented fish. Pond nursery research focused on improved management of zooplankton populations should improve survival levels. Better cleaning of the harvest basin prior to harvest and incorporation of a different harvesting technique (e.g., fish pump) may increase efficiency of fish removal and minimize stress and injury. Tank nursery research on identification of suitable environmental and nutritional parameters should result in higher production levels of normally pigmented fish.

Results of studies on the salinity tolerance of southern flounder indicated that this species is euryhaline. However, recently metamorphosed juveniles (50 days old) appear less tolerant to freshwater conditions than older (220-day-old) juveniles which exhibited 100% survival at salinities ranging from 0 to 10 g/L. Similarly, Daniels et al. (1996) reported 0% survival for larvae undergoing metamorphosis at 0 g/L but 59% survival at 20 g/L. Longer term studies will be required to further define the salinity requirements of different life stages and especially the effects on growth and survival rates. Mortality of southern flounder due to infestations of *Amyloodinium sp.* and *Argulus sp.* have been noted in our laboratory in high salinity culture tanks. Rearing of flounder in low salinity to freshwater conditions may avoid similar problems with these parasites. Additionally, if this species can be successfully grown on non-coastal sites, substantial saving in land costs will occur.

Results of our weaning studies suggest that it may be easier to train young juveniles (77 days old) to accept dry diets, than older juveniles (220 days old). The older fish required 106 days to transfer to dry diets as compared to 14 days for the young fish. Additional research should focus on improvement of weaning techniques and identification of practical diets suitable for producing food-size fish and cultured broodstock.

In summary, studies to date indicate that the southern flounder is a suitable candidate for culture. However, additional research is needed to identify the performance of various life stages in different rearing systems and to identify optimal and acceptable environmental conditions and satisfactory diets. Such research will need to be coupled with marketing studies to determine the economic potential for southern flounder aquaculture.

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## LITERATURE CITED

Ackerman, J. 1997. Recreating the bounty of the sea. Boston Sunday Globe, March 6, 1997, section F, pp. 1, 4. Boston, MA.

Arnold, C.R., W.H. Bailey, T.D. Williams, J. Johnson, and J.L. Lasswell. 1977. Laboratory spawning and larval rearing of red drum and southern flounder. Southeast. Assoc. Fish Wildl. Agencies 31: 437-440.

Bearden, C.M. 1961. Common marine fishes of South Carolina. Contrib. Bears Bluff Lab. No. 34, 47 p.

Berlinsky, D.L., W. King, T.I.J. Smith, R.D. Hamilton, J. Holloway, Jr., and C.V. Sullivan. 1996. Induced ovulation of southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. J. World Aquacult. Soc. 27(2): 143-152.

Brisbal, G.A. and D.A. Bengston. 1993. Reversed asymmetry in laboratory reared summer flounder. Prog. Fish-Cult. 55: 106-108.

Dahlberg, M.D. 1972. An ecological study of Georgia coast fishes. Fish. Bull., U.S. 70:323-353.

Daniels, H.V., D.L. Berlinsky, R.G. Hodson, and C.V. Sullivan. 1996. Effects of stocking density, salinity, and light intensity on growth and survival of southern flounder (*Paralichthys lethostigma*) larvae. J. World Aquacult. Soc. 27(2): 153-159.

Denson, M.R. and T.I.J. Smith. 1997. Effects of diet and light intensity on survival, growth and pigmentation of southern flounder (*Paralichthys lethostigma*). J. World Aquacult. Soc. 28(4): 366-373.

Fukusho, W., M. Okauchi, H. Tanaka, S.I. Wahyuni, P. Kraisingdecha, and T. Watanabe. 1985. Food value of a rotifer *Brachionus plicatilis*, cultured with *Tetraselmis tetrathele* for larvae of a flounder *Paralichthys olivaceus*. Bull. Natl. Res. Inst. Aquacult. No. 7: 29-36.

Ginsburg, I. 1952. Flounders of the genus *Paralichthys* and related genera in American waters. U.S. Fish. Wildl. Serv., Fish. Bull. 71: 267-351.

Gutherz, E. J. 1967. Field guide to the flatfishes of the family Bothidae in the western North Atlantic. U.S. Fish. Wildl. Serv., Bur. Commer. Fish., Circ. 263, 17 p.

Harrell, R.M., J.H. Kerby, and R.V. Minton. 1990. Culture and propagation of striped bass and its hybrids. Striped Bass Committee, Southern Div., American Fisheries Society, Bethesda, MD, USA. 323 p.

Henderson-Arzapalo, A., R.L. Colura, and A.F. Maciorowski. 1988. Temperature and photoperiod induced maturation of southern flounder. Management Data Ser. No. 154, Texas Parks and Wildlife Dept., Austin, TX.

Holmefjord, I., J. Gulbrandson, I. Lein, T. Refstie, P. Leger, T. Harboe, I. Huse, P. Sorgeloos, S. Boola, Y. Olsen, K.I. Reitan, O. Vadstein, G. Oiem, and A. Danielsberg. 1993. An intensive approach to Atlantic halibut fry production. J. World Aquacult. Soc. 24(2): 275-284.

Jenkins, W.E., T.I.J. Smith, C.V. Sullivan, and D.L. Berlinsky. 1997. Production of southern flounder (*Paralichthys lethostigma*) juveniles in an outdoor nursery pond. J. World Aquacult. Soc. 28(2): 211-214.

Lasswell, J.L., B.W. Lyons, and W.H. Bailey. 1978. Hormone-induced spawning of southern flounder. Prog. Fish-Cult. 40: 154.

McCarty, C.E., J.G. Geiger, L.N. Sturmer, B.A. Gregg, and W.P. Rutledge. 1986. Marine finfish culture in Texas: a model for the future, pp. 249-262. *In*: R.H. Stroud (ed.), Fish Culture in Fisheries Management. American Fisheries Society, Bethesda, MD.

Minkhoff, G. and A.P. Broadhurst. 1994. Intensive production of turbot, *Scophthalmus maximus*, fry, pp. 14-31. *In*: P. Lavens and R.A.M. Remmerswaal (eds.), Turbot Culture: Problems and Prospects.

European Aquaculture Society, Oostende, Belgium.

Music, J.L. and J.M. Pafford. 1984. Population dynamics and life history aspects of major marine sportfishes in Georgia's coastal waters. Ga. Dept. Natur. Resour., Contrib. Ser. No. 38, 382 p.

National Marine Fisheries Service. 1995. New York Fulton fish market primary wholesale selling prices. Fishery Market News, January to October, New York, NY.

Powell, A.B. and F.J. Schwartz. 1977. Distribution of paralichthid flounders (Bothidae:*Paralichthys)* in North Carolina estuaries. Chesapeake Sci. 18: 334-339.

Powell, A.B. and F.J. Schwartz. 1979. Food of *Paralichthys dentatus* and *P. lethostigma* (Pisces:Bothidae) in North Carolina estuaries. Estuaries 2: 276-279.

Seikai, T. 1985a. Influence of feeding periods of Brazilian *Artemia* during larval development of hatchery-reared flounder *Paralichthys olivaceus*. Bull. Jpn. Soc. Sci. Fish. (4): 521-527.

Seikai, T. 1985b. Reduction in occurrence of albinism in juvenile flounder *Paralichthys olivaceus* hatchery reared on wild zooplankton. Bull. Jpn. Soc. Sci. Fish. 51(8): 1261-1267.

Seikai, T. and J. Matsumoto. 1994. Mechanism of pseudoalbinism in flatfish: an association between pigment cells and skin differentiation. J. World Aquacult. Soc. 25(1): 78-85.

Smigielski, A.S. 1975. Hormone-induced spawning of the summer flounder and rearing of larvae in the laboratory. Prog. Fish-Cult. 37(1): 3-8.

Smith, T.I.J. and W.E. Jenkins. 1996. Regional development of hybrid striped bass aquaculture in the southeastern United States, pp. 175-179. *In:* T. G. Heggbergett, J. G. Woiwode, and R .J. Wolotira Jr. (eds.), The Role of Aquaculture in World Fisheries (Theme 6) . Proceedings World Fisheries Conference. Oxford & IBH Publ. Co. Pvt. Ltd., New Delhi, India.

Smith, T.I.J., W.E. Jenkins, J.M. Whetstone, and A.D. Stokes. 1996. A guide to pond culture of hybrid striped bass. South Carolina Sea Grant Extension Program, Charleston, SC. 41 p.

Spedicato, M.T., G. Lembo, and P. Trotta. 1994. Larval and post larval rearing of *Paralichthys olivaceus* (Temminck and Schlegel): the first experience in Italy, pp. 292-301. *In:* P. Lavens and R.A.M. Remmerswaal ( eds.), Turbot Culture: Problems and Prospects. European Aquaculture Society, Oostende, Belgium.

Stokes, G.M. 1977. Life history studies of southern flounder (*Paralichthys lethostigma*) and gulf flounder (*P. albigutta*) in the Aransas Bay area of Texas. Texas Parks Wildl. Dept., Tech. Ser. No. 25, 37 p.

Støttrup, J.G. and Y. Attramadal. 1992. The influence of different rotifer and *Artemia* enriched diets on growth, survival and pigmentation in turbot (*Scophthalmus maximus* L.) larvae. J. World Aquacult. Soc. 23(4): 307-316. Tucker, C.S. and E.W. Robinson. 1990. Channel Catfish Farming Handbook. Van Nostrand Reinhold, New York, NY. 454 p.

Waters, E.B. 1996. Sustainable flounder culture and fisheries. North Carolina Sea Grant Publ. UN-SG-96-14, Raleigh, NC. 12 p.

Wenner, C.A., W.A. Roumillat, J.E. Moran, Jr., M.B. Maddox, L.B. Daniel, III, and J.W. Smith. 1990. Investigations on the life history and population dynamics of marine recreational fishes in South Carolina: part I, southern flounder *Paralichthys lethostigma*. Report to USFWS, Atlanta, GA, for Project F-37, pp. 21-35.

# LICENSING AND REGULATION OF VETERINARY BIOLOGICS FOR FISH IN THE UNITED STATES

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## ABSTRACT

The U.S. Department of Agriculture (USDA) regulates biologics for fish, including vaccines, bacterins, and diagnostic test kits, produced in, imported into, or exported from the United States. The regulatory process is designed to ensure that biologics under USDA jurisdiction are not contaminated, worthless, dangerous, or harmful. The Animal and Plant Health Inspection Service (APHIS), an agency within the USDA, licenses and inspects biologics production facilities, and licenses and tests veterinary biological products. Veterinary biological products should be pure, safe, potent, and efficacious. A biologics-producing firm located in the United States may sell its products provided the firm possesses a valid U.S. Veterinary Biological Product License for each product produced for sale, as well as a valid U.S. Veterinary Biologics Establishment License. A permittee (i.e., the legal representative in the United States of a biologics-producing firm located outside the United States) may import biologics into the United States provided the permittee possesses a valid U.S. Veterinary Biological Product Permit. Biologics available in the United States for fish are manufactured by Alpharma NW Inc., Aqua Health Ltd., and DiagXotics. Monovalent and multi-faction bacterins (i.e., antigenic suspensions of inactivated bacterial organisms) are available for the vaccination of fish to aid in the prevention of furunculosis caused by *Aeromonas salmonicida*, enteric septicemia of catfish caused by *Edwardsiella ictaluri*, columnaris disease caused by *Flavobacterium columnare*, vibriosis caused by *Vibrio anguillarum* and *V. ordalii*, cold water vibriosis caused by *V. salmoninarum*, and enteric redmouth diseases caused by *Yersinia ruckerii*. Qualitative and quantitative test kits to diagnose the presence of the bacterial kidney disease antigen *Renibacterium salmoninarum* in fish are also available in the United States. A bacterin recommended as an aid in the prevention of winter ulcers caused by *V. viscosus* is produced in the United States for export only.

## INTRODUCTION

The U.S. Department of Agriculture (USDA) regulates biologics for fish produced in, imported into, transported through, or exported from the United States. Veterinary biologics include viruses, serums, toxins, and analogous products (e.g., vaccines, bacterins, allergens, antibodies, antitoxins, toxoids, immunostimulants, cytokines, etc.) which act through an immune mechanism to prevent, diagnose, manage, or cure disease of animals.

## DISCUSSION

Biologics currently available for fish in the Unites States include bacterins and diagnostic test kits. Bacterins (i.e., an antigenic suspension of inactivated bacterial organisms) are used for the vaccination of fish as an aid in the prevention of furunculosis caused by *Aeromonas salmonicida*, enteric septicemia of catfish caused by *Edwardsiella ictaluri*, columnaris disease caused by *Flavobacterium columnare*, vibriosis caused by *Vibrio anguillarum* and *Vibrio ordalii*, cold water vibriosis caused by *Vibrio salmoninarum*,

and enteric redmouth diseases caused by *Yersinia ruckerii*. Qualitative and quantitative test kits to diagnose the presence of the bacterial kidney disease antigen *Renibacterium salmoninarum* in fish are also available in the United States. A bacterin recommended as an aid in the prevention of winter ulcers caused by *Vibrio viscosus* is produced in the United States for export only. Depending on the specific biological product being considered, bacterins may contain a single fraction or multiple fractions, and may be recommended for administration to fish by immersion, by injection, or by ingestion.

The regulatory process is designed to ensure that all biologics under USDA jurisdiction are pure, safe, potent, and efficacious, and not worthless, contaminated, dangerous, or harmful. The Animal and Plant Health Inspection Service (APHIS), an agency within the USDA, licenses and inspects biologics production facilities, and licenses and tests biologics produced in licensed biologics-manufacturing facilities.

The Center for Veterinary Biologics (CVB) is the veterinary biologics regulatory program within APHIS and is composed of three units with defined functions. The Licensing and Policy Development (CVB-LPD) unit establishes licensing standards and policy; reviews prelicense documentation; reviews test methods, outlines of production and labels; and issues, suspends, or revokes licenses and permits. The Inspection and Compliance (CVB-IC) unit inspects production facilities, methods, and records; releases serials (lots or batches) of biologics for distribution in the marketplace; performs post-release product surveillance; and investigates suspected law violators and consumer complaints. The Laboratory (CVB-L) unit develops test methods, standards, and reagents; performs prelicense, surveillance, and field problem testing; and trains personnel from other laboratories.

The authorities and procedures for the regulation of biologics are defined in a variety of published documents, including the Virus-Serum-Toxin Act of 1913 (amended in 1985), Title 9 of the Code of Federal Regulations, Veterinary Biologics Memorandums, Veterinary Biologics Notices, Veterinary Biologics General Licensing Considerations, and Supplemental Assay Methods. Further sources of information and guidance include: the semi-annual Veterinary Biological Products publication listing the licensees, permittees, and veterinary biologics produced, the CVB internet home page, and APHIS-sponsored public meetings.

Veterinary biologics eligible for distribution and sale in the United States may be manufactured in facilities located either in the United States or abroad. In order to sell a veterinary biologic in the United States, a biologics manufacturer located in the United States must possess two types of Federal licenses: a U.S. Veterinary Biologics Establishment License for the production facility, and a separate U.S. Veterinary Biological Product License for each biological product. In order to import from abroad and sell a veterinary biological product in the United States, a foreign veterinary biologics manufacturer's legal representative (permittee) in the United States must possess a U.S. Veterinary Biological Product Permit for the biologic(s) to be imported. With only minor differences, the licensing process for domestically produced veterinary biologics is the same as the permitting process for veterinary biologics imported from abroad.

The applicant for an establishment license or a product permit should submit the following documents to the CVB:

1. Application for U.S. Veterinary Biologics Establishment License (APHIS Form 2001): a one-page document indicating general information regarding the domestic biologics-manufacturing establishment.
2. Application for U.S. Veterinary Biological Product Permit (APHIS Form 2005): a one-page document completed by the permittee of a foreign biologics-manufacturing establishment indicating general information regarding the permittee, the foreign biologics producer, and the biological product(s).
3. Articles of Incorporation: a legal document indicating the business operating status of the manufacturing establishment.
4. Water Quality Statement: a document required for domestic veterinary biologics manufacturers only indicating the manufacturing establishment's status regarding applicable

U.S. effluent water quality control standards.

5. Application for U.S. Veterinary Biological Product License (APHIS Form 2003): a one-page document for domestically produced veterinary biologics indicating general biological product information.
6. Qualifications of Veterinary Biologics Personnel (APHIS Form 2007): a one-page document indicating specific information regarding the educational and work background of employees involved in biologics production.
7. Facilities Documents: blueprints, plot plans, and legends describing the biologics production facilities.

In support of the product license or permit applications, the applicant should prepare in an acceptable manner and submit to Licensing and Policy Development the following items (some variation may exist depending on the particular veterinary biological product being considered):

1. Outline of Production and Related Special Outlines: documents describing the protocol for manufacturing and testing of a particular biologic.
2. Master Seed Purity and Identity Test Report: the Veterinary Biologics Production and Test Report form (APHIS Form 2008) indicating the test results for the organism selected and permanently stored at a specified passage level from which all additional passages are derived.
3. Master Cell Stock Purity, Stability, and Non-tumorigenic Quality Test Report: the Veterinary Biologics Production and Test Report form (APHIS Form 2008) indicating the test results for the cells within a specific passage level range used to grow seed organisms for biologics production.
4. Backpassage Test Report: results of reversion to virulence studies for conventional modified live or live recombinant-derived vaccines indicating the Master Seed's genetic stability and reversion to virulence potential following administration to the host animal.
5. Efficacy Report: study results indicating the effectiveness of the veterinary biological product to perform as indicated on the product label. Vaccines recommended as an aid in the prevention of a specific fish disease are typically evaluated for efficacy by vaccination-challenge studies. The vaccine (produced with the lowest antigen level and at the highest passage level from Master Seed approved in the filed Outline of Production) should be administered according to label directions (e.g., injection, immersion, or oral) to the youngest age or smallest size fish for which the product shall be recommended. After an appropriate post-vaccination observation period, the vaccinated fish and other non-vaccinated control fish are challenged with a virulent strain of microorganism for which protection is recommended, and all post-challenge findings are accurately recorded. The precise challenge method and the criteria for determining protection vary with the immunizing agent. For products with two or more fractions, data should be submitted to evaluate any in vivo or in vitro interference of the various fractions.
6. Serial Purity, Safety, and Potency Test Report: the Veterinary Biologics Production and Test Report form (APHIS Form 2008) indicating all required test results for each of at least three consecutively produced prelicense serials (batches or lots) of finished product:
    a. Purity test results indicate if extraneous viable bacteria and fungi are present in the finished product. The permittee of imported veterinary biologics is charged a monetary fee if APHIS conducts additional testing of the finished biological product for exotic viruses.
    b. Laboratory safety test results indicate if there are any adverse reactions attributable to the vaccination of susceptible fish with the biological product during the pre-challenge observation period.
    c. Potency test results indicate the relative strength of the biological product, and are designed to correlate with the approved host animal vaccination-challenge efficacy study. Potency tests for killed viral or killed

bacterial products typically utilize laboratory animal or host animal evaluations or else quantitative in vitro methods. The potency of live virus and bacterial vaccines is typically measured by means of bacterial counts or virus titrations. The bacterial count of a live bacterial vaccine must be sufficiently greater than that shown to be protective in the immunogenicity (efficacy) test to ensure that at any time prior to the expiration date the count will be at least twice that used in the immunogenicity test. The virus titer of a live viral vaccine at release should be at least 1.2 logarithms greater than that shown to be protective in the immunogenicity test to ensure that at any time prior to the expiration date the titer will be at least 0.7 logarithms greater than that used in the immunogenicity test.

d. Other Outline of Production finished product test results indicate specific required information, e.g., microorganism identity, residual-free formaldehyde, viricidal activity, etc.

7. Field Safety Report: study results indicating the level of unsuspected adverse product-related reactions that may not have been observed during product development. Two or more prelicense serials are tested at three or more distinct geographic locations on a large number of appropriately sized fish that do not belong to the manufacturer. The manufacturer receives authorization to conduct field safety studies only after submission of acceptable efficacy data and satisfactory testing results of three consecutively produced prelicense serials. The field study is approved only if the test conditions are adequate to prevent the spread of disease, and the firm has obtained permission from the proper animal health authorities for each state where the tests will take place. Before beginning the field safety test, the firm should submit to Licensing and Policy Development for review a detailed protocol indicating the proposed observation and recording methods.
8. Product Stability Report: results of studies validating product shelf life (i.e., expiration dating).
9. Label: the insert, container label, and carton label indicating the true product name, the name and address of the producer (and also the importer for imported products), the establishment license or permittee number, the recommended storage temperature, the full instructions for use, the withdrawal time if the biologic is administered to food animals, the expiration date, the serial identification number, the recoverable quantity and number of doses, the presence of any antibiotic used as a preservative, the indication to use the entire contents of a multi-dose container when the container is first opened, the recommendation to burn the container and unused contents of all live organism products, and any special restrictions. The label may not contain any information which is false or misleading. All label claims must be supported by data submitted and filed by Licensing and Policy Development as acceptable.

Before issuing an establishment license or permit for general distribution and sale, APHIS will conduct an on-site inspection of the biologics production facilities and equipment to determine that these are acceptable for producing, testing, and distributing veterinary biologics using good manufacturing procedures and good laboratory techniques. The permittee for a foreign biologics manufacturer is charged a monetary fee to pay for the on-site inspection of a foreign biologics production facility. Biologics manufacturers should use good sanitary measures in compliance with Federal regulations and the Outline of Production. At the prelicense inspection, APHIS reviews all aspects of the manufacturing process, including accurate record keeping and product sampling. Following submission by the firm to APHIS of satisfactory results for all required prelicense serial release tests, APHIS will conduct confirmatory prelicense testing of representative samples of three consecutively produced prelicense serials at the CVB-L. APHIS will issue the appropriate establishment and product licenses or permit only after all prelicensing requirements have been satisfied.

There are several types of U.S. Veterinary Biological Product Licenses and U.S. Veterinary Biological Product Permits. A regular biologics product license authorizes the distribution of a veterinary biological product manufactured in the United States, with or without restrictions (e.g., for use by or under the supervision of a veterinarian only, intra-state distribution limited to authorized recipients or approved laboratories, use only on premises having a history of the disease, for export only, etc.). A conditional product license is issued in an expedited procedure to make a biologic needed (e.g., in an emergency or limited market situation) available following the demonstration of product purity and safety (even though product efficacy and potency studies remain in progress). A permit for general distribution and sale allows for the importation into the United States and distribution (with or without restrictions) of a specified biologic or biologics. Permits may also be issued to allow the importation of biologics into the United States for research and evaluation purposes or for transit shipment only.

## EPILOGUE

Biological products for vaccinating fish are currently available for sale in the United States from two companies: Alpharma NW Inc., Bellevue, Washington, telephone (206) 882-0448, and Aqua Health U.S.A., Buhl, Idaho, telephone (208) 543-5369. Test kits for the diagnosis of bacterial kidney disease antigen in fish are available from DiagXotics, Inc., Wilton, Connecticut, telephone (203) 762-0279.

Qualified personnel at APHIS' CVB are available to assist biologics manufacturers and permittees in the application process. For further information regarding the regulation of veterinary biologics, contact: U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Center for Veterinary Biologics, Licensing and Policy Development, 4700 River Road, Unit 148, Riverdale, Maryland 20737-1231; telephone: (301) 734-8245, fax (301) 743-8910. Information regarding the CVB is available from the Internet web site <http://www.aphis.usda.gov/vs/cvb>.

# EFFECTS OF REARING CONDITIONS ON BLIND SIDE HYPERMELANOSIS IN JAPANESE FLOUNDER

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## ABSTRACT

The effects of light direction, intensity, and bottom substrate on the hypermelanosis in Japanese flounder were examined. Hatchery-produced juvenile or young flounder were kept in aquaria with transparent tops and bottoms to illuminate the fish with upward and downward light. When the bottom of the aquarium was a transparent plastic plate, hypermelanosis occurred in all the fish tested regardless of light direction or intensity (downward/upward illumination: 1300/1100, 1300/60, and 150/7 lux). However, only 14% of the fish showed hypermelanosis in the aquarium with a sandy bottom and no upward light. Percentage occurrence of fish with hypermelanosis decreased drastically when the bottom was covered with glass sand, even if the fish were exposed to a high intensity of upward light (1400 lux). Similar trends were observed in the enlargement of the blind side pigmented area. None of the fish showed visible expansion of the pigmented area in the aquarium with sand or glass sand on the bottom; however, the pigmented area was enlarged in half of the fish in the aquarium with a transparent plastic plate bottom. From these results, it is considered that, not light, but the presence of sand on the aquarium bottom is the primary cause of blind side hypermelanosis in Japanese flounder.

## INTRODUCTION

The Japanese flounder *Paralichthys olivaceus* is one of the most important mariculture fish species in Japan. The wild fish of this species generally has a white blind side, while almost all the cultured flounder show a dark pigmented area on their blind side (ambicoloration or hypermelanosis on the blind side). This color anomaly is a serious problem in flounder culture, because it usually decreases the market price of the fish.

Norman (1934) divided hypermelanosis of flatfishes into three types by its characteristics: staining, spotting, and true ambicoloration. The staining type is the most common in cultured Japanese flounder (Yamamoto and Oda 1991). Some factors such as illumination on the blind side, food, and stocking density are considered to affect this type of hypermelanosis in Japanese flounder (Seikai 1991, Suzuki 1994, Takahashi 1994). Among these factors, illumination to the blind side seems to be the most plausible, because there are many studies not only on Japanese flounder (Seikai 1991) but also on other flatfish species which showed a relationship between light and hypermelanosis (Cunningham 1891, 1893, 1895, Osborn 1940, 1941, Stickney and White 1975). However, because most of the studies were conducted in aquarium in which the bottom was covered with sand to prevent upward light, the sandy substrate may have affected their results. In this study, rearing experiments were conducted to determine if light direction and intensity or bottom substrate are the most important factors for hypermelanosis in cultured Japanese flounder.

## MATERIALS AND METHODS

Fish of about 1g body weight were obtained from commercial hatcheries, and were kept in a tank with a recirculating seawater system until the start of experiments.

Experiments were carried out in aquaria equipped with a closed recirculating system (Fig.

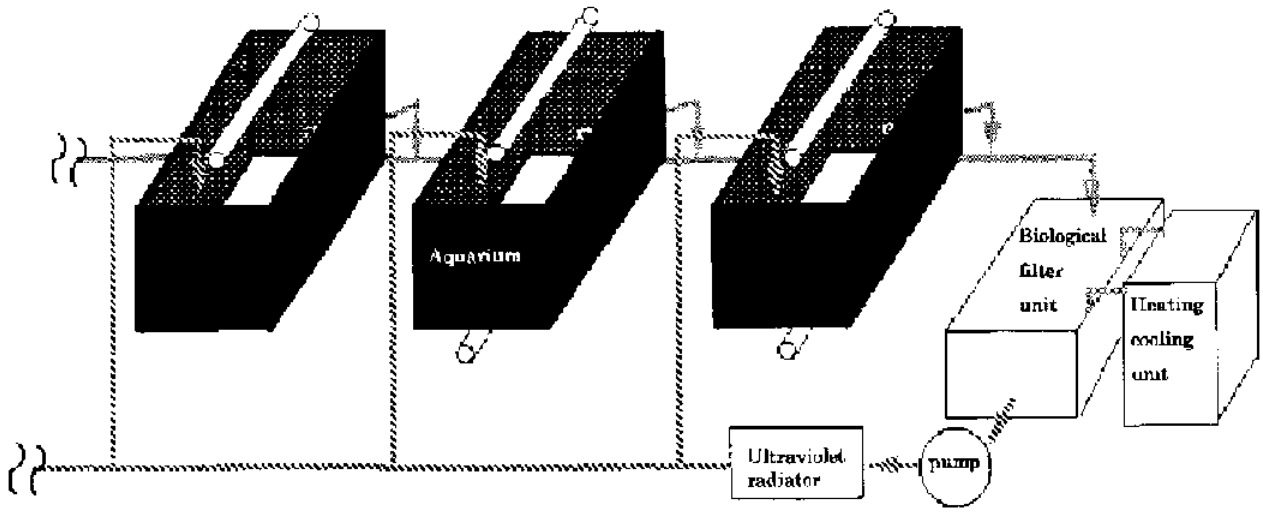


**Figure 1.** Experimental setup.

| Bottom material of aquarium | Light intensity (lux) | | Percentage of hypermelanosis fish (%) | Survival rate (%) | Specific growth rate (%) |
|---|---|---|---|---|---|
| | Downward | Upward | | | |
| Transparent plate | 150 | 7 | 100 | 93 | 2.1 |
| | 1300 | 60 | 100 | 80 | 2.3 |
| | 1300 | 1100 | 100 | 93 | 2.2 |
| Sand (1cm thickness) | 200 | 0 | 7 | 80 | 1.8 |
| | 1900 | 0 | 14 | 50 | 1.4 |

**Table 1.** Effects of downward and upward light intensity on the blind side hypermelanosis of Japanese flounder reared with or without sand on the bottom of the aquarium, experiment 1.

1). The aquaria with transparent acrylic bottoms were placed on transparent acrylic plates. The insides of aquarium walls were made of matted black vinyl chloride plates, and the top was covered with a transparent acrylic plate. Downward and upward illumination was provided with fluorescent lights installed above and below the aquaria. These lights were turned on for 12 h/day. Fish were fed with a commercial pelleted diet twice a day for 5 days/wk during the experimental period. Temperature was maintained at 23 °C.

Experiment 1 was designed to examine the effect of light intensity and direction on the hypermelanosis of the flounder in transparent plastic bottom aquaria with or without sand. Experimental conditions are shown in Table 1. The bottoms of two aquaria were covered with a 1-cm layer of coarse sand (particle size 0.5 - 1.0 mm). Fifteen fish of about 6 g body weight without visible pigmentation on the blind side were reared for 16 wk. At the end of the experiment, all surviving fish were anesthetized, and photographs of their blind sides were taken individually to examine the pigmentation.

In experiments 2 and 3, three aquaria with upward light and different bottom conditions were prepared as follows (Tables 2 and 3): (1) a transparent plastic plate and strong upward light, (2) a white opaque plate and weak upward light, (3) similar light conditions as the first, but the bottom was covered with a 1-cm layer of transparent glass sand (particle size 0.5 - 1.0 mm).

| Bottom material of aquarium | Light intensity (lux) | | Percentage of hypermelanosis fish (%) | Survival rate (%) | Specific growth rate (%) |
|---|---|---|---|---|---|
| | Downward | Upward | | | |
| Transparent plate | 1800 | 1000 | 88 | 68 | 2.9 |
| White opaque plate | 1800 | 10 | 100 | 72 | 2.9 |
| Glass sand | 1900 | 1400 | 27 | 88 | 3.0 |

**Table 2.** Effects of upward light and the presence of sandy substrate on the bottom of the aquarium on the blind side hypermelanosis of Japanese flounder, experiment 2.

| Bottom material of aquarium | Light intensity (lux) | | Percentage of hypermelanosis fish (%) | Survival rate (%) | Specific growth rate (%) |
|---|---|---|---|---|---|
| | Downward | Upward | | | |
| Transparent plate | 1800 | 1000 | 88 | 85 | 3.3 |
| White opaque plate | 1800 | 10 | 100 | 60 | 3.4 |
| Glass sand | 1900 | 1400 | 18 | 85 | 3.4 |

**Table 3.** Effects of upward light and the presence of sandy substrate on the bottom of the aquarium on the blind side hypermelanosis of Japanese flounder, experiment 3.

Twenty-five fish of about 4 g body weight were reared for 12 wk in experiment 2 and 20 fish of about 1 g were reared for 16 wk in experiment 3. None of the fish used in the experiments had visible pigmentation at the start. Fish with hypermelanosis were described in experiment 1.

Experiment 4 was carried out to examine the effect of bottom conditions on the enlargement of the dark pigmented area on the blind side with three aquaria prepared as follows: (1) bottom of transparent plate with strong upward light, (2) bottom covered with a 1-cm layer of transparent glass sand and strong upward light, (3) bottom covered with a 1-cm layer of coarse sand to prevent upward light (Table 4). Fish of about 25 g body weight, all of which had partial dark pigmentation on their blind side, were reared for 4 wk. Photographs of the blind side of individual fish were taken at the start and at the end of the experiment and the changes in pigmented area were compared.

## RESULTS AND DISCUSSION

The results of experiment 1 are shown in Table 1. In the aquarium without sand on the bottom, all fish showed hypermelanosis on the blind side at the end of the experiment, regardless of light intensity. However, only 7 and 14% of the fish showed hypermelanosis in the aquaria with sand on the bottom (Fig. 2). Specific growth rate of the fish was lower in the aquaria with a sand bottom than those without sand.

The results of experiments 2 and 3 were similar to each other (Tables 2, 3). Namely, 100% of fish had hypermelanosis in the aquarium with the white opaque plate bottom, and 88% in the aquarium with the transparent plate bottom. In contrast to these, less than 30% of fish showed

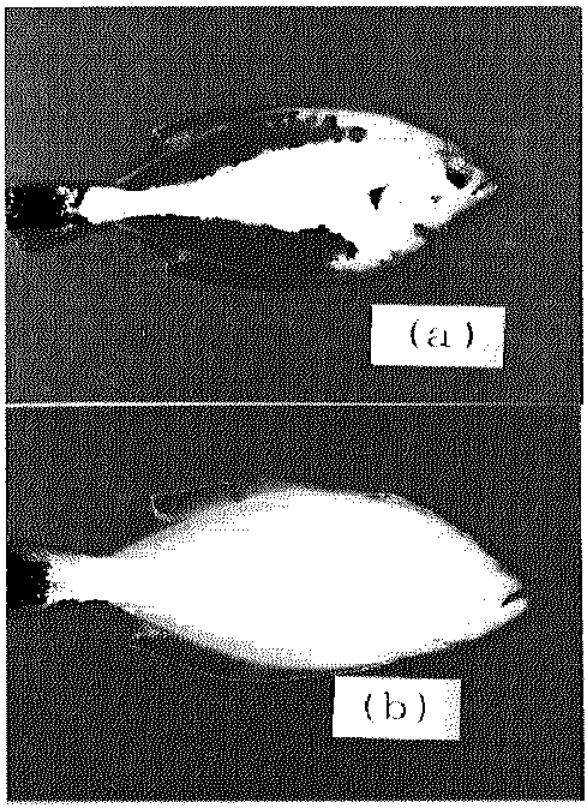


**Figure 2.** Photographs of the blind side of Japanese flounder at the end of experiment 1: a - Fish showing typical hypermelanosis, reared in an aquarium with a transparent acrylic plate on the bottom. b - Fish without visible pigmentation on the blind side, reared in aquarium with coarse sand on bottom.

dark pigmentation on the blind side with glass sand on the bottom in spite of the strong upward light. Specific growth rate was almost the same among treatments in both experiments.

Sand on the bottom of the aquarium was also effective for preventing enlargement of the pigmented area on the blind side (Table 4). No fish showed enlargement of their pigmented area in the aquaria with sand or glass sand on the bottom, while half of the fish enlarged their pigmented area in the aquarium with a transparent plate bottom. None of the fish died during the experimental period under any conditions, and the specific growth rate varied with treatment.

Previous papers concerning the pigmentation on the blind side in flatfish species suggested that light is the primary factor for such an abnormal coloration. However, from the results of this study, it is better to consider that, not light, but the presence of sandy substrate on the bottom of the culture tank has an important role in this phenomenon. In this study, sandy substrate on the bottom prevented the occurrence of hypermelanosis as well as its enlargement. As there is no other information that supports our results, more research is needed to determine how much the occurrence of hypermelanosis depends on sandy substrate or light. Furthermore, sand on the bottom of culture tanks is not considered to be practical, because it will easily cause deterioration of the culture environment by producing anaerobic areas. Therefore, alternative substrates will be required

| Bottom material of aquarium | Light intensity (lux) | | Percentage of fish with hypermelanosis area enlargement (%) | Survival rate (%) | Specific growth rate (%) |
|---|---|---|---|---|---|
| | Downward | Upward | | | |
| Transparent plate | 1300 | 400 | 43 | 100 | 0.5 |
| Glass sand | 1500 | 300 | 0 | 100 | 0.1 |
| Sand | 1300 | 0 | 0 | 100 | 0.6 |

**Table 4.** Effects of bottom substrate, light intensity (lux), and direction on the enlargement of blind side hypermelanosis of Japanese flounder, experiment 4.

for practical usage.

Most of the pigmentation on the blind side of the flounder was shown at a margin of the trunk, caudal peduncle, and basement of pectoral fin in this study (Fig. 2), and its location was the same as generally seen in cultured Japanese flounder (Seikai 1991, Yamamoto and Oda 1991).

## ACKNOWLEDGMENT

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## LITERATURE CITED

Cunningham, J. T. 1891. An experiment concerning the absence of color from the lower sides of flat-fishes. Zool. Anz. 14: 27-32.

Cunningham, J. T. 1893. Researches on the coloration of the skins of flat-fishes. J. Mar. Biol. Assoc. U. K. 3 (N. S.): 111-118.

Cunningham, J. T. 1895. Additional evidence on the influence of light in producing pigments on the lower sides of flat fishes. J. Mar. Biol. Assoc. U.K. 4 (N. S.): 53-59.

Norman, J. R. 1934. A systematic monograph of the flatfishes (Heterosomata). Volume 1. Brit. Mus., London. 459 p.

Osborn, C. M. 1940. The experimental production of melanin pigment on the lower surface of summer flounders (*Paralichthys dentatus*). Proc. Natl. Acad. Sci. U.S.A. 26: 155-161.

Osborn, C. M. 1941. Studies on the growth of integumentary pigment in the lower vertebrates. I. The origin of artificially developed melanophores on the normally unpigmented ventral surface of the summer flounder (*Paralichthys dentatus*). Biol. Bull. (Woods Hole) 81: 341-351.

Seikai, T. 1991. Influences of fluorescent light irradiation, ocular side pigmentation, and source of fishes on the blind side pigmentation in the young Japanese flounder, *Paralichthys olivaceus*. Suisanzoshoku 39: 173-180 (in Japanese).

Stickney, R. R. and D. B. White. 1975. Ambicoloration in tank cultured flounder, *Paralichthys dentatus*. Trans. Am. Fish. Soc. 104: 158-160.

Suzuki, N. 1994. Ultrastructure of the skin on reverse side of hatchery-reared Japanese flounder, *Paralichthys olivaceus*, with reference to the pigmentation. Bull. Nansei Natl. Fish. Res. Inst. 27: 113-128 (in Japanese).

Takahashi, Y. 1994. Influence of stocking density and food at late phase of larval period on hypermelanosis on the blind body side in juvenile Japanese flounder. Nippon Suisan Gakkaishi 60: 593-598 (in Japanese).

Yamamoto, S. and T. Oda. 1991. The effects of several diets on survival, growth and color anomalies in juvenile flounders *Paralichthys olivaceus*. Bull. Fish. Exp. Sta. Okayama Pref. 6: 100-108 (in Japanese).

# MICROBIOLOGY OF EARLY LARVAL STAGES OF SUMMER FLOUNDER *PARALICHTHYS DENTATUS* GROWTH IN A RECIRCULATING WATER SYSTEM

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## ABSTRACT

Finfish in early larval stages of growth can suffer high mortality in aquacultural facilities because of diseases and nutritional problems. Recent studies suggest that bacteria associated with the live feed and hatchery environments that colonize finfish can have beneficial or detrimental effects on fish health. A local commercial facility that grows summer flounder in a recirculating water system has been the subject of microbiological studies for their first four production runs. The culture of summer flounder is in its infancy and the microbiology of these fish is not well characterized. Samples of fish, tank water, and feed collected at times of change in feeding regime, metamorphosis and episodic high mortality and disease events were analyzed for different bacteria. Growth media targeting total heterotrophs, total vibrios and *Vibrio anguillarum* were used to enumerate and isolate bacteria. Isolates were identified to species and/or genus. Differences and similarities in microbial community diversity and abundance at different life stages and feeding regimes were noted. The results provide an initial database for determining the role of bacteria in the onset of disease and the health of early stages of summer flounder growth.

## INTRODUCTION

Aquaculture is becoming widespread and growing rapidly in northern New England, USA, and throughout the world. Among many uncertainties, one of the biggest is the incidence of disease in the fish being cultured. Diseases can cause significant fish mortalities, especially in early life stages, and such events are obviously catastrophic to any industry.

Bacterial pathogens that cause diseases in fish often enter the host with ingested food or feces and colonize the intestinal tract (Romalde et al. 1996). The bacterial diversity is enormous in fish tissue and hatchery environments (Muroga et al. 1987, Nicolas et al. 1989, Sorgeloos 1994), making it difficult to identify pathogens or monitor for predicting the onset of disease. Prophylactic and direct treatment of diseases often involves use of antibiotics and vaccination (Cahill 1990, Joosten et al. 1995). There are many disadvantages to using antibiotics, including the potential for evolution of drug resistant strains (Kapetanaki et al. 1995), harmful effects on fish eggs (Munro et al. 1995), negative effects of seawater (Barnes et al. 1995), and complex governmental regulations. In recirculating aquaculture systems (RAS), the use of antibiotics is even more limited because of the need for establishing stable microbial communities on biofilters needed for removing wastes. An alternative approach to disease management is the use of probiotic bacteria. This approach employs use of the beneficial or benign natural microflora associated with healthy fish to establish and maintain a

microflora that can suppress potential pathogens and promote fish growth. Inhibition can be accomplished by production of toxins (Fouz et al. 1995), siderophore production (Pybus et al. 1994) or by competitive exclusion of pathogens (Smith and Davey 1993).

Great Bay AquaFarms (GBA) is a land-based RAS facility located in Portsmouth, New Hampshire, that is unique in North America for the combination of system and the cultivation of summer flounder *Paralichthys denatus*. Different aspects of the culture and diseases of two other biologically similar flatfish species—Japanese flounder and turbot— have been studied. However, little is known about the microbiology of summer flounder, especially in an RAS. An early study identified *Vibrio anguillarum* as a common pathogen associated with kidney tissue in dead fish, both feral and cultured, from the coasts of New Hampshire and Maine (Strout et al. 1978). More recent work in New Hampshire has focused largely on the incidence and ecology of human pathogenic *Vibrio* sp. in the Great Bay estuary (Jones et al. 1991, O'Neill et al. 1992, Jones et al. 1997 ), the source of water for GBA culture tanks. The purpose of this study was to determine the effects of intestinal microflora and the culture environmental conditions on the health and survival of larval summer flounder.

## METHODS

Great Bay AquaFarms, Inc. is a commercial hatchery dedicated to the culture of summer flounder. Young larvae are grown from fertilized eggs, provided by brood fish on site, in recirculating culture tanks until the juveniles reach a size of 5-10 g (5-8 cm), at which time they are transferred to on-growing operations. The focus of this study was the fourth production run since the start of GBA in 1996, which began on 22 March 1997. The conditions in the rearing tanks were subject to many changes during the 100-day study, including feeding regime, tank disinfection and cleaning, and movement of fish between tanks. Larvae were fed algae and rotifers in eight larval rearing tanks for the first 20 days, then *Artemia* nauplii followed by enriched *Artemia* for the next ~20 days prior to metamorphosis. After 35-40 days, metamorphosed fish were transferred to 12 weaning tanks and fed artificial feed weaning diets.

Samples for microbiological analyses were taken from different tanks on a weekly basis. The justification for not sampling specific tanks in a consistent fashion was that fish reared in specific larval tanks were mixed into different weaning tanks, and some weaned fish were remixed between weaning tanks. These factors made it difficult to conduct analyses under controlled experimental conditions, so sampling was eventually focused on tanks with clearly distinguishable healthy and unhealthy fish. Sampling for sick and healthy fish involved paired fish samples from the same tank on any given sample date. Tank water temperature remained relatively constant, ranging from 16.4 to 19.9°C. Salinity ranged from 18 to 32 ppt.

Accurate estimates of fish densities in all rearing tanks were not available, so percent survival could not be calculated. The densities in tanks ranged from 100,000 to 200,000 fish in tanks not affected by disease, and substantially lower in tanks where disease had been present. Assessment of the degree of mortality of fish was based on quantifying dead fish on a daily basis in each tank. Sick fish were identified by altered pigmentation and feeding behavior.

Fish, feed, and water samples were collected using sterile containers and transported on ice to the Jackson Estuarine Laboratory for analysis. Samples were processed within 2 h of collection. The fish were anesthetized, measured, surface sterilized, and ground with a mortar and pestle. Tissue, water and feed samples were diluted in sterile buffered peptone water and aliquots from a range of dilutions were collected onto membrane filters and placed on different agar media. Total heterotrophs were cultured from 2216E medium, total vibrios were cultured from thiosulfate-citrate-bile salts-sucrose (TCBS) medium, and *V. anguillarum* was cultured from VAM agar (Alsina et al. 1994), all incubated at room temperature (18-22°C). Focus was placed on vibrios because they have been shown to be important in other aquacultural settings both as agents of disease and as beneficial 'probiotic' organisms, and are the dominant bacteria in the intestines of larval and juvenile marine fish (Muroga et al. 1987). In addition, the source water from the Great Bay estuary is known

to have abundant vibrios (Jones et al. 1997), particularly during the time of the production run under study.

Dominant and unique colonies on all plates were identified using an identification scheme similar to Muroga et al. (1987). Colony morphology and color plus carbohydrate utilization reactions were noted. Isolated colonies were re-streaked onto TSA medium and the colony morphology and color plus pigment production were noted for re-grown isolates. Cell morphology, motility, oxidase reaction, and gram reaction were determined. Gas production, growth, and acid production with single carbon sources were determined along with amino acid decarboxylase reactions. Growth at different salinities and temperatures were also used to identify bacterial isolates.

## RESULTS

Figure 1 illustrates the dynamics of mortality in all of the larval-rearing and weaning tanks. The first spike in fish mortality occurred within a week after feeding on *Artemia that* began on day 21. The salinity in the tanks dropped from ~ 25 ppt to 18 ppt between days 27 and 29, when 9.7 cm of rain fell in 48 h, dropping the salinity of the source estuarine water in the process. A consistent, medium level of mortality persisted in some of the tanks during the first 3 wk of weaning diet, followed by a slight drop in mortality rate. The persistent mortality in tanks after day 60 was nearly all associated with delayed mortality in tanks that had shown good survival early in the weaning period.

Total vibrio concentrations increased dramatically in the rearing tanks after day 20 when *Artemia* feeding began (Fig. 2). The highest concentration of vibrios occurred during the time when the first heavy mortality occurred. Total heterotroph concentrations increased only after day 30, following the spike in vibrio numbers. A small

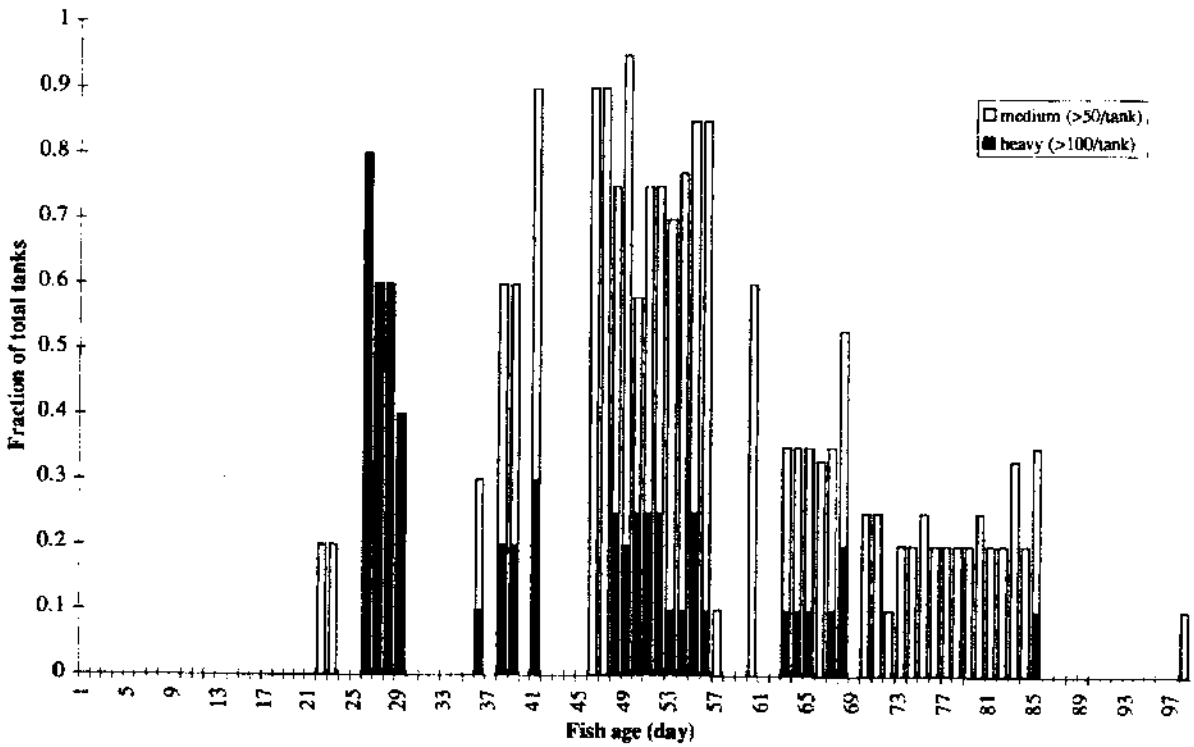


**Figure 1.** Degree of fish mortality in larval (0-40 days) and weaning (42-98 days) tanks.

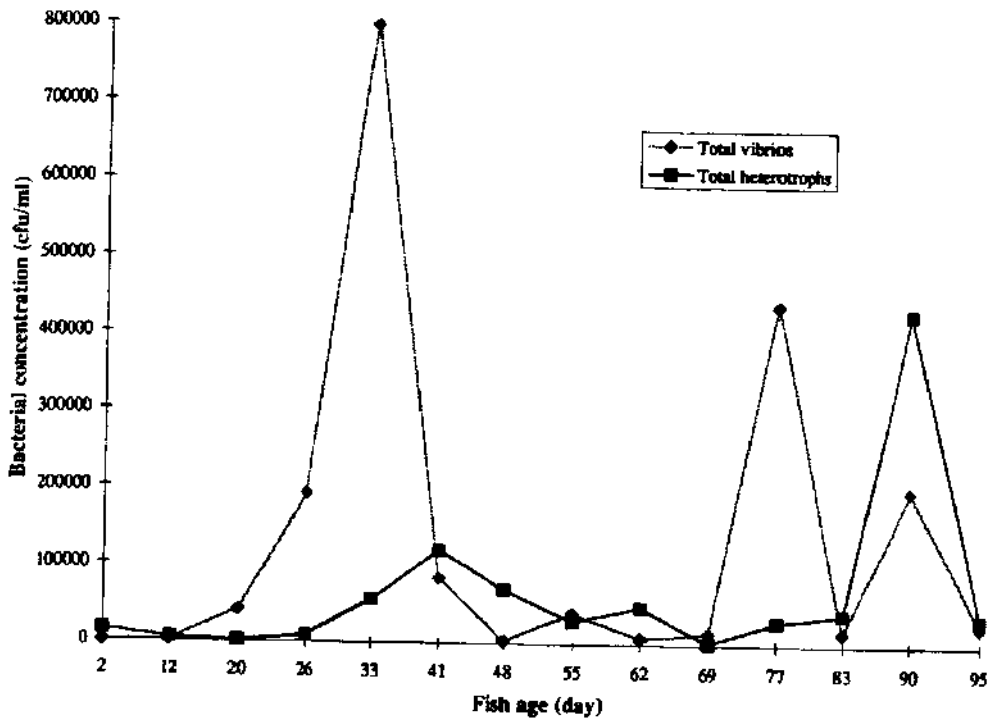


**Figure 2.** Bacterial concetrations in larval and weaning tanks. cfu = colony-forming units.

peak in concentrations of putative (yellow colonies on VAM agar) *V. anguillarum* coincided with the total vibrio peak (data not shown).

Relatively low concentrations of all bacteria were apparent in the tank water from day 45 to day 69, followed immediately by a second large peak in total vibrio concentrations (Fig. 2). This second vibrio peak occurred at the beginning of June, when estuarine temperatures began to increase above 15°C and microbial communities dramatically change, typically characterized by significant increases in the diversity and population sizes of *Vibrio* sp. (O'Neill et al. 1992). The salinity in the culture tanks also increased from the low of 18 ppt on day 29 to 32 ppt on day 70. The second peak in total vibrios also corresponded with the onset of another incidence of elevated mortality, nearly all of which occurred in tanks that had relatively healthy fish early in the weaning phase. Thus, the microbial dynamics in the fish tanks had some relationship to the occurrence of disease/mortality in the fish. Peaks in total heterotrophs, total vibrios, and *V. anguillarum* also occurred on day 90 post-hatch.

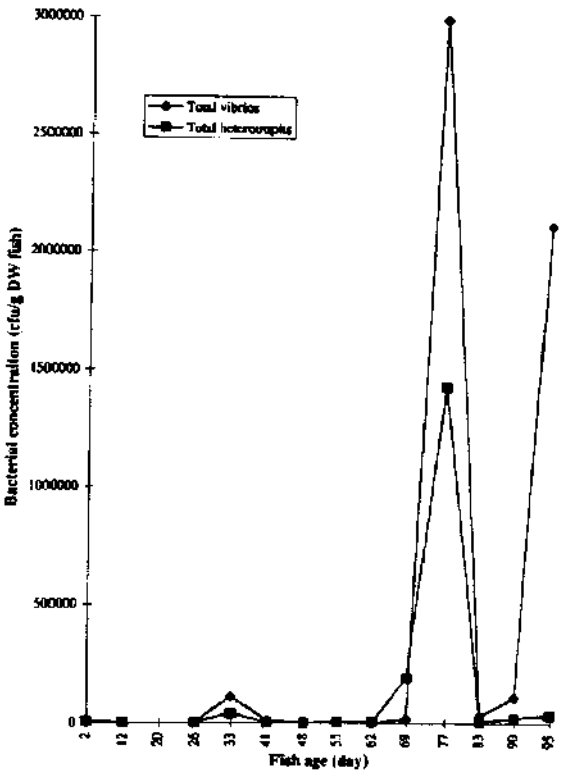


**Figure 3.** Bacterial concentrations in "healthy" fish. cfu = colony forming units; DW = dry weight.

The delay in onset of increases in total heterotroph populations relative to total vibrios was not seen in the healthy fish. Simultaneous peaks in concentrations for both total heterotrophs and total vibrios occurred on days 33, 77, and 95 (Fig. 3), a three-peak pattern similar to the microbial dynamics in the rearing tanks. A comparison of total vibrios in healthy and unhealthy fish taken from the same tanks on five sample dates from day 41 through day 83 showed unhealthy fish had higher concentrations of total vibrios than healthy fish on four of the five sample dates, with overall average concentrations in unhealthy fish >10 times higher than in unhealthy fish (Fig. 4). In contrast, total heterotroph concentrations were higher in healthy fish in the first four of the five samples (Fig. 5). Concentrations of total heterotrophs in unhealthy fish were much higher in the fifth sample and the overall averages for unhealthy and healthy fish were similar. The TCBS medium for recovery of total

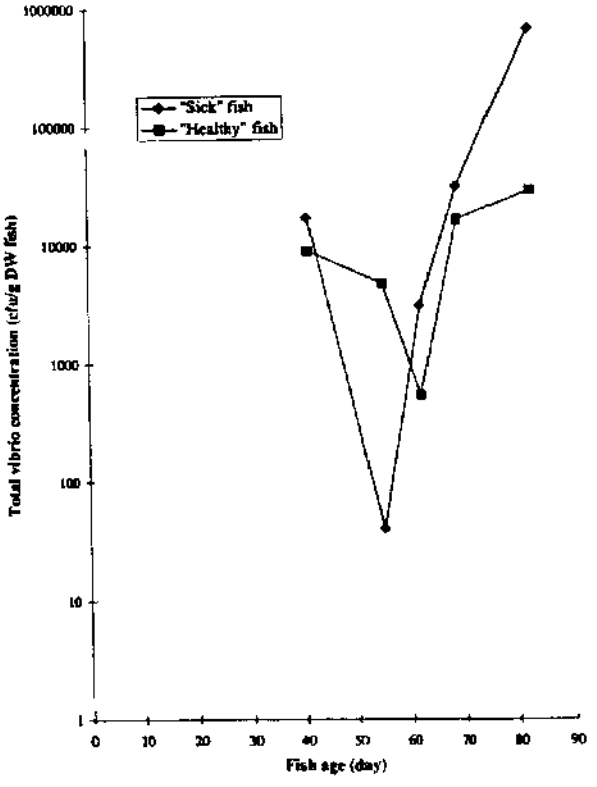


**Figure 4.** Total vibrio concentrations in "healthy" and "sick" fish. cfu = colony forming units; DW = dry weight.

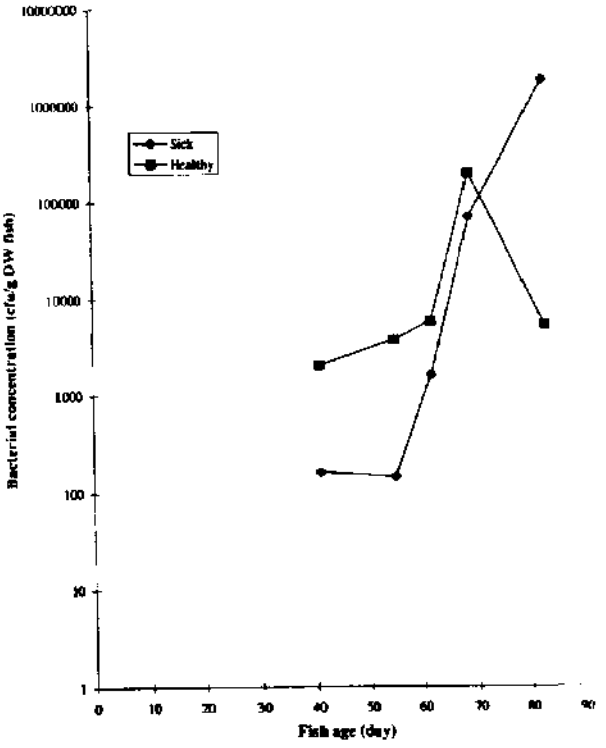


**Figure 5.** Total hetrotrophic bacterial concentrations in "healthy" and "sick" fish. cfu = colony forming units; DW = dry weight.

vibrios recovered higher numbers of bacteria than the 2216E medium.

Predominant bacterial isolates from live feed and tank water were identified to species and/or genus (Table 1). The results are biased toward isolation of vibrios because of the isolation media used. The feed had a more predominant presence of *Vibrio* sp. although vibrios occurred in both the feed and the water. Numerous bacteria were present in the rotifers and the *Artemia*. The tank water contained many species, with major changes in composition accompanying changes in the feed and tank environment. *Moraxella* sp. was the most consistently prevalent organism. Otherwise, there were few similarities between isolates from the tank water and the feeds.

## DISCUSSION

Bacterial numbers and species composition varied widely during the early stages of sum-

| Age: Feed: | 1-20 days Rotifers/algae | 21-33 days Artemia |
|---|---|---|
| **Live feed** | *Moraxella* sp. | *Moraxella* sp. |
| | *Vibrio* sp. I | *Vibrio* sp. I |
| | *Vibrio* sp. III | *Vibrio* sp. III |
| | *V. alginolyticus* | *V. damsela* |
| | Enterobacteriaceae | Enterobacteriaceae |
| | *Flavobacterium* sp. | *V.* parahaemolyticus |
| | | *Aeromonas* sp. |
| **Tank water** | *Acinetobacter* sp. | *Argobacterium* sp. |
| | *Aeromonas* sp. | Enterobacteriaceae |
| | *Moraxella* sp. | *Moraxella* sp. |
| | *Pseudomonas* sp. III/IV | *Pseudomonas* sp. I/II |
| | *V. fluvialis* | *Vibrio* sp. I |
| | *V. anguillarum* | *V. alginolyticus* |
| | | *V. parahaemolyticus* |

**Table 1.** Bacterial species in live feed and tank water for summer flounder at Great Bay Aquafarms.

mer flounder growth at GBA. Factors that could have affected the abundance and succession of bacterial species include the microflora of the live feed, nutritional differences in feeds, fish growth and changes in physiology, seasonal changes in source water, the transfer of fish between tanks, and environmental conditions in the culture system. Others have reported similar microbial community dynamics and species composition in a variety of cultured finfish (Campbell and Buswell 1983, Muroga et al. 1987, Nicolas et al. 1989, Sorgeloos 1994). The similarity in species diversity and abundance of different bacteria with other studies suggests that there are no unique microbiological characteristics of summer flounder or northern New England culture conditions.

There were peaks in both total heterotrophs and total vibrios that corresponded roughly with elevated mortality episodes. These peaks were observed in both the tank water and the fish tissue. Munro et al. (1995) clearly demonstrated that *V. anguillarum* is a pathogen of larval turbot, and Nicolas et al. (1989) reported a domination of the rotifer microflora by *Vibrio* sp. associated with high mortality of larval turbot. In this study, vibrios were associated with unhealthy fish, but also with healthy fish and their tank water, even in high numbers at certain times during the early growth stages of the summer flounder. However, fish considered healthy were present in tanks that also contained unhealthy fish, making cross contamination highly probable. The simple presence of vibrios is apparently not a clear indication of disease potential in summer flounder. However, the general trend of higher numbers of total vibrios in unhealthy compared to healthy fish suggests that total vibrio counts may provide a better reflection of disease than total heterotroph counts. The earlier occurrence of a peak in total vibrios compared to total heterotrophs in tank water just prior to the first episode of high mortality suggests that monitoring total vibrio concentrations in tank water may be useful in predicting disease.

The microflora of the feed and culture environment was dominated by *Vibrio* sp. The use of traditional culture methods provides results that are strongly influenced by the composition of the isolation media and the isolation conditions used. Because heterotrophs other than vibrios were also present, the lower numbers of bacteria recovered on 2216E medium compared to TCBS suggests that a better medium for recovery of total heterotrophs is needed. Others have reported that *Vibrio* sp. are the dominant bacteria in the intestines of larval and juvenile marine fish (Muroga et al. 1987). The use of TCBS and VAM agars in this study anticipated

this, biasing the results in order to provide isolates that may be useful in future studies on probiotic bacteriaand pathogens. The phylogeny of the fish pathogens and general microflora would be better determined using molecular methods (Amann et al. 1995), although this was clearly not the purpose of this study.

There were a few apparent differences between the fish and tank water microflora, as well as in the abundance of bacteria in healthy and unhealthy fish. These preliminary observations suggest that detection of the selection of bacterial species in fish both during colonization of the fish intestine from live feed and during disease episodes may be difficult using the methods in this study. More detailed data on abundance of bacterial species and speciation of isolates from healthy and unhealthy fish during the targeted production run and other runs at GBA are currently being analyzed, and the results will hopefully provide clearer results for a future publication. Further work and refinement of methods are needed to more clearly identify probiotic and pathogenic bacteria. This study also suggests that further work should be done to better understand the relationship between the microflora of the live feed and the eventual colonization of larval and juvenile fish.

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## LITERATURE CITED

Alsina, M., J. Martinez-Picado, J. Jofre, and A.R. Blanch. 1994. A medium for presumptive identification of *Vibrio anguillarum*. Appl. Environ. Microbiol. 60: 1681-1683.

Amann, R., I.W. Ludwig, and K.H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59: 143-169.

Barnes, A.C., T.S. Hastings, and S.G.B. Amyes. 1995. Aquaculture antibacterials are antagonized by seawater cations. J. Fish Dis. 18: 463-465.

Cahill, M.M. 1990. Bacterial flora of fishes: a review. Microb. Ecol. 19: 21-41.

Campbell, A.C. and J.A. Buswell. 1983. The intestinal microflora of farmed Dover sole (*Solea solea*) at different stages of fish development. J. Appl. Bacteriol. 55: 215-223.

Fouz, B., B. Novoa, A.E. Toranzo, and A. Figueras. 1995. Histopathological lesions caused by *Vibrio damsela* in cultured turbot, *Scophthalmus maximus* (L.): inoculations with live cells and extracellular products. J. Fish Dis. 18: 357-364.

Jones, S.H., K.R. O'Neill, and T.L. Howell. 1991. Differential elimination of indicator bacteria and pathogenic *Vibrio* sp. from Maine oysters (*Crassostrea virginica*) in a commercial controlled purification facility. J. Shellfish Res. 10: 105-112.

Jones, S.H., R. Langan, W.H. McDowell, and B.W. Summer-Brason. 1997. Influence of organic and nutrient pollution on fecal-borne and indigenous bacteria in estuarine environments. Final report, National Estuarine Research Reserve System, Sanctuaries and Research Div., OCRM, NOAA, Silver Spring, MD.

Joosten, P.H.M., M. Aviles-Trigueros, P. Sorgeloos, and J.H.W.M. Rombout. 1995. Oral vaccination of juvenile carp (*Cyprinus carpio*) and gilthead seabream (*Sparus aurata*) with bioencapsulated *Vibrio anguillarum* bacterin. Fish Shellfish Immunol. 5: 289-299.

Kapetanaki, M., J. Kerry, M. Hiney, C.O'Brien, R. Coyne, and P. Smith. 1995. Emergence, in oxytetracycline-free marine mesocosms, of microorganisms capable of colony formation on oxytetracycline-containing media. Aquaculture 134: 227-236.

Munro, P.D., A. Barbour, and T.H. Birkbeck. 1995. Comparison of the growth and survival of larval turbot in the absence of culturable bacteria with those in the pres-

ence of *Vibrio anguillarum*, *Vibrio alginolyticus*, or a marine *Aeromonas* sp. Appl. Environ. Microbiol. 61: 4425-4428.

Muroga, K., M. Higashi, and H. Keitoku. 1987. The isolation of intestinal microflora of farmed red seabream and black seabream (*Acanthopagrus schlegeli*) at larval and juvenile stages. Aquaculture 65: 79-88.

Nicolas, J.L., E. Robic, and D. Ansquer. 1989. Bacterial flora associated with a trophic chain consisting of microalgae, rotifers and turbot larvae: influence of bacteria on larval survival. Aquaculture 83: 237-248.

O'Neill, K.R., S.H. Jones, and D.J. Grimes. 1992. Seasonal incidence of *Vibrio vulnificus* in the Great Bay estuary of New Hampshire and Maine. Appl. Environ. Microbiol. 58: 3257-3262.

Pybus, V., M.W. Loutit, I.L. Lamont, and J.R. Tagg. 1994. Growth inhibition of the salmon pathogen *Vibrio ordalii* by a siderophore produced by *Vibrio anguillarum* strain VLA355. J. Fish Dis. 17: 311-324.

Romalde, J.L., B. Margarinos, S. Nunez, J.L. Barja, and A.E. Toranzo. 1996. Host range susceptibility of *Enterococcus* sp. strains isolated from diseased turbot: possible routes of infection. Appl. Environ. Microbiol. 62: 607-611.

Smith, P. and S. Davey. 1993. Evidence for the competitive exclusion of *Aeromonas salmonicida* from fish with stress-induced furunculosis by a fluorescent pseudomonad. J. Fish Dis. 16: 521-524.

Sorgeloos, P. 1994. State of the art in marine fish larviculture. World Aquacult. 25: 34-37.

Strout, R.G., E.S. Sawyer, and B.A. Coutermarsh. 1978. Pathogenic vibrios in confinement-reared and feral fishes of the Maine-New Hampshire coast. J. Fish. Res. Board Can. 35: 403-408.

# NUTRITIONAL REQUIREMENTS IN BROODSTOCK OF MARINE FISHES

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## ABSTRACT

The present work reviews the relationship between broodstock nutrition and quality of egg and larvae in marine fish. Nutrients in the diets have profound effects on gonadal development in fish. Egg production, hatching rate, and larval survival are negatively affected by deficiency in nutrients such as n-3 highly unsaturated fatty acids and a few vitamins in the diet. Protein quality and quantity also have an effect on egg quality. Effective broodstock diet, however, cannot be developed as long as the nutritional requirements of broodstock remain obscure. Supplementation of components to the diet for growth may be required for further enhancement of the nutritional quality of broodstock diets. More research effort is needed on broodstock nutrition and reproductive physiology for the improvement of seed production.

## INTRODUCTION

Gonadal development in several species of fish is greatly affected by broodstock nutrition. During the last decade, increasing attention has been paid to the role of individual nutrient components in broodstock diets (Bromage 1995). Nutritional studies in broodstock of marine fish have been conducted mainly on sea breams—red sea bream *Pagrus major* and gilthead sea bream *Sparus aurata*. Little is known about the nutritional requirements of broodstock in other species such as flounder in spite of the importance of these species in aquaculture. It is important to review data and current problems affecting broodstock nutrition for future research. The major groups of feed components which have been previously studied are essential fatty acids (EFA), proteins, and several vitamins. Table 1 shows effects of the feed components on egg and larval quality in marine fish. The purpose of this paper is to summarize and discuss current information on the nutritional requirements of broodstock and to suggest areas for further research.

### Essential fatty acids

Lipids play a major role as membrane constituents and energy reserves in fish embryos, and n-3 highly unsaturated fatty acids (n-3 HUFA), in particular docosahexaenoic acid (DHA), are essential for larval development (Watanabe 1993, Furuita *et al.* 1996a, b). When red sea bream broodstock were fed a diet containing a high content of corn oil (an EFA-deficient diet) before and during spawning, the percentage of viable eggs, hatching rate, and normal larvae were significantly lower than those of the control (Fig. 1) (Watanabe *et al.* 1984a). There was also a direct correlation between the level of broodstock dietary n-3 HUFA and larval growth in gilthead sea bream (Tandler *et al.* 1995). Larvae from broodstock fed a diet in which n-3 HUFA was completely excluded had a 34% growth retardation compared to larvae from broodstock fed a high n-3 HUFA diet (15 mg/g diet). While there was no significant effect of dietary n-3 HUFA levels on 32-day survival, the swimbladder inflation rate was affected significantly. The fatty acid composition of eggs is directly affected by the fatty acid composition of the broodstock (Mourente and Odriozola 1990). Some fatty acids affect the egg quality of the Japanese flounder *Paralichthys olivaceus* (Fig. 2) and the Atlantic halibut *Hippoglossus hippoglossus* (Parrish *et al.* 1994). However, it is often observed that the n-3 HUFA content of red sea bream eggs has no relation to egg quality (Watanabe 1985).

| Feed component | Fish | Eggs | | Larvae | | Reference |
|---|---|---|---|---|---|---|
| | | Viability | Hatching | Survival | Normality | |
| **Lipid** | | | | | | |
| N-3 HUFA | *Pagrus major* | + | + | + | + | Watanabe *et al.* (1984a) |
| (squid oil or | *Sparus aurata* | + | + | + | | Tandler *et al.* (1995) |
| pollack liver oil, etc.) | | + | ± | + | + | Fernandez-Palacios *et al.* (1997) |
| | *Dicentrarchus labrax* | + | + | | | Navas *et al.* (1995) |
| High n-3 HUFA | *Sparus aurata* | + | ± | - | - | Fernandez-Palacios *et al.* (1995) |
| Corn oil (EFA-def) | *Pagrus major* | - | - | - | - | Watanabe *et al.* (1984a) |
| Soy oil (EFA-def) | *Sparus aurata* | - | - | - | - | Tandler *et al.* (1995) |
| Phospholipids | *Pagrus major* | + | + | + | + | Watanabe *et al.* (1991b) |
| | *Paralichthys olivaceus* | ± | ± | ± | ± | Cited in Takeuchi (1997) |
| **Protein** | | | | | | |
| Cuttlefish meal | *Pagrus major* | + | + | + | + | Watanabe *et al.* (1984a) |
| Squid meal | *Sparus aurata* | + | + | + | + | Fernandez-Palacios *et al.* (1997) |
| Low protein | *Pagrus major* | - | - | - | - | Watanabe (1985) |
| | *Dicentrarchus labrax* | - | - | - | - | Cerda *et al.* (1994) |
| **Vitamin C** | *Sardinops melanosticta* | + | + | | | Akiyama *et al.* (1990) |
| | *Gadus morhua* | ± | ± | ± | ± | Mangor-Jensen *et al.* (1994) |
| **Vitamin E** | *Pagrus major* | + | + | + | + | Watanabe *et al.* (1991b) |
| | *Sparus aurata* | + | + | + | + | Fernandez-Palacios *et al.* (1996) |
| | *Paralichthys olivaceus* | ± | ± | ± | ± | Cited in Takeuchi (1997) |
| **Carotenoid** | *Pagrus major* | + | + | + | + | Watanabe *et al.* (1991b) |
| | *Seriola quinqueradiata* | + | + | + | + | Verakunpiriya *et al.* (1997b) |

+ = positive effect; - = negative effect; ± = no effect was observed.

**Table 1.** Effect of broddstock diet on egg quality in marine fishes.

| | High protein | Cuttlefish meal | Raw krill* | Corn oil** |
|---|---|---|---|---|
| Egg production (x $10^4$/fish) | 149.5 | 121.6 | 202.1 | 58.7 |
| Viable eggs (%) | 49.1 | 68.6 | 82.1 | 18.2 |
| Hatching rate (%) | 83.1 | 93.7 | 90.3 | 27.3 |
| Normal larvae (%) | 51.6 | 82.2 | 91.2 | 24.0 |
| Final productivity of larvae from total egg produced (%) | 21.1 | 52.8 | 68.1 | 1.2 |

* This group had been fed a high protein diet shortly before spawning.

** This group had been fed a cuttlefish meal diet shortly before spawning.

**Table 2.** Effect of broodstock diet on spawning and egg quality in red sea bream.

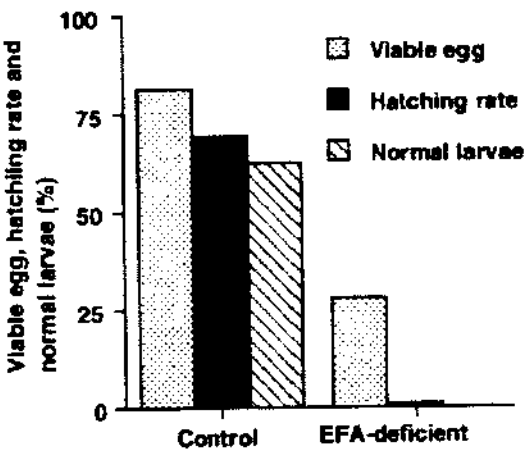


**Figure 1.** Effect of dietary EFA on egg quality of red sea bream (drawn from data in Watanabe et al. 1984a).

In fish like the red sea bream which feed during spawning, egg quality is affected by diets given shortly before spawning (Watanabe *et al.* 1984c). The egg quality of red sea bream fed a fish meal diet is improved by feeding them raw krill and the egg quality of broodstock fed a cuttlefish meal diet was reduced by feeding an EFA-deficient diet (Table 2). Changes in egg composition and egg and larval quality after a change in the broodstock diet occurred within 15 days (Fig. 3) (Tandler *et al.* 1995).

In conclusion, the nutritional value of lipids in the broodstock diet has a considerable effect on egg and larval quality. Mobilization of body stores of EFA during spawning can probably only compensate for minor deficiencies in the diet. For optimum larval growth, survival, and swimbladder inflation rate in gilthead sea bream, the broodstock diet must include at least 15 mg/g diet of n-3 HUFA, with 50-60% DHA (Tandler *et al.* 1995). Fernandez-Palacios *et al.* (1995) also suggested that egg quality in gilthead sea bream can be improved by increasing the n-3 HUFA level to 1.6%. This is similar to the levels reported for red sea bream by Watanabe and co-workers. However, a high level of dietary n-3 HUFA is likely to have a negative effect on larval survival of gilthead sea bream (Fernandez-Palacios *et al.* 1995). Furthermore, recent studies suggest the importance of the ratio of n-3 series to n-6 series HUFA in broodstock diet and that efforts should be directed toward establishing the optimum ratio of DHA/ eicosapentanoic acid/ arachidonic acid in the diet (Bell *et al.* 1997).

**Protein**

The protein level and quality in diets for brood fish affect the reproductive performance. Egg production is reduced both in red sea bream (Watanabe *et al.* 1984a) and sea bass

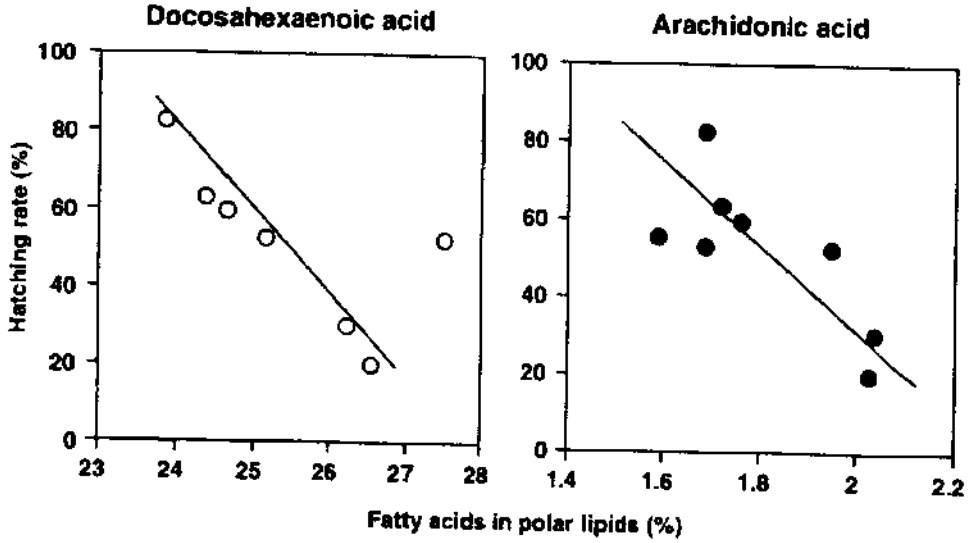


**Figure 2.** Fatty acid levels in eggs and hatching rates of Japanese flounder.

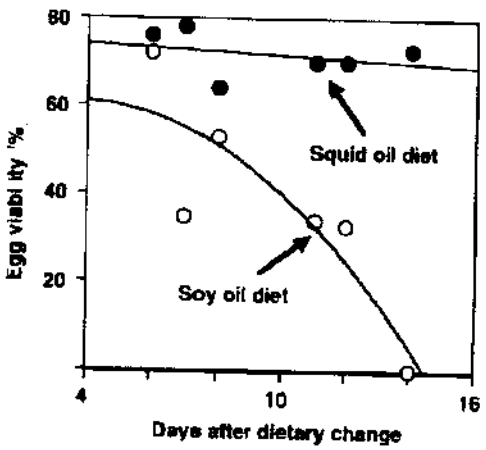


**Figure 3.** Effect of changes in dietary lipid on egg viability of gilthead sea bream (redrawn from Tandler et al. 1995).

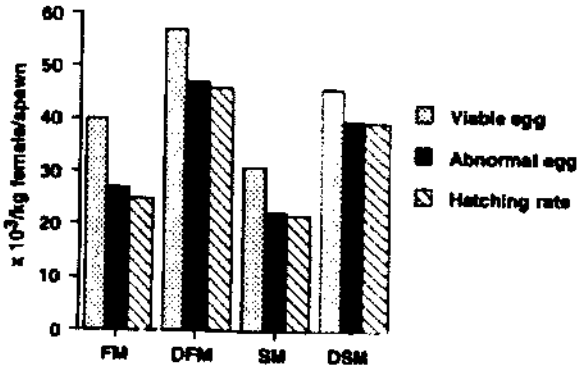


**Figure 4.** Spawn production of gilthead sea bream broodstock (redrawn from Fernandez-Palacios et al. 1997). FM denotes fish meal; DFM = defatted fish meal; SM = squid meal; DSM = defatted squid meal.

*Dicentrarchus labrax* (Cerda *et al.* 1994) by lowering the protein level from 50 to 35%. An optimum protein level for broodstock diet was estimated to be around 45% for red sea bream (Watanabe *et al.* 1984a). Cuttlefish meal and squid meal are superior to fish meal as protein sources for red sea bream (Watanabe *et al.* 1984b) and gilthead sea bream (Fernandez-Palacios *et al.* 1997) (Fig. 4). Watanabe *et al.* (1991a) showed that the effective component of cuttlefish meal is contained in nonfat-soluble fraction. Tandler *et al.* (1995) evaluated the importance of squid meal protein extract for gilthead sea bream by replacing it with equal amounts of casein or wheat gluten. The results suggest that the positive effect of squid protein could be attributed to its similarity in essential amino acid (EAA) composition to the egg protein. Based on this information, it was possible to improve the wheat gluten diet by supplementing it with an EAA profile which resembles that of the egg. Such diets resulted in a doubling of survival at 15 days compared with the wheat gluten diet

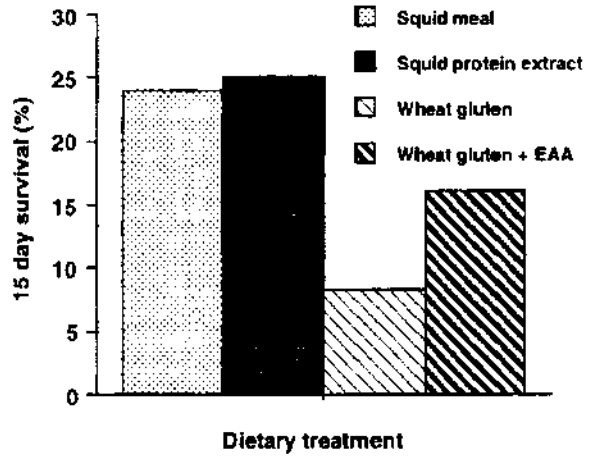


**Figure 5.** Effect of dietary protein on larval survival at day 15 after hatching. During 15 days of spawning, broodstock were fed diets containing a full squid protein extract or a wheat gluten-based diet or a wheat gluten-based diet supplemented with essential amino acids (EAA) which resembled those of the gilthead sea bream egg (redrawn from Tandler et al. 1995).

(Fig. 5). However, this was still lower than the full squid meal diet. The effect of protein supplementation of broodstock diets on egg quality was not found with changes in amino acid profiles (Tandler *et al.* 1995). Moreover, changes in the amino acid composition of eggs were small despite marked changes in protein quality of the broodstock diet (Tandler *et al.* 1995). Tandler *et al.* (1995) suggested that reduction in egg quality from broodstock fed an imbalanced EAA diet resulted from a change of the concentration at the vitellogenin (Vg) binding sites. For improvement of egg and larval quality, the protein should have a similar EAA composition to the egg protein and broodstock diet should contain 45-50% protein. Balanced dietary protein promotes Vg synthesis and uptake, which lead to high fecundity and egg quality (Tandler *et al.* 1995).

**Vitamin C (ascorbic acid)**

Ascorbic acid (AsA) is important in the process of sexual maturation as it plays a part in the biosynthesis process of gonadal steroid hormones (Sandnes 1984). Since AsA is essential for the biosynthesis of collagen in connective tissue, the AsA content in eggs before spawning is critical for normal development of the newly hatched larvae in seed production (Ikeda 1985). A diet containing very low levels of AsA has negative effects on the Japanese parrotfish *Oplegnathus fasciatus* (Ishibashi *et al.* 1994) and sardine *Sardinops melanosticta* (Akiyama *et al.* 1990). Ishibashi *et al.* (1994) showed that the gonadosomatic index (GSI) of female parrotfish was correlated to the AsA level in the diet, although the AsA content in the gonad was not correlated to the dietary AsA level. The number of eggs spawned by sardine broodstock fed a diet containing 8 mg/100 g AsA was significantly lower compared to those fed a diet containing 320 mg/100 g AsA (Akiyama *et al.* 1990). In the cod *Gadus morhua*, differences in free amino acid profile, egg strength, and neutral buoyancy were found between treatments of different levels of AsA in the diet, whereas no effects on vital parameters such as the fertilization rate and survival rate were observed (Mangor-Jensen *et al.* 1994).

**Vitamin E**

Vitamin E (VE) is known to be essential for reproduction of freshwater fishes such as ayu *Plecoglossus altivelis*, common carp *Cyprinus carpio*, and rainbow trout *Oncorhynchus mykiss* (Watanabe 1985). The viability and hatchability of red sea bream eggs (Watanabe *et al.* 1991b) (Fig. 6) and gilthead sea bream eggs (Fernandez-Palacios *et al.* 1996) was improved by raising dietary VE levels. In Japanese flounder, rates of fertilization and hatching were not affected by the addition of VE to the diet although egg production increased compared to the control (Takeuchi 1997). It is not clear if this phenomenon resulted from differences in VE requirement between flounder and sea breams or other factors. However, VE is suggested to play an important role in eggs and larvae of flounder since VE content in eggs is usually high but quickly decreases after hatching (Takeuchi 1997). Further studies are necessary to clarify the role of dietary VE in broodstock nutrition and egg production in the flounder and other species.

**Astaxanthin**

Feeding red sea bream frozen raw krill shortly before spawning is known to improve the

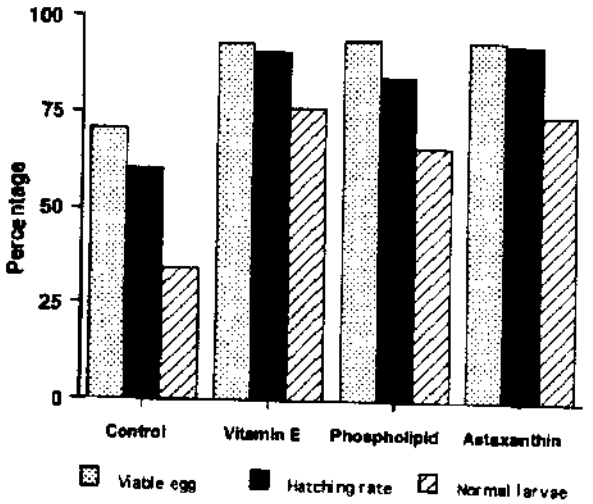


**Figure 6.** Effects of dietary supplemenation with vitamin E, phospholipid, and astaxanthin on egg and larval quality in red sea bream (drawn from data in Watanabe et al. 1991b).

egg quality. The effective components in krill were found in both the polar and nonpolar lipid fractions (Watanabe *et al.* 1991a, b). The krill oil contains polar and nonpolar fractions, mainly composed of phosphatidylcholine and triglyceride, respectively. This led to the postulation that the attributive components might be phosphatidylcholine in the polar and astaxanthin in the nonpolar fractions (Fig. 6). The egg quality of red sea bream was improved by a supplement of synthesized astaxanthin (Watanabe and Kiron 1995).

Astaxanthin, along with other carotenoids, vitamin E, and phospholipids are thought to act as quenchers or scavengers of singlet oxygens or other free radicals, i.e., they absorb the energy of these compounds within their extensive double bond structure, effectively preventing reactive damage to other molecules, particularly polyunsaturated fatty acids (Watanabe and Kiron 1995).

Recently, Verakunpiriya *et al.* (1997a) investigated whether addition of krill meal in a pelleted diet can improve the spawning performance of yellowtail *Seriola quinqueradiata* broodstock. Consequently, they observed that egg production and quality decreased with an increase of krill meal in the diets. They stated that an overdose of astaxanthin contained in krill meal may have negatively affected spawning performance. Verakunpiriya *et al.* (1997b) examined the supplementation effect of astaxanthin on the spawning performance of yellowtail by feeding diets containing various levels of synthesized astaxanthin. The results indicated that optimum astaxanthin level in broodstock diet for yellowtail is around 30 ppm.

The nutritional value of the broodstock diet considerably affects egg and larval quality. However, effective broodstock diet cannot be developed so long as the nutritional requirements of broodstock remain obscure. In particular, the effect of micronutrients such as AsA, VE, and astaxanthin on spawning performance and egg quality is different among species. Studies on other nutrients, such as vitamin A, are not available despite its important role in development. When little is known of the specific broodstock nutritional requirements, a practical composition of broodstock diet could be based on the general requirement of each species. Supplementation of components such as vitamins may be required for further enhancement of the nutritional quality of broodstock diets. More research effort is required on broodstock nutrition, oocyte maturation, and larval development for the improvement of mass production technology.

## LITERATURE CITED

Akiyama, T., M. Shiraishi, T. Yamamoto, and K. Hirose. 1990. Effects of dietary ascorbic acid at the maturation and spawning time in sardine *Sardinops melanosticta*. Abstract. Meet. Jpn. Fish. Sci., Tokyo, p.110. [In Japanese].

Bell, J. G., B. M. Farndale, M. P. Bruce, J. M. Navas, and M. Carillo. 1997. Effects of broodstock dietary lipid on fatty acid compositions of eggs from sea bass (*Dicentrarchus labrax*). Aquaculture 149: 107-119.

Bromage, N. R. 1995. Broodstock management and seed quality- general considerations, pp. 1-24. *In:* N. R. Bromage and R. J. Roberts (eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford.

Cerda, J., M. Carrillo, S. Zanuy, J. Ramos, and M. de la Higuera. 1994. Influence of nutritional composition of diet on sea bass, *Dicentrarchus labrax* L., reproductive performance and egg and larval quality. Aquaculture 128: 345-361.

Fernandez-Palacios, H., M. Izquierdo, L. Robaina, A. Valencia, M. Salhi, and J. M. Vergara. 1995. Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata* L.). Aquaculture 132: 325-337.

Fernandez-Palacios, H., M. S. Izquierdo, M. Gonzalez, L. Robaina, and A. Valencia. 1996. Combined effect of dietary tocopherol and n-3 HUFA on egg quality of gilthead sea bream broodstock (*Sparus aurata*). Abstract. Int. Symp. Nutr. Feed. Fish, College Station, TX.

Fernandez-Palacios, H., M. S. Izquierdo, L. Robaina, A. Valencia, M. Salhi, and D. Montero. 1997. The effect of dietary protein and lipid from squid and fish meals on egg quality of broodstock for gilthead seabream (*Sparus aurata*). Aquaculture 148: 233-246.

Furuita, H., T. Takeuchi, M. Toyota, and T. Watanabe. 1996a. EPA and DHA requirements in early juvenile red sea bream using HUFA enriched *Artemia* nauplii. Fish. Sci. 62: 246-251.

Furuita, H., T. Takeuchi, T. Watanabe, H. Fujimoto, S. Sekiya, and K. Imaizumi. 1996b. Requirements of larval yellowtail for eicosapentaenoic acid, docosahexaenoic acid and n-3 highly unsaturated fatty acids. Fish. Sci. 62: 372-379.

Ikeda, S. 1985. Vitamins, pp. 43-53. *In:* Y. Yone (ed.), Fish Nutrition and Diets. Koseisha Koseikaku, Tokyo. [In Japanese].

Ishibashi, Y., K. Kato, S. Ikeda, O. Murata, T. Nasu, and H. Kumai. 1994. Effect of dietary ascorbic acid supplementation on gonadal maturation in Japanese parrot fish. Suisanzoshoku 42: 279-285.

Mangor-Jensen, A., J. C. Holm, G. Rosenlund, O. Lie, and K. Sandnes. 1994. Effects of dietary vitamin C on maturation and egg quality of cod *Gadus morhua* L. J. World Aquacult. Soc. 25: 30-40.

Mourente, G. and J. M. Odriozola. 1990. Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus surata* L.). Fish Physiol. Biochem. 8: 93-101.

Navas, J. M., M. Thrush, J. Ramos, M. Bruce, M. Carrillo, S. Zanuy, and N. Bromage. 1995. The effect of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass (*Dicentrarchus labrax*). Proc. Fifth Int. Symp. Reprod. Physiol. Fish, Austin, TX.

Parrish, C. C., J. A. Brown, E. S. Daniel, and D. C. Somerton. 1994. Fatty acid composition of Atlantic halibut eggs in relation to fertilization. Bull. Aquacult. Assoc. Canada 94: 36-38.

Sandnes, K. 1984. Some aspects of ascorbic acid and reproduction in fish, pp. 206-212. *In:* I. Wegger, F. J. Tagwerker, and J. Moustgaard (eds.), Ascorbic Acid in Domestic Animals. The Royal Danish Agriculture Society, Copenhagen.

Takeuchi, T.. 1997. Nutritional requirement for improvement of rearing seed production, pp. 96-106. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseisha Koseikaku, Tokyo. [In Japanese].

Tandler, A., M. Harel, W. M. Koven, and S. Kolkovski. 1995. Broodstock and larval nutrition in gilthead seabream *Sparus aurata* - new findings on its mode of involvement in improving growth, survival and swimbladder inflation. Isr. J. Aquacult. 47: 95-111.

Verakunpiriya, V., K. Watanabe, K. Mushiake, K. Kawano, T. Kobayashi, I. Hasegawa, V. Kiron, S. Satoh, and T. Watanabe. 1997a. Effect of krill meal supplementation in soft-dry pellets on spawning and quality of egg of yellowtail. Fish. Sci. 63: 433-439.

Verakunpiriya, V., K. Mushiake, K. Kawano, and T. Watanabe. 1997b. Supplemental effect of astaxanthin in broodstock diets on the quality of yellowtail eggs. Fish. Sci. 63: 816-823.

Watanabe, T. 1985. Importance of the study of broodstock nutrition for further development

of aquaculture, pp. 395-414. *In:* C. B. Cowey, A. M. Mackie, and J. G. Bell (eds.), Nutrition and Feeding in Fish. Academic Press, London.

Watanabe, T. 1993. Importance of docosahexaenoic acid in marine larval fish. J. World Aquacult. Soc. 24: 152-161.

Watanabe, T. and V. Kiron. 1995. Red sea bream (*Pagrus major*), pp. 398-413. *In:* N. R. Bromage and R. J. Roberts (eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford.

Watanabe, T., T. Arakawa, C. Kitajima, and S. Fujita. 1984a. Effect of nutritional quality of broodstock diets on reproduction of red sea bream. Bull. Jpn. Soc. Sci. Fish. 50: 495-501.

Watanabe, T., A. Itoh, C. Kitajima, and S. Fujita. 1984b. Effect of dietary protein levels on reproduction of red sea bream. Bull. Jpn. Soc. Sci. Fish. 50: 1015-1022.

Watanabe, T., A. Itoh, A. Murakami, Y. Tsukashima, C. Kitajima, and S. Fujita. 1984c. Effect of nutritional quality of diets given to broodstock on the verge of spawning on reproduction of red sea bream. Bull. Jpn. Soc. Sci. Fish. 50: 1023-1028.

Watanabe, T., M.-J. Lee, J. Mizutani, T. Yamada, S. Satoh, T. Takeuchi, N. Yoshida, T. Kitada, and T. Arakawa. 1991a. Effective components in cuttlefish meal and raw krill for improvement of quality of red seabream *Pagrus major* eggs. Nippon Suisan Gakkaishi 57: 681-694.

Watanabe, T., T. Fujimura, M.-J. Lee, K. Fukusho, S. Satoh, and T. Takeuchi. 1991b. Effect of polar and nonpolar lipids from krill on quality of eggs of red seabream *Pagrus major*. Nippon Suisan Gakkaishi 57: 695-698.

# SEQUENTIAL UTILIZATION OF FREE AMINO ACIDS, YOLK PROTEIN, AND LIPIDS BY DEVELOPING EMBRYOS AND LARVAE IN BARFIN FLOUNDER *VERASPER MOSERI*

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## ABSTRACT

Changes in contents of free amino acids (FAA), lipovitellin (Lv) which is the major yolk protein in ovulated egg, and lipids were examined in developing embryos and larvae of barfin flounder *Verasper moseri* to elucidate the sequential utilization of these nutrient stocks before first feeding. Hatching takes place on the 10th day after fertilization at a water temperature of 8°C, and the hatched larvae almost absorb their entire yolk sacs within 11 days after hatching. Total FAA content showed no change during the first 4 days, then decreased to about 13% of the initial level by the 13th day after fertilization. During the process, non-essential amino acids tended to decrease faster than essential amino acids. The lipovitellin content, measured by quantitative immunodiffusion using antiserum against 170 kDa Lv of ovulated eggs, was approximately stable during the 13 days after fertilization, then decreased rapidly until the end of yolk sac absorption. Phospholipids, which seemed to be bound with Lv apo-proteins, decreased gradually after hatching, coinciding with the decrease of Lv. From these results, we consider the following four periods for the sequential nutrient utilization in barfin flounder embryos and larvae: (1) before FAA utilization period, 0-4th day; (2) FAA utilization period, 4-10th day; (3) switching period, 10-13th day; and (4) Lv and phospholipid utilization period, 13-21st day.

## INTRODUCTION

Yolk nutrient stocks of a teleost egg are utilized as a source for energy metabolism and for embryonic body construction as in other oviparous animals. Generally in fish, carbohydrate, lipid, and protein are consumed prior to hatching for energy production, while lipid and protein catabolism predominates after hatching (Heming and Buddington 1988). Especially neutral lipids, such as triglyceride (TG) and wax ester, are considered to be the most important energy reserves in fish eggs (Vetter and Hodson 1983, Heming and Buddington 1988).

Recently, in some marine pelagic egg spawners, free amino acids (FAA) are suggested to be consumed as an important fuel during the energy metabolism of developing embryos and larvae (for review see Rønnestad and Fyhn, 1993). In Atlantic cod (Finn et al. 1995a) and Atlantic halibut (Finn et al. 1995c), whose eggs have no visible oil globules, amino acids (FAA and protein) are considered to be the main substrate for energy metabolism. However, it is difficult to directly determine the protein utilization by biochemical measurements of whole eggs and larvae, because it determines only the net sum of a declining yolk protein and an increasing body tissue protein (Heming and Buddington 1988).

Matsubara and Koya (1997) demonstrated the occurrence of yolk proteolysis during oocyte maturation in barfin flounder that spawn pelagic eggs having no visible oil globule. The maturation-associated yolk proteolysis provides FAA and monomeric lipovitellin (Lv, molecular mass: 170kDa) in matured eggs. In the present study, we analyzed quantitative change of FAA and Lv during development in barfin flounder to clarify the pattern of these nutrient stocks. Furthermore, we analyzed quantitative change of phospholipids (PL) and TG, which are suggested to be the major lipid classes being catabolized by embryos and larvae in Atlantic halibut (Rainuzzo et al. 1992) and Atlantic cod (Fraser et al. 1988, Finn et al. 1995b).

## MATERIALS AND METHODS

The adult male and female barfin flounders used in the present study were kept in a 40- kL aquaria at Akkeshi Station, Japan Sea-Farming Association (JASFA), in Hokkaido. A total of five groups of fertilized eggs (A to E) were obtained from different females during the April spawning season in 1995 and 1996. Eggs were artificially fertilized and incubated in a flow-through hatching cylinder at a temperature of 8°C. Under these conditions, hatching occurred on the 10th day after fertilization, and the hatched larvae absorbed their yolk sacs within 11 days after hatching. Eggs and unfed larvae were sampled at the time just before fertilization, and on the 2nd, 4th, 6th, 8th, 10th, 13th, 16th, and 19th day after fertilization. Measurement of Lv, ammonia, and lipid contents were carried out on all five series (A to E), while FAA content was measured on three series (A to C).

For the analyses of FAA and ammonia contents, samples of 10 eggs or larvae were homogenized in 0.25 ml of 6% trichloroacetic acid. After centrifugation at 10,000 rpm for 10 min, the supernatants were collected and mixed with 0.5 ml of diethyl ether. The mixture was then centrifuged again at 2500 rpm for 3 min and the lower phase was used for FAA and ammonia analyses. Amino acids were analyzed using a Shimadzu LC-10A analyzing system as described by Matsubara and Koya (1997). Ammonia concentration was determined by the phenol-hypochlorite method (Ammonia Test Kit, Wako).

For the measurement of Lv content, samples of eggs and larvae were homogenized at 10 individuals/ml in 0.9% NaCl solution. After centrifugation at 10,000 rpm for 10 min, the supernatants were collected. The Lv concentrations in egg and larvae homogenates were determined by the method of Mancini et al. (1965) using antiserum against 170 kDa Lv of ovulated eggs as described by Matsubara and Koya (1997).

Lipids were extracted with 0.3 ml of ethanol-diethyl ether (3:1) from the samples of 10 eggs or larvae. The supernatants were collected after centrifugation at 6000 rpm for 10 min. Phospholipid (PL) and triglyceride (TG) in the supernatants were quantified using enzymatic procedures (Phospholipid B-Test Kit and Triglyceride G-Test Kit, Wako).

Statistical analysis was carried out by Duncan's multiple range test. Significance was accepted at $p < 0.05$.

## RESULTS AND DISCUSSION

The total FAA content in an ovulated but unfertilized egg of barfin flounder was 267 nmol/egg, corresponding to about 35 µg/egg (Fig. 1). The FAA in an egg is suggested to be provided by degradations of yolk proteins during the final oocyte maturation (Matsubara and Koya 1997). The increased FAA seems to play an important role in acquiring the buoyancy of eggs as an osmotic effecter for oocyte hydration. The FAA content of eggs showed no change during the first 4 days after fertilization, then decreased rapidly to about 13% of the initial level by the 13th day. The 4th-day embryo almost completed epiboly and was at the stage of early somite formation, and the 13th-day larva was at the stage of appearance of pectoral fins.

The ammonia content in an unfertilized egg was 6.4 nmol/egg (Fig. 2). It increased rapidly from the 4th to 8th day after fertilization and reached a peak of 44 nmol/egg, coinciding with

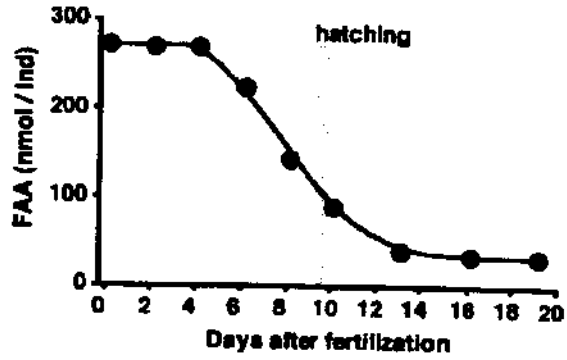


**Figure 1.** Changes in the total content of 16 free amino acids (FAA) of developing eggs and larvae in barfin flounder. Data are presented as mean of three samples (series A-C). Hatching is represented by a vertical shaded bar.

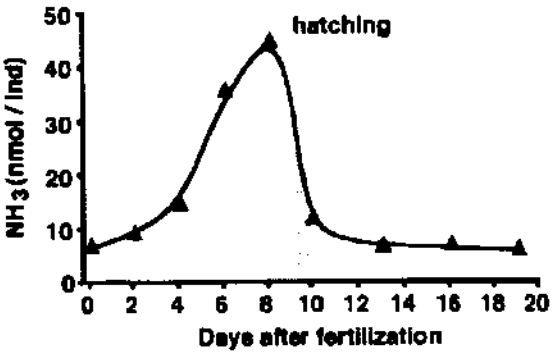


**Figure 2.** Ammonia content of developing eggs and larvae in barfin flounder. Data are presented as mean of five samples (series A-E). Hatching is represented by a vertical shaded bar.

the decrease of total FAA content (Fig. 2). From these results, FAA seems to be utilized as substrates in energy metabolism with the production of ammonia as other pelagic eggs of marine teleosts (see review by Rønnestad and Fyhn 1993). After hatching, the larval ammonia content decreased rapidly. The decrease of ammonia content after hatching is suggested to be due to excretion (Rønnestad and Fyhn1993).

Figure 3 shows changes in the contents of individual FAA of developing eggs and larvae. Among these, leucine, alanine, lysine, and serine were quantitatively dominant. We classified leucine, threonine, lysine, valine, isoleucine, arginine, histidine, methionine, phenylalanine, and tyrosine as essential amino acids (EAA) according to the classification of Wilson (1985). Although the content of all FAA decreased following the progression of development, decline of each FAA was not equally shared between EAA and non-essential amino acids (NEAA). The contents of all NEAA showed rapid decrease compared with EAA, and decreased to less than 10% of initial level by hatching. On the other hand, most EAA seemed to decrease at a slower rate than NEAA, especially in tyrosine and phenylalanine. There likely seems to be selective utilization of FAA during the egg stages in barfin flounder as mentioned by Rønnestad et al. (1993). However, no selective utilization of NEAA was observed in some marine fish such as Atlantic cod (Rønnestad and Fyhn 1993, Finn et al. 1995a).

In the present study, we also measured Lv contents in eggs and larvae using quantitative immunodiffusion (Fig. 4). The Lv content of unfertilized egg was 82 μg/egg and approximately stable during the 13 days after fertilization. The Lv content then began to decrease significantly until the end of yolk sac absorption. Thus, utilization of Lv for body protein synthesis and substrates for energy supply is suggested to occur during the late stage of development in barfin flounder. The beginning of Lv utilization from the 13th day after fertilization coincides well with the end of total FAA decrease. Therefore, it is suggested that the source of amino acid supply of barfin flounder larva shifts from the FAA pool to Lv at the time of exhaustion of the FAA pool as mentioned by Rønnestad et al. (1993).

Generally, TG and wax ester (neutral lipids) are the most important energy reserves of developing fish on a caloric basis. In contrast, some marine species, such as Atlantic cod and Atlantic halibut which have PL-rich eggs and relatively low levels of total lipid, appear to use PL as a major lipid substrate in developing embryos and larvae (Fraser et al. 1988, Rainuzzo et al. 1992, Finn et al. 1995b, c). The contents of PL and TG in an unfertilized egg of barfin flounder were 14.9 μg/egg and 2.0 μg/egg, respectively (Fig. 5). The PL content was approximately stable before hatching, then gradually decreased until the 19th day. The content of PL on the 19th day was significantly lower ($p<0.05$) than those of egg stages. On the other hand, no significant change occurred in TG content during the 19 days. Thus, the barfin flounder larvae also use PL as a major lipid substrate. Nakagawa and Tsuchiya (1971, 1972) describe two states of major lipid in eggs of rainbow trout: one is free lipids accumulated in oil globules, and the other is bound lipids binding to lipoproteins. However, barfin flounder eggs have no oil globule, and Lv contains about 15% of PL and 4% of TG (Matsubara and Sawano 1995). In addition, the decrease of PL coincides well with the decrease of Lv after hatching (Figs. 4, 5). Therefore, most of the PL in barfin flounder egg is thought to bind

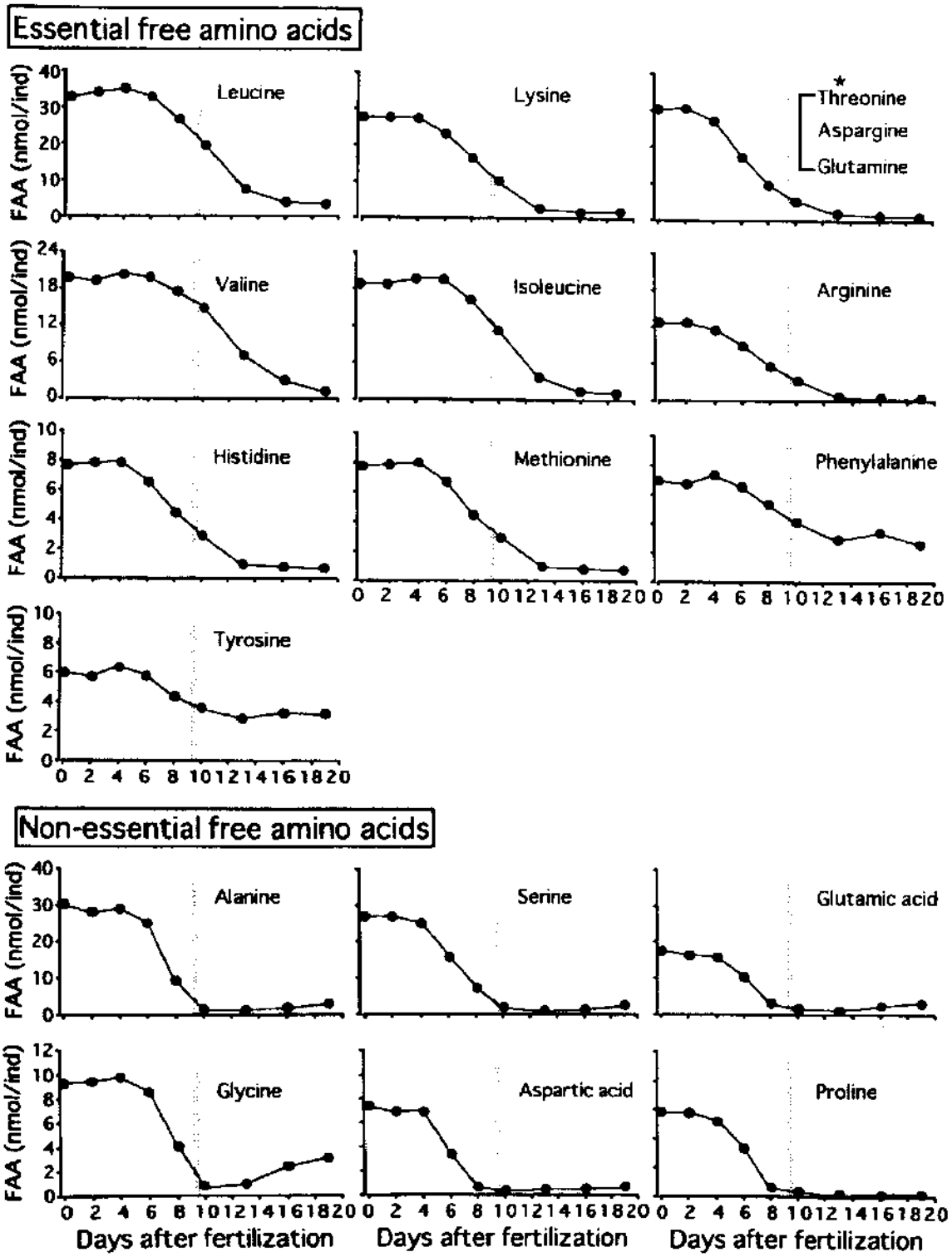


**Figure 3.** Changes in the content of individual free amino acids (FAA) of developing eggs and larvae in barfin flounder. Hatching is represented by vertical shaded bars. *This fraction was a mixture of threonine, asparagine, and glutamine. We measured the content of this fraction by using threonine as standard.

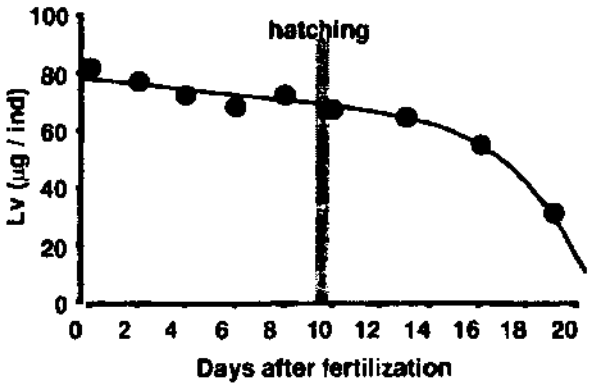


**Figure 4.** Changes in lipovitellin (Lv) content of developing eggs and larvae in barfin flounder. Hatching is represented by a vertical shaded bar.

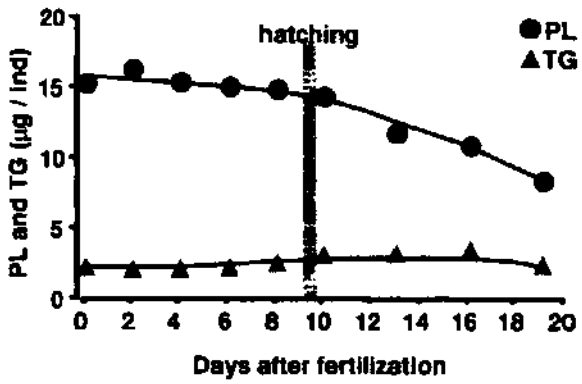


**Figure 5.** Changes in the contents of phospholopid (PL) and triglyceride (TG) of developing eggs and larvae in barfin flounder. Hatching is represented by a vertical shaded bar.

to Lv apoprotein and to become available after Lv degradation.

From these results on the analysis of biochemical composition of eggs and larvae in barfin flounder, we consider the following four periods for the sequential nutrient utilization in barfin flounder embryos and larvae: (1) before FAA utilization period, 0-4th day; (2) FAA utilization period, 4-10th day; (3) switching period, 10-13th day; and (4) Lv and PL utilization period, 13-21st day. Furthermore, many parts of these nutrient stocks are suggested to be supplied by Lv and other yolk proteins. These findings provide useful information for improvement in nutrition of embryos and larvae before first feeding in aquaculture, as well as for research in clarifying the nutritional requirement in developing larvae.

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## LITERATURE CITED

Finn, R.N., H.J. Fyhn, and M.S. Evjen. 1995a. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). 1. Respiration and nitrogen metabolism. Mar. Biol. 124: 355-369.

Finn, R.N., J.R. Henderson, and H.J. Fyhn. 1995b. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). 2. Lipid metabolism and enthalpy balance. Mar. Biol. 124: 371-379.

Finn, R.N., I. Rønnestad, and H.J. Fyhn. 1995c. Respiration, nitrogen and energy metabolism of developing yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.). Comp. Biochem. Physiol. 111A: 647-671.

Fraser, A.J., J.C. Gamble, and J.R. Sargent. 1988. Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). Mar. Biol. 99: 307-313.

Heming, T.A. and R.K. Buddington. 1988. Yolk absorption in embryonic and larval fishes, pp. 407-446. *In:* W.S. Hoar. and D.J. Randall. (eds.), Fish Physiology. Vol. 11A. Academic Press, New York.

Mancini, G., A.O. Carbonara, and J.F. Helemans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2: 235-254.

Matsubara, T. and Y. Koya. 1997. Course of proteolytic cleavage in three classes of yolk proteins during oocyte maturation in barfin flounder *Verasper moseri*, a marine teleost spawning pelagic eggs. J. Exp. Zool. 278: 189-200.

Matsubara, T. and K. Sawano. 1995. Proteolytic cleavage of vitellogenin and yolk proteins during vitellogenin uptake and oocyte maturation in barfin flounder (*Verasper moseri*). J. Exp. Zool. 272: 34-45.

Nakagawa, H. and Y. Tsuchiya. 1971. Studies on rainbow trout egg. III. Determination of lipid composition of oil globule and lipoprotein. J. Fac. Fish. Anim. Husb. Hiroshim. Univ. 10: 11-19.

Nakagawa, H. and Y. Tsuchiya. 1972. Studies on rainbow trout egg. IV. Changes of yolk content during embryogenesis. J. Fac. Fish. Anim. Husb. Hiroshim. Univ. 11: 111-118.

Rainuzzo, J.R., K.I. Reitan, and L. Jørgensen. 1992. Comparative study on the fatty acid and lipid composition of four marine fish larvae. Comp. Biochem. Physiol. 103B: 21-26.

Rønnestad, I. and H.J. Fyhn. 1993. Metabolic aspect of free amino acids in developing marine fish eggs and larvae. Rev. Fish. Sci. 1: 239-259.

Rønnestad, I., E.P. Groot, and H.J. Fyhn. 1993. Compartmental distribution of free amino acids and protein in developing larvae of Atlantic halibut (*Hippoglossus hippoglossus*). Mar. Biol. 116: 349-354.

Vetter, R.D. and R.E. Hodson. 1983. Energy metabolism in rapidly developing marine fish egg, the red drum (*Sciaenops ocellata*). Can. J. Fish. Aquat. Sci. 40: 627-634.

Wilson, R.P. 1985. Amino acids and protein requirement of fish, pp. 1-16. *In:* C.B. Cowey, A. Mackie, and J.B. Bell. (eds.), Nutrition and Feeding in Fish. Academic Press, London.

# EFFECTS OF MICROALGAE AND LIVE DIET TYPE ON THE GROWTH OF FIRST-FEEDING WINTER FLOUNDER *(PLEURONECTES AMERICANUS)*

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## ABSTRACT

The addition of microalgae to larval rearing systems ("green water" treatment), has been shown to enhance the growth and survival of certain marine fish species. Along with the presence or absence of microalgae, diet type affects larval growth, and several studies have demonstrated that cultured live food organisms (e.g. rotifers and brine shrimp) are nutritionally inferior to wild zooplankton as a first food for marine finfish larvae. In a 2 x 2 factorial design experiment that lasted for five weeks from first feeding, we examined the effects of green water, clear water, wild zooplankton and cultured rotifers (*Brachionus plicatilis*) on the growth of winter flounder (*Pleuronectes americanus*) larvae. Results from the two way analysis of variance indicated that there was no significant interaction ($P=0.80$) between the two factors (presence/absence of microalgae, wild/cultured prey). Therefore, we considered the two factors independently of each other. At any time, fish in the green water treatments were significantly longer ($P<0.05$) than those in clear water treatments. There were no differences ($P>0.05$) in larval lengths between food types within either green or clear water. The mean instantaneous growth rates (length increases per week) were 15.4, 14.2, 12.2, and 9.6% for green water/wild zooplankton, green water/rotifers, clear water/rotifers, and clear water/wild zooplankton, respectively. Results of this study indicate that green water enhances the growth of winter flounder larvae, and there is little, if any, difference between wild zooplankton and rotifers as a first feeding diet.

## INTRODUCTION

Domestic and overseas demand for high quality flatfish, combined with the declining harvest from wild populations, has greatly increased interest in the culture of various flounder species (Waters 1996). Those being considered for commercial aquaculture along the Atlantic coast of the United States include summer flounder (*Paralichthys dentatus*), yellowtail flounder (*Pleuronectes ferruginea*), and southern flounder (*Paralichthys lethostigma*). In addition to these, winter flounder (*Pleuronectes americanus*) is also being considered because many of the techniques for culturing this species have been developed. These include the technique for the artificial spawning of captive broodstock (Smigielski and Arnold 1972), larval rearing (Smigielski 1975; Rogers 1976; Laurence 1977; Klein-MacPhee et al. 1982, 1993), and the successful weaning of juveniles onto formulated diets (Lee and Litvak 1996).

The problems that have impeded commercial culture of many finfish species center around low larval survival and growth, particularly at the time of first feeding. The use of live food in the culturing of the early life stages of marine fish larvae, including winter flounder, is currently consid-

| Week | Algal Presence Green vs. Clear | Food Type Rotifers vs. Wild |
|---|---|---|
| | *p-value* | *p-value* |
| 1 | **0.0047** | **0.7459** |
| 1 | **0.0006** | **0.7558** |
| 3 | **0.0071** | **0.9832** |
| 4 | **0.0034** | **0.9748** |
| 5 | **0.0064** | **0.6046** |

**Table 1.** Analysis of algal presence and food type on winter flounder growth. P-values derived from unpaired T-tests.

| Week | Green | | Clear | | Rotifers | | Wild | |
|---|---|---|---|---|---|---|---|---|
| | mean +/-sd | %/wk | mean +/-sd | %/wk | mean +/-sd | %/wk | mean +/-sd | %/wk |
| 1 | 5.1 +/-0.10 | 17 | 4.5 +/-0.17 | 5 | 4.7 +/-0.45 | 10 | 4.8 +/-0.26 | 12 |
| 2 | 6.2 +/-0.38 | 19 | 5.3 +/-0.21 | 17 | 5.8 +/-0.76 | 20 | 5.6 +/-0.38 | 15 |
| 3 | 7.4 +/-0.50 | 18 | 5.9 +/-0.41 | 10 | 6.5 +/-0.85 | 12 | 6.6 +/-1.07 | 15 |
| 4 | 8.1 +/-0.26 | 9 | 6.6 +/-0.45 | 10 | 7.2 +/-0.66 | 10 | 7.2 +/-1.16 | 10 |
| 5 | 9.1 +/-0.64 | 11 | 7.0 +/-0.60 | 6 | 7.5 +/-1.07 | 4 | 8.1 +/-1.5 | 12 |
| Mean instantaneous growth rate | | 15 | | 10 | | 11 | | 13 |

**Table 2.** Mean lenghts (mm) and instantaneous growth rates (%/wk) for replicates of green water, clear water, rotifer, and wild zooplankton treatments. sd = standard deviation.

| Week | Green/Wild | | Green/Rotifers | | Clear/Wild | | Clear/Rotifers | |
|---|---|---|---|---|---|---|---|---|
| | mean +/-sd | %/wk | mean +/-sd | %/wk | mean +/-sd | %/wk | mean +/-sd | %/wk |
| 1 | 5.1 +/-0.10 | 17 | 5.2 +/- * | 19 | 4.6 +/-0.00 | 7 | 4.5 +/-0.30 | 5 |
| 2 | 6.0 +/-0.10 | 16 | 6.6 +/- * | 24 | 5.3 +/-0.00 | 14 | 5.4 +/-0.40 | 18 |
| 3 | 7.4 +/-0.70 | 21 | 7.4 +/- * | 11 | 5.7 +/-0.10 | 7 | 6.1 +/-0.60 | 12 |
| 4 | 8.2 +/-0.30 | 10 | 7.9 +/- * | 7 | 6.3 +/-0.40 | 10 | 6.9 +/-0.40 | 12 |
| 5 | 9.3 +/-0.80 | 13 | 8.7 +/- * | 10 | 7.0 +/- 0.90 | 10 | 7.0 +/-0.50 | 14 |
| Mean instantaneous growth rate | | 15 | | 14 | | 10 | | 12 |

**Table 3.** Mean lenghts (mm) and instantaneous growth rates (%/wk) for all four treatments. sd = standard deviation. * = no sd due to loss of a replicate.

ered obligatory. The most widely used live food organisms are cultured rotifers, such as *Brachionus plicatilis*, and brine shrimp (*Artemia salina*) nauplii. While the use of these two prey species is common, they are relatively expensive because of the labor involved in their production (Ehrlich and Rust 1989). Le Ruyet et al. (1993), for example, have calculated that live prey feeding (mainly *Artemia*) represented 79% of the total production cost of a 45 day old sea bass. A second, and critical disadvantage of cultured live foods (*Brachionus* and *Artemia*), is that they do not provide optimal larval nutrition, largely due to low levels of essential fatty acids (Watanabe et al. 1983a, Witt et al. 1984, Leger et al. 1986, van der Meeren et al. 1993). For this reason, fish larvae reared on cultured foods often exhibit abnormal development, poor growth, and low survival (Watanabe et al. 1980, Fujita et al. 1980, van Ballaer et al. 1985, Izquierdo et al. 1989, Koven et al. 1990, van der Meeren 1991c). Methods of improving the nutritional quality of cultured foods, via enrichment with highly unsaturated fatty acids (HUFA), are available (Watanabe et al. 1983b), but they add to the cost of live food production. Natural (wild) live foods (primarily copepods) have been used with greater success (Naas et al. 1987, Ellertsen et al. 1981, van der Meeren 1991b, Le Ruyet et al. 1993), because they are generally richer in essential fatty acids (Pedersen 1993). In Atlantic cod for example, larvae fed diverse assemblages of wild zooplankton in both semi-intensive (van der Meeren 1991b, Otterå 1993, van der Meeren and Naess 1993) and extensive systems (Øiestad et al. 1985, Skjolddal et al. 1990, Bløm et al. 1991) have generally displayed good survival and growth.

In addition to the use of live larval food organisms, whether cultured or wild, the addition of microalgae species to larval rearing tanks ("green water" treatment) has been widely accepted as a technique for commercial marine finfish production. The addition of microalgae has enhanced larval growth and survival of a number of species, including turbot (Howell 1979; Scott & Middleton 1979; Jones et al. 1981; Bromley & Howell 1983; Reitan et al. 1993), halibut (Naas et al. 1992; Bergh et al. 1994), summer flounder (Alves et al. 1997), cod (Pedersen et al. 1989; van der Meeren 1991a), and grunion (Vasquez-Yeomans et al. 1990). While the mechanism(s) by which the microalgae improve growth and survival remains unclear, and may differ among both microalgal and fish species, several hypotheses have been proposed to explain their positive effects at first-feeding. They may provide nutritional benefits either directly via ingestion and absorption (Moffatt 1981), or indirectly by increasing the amounts of essential fatty acids in the rotifers being fed to the fish larvae (Reitan et al. 1993). Microalgae may also trigger digestion processes in the larvae (Hjelmeland et al. 1988). In addition to nutritional benefits, microalgae may inhibit pathogenic bacteria (Austin et al. 1992), influence the establishment of intestinal microflora (Skjermo & Vadstein 1993), and stabilize water quality (Houde 1975, 1978). It has also been suggested by Naas et al. (1992) that microalgae can change ambient light conditions in the larval tanks, which may, in turn, lead to an increase in the consumption of zooplankton at first-feeding.

Because first-feeding diet (live food type) and the addition of microalgae have both been shown to influence larval growth and survival, and because we are unaware of any published work that has examined these variables for winter flounder, we set out to determine if live food type and the presence or absence of microalgae effected the growth and survival of winter flounder larvae. In this research, the following null hypotheses were tested: (1) the addition of microalgae to larval rearing systems has no effect on the growth of winter flounder; and (2) there is no difference in the growth between first feeding winter flounder larvae fed live, laboratory cultured rotifers, and those fed a diet of wild zooplankton.

## MATERIALS AND METHODS

A 2 X 2 factorial design experiment with two replicates per treatment was initiated in May 1996 and lasted for five weeks from first feeding to metamorphosis. Two hundred, five day post-hatch winter flounder larvae were stocked into each of eight 20 liter, tapered, round, gray, plastic aquaria measuring 32 cm high, with a 43 cm diameter top and 35 cm diameter bottom. A green water starvation treatment served as the control. Treatments were static with 50% water change (10 liters) every third day. Replacement seawater was filtered

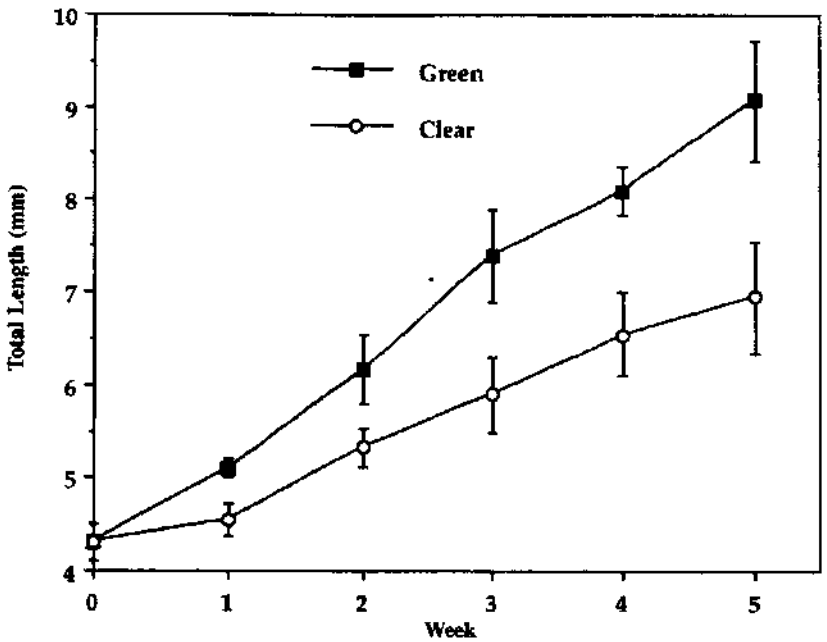


**Figure 1.** Winter flounder growth in the presence or absence of microalgae. Vertical bars represent +/- 1 standard deviation.

to remove particles greater than 5 μm and treated with UV light prior to addition to the experimental aquaria. Aquaria were submerged in a 13 cm deep flow through water table to maintain ambient seawater temperature. Temperature ranged from 8 to 15°C, with a daily average of 10°C over the course of the experiment. Salinity was maintained at 30-32 ppt. Larvae were exposed to 24 hours light using a 35 Watt fluorescent light suspended 76 cm above the aquaria.

Semi-continuous cultures of the microalga *Isochrysis galbana* (Tahitian strain) were maintained in 80 L fiberglass cylinders, and were provided with $f_2$ media (Guillard and Ryther 1962). Rotifers (L-type) were cultured in identical cylinders on a diet of *Isochrysis galbana* and dry yeast (at 1g/million rotifers/d). Cultured rotifers were fed to respective larval fish treatments at an average daily rate of 2600/l. Wild zooplankton were harvested from Portsmouth Harbor, New Hampshire by towing an 80 micron plankton net through the top two meters. Collected plankton were sieved through a 200 μm and a 48 μm screen, and counted. Wild zooplankton, consisting of approximately 90% copepod nauplii, were fed to respective treatments at an average daily rate of 2100/l. All treatments were visually inspected prior to feeding, and it was determined that larvae were fed to satiation based upon the presence of residual planktors. Three liters of *Isochrysis galbana* were added to treatments receiving green water every third day (with water changes) at a density of 200,000 cells/ml.

A random sample of ten larvae from each replicate in each treatment were measured to the nearest 0.5 mm (total length) each week. Mortalities were not replaced throughout the duration of the experiment. Mean length per replicate was used as the response variable. A two-way analysis of variance (ANOVA) was used to determine if there was any interaction between the two factors (prey type, presence/ absence of microalgae). Where possible, unpaired t-tests were used to compare lengths of larvae raised in different combinations of the two factors. Instantaneous growth rate, G (%/wk) was calculated using Ricker's (1979) formula: $G = (\ln Y_{i+1} - \ln Y_i)/(t_{i+1} - t_i)$, where $Y_i$ is length at time $t_i$. Survival between treatments was compared using one-way analysis of variance (ANOVA).

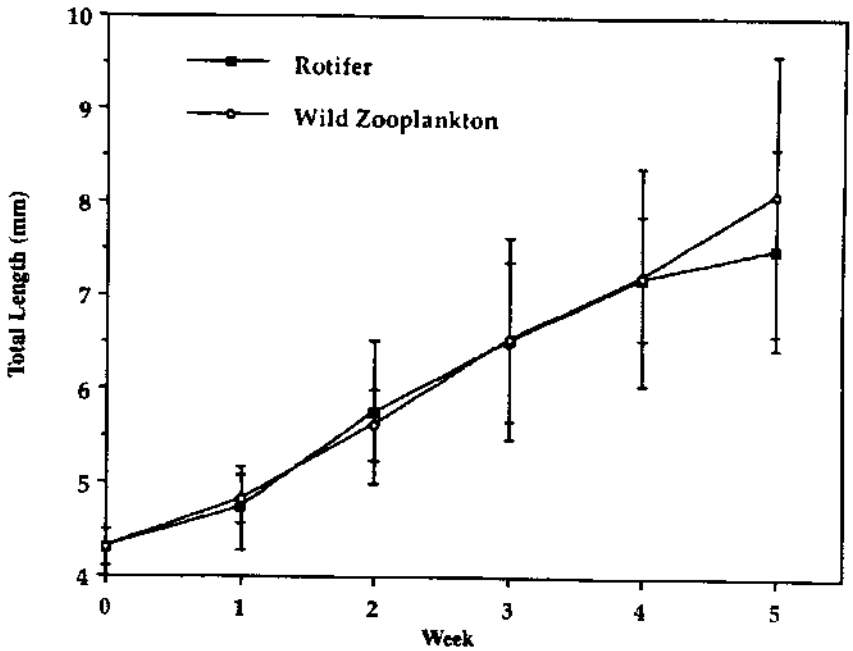


**Table 2.** Effects of food type on winter flounder growth. Vertical bars represent +/- 1 standard deviation.

## RESULTS

Results from the two way analysis of variance indicated that there was no significant interaction (P=0.80) between the two factors (presence/absence of microalgae, wild/cultured prey). Because of this lack of interaction, we were able to consider the two factors independently of one another. Larvae in treatments receiving microalgae (green water) were compared to in their absence (clear water). In this comparison, which disregarded food type, a highly significant difference (P<0.01) in total length was found in each week of the experiment (Table 1). Figure 1 shows the trends in mean length over the five weeks for larvae reared in the presence and absence of microalgae. Larvae in green water treatments grew to a mean length of 9.1 ± 0.64 mm at a mean instantaneous growth rate of 15%/week, while final mean length of those in clear water was 7.0 ± 0.60 mm, with a mean instantaneous growth rate of 10%/week (Table 2).

No significant difference (P>0.05) in length was found between larvae in replicates receiving cultured rotifers and those which were fed wild zooplankton, regardless of algal presence (Table 1). Figure 2 illustrates trends in length of larvae that were reared on cultured rotifers and wild zooplankton. Larvae in treatments fed rotifers grew to a mean length of 7.5 ± 1.1 mm at a mean instantaneous growth rate of 11%/week, while those fed wild zooplankton grew to a mean length of 8.1 ± 1.5 mm at a mean instantaneous growth rate of 13%/week (Table 2).

The loss of a replicate in the green water/rotifer treatment precluded us from making statistical comparisons between this treatment and others. We were however, able to compare final mean lengths of larvae reared in two combinations of food type and the presence/absence of microalgae. Final mean length of larvae raised in the clear water/rotifer combination (7.0 mm) was not significantly different (P>0.05) from that of larvae raised in the clear water/wild prey combination (7.0 mm) (Table 3). The final mean length of larvae raised in the green water/wild prey combination (9.3 mm) was significantly longer (P<0.05) than that of larvae in the clear water/wild prey combination (7.0 mm) (Table 3).

There was no significant difference (P>0.05) between final mean survival values which ranged from 13.5 to 22.0% (Figure 3). All larvae in the control (green water with no food) died by week two.

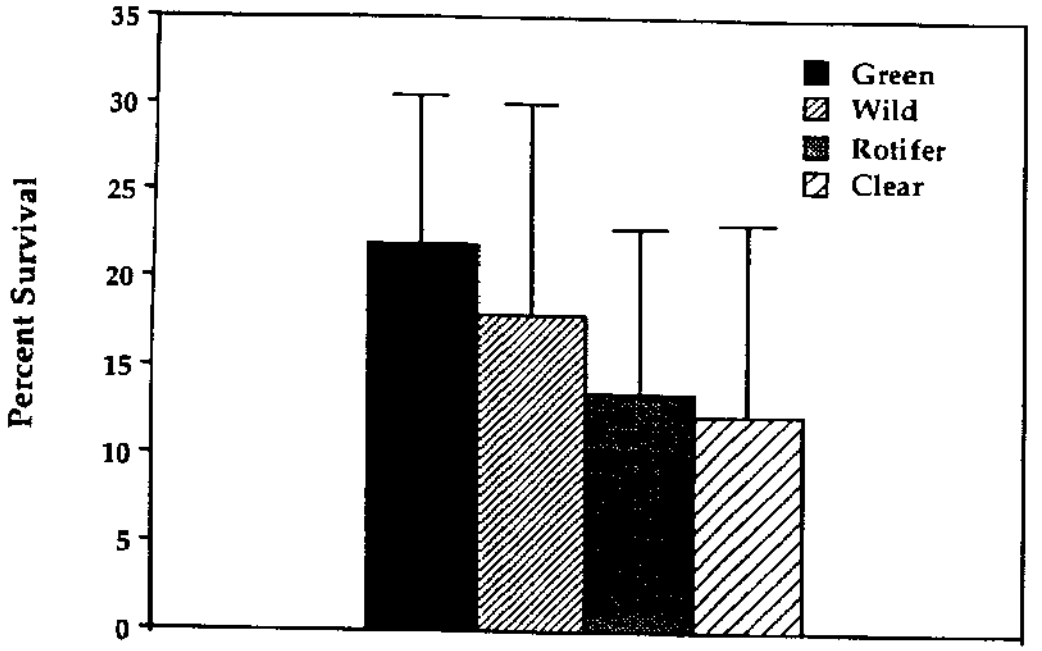


**Table 3.** The effects of food type and algal presence on winter flounder survival after five weeks. Vertical bars represent +/- 1 standard deviation.

## DISCUSSION

Growth of winter flounder larvae has been studied extensively, and has been found to vary with both temperature (Laurence 1975) and prey density (Laurence 1977). In general, growth in length of both laboratory-reared and wild-caught larvae is curvilinear (Pearcy 1962a; Bertram et al. 1996; Jerald et al. 1993), with growth being quickest during the first weeks after hatching, and then slowing as the fish approach metamorphosis. Although the weekly mean sizes we found in this study varied between treatments, all of our larvae grew at rates generally comparable to those reported for winter flounder larvae in nature (Pearcy 1962a), in the laboratory (Laurence 1975, 1977; Chambers and Leggett 1987; Jerald et al. 1993; Bertram et al. 1996), and in in-situ mesocosms (Laurence et al. 1979).

Results from this study demonstrated that the presence of microalgae significantly improved the growth of larval winter flounders. Similar results have been found for the larvae of other species, including turbot (*Scophthalmus maximus*), halibut (*Hippoglossus hippoglossus*), summer flounder (*Paralichthys dentatus*), cod (*Gadus morhua*), and grunion (*Leurestes tenuis*) (Howell 1979; Scott & Middleton 1979; Jones et al. 1981; Bromley & Howell 1983; Pedersen et al. 1989; Vasquez-Yeomans et al. 1990; van der Meeren 1991a; Naas et al. 1992; Reitan et al. 1993; Bergh et al. 1994; Alves et al. 1997). As seen in Table 1 and Figure 1, larvae reared in green water were significantly longer than those in clear water in each week of the experiment. Differences in instantaneous growth rates were most pronounced at the end of the first week of the experiment (17% vs. 5% for green water and clear water treatments, respectively). This suggests that the presence of microalgae enhanced larval growth within the first week of exogenous feeding. Although not quantified in this experiment, we noted that larvae receiving microalgae initiated feeding sooner than larvae in clear water. A similar observation was made for halibut larvae reared in green water (Naas et al. 1992), where enhanced first-feeding was ascribed to the microalgae effecting ambient light levels in the culture tank, which in turn improved larval feeding efficiency. It has also been suggested that microalgae may stimulate enzymatic activity of the larva's gut during first feeding (Hjelmeland et al. 1988), or supply exogenous enzymes that assist the larvae in their digestion of zooplankton (Bromage and Roberts 1995). If, as

we expect, our winter flounder larvae in green water initiated feeding earlier than those in clear water, and if the microalgae triggered the digestion processes, it could account for the significant difference in length (and growth rate) we observed at the end of week 1.

The benefits of microalgae may also have resulted from direct ingestion of the algae, which has been observed in a number of marine fish larvae, including northern anchovy (Moffatt 1981), turbot (Howell 1979; Last 1979), halibut (Reitan et al. 1993) cod (van der Meeren 1991a) and wild-caught winter flounder (Pearcy 1962b). Both the mechanism of micoalgal ingestion, which may involve either drinking or filter-feeding, and the nutritive value of the ingested microalgae are open to speculation (Van der Meeren 1991a). Studies with larval cod (van der Meeren 1991a), turbot (Howell 1979) and halibut (Reitan et al. 1993) suggest that assimilation of the microalgae by the larval gut is low. Despite this, Tytler et al. (1997) found that turbot larvae had chlorophyll containing apical vacuoles in the gut enterocytes 3 days after hatching, and they suggested that although assimilation efficiency was low, the larvae may obtain small amounts of essential fatty acids, amino acids, and carotenoids from the microalgal cell pigments. Microalgae may also enter the larval gut indirectly through ingesting microalgae-fed rotifers (Reitan et al. 1993). It has also been suggested that the addition of microalgae leads to the establishment of an early larval intestinal microflora (Skjermo and Vadstein 1993; Bergh et al. 1994). This in turn, may enable the digestion of algal cells (Rimmer and Wiebe 1987), may provide amino acids, fatty acids and vitamins (Kashiwada and Teshima 1966; Fong and Mann 1980; Ringø et al. 1992), and may inhibit bacterial pathogens (Olsson et al. 1992).

Apart from stimulating first feeding and/or providing either direct or indirect nutrition , microalgae may also act to control bacterial growth in tanks by releasing natural bacteriostatic agents. Austin et al. (1992) for example, found that the exudates from one species of algae (*Tetraselmis suecica*) inhibited certain bacterial fish pathogens. The microalgae may also stabilize water quality by absorbing waste products and producing oxygen (Houde 1975, 1978). Because both bacteriostatic agents and water quality would effect survival, it is possible that the microalgae was responsible for the tendency (not statistically significant) for survival to be higher in green water treatments than in clear water treatments (Figure 3). Survival estimates from this study ranged from about 13.5-22% at the end of 5 weeks which are lower than the approximate 34% reported by Laurence (1977) for winter flounder larvae raised at 8°C and provided with 3000 wild zooplankters/l. Our lower observed percentages may have resulted from our fluctuating, and slightly warmer, incubation temperatures.

We found no significant difference ($p>0.05$) in the final mean lengths of larvae, or in percent survival, between the two live prey treatments. Among the components of any larval fish diet, it is well documented that fatty acids, particularly n-3 highly unsaturated fatty acids (HUFA), are important to the nutrition of marine fish larvae (Watanabe et al. 1983b; Van Ballaer et al. 1985; Koven et al. 1990), including winter flounder (Klein-MacPhee et al. 1980). Because wild zooplankton are typically rich in these essential fatty acids compared to cultured live food organisms (Watanabe et al. 1980, 1983a; van Ballaer et al. 1985; Leger et al. 1986; Naess et al. 1995), experiments in which marine fish larvae have been fed cultured prey and wild zooplankton have generally shown that growth and survival are higher in those fed wild zooplankton (Skjolddal et al. 1990). It has also been shown that some larvae (e.g. turbot) select wild zooplankton over the rotifer *Brachionus plicatilis* if given a choice (van der Meeren 1991c). Our finding that there was no significant difference in lengths or survival between larvae fed cultured rotifers and those fed wild zooplankton suggests that the two food types were similar in their nutritional value. Reitan et al. (1993) have shown that rotifers fed *Isochrysis galbana* (T. Iso) have relatively high levels of lipids and 22:6 n-3 highly unsaurated fatty acid (HUFA) compared to those in clear water, and that turbot larvae fed these microalgae-enriched rotifers have higher growth and survival than those fed rotifers grown in clear water. Presumably this was due to the microalgae providing a source of micronutrients and HUFA to the larvae, both of which are essential for growth and survival (Fukusho et al. 1984; Brown et al. 1997). Moreover, Reitan et al. (1993) found that

total lipids and fatty acids remained relatively high in uneaten rotifers living in green water systems because they were consuming the microalgae. Thus culture tanks receiving microalgae promoted a continuous supply of highly nutritious rotifers for the fish larvae. Because *Isochrysis galbana* is known to have relatively high levels of essential fatty acids (Brown et al. 1997), and because a number of authors have shown that the levels of n-3 HUFA can be increased in cultured food organisms, including rotifers, by feeding them unicellular marine algae rich in n-3 HUFA (Kitajima et al. 1979; Scott and Middleton 1979; Koven et al. 1990; Reitan et al. 1993), we believe our rotifers were enriched to levels comparable to the wild zooplankton we used, thereby accounting for similar performance of the larvae fed these two diets. Comparisons of larval lengths from the experiment support this theory of microalgal enrichment (Table 3). We found, for example, that larvae fed both cultured rotifers and wild zooplankton in the presence of microalgae were larger than those fed cultured rotifers and wild zooplankton in the absence of microalgae, at every weekly time interval. While the microalgae may have been having a number of effects (see above), it is possible that it may have been improving the nutritional value of both the rotifers and wild zooplankton such that they were nutritionally equivalent. We also found that there was no significant difference in the mean lengths of larvae fed rotifers and wild zooplankton in the absence of microalgae. This result also suggests that the two diets were nutritionally equivalent. In this case, however, the equivalence was probably due to the rotifers having been fed microalgae as they were being cultured.

In this five week experiment, we found that larvae in the green water treatments grew to larger mean lengths than larvae in the clear water treatments, regardless of food type. We also found that there was a tendency, although not statistically significant, for survival to be higher in green water treatments than in clear water treatments. These results indicate that microalgae should be used when culturing winter flounder larvae. This may be particularly important during the first week following yolk-sac absorption, as indicated by the greatest disparity in the instantaneous instantaneous growth rate of larvae in green water treatments (17%/wk) compared to those in clear water treatments (5%/wk) for this early period. Results of the study also suggest that there is little difference between wild zooplankton and cultured rotifers as a first feeding diet for winter flounder larvae. We note, however, that our rotifers were almost certainly enriched, particularly in essential fatty acids, by the microalgae with which they were cultured, and that their tendency, although not statistically significant, for survival to be higher in green water treatments than in clear water treatments. These results indicate that microalgae should be used when culturing winter flounder larvae. This may be particularly important during the first week following yolk-sac absorption, as indicated by the greatest disparity in the instantaneous growth rate of larvae in green water treatments (17%/wk) compared to those in clear water treatments (5%/wk) for this early period. Results of the study also suggest that there is little difference between wild zooplankton and cultured rotifers as a first feeding diet for winter flounder larvae. We note, however, that our rotifers were almost certainly enriched, particularly in essential fatty acids, by the microalgae with which they were cultured, and that this presumed nutritional quality was probably maintained over time by the addition of microalgae to the larval fish cultures. It is likely that rotifers grown in the absence of microalgae would not promote comparable growth.

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## LITERATURE CITED

Alves, D., D.A. Bengtson and J.L. Specker. 1997. Investigations into the causes of larval mortality in cultured summer flounder (*Paralichthys dentatus* L.). Symposium on Marine Finfish and Shellfish Aquaculture, Marine Stock Enhancement, and Open

Ocean Engineering. Univ. of New Hampshire, Sept. 1997. (abstract).

Austin, B., E. Baudet and M. Stobie. 1992. Inhibition of bacterial pathogens by *Tetraselmis suecica*. J. Fish. Diseases, 15: 55-61.

Bergh, Ø., K.E. Naas and T. Harboe. 1994. Shift in the intestinal microflora of Atlantic halibut (*Hippoglossus hippoglossus*) larvae during first feeding. Can. J. Fish. Aquat. Sci., 51: 1899-1903.

Bertram, D.F., T.J. Miller and W.C. Leggett. 1996. Individual variation in growth and development during the early life stages of winter flounder, *Pleuronectes americanus*. Fish. Bull. U.S., 95: 1-10.

Bløm, G., H. Otterå, T. Svåsand, T.S. Kristensen, and B. Serigstad. 1991. The relationship between feeding conditions and production of cod fry (*Gadus morhua* L.) in a semi-enclosed marine ecosystem in western Norway, illustrated by use of a consumption model. ICES Mar. Sci. Symp., 192: 176-189.

Bromage N. R., and R. J. Roberts. 1995. Broodstock Management and Egg Larval Quality. Blackwell Science Ltd., Oxford, England, 424 pp.

Bromley, P.J. and B.R. Howell. 1983. Factors influencing the survival and growth of turbot larvae, *Scophthalmus maximus* L., during the change from live to compound feeds. Aquaculture, 31: 31-40.

Brown, M.R., S.W. Jeffrey, J.K. Volkman and G.A. Dunstan. 1997. Nutritional properties of microalgae for mariculture. Aquaculture, 151: 315-331.

Chambers, R.C. and W.C. Leggett. 1987. Size and age at metamorphosis in marine fishes: an analysis of laboratory-reared winter flounder (*Pseudopleuronectes americanus*) with a review of variation in other species. Can. J. Fish. Aquat. Sci., 44: 1936-1947.

Ehrlich, K., M. Cantin, and M. Rust. 1989. Growth and survival of larval and post larval smallmouth bass fed a commercially prepared dry feed and/or *Artemia* nauplii. J. World Aqua., 20:1-11.

Ellertsen, B., E. Moksness, P. Solemdal, S. Tilseth, T. Westgard, and V. Øiestad. 1981. Growth and survival of cod larvae in an enclosure. Experiments and a mathematical model. Rapp. P.-V. Reun. Cons. int. Explor. Mer, 178: 45-57.

Fong, W. and K.H. Mann. 1980. Role of gut flora in the transfer of amino acids through a marine food chain. Can. J. Fish. Aquat. Sci., 37: 88-96.

Fujita, S., T. Watanabe, and C. Kitijima. 1980. Nutritional quality of *Artemia* from different localities as a living feed for marine fish from the viewpoint of essential fatty acids. In: G. Personne, P. Sorgeloos, O. Roels, and E. Jaspers (Editors). The Brine Shrimp, *Artemia*.: Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium, p. 277-290.

Fukusho, K., M. Okauchi, S. Nuraini, A. Tsujigado and T. Watanabe. 1984. Food value of a rotifer *Brachionus plicatilis*, cultured with *Tetraselmis tetrathele* for larvae of red seabream *Pagrus major*. Bull. Japan. Soc. Sci. Fish., 50: 1439-1444.

Guillard, R. R. L., and J. H. Ryther. 1962. Studies on marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacae* (Cleve). Gran. Can. J. Microbiol. 8:229-239.

Hjelmeland, B.H. Pedersen, and E. M. Nilssen. 1988. Trypsin content in intestines of herring larvae (*Clupea harengus*), ingesting inert polystyrene spheres or live crustacea prey. Mar. Biol., 98: 331-335.

Houde, E.D. 1975. Effects of stocking density and food density on survival, growth and yield of laboratory-reared larvae of seabream *Archosargus rhomboidalis* (L.) (Sparidae). J. Fish. Biol., 7: 115-127.

Houde, E.D. 1978. Critical food concentrations for larvae of three species of subtropical marine fishes. Bull. Mar. Sci., 28: 395-411.

Howell, B.R. 1979. Experiments on the rearing of larval turbot, *Scophthalmus maximus* L. Aquaculture, 18: 215-225.

Izquierdo, M.S., T. Watanabe, T. Takeuchi, T. Arakawa, and C. Kitijima.1989. Requirements of larval red seabream *Pagrus major* for essential fatty acids. Nippon Suisan Gakkaishi, 55: 859-867.

Jerald, A., S.L. Sass and M.F. Davis. 1993. Early growth, behavior and otolith development of the winter flounder, *Pleuronectes americanus*. Fish. Bull. U.S., 91: 65-75.

Jones, A., R.A. Prickett and M.T. Douglas. 1981. Recent developments in techniques for rearing marine flatfish larvae, particularly turbot (*Scophthalmus maximus* L.), on a pilot commercial scale. Rapp. P.-V. Reun. Cons. Int. Explor. Mer, 178: 522-526.

Kashiwada, K. and S. Teshima. 1966. Studies on the production of B-vitamins by intestinal bacteria of fish - I. Nicotinic acid, pantothenic acid and vitamin $B_{12}$ in carp. Bull. Japan. Soc. Sci. Fish., 32: 961-966.

Kitajima, C., S. Fujita, F. Oowa, Y. Yone and T. Watanabe. 1979. Improvement of dietary value for red seabream larvae of rotifers, *Brachionus plicatilis*, cultured with baker's yeast, *Saccharomyces cerevisiae*. Bull. Japan. Soc. Sci. Fish., 45: 469-471.

Klein-MacPhee, G., W.H. Howell and A.D. Beck. 1980. International study on *Artemia* VII. Nutritional value of five geographical strains of *Artemia* to winter flounder *Pseudopleuronectes americanus* larvae. In: G. Personne, P. Sorgeloos, O. Roels, and E. Jaspers (Editors). The Brine Shrimp, *Artemia*.: Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, Belgium, p. 305-312.

Klein-MacPhee, G., W.H. Howell and A.D. Beck. 1982. Comparison of a reference strain and four geographical strains of Artemia as food for winter flounder, *Pseudopleuronectes americanus*, larvae. Aquaculture, 29: 279-284.

Klein-MacPhee, G., B.K. Sullivan and A.A. Keller. 1993. Using mesocosms to assess the influence of food resources and toxic materials in larval fish growth and survival. Amer. Fish. Soc. Symp., 14: 105-116.

Koven, W.M., A. Tandler, G. W. Kissil, D. Sklan, O. Freizlander, and M. Harel. 1990. The effect of dietary (n-3) polyunsaturated fatty acids on growth, survival and swim bladder development in *Sparus auratus* larvae. Aquaculture, 91: 131-141.

Last, J.M. 1979. The food of larval turbot, *Scophthalmus maximus* L. from the west central North Sea. J. Cons. Int. Explor. Mer, 38: 308-313.

Laurence, G.C. 1975. Laboratory growth and metabolism of the winter flounder *Pseudopleuronectes americanus* from hatching through metamorphosis at three temperatures. Mar. Biol., 32: 223-229.

Laurence, G.C. 1977. A bioenergetic model for the analysis of feeding and survival potential of winter flounder, *Pseudopleuronectes americanus*, larvae during the period from hatching to metamorphosis. Fish. Bull. U.S., 75: 529-546.

Laurence, G.C., T.A. Halavik, B.R. Burns and A.S. Smigielski. 1979. An environmental chamber for monitoring "in-situ" growth and survival of larval fishes. Trans. Amer. Fish. Soc., 108: 197-203.

Lee, G. W. and M. K. Litvak. 1996. Weaning wild young-of-the-year winter flounder, *Pleuronectes americanus* (Walbaum) on a dry diet: Effects on growth, survival, and feed efficiency ratios. J. World Aqua. Soc., 27(1): 30-38.

Leger, P., D.A. Bengtson, K.L. Simpson, and P. Sorgeloos. 1986. The use and nutritional value of *Artemia* as a food source. Oceanogr. Mar. Biol. Ann. Rev., 24: 521-623.

Le Ruyet, J.P., J.C. Alexandre, L.Thebaud, and C. Mugnier. 1993. Marine fish larvae feeding. Formulated diets or live prey? J. World Aqua. Soc., 24: 211-224.

Moffatt, N.M. 1981. Survival and growth of northern anchovy larvae on low zooplankton densities as affected by the presence of a *Chlorella* bloom. Rapp. P. -V. Reun. Cons. Int. Explor. Mer, 178: 475-480.

Naas, K.E., L. Berg, J. Klungsøyr, and K. Pittman. 1987. Natural and cultivated zooplankton as food for halibut (*Hippoglossus hippoglossus*) larvae. ICES C.M. 1990/F:17, 23 pp. (in mimeo).

Naas, K. E., T. Naess, and T. Harboe. 1992. Enhanced first feeding of halibut larvae (*Hippoglossus hippoglossus*) in greenwater. Aquaculture, 105: 143-156.

Naess, T., M. Germain-Henry, K.E. Naas. 1995. First feeding Atlantic halibut (*Hippoglossus hippoglossus*) using different combinations of *Artemia* and wild zooplankton. Aquaculture, 130: 235-250.

Olsson, J.C., A. Westerdahl, P.L. Conway and S. Kjelleberg. 1992. Intestinal colonization potential of turbot (*Scophthalmus maximus*) and dab (*Limanda limanda*) associated bacteria with inhibitory effects against *Vibrio anguillarum*. Appl. Environ. Microbiol., 58: 551-556.

Otterå, H. 1993. Feeding, growth and survival of cod (*Gadus morhua* L.) reared in replicate plastic enclosures. Can. J. Fish. Aquat. Sci., 50: 913-924.

Pearcy, W.G. 1962a. Ecology of an estuarine population of winter flounder, *Pseudopleuronectes americanus* (Walbaum). III. Distribution, abundance, growth, and production of juveniles; survival of larvae and juveniles. Bull. Bingham Oceanogr. Collect. Yale Univ., 18: 39-64.

Pearcy, W.G. 1962b. Ecology of an estuarine population of winter flounder, *Pseudopleuronectes americanus* (Walbaum). IV. Food habits of larvae and juveniles. Bull. Bingham Oceanogr. Collect. Yale Univ., 18: 65-78.

Pedersen, T. 1993. Aspects of start-feeding and rearing of cod (*Gadus morhua* L.) juveniles employed in enhancement studies of coastal cod populations. Doctoral Dissertation. Norwegian College of Fishery Science, Univ. of Tromso, Tromso, Norway. 141 p.

Pedersen, T., J.E. Eliassen, H.C. Eilertsen, K.S. Tande, and R.E. Olsen. 1989. Feeding, growth, lipid composition, and survival of larval cod (*Gadus morhua* L.) in relation to environmental conditions in an enclosure at 70o in northern Norway. Rapp.- V. Reun. Cons. perm. int. Explor. Mer, 191: 409-420.

Reitan, K.I., J.R. Rainuzzo, G. Oie and Y. Olsen. 1993. Nutritional effects of algal addition in first-feeding of turbot (*Scophthalmus maximus* L.) larvae. Aquaculture, 118: 257-275.

Ricker, W. E. 1979. Growth rates and models. In: W. S. Hoar, D. J. Randall and J. R. Brett, (Editors). Fish physiology, Vol. VIII. Bioenergetics and growth. Academic Press, New York, USA. p. 677-743

Rimmer, D.W. and W.J. Wiebe. 1987. Fermentative microbial digestion in herbivorous fishes. J. Fish. Biol., 31: 229-236.

Ringø, E., P.D. Sinclair, H. Birkbeck and A. Barbour. 1992. Production of eicosapentaenoic acid (20:5 n-3) by *Vibrio pelagius* isolated from turbot (*Scophthalmus maximus* L.) larvae. Appl. Environ. Microbiol., 58: 3777-3778.

Rogers, C. A. 1976. Effects of temperature and salinity on the survival of winter flounder embryos. Fish. Bull. U.S., 74: 52-58.

Scott, A.P. and C. Middleton. 1979. Unicellular algae as a food for turbot (*Scophthalmus maximus* L.) larvae - the importance of dietary long-chain polyunsaturated fatty acids. Aquaculture, 18: 227-240.

Skjermo, J. and O. Vadstein. 1993. The effect of microalgae on skin and gut bacterial flora of halibut larvae. In: H. L.A. Reinertsen, L.A. Dahle, L. Jørgensen and T. Tvinnereim (Editors). Proceeding from International Conference on Fish Farming Technology, Trondheim, Norway, August, 1993, p.61-67.

Skjolddal, L.H., T. Harboe, T. Nåess, K.E. Nass, and H. Rabben. 1990. A comparison of growth rates of hailbut larve fed wild zooplankton and enriched Artemia. ICES C.M. 1990/F:60 (In Mimeo).

Smigielski, A. S. and C. R. Arnold. 1972. Separating and incubating winter flounder eggs. Prog. Fish Cult., 34:113.

Smigielski, A.S. 1975. Hormone induced ovulation of the winter flounder, *Pseudopleuronectes americanus*. Fish. Bull. U.S., 73: 431-438.

Tytler, P., J. Ireland and L. Murray. 1997. A study of the assimilation of fluorescent pigments of microalgae *Isochrysis galbana* by the early stages of turbot and herring. J. Fish Biol., 50: 999-1009.

van Ballaer, E., F. Amat, F. Hontoria, P. Leger, and P. Sorgeloos. 1985. Preliminary results and nutritional evaluation of w-3-HUFA enriched *Artemia* nauplii for larvae of the sea bass, *Dicentrarchus labrax*. Aquaculture, 49: 223-229.

van der Meeren, T. 1991a. Algae as first food for cod larvae, *Gadus morhua* L.: filter feeding or ingestion by accident? J. Fish Biol., 39: 225-237.

van der Meeren, T. 1991b. Production of marine fish fry in Norway. J. World Aqua. Soc., 22: 37-40.

van der Meeren, T. 1991c. Selective feeding and predictive food consumption in turbot larvae (*Scopthalmus maximus* L.) reared on the rotifer *Brachionus plicatilis* and natural zooplankton. Aquaculture, 93: 35-55.

van der Meeren, T. and T. Naess. 1993. How does cod (*Gadus morhua*) cope with variability in feeding conditions during early larval stages? Marine Biology, 116: 637-647.

van der Meeren, T., J. Klungsoyr, S. Wilhelmsen, and P. G. Kvenseth. 1993. Fatty acid composition of unfed cod larvae (*Gadus morhua* L.) and cod larvae feeding on natural zooplankton in large enclosures. J. World Aqua. Soc., 24: 167-185.

Vasquez-Yeomans, L., E. Carrillo-Barrios-Gomez and E. Sosa-Cordero. 1990. The effect of the nanoflagellate Tetraselmis suecica on the growth and survival of grunion, *Leurestes lenuis*, larvae. Environ. Biol. Fish., 29: 193-200.

Watanabe, T., F. Oowa, C. Kitijima, and S. Fujita. 1980 Relationship between dietary value of brine shrimp *Artemia salina* and their content of n-3 highly unsaturated fatty acids. Bull. Japan. Soc. Sci. Fish., 46: 35-41.

Watanabe, T., C. Kitajima, and S. Fujita. 1983a. Nutritional values of live organisms used in Japan for mass propogation of fish: a review. Aquaculture, 34: 115-143.

Watanabe, T., T. Tamiya, A. Oka, M. Hirata, C. Kitajima, and S. Fujita. 1983b. Improvement of dietary value of live foods for fish larvae by feeding them on highly unsaturated fatty acids and fat soluable vitamins. Bull. Jap. Soc. Sci. Fish., 49: 471-479.

Waters, E.B. 1996. Sustainable flounder culture and fisheries. Univ. of North Carolina Sea Grant College Program, Raleigh, N.C., Pub. No. UNC-SG-96-14, 12 pp.

Witt, U., G. Quantz, D. Kuhlmann, and G. Kattner. 1984. Survival and growth of turbot larvae *Scopthalamus maximus* L. reared on different food organisms with special regard to long-chain polyunsaturated fatty acids. Aqua. Engin., 3: 177-190.

Øiestad, V., P.G. Kvenseth, and A. Folkvord. 1985. Mass production of Atlantic cod juveniles (*Gadus morhua*) in a Norwegian saltwater pond. Trans. Amer. Fish. Soc., 114: 590-595.

# DEVELOPMENTAL PROCESS OF DIGESTIVE ORGANS AND THEIR FUNCTIONS IN JAPANESE FLOUNDER *PARALICHTHYS OLIVACEUS*

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## ABSTRACT

To elucidate the developmental process of the digestive function of the pancreas and the intestine during the larval stage of Japanese flounder, distribution of aminopeptidase and trypsinogen was traced by immunohistochemical methods. Aminopeptidase was detected from the brush border of the posterior intestine at hatching. At 2 days post hatching (dph), the rectum had differentiated morphologically, and both the brush border of the intestine and rectum showing a strong reaction to anti-aminopeptidase antibody (anti-rAmp), but the reaction to the antibody in the rectum was reduced at 3 dph. Thus, the intestinal epithelial cells of flounder larvae had already started to synthesize digestive enzymes onto the brush border at hatching, and the functional differentiation of the rectum from the intestine occurs at the first feeding at 3 dph. Trypsinogen was detected in the pancreas beginning at 2 dph. The trypsinogen secretion into the pancreatic duct was found at 3 dph. Thus, the pancreas of flounder larva acquires exocrine function by the time of the first feeding at 3 dph. The pancreas was a small compact organ at 3 dph, and it started to elongate along the veins of the intestine at 20 dph. Thus, the pancreas of the flounder completes the formation from the compact-type organ of larva to the diffuse-type organ of the adult at metamorphosis, at the time of development of the gastric glands in the stomach wall. Therefore, the digestive organs of the Japanese flounder, other than the stomach (pancreas, intestine and rectum), have acquired the digestive ability by the time of first feeding and their digestive system becomes fully developed after metamorphosis.

## INTRODUCTION

Japanese flounder *Paralichthys olivaceus* is an important species in both aquaculture and commercial fisheries in Japan. The recent increase in the intensive production of juveniles for seeding has necessitated a more detailed understanding of the early development of flounder larvae.

Since the gastric glands of Japanese flounder have not fully developed until metamorphosis (Miwa et al. 1992), the pancreas is the sole exocrine organ responsible for secreting digestive enzymes during the larval stage. In summer flounder *Paralichthys dentatus* larvae, the epithelial cells of the intestine and the rectum absorb lipid and protein, respectively (Bisbal and Bengtson 1995). This absorption of protein by rectal cells indicates intercellular digestion via pinocytosis (Watanabe 1981, Watanabe 1982, Georgopoulou et al. 1986, Govoni et al. 1986). In turbot *Scophthalmus maximus* larvae, aminopeptidase activity was found on the intestinal brush border (Cousin et al. 1987). In the larval stage of flounder, therefore, ingested food is passed directly into the intestine, where it is digested by the pancreatic enzymes and the enzymes of the intestinal brush border and undigested proteins are absorbed by rectal cells.

Because marine fish larvae cannot properly utilize artificial diets due to their undeveloped digestive organs (Graff and Sorenson 1970, Braid and Shell 1981, Baragi and Lovell 1986, Beccaria et al. 1991), rotifers and brine-shrimp nauplii are essential for rearing larvae. In order to establish an efficient rearing system for flounder larvae including the development of complete artificial diets for early larvae, it is important to understand their digestive ability at the larval stage. In this study, to elucidate the developmental process of the digestive function of the intestine, rectum, and pancreas during the larval stage of Japanese flounder,

distribution of aminopeptidase and trypsinogen and absorption of rotifer proteins derived from rotifers were traced by immunohistochemical methods. In addition, the formation process of diffuse pancreas was followed.

## MATERIALS AND METHODS

### Larvae

Larvae of the Japanese flounder *Paralichthys olivaceus* were kept in a tank supplied with running seawater (17±1°C). The larvae were fed on rotifers *Brachionus plicatilis* from 3 to 30 dph, on brine-shrimp nauplii *Artemia* sp. from 15 to 45 dph, and on an artificial diet (Kyowa A-250, Japan) from 20 dph.

### Immunohistochemistry

Larvae (n=10) were fixed with 10% formalin in 10 mM Tris-buffered saline (TBS) pH 7.5 for 24 h at 0, 1, 2, 3, 10, 20, 30, and 45 dph. Fixed samples were dehydrated through a graded ethanol series, embedded in paraffin, and cut into serial sections 6 μm thick.

Sections were stained immunohistochemically using anti-eel trypsinogen antibody (anti-eTrg),

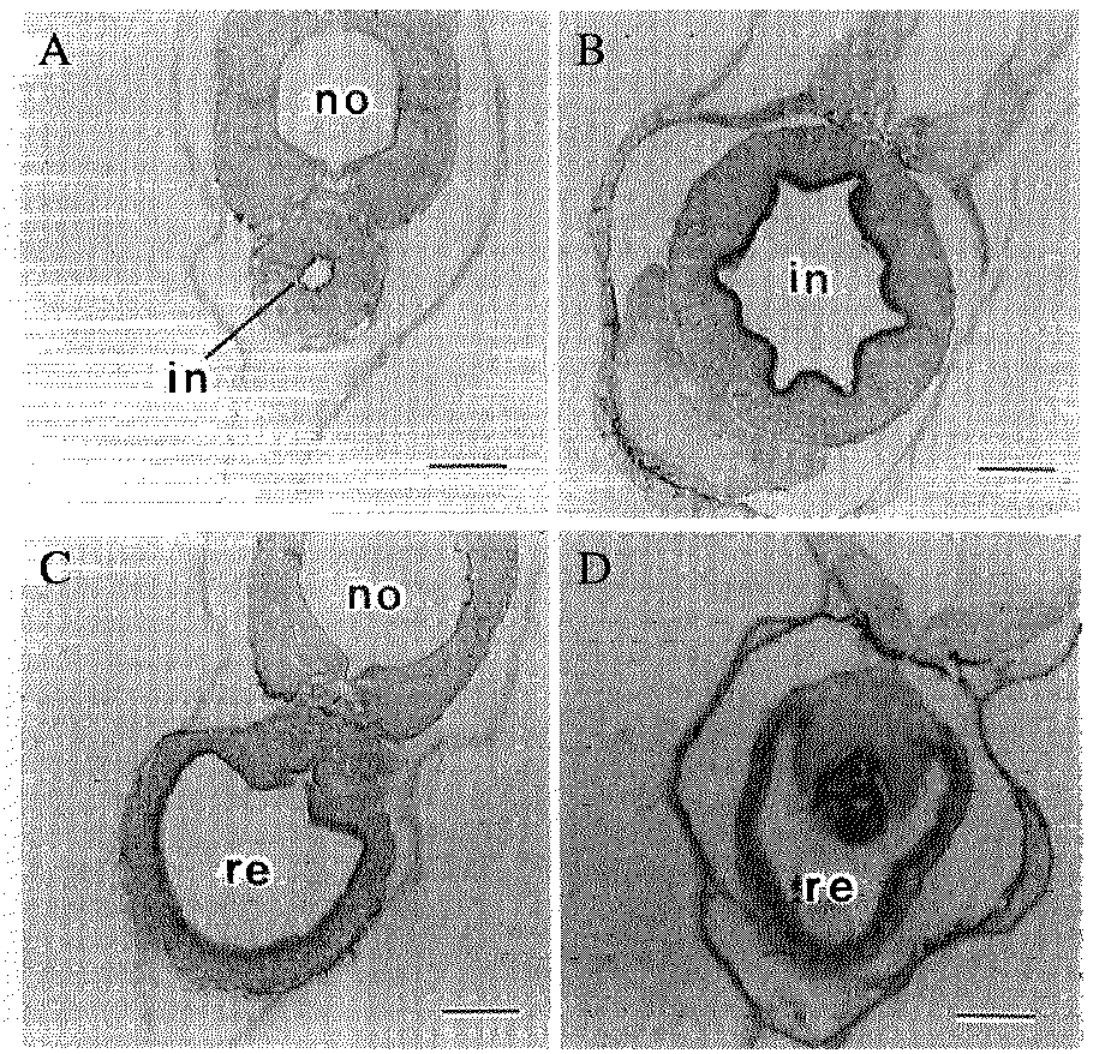


**Figure 1.** Distribution of aminopeptidase in the digestive tract and absorption of proteins by rectal cells of early Japanese flounder larvae. Sections were immunostained with anti-rAmp (A-C) and anti-Rot (D). A; posterior instestine at hatching. B; anterior intestine at 3 dph. C,D; rectum at 3 dph. in = intestine; no = notochord; re = rectum. Scale bars indicate 25 μm.

anti-red sea bream aminopeptidase (anti-rAmp), and antibody against whole soluble proteins of rotifers (anti-Rot), and were developed by Histofine SAB-PO kit (Nichirei, Japan).

## RESULTS AND DISCUSSION

### Development of the digestive function of gut

The intestine of newly hatched Japanese flounder larvae was a narrow duct consisting of a smooth single-layered epithelium with no mucosal folds. The brush border of the intestinal epithelial cells was still indistinct, but slight immunochemical staining for aminopeptidase was detected in the brush border of the posterior intestine (Fig.1A). At 1 dph, the brush border of the anterior intestine also exhibited weak immunoreaction to anti-rAmp. The rectum had differentiated morphologically from the intestine at 2 dph, and the brush border of the intestine and the rectum gave strong signals to anti-rAmp. The intestinal epithelia had formed mucosal folds at 3 dph. The brush border of the intestine showed strong signals to anti-rAmp, but the signals in the rectum had been reduced (Fig. 1B and C).

After the first feeding of rotifers at 3 dph, strong immunohistochemical staining for proteins derived from rotifers was detected from the epithelial cells of the larval rectum (Fig.1D). This indicates that rectal cells of Japanese flounder larvae have acquired the ability to absorb dietary proteins via pinocytosis at 3 dph.

Accordingly, the intestinal epithelial cells have already started to synthesize digestive enzymes onto the brush border at hatching, and the functional differentiation of the rectum from the intestine occurs by the time of first feeding at 3 dph in Japanese flounder.

In summer flounder *Paralichthys dentatus* larvae, mucosal folds were formed in the intestine at 4 dph and active pinocytotic features were observed in the rectal cells at this stage (Bisbal and Bengtson 1995). Thus, the differentiation of the gut appears to be synchronized with the onset of exogenous feeding in flounder.

### Development of exocrine pancreas

At hatching, the larval gut of Japanese flounder was a simple tube without accessory organs. The primordia of the pancreas and the liver had differentiated from the gut at 1 dph (Fig. 2A). Slight immunochemical staining from trypsinogen was detected in the pancreas beginning at 2 dph (Fig. 2B). The immunoreaction to anti-cTrg increased from 2 to 3 dph. Trypsinogen secretion into the pancreatic duct was found at 3 dph (Fig. 2C). Thus, the pancreas of flounder larvae acquires exocrine function by the time of first feeding at 3 dph.

Beccaria et al. (1991) classified the developmental process of pancreatic primordia in fish as four types that were based on morphological observations. The pancreas of Japanese flounder shows a similar developmental type to sea bass *Dicentrarchus labrax*.

The pancreas of Japanese flounder was a compact organ localized around the gallbladder at 3 dph (Fig. 2D). The intestine had coiled and the pancreas slightly elongated posteriorly at 10 dph. The coiling of the intestine was more pronounced at 20 dph. The pancreas was mainly localized around the gallbladder but the posterior part of the pancreas had begun to elongate along the vein on the intestine at this time (Fig. 2E).

At 30 dph, the pyloric appendages had differentiated from the anterior part of the intestine and the bile duct had elongated. The pancreas was distributed from the vicinity of the gallbladder to the proximal part of the pyloric appendages. In addition, the posterior part of the pancreas had elongated further along the vein on the intestine.

At 45 dph (completion of metamorphosis), the gastric glands had developed in the stomach wall. The pancreas was localized around the proximal part of the pyloric appendages, along the bile duct and along the veins running to the porta hepatis from the stomach, pyloric appendages, spleen, and intestine (Fig. 2F). At this stage, the pancreas becomes similar in structure to the diffuse pancreas of the adult flounder (Kurokawa and Suzuki 1995). Thus, the Japanese flounder pancreas completes the transition from the compact-type organ of larva to the diffuse-type organ of adult at metamorphosis.

The formation process of the diffuse-pancreas of sea bass *D. labrax* was observed using scanning electron microscopy (Diaz et al. 1989). The pancreas of sea bass also be-

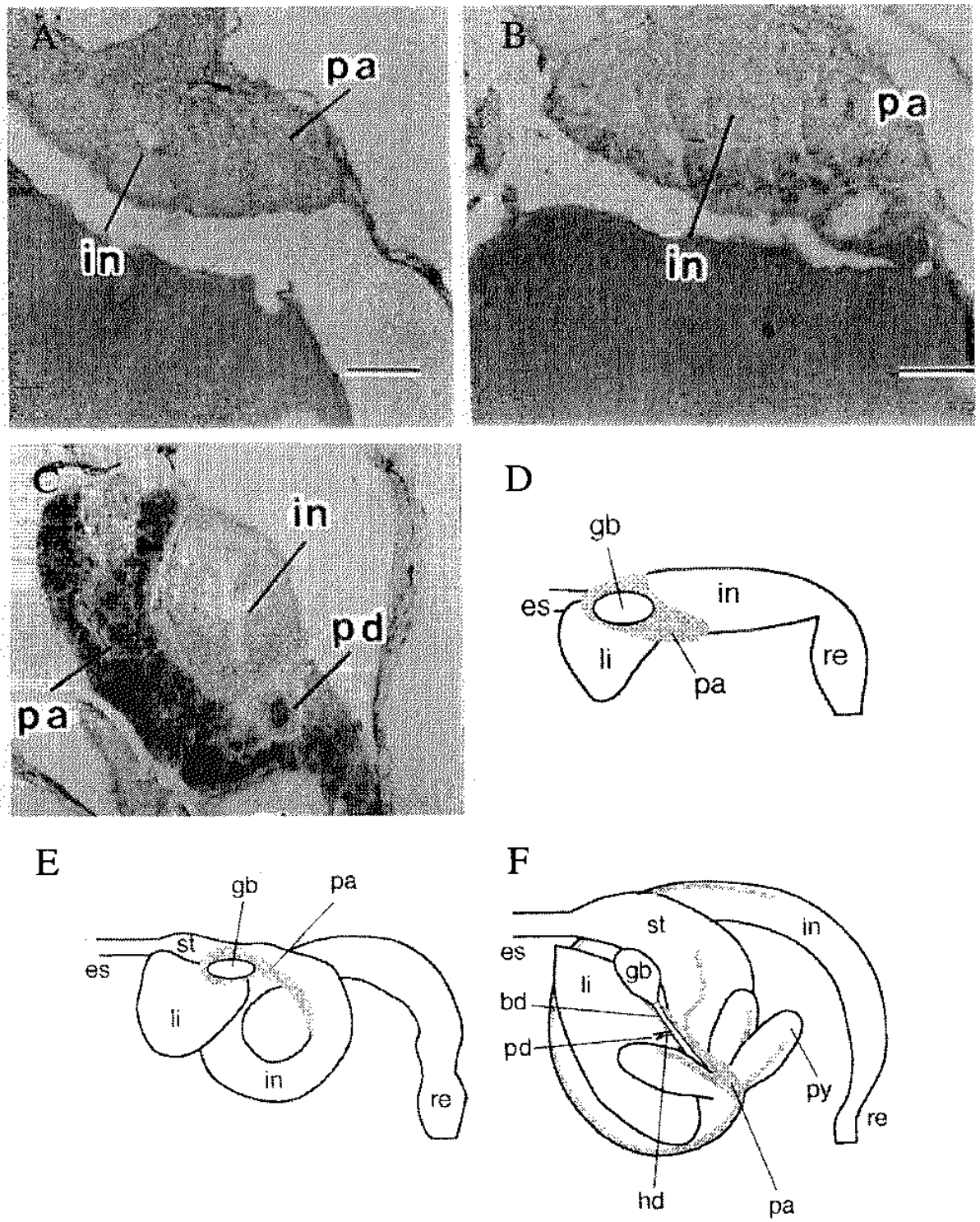


**Figure 2.** Distribution of trysinogen in the pancreas of early Japanese flounder larvae and formation of diffuse pancreas. Sections were immunostained with anti-eTrg (A-C). The morphology of the pancreas was reconstructed from serial sections and is represented schematically (D-F). Shaded areas indicate pancreatic tissue. A; 1 dph. B; 2 dph. C, D; 3 dph. E; 30 dph. F; 45 dph (completion of metamorphosis). bd = bile duct; es = esophagus; gb = gallbladder; hd = hepatic duct; in = intestine; li = liver; pa = pancreas; pd = pancreatic duct; py = pyloric appendages; re = rectum; st = stomach. Scale bars indicate 25 $\mu$m.

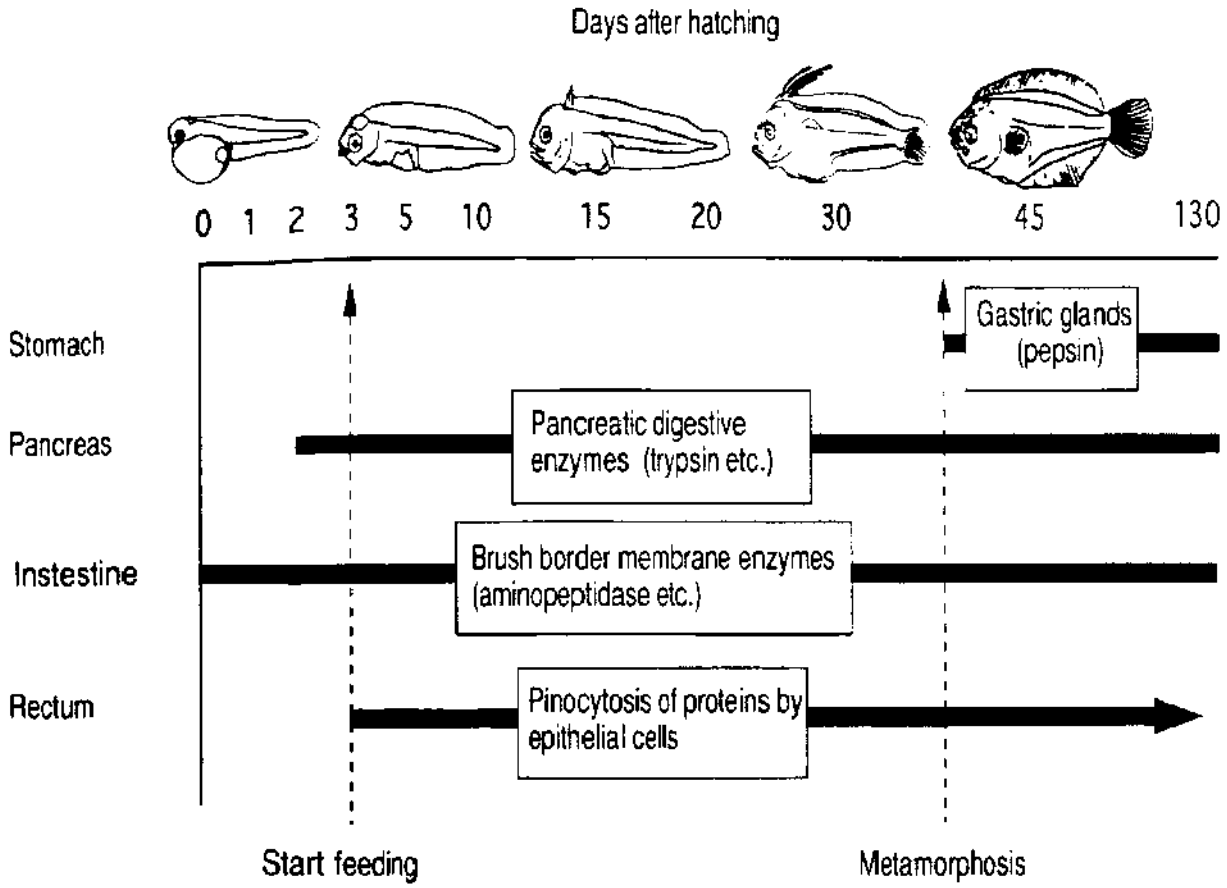


**Figure 3.** Schematic illustration showing the development of digestive functions during larval and juvenile stages in Japanese flounder.

comes a diffuse organ by the juvenile stage. It appears, therefore, that the pancreas of teleosts which possess a diffuse pancreas commonly completes development into a diffuse organ by the juvenile stage. However, the biological significance of the transformation from compact to diffuse pancreas is unclear.

The developmental process of the digestive functions during the larval stage of Japanese flounder could be summarized as shown in Figure 3. The intestine of the Japanese flounder have expressed digestive enzymes on the brush border membrane at hatching, and the functional differentiation of rectum from intestine occurs at 3 dph. The pancreatic cells begin synthesis of digestive enzymes at 2 dph and secretion of enzymes into the intestine at 3 dph. Thus, the digestive organs of flounder larvae, other than stomach (intestine, rectum, and pancreas), have acquired digestive functions by the onset of exogenous feeding at 3 dph. Therefore, we concluded that the acquisition of digestive functions in the intestine, rectum, and pancreas are
presumably a requirement for larvae to start feeding.

The pancreas completes the transformation from a compact-type organ of larva to a diffuse-type organ at metamorphosis. It is known that the gastric glands differentiate and begin synthesis of pepsinogen at metamorphosis in Japanese flounder (Miwa et al. 1992). Therefore, the digestive system of Japanese flounder becomes fully developed in the early juvenile stage flounder following metamorphosis. This may be one reason why artificial diets can be utilized by juveniles but not by larvae.

## LITERATURE CITED


Baragi, V. and R.T. Lovell. 1986. Digestive enzyme activities in striped bass from first feeding through larva development. Trans. Am. Fish. Soc. 115: 478-484.

Beccaria, C., J.P. Diaz, R. Connes, and B. Chatain. 1991. Organogenesis of the exocrine pancreas in the sea bass, *Dicentrarchus labrax* L., reared extensively and intensively. Aquaculture 99: 339-354.

Bisbal, G.A. and D.A. Bengtson. 1995. Development of the digestive tract in larval summer flounder. J. Fish Biol. 47: 277-291.

Braid, M.R. and E.W. Shell. 1981. Incidence of cannibalism among striped bass fry in an intensive culture system. Prog. Fish-Cult. 43: 210-212.

Cousin, J.C.B., F. Baudin-Laurencin, and J. Gabaudan. 1987. Ontogeny of enzymatic activities in fed and fasting turbot, *Scophthalmus maximus* L. J. Fish Biol. 30: 15-33.

Diaz, J.-P., R. Connes, P. Divanach, and G. Barnabe. 1989. Développement du foie et du pancréas du loup, *Dicentrarchus labrax*: I. Etude de la mise en place des organes au microscope électronique à balayage. Ann. Sci. Nat. Zool. 10: 87-98.

Georgopoulou, U., M.F. Sire, and J.M. Vernier. 1986. Absorption intestinale des protéins sous forme macromoléculaire et leur digestion chez la Truite arc-en-ciel. Étude ultrastructurale et biochimique en relation avec la premiére prise de nourriture. Can. J. Zool. 64: 1231-1240.

Govoni, J.J., G.W. Boehlert, and Y. Watanabe. 1986. The physiology of digestion in fish larvae. Environ. Biol. Fishes 16: 59-77.

Graff, D.R. and L. Sorenson. 1970. The successful feeding of a dry diet to esocids. Prog. Fish-Cult. 32: 31-35.

Kurokawa, T. and T. Suzuki. 1995. Structure of the exocrine pancreas of flounder (*Paralichthys olivaceus*): immunological localization of zymogen granules in the digestive tract using anti-trypsinogen antibody. J. Fish Biol. 46: 292-301.

Miwa, S., K. Yamano, and Y. Inui. 1992. Thyroid hormone stimulates gastric development in flounder larvae during metamorphosis. J. Exp. Zool. 261: 424-430.

Watanabe, Y. 1981. Ingestion of horseradish peroxidase by the intestinal cells in larvae or juveniles of some teleosts. Bull. Jpn. Soc. Sci. Fish. 47: 1299-1307.

Watanabe, Y. 1982. Intracellular digestion of horseradish peroxidase by the intestinal cells of teleost larvae and juveniles. Bull. Jpn. Soc. Sci. Fish. 48: 37-42.

# A/E RATIO PROFILES OF THE ESSENTIAL AMINO ACID REQUIREMENTS AMONG VARIOUS FINFISH SPECIES

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## ABSTRACT

Dietary essential amino acid requirements have been determined in several fishes. Recently, some researchers have applied a new method based on the idea that there should be a correlation between whole body amino acid composition and the dietary amino acid requirement. For instance, only one amino acid requirement can be determined by growth data and the other nine can be estimated as being proportional to the whole body amino acid composition. The authors think that this new method is problematic, because the essential amino acid requirement profiles based on fish growth assay appear to be different among fish species than those of body amino acid compositions. In order to evaluate the dissimilarity of essential amino acid requirements among fish species, we used the "A/E ratio" defined as follows: A/E ratio equals to {[Each essential amino acid (by weight)] divided by [All essential amino acids (by weight)]}. By using this index, fish growth stages and water temperatures do not affect the evaluation compared with the absolute values of the amino acid requirements. From the diagram obtained based on the A/E ratio profiles through the Fitch-Margoliash method, close similarities of essential amino acid requirements were found between carp and catla belonging to the family Cyprinidae, and among chinook salmon, chum salmon, and coho salmon in the family Salmonidae. This suggests the occurrence of specificity in amino acid requirements among each fish family, and we suggest that growth experiments concerning essential amino acid requirements should be conducted on at least one fish species per family.

## INTRODUCTION

From the viewpoint of practical fish culture, protein is the most important constituent in fish feed, not only as the material for structural elements of animals, but also as the main energy source. The subject of dietary protein must be dealt with in regard to both quality and quantity. Fish body protein is composed of approximately 20 distinct amino acids. The amino acids which are not synthesized entirely or sufficiently for fish needs must be supplied through fish feed. These are called the essential amino acids.

The dietary essential of 10 amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine has been estimated for fishes such as salmonids (Halver et al. 1957, Halver and Shanks 1960, Shanks et al. 1962, Akiyama et al. 1985), European eel *Anguilla anguilla* and Japanese eel *Anguilla japonica* (Arai et al. 1972), common carp *Cyprinus carpio* (Nose et al. 1974), red sea bream *Pagrus major* (Yone 1976), and tilapia *Tilapia zillii* (Mazid et al. 1978), based on growth response. In addition, the tracer experiments using $^{14}C$ have shown that plaice

*Pleuronectes platessa*, sole *Solea solea* (Cowey et al. 1970), and sea bass *Dicentrarchus labrax* (Wilson 1989) require the same 10 amino acids. It is considered at present that these 10 amino acids are essential for all fish species.

One of the important factors in determining the efficiency of protein utilization for fish is the composition of essential amino acids in diet. It is very important to clarify whether the patterns of amino acid requirement are identical or the specificity exists among species. The present study was carried out focusing on the presence of specificity in requirement of the essential amino acids using already published data.

Since the studies on qualitative and quantitative amino acid requirements in fish commenced in the United States in the 1950s, data for various fishes have been accumulated using different methods. The quantitative requirement was determined based on growth responses to dietary graded levels of a considered amino acid especially early in the studies.

Recently, however, some researchers have applied a new method based on the idea that there should be a correlation between whole body amino acid pattern and amino acid requirement. In amino acid nutrition for swine, the concept of an ideal protein balance is proposed, which is based on the idea that there should be a correlation between the body amino acid composition and the dietary amino acid requirement (Agricultural Research Council 1981). Based on this theory, Wilson and Poe (1985) and Wilson (1993) introduced a new method for estimating the amino acid requirement into fish nutrition: i.e., only the lysine requirement is determined from growth assays in feeding experiments and the other nine essential amino acid requirements are estimated as being proportional to the whole body amino acid composition pattern including lysine which is normally the first limiting amino acid in most feedstuffs. This method was also recently applied to red drum (Moon and Gatlin 1991) and juvenile Japanese flounder (Forster and Ogata, pers. commun.).

Considering that body amino acid patterns are nearly identical among fish species, as shown below by a graphical method, there would be little difference in the patterns of requirement if they were estimated by this new method. The authors doubt if the new method using the body amino acid composition is scientifically reliable, and aim to evaluate in detail the dissimilarity of essential amino acid requirement profiles among different finfish species.

## MATERIALS AND METHODS

### (a) Data for essential amino acid requirements

The complete quantitative requirement for 10 essential amino acids has already been determined for chinook salmon *Oncorhynchus tshawytscha* (Halver et al. 1958, 1959, Delong and Halver 1962, Chance et al. 1964, Halver 1965, Klein and Halver 1970), coho salmon *O. kisutch* (Arai and Ogata 1993), chum salmon *O. keta* (Akiyama and Arai 1993), channel catfish *Ictalurus punctatus* (Wilson and Poe 1985), common carp (Akiyama et al. 1997), catla *Catla catla* (Ravi and Devaraj 1991), Nile tilapia *Oreochromis niloticus* (Santiago and Lovell 1988), milkfish *Chanos chanos* (Borlongan and Coloso 1993), and Japanese eel (Akiyama et al. 1997). As these studies have been conducted by the same laboratory or research groups for each fish species, under almost identical experimental conditions for the determination of all 10 essential amino acids, i.e., similar basal diets, feeding levels, and environmental conditions (water quality and temperature used), test fish ages, sizes, etc., they give the most appropriate data for comparing the difference of requirements among fish species.

In traditional methods based on growth assays, fish must be fed graded levels of specified amino acid in test diets containing either only crystalline amino acids or a mixture of casein, gelatin, crystalline amino acids, and other nitrogen sources. The rearing experiments are repeated at least 10 times for 10 essential amino acids to complete a series of study in the one species, and the requirement values are estimated based on the conventional growth response curve. It is labor intensive and expensive. But we should note that only the data obtained from well-defined experimental conditions can be utilized for precise comparison of the profiles of essential amino acid requirements among different finfish species.

### (b) A/E ratio, as a tool for comparing the mode

**of essential amino acid requirements**

The sum of essential amino acids in dietary crude protein varies widely from 24% for coho salmon to 39% for catla and Japanese eel, and the average value among nine species is approximately 34%. These values are much lower than those of feedstuffs commonly used in fish feeds, which are around 50% in fish meal, soybean meal, and corn gluten meal.

Therefore, it is quite questionable to directly compare each absolute value of essential amino acid requirement among fish species due to the differences of protein level in test diet and the sum of essential amino acids in dietary crude protein by fish species. To overcome this difficulty, Arai (1981) has introduced a concept of the A/E ratio into the field of fish nutrition study as a useful tool for evaluating amino acid balance in dietary protein from the results of feeding experiments using juvenile coho salmon. The efficacy of this index has been reconfirmed by the feeding experiments of cherry salmon *Oncorhynchus masou masou*, and amago salmon *O. masou ishikawae* (Ogata et al. 1983). The A/E ratio is defined as [(each essential amino acid content/total essential amino acid content including cystine and tyrosine)x1000]. This index is not regarded as an absolute value of quantitative requirement but gives attention to the relative balance among the 10 essential amino acids. Accordingly, the A/E ratios of 10 amino acid requirements were calculated for the nine fish species, to allow a standardized comparison.

**(c) Calculating the dissimilarity indices from the A/E ratio profiles**

For each pair of fish among nine species for which 10 A/E ratios of essential amino acid requirements are known, the dissimilarity or distance index was calculated as follows,

$$D_1(a,b)=\sum_{i=1}^{10}\frac{|R_{ai}-R_{bi}|}{2} \quad (1)$$

or

$$D_2(a,b)=\sqrt{\sum_{i=1}^{10}\frac{(R_{ai}-R_{bi})^2}{2}} \quad (2)$$

where "a" and "b" represented the two fish species being compared, and $R_{ai}$ indicates the A/E ratio of the requirement of i-th essential amino acid for the fish species "a."

The $D_1$ defined in equation (1) is a distance index introduced by Prevosti et al. (1975) and is essentially the same measure as the Manhattan distance (Sneath and Sokal 1973) except for the standardization factor of 1/2. The $D_2$ defined in equation (2) is a distance index introduced by Rogers in1972 (Nei 1987), and is essentially the same measure as the Euclidean distance (Sneath and Sokal 1973) except for the standardizing factor of 1/2. Both of the distances are quite frequently used for evaluating the degree of dissimilarity between two sets of continuous characters. For the species "a" and "b" with identical A/E ratios for all the 10 essential amino acids, both $D_1$ (a,b) and $D_2$ (a,b) are equal to zero. The values of $D_1$ and $D_2$ increase with increasing dissimilarity between the two A/E ratio profiles and the maximum value possible is 1 for both $D_1$ and $D_2$.

The respective $D_1$ and $D_2$ values for the A/E ratios of amino acid requirement were assembled into a species-by-species dissimilarity matrix of 8 x 8 dimensions. Essentially the same procedure was used to analyze the A/E ratio profiles of the whole body amino acid compositions for the 12 fish species.

**(d) Visualization of the dissimilarities among A/E ratio profiles**

To visualize the dissimilarity relation of A/E ratio profiles among the fish species, dissimilarity diagrams were drawn based on the dissimilarity matrices. The PHYLIP 3.5c computer package (Felsenstein 1993) was used for this purpose. Both the Fitch-Margoliash method (Fitch and Margoliash 1967) and the neighbor-joining method (Saitou and Nei 1987) were applied to each matrix.

## RESULTS AND DISCUSSION

As for the A/E ratio of the essential amino acid requirements (Table 1), Cyprinidae, Nile tilapia, and Japanese eel show rather lower values in arginine requirement. Isoleucine and leucine are highly required by Japanese eel and milkfish. Threonine requirements in salmonidae and of channel catfish are low, and tryptophan

| | SALMONIDAE | | | AMEIURIDAE | CYPRINIDAE | | CICHLIDAE | CHANIDAE | ANGUILLIDAE |
|---|---|---|---|---|---|---|---|---|---|
| Amino acids ↓ | Chum salmon | Chinook salmon | Coho salmon | Channel catfish | Common carp | Catla | Nile tilapia | Milk-fish | Japanese eel |
| Arginine | 183 | 177 | 131 | 143 | 120 | 125 | 124 | 140 | 115 |
| Histidine | 46 | 53 | 37 | 51 | 59 | 64 | 51 | 53 | 53 |
| Isoleucine | 67 | 65 | 49 | 86 | 70 | 61 | 92 | 107 | 102 |
| Leucine | 108 | 115 | 139 | 117 | 92 | 96 | 100 | 136 | 135 |
| Lysine | 140 | 147 | 156 | 171 | 159 | 162 | 151 | 107 | 135 |
| Met+Cys | 86 | 118 | 111 | 78 | 87 | 87 | 95 | 87 | 81 |
| Phe+Tyr | 177 | 150 | 184 | 166 | 182 | 161 | 164 | 139 | 148 |
| Threonine | 86 | 65 | 82 | 74 | 109 | 128 | 111 | 120 | 102 |
| Tryptophan | 21 | 15 | 20 | 17 | 22 | 25 | 30 | 16 | 28 |
| Valine | 86 | 94 | 77 | 98 | 101 | 92 | 83 | 95 | 102 |
| Total | 1000 | 999 | 999 | 1001 | 1001 | 1001 | 1001 | 1000 | 1001 |

**Table 1.** A/E ratios of dietary amino acids requirements for nine fish species.

| Amino acids ↓ | Chum salmon | Chinook salmon | Coho salmon | Cherry salmon | Rainbow trout | Atlantic salmon | Channel catfish | Common carp | Nile tilapia | Yellow-tail | Milk-fish | Jap. eel |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Arginine | 115 | 119 | 115 | 119 | 123 | 126 | 132 | 124 | 137 | 125 | 124 | 133 |
| Histidine | 67 | 44 | 58 | 46 | 57 | 57 | 43 | 45 | 55 | 57 | 50 | 76 |
| Isoleucine | 77 | 79 | 71 | 76 | 83 | 84 | 85 | 75 | 79 | 78 | 87 | 82 |
| Leucine | 140 | 155 | 144 | 145 | 146 | 147 | 146 | 136 | 143 | 142 | 158 | 145 |
| Lysine | 167 | 165 | 166 | 170 | 163 | 176 | 168 | 171 | 170 | 161 | 156 | 163 |
| Met+Cys | 80 | 82 | 92 | 86 | 71 | 53 | 75 | 75 | 75 | 86 | 68 | 77 |
| Phe+Tyr | 147 | 148 | 146 | 158 | 149 | 149 | 147 | 157 | 136 | 150 | 147 | 137 |
| Threonine | 90 | 86 | 98 | 89 | 92 | 94 | 87 | 104 | 86 | 88 | 93 | 77 |
| Tryptophan | 29 | 10 | 27 | 16 | 18 | 18 | 15 | 20 | 16 | 23 | 21 | 13 |
| Valine | 88 | 112 | 83 | 93 | 98 | 97 | 102 | 94 | 103 | 91 | 96 | 96 |
| Total | 1000 | 1000 | 1000 | 998 | 1000 | 1001 | 1000 | 1001 | 1000 | 999 | 1000 | 999 |

**Table 2.** A/E ratios of the essentaial amino acid composition in the whole body tissue of 12 fish species.

requirements of Nile tilapia and Japanese eel are high when compared with those of the other fishes. These findings suggest a certain degree of consistency of requirement within family or dissimilarity among families from a viewpoint of relative balance. On the other hand, the A/E ratios of essential amino acid compositions in the whole body tissue of fish species (Table 2) do not seem to display any remarkable variability in contrast to the A/E ratios of requirements. This point is quantitatively examined below.

Table 3 shows two dissimilarity matrices obtained from the A/E ratio profiles of the essential amino acid requirements: above the diagonal, the $D_1$ indices; and below the diagonal, $D_2$ indices. Table 4 shows two dissimilarity matrices obtained from A/E ratio profiles of whole body amino acid composition. The $D_1$ values are in the range from 0.0390 to 0.1320 in Table 3, whereas in the range of 0.0195 to 0.0615 in Table 4. This indicates that the dissimilarity indices are larger for the dietary essential amino acid requirements than for the body amino acid compositions. The same tendency is also observed in the $D_2$ indices.

To visualize the dissimilarities of A/E ratio profiles among the fish species, dissimilarity diagrams were drawn based on the four dissimilarity

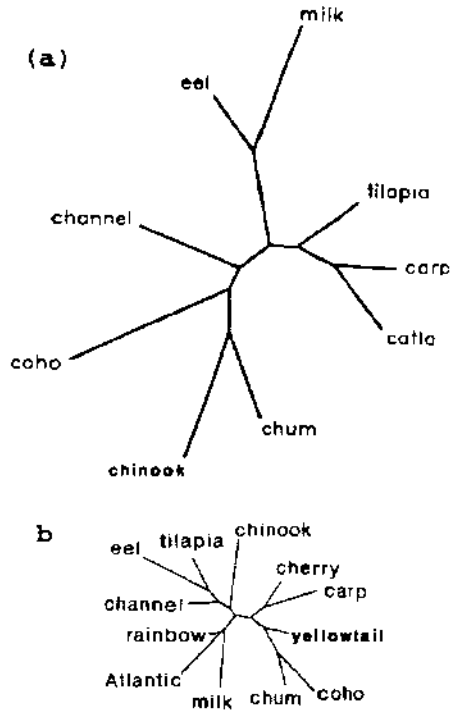


**Figure 1.** Two dissimilarity diagrams, drawn to visualize the dissimilarity relation of A/E ratio profiles among the fish species, based on the dissimiliarity matrices of D1 in Tables 3 and 4. (a), for the essential amino acid requirements; and (b), for the whole body amino acid compositions. The two diagrams were drawn with the use of the Fitch-Margoliash (1967) algorithm.

| Species | Chum salmon | Chinook salmon | Coho salmon | Channel catfish | Common carp | Catla | Nile tilapia | Milk- fish | Japanese eel |
|---|---|---|---|---|---|---|---|---|---|
| Chum salmon | | 0.0615 | 0.0860 | 0.0755 | 0.0795 | 0.0925 | 0.0835 | 0.1190 | 0.1075 |
| Chinook salmon | 0.0352 | | 0.0955 | 0.0770 | 0.1120 | 0.1090 | 0.1050 | 0.1195 | 0.1140 |
| salmon | 0.0505 | 0.0505 | | 0.0915 | 0.0915 | 0.1035 | 0.0945 | 0.1320 | 0.1145 |
| Channel catfish | 0.0419 | 0.0455 | 0.0466 | | 0.0760 | 0.0840 | 0.0730 | 0.0975 | 0.0820 |
| Common carp | 0.0527 | 0.0633 | 0.0508 | 0.0401 | | 0.0390 | 0.0520 | 0.1125 | 0.0820 |
| | 0.0567 | 0.0655 | 0.0560 | 0.0488 | 0.0229 | | 0.0510 | 0.1045 | 0.0930 |
| Nile tilapia | 0.0514 | 0.0590 | 0.0513 | 0.0396 | 0.0271 | 0.0295 | | 0.0905 | 0.0660 |
| Milkfish | 0.0636 | 0.0681 | 0.0724 | 0.0626 | 0.0651 | 0.0621 | 0.0491 | | 0.0555 |
| Japanese eel | 0.0636 | 0.0661 | 0.0594 | 0.0443 | 0.0489 | 0.0507 | 0.0358 | 0.0322 | |

**Table 3.** Dissimiliarity matrices of the A/E ratio profiles of dietary essential amino acid requirements (above the diagonal, $D_1$ indices; below the diagonal, $D_2$ indices).

| Species | Chum salmon | Chinook salmon | Coho salmon | Cherry salmon | Rainbow trout | Atlantic salmon | Channel catfish | Common carp | Nile tilapia | Yellow-tail | Milk-fish | Japanese eel |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chum salmon | - | 0.0480 | 0.0240 | 0.0350 | 0.0340 | 0.0485 | 0.0460 | 0.0425 | 0.0450 | 0.0245 | 0.0480 | 0.0455 |
| Chinook salmon | 0.0295 | - | 0.0540 | 0.0310 | 0.0360 | 0.0525 | 0.0280 | 0.0485 | 0.0400 | 0.0395 | 0.0400 | 0.0525 |
| Coho salmon | 0.0136 | 0.0298 | - | 0.0370 | 0.0400 | 0.0535 | 0.0550 | 0.0455 | 0.0540 | 0.0285 | 0.0530 | 0.0615 |
| Cherry salmon | 0.0205 | 0.0182 | 0.0185 | - | 0.0320 | 0.0435 | 0.0310 | 0.0245 | 0.0390 | 0.0245 | 0.0440 | 0.0525 |
| Rainbow trout | 0.0168 | 0.0188 | 0.0225 | 0.0170 | - | 0.0195 | 0.0240 | 0.0345 | 0.0300 | 0.0225 | 0.0210 | 0.0365 |
| Atlantic salmon | 0.0260 | 0.0285 | 0.0333 | 0.0273 | 0.0160 | - | 0.0345 | 0.0420 | 0.0395 | 0.0390 | 0.0325 | 0.0510 |
| Channel catfish | 0.0264 | 0.0154 | 0.0286 | 0.0172 | 0.0137 | 0.0208 | - | 0.0365 | 0.0210 | 0.0365 | 0.0330 | 0.0365 |
| Common carp | 0.0228 | 0.0257 | 0.0220 | 0.0154 | 0.0176 | 0.0228 | 0.0194 | - | 0.0435 | 0.0360 | 0.0425 | 0.0610 |
| Nile tilapia | 0.0246 | 0.0215 | 0.0285 | 0.0238 | 0.0162 | 0.0219 | 0.0131 | 0.0245 | - | 0.0365 | 0.0460 | 0.0295 |
| Yellowtail | 0.0129 | 0.0226 | 0.0143 | 0.0136 | 0.0135 | 0.0271 | 0.0184 | 0.0192 | 0.0193 | - | 0.0355 | 0.0420 |
| Milkfish | 0.0245 | 0.0205 | 0.0274 | 0.0226 | 0.0120 | 0.0203 | 0.0168 | 0.0240 | 0.0220 | 0.0200 | - | 0.0515 |
| Japanese eel | 0.0231 | 0.0302 | 0.0307 | 0.0305 | 0.0212 | 0.0285 | 0.0262 | 0.0348 | 0.0182 | 0.0218 | 0.0274 | - |

**Table 4.** Dissimilarity matrices of the A/E ratio profiles of the whole body amino acid composition (above the diagonal, $D_1$ indices; below the diagonal, $D_2$ indices).

matrices in Tables 3 and 4. A typical pair of such diagrams is shown in Figure 1(a) and (b), for the requirements and the compositions, respectively, which were drawn with the Fitch-Margoliash (1967) algorithm, from dissimilarity matrices of $D_1$. In this type of diagram, the sum of the length of branches along the path connecting each pair of species corresponds to the estimate of the dissimilarity index best suited to the original dissimilarity matrix data.

It is obvious that the size of diagram (a) is about twice the size of diagram (b), indicating higher variability of A/E ratio profiles of dietary amino acid requirements than body amino acid compositions. Some researchers may argue that this difference in diagram size between (a) and (b) is the reflection of larger experimental error in the process of determining the dietary requirements than in determining the body amino acid compositions. It should be noted, however, that the species are not randomly distributed in Figure 1(a). Although the distances along the path connecting species do not seem to perfectly correspond to the genetic distances inferred from the phylogenetic relations suggested by Greenwood et al. (1966), there are clusters of species corresponding to phylogenetic classifications. Carp is neighboring with catla, both of which belong to the same family Cyprinidae. Further, chum salmon, chinook salmon, and coho salmon in the family Salmonidae are located close to one another. This cluster of salmonid species reflects the higher similarity of A/E ratios of requirements, in spite of the slight differences in experimental factors such as the main nitrogen sources and the amino acid composition of basal diets, and rearing temperatures. Whereas, in diagram (b) from the A/E ratios of whole body tissue composition, the fish species are randomly located irrespective of classification into families or phylogenetic categories and the distance among fishes is much shorter. It indicates that the essential amino acid compositions of whole body have smaller variations than the dietary requirements among fish species, as already reported by Wilson and Cowey (1985). Exactly the same topologies were obtained in the dissimilarity diagrams drawn using the neighbor-joining method (Saitou and Nei 1987) instead of the Fitch-Margoliash (1967) method.

It may be true that the use of amino acid composition profile for the whole body of fish, especially where dietary requirement data are not available, can be a useful tool in feed management, and it is certainly expected to be less time consuming and less expensive than the traditional method with repeated feeding experiments. The determined levels of essential amino acid requirement based on the concept of an "ideal" protein, however, would be similar among all fish species, because there are little differences in the body amino acid composition among species. Our findings deduced from growth data indicate that specificities of amino acid requirements among species or families exist as a concern in the balance (A/E ratio) of dietary amino acids. We therefore cannot deny the necessity for traditional methods in determining essential amino acid requirements by feeding experiments. We suggest that growth experiments concerning essential amino acid requirements should be conducted on at least one fish species in a family. Further, accurate studies are needed to ascertain specificity of essential amino acid requirements among different finfish species.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

Agricultural Research Council. 1981. Protein and amino acid requirements, pp. 67-124. *In:* Agricultural Research Council Working Party (ed.), The Nutrient Requirements of Pigs. Commonwealth Agricultural Bureaux, Slough, England.

Akiyama, T. and S. Arai. 1993. Amino acid requirements of chum salmon fry and supplementation of amino acid to diet, pp. 35-48. *In:* M. R. Collie and J. P. McVey (eds), Proceedings of the 20th U.S.-Japan Symposium on Aquaculture Nutrition. Newport, OR.

Akiyama, T., S. Arai, T. Murai, and T. Nose. 1985. Threonine, histidine and lysine

requirements of chum salmon fry. Nippon Suisan Gakkaishi 51: 635-639.

Akiyama, T., I. Oohara, and T. Yamamoto. 1997. Comparison of essential amino acid requirements with A/E ratio among fish species (review paper). Fish. Sci. 63: 963-970.

Arai, S. 1981. A purified test diet for coho salmon, *Oncorhynchus kisutch*, fry. Nippon Suisan Gakkaishi 47: 547-550.

Arai, S., T. Nose, and Y. Hashimoto. 1972. Amino acids essential for the growth of eels, *Anguilla anguilla* and *A. japonica*. Nippon Suisan Gakkaishi 38: 753-759.

Arai, S. and H. Ogata. 1993. Quantitative amino acid requirements of fingerling coho salmon, pp. 19-28. *In:* M. R. Collie and J. P. McVey (eds), Proceedings of the 20th U.S.-Japan Symposium on Aquaculture Nutrition, Newport, OR.

Borlongan, I. G. and R. M. Coloso. 1993. Requirements of juvenile milkfish (*Chanos chanos* Forsskal) for essential amino acids. J. Nutr. 123: 125-132.

Chance, R. E., E. T. Mertz, and J. E. Halver. 1964. Nutrition of salmonoid fishes XII. Isoleucine, leucine, valine and phenylalanine requirements of chinook salmon and interrelations between isoleucine and leucine for growth. J. Nutr. 83: 177-185.

Cowey, C. B., J. W. Adron, and A. Blair. 1970. Studies on the nutrition of marine flatfish. The essential amino acid requirements of plaice and sole. J. Mar. Biol. Assoc. U.K. 50: 87-95.

Delong, D. C. and J. E. Halver. 1962. Nutrition of salmonoid fishes X. Quantitative threonine requirements of chinook salmon at two water temperatures. J. Nutr. 76: 174-178.

Felsenstein, J. 1993. PHYLIP (Phylogeny inference package) Version 3.5c. Dept. Genetics, SK-50, Univ. Washington, Seattle.

Fitch, W. M. and E. Margoliash. 1967. The construction of phylogenetic trees. Science 155: 279-284.

Greenwood, P. H., D. E. Rosen, S. H. Weitzman, and G. S. Myers. 1966. Genetic studies of teleostean fishes, with a provisional classification of living forms. Bull. Amer. Mus. Nat. Hist. 131: 339-456.

Halver, J. E. 1965. Tryptophan requirements of chinook, sockeye and silver salmon. Fed. Proc. 24: 169.

Halver, J. E., D. C. Delong, and E. T. Mertz. 1957. Nutrition of salmonoid fishes V. Classification of essential amino acids for chinook salmon. J. Nutr. 63: 95-105.

Halver, J.E., D. C. Delong, and E. T. Mertz. 1958. Threonine and lysine requirements of chinook salmon. Fed. Proc. 17: 478.

Halver, J. E., D. C. Delong, and E. T. Mertz. 1959. Methionine and cystine requirements of chinook salmon. Fed. Proc. 18: 2076.

Halver J. E. and W. E. Shanks. 1960. Nutrition of salmonoid fishes VIII. Indispensable amino acids for sockeye salmon. J. Nutr. 72: 340-346.

Klein, R. G. and J. E. Halver. 1970. Nutrition of salmonoid fishes: arginine and histidine requirements of chinook salmon and coho salmon. J. Nutr. 100: 1105-1110.

Mazid, M. A., Y. Tanaka, T. Katayama, K. L. Simpson, and C. O. Chichester. 1978. Metabolism of amino acids in aquatic animal-III. Indispensable amino acids for *Tilapia zillii*. Nippon Suisan Gakkaishi 44: 739-742.

Moon, H. Y. and D. M. Gatlin III. 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95: 97-106.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia Univ. Press, New York. 512 p.

Nose, T., S. Arai, D. Lee, and Y. Hashimoto. 1974. A note on amino acids essential for growth of young carp. Nippon Suisan Gakkaishi 40: 903-908.

Ogata, H., S. Arai, and T. Nose. 1983. Growth responses of cherry salmon *Oncorhynchus masou* and amago salmon *O. rhodurus* fry fed purified casein diets supplemented with amino acids. Nippon Suisan Gakkaishi 49: 1381-1385.

Prevosti, A., J. Ocana, and G. Alonso. 1975. Distances between populations of

*Drosophila subobscura* based on chromosome arrangement frequencies. Theor. Appl. Genet. 45: 231-241.

Ravi, J. and K. V. Devaraj. 1991. Quantitative essential amino acid requirements for growth of catla, *Catla catla* (Hamilton). Aquaculture 96: 281-291.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 45: 406-425.

Santiago, C. B. and R. T. Lovell. 1988. Amino acid requirements for growth of Nile tilapia. J. Nutr. 118: 1540-1546.

Shanks, W. E., G. D. Gahimer, and J. E. Halver. 1962. The indispensable amino acids for rainbow trout. Prog. Fish-Cult. 24: 68-73.

Sneath, H. P. A. and R. R. Sokal. 1973. Numerical Taxonomy. W. H. Freeman and Company, San Francisco, CA. 573 p.

Wilson, R. P. 1989. Amino acids and protein, pp. 111-151. *In:* J. E. Halver (ed), Fish Nutrition, 2d Edition. Academic Press, San Diego, CA.

Wilson, R. P. 1993. Amino acid nutrition of fish: a new method of estimating requirement values, pp. 49-54. *In:* M. R. Collie and J. P. McVey (eds), Proceedings of the 20th U.S.-Japan Symposium on Aquaculture Nutrition, Newport, OR.

Wilson, R. P. and C. B. Cowey. 1985. Amino acid composition of whole body tissue of rainbow trout and Atlantic salmon. Aquaculture 48: 373-376.

Wilson, R. P. and W. E. Poe. 1985. Relationship of whole body and egg essential amino acid patterns to amino acid requirement patterns in channel catfish, *Ictalurus punctatus*. Comp. Biochem. Physiol. 80B: 385-388.

Yone, T. 1976. Nutritional studies of red sea bream. Rep. Fish. Res. Lab. Kyushu Univ. 3: 87-101.

# DEVELOPING A STOCK ENHANCEMENT PROGRAM BASED ON ARTIFICIAL SEEDLINGS: ACTIVITIES OF THE JAPAN SEA-FARMING ASSOCIATION (JASFA) IN THE LAST DECADE

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## ABSTRACT

The Japan Sea-Farming Association (JASFA) was established in 1963 as the Seto Inland Sea Farming Association and reorganized in 1979 as JASFA. JASFA has been engaged in the task of developing techniques relating to the farming fishery process. The term of farming fishery, in Japanese Saibai-Gyogyou, means the ideal fishery system which is composed of stock enhancement and fishery management. Farming fishery is based on the artificial seedlings technique which was constructed on some components, i.e., broodstock management, induced spawning, incubation of fertilized eggs, and rearing of fry and juveniles. Stock enhancement of the farming fishery was constructed on the intermediate rearing in nursery grounds to acclimatize artificial juveniles to the natural environment in releasing areas, seed release, management for released artificial seed in pre-recruit periods, and fishery management.

## INTRODUCTION

Oshima (1984) reviewed the historical development of the Japan Sea-Farming Association as follows. In 1961, the Japan Fisheries Agency (JFA) established a plan to promote coastal fisheries by developing stock enhancement technology utilizing potential and untapped productivity of the sea. The plan was put into action in 1962, and the Seto Inland Sea was selected as a model littoral zone of stock enhancement for the ranching of juveniles. The Seto Inland Sea Culture Fishery Center was established as the base of operation for the intended technological development. Furthermore, the Seto Sea Fish Farming Association was established in 1963, which operated the center by commission from the government. This name was derived from the abbreviation of "Fish Farming Promotion Actualization Center." This is the first time that "fish farming" was used. Recently, the term "farming fisheries" and "sea farming" have been used to express fish farming. In this paper, farming fisheries consists of stock enhancement based on artificial seedlings. In 1978, the Seto Inland Sea Fish Farming Association was reorganized and renamed the Japan Sea-Farming Association (JASFA) to develop the needed technology and to overcome the transitional period of financial difficulties. JASFA is mandated by JFA to develop stock enhancement techniques based on artificial seed production. The system and administrative roles of farming fisheries are summarized in Figure 1. The national government has been engaged in the technological development of highly migratory and migratory species. The prefectural governments are playing important roles in the development and commercialization of migratory and nearshore species. Public corporations and fishery cooperatives are organizations in charge of operating farming fisheries for coastal species, except for the technological development of some species such as the Japanese spiny lobster. The national government takes responsibility for the technological development of nearshore species such as the Japanese lobster because of the difficulties and high risks involved which are beyond the capabilities of prefectural governments (Matuoka 1996).

Figure 2 shows the locations of 16 national sea-farming centers operated by JASFA. National centers are located over a wide area ranging from the Akkesi Station, Hokkaido, in the subarctic zone (close to latitude 43°N), to the Yaeyama Station,

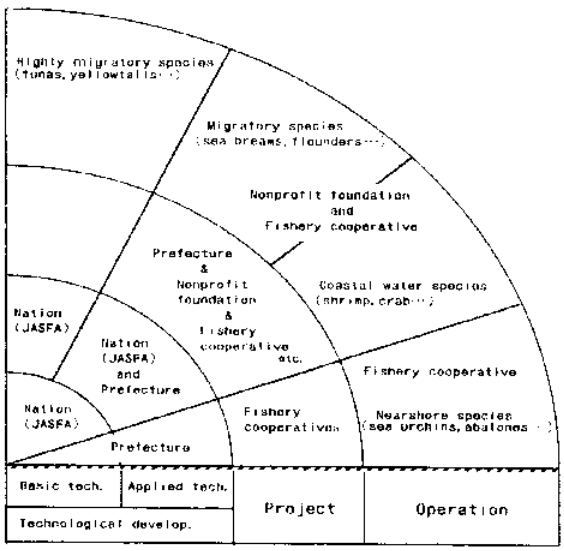


**Figure 1.** Schematic explanation of roles in farming fisheries in Japan (after Japan Fisheries Agency). JASFA denotes Japan Sea-Farming Association.

Okinawa, in the subtropical zone (close to 24°N). Thirty-nine prefectural governments have been constructed and are operating 53 prefectural sea-farming centers ( Fig. 3). Public corporations and fishery cooperatives have constructed sea-farming centers.

## Process of technological development in farming fisheries

The process of technological development in farming fisheries is schematically described in Figure 4, which shows the case of Atlantic bluefin tuna (Fushimi et al., in press.). This figure focuses on artificial seed production. The technique of artificial seed production is composed of four parts, i.e., broodstock management, induced maturation and spawning, larval rearing, and live feed culture. Artificial seed produced in sea-farming centers are transported to release areas, and then are reared in nursery grounds to acclimatize to the natural environment, or released immediately if the size of juveniles is adequate for survival in the natural environment. Fishery management methodology has to apply to artificial seed in pre-recruit and post-recruit periods in order to maintain optimal yield from them.

## Progress of artificial seed production, release, and catch

The technology of artificial seed production is making steady progress. In 1995, seed for stock enhancement was produced by 284 facilities for 80 species. The total production number was 3640 million individuals and the total release number was 11 billion individuals including natural seed (Morita 1997). The numbers of artificial seed production and release in 1995 were 3600 million for 80 species and 3000 million for 69 species, respectively. The role of JASFA, as shown in Figure 1, is the technological development for highly migratory species, migratory species, and coastal water

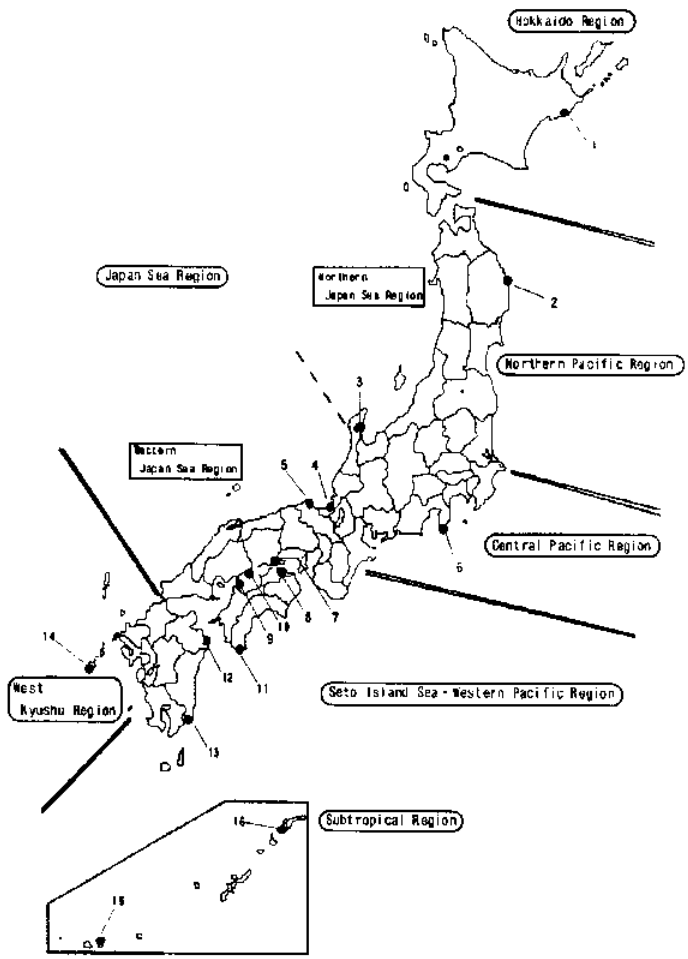


**Figure 2.** Locations of the JASFA Stations.
1. Akkesi Stn. 2. Miyako Stn. 3. Notojima Stn. 4. Obama Stn. 5. Miyazu Stn. 6. Minami-Izu Stn. 7. Tamano Stn. 8. Yashima Stn. 9. Hakatajima Stn. 10. Momoshima Stn. 11. Komame Stn. 12. Kamiura Stn. 13. Shibushi Stn. 14. Goto Stn. 15. Yaeyama Stn. 16. Amami Stn.

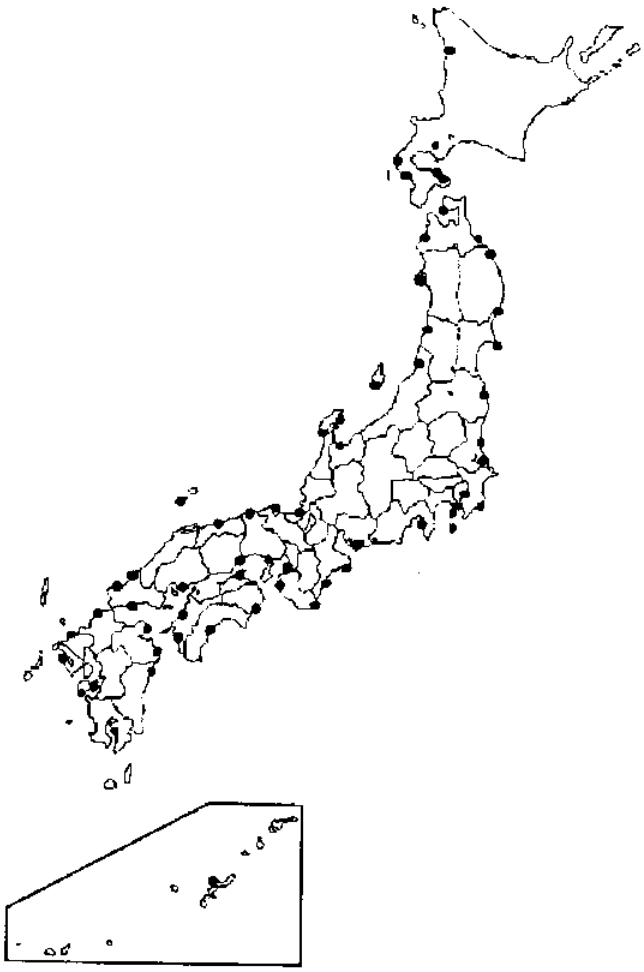

**Figure 3.** Locations of prefectural sea-farming centers.

species. Target species number and number of seed production of JASFA in 1995 were 36 species and 120 million individuals, respectively, excluding Mollusca and Echinodermata (JASFA 1997a). Public corporations and fishery cooperatives engaged in intermediate rearing and releasing operations numbered 1387.

Over 1 million seed each are produced for 33 species, and over 10 million seed each for 11 species. They are three species of Pisces: Japanese flounder *Paralichthys olivaceus*, red sea bream *Pagrus major*, and black sea bream *Acanthopagrus schlegeli;* three species of Crustacea: kuruma prawn *Penaeus japonicus*, swimming crab *Portunus trituberculatus*, and speckled shrimp *Metapenaeus ensis*; four species of Mollusca: scallop *Patinopecten yesoensis*, short-neck clam *Tapes philippinarum*, Yeso abalone *Nordotis discus hannai*, and disk abalone *Nordotis discus discus*; and one species of Echinodermata: northern green sea urchin *Strongylocentrotus intermedius*. Annual fluctuations of the number of seed production, release, and catch in some species are described as follows:

**Japanese flounder *Paralichthys olivaceus***

Figure 5 shows annual fluctuations of the number of seed production, release, and catch of Japanese flounder. The numbers of seed production and release are increasing steadily, and quantity of seed production and releases have surpassed that of red sea bream in 1995. Quantity of seed production in 1995 was 31 million individuals and release was 23 million individuals, respectively. Mean annual seed production and release numbers are 19 million and 13 million individuals, respectively. Mean annual catch is 6800 tons, which fluctuated between 5100 (1990) to 8200 (1986) tons, and catch has been increasing since 1991.

**Red sea bream *Pagrus major***

The technological development of farming fisheries in Pisces is represented by red sea bream, and good results in the technological development of this fish have been leading new trials for another species. The numbers of seed production, release, and catch of red sea bream are shown in Figure 6. Mean annual seed production and release numbers are 26 million and 19 million individuals, respectively.

Mean annual catch is 14,000 tons, and annual catch fluctuated between 13,000 tons (1988) to 16,000 tons (1984). Recently, it has become apparent that sport fisheries land similar quantities; thus, regulation and symbiosis with sport fishing are new problems to solve ( Imai 1994, Imai et al. 1994, Imai 1996, and Shinoda 1997).

**Black sea bream *Acanthopagrus schlegeli***

The numbers of seed production, release, and catch of black sea bream are shown in Figure 7. Mean annual seed production and release numbers are 9 million and 6 million individuals, respectively.

Mean annual catch is 3900 tons, and annual catch fluctuated between 3600 tons (1994) to 4,300 tons (1984). This fish encounters the same

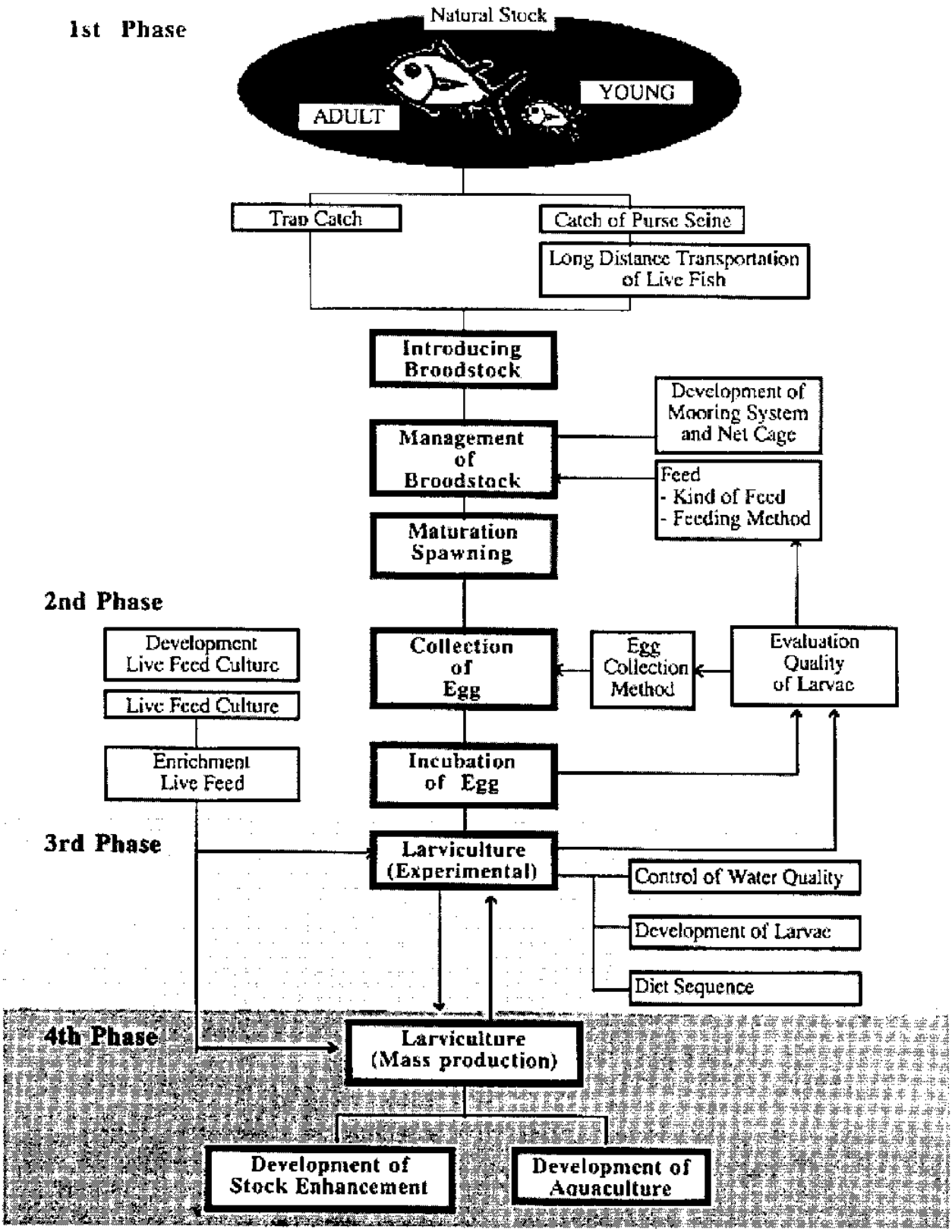


**Figure 4.** Schematic explanation of technological development in stock enhancement of Atlantic bluefin tuna (after Fushimi et al., in press).

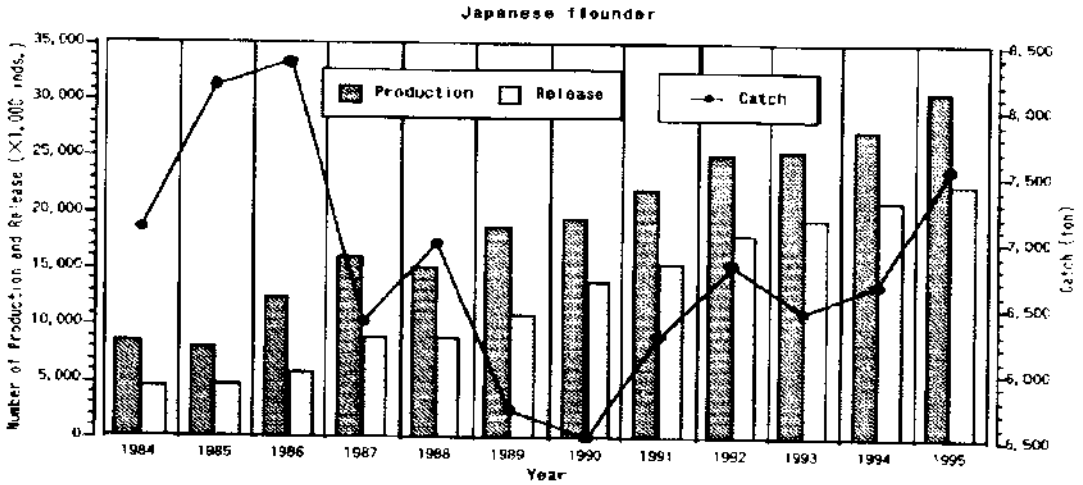


**Figure 5.** Annual fluctation of seed production, release and catch of Japanese flounder *Paralichthys olivaceus*.

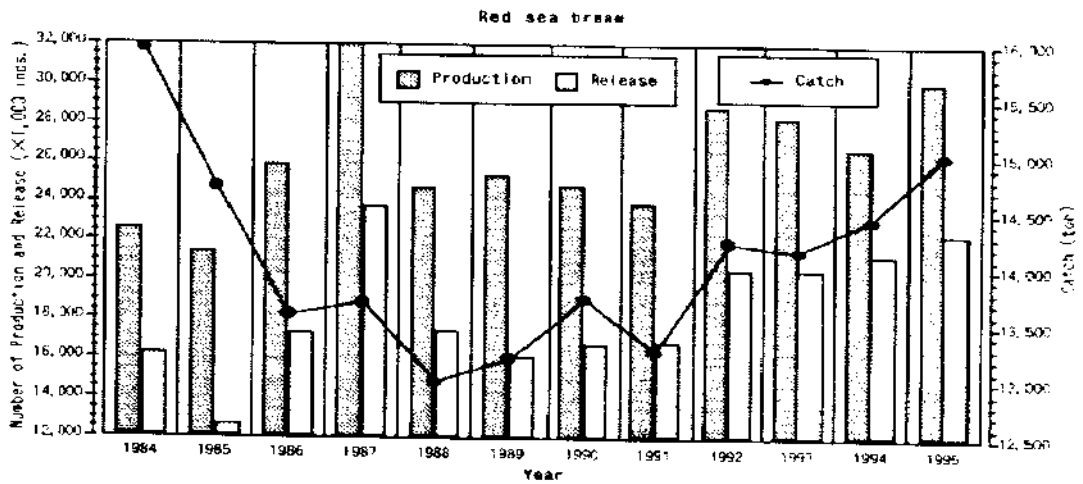


**Figure 6.** Annual fluctation of seed production, release and catch of red sea bream *Pagrus major*.

problems as red sea bream, i.e., regulation and symbiosis with sport fishing.

### Kuruma prawn *Penaeus japonicus*

The technological development in farming fisheries of kuruma prawn has attained the role of pioneer in this field accompanied by red sea bream. The first guidebook publication of the kuruma prawn farming fishery was issued by JASFA in 1986 (Kurata et al. 1986).

The numbers of seed production, release, and catch of the kuruma prawn are shown in Figure 8. Mean annual seed production and release numbers are 510 million and 305 million individuals, respectively.

Mean annual catch is 3000 tons, and annual catch fluctuated between 2300 tons (1993) to 3400 tons (1984). It seems that abundance of the kuruma prawn has been recovering by farming fisheries, because the mean annual catch had

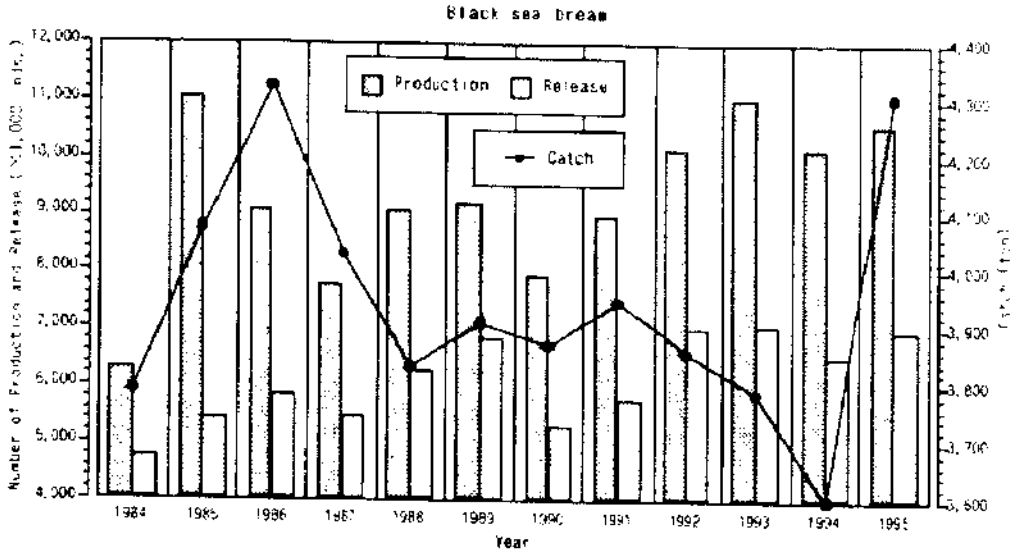


**Figure 7.** Fluctuation of seed production, release and catch of black sea bream *Acanthopagrus schlegeli*.

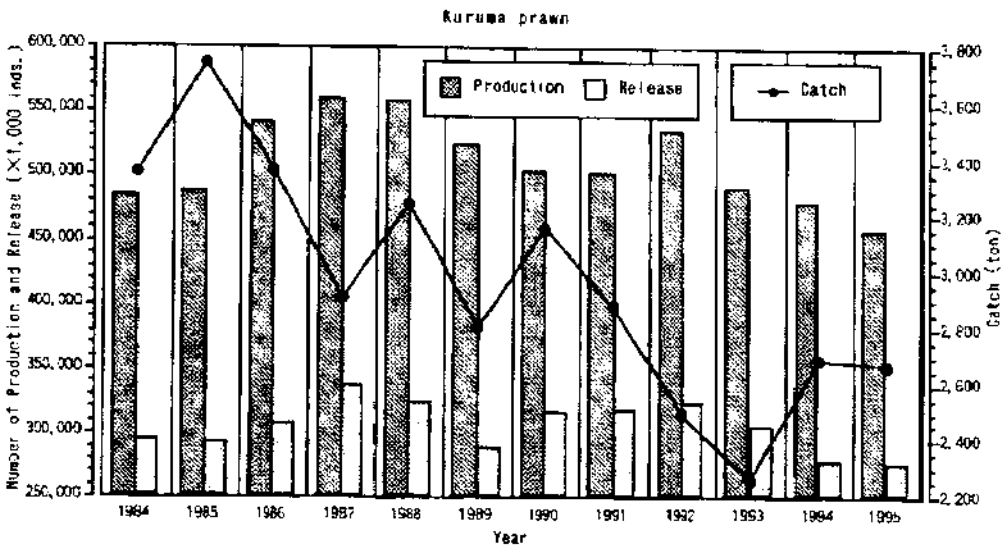


**Figure 8.** Annual fluctuation of seed production, release and catch of kuruma prawn *Penaeus japonicus*.

declined to 1000 tons in the late 1960s. The case of Hamana Lake, a brackish lake in Shizuoka prefecture, is well known (Fushimi 1983).

### Swimming crab *Portunus trituberculatus*

The technological development of farming fisheries in the swimming crab has played an important role in this field, too. A monograph and manual of seed production was published by JASFA recently (Hamasaki 1996, JASFA 1997b).

The numbers of seed production, release, and catch of the swimming crab are shown in Figure 8. Mean annual seed production and release

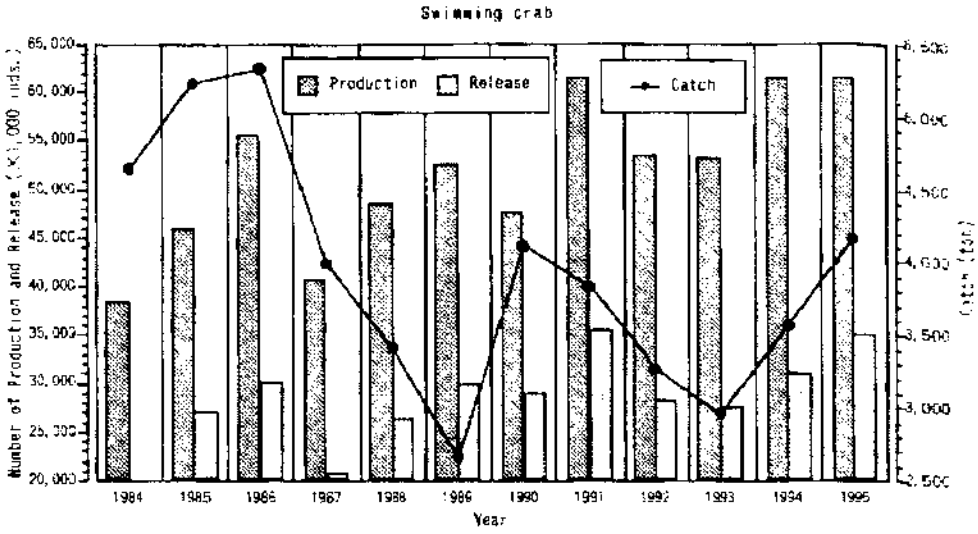


**Figure 9.** Annual fluctuation of seed production, release and catch of swimming crab *Portunus trituberculatus*

numbers are 52 million and 28 million individuals, respectively.

Mean annual catch is 3900 tons, and annual catch has fluctuated between 3000 tons (1993) to 5300 tons (1986). Mean annual catch in the late 1960s declined to nearly 1000 tons, thus abundance of the swimming crab has recovered by farming fisheries, too.

### New frontier of farming fisheries

Exploitation of the field of farming fisheries has been continuing within JASFA, using the accumulated experiences and knowledge of over 30 yr. Technological developments in various JASFA activities are making steady progress, some of which are briefly described below.

### Development of biocontrol for seed production of the swimming crab

Results of seed production of the swimming crab is influenced by the flora of microorganisms. Bacterial strain PM-4, isolated from a crustacean culturing pond, improved the growth of swimming crab larvae and repressed growth of *Vibrio anguillarum* in seawater. Methodology to apply this finding has been developed, and production of swimming crab larvae was greatly increased by adding the bacterial strain PM-4 to their culture water (Nogami and Maeda 1992, Nogami et al., in press). It is expected that this will be used in crustacean seed production.

### Development of rearing larvae of Japanese spiny lobster *Panulirus japonicus*

Since 1899, many Japanese marine biologists have tried to rear phyllosoma of *P. japonicus; the* first success to 3 instar was attained in 1958 (Nonaka et al. 1958). After that, the rearing period was gradually improved, and last stage phyllosoma was attained in 1981 (Inoue 1981). The first successful rearing of juveniles was realized in 1989 (Yamakawa et al. 1989, Kittaka and Kimura 1989). Success in rearing larvae of *P. japonicus* was not reproduced due to difficulties in rearing. JASFA established the Minami-Izu Station in 1988, to engage in the development of rearing larvae of *P. japonicus*. Subsequently, the JASFA Minami-Izu Station has attained the complete rearing of phyllosoma of the lobster. It seems that development in hardware of the rearing system for phyllosoma is the main reason for this success ( JASFA 1993). The JASFA Minami-Izu Station had produced 134 puerulii and 48 juveniles in 1994, and 284 puerulii and 114 juveniles were produced during 1990-1996.

**Development of broodstock management and rearing larvae of Pacific bluefin tuna *Thunnus thynnus***

In JASFA, the development of broodstock management of Pacific bluefin tuna (PBT) had started in 1985 at the Yaeyama Station in Okinawa, established in 1985. Broodstock of PBT was reared in net cages, and we observed very rapid growth rate, but very low survival rate, due to high water temperature in the subtropical area. The JASFA Amami Station further north was established in 1995, and has been engaged in the development of broodstock management of PBT.

The first spawning success of PBT broodstock of JASFA was attained in 1997. The first successful spawning was observed for the 9-10 age group reared in 40-m round-shaped net cages 10 m deep on 13 May 1997, and 1,500,000 fertilized eggs were collected from this broodstock. Spawning of 7 age groups had been induced by rising water temperature in early July 1997, and 5,600,000 fertilized eggs were collected in just 2 days. Subsequently, egg quality of these fertilized eggs was examined. Experimental rearing of PBT larvae was begun at the Amami Station in 1997 (Yamazaki 1997).

**Development of farming fisheries of Pacific herring (resident type) *Clupeia pallasi***

Pacific herring *Clupeia pallasi* has shown drastic stock abundance fluctuation, especially in the Hokkaido-Sakhalin stock. Local stock of Pacific herring (resident type, RT herring) has been inhabiting off eastern Hokkaido; their spawning ground is distributed in the *Zostera* zone of brackish lakes, i.e., Notsuke Bay, Furen-ko, Akkesi Bay, and Yudo-numa, and their migrating area is limited to the coastal area of eastern Hokkaido. The JASFA Akkesi Station, established in 1981, has been engaged in the technical development of farming fisheries for RT herring since 1983 at Notsuke-ko. Population parameters of released RT herring were estimated recently, and the stock abundance of RT herring is recovering since artificial seed release was begun, with an estimated recovery rate at 6%. It is a successful example of the technological development carried out by the JASFA Akkesi Station on seed production, intermediate rearing, large scale marking techniques for otholith using Alizarin-complexone (ALC), application of statistical survey techniques for the fisheries market, and foundation of a cooperative system for stock enhancement trials by fishery cooperatives, administration, and research.

**Development of seed production and release of coonstripe shrimp *Pandalus hypsinotus***

Coonstripe shrimp *Pandalus hypsinotus* is one of the important target species for the deep sea pot fisheries, and the JASFA Obama Station, established in 1983, has been engaged in the technical development of this shrimp. The JASFA Obama Station has developed techniques for artificial seed production and subsequent release in 200 to 300m water depths. Survival rate of seed production and density of post-larvae have been consistently attained at 70% and 7000 individuals/$m^3$, respectively. Experimental artificial seed release has been carried out at Toyama Bay in 200 to 300m depths, and results of this experiment point to the success of stock abundance recovery by artificial seed release.

Many species of the *Pandulus* group are important commercial fisheries, and technological developments by the JASFA Obama Station have attracted the attention of people concerned with the the deep sea pot fishery. The technique of seed production for sandfish *Arctocopous japonicus* developed by the JASFA Notojima Station is unique. Larvae have been reared by using natural plankton, composed mainly of Copepodite, collected by nightlighting. Trials of stock enhancement based on seed release have been continued in Akita Prefecture, because of the drastic decline in abundance.

According to this brief overview of activities in farming fisheries, it is evident that the presence of farming fisheries is essential for exploiting and maintaining marine resources by the Japanese coastal fisheries. This review focuses on an overview of the main activities and some new frontiers. We have faced many problems to solve in order to establish the needed technology of farming fisheries, and continuing efforts are required.

## ACKNOWLEDGMENTS

I wish to express sincere thanks to Dr. Izumi Nakamura and Mrs. Reiko Nakamura, Kyoto University, for editing scientific and English names of aquatic organisms. I thank the technical staff of JASFA, particularly Kinya Nogami, Tetsuo Morita, Syukei Masuma, Shigenori Suzuki, Masato Aritaki, Keita Hattori, Taizo Morioka, and Shintaro Sekine for helpful discussions.

## LITERATURE CITED

Fushimi, H. 1983. Stock enhancement of Kuruma prawn (*Penaeus japonicus*) in Hamana-ko (lagoon), pp. 236-253.. *In:* Y. Oshima ( ed. ), Tsukuru Gyogyo, latest edition. [In Japanese].

Fushimi, H., K. Kani, H. Nhhala, S. Nakamura, A. Abrehouch, K. Chebaki, and A. Berraho. In press. Attempt on resources enhancement of Atlantic bluefin tuna, present status and future perspective of Japan-Moroccan cooperative project for aquaculture of Atlantic bluefin tuna. ICCAT Special Publication on ICCAT 25th Anniversary International Tuna Symposium.

Hamasaki, K. 1996. Study on the reproduction and development of the swimming crab, *Portunus trituberculatus*. JASFA Spec. Bull. 8, 124 pp.

Imai, T. 1994. Present status of fishing activities on red sea bream with the pleasure boats in Kanagawa Prefecture. Saibai Giken 23(1): 85-93. [In Japanese].

Imai, T. 1996. Estimates of return rates of red sea bream seeds released in Kanagawa Prefecture. Saibai Giken 25(1): 59-784. [In Japanese].

Imai, T., H. Takama, and I. Shibata. 1994. Estimaties of the total amount of red sea bream caught by recreational party boats in Kanagawa Prefecture. Saibai Giken 23(1): 77-83. [In Japanese].

Inoue, M. 1981. Study on rearing of phyllosoma of the Japanese spiny lobster, *Panulirus japonicus*. Spec. Bull. Kanagawa Pref. Fish. Exp. Stn. 1, 91 pp. [In Japanese].

JASFA (Japan Sea-Farming Association). 1993. Development of larviculture of the Japanese spiny lobster, *Panulirus japonicus*. Tech. Rep. 55, 128 pp. [In Japanese ].

JASFA (Japan Sea-Farming Association ). 1997a. Annual report of Heisei 7 nenndo. 365 pp. [In Japanese].

JASFA (Japan Sea-Farming Association). 1997b. Principles and practice in mass-larviculture of the swimming crab, *Portunus trituberculatus*. Saibai-gyogyou Gijutu Ser. 3, 181 pp. [In Japanese].

Kittaka, J. and K. Kimura. 1989. Culture of the Japanese spiny lobster, *Panulirus japonicus* from egg to juvenile stage. Jpn. J. Sci. Fish. 55(6): 963-970.

Kurata, H., F. Hiyama, and H. Fushimi, Editors. 1986. Guide book of the kuruma prawn farming fishery. Saibai-sosho 1, JASFA, 306 pp. [In Japanese].

Matuoka, T. 1996. Present state and prospects of Japan's sea farming. Abstract. The International Symposium on Marine Ranching, Ishikawa.

Morita, T. 1997. Number of hatchery-reared seeds and its release in the period April 1995-March 1996. Saibai 82: 17-25. [In Japanese].

Nogami, K. and M. Maeda. 1992. Bacteria as biocontrol agents for rearing larvae of the crab *Portunus trituberculatus*. Can. J. Fish. Aquat. Sci. 49: 2373-2376.

Nogami, K. K. Hamasaki, M. Maeda, and K. Hirayama. In press. Biocontrol method in aquaculture for the swimming crab larvae *Portunus trituberculatus*. Special Bulletin of the Faculty of Fisheries, Nagasaki University.

Nonaka, M., Y. Oshima, and R. Hirano. 1958. Rearing and ecdysis of phyllosoma of the Japanese spiny lobster, *Panulirus japonicus*. Suisan-zosyoku 5(3): 13-15. [In Japanese].

Oshima, Y. 1984. Status of "fish farming" and related technological development in the cultivation of aquatic resources in Japan. *In:* I.C. Liao and R. Hirano (eds.), Proceedings of ROC-JAPAN Symposium on Mariculture. Tungkang Mar. Lab. Conf. Proc. 1: 1-11.

Shinoda, M. 1997. Sports fishing and commercial fisheries of the red sea bream "Madai" in the sea off Kyoto Prefecture. Aquabiology 19(4): 321-325. [In Japanese].

Yamakawa, T., M. Nishimura, H. Matsuda, A. Tsujigado, and N. Kamiya. 1989. Complete larval rearing of the Japanese spiny lobster, *Panulirus japonicus*. Jpn. J. Sci. Fish. 55(4): 745.

Yamazaki, H. 1997. Spawning success of the Pacific bluefin tuna in Amami Station, JASFA. Saibai 83: 1. [In Japanese].

# EFFECTS OF COVERING A TIDAL FLAT WITH SAND FOR STOCK ENHANCEMENT OF TONGUEFISH: A FEASIBILITY STUDY AT ARIAKE SOUND IN KYUSHU, JAPAN

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## ABSTRACT

Ariake Sound is characterized by a high tidal range of about 6 m at the innermost part, and is known to have high productivity of commercially important species. However, the production of certain species has shown decreasing trends due to overfishing and deterioration in environmental conditions. Tonguefish are important species for gill net and trawl fisheries in this sound because of their high commercial values, but the annual catch of *Cynoglossus abbreviatus* has been decreasing markedly during the last decade. We assumed that covering the muddy tidal flat with sand as a means of habitat restoration would enhance the stock of these fish. In order to study the effects of sand covering on growth and survival of tonguefish *C. abbreviatus* and *C. joyneri* juveniles, we carried out periodic samplings by small beam trawl at the innermost part of the sound. A sand-covered area made in 1991, at about the lowest low water level, to increase the production of short-neck clams was selected as the survey area. The gear was towed along the lines set on the sand-covered area and a nearby muddy area as a control. The periodic samplings revealed that occurrence of *C. abbreviatus* in the sand-covered area increased with growth, but was not the case for *C. joyneri*. Since the larger juveniles of *C. abbreviatus* changed their prey animals from copepods to gammarids and mysids which were known to be abundant in the sandy area, it was suggested that covering the mud with sand provided beneficial effects at least for the growth and survival of this species.

## INTRODUCTION

Ariake Sound in Kyushu is characterized by a wide tidal range of 6 m at the innermost part, and a large (263 km$^2$ ) tidal flat that accounts for 40% of the Japanese tidal flats (Sugano 1981). High productivity of this sound due to these topographic features supports various kinds of fisheries including laver culture whose annual output is about 40 billion yen (ca. $330 million).

Fauna in the sound is unique and many species exist only here in Japan. Some of these species are regarded as continental relics, including the tonguefish *Cynoglossus abbreviatus*. The fishing of tonguefish is conducted only in the sound and a part of the Seto Inland Sea (Ohsaka and Koshiishi 1997). Another tonguefish, *C. joyneri*, inhabits the coast in the southern part of Japan, but some taxonomic studies are still ongoing since some morphological differences were found between the fish in the sound and in other waters. These two species, together with *C. robustus*, are important species for gill net and trawl fisheries in the sound because of their high commercial value, but the annual catch of these fishes has been decreasing during the last decade.

The decreasing trends in catches of tonguefish and other commercially important species can be attributed to overfishing and deterioration in environmental conditions. The reduction of sandy tidal flats is thought to be one of the serious environmental changes. An attempt to cover a muddy tidal flat with sand to restore the production of the short-neck clam has been carried out, and some positive achievements have been demonstrated (Ueda and Yamasita 1997). The sand covering of the muddy flat is predicted to make conspicuous changes in terms of the burrowing condition and food organism distribution for tonguefish juveniles that inhabit the tidal flat as their nursery ground. This research is to study the effect of this manipulation on the enhancement of these

fish. We hypothesized that covering the muddy flat with sand produces positive effects on the growth and survival of tonguefish by means of beneficial change in feeding conditions.

## MATERIALS AND METHODS

A sand-covered area made from 1991 through 1995 in an attempt to increase the production of short-neck clams was selected as the survey area. The muddy area of 300 m by 900 m at about the lowest low water level was covered with a sand layer 40 cm thick (Fig. 1). Sampling was carried out on the days of the spring tide of May, June, and August in 1994 and 1995. A beam trawl net with a 2-m-mouth width and 2.1-mm-mesh aperture was used as the sampling gear. The net was towed by a boat along the two 200-m lines, one set on the sand-covered area made in 1991 and the other set on a nearby muddy area as a control (Fig. 1). Samplings by the beam trawl net along each line were performed five times serially, two times at flood tide, one time at high tide, and two times at ebb tide when the water depths were about 2, 3.5, and 5 m, respectively. Since towing a beam trawl net by boat could not be performed properly when the water depth decreased below 2 m, a small set net with a 10-m wing was also used to catch fishes.

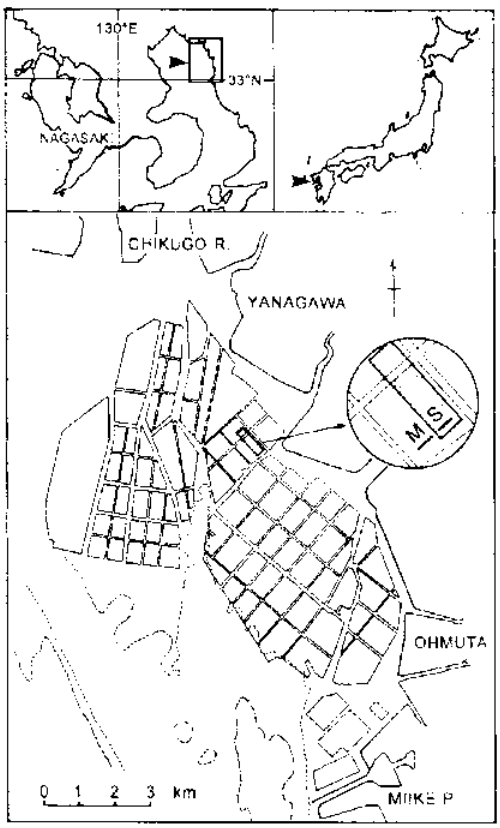


**Figure 1.** Map of Ariake Sound. Screened area off Yanagawa City indicates the sand-covered area. The lines for beam trawl are indicated within the circle where S denotes the line in the sand-covered area, and M denotes the line in the muddy control area.

Some sediment samples were collected to analyze the particle size by wet sieving and the distribution of possible prey for tonguefish. The digestive tract contents of tonguefish collected in the previous survey were examined to study prey animals.

## RESULTS AND DISCUSSIONS

### Occurrence, distribution, and growth of tonguefish at the northeastern part of the sound

Our previous survey on the distribution of tonguefish from 1990 thorough 1993 revealed that four species of Cynoglossidae juveniles, i.e., *Cynoglossus robustus*, *C. abbreviatus*, *C. joyneri*, and *C. interruptus*, occurred in water shallower than 25 m at the northeastern part of the sound. Within the intertidal zone, *C. abbreviatus* and *C. joyneri* were numerically dominant, so we focused our study on these two species. From the occurrence of juveniles less than 15 mm, we predicted that the periods for settlement of *C. abbreviatus* and *C. joyneri* were from March to May and from July to October, respectively. Older 0-group (0-yr-old) *C. abbreviatus* seemed to migrate offshore or into deeper parts of the sound, because the density of the juveniles in the shallow area decreased to nearly zero in winter (Fig. 2a). This seasonal migration was confirmed by the information obtained through a questionnaire on tonguefish occurrence sent out to fishermen (Ohsaka and Koshiishi 1995). Contrary to this, seasonal change in the density of *C. joyneri* was rather low in general. Though there was a certain depth migration, 0-group *C. joyneri* inhabited the area shallower than 10 m during their first year (Fig. 2b). Mean body length of 1-yr-old *C. abbreviatus* collected in the early settling season was about 150 mm and that of *C. joyneri* was 130 mm (Koshiishi et al. 1994).

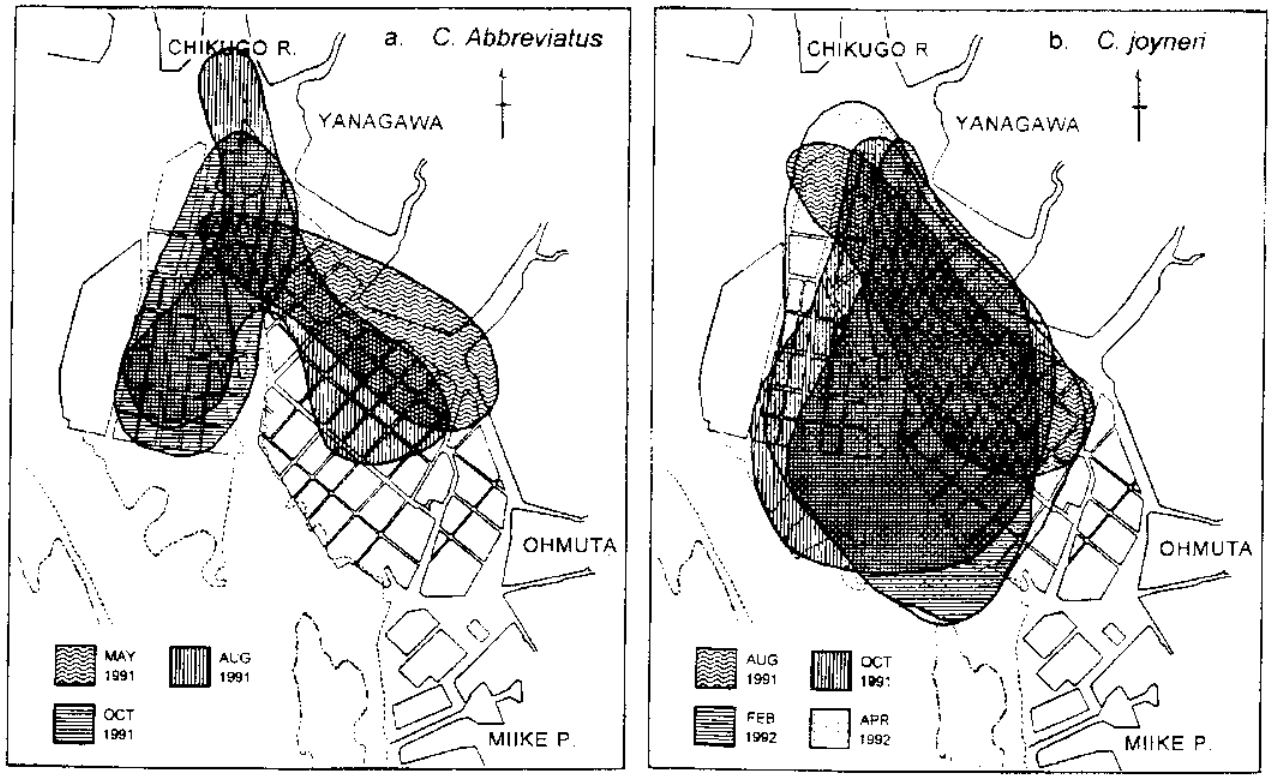


**Figure 2.** Seasonal change in main distribution area of 0-group tonguefish in northeastern part of Ariake Sound. a: *Cynoglossus abbreviatus*; b: *Cynoglossus joyneri*. The dotted line indicates 5-m isodepth. Five sampling surveys were carried out from May 1991 through April 1992. Since the number of 0-group *C. abbreviatus* collected in February was few, no illustration was presented.

## Distribution of tonguefish in the sand-covered area

The ground levels of the sand-covered area and the control (muddy) area were about 50 cm and 10 cm above the lowest low water level, respectively. More than half of the sediment on the sand-covered area consisted of medium and coarse sand, and about 70% of that in the control area consisted of particles less than 63 mm (Fig. 3). About 25 thousand fish of ca. 50 species were collected in our survey in 1994 and 1995 (Table 1). The fact that more than 90% of these fish were juveniles confirmed the importance of the tidal flat as a nursery habitat for fish, as pointed out previously by Uchida and Tsukahara (1955). Among these species, several gobiidae species were numerically dominant. Cynoglossidae species were also collected in relatively large numbers.

Though the lines on the sand-covered area and the muddy control area were set closely, only 50 m apart, the majority of each demersal fish

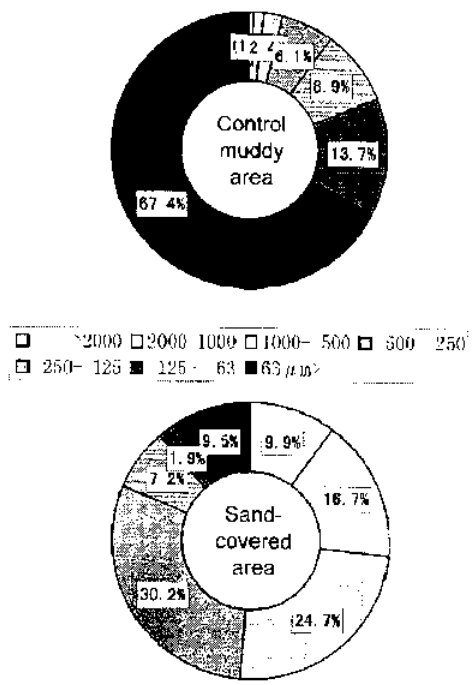


**Figure 3.** Particle size composition of the sand-covered area and nearby muddy control area in 1994.

**Table 1.** Fish species collected by small beam trawl net and set net in the sand-covered area and muddy control area in 1994 and 1995.

| | |
|---|---|
| Clupeidae | *Sardinella zunasi* |
| | *Konosirus punctatus* |
| | *Ilisha elongata* |
| Engraulididae | *Engraulis japonicus* |
| | *Coilia nasus* |
| Congridae | *Conger myriaster* |
| Salangidae | *Salanx ariakensis* |
| Plotosidae | *Plotosus lineatus* |
| Synodontidae | sp. |
| Belonidae | sp. |
| Hemiramphidae | sp. |
| Syngnathidae | sp. |
| Mugilidae | sp. |
| Atherinidae | *Hypoatherina valenciennei* |
| Leiognathidae | sp. |
| Sciaenidae | *Nibea albiflora* |
| | *Argyrosomus argentatus* |
| Trichiuridae | *Trichiurus lepturus* |
| Centrolophidae | *Psenopsis anomala* |
| Stromateidae | sp. |
| Gobiidae | *Acentrogobius pflaumii* |
| | *Favonigobius gymnauchen* |
| | *Silhouettea dotui* |
| | *Tridentiger barbatus* |
| | *Tridentiger nudicervicus* |
| | *Tridentiger bifasciatus* |
| | *Glossogobius olivaceus* |
| | *Chaenogobius uchidai* |
| | *Acanthogobius flabimanus* |
| | *Acanthogobius hasta* |
| | *Amblychaeturichthys hexanema* |
| | *Apocryptodon punctatus* |
| | *Ctenotrypauchen microcephalus* |
| | *Taenioides cirratus* |
| | *Taenioides rubicundus* |
| Platycephalidae | *Cociella crocodila* |
| | *Platycephalus indicus* |
| Callionymidae | *Repomucenus richardsonii* |
| | *Repomucenus valenciennei* |
| Paralichthyidae | *Paralichthys olivaceus* |
| | *Pseudorhombus arsius* |
| Pleuronectidae | *Pleuronichthys cornutus* |
| | *Pleuronichthys sp.* |
| | *Kareius bicoloratus* |
| Soleidae | *Zebrias zebra* |
| | sp. |
| Cynoglossidae | *Cynoglossus lighti* |
| | *Cynoglossus abbreviatus* |
| Tetraodontidae | *Takifugu xanthopterus* |
| | *Takifugu rubripes* |

species was collected, throughout the survey period, in either the sand-covered area or control area (Fig. 4), indicating the strong effect of sediment condition on their distribution. As for Gobiidae species, almost all *Favonigobius gymnauchen* were collected in the sand-covered area while *Acentrogobius pflaumii* were collected in the control area. The tendency of one-sided catch in these species was recognized regardless of their size, or age. On the other hand, density ratios between the sand and control areas for juveniles of *Acanthogobius hasta* drastically increased with growth (Table 2). The juveniles collected in the sand-covered area were about 10% of those in the control area when less than 20 mm in body length, but became nearly 100% when they exceeded 60 mm.

As for Pleuronectiformes, almost all Japanese flounder *Paralichthys olivaceus* and stone flounder *Kareius bicoloratus* were collected in the sand-covered area (Fig. 4). Contrary to this, only a few *C. joyneri* juveniles were collected in the sand-covered area (Fig. 5). Though a few small juveniles were collected in the sand-covered area in August, the older 0-group fish were collected in the control area without exception. The juveniles of *C. abbreviatus* showed a similar distribution pattern to *A. hasta*. The percentage of juveniles collected in the sand-covered area increased with growth (Fig. 5, Table 2).

Figure 6 shows the body length fequency of two tonguefish species caught by the two sampling gears in 1994 and 1995. In June, the average body length of *C. abbreviatus* collected by beam trawl net in the sand-covered area was larger than that in the control area, and the differences were significant in both years. This is very interesting because no such results were obtained for *C. joyneri*. In August, the number of *C. abbreviatus* collected by beam trawl net decreased markedly, and the number collected by set net increased in turn. It is noteworthy that more than half of the *C. abbreviatus* collection by set net occurred when the water depth decreased to less than 1 m of ebb tide. These results suggested that *C. abbreviatus* juveniles expand their habitat from the muddy tidal flat to the sandy flat with growth. The increase in number of fish collected by set net in August may indicate that

Table 2. Mean density (N/100m²) of 0-group fish caught by beam trawl on the days of spring tide and respective mean body length.

| | 1994 | | | 1995 | | |
|---|---|---|---|---|---|---|
| | MAY | JUN | AUG | MAY | JUN | AUG |
| *Acanthogobius hasta* | | | | | | |
| Sand-covered area (S) | 1.6 | 1.2 | 0.3 | 10.2 | 13.6 | 1.1 |
| Control (muddy) area (M) | 15.2 | 5.8 | 0.3 | 157.2 | 26.7 | 1.4 |
| S/M (%) | 10 | 21 | 106 | 7 | 51 | 77 |
| Mean body length (mm) | 17.5 | 45.4 | 69.2 | 13.7 | 32.5 | 64.5 |
| *Cynoglossus abbreviatus* | | | | | | |
| Sand-covered area (S) | 0.3 | 0.1 | 0.1 | 0.2 | 0.8 | 0.2 |
| Control (muddy) area (M) | 8.9 | 4.3 | 0.3 | 2.0 | 1.7 | 0.6 |
| S/M (%) | 4 | 2 | 15 | 8 | 46 | 36 |
| Mean body length (mm) | 22.6 | 45.0 | 113.4 | 15.7 | 31.8 | 110.7 |

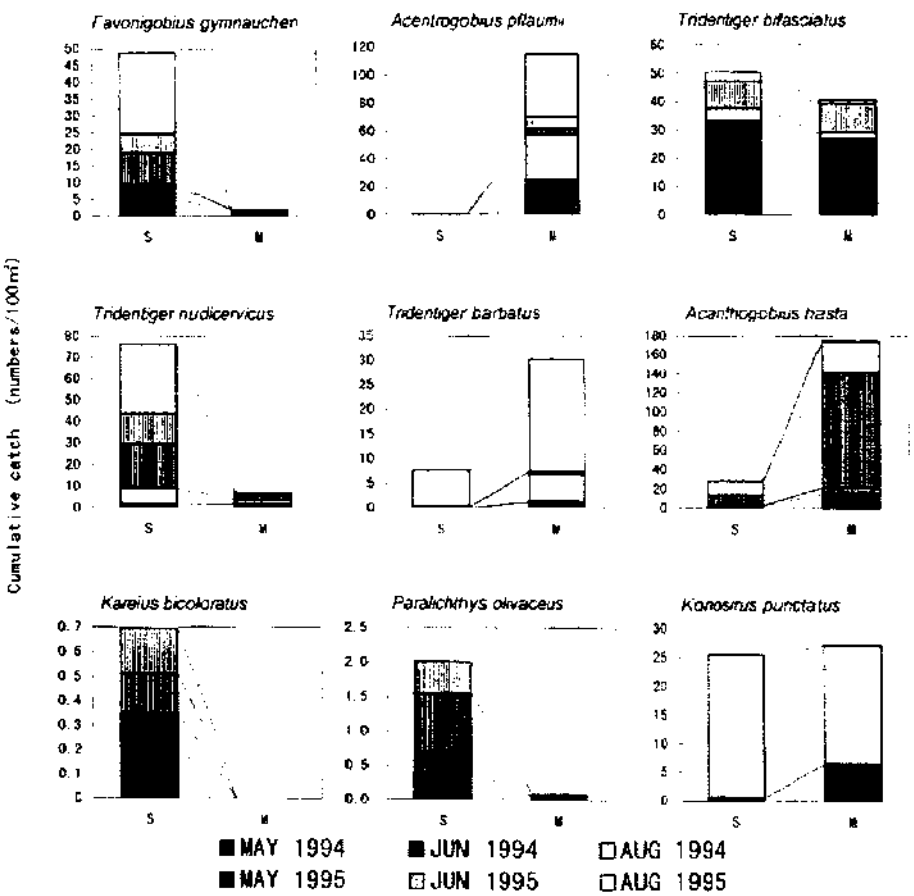


**Figure 4.** Cumulative catches of nine fish species by six serial samplings carried out in the sand-covered are (S) and nearby muddy control area (M). Average number per unit area of five to six towings in each sampling series was cumulated.

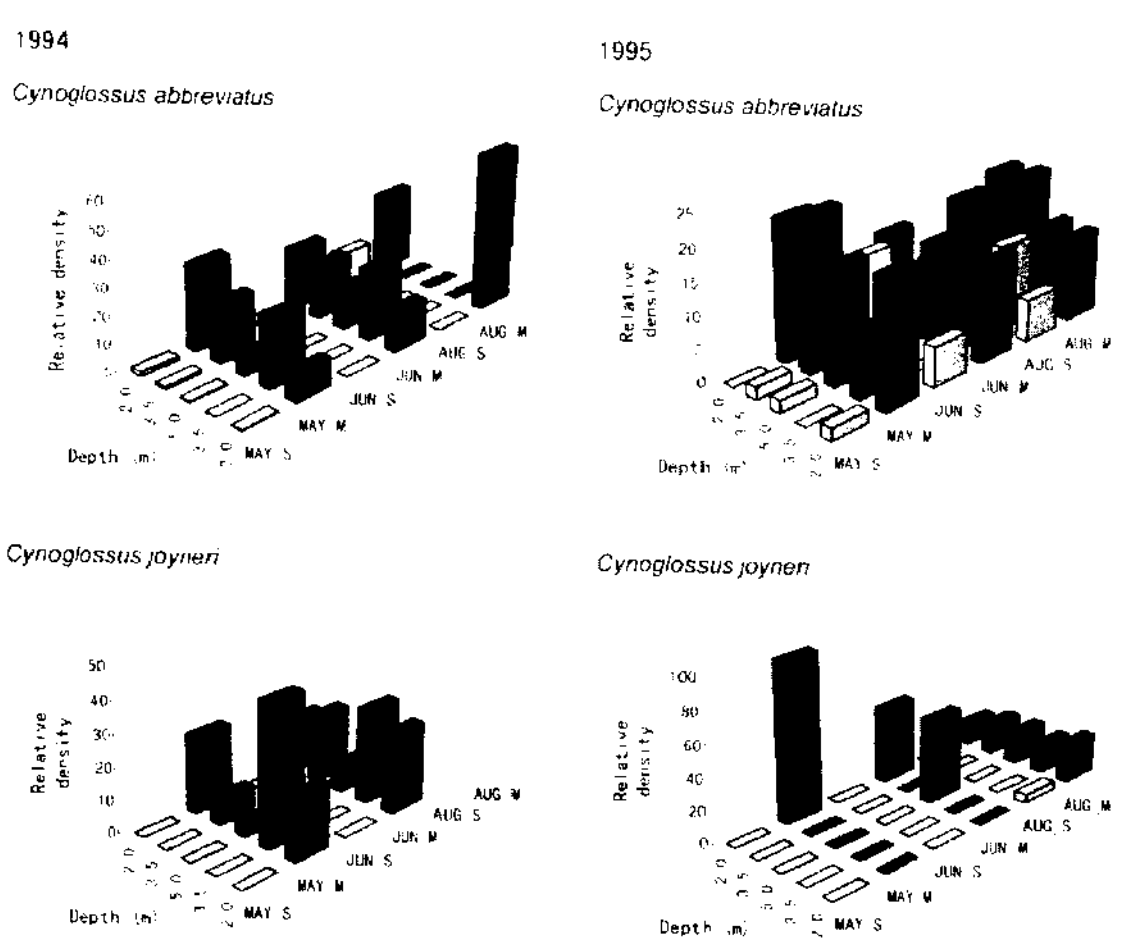


**Figure 5.** Change in relative density of tonguefish in the sand-covered area (S) and the muddy control area (M) with tidal level in May, June, and August. Total catch of 10 towings, five in the sand-covered area and five in the muddy control area towed at different tidal levels, was defined as 100.

migration synchronized with the tidal cycle was not clear in May or June when the juveniles were still small. Compared with *C. abbrebiatus*, the reverse pattern was true for *C. joyneri*. Few 0-group fish exceeding 100 mm in body length were caught in the sand-covered area, and the juveniles collected by set net were the smaller ones.

Our laboratory experiment showed that the *C. abbreviatus* juveniles of about 40 mm in body length could burrow in fine sand, but could not when the bottom was coarse sand (Ohsaka et al. 1997). This experiment also showed that the range of particle size in which 0-group *C. abbreviatus* could burrow widened with growth. The juveniles of 70 mm were found to show high burrowing rates in coarse sand as well as in fine sand. Figure 7 shows the relative ratios of the densities of 0-group tonguefish in sandy and muddy areas which was calculated from the number of fish caught by beam trawl net in our previous survey in 1991 and 1992. Sediment was classified into two categories, i.e., sand and mud, along the lines of towing. The ratio of 0-group fish caught in sandy sediment tended to increase with growth in both *C. abbreviatus* and

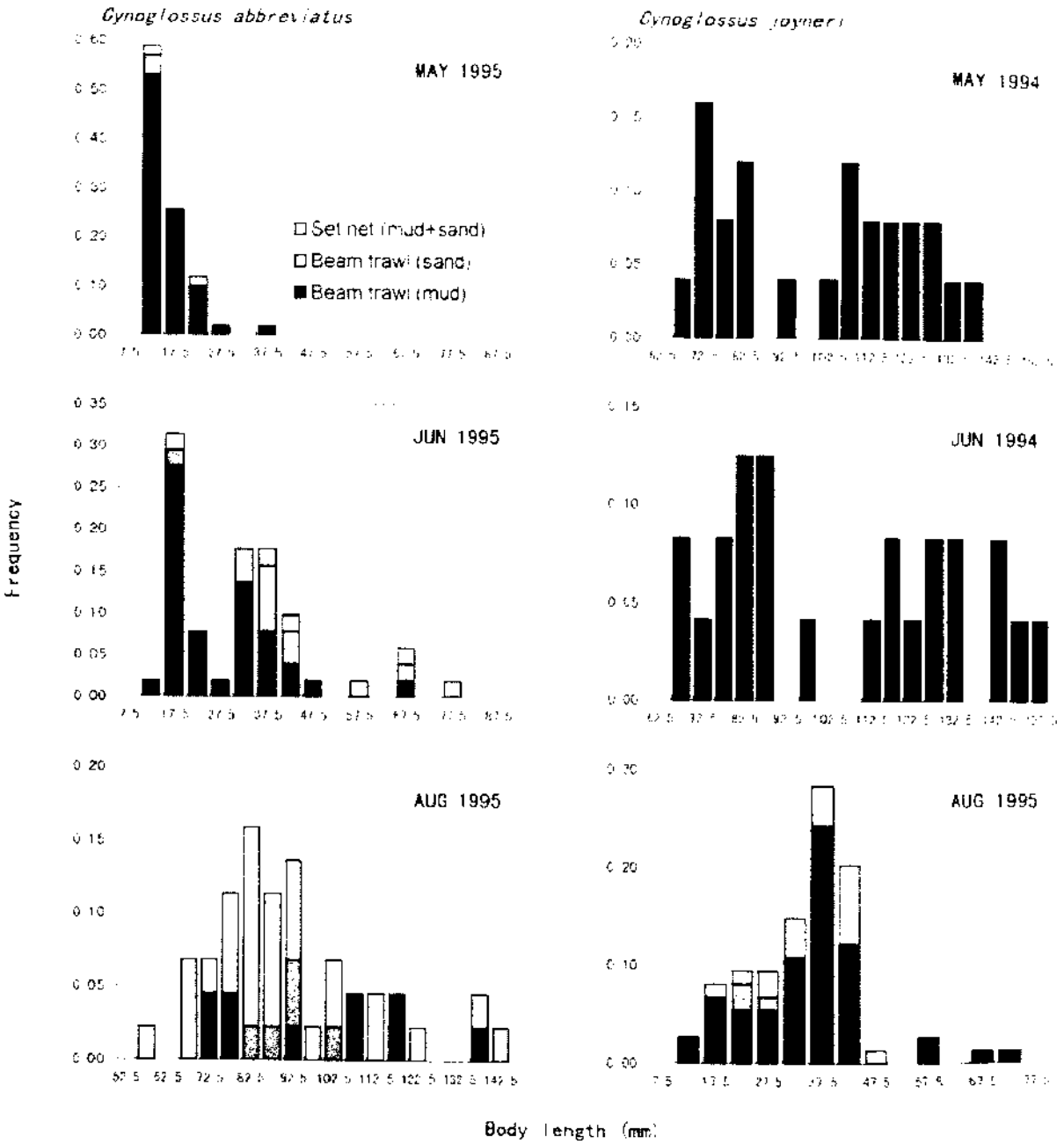


**Figure 6.** Body length composition of 0-group tonguefish caught by beam trawl net and set net in daytime in the sand-covered area and the muddy control area.

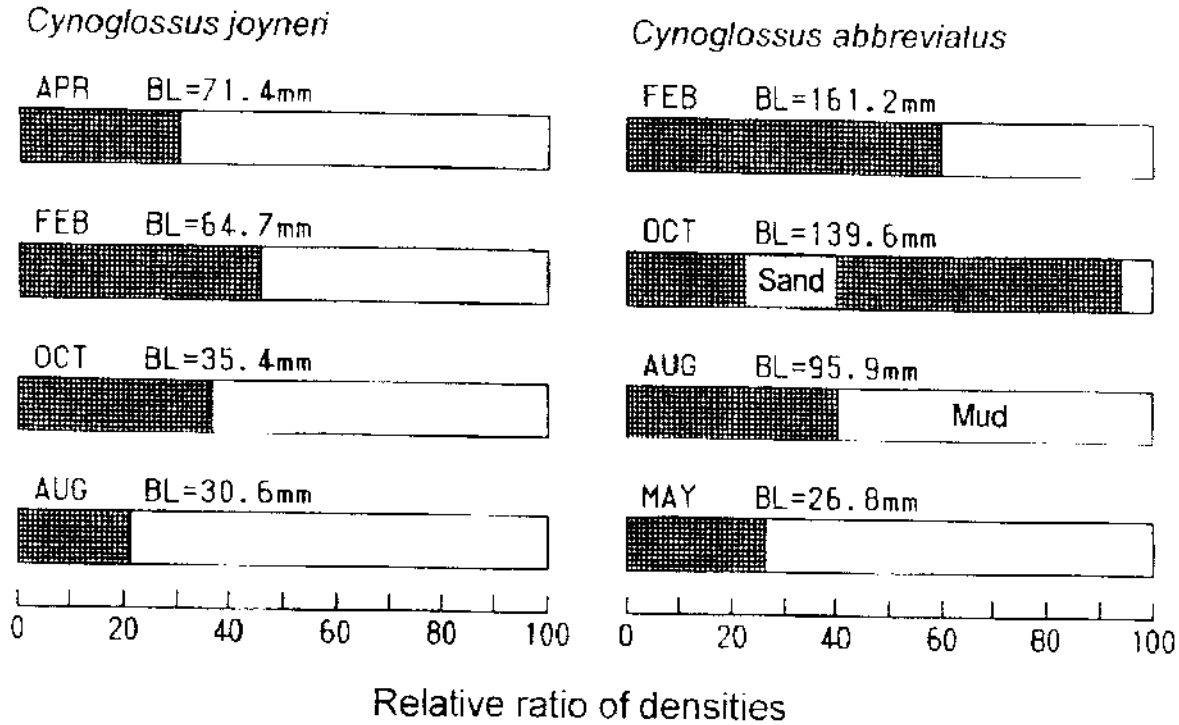


**Figure 7.** Relative ratio of the densities of 0-group tonguefish caught by beam trawl net in our previous surveys in 1991 and 1992. Data from 25-35 samplings along lines distributed in northeastern part of Ariake Sound in each month were used.

*C. joyneri*. However the tendency was much clearer in *C. abbreviatus* than *joyneri*. These results coincided with the pattern of distribution of tonguefish juveniles in the sand-covered area.

**Prey animals of the two tonguefish and their distribution**

Prey animals of both *C. abbreviatus* and *C. joyneri* consisted of small crustaceans such as copepods, gammarids, and polychaetes (Fig. 8). Both species preyed mainly on copepods when their body lengths were smaller than 50 mm. *C. abbreviatus* preyed primarily on harpacticoid copepods compared to *C. joyneri* whose prey primarily consisted of calanoid copepods. The importance of copepods as prey had decreased in both species when their body lengths exceeded 50 mm. Gammarids and mysids became the main prey of *C. abbreviatus*. Contrary to *C. abbreviatus*, *C. joyneri* of over 50 mm preyed primarily on polychaetes.

We tried to compare the amount of food organisms distributed in the sand-covered area and control area. Using several samplers such as the core sampler, grab, and sled net, four series of sampling were carried out from May through July in 1995 and 1996. Unfortunately, no clear distribution pattern was found in gammarids, mysids, and cumaceas. However, a larger number of harpacticoid copepods was always found in the sand-covered area, and the reverse results were found for polychaetes.

**Effects of sand-covering manipulation for tonguefish growth and survival**

Our sampling survey revealed that *C. abbreviatus* inhabited the sand-covered area, and they passed by in tidal migration when their body

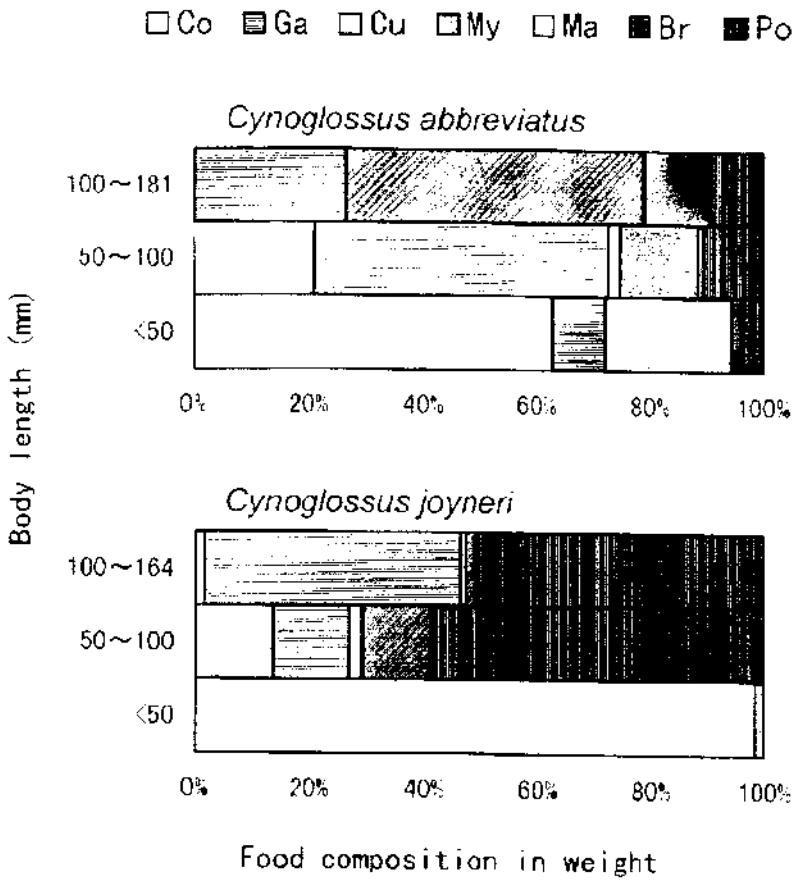


**Figure 8.** Weight composition of the prey animals by size of two 0-group tonguefish. The composition was calculated by point method. Co: Copepoda; Ga: Gammaridae; Cu. Cumacea; My: Mysidacea; Ma: Macrura; Br: Brachyura; Po: Polychaeta.

length exceeded 40 mm. In order to prove some beneficial effects of a sand-covering manipulation for the growth and survival of 0-group *C. abbreviatus*, the next two points must be elucidated: (1) the density of available prey in the sand-covered area is higher than the nearby muddy flat area; and (2) the 0-group fish in the sand-covered area actually preyed on food organisms inhabiting the area.

As for gammarid and mysid density, our data showed no consistent difference in densities between the sand-covered area and the control area. But this may be partly because the sampling size was not large enough and the particle size of the sand used for the short-neck clam project was too large for these crustaceans. Generally speaking, small benthic crustacean densities are higher in sandy sediments than muddy sediments (Horikoshi and Kikuchi 1976, Kikuchi 1985). We believe that the first point will be clarified if more samples are analyzed.

To illustrate the second point, *C. abbreviatus* juveniles collected by a 24-h serial sampling in June were analyzed for digestive tract contents (Table 3). All juveniles caught in the daytime and at night were analyzed together, since there was no clear diurnal change in the whole contents. Time interval of the sampling was changed from 1 to 4 h according to tidal periodicity. There was no difference in the digestive tract fullness index between the fish collected in the sand-covered area and the control area when fish

Table3. Fullness index of digestive tract (dry content weight / dry body weight, %) of *C.abbreviatus* caught in the sand-covered area and the control area in June, 1995. The prey found as intact appearance was classified as undigested.

| Body length | Sand-covered area | | | Control (muddy) area | | |
|---|---|---|---|---|---|---|
| (mm) | No. | Whole | Undigested | No. | Whole | Undigested |
| 14.0～40.0 | 54 | 2.93 | 0.59 | 26 | 2.24 | 0.68 |
| 40.1～78.6 | 12 | 2.91 | **0.06** | 13 | 2.28 | **0.02** |

smaller than 40 mm were compared. However, for the larger juveniles, the amount of undigested contents of juveniles collected in the sand-covered area was clearly larger than that collected in the control area as shown in the fullness index of % dry weight of undigested content/dry body weight. Because the time interval was relatively short, we supposed that the amount of undigested prey in the digestive tract closely represented that of the prey ingested in the area where the juveniles were collected. Thus, the second point is partially explained.

Though our data is limited, we consider that covering the muddy flat with sand provided beneficial effects for the growth and survival of *C. abbreviatus*.

## LITERATURE CITED

Horikoshi, M. and T. Kikuchi. 1976. Benthos, pp. 149-437. *In:* M. Iwashita, M. Hoshino, S. Horibe, J. Masuzawa, and S. Motoda (eds.), Algae and Benthos. Tokai Daigaku Shuppan Kai, Tokyo. [In Japanese].

Kikuchi, T. 1985. Sediment bottom ecosystem and benthos. Fish. Eng. 22(1): 25-33. [In Japanese].

Koshiishi, Y., Y. Ohsaka, Y. Sudo, and R. Ikemoto. 1994. Relationship between depth migration and environmental conditions of some 0-group flatfishes, pp. 64-75. *In:* Reports of the National Fisheries Research Institute on Coastal Fishing Ground Improvement and Development for 1992. Fisheries Agency, Tokyo. [In Japanese].

Ohsaka, Y. and Y. Koshiishi. 1995. Results of a questionnaire on fisheries and distribution of tonguefishes in Ariake Sound sent out to fishermen. News Seikai Natl. Fish. Res. Inst. (80): 22-25. [In Japanese].

Ohsaka, Y. and Y. Koshiishi. 1997. Kourai-akashitabirame (*Cynoglossus abbreviatus*), pp. 190-196. *In:* Biology of threatened and/or possibly threatened aquatic animals. Japan Fisheries Resources Conservation Association, Tokyo. [In Japanese].

Ohsaka, Y., Y. Koshiishi, and M. Sano. 1997. Studies on fishing ground development for tonguefishes by sediment improvement, pp. 66-73. *In:* Reports of the National Fisheries Research Institute on Coastal Fishing Ground Improvement and Development for 1995. Fisheries Agency, Tokyo. [In Japanese].

Sugano, T. 1981. Natural Guide of Ariake Sea. Tokai Daigaku Shuppan Kai, Tokyo. 194 p. [In Japanese].

Uchida, K. and H. Tsukahara. 1955. The fish-fauna of Ariake Sound. Bull. Biogeogr. Soc. Jpn. 16-19: 292-302. [In Japanese].

Ueda, T. and T. Yamasita. 1997. The case of development of Japanese littleneck clam *Ruditapes philippinarum* culture ground. Fish. Eng. 33(3): 213-218. [In Japanese].

# PROSPECTS IN STOCK ENHANCEMENT OF JAPANESE FLOUNDER

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## ABSTRACT

According to fisheries statistics, there has been no significant increase in commercial catch of the Japanese flounder *Paralichthys olivaceus* during the past 40 yr even though releases of hatchery-reared juveniles started in 1977 and the numbers of juveniles released increased linearly to 22.6 million in total by 1995. The extensive studies conducted in many institutions to improve performance of the stock enhancement program indicate that: (1) the adaptability of reared juveniles to the natural environment is poor, (2) their mortality rate just after being released is extremely high due to cannibalism and predation from various animals, and (3) their growth depends on the availability of food organisms at the release site which may be limited and fluctuates annually. Recently, however, positive achievements have been obtained in some areas where larger-sized juveniles are released and strict management of the mixed stocks of released and wild is observed. On the other hand, mass releases of the hatchery-reared fishes are alleged to cause a variety of problems including: (1) spread of pathogens, (2) limited numbers of broodfish decrease genetic diversity, (3) genetic constitution and fitness of wild stocks are changed or diminished, and (4) impacts of mass releases on ecosystems are not well understood. In order to make stock enhancement not only economically but also scientifically sound, conservation of the biodiversity cannot be ignored. As a countermeasure to these issues, the Japanese government has initiated a new project to clarify genetic effects of stock enhancement on natural populations and interactions between the released and native populations

## INTRODUCTION

Faced with declining marine fish populations worldwide and an expanding world population, marine fish enhancement has been attracting global attention (Blankenship and Leber 1995). In Japan, stock enhancement programs were initiated by the government of Japan as a national project in the early 1960s in order to restore the stocks of commercially important marine species whose populations were declining due to overfishing, pollution, habitat degradation, or human influences. Technology developed in these projects has enabled us to produce large numbers of marine fish and shellfish larvae beyond vulnerable juvenile stages.

Mass releases of the hatchery-reared juveniles of Japanese flounder *Paralichthys olivaceus* widely distributed in coastal waters of Japan from Hokkaido to Kyushu began in 1977. Since then, numbers of juveniles released in all areas increased linearly to 22.6 million in total by 1995 as shown in Figure 1 (Fisheries Agency of Japan 1997). However, fisheries catch statistics (Ministry of Agriculture, Forestry and Fisheries 1997) show no significant change in commercial catch of this species during the past 40 yr.

In the case of stock enhancement of chum salmon *Oncorhynchus keta*, more than half a century of hatchery releases in Hokkaido produced no evidence of an increased yield until the 1960s. But as the annual number of releases increased from 0.5 billion in the 1970s to 1 billion in 1982, the total numbers of catch started to increase gradually and reached up to 440% over the historic record of the pre-hatchery release period with the return rate of about 3% in 1990 (Kaeriyama 1994). Based on this achievement, some people believe the numbers of flounder released are not sufficient to expect a substantial increase in commercial catch.

In order to improve the performance of stock enhancement of flounder, extensive studies have been conducted in many national and

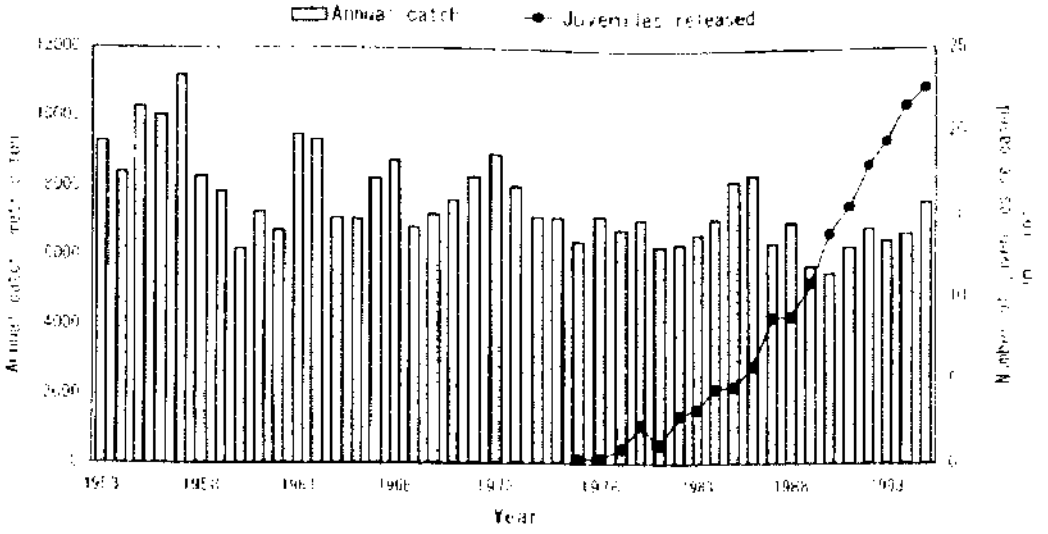


**Figure 1.** Annual catch of Japanese flounder and number of juveniles released in Japan.

prefectural institutions, and universities. In this review paper, we examined the progress to date to clarify why stock enhancement of flounder has not shown clear-cut evidence for increased yields nationwide. Also, the future direction of this program is discussed including whether or not a large increase in numbers (up to billions) of juveniles released is feasible or has potential to obtain results similar to chum salmon.

## PROGRESS TO DATE

As mentioned earlier, releases of over 20 million juveniles in waters along the entire Japan coast have shown no positive effect on commercial landings of this species. However, if the relationship between annual catch and number of juveniles released is examined by region (regions 1-8, Fig. 2), we can see a somewhat different picture. Annual catches show increasing trends in region 4, Southern Pacific Ocean, and region 8, Seto Inland Sea, as the numbers of juveniles released have increased (Figs. 3, 4). A common feature in these two regions is that the level of annual catches before the start of mass-releases was rather low compared with the remaining regions. To understand differences in results of juvenile flounder releases in regions 4 and 8 and other regions throughout Japan, it is instructive to examine progress made in the studies on biology and ecology of this species, and on fisheries management practices.

## QUALITY AND FITNESS TO THE NATURAL ENVIRONMENT

Mass production of fry or juveniles is made possible by providing sufficient foods under intensive condition and by isolating them from predators. As a consequence, physically weak individuals which cannot survive in the wild and those having deformity and abnormal coloration of the body, etc. are produced. The most commonly observed problems in the production of flounder juveniles used to be albinism and ambicoloration (Seikai 1997). Also, it has been found that the juveniles reared with a formulated feed contain much higher levels of free non-essential amino acids in the muscle. Since free amino acids, especially non-essentials, stimulate the olfactory and gustatory senses of crustaceans, it is presumed that they are more vulnerable to predators. However, the composition of free amino acids can be changed within a week by feeding mysids instead of a formulated feed (Yoshinaga 1996). Likewise, quality of reared fish is improving due to progress in rearing techniques and improvements in feeds.

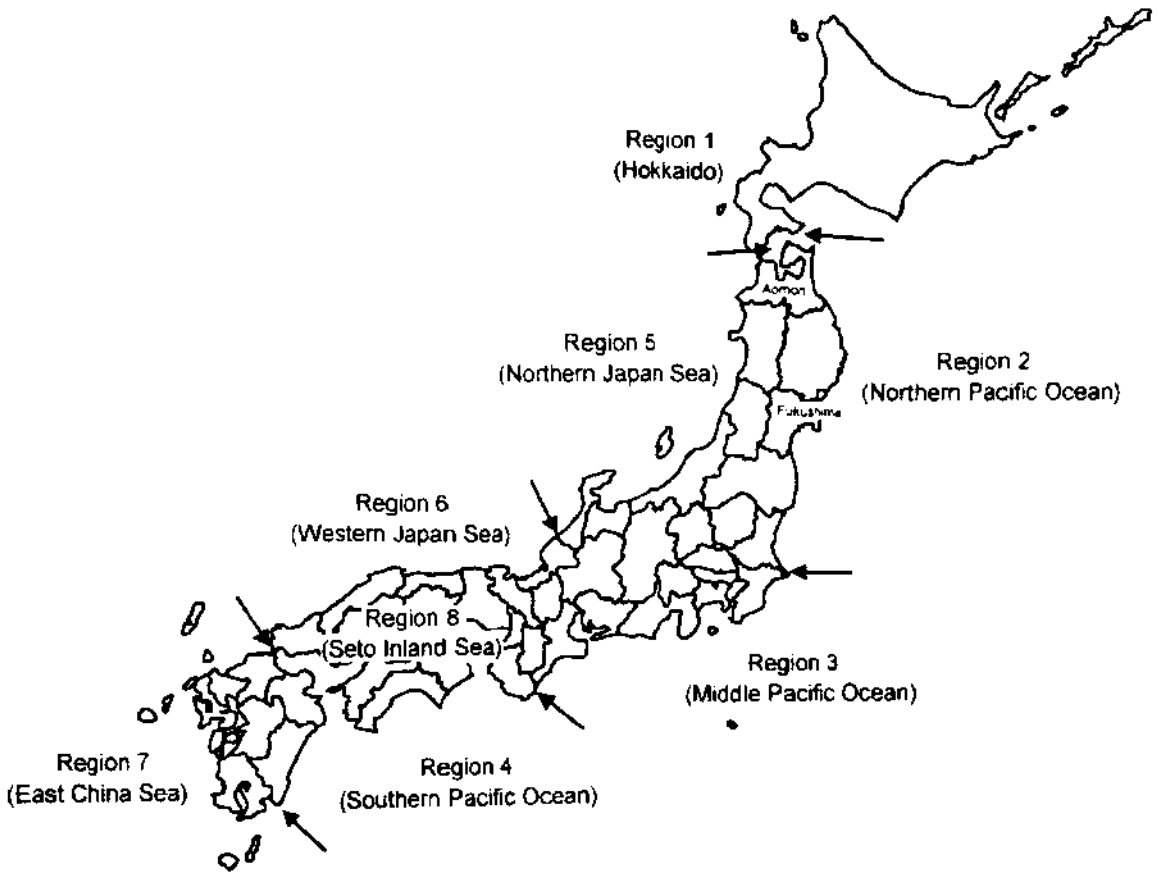


**Figure 2.** Coastal waters around Japan divided in 8 regions.

The life history of Japanese flounder has been well elucidated by exhaustive field and experimental studies (Minami 1997, Noichi 1997, Tanaka 1997). Generally speaking, the juveniles less than 70 mm in total length (TL) prey mainly on mysids and then become piscivorous depending on the availability of food organisms (Noichi 1997). According to changes in feeding behavior, they disperse from nursery grounds in coastal waters to offshore (Koshiishi et al. 1991). In addition, physiological and behavioral studies indicate that the released fish show poor swimming ability, peculiar feeding behavior, and lack of predator avoidance, which result in poor survival after release (Furuta 1996). Although the ecological mechanisms are not completely understood, it is reported that rearing fish under less intensive conditions or simulated natural conditions for a short period before release improves their fitness to the natural environment (Yamashita 1997).

## ENVIRONMENTAL CONDITION OF RELEASE SITE

Growth of the released juveniles depends on the availability of food organisms such as mysids on the nursery ground, although mortality by starvation may be insignificant (Yamashita et al. 1994). Koshiishi et al. (unpublished data) have shown that abundance of mysids substantially varies annually and seasonally as shown in Figure 5. Also, it has been reported by Koshiishi et al. (1988) that density of mysids at the release site is drastically decreased within a day after mass releases of juveniles. Thus, it is apparent that the carrying

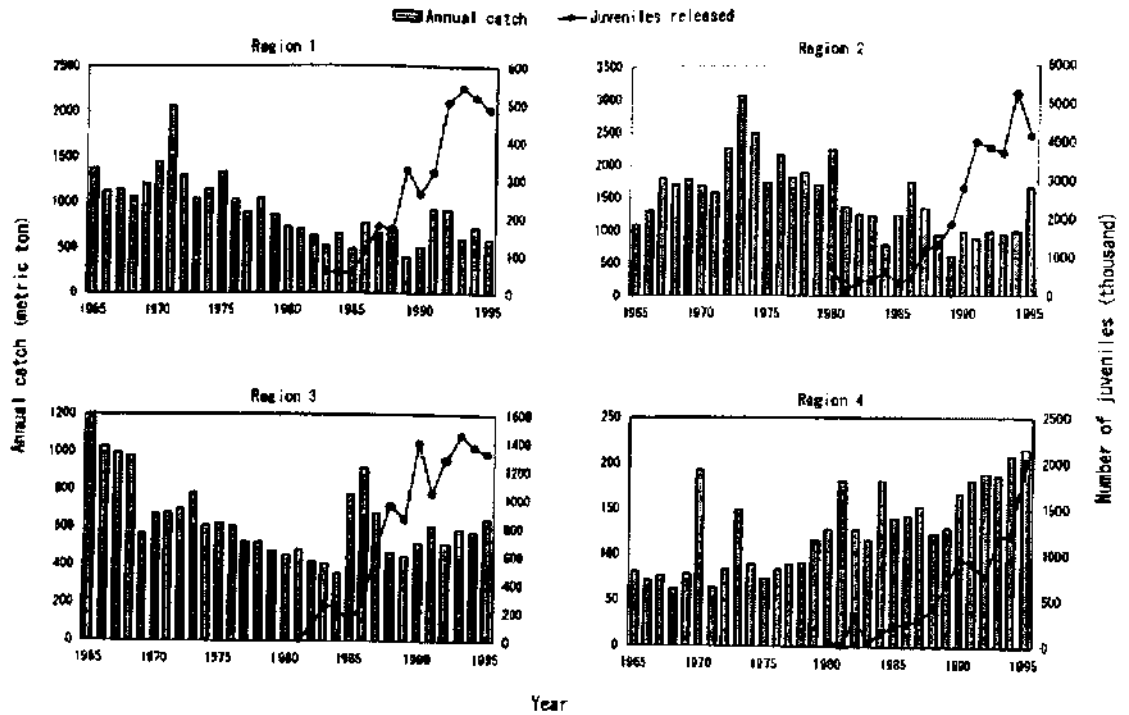


**Figure 3.** Annual catches of Jananese flounder and number of juveniles released in regions 1-4.

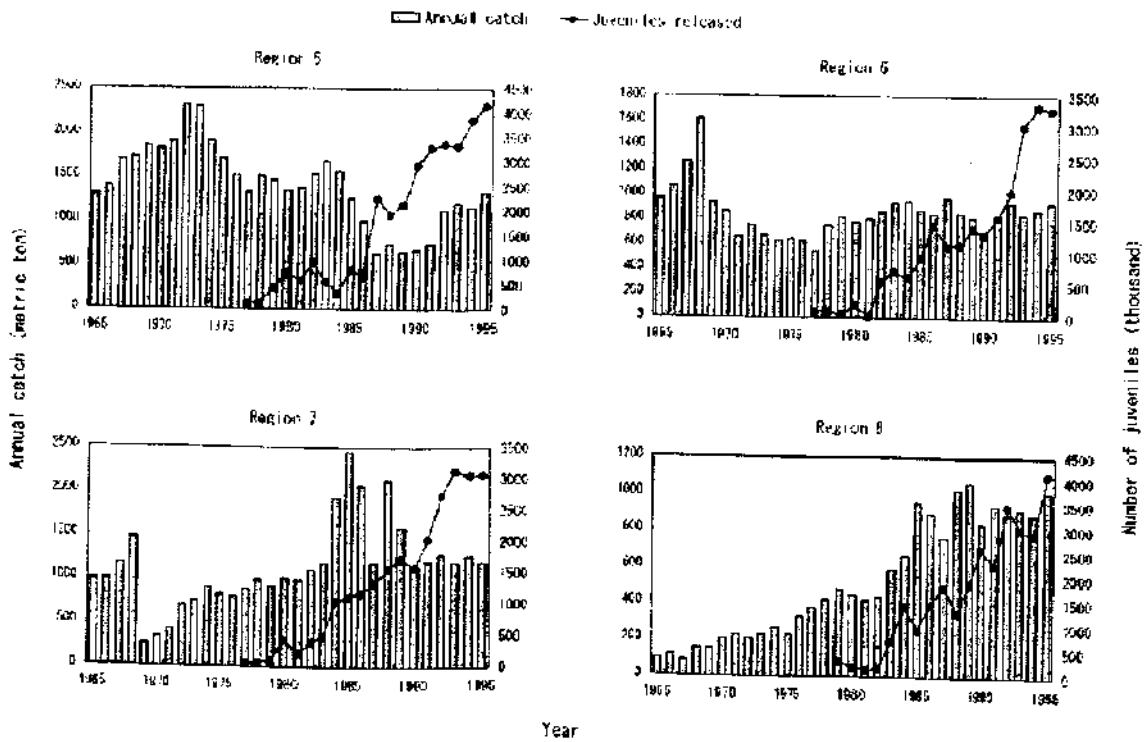


**Figure 4.** Annual catches of Japanese flounder and number of juveniles released in regions 5-8.

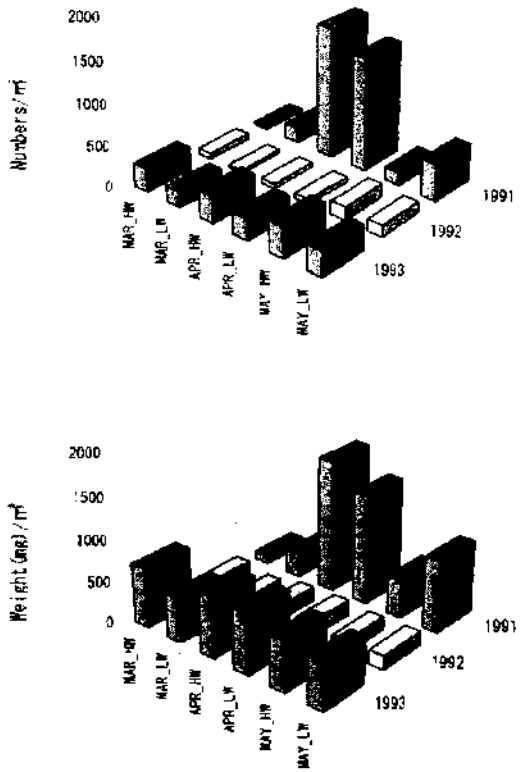


**Figure 5.** Annual and seasonal changes in the density of mysids

capacity of the nursery ground is limited and fluctuates annually. The availability of food organisms is a crucial factor to sustain good growth of juvenile flounder since they stay in particular nursery grounds until becoming piscivorous. This early marine-life behavior of Japanese flounder is different from chum salmon.

A major cause of the high mortality after the release of flounder is known to be predation by crustaceans and fishes including wild flounder (Yamashita et al. 1993), so that the presence of sandy ground providing a hiding place is also a critical element. To minimize predation problems, various measures are being practiced such as releasing small-sized juveniles (30-50 mm in TL) before wild flounder appear on the nursery ground, or raising juveniles to larger sizes (up to 100 mm in TL) which are less vulnerable to predation (Yamashita et al. 1993).

## FISHERY MANAGEMENT

High survival and growth can be expected if large-sized juveniles are released according to the carrying capacity of a target nursery area having suitable habitat at a time when food organisms are abundant. Even though these factors are taken into consideration and the best methods are

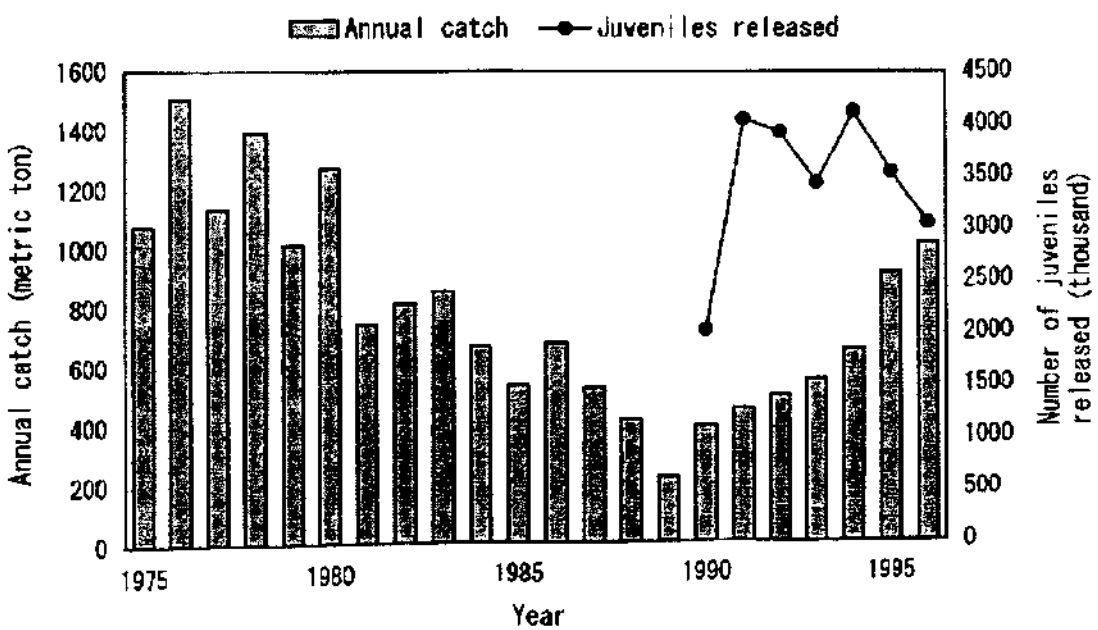


**Figure 6.** Annual catch of Japanese flounder and number of juveniles released in Aomori Prefecture.

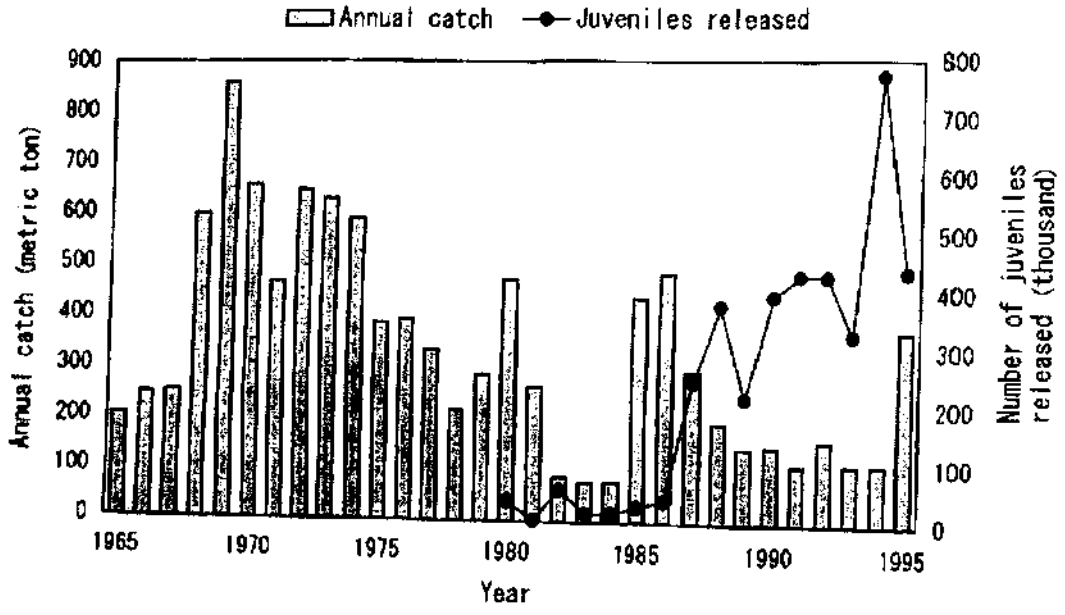


**Figure 7.** Annual catch of Japanese flounder and number of juveniles released in Fukushima Prefecture.

employed in releasing juveniles, the results differ among regions. One of the reasons for the lack of success in many regions seems to be the bycatch of less valuable small-sized fish in commercial fishing.

As mentioned earlier, positive results for releasing juvenile flounder have been obtained in certain regions like the Seto Inland Sea. Additionally, positive relationships between the annual catches and numbers of animals being released in the other species such as red sea bream and crustaceans have been achieved in this region (Ogawa 1995). The Seto Inland Sea is a relatively closed area which limits migration of released animals out of this region. Also, self-imposed regulations by fishermen are well abided to not harvest animals less than certain sizes and to re-release them if captured.

So far, the most successful achievement has been attained in Aomori Prefecture. The annual catch of Japanese flounder in Aomori Prefecture used to be the highest in Japan (about 15% of the total catch in Japan) and it is designated as the prefecture's fish. However, the annual catch of over 1500 tons in 1976 dropped to 224 tons in 1989 (Fig. 6). In response to the drastic decline, the prefectural government implemented a guideline for fisheries management of this species with a consensus of the fishermen after laborious dialogues through the Fisheries Cooperative Associations. The guideline contains various regulations to protect the broodstock and juveniles of both native and released flounder, and designation of the important nursery grounds as a sanctuary. In accordance with implementation of this guideline, 2-4 million juveniles about 50 mm in TL have been produced using funds contributed by the fishermen (4% of income from flounder fishing) and have been released annually since 1990. The minimum size to be harvested was raised gradually from 25 cm in 1990 to 35 cm in 1995. As a result of these efforts, the annual catch of flounder has been linearly increasing and it surpassed the 1000-ton level (almost 5-fold increase in 7 yr) in 1996 (Aomori Prefecture 1997).

These results indicate that fisheries management is a key factor for successful stock enhancement activity. Also, the fact that positive results have been obtained in regions 4 and 8, where the annual catches before the start of stocking were relatively small or in Aomori Prefecture where the stock was depleted, indicate

the importance of the initial stock size for a successful stocking program, which in turn may indicate that the carrying capacity for juvenile flounder is limited and varies from region to region. Thus, unlike the chum salmon project, large increases in numbers of juvenile flounder for release in many regions may not be feasible. However, a substantial increase in size may be effective in certain areas if the production of larger juveniles becomes cost effective.

## FUTURE DIRECTION

In Fukushima Prefecture, about 0.2-0.4 million juveniles having 70-100 mm in TL have been released annually since 1987 but so far have produced no evidence of an increased yield (Fig. 7). However, the detailed market survey along with field study indicate that the recapture rate of released flounder by year class was in a range of 16-31% with average value of 24% for 4 yr (1987 through 1990) which is almost 8-fold higher than the return rate of chum salmon. The reasons for attaining a high recapture rate in Fukushima Prefecture are reported to be that the survival of released juveniles is high due to use of large-sized fish; mysids are abundant around the release sites; fishing effort for flounder is intensive; and the fishermen refrain from harvesting flounder less than 30 cm in TL (Fujita et al. 1993). They also made a cost-benefit analysis for the entire operation in Fukushima Prefecture, and found that the flounder stocking resulted in annual profits of $410-670 thousand assuming wholesale prices of flounder for 1-yr-old fish and for 2-yr-old fish were $21 and $33/kg, respectively, and the benefit was 2-3 times higher than the entire costs of juvenile production and release. This result agrees very well with that of a market survey conducted by the Japan Sea Farming Association which indicates that stock enhancement of flounder can pay off if the recapture rate exceeds 20% (Furusawa 1994). Fujita et al. (1993) suggested that much higher profits can be obtained if harvest restrictions of 0-yr-old flounder are more strictly observed by fishermen because its wholesale price is only about $4/kg.

These results suggest that in certain cases, stock enhancement can be economically feasible even if it does not result in increased yield. An economically beneficial effect may be found in other areas as well if a cost-benefit analysis is conducted. Thus, encouraging prospects do exist in a stock enhancement program for Japanese flounder, although fish stocking may not be a panacea to the problem of declining populations.

As fisheries resources management has developed and expanded, the use of and need for cultured fishes have increased (Schramm and Piper 1995). However, mass releases of cultured fish have been alleged to cause a variety of problems such as: spread of pathogens; limited numbers of the broodfish decreases genetic diversity; genetic constitution and fitness of wild stock are changed or diminished; and so forth (Edward and Nickum 1993). Also, conservation of the species and ecological diversities became an international issue after the Convention on Biological Diversity came into effect. Under these circumstances, many symposia or workshops related to these issues have been held worldwide.

For instance, the US-Japan Natural Resources (UJNR) Aquaculture Panel held the Symposium on Interaction between Cultured Species and Naturally Occurring Species in the Environment in Alaska in 1993, and the International Symposium and Workshop on the Uses and Effects of Cultured Fishes in Aquatic Ecosystems were held in New Mexico in 1994. In order to make a stock enhancement program not only economically but also scientifically sound, more academic information on the impact of mass releases of hatchery-reared fish is needed. The Agriculture, Forestry and Fisheries Research Council of Japan has just initiated a new project "Effect of Fish Stock Enhancement on Biodiversity." In this project, we plan to conduct studies on the genetic constitution and ecological effects of stock enhancement on the native populations of Japanese flounder, and to develop technology for stock enhancement minimizing adverse effects on biodiversity. Also, a joint project on flounder between Japan and the USA is now ongoing and significant scientific contributions from these projects are expected.

A put and take fishery which is supposed to have less impact on the genetic diversities of natural populations can be one of the future directions for stock enhancement programs. However, this practice will permanently depend on a stocking program like the chum salmon project.

## CONCLUSIONS

- Unlike the chum salmon project, large increases in numbers of juvenile flounder for release may not be feasible because of the limited carrying capacity.
- If the stock enhancement program is carried out where the initial stock size is relatively small or the stock is depleted, clear-cut evidence for increased yield can be obtained even at the present level of releases.
- As far as a high valued fish like flounder is concerned, if the recapture rate exceeds 20%, mass-release is economically feasible even though no increase in the catch is obtained.
- In either case, stock management by fishermen is a must, especially in the restriction of harvesting undervalued small fish.
- In order to make stock enhancement a sustainable program, impacts of mass releases on the ecosystem must be scientifically elucidated.

## LITERATURE CITED


Aomori Prefecture. 1997. Fisheries Statistics of Aomori Prefecture. Aomori Prefectural Government, Aomori. 197 p. [In Japanese].

Blankenship, H.L. and K.M. Leber. 1995. A responsible approach to marine stock enhancement, pp. 167-175. *In:* H.L. Schramm and R.G. Piper (eds.), Uses and Effects of Cultured Fishes in Aquatic Ecosystems. American Fisheries Society, Bethesda, MD.

Edward, G.B. and J.G. Nickum. 1993. Use of propagated fishes in Fish and Wildlife Service programs. UJNR Tech. Rep. No. 22: 41-44.

Fisheries Agency of Japan. 1997. Statistics on Production and Release of the Seeds for Stock Enhancement. The Japan Sea Farming Association, Tokyo. 412 p. [In Japanese].

Fujita, T., T. Mizuno, and Y. Nemoto. 1993. Stocking effectiveness of Japanese flounder *Paralichthys olivaceus* fingerlings released in the coast of Fukushima Prefecture. Saibai Giken 22(1): 67-73. [In Japanese].

Furusawa, T. 1997. Key problems of sea-farming associated with its perspective, pp. 117-126. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseisha Koseikaku, Tokyo. [In Japanese].

Furuta, S. 1996. Predation of juvenile Japanese flounder (*Paralichthys olivaceus)* by diurnal piscivorous fish: field observation and laboratory experiments, pp. 285-296. *In:* Y. Watanabe, Y. Yamashita, and Y. Oozeki (eds.), Survival Strategies in Early Life Stages of Marine Resources. A.A. Balkema, Rotterdam.

Kaeriyama, M. 1994. Trend of chum salmon production in Japan. Kaiyo Monthly 26(8): 471-473. [In Japanese].

Koshiishi, Y., T. Fujii, M. Noguchi, and Y. Hirota. 1988. Estimated amount of feeding by released 0-group flounder (*Paralichthys olivaceus)* and other demersal fishes on mysids in shallow waters off Igarashi-Hama, Niigata. Marine Ranching Program Progress Report on Flounder Production 3: 253-267. Seikai Natl. Res. Fish. Inst., Nagasaki. [In Japanese].

Koshiishi, Y., H. Itano, and Y. Hirota. 1991. Artificial stock-size improvement of the flounder *Paralichthys olivaceus:* present status of technological achievement. U.S. Dept. Commerce, NOAA Tech. Rep. NMFS 102: 33-43.

Minami, T. 1997. Life history, pp. 9-24. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseisha Koseikaku, Tokyo. [In Japanese].

Ministry of Agriculture, Forestry and Fisheries. 1997. Annual Statistics of Fisheries and Aquaculture Production. 309 p. [In Japanese].

Noichi, T. 1997. Early life history, pp. 25-40. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseisha Koseikaku, Tokyo. [In Japanese].

Ogawa, T. 1995. Trends in number of the seeds released and annual catches. News Nansei Natl. Fish. Res. Inst. 55: 8-9. [In Japanese].

Schramm, H.L. and R.G. Piper. 1995. Preface, pp.1. *In:* H.L. Schramm and R.G. Piper (eds.), Uses and Effects of Cultured Fishes in Aquatic Ecosystems. American Fisheries Society, Bethesda, MD.

Seikai, T. 1997. Mechanism of metamorphosis, pp. 63-73. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseisha Koseikaku, Tokyo. [In Japanese].

Tanaka, M. 1997. Ecological significance of metamorphosis, pp. 52-62. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseisha Koseikaku, Tokyo. [In Japanese].

Yamashita, Y. 1997. Ecology and releasing techniques, pp. 107-116. *In:* T. Minami and M. Tanaka (eds.), Biology and Stock Enhancement of Japanese Flounder. Koseisha Koseikaku, Tokyo. [In Japanese].

Yamashita, Y., S. Nagahora, H. Yamada, and D. Kitagawa. 1994. Effects of release size on survival and growth of Japanese flounder *Paralichthys olivaceus* in coastal waters off Iwate Prefecture, northeastern Japan. Mar. Ecol. Prog. Ser. 105: 269-276.

Yamashita, Y., K. Yamamoto, S. Nagahora, K. Igarashi, Y. Ishikawa, O. Sakuma, H. Yamada, and Y. Nakamoto. 1993. Predation by fishes on hatchery-raised Japanese flounder, *Paralichthys olivaceus*, fry in the coastal waters of Iwate Prefecture, northeastern Japan. Suisanzoshoku 41: 497-505. [In Japanese with English abstract].

Yoshinaga, T. 1996. Comparison of reared and wild juveniles of Japanese flounder - difference in free amino acid composition in the muscle. Yoshoku No.10:112-113. [In Japanese].

# REPRODUCTIVE MECHANISMS IN *MACROBRACHIUM ROSENBERGII* AND *PENAEUS JAPONICUS*: ENDOCRINOLOGICAL RESEARCH AND POTENTIAL APPLICATIONS IN AQUACULTURE

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## ABSTRACT

The giant freshwater prawn *Macrobrachium rosenbergii*, cultured extensively throughout South Asia, and the kuruma prawn *Penaeus japonicus*, targeted principally in Japan and Taiwan, are species of commercial importance which have been widely studied in terms of basic physiological function. In Japan, *P. japonicus* is additionally a significant target of artificial seed production operations for restocking of coastal areas. In order to ensure a sustainable means of artificial seed production for significant crustacean species, it is important to effectively control female molting and reproduction under artificial conditions. In decapod Crustacea, the physiological processes of molting and reproduction are linked and are under hormonal control. The role of ecdysteroids which serve as molting hormones are well-established, but the physiological significance of juvenile hormone-related substances is just beginning to become clear. Endocrinological research in *M. rosenbergii* and *P. japonicus* can be potentially applied to aquaculture operations in the future, including artificial seed production programs for *P. japonicus*.

## INTRODUCTION

The establishment of sustainable prawn culture depends on many factors and requires the integration of various fields of expertise. At present, inducing reproduction in captivity and controlling disease remain obstacles to successful culture, and solving these technological problems will depend greatly on basic research relating to the biology and physiology of the animals being targeted. The giant freshwater prawn *Macrobrachium rosenbergii*, cultured extensively throughout South Asia (Chavez Justo 1991), and the kuruma prawn *Penaeus japonicus*, targeted principally in Japan and Taiwan (Liao and Chen 1994), are species of commercial importance which have been widely studied in terms of basic physiological function. While widespread viral infection has become a problem of increasing magnitude in the culture of saltwater *Penaeus* species, disease outbreak has not been of significant concern in freshwater species such as *M. rosenbergii*. However, in all prawn species, it is important to be able to effectively control molting and reproduction under artificial conditions in order to produce larval seed for further aquacultural growout. In Japan, *P. japonicus* is additionally a significant target of artificial seed production operations for restocking of coastal areas.

This paper addresses the current state of endocrinological research in *M. rosenbergii* and *P. japonicus*, focusing on the roles of ecdysteroids and juvenoids in molting and reproduction, and discusses how basic research in this area can be potentially applied to aquaculture operations in the future. The status of artificial seed production programs for *P. japonicus* and current aquaculture in Japan in this context are also discussed.

## REPRODUCTIVE ENDOCRINOLOGY IN *M. ROSENBERGII* AND *P. JAPONICUS*

### Background

In decapod Crustacea including prawns, shrimps, lobsters and crabs, the physiological

processes of molting and reproduction are inextricably linked and under hormonal control. Ecdysteroids such as 20-hydroxyecdysone serve as "molting hormones" in Crustacea and are excreted from a tissue known as the Y-organ. On the other hand, peptide substances such as molt-inhibiting hormone (MIH) and vitellogenesis-inhibiting hormone (VIH) originating in the sinus gland complex of the eyestalks exert negative influence on molting and ovarian development. There is evidence for the existence of positive stimulatory factors, including a putative vitellogenesis-stimulating hormone (VSH), vitellogenis-stimulating ovarian hormone (VSOH), and molt-stimulating hormone (MSH) which may possibly be secreted at the brain and thoracic ganglion (Takayanagi et al. 1986, Meusy and Payen 1988); however, such factors have not been sufficiently isolated and identified. A general scheme for the control of molting and reproduction in Crustacea is shown in Figure 1. Structures of representative ecdysteroids in *M. rosenbergii* are shown in Figure 2.

In insects, juvenile hormone (JH), a larval developmental hormone, also appears in the adult female to stimulate yolk protein production and uptake. To date, JH itself has not been identified in any crustacean species. Methyl farnesoate (MF), the un-epoxidated precursor of JH, has been found in a limited number of crustacean species such as the American lobster *Homarus americanus* (Tsukimura and Borst 1992) and the spider crab *Libinia emerginata* (Laufer et al. 1987). In previous studies of this author and co-workers, MF was detected in *M. rosenbergii* (Wilder et al. 1995) but was not found in *P. japonicus*. MF has been shown to be secreted from the mandibular organs (Sagi et al. 1991). While MF is considered to be the crustacean equivalent of JH, its role in crustacean reproduction remains unclear. MF may possibly function as a VSH to stimulate yolk protein production and uptake as suggested in Figure 1. The structure of MF is shown in Figure 3

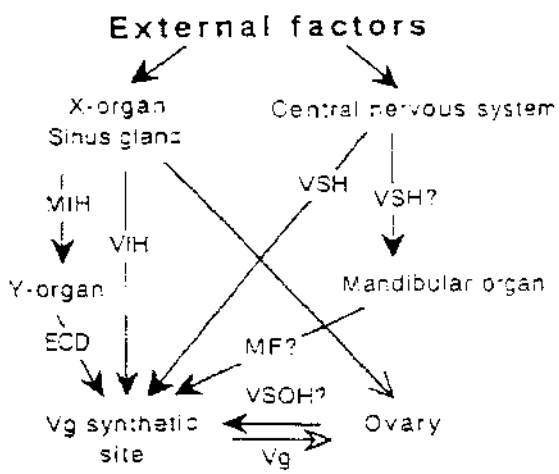


**Figure 1.** General scheme for the endocrinological control of molting and reproduction in Crustacea. Abbreviations are indicated in the figure for molt-inhibiting hormone (MIH), vitellogenesis-inhibiting hormone (VIH), ecdysteroid (ECD), vitellogenesis-stimulating hormone (VIH), methyl farnesoate (MF), and vitellogenesis-stimulating ovarian hormone (VSOH).

### Ecdysteroids and juvenoids in *M. rosenbergii* and *P. japonicus*

In both *M. rosenbergii* and *P. japonicus*, an ecdysteroid surge occurs in the hemolymph in the late pre-molt stage and the predominant ecdysteroid species is observed to be 20-hydroxyecdysone with lesser amounts of highly polar ecdysteroids (high polarity products (HPP)). In *M. rosenbergii*, peak titers are about 40 ng/ml (Okumura et al. 1992), and in *P. japonicus*, these levels reach nearly 200 ng/ml (Okumura et al. 1989). 20-hydroxyecdysone is generally considered to be the active form of the hormone in most crustacean species, and it regulates the molting cycle.

In addition to involvement in molting, ecdysteroids are found in newly laid eggs and mature ovaries of numerous insect and crustacean species. In general, eggs ecdysteroids during the early embryonic stages are ovarian in origin and serve as a stock for purposes of early development until embryonic prothoracic glands or Y-organs differentiate and produce ecdysteroids *de novo* (Spindler et al. 1987). In *M. rosenbergii*, ecdysteroids are present in mature ovaries (25 ng/g) and newly spawned eggs (16.9 ng/g) (Wilder et al. 1990). These ecdysteroids are accumulated in ovaries during the reproductive molt cycle (molt cycle accompanied by maturation of the ovaries) to levels of 50 ng per ovary. In contrast, during the common molt cycle (molt cycle in which ovaries

## Ecdysteroid structures

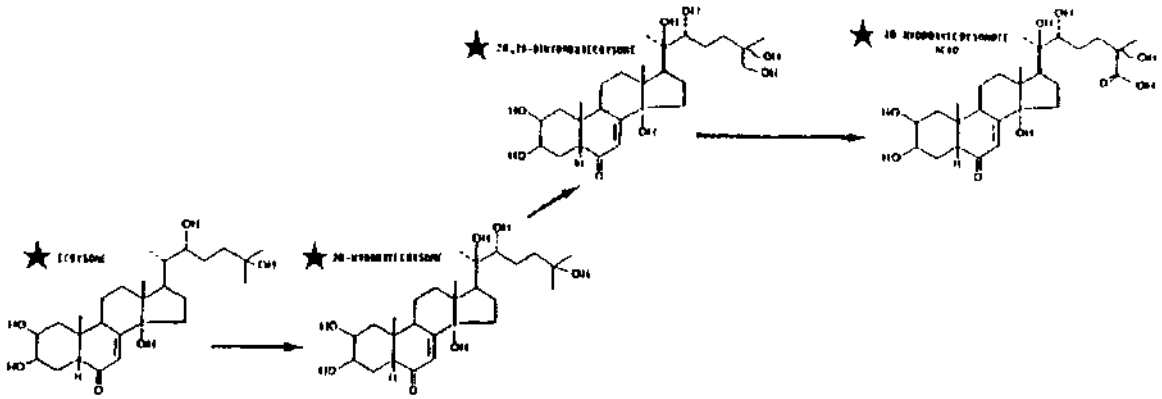

**Figure 2.** Structures of representative ecdysteroids in *M. rosenbergii* including the active form of the hormone, 20-hydroxyecdysone, precursor ecdysone, and metabolites 20,26-dihydroxyecdysone and 20-dihydroxyecdysonoic acid.

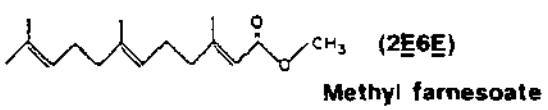


**Figure 3.** Structure of crustacean juvenoid substance, methyl farnesoate (MF).

remain immature), ecdysteroid content is 1.5 ng per ovary (Wilder et al. 1991) (Fig. 4). This ovarian ecdysteroid accumulation which occurs in synchronization with molting may signify a role for these ecdysteroids in inducing germinal vesicle breakdown (GVBD) and subsequent ovulation.

This author has examined *M. rosenbergii* and *P. japonicus* for the presence in the hemolymph of juvenoid substances including juvenile hormone III and methyl farnesoate (MF). MF was present in females during both the reproductive and common molt cycles and in males in *M. rosenbergii* (Wilder et al. 1995), but was not detectable in *P. japonicus* (Wilder and Aida 1995). In *M. rosenbergii*, MF fluctuated during the molt cycle without connection to ovarian development, being highest in the early pre-molt stages (Wilder et al. 1995). These results suggest that MF may

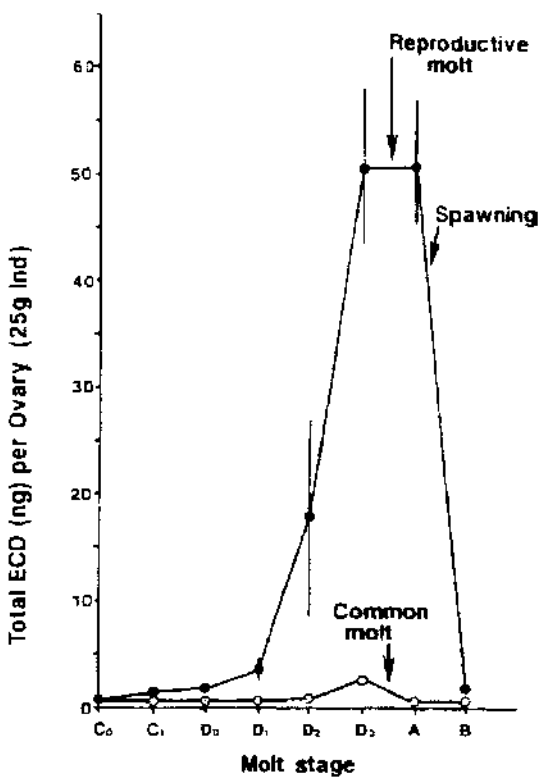


**Figure 4.** Ovarian ecdysteroid accumulation in ovaries during the reproductive molt cycle and ecdysteroid content during the common molt cycle in *M. rosenbergii* on basis of a 25-g individual. Ecdysteroids are abbreviated as ECD.

be involved in the molting process. In *Artemia*, MF has been found to elevate Na/K-ATPase activity in larval homogenates, additionally suggesting a role in osmoregulation (Ahl and Brown 1991).

In a separate investigation, this author and co-workers tested the effects of MF injection on vitellogenin production in eyestalk-ablated juvenile *M. rosenbergii* (Wilder et al. 1994). Although vitellogenesis in *M. rosenbergii* will not be discussed in detail here, there were no observed increases in vitellogenin production in response to MF treatment. These results indicate that MF alone could not promote increased vitellogenin production, but do not rule out a role for MF in crustacean reproduction. Whether MF plays a role in inducing patency of the ovarian follicles, thereby allowing the uptake of vitellogenin in developing oocytes, as JH does in insects (Davey and Huebner 1974, Davey et al. 1993), needs to be further addressed.

### Current perspectives in endocrinological research

At present, it is well-established that 20-hydroxyecdysone and other ecdysteroids function as molting hormones in Crustacea, but it is still unclear what role ecdysteroids play in conjunction with juvenoid substances in stimulating reproductive processes. Much progress has been achieved recently in the isolation and identification of eyestalk hormones, particularly of MIH. A Japanese group has succeeded in isolating a putative MIH in *P. japonicus* (Yang et al. 1996) and has demonstrated it to have molt-inhibiting activity by assessing ecdysteroid synthetic activity under Y-organ culture. The nature of VIH in both *M. rosenbergii* and *P. japonicus* and MIH in *M. rosenbergii* remains unclarified. More information concerning the structures of these hormones and how titers fluctuate during the molt cycle should greatly improve knowledge of endocrinological mechanisms controlling molting and reproduction. It is also of importance to further elucidate the physiological roles of MF, and to determine whether putative brain and ovarian factors are involved in hormonal processes. Finally, an understanding of how such mechanisms operate in context of environmental factors is expected to contribute significantly to the development of techniques for controlling maturation and reproduction in captivity.

The remainder of this paper will introduce the current status of *P. japonicus* culture in Japan, and artificial seed production programs for this species and related applied research being carried out by the Japan Sea-Farming Association (JASFA). The author is engaged in a combined farming systems project in the Mekong Delta region of Vietnam, focusing on *M. rosenbergii* seed production and aquaculture, but this will not be discussed in detail.

## *PENAEUS JAPONICUS* CULTURE IN JAPAN

The following information is based on a 1995 Fiscal Year Report of the Norinchukin Bank, Fisheries Division (see Fujiwara 1995). In 1995, total marine culture production in Japan was approximately 1.284 million tons with a market value of ¥575.6 billion. Major species of interest in Japan include yellowtail, sea bream, flounder, and the kuruma prawn *P. japonicus*. Of this total production, *P. japonicus* culture accounted for approximately 2000 tons valued at ¥13.514 billion. During 1991-1994, production of *P. japonicus* decreased from nearly 2500 to 1500 tons due to severe viral outbreaks in western Japan, but evidence of recovery is beginning to be seen as causes of viral outbreak have come to be elucidated. The number of operators in total has remained fairly constant during this period, around 150-160 enterprises nationwide. Table 1 summarizes changes in enterprise number, production volume, and market volume from 1991 to 1995.

*P. japonicus* culture began as early as 1962 with the development of artificial propagation techniques, and production levels peaked in 1988 at about 3020 tons. Typically, culture operations are begun in the spring, between March and May, and prawns are reared to market size of about 30-50 g by the end of the year or following spring. Culture is thus carried out on the basis of yearly cycles. Common feeds include minced sardine, squid or clam, and artificial pellets. Culture is focused predominantly in western Japan, Kyushu, and Okinawa. In 1994, of a total of 151 operators, 52 were based in Kumamoto Prefecture, 25 in

| | Year | | | | |
|---|---|---|---|---|---|
| | 1991 | 1992 | 1993 | 1994 | 1995 |
| No. enterprises | 161 | 156 | 163 | 151 | - |
| Production volume (tons) | 2,491 | 2,187 | 1,712 | 1,519 | *2,000 |
| Market volume (billions of yen) | 17.178 | 17.144 | 15.93 | 12.978 | 13.514 |

*estimated

**Table 1.** Changes in enterprise number, production volume and market volume for kuruma prawn culture in Japan during 1991-1995.

| Prefecture | No. enterprises | Total area (m²) | Average area (m²) |
|---|---|---|---|
| Kumamoto | 52 | 1,549,000 | 30,000 |
| Kagoshima | 25 | 997,000 | 40,000 |
| Okinawa | 20 | 715,000 | 36,000 |
| Ehime | 15 | 642,000 | 49,000 |
| Yamaguchi | 13 | 581,000 | 39,000 |

**Table 2.** Number of enterprises engaging in kuruma prawn culture, and total and average areas under operation.

Kagoshima, 20 in Okinawa, 15 in Ehime, and 13 in Yamaguchi. Table 2 summarizes these figures along with total and average area per enterprise under culture. Three forms of culture are typically practiced: artificial pond culture, net culture, and tank culture. Artificial pond culture and tank culture are most predominant with only a minority of operators engaged in net culture.

In 1992, widespread viral outbreak occurred as a result of the introduction of infected seed imported from China. Causes of viral outbreak have since been elucidated, and control measures, such as the disinfecting of culture ponds, have helped bring the situation under control. Operators have had to rely increasingly more on domestic sources of seed. In Okinawa, parent prawns obtained from Miyazaki, Oita, and Nagasaki Prefectures are used to secure seed. While there is some technical assistance and cooperation carried out between governmental agencies and private operators, the former is not permitted to produce and sell seed to the private sector; therefore, culturists need to rely on other private sources for obtaining seed. Government-sponsored projects relating to artificial seed production are implemented explicitly for purposes of coastal restocking or on an experimental basis. These programs will be introduced in the next section.

## ARTIFICIAL SEED PRODUCTION PROGRAMS FOR *P. JAPONICUS* IN JAPAN

### Current status

Artificial seed production programs for *P. japonicus* are implemented by the Japan Sea-Farming Association (JASFA), an auxiliary organization of the Japan Fisheries Agency. Programs relating to kuruma prawn production are mainly carried out at JASFA's Momoshima Station in Hiroshima Prefecture, and Shibushi Station in Kagoshima Prefecture. Actual operations are principally carried out at Shibushi while work relating to the cultivation of female spawners is being done at Momojima.

Figure 5 shows statistics for artificial seed production and release during the years 1977-1995. Production levels have generally ranged between 400 and 600 million seeds/yr, with figures for actual release fluctuating around 300 million seeds/yr (JASFA statistics, personal communication, M. Kobayashi, Japan Sea-Farming Association, Kanda, Tokyo). At present, JASFA relies entirely on the

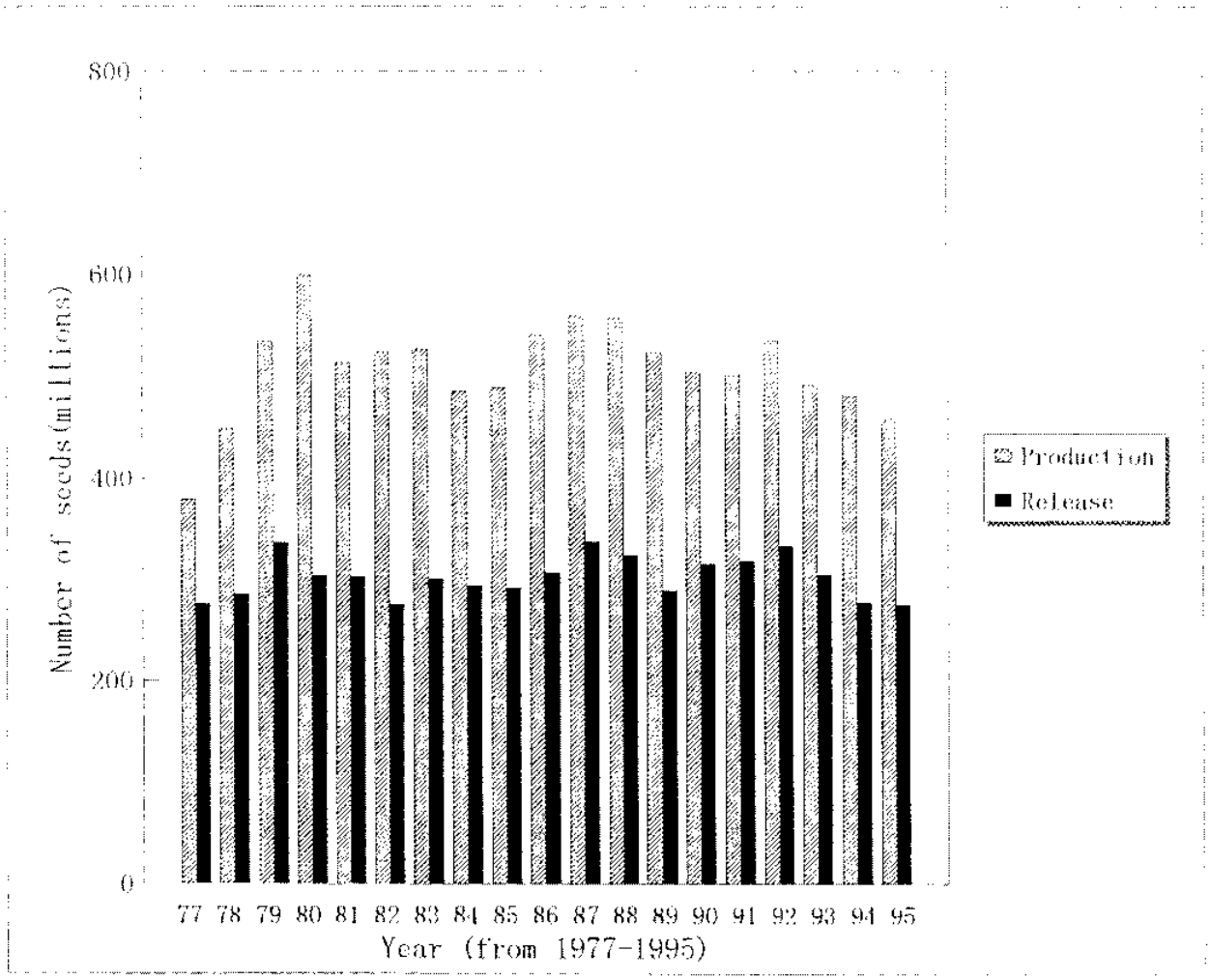


**Figure 5.** Statistics for artificial seed production and release of *P. japonicus* in Japan from 1977 to 1995 (courtesy of the Japan Sea-Farming Association).

use of natural spawners for obtaining seeds which are generally raised to a size of 12-18 mm before release, although "large-size" seeds from 20-40 mm are occasionally produced. Seed production operations are conducted principally from April to September, when water temperature is warm (about 26°C) and parent spawners are readily available. In general, prawns with developed ovaries are purchased from commercial fishermen, and are brought to JASFA premises while chilled slightly or kept on sawdust, and are then put into stocking tanks. With temperature in the stocking tanks raised back to higher temperatures, spawning usually occurs in the evening or by the following day. At Shibushi in 1995, purchasing operations were carried out 20 times between 25 April and 7 September, during which time 6283 parent prawns were obtained (Miyajima 1995), and actual seed production operations were implemented on a total of 15 occasions. Of total prawns purchased, 6120 individuals survived transportation to Shibushi Station, and 2005 individuals actually spawned at an average spawning rate of 39.4%. This yielded an initial total of 236 million seeds with a final harvest figure of 92 million seeds. Table 3 shows actual figures for each spawning occasion, with size of tank used, date, number of seeds obtained, and density for initial stocking and harvest.

| Prod. No. | Tank size (m3) | Initial Production Date | Seed no. | Density ($10^4$/m³) | Final Harvest Date | Seed no. | Density ($10^4$/m³) | Body length (mm) | Survival (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2,500 | 26-27 Apr. | 34,000,000 | 3.4 | 19 May | 5,850,000 | 0.5 | 8.1 | 17.2 |
| 2 | 400 | 7-9 May | 8,070,000 | 3.8 | 12 June | 6,390,000 | 1.6 | 14.4 | 79.2 |
| 3 | 400 | 11 May | 3,020,000 | 1.5 | 12 May | - | - | - | - |
| 4 | 400 | 19-21 May | 7,300,000 | 3.7 | 6 June | 2,660,000 | 0.7 | 17.9 | 27.4 |
| 5 | 2,500 | 25-26 May | 11,480,000 | 1.1 | 30 May | - | - | - | - |
| 6 | 2,500 | 8 June | 66,920,000 | 6.7 | 12 July | 31,420,000 | 1.8 | 14.5 | 46.9 |
| 7 | 400 | 30 June | 18,640,000 | 7.2 | 4 Aug | 10,210,000 | 2.6 | 14.5 | 54.7 |
| 8 | 400 | 30 June | 18,410,000 | 7.1 | 7 Aug. | 9,200,000 | 2.3 | 14.8 | 50.0 |
| 9 | 2,500 | 29 July | 32,090,000 | 3.2 | 31 Aug. | 16,340,000 | 0.9 | 13.7 | 51.0 |
| 10 | 400 | 11-12 Aug. | 4,230,000 | 1.7 | - | - | - | - | - |
| 11 | 400 | 11-12 Aug. | 4,430,000 | 1.8 | - | - | - | - | - |
| 12 | 400 | 19-20 Aug. | 3,210,000 | 1.2 | - | - | - | - | - |
| 13 | 400 | 19-20 Aug. | 3,150,000 | 1.2 | - | - | - | - | - |
| 14 | 400 | 7 Sept. | 11,000,000 | 4.4 | 21 Sept. | 6,170,000 | 1.5 | 6.9 | 56.1 |
| 15 | 400 | 7 Sept. | 10,680,000 | 4.3 | 21 Sept. | 4,010,000 | 1.0 | 4.9 | 37.5 |
| | | total | 236,630,000 | | total | 92,250,000 | avg | 12.2 | 46.7 |

**Table 3.** Artificial seed production at JASFA Shibushi Station in 1995: initial production, density, body length and final harvest values.

Operations were similar in the previous two years of 1993-1994 (Sato 1993, Sato and Yoseta 1994).

Transport of artificial seeds is usually done by truck, and seeds are supplied to various prefectural users. Some trips take up to 17-18 h. Shipping density ranges from 5.6 - 55.7 million individuals/m$^3$ and is adjusted according to length of the trip and size of the seed. Temperature is kept between 19.0-22.5°C in order to suppress metabolism. Mortality during shipping is virtually nil and seeds are observed to be in good condition upon arrival.

At present, it is not difficult to secure parent females during mid-spring to early autumn; however, the availability of spawners obtained from natural sources makes it difficult to conduct operations earlier than April. In addition, while seed production is generally successful based on placing these females in holding tanks prior to spawning and collecting the seed, it is difficult to control spawning time or to synchronize the spawning of many individuals which would make operations more efficient. It is still therefore necessary to improve technology in order to provide a stable supply of seed for coastal restocking. JASFA is engaging in basic and applied research in order to address these problems. It also remains difficult to assess the effectiveness of seed release programs. At present, JASFA is investigating means of marking seed destined for release in order to determine the proportions of artificially-produced prawns in the natural habitat. This manuscript will not discuss these areas in detail, but one potential means of marking is uropod-cutting, whereby the regenerated uropod differs in color and pattern from uncut ones, making individuals of artificial origin distinguishable (Miyajima et al. 1996).

## RESEARCH RELATING TO SEED PRODUCTION IN *P. JAPONICUS*

### Background

The Momoshima Station of JASFA has developed a biopsy method for determining the state of ovarian development in *P. japonicus* (Miyajima and Matsumoto 1996). It was previously necessary to rely on assessing prawns for maturity by observing the visible development of the ovaries, and classifying them into A, B, C, and D ranks based on relative size and visual appearance of the ovaries. The A and B ranks in which ovaries are enlarged were considered mature and the C and D stages in which ovaries were still elongated were considered immature (Miyajima and Matsumoto 1996). However, with these methods, it was difficult to observe fine differences in ovarian maturity which would serve as an index for spawning potential. The biopsy methods permit detailed observations of developing oocytes. In this method, a syringe is inserted into the ovaries via the soft area between the first abdominal segment and the carapace. Oocytes are then collected and positioned onto a glass slide and observed under light microscopy at a magnification of 100-200X. Oocytes can be differentiated into

three stages which correlate with histological examination. In the third yolk globule stage, cortical alveoli cannot be seen, but other features are similar to those of the early maturation stage. In this stage, the cortical alveoli become apparent. Finally, in the maturation stage, the cortical alveoli become elliptical.

These methods are a useful tool in selecting spawners for seed production operations and in implementing experiments relating to induction of maturation. In JASFA during 1995 (Miyajima 1995), a series of experiments carried out at the Momoshima Station relating to artificial maturation in context of selection of prawns, transport conditions, and rearing conditions are highlighted. These studies, implemented in order to develop technology for the control of maturation and spawning, are briefly described below.

## Development of technology for the control of maturation and spawning

### *Stimulation of spawning*

In part (1) of this study, the relationship between maturation stage of the ovary and spawning rate and the effects of eyestalk ablation on inducing maturation were examined (below, see Miyajima 1995). One-yr-old females showing A rank ovaries were selected and biopsied in order to classify prawns into one of the three maturation stages (third yolk globule, early maturation, and mature) as described above. Prawns were put into individual aquariums maintained at 25°C. Spawning rates, number of eggs per batch, and hatching rates were observed for each group. In yolk globule prawns, 7.1% of the individuals spawned while in the latter two groups, this was 100% and 75%, respectively. Egg batches ranged between 200,000 and 300,000 eggs per prawn for those individuals which spawned, and hatchout rates were 32.2%, 57.1%, and 78.9%, respectively, for the three maturation stages given above. These results are shown in Table 4. Fifteen additional individuals in the third yolk globule stage were unilaterally eyestalk ablated (right-side). In these individuals, there was a 40% spawning rate with about 200,000 eggs per batch and 73.7% hatching. In individuals not ablated, spawning was 7.1% with no differences between the ablated group regarding batch-size and hatching rate. These results demonstrated that biopsy can be used to reliably select prawns which will spawn, and that unilateral ablation is effective in increasing spawning rates in individuals prior to reaching the maturation stages.

In part (2) of this study, the use of environmental factors to induce spawning in the third yolk globule stage females was examined. Fifteen 1-yr-old females with A rank ovaries in this stage were selected and used experimentally for a period of 4-7 days. Three groups with differing light conditions, 24 h lights-on, 14 h lights-on, and 0 h lights-on were maintained between 18.6-24.2°C. As a result, one individual in the 14 h lights-on group only spawned during the experimental period (6.7%). In all groups, clear development of the cortical alveoli was not seen, but in contrast, there was degeneration of the oocytes in 10%, 33.3%, and 80.0% of the individuals in the 24, 14, and 0 h lights-on groups, respectively. These results indicated that shortening day length has adverse effects on ovarian maturation, and suggested that light treatment can be used to control maturation processes.

### *Induction of maturation*

In order to secure seed at specific, desired times, it is considered essential to elucidate the environmental factors that are involved in controlling female maturation. In this experiment, the effects of light and temperature on ovarian maturation were examined (below, see Miyajima 1995). The study was conducted during the season in which female prawns are not normally observed to mature. Three groups—20°C lights-on 14 h, 25°C lights-on 14 h, and 20°C natural day length—

| Ovarian stage | No. prawns | No. spawners | Spawning rate | No. days | Egg batch size | Hatching rate |
|---|---|---|---|---|---|---|
| 3rd yolk globule | 14 | 1 | 7.1% | 1 | 261,000 | 32.2% |
| Early maturation | 22 | 22 | 100.0% | 1 | 214,000±101,000 | 57.1±36.2% |
| Maturation | 4 | 3 | 75.0% | 1 | 296,000±84,000 | 78.9±19.2% |

**Table 4.** Spawning rates, number of eggs per batch and hatching rates for spawning stimulation experiment in kuruma prawn at JASFA.

were employed. Prawns were reared for a period of about 2 months and then examined for ovarian maturation and evidence of mating (deposition of sperm case). Mating rates were 50.0%, 83.7%, and 93.8% in groups 1, 2, and 3, respectively. Maturation rates were 6.0%, 26.5%, and 0.0%. Thus, treatment 2 was most effective. Prawns with mature ovaries were further examined by biopsy for the presence of the cortical alveoli, but no individuals exhibited this. Actual spawning rates were 0.0% and 42.9% in groups 1 and 2. Thus, only group 2 individuals spawned with hatchout rates of 86.4%. This is the first time, however, that females were induced to mature and spawn outside of their normal spawning season without using eyestalk ablation, by manipulating environmental parameters.

## PERSPECTIVES ON ARTIFICIAL SEED PRODUCTION AND CONCLUSIONS

The above studies carried out by JASFA have demonstrated that it is possible to control maturation and spawning in *P. japonicus* by understanding the effects of the environment on these processes. At present, JASFA is cooperating with universities and other research organizations to increase knowledge of mechanisms of maturation and to improve existing technology. In fish, knowledge of the interaction of the environment and endocrinology of significant species has formed a basis for the development of useful technology. In many species, it is known that following ovarian maturation, the secretion of steroid hormones which serve as maturation-inducing substance (MIS) is triggered by environmental cues. Other basic knowledge has allowed the development of hormonal treatments to stimulate final maturation and spawning, such as in the use of human chorionic gonadotropin. In Crustacea, while much progress has been achieved in elucidating hormonal mechanisms, much remains to be elucidated on how environmental factors influence the secretion of hormones which control molting and reproductive processes. Similar to fish, whether an MIS exists is still unclear. In the future, it will be important to link basic studies to explain observations and results of fieldwork and practical experiments. Cooperation between persons working in these respective areas should be actively pursued.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

Ahl, J.S.B. and J.J. Brown. 1991. The effect of juvenile hormone III, methyl farnesoate, and methoprene on Na/K-ATPase activity in larvae of the brine shrimp, *Artemia*. Comp. Biochem. Physiol. 100A: 155-158.

Chavez Justo, C. 1990. Ecology, reproduction, and culture of the giant freshwater prawn, *Macrobrachium rosenbergii*, pp. 163-191. *In:* C. Chavez Justo (ed)., The Aquaculture of Shrimp, Prawn and Crayfish in the World. Midori Shobo Publ., Tokyo. (in Japanese)

Davey, K.G. and E. Huebner. 1974. The response of the follicle cells of *Rhodnius prolixus* to juvenile hormone and antigonadotropin in vitro. Can. J. Zool. 52: 1407-1412.

Davey, K.G., V.L. Sevala, and D.R.B. Gordon. 1993. The action of juvenile hormone and antigonadotropin on the follicle cells of *Locusta migratoria*. Invertebr. Reprod. Dev. 24:39-36.

Fujiwara, T. 1995. Shuyo yoshokugyo sanchi chosa hokuku. Annual fiscal report of the Norinchukin (Agricultural) Bank, 1-13-2 Yurakucho, Chiyoda-ku, Tokyo, 100-0006, Japan. 106 p. (In Japanese)

Laufer, H., D. Borst, F.C. Baker, C. Carrasco, M. Sinkus, C.C. Reuter, L.W. Tsai, and D.A. Schooley. 1987. Identification of a juvenile hormone-like compound in a crustacean. Science 235: 202-205.

Liao, I.C. and Y-H. Chien. 1994. Culture of kuruma prawn (*Penaeus japonicus* Bate) in Asia. World Aquacult. 25(1): 18-33.

Meusy, J-J. and G.G. Payen. 1988. Female reproduction in malacostracan Crustacea. Zool. Sci. 5:217-265.

Miyajima, Y. 1995. Artificial seed production in kuruma prawn at Momoshima Station, pp. 43-51. *In:* Nippon saibai gyogyo kyokai jigyo-nenpo. Annual report of the Japan Sea-Farming Association (JASFA), 3-14-8 Uchikanda, Chiyoda-ku, Tokyo 101-0047, Japan. (In Japanese)

Miyajima, Y. and Matsumoto, A. 1996. Maturity classification using biopsy in pond-reared broodstock of kuruma prawn *Penaeus japonicus* and efficient egg removal. Saibai Giken 25: 37-40. (In Japanese)

Miyajima, T., K. Toyota, Y. Hamanaka, and H. Komaki. 1996. Efficiency of uropod cutting for marking young kuruma prawn *Penaeus japonicus*. Saibai Giken 25: 41-46.

Mizuta, Y. 1995. Artificial seed production in kuruma prawn, pp. 42-43. *In:* Nippon saibai gyogyo kyokai jigyo-nenpo. Annual report of the Japan Sea-Farming Association (JASFA), 3-14-8 Uchikanda, Chiyoda-ku, Tokyo 101-0047, Japan. (In Japanese)

Nishi, A. 1995. Promotion of kuruma prawn artificial seed production at Shibushi Station, pp. 336-338. *In:* Nippon saibai gyogyo kyokai jigyo-nenpo. Annual report of the Japan Sea-Farming Association (JASFA), 3-14-8 Uchikanda, Chiyoda-ku, Tokyo 101-0047, Japan. (In Japanese)

Okumura, T., K. Nakamura, K. Aida, and I. Hanyu. 1989. Hemolymph ecdysteroid levels during the molt cycle in the kuruma prawn *Penaeus japonicus*. Nippon Suisan Gakkaishi 55: 2091-2098.

Okumura, T., C.H. Han, Y. Suzuki, and K. Aida. 1992. Changes in hemolymph vitellogenin and ecdysteroid levels during the reproductive and non-reproductive molt cycles in the giant freshwater prawn, *Macrobrachium rosenbergii*. Zool. Sci. 9: 37-45.

Sagi, A., E. Homola, and H. Laufer. 1991. Methyl farnesoate in the prawn *Macrobrachium rosenbergii*: synthesis by the mandibular organ *in vitro* and titers in the hemolymph. Comp. Biochem. Physiol. 99B: 879-882.

Sato, H. 1993. Promotion of kuruma prawn artificial seed production at Shibushi Station, pp. 361-362. *In:* Nippon saibai gyogyo kyokai jigyo-nenpo. Annual report of the Japan Sea-Farming Association (JASFA), 3-14-8 Uchikanda, Chiyoda-ku, Tokyo 101-0047, Japan. (In Japanese)

Sato, H. and K. Yoseta. 1994. Promotion of kuruma prawn artificial seed production at Shibushi Station, pp. 324-326. *In:* Nippon saibai gyogyo kyokai jigyo-nenpo. Annual report of the Japan Sea-Farming Association (JASFA). (In Japanese)

Spindler, K.D., A. Van Wormhoudt, D. Sellos, and M. Spindler-Barth. 1987. Ecdysteroid levels during embryogenesis in the shrimp, *Palaemon serratus* (Crustacea Decapoda): quantitative and qualitative changes. Gen. Comp. Endocrinol. 66: 116-122.

Takayanagi, H., Y. Yamamoto, and N. Takeda. 1986. An ovary-stimulating factor in the shrimp, *Paratya compressa*. J. Exp. Zool. 240: 203-209.

Tsukimura, B. and D.W. Borst. 1992. Regulation of methyl farnesoate in the hemolymph and mandibular organ of the lobster, *Homarus americanus*. Gen. Comp. Endocrinol. 86: 297-303.

Wilder, M.N. and K. Aida. 1995. Crustacean ecdysteroids and juvenoids: chemistry and physiological roles in two species of prawn, *Macrobrachium rosenbergii* and *Penaeus japonicus*. Isr. J. Aquacult. Bamidgeh. 47: 129-136.

Wilder, M.N., T. Okumura, K. Aida, and I. Hanyu. 1990. Ecdysteroid fluctuations during embryogenesis in the giant freshwater prawn, *Macrobrachium rosenbergii*. Gen. Comp. Endocrinol. 80:93-100.

Wilder, M.N., T. Okumura, and K. Aida. 1991. Accumulation of ovarian ecdysteroids in synchronization with gonadal development in the giant freshwater prawn, *Macrobrachium rosenbergii*. Zool. Sci. 8: 919-927.

Wilder, M.N., T. Okumura, Y. Suzuki, N. Fusetani, and K. Aida. 1994. Vitellogenin production induced by eyestalk ablation in the giant freshwater prawn *Macrobrachium rosenbergii* and trial methyl farnesoate administration. Zool. Sci. 11: 45-53

Wilder, M.N., S. Okada, N. Fusetani, and K. Aida. 1995. Hemolymph profiles of juvenoid substances in the giant freshwater prawn *Macrobrachium rosenbergii* in relation to reproduction and molting. Fish. Sci. 61: 175-176.

Yang, W-J., K. Aida, A. Terauchi, H. Sonobe, and H. Nagasawa. 1996. Amino acid sequence of a peptide with molt-inhibiting activity from the kuruma prawn *Penaeus japonicus*. Peptides 17: 197-202.

# PRIMARY PRODUCTIVITY OF SANDY SHORES

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## ABSTRACT

Many kinds of aquatic organisms are found inhabiting the surf zone around exposed sandy shores and, in particular, plankton feeders such as the sandy beach clam is an important species contributing to fishery resources. This fact shows that the biomass of phytoplankton in these areas is abundant, which comes from primary production. Thus, it may be possible to establish no-feed aquaculture and nursery culture of bivalves by using abundant, natural phytoplankton as feed in such areas. However, because sandy shores are often utilized for various human activities, the beach shape is sometimes artificially modified.. It is therefore necessary to clarify the mechanisms which support primary production in exposed sandy shores in order to maintain and improve biological production in harmony with a variety of coastal uses. In this context, we have investigated primary production and characteristics of nutrients in the surf and outer turbulent zones of an exposed sandy shore located in Ibaraki Prefecture, Japan. In general, it has been shown that the primary production rate in the ocean strongly depends on light intensity and water temperature. However, the results of the present investigation on exposed sandy shores suggest that the most important factor regulating primary production is nutrient concentration. Therefore, understanding the dynamics and mechanisms of nutrient supply is considered an important step in evaluating primary production. Moreover, it has been shown that productivity is high in the surf zone as well as offshore. It is thought that the physical characteristics of the surf zone, i.e., turbulence of water caused by waves, run-up of seawater, infiltration of run-up water in sand, and exudation of underground water, are related to high primary production.

## INTRODUCTION

It is thought that biota are poor in the surf zone on exposed sandy shores because the turbulence of seawater is violent, and there are no steady adhesion bases. However, many kinds of aquatic organisms inhabit such areas, and the sandy beach clams are important fishery resources on sandy shores. The fact that there is an abundance of organisms of low trophic levels, such as plankton feeders, are inhabited shows that the phytoplankton biomass is enough to support these organisms.

However, primary production research around the surf zone on exposed sandy shores has been rare (Brown and McLachlan, 1990). Moreover, the supply mechanism of nutrients which is an important factor to measure the primary production is not well known. The main characteristics of exposed sandy shores are as follows. First, seawater and sediments are always turbulent because of waves breaking against the beach. Second, seawater infiltrates the sand through run-up on the beach. Therefore, it is thought that the substance exchange between three spheres (land, hydrosphere, and atmosphere) is active. It is very interesting to know how nutrients are supplied in such environmental conditions.

Sandy shores are utilized for various human activities. From the fishery point of view, sandy shores may be utilized as fishing grounds.

no-feed aquaculture grounds and as bivalve nursery culture grounds which take adavantage of abundant phytoplankton as feed. Moreover, in reference to land development and coastal protection, beach shape is modified artificially. It is therefore necessary to clarify the mechanisms of substance cycling which supports primary production on exposed sandy shores in order to maintain and improve biological production in harmony with a variety of coastal uses.

We have been studying these mechanisms since 1992. The temporal and spatial variation of both phytoplankton biomass and nutrient concentration in the surf zone were researched from 1992 to 1994. Primary production has been measured in the surf zone and offshore area every season since 1995. Moreover, research concerning the behavior of the underground water around the beach began in 1996. Here, the research results of the variation of phytoplankton biomass, nutrients, and primary production in the surf zone and offshore area are introduced.

## METHODS

The research area is Kashima-nada, located in the southern part of Ibaraki Prefecture, Japan, on the Pacific Ocean as shown in Figure 1. Kashima-nada is an exposed and shallow sandy shore, and its total length along the shoreline from the Oharai beach at the northern end to the Hasaki beach in the southern end is about 80 km. It is one of the major sandy beaches in Japan. The beach is divided into two parts, the north and the south sides, with the Kashima Port in the middle. The beach is flat and wide, but recently the coastal erosion has occured in places, especially in the centeral part.

Field research concerning variation of chlorophyll *a* and nutrients around the surf zone was carried out at the research pier near the Hasaki Oceanographical Research Station (HORS), Port and Harbor Research Institute, Ministry of Transport, as shown in Figure 2. The sandy beach there is very flat and wide. The nearshore also has a gentle bottom slope and wide surf zone. Shoreline water and both sea surface and bottom water at the offshore end of the pier, 380 m offshore from the shoreline, 5 m in depth, was sampled in order to determine chlorophyll *a* concentration and size distribution of phytoplankton. Because we wanted to obtain detailed knowledge about the temporal variation of phytoplankton concentration in this research, the seawater was sampled approximately 500 times in 3 yr from 1992 to 1994. Nutrient concentration was determined from 1993

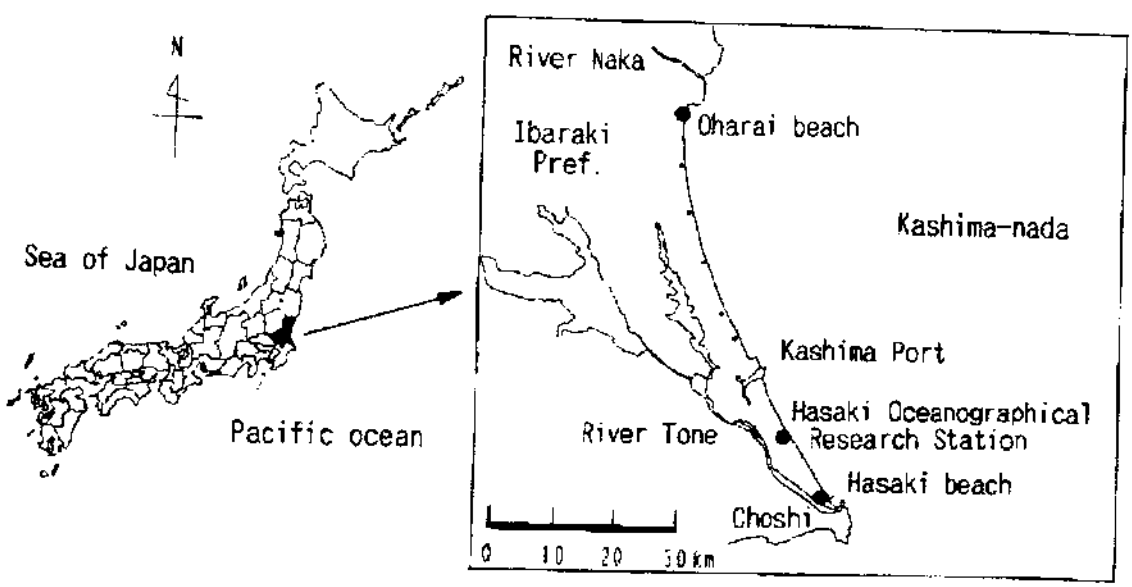


**Figure 1.** Location of Kashima-nada and the Hasaki Oceanographical Research Station.

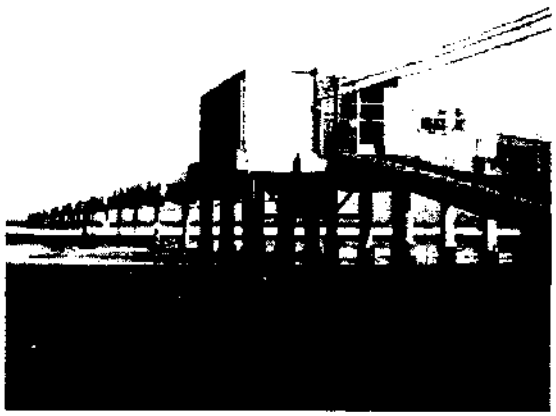

**Figure 2.** Photograph of the the Hasaki Oceanographical Research Station.

on. The size distribution of phytoplankton was measured once a week from 1993 to 1994.

Chlorophyll *a* distribution was examined along the beach from Oharai beach to Hasaki beach to characterize phytoplankton biomass in the surf zone on Kashima-nada. This research was carried out eight times from 1992 to 1994.

Primary production was measured by the site method (pseudosite method in stormy weather) at Sta.3 (1.7 miles offshore from HORS, in 10-m depths) and Sta. 6 (7.7 miles offshore from HORS, in 40-m depths) as shown in Figure 3. First, vertical distribution of light quantum in the sea was measured. Next, sea surface water and seawater of each depth of quantum number at 50, 25, 10, and 1% in comparison with the surface water were sampled, and these water samples were divided into 1-L transparent polycarbonate bottles. These bottles were hung at original depths after carbon-13 ($^{13}C$) reagent was added and phytoplankton in the bottles was cultured for 3 or 4 h. Finally, the photosynthetic rate was estimated by measuring the quantity of $^{13}C$ uptake by phytoplankton while culturing. Additionally, water temperature, salinity, and the concentration of both chlorophyll *a* and nutrients were measured at many points in this area including the research position.

On the pier of HORS, the photosynthetic rate of the surface water at the shoreline part (1 m in depth) and both surface water and bottom water at the offshore end of the pier (5 m in depth) were similarly measured by the site method. The water temperature, salinity, and concentration of both chlorophyll *a* and nutrients were also measured.

In the offshore area, primary production was measured eight times around noon on a day in July, August, and November 1995; February, May, July, and November 1996; and May 1997. In the surf zone, the research was carried out five times on the same day or one of the same days as offshore research.

Chlorophyll *a* was analyzed as follows. After water samples were filtered through a 1-μm glass fiber filter and the pigments in the particle which had been caught on the filter were extracted with acetone, concentration of the pigment was determined by the spectrum method (Lorenzen 1967)

Five kinds of nutrients (nitrate, nitrite, ammonium, phosphate, and silicate) were determined with an autoanalyzer (TRAACS-800, Bran+Ruebbe Co.) by absorption photometry. Stable isotope $^{13}C$ was determined with $^{13}C$ analyzer (Nippon-Bunkho Co.) located at the National Research Institute of Fisheries Science.

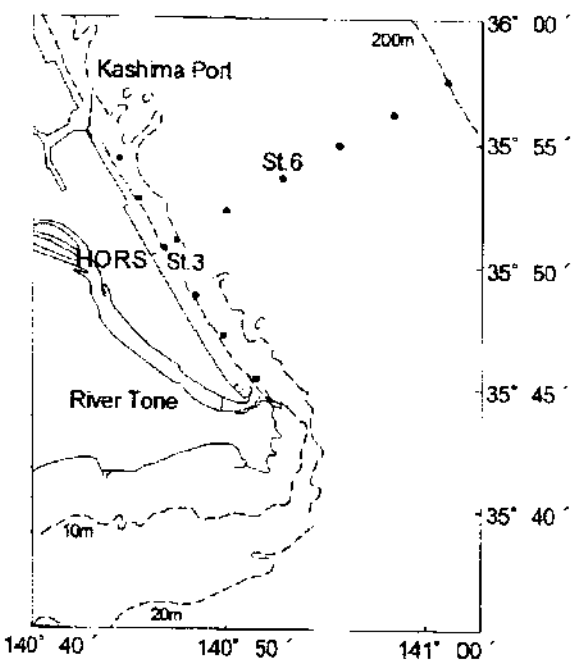


**Figure 3.** Location of the field research on offshore area.

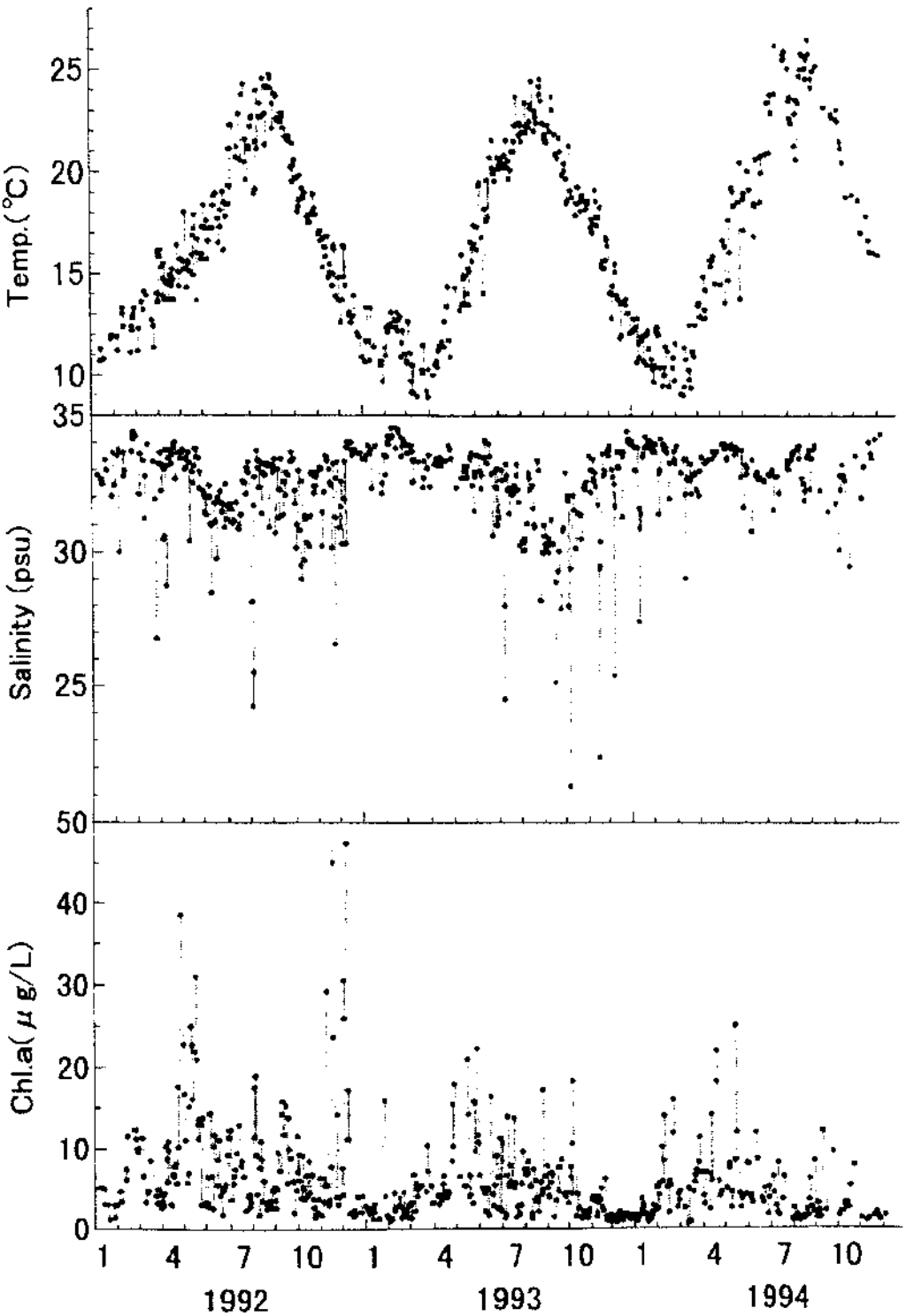


**Figure 4.** The temporal variation of temperature, salinity and chlorophyll *a* at the shoreline of the Hasaki Oceanographical Research Station.

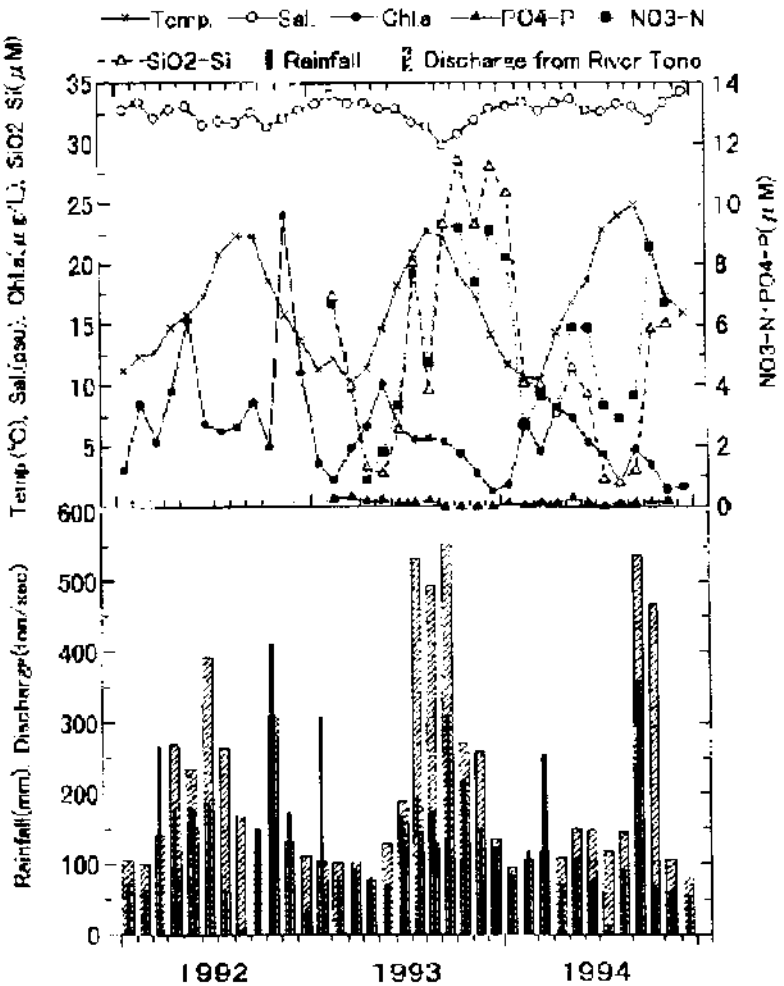


**Figure 5.** The variation of temperature, salinity, chlorophyll *a*, nitrate, phosphate and silicate at the shoreline of the Hasaki Oceanographical Research Station; discharge from River Tone; and rainfall at the Choshi Weather Station. Rainfall is showed as sum, and others are showed as mean value.

## Temporal and spatial variation of phytoplankton biomass and nutrient concentration in the surf zone

Figure 4 shows the temporal variation of chlorophyll *a* at the shoreline of HORS from 1992 to 1994. Monthly mean values of the water temperature, salinity, chlorophyll *a*, and nutrient concentration at the shoreline of HORS are shown in the upper part of Figure 5; total rainfall at Choshi and mean value of discharge from the River Tone (Ministry of Construction 1994) observed at 76 km above the river mouth are in the lower part of Figure 5.

Chlorophyll *a* varied from about 1 to 20 μg/L. The annual mean value of chlorophyll *a* at the shoreline from 1992 to 1994 was 9.5, 4.6, and 4.8 μg/L, respectively, and the biomass from 1993 to 1994 was low compared with that in 1992. Bivalve juveniles such as the Japanese surf clam *Pseudocardium sachalinensis* appeared abundantly in this area in 1993 where it has grown well. There is a possibility that the chlorophyll *a* decrease after 1993 was caused by ingestion pressure by these clams.

Chlorophyll *a* concentration showed a tendency to be high at the shoreline compared with the offshore end of the pier. Chlorophyll *a* concentration was very high during one month from the end of April to the end of May, and it was thought that this phenomenon was due to a spring

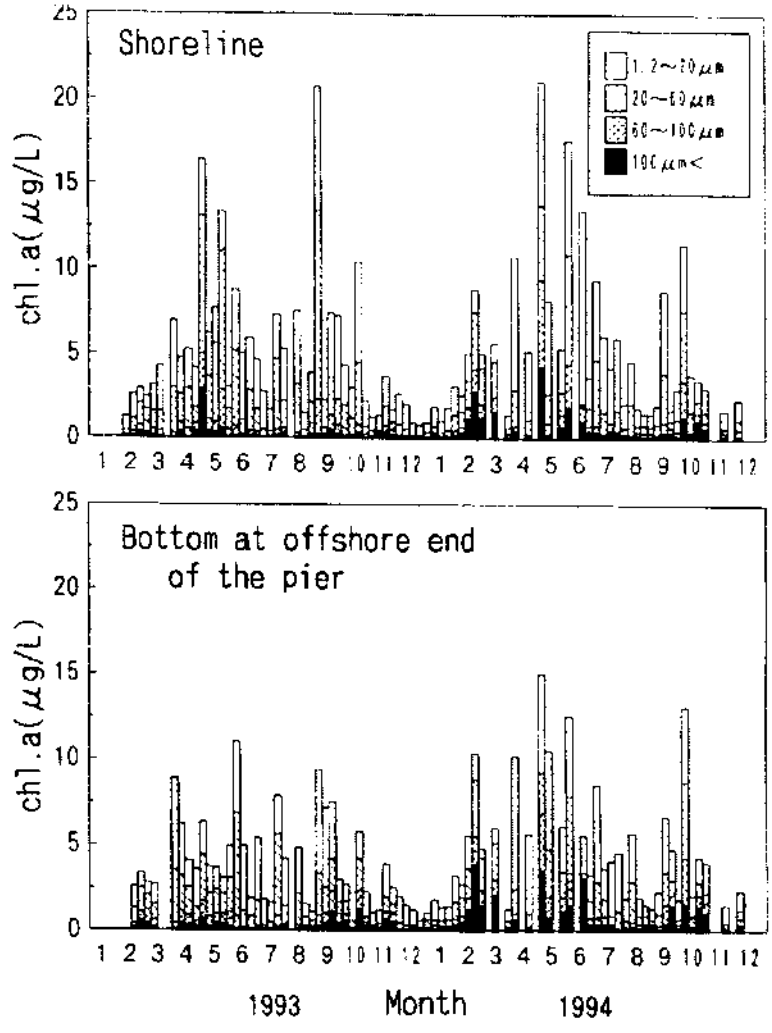


**Figure 6.** The variation of chlorophyll *a* concentration which is classified according to the size at the shoreline of the Hasaki Oceanographical Research Station and the bottom water of the offshore end of the pier.

phytoplankton bloom. Chlorophyll *a* concentration changed drastically over a short term. Such a change occurred because of a sudden change in weather or oceanographic phenomena such as wave, current, wind, or discharge from the River Tone.

Though floating diatoms were dominant in the suspended matter, many fecal pellets and detritus were seen.

The variation of the nutrient concentration at the shoreline is described as follows. Usually, concentration of nitrate, silicate, and phosphate was less than 10, 20 and 0.4 μM, respectively. However, a very high value was often seen in diurnal variation, and it was thought that this depended on sudden changes in weather as well as the chlorophyll *a* variation. The nutrient concentration decreased during the spring bloom because of uptake by phytoplankton. That was exhausted in the summer and recovered gradually in the autumn. At HORS, nutrient concentration at the shoreline was a little higher than at the offshore end of the pier.

It is believed that the main supply sources of nutrients are the offshore bottom water, the inland waters such as the river water, and the beach

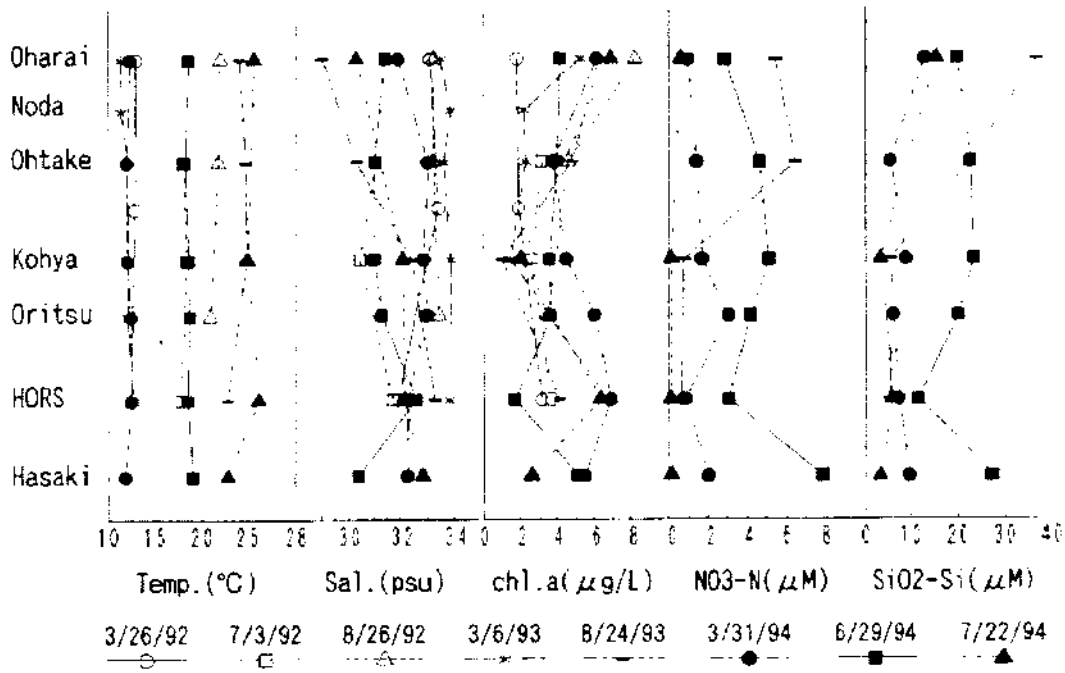


**Figure 7.** The variation of temperature, salinity, chlorophyll *a*, nitrate and silicate at some shoreline points along the Kashima-nada.

underground water, in addition to the regenerated nutrients in the ecosystem. A correlation was seen between salinity and both the concentration of nitrate and silicate; therefore, it was thought that the influence of the inland water was a strong supply source of nutrients for the surf zone.

Figure 6 shows the annual variation of chlorophyll *a* concentration that is classified according to phytoplankton size at both the shoreline and the bottom at the offshore end of the pier. Phytoplankton size was large during the winter and spring months, especially the spring bloom, and dominant size was 20-60 μm. It was shown that the size was smaller in summer. At the offshore end of the pier, the mean size of phytoplankton was larger than at the shoreline and it was thought that large suspended particles were disposed to sink although turbulence of the water was violent.

Figure 7 shows the chlorophyll *a* distribution of the shoreline water along Kashima-nada. Chlorophyll *a* concentration was high in both the north and south, but low in the central part. The beach at both ends is flat and wide where sand is fine, while the beach in the center part has a steep incline where sand is coarse. The River Naka flows into the north end of the Kashima-nada and the River Tone flows into its south end. There is a tendency for river water to go southward after flowing into the sea. Therefore, in the southern part with few influences of the river water, salinity was high and nutrient concentration was low. However, the level of chlorophyll *a* was high at HORS. This suggests that primary production is being influenced by the shape of the sand, in addition to the influence of river water.

**Estimates of primary production**

Figure 8 shows the optical quantum vertical distribution at the observation points (Sta. 3 and Sta. 6) in the offshore area of Kashima-nada. Light transmittance short in winter and spring and long in summer and autumn. During the entire research period, light reached the seabed and the value was from 2.5 to 12% compared with the surface at Sta. 3. Therefore, it can be said that all layers were productive, euphotic zones. At Sta. 6, the compensation depth, depths at 1% of light intensity

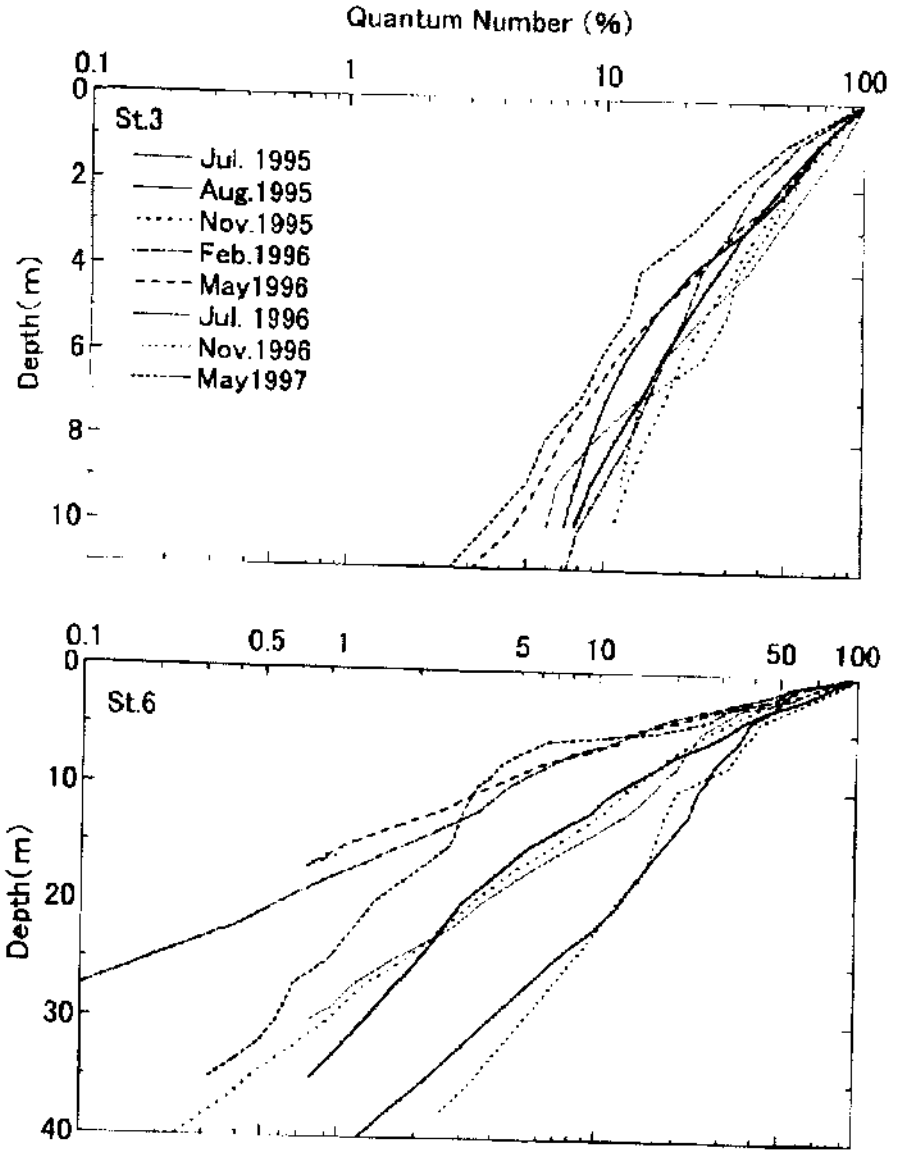


**Figure 8.** The vertical distribution of the optical quantum number at Sta. 3 and Sta. 6.

at the sea surface, varied from 15 to 35 m.

Chlorophyll *a*, measured when primary production was measured, showed the same tendency as the previous research on the surf zone. In summary, concentration was high from early spring to around May and low, around 1 μg/L, from the end of the rainy season, June and July, to August in both the surf zone and the offshore. A high concentration layer, from 10 to 40 μg/L, existed widely from 0 to 10 m in depth, and the seawater was brown, in May 1997.

Figure 9 shows an example of the vertical distribution of the nutrient concentration at Sta. 3 and Sta. 6. The nutrient concentration in the offshore was vertically the same in autumn and winter. On the other hand, it was exhausted during July and August in the upper part of the pycnocline because the supply of nutrients was cut off with stratification

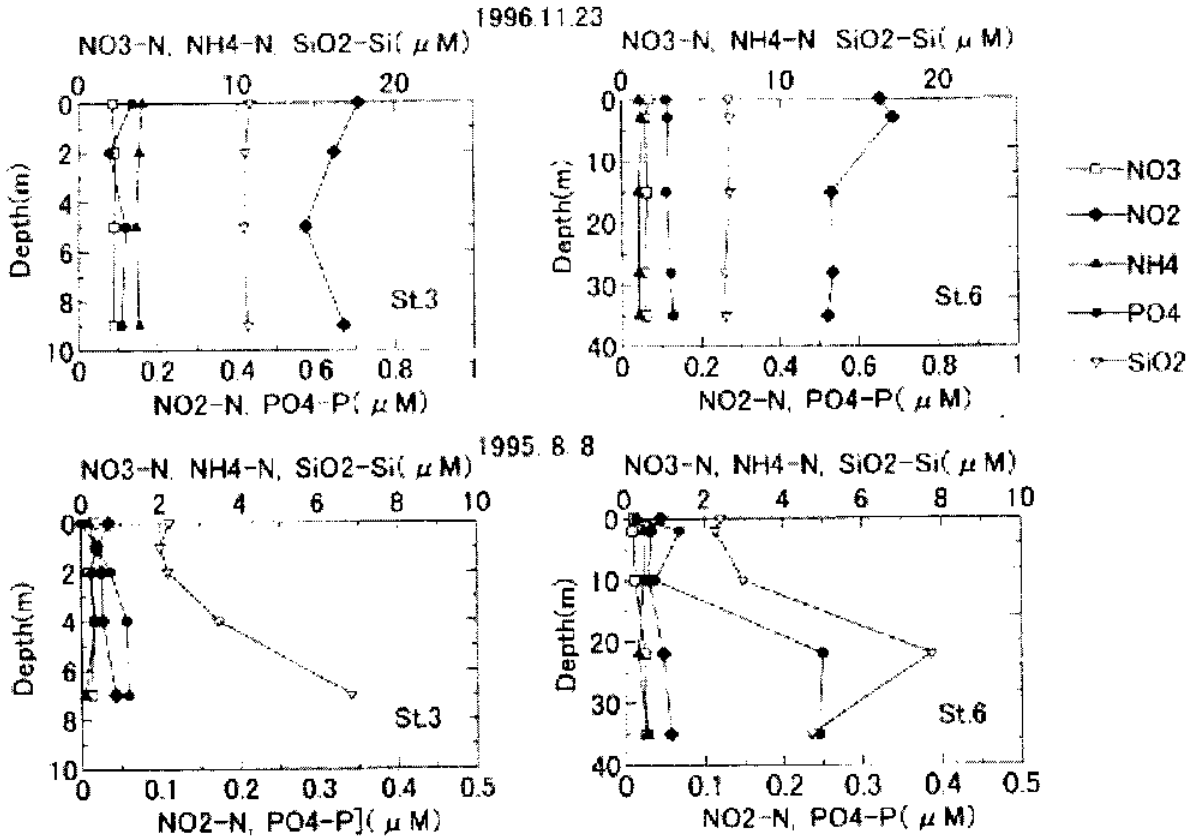


**Figure 9.** The vertical distribution of the nutrients at Sta. 3 and Sta. 6 in August 1995 and November 1996.

after large nutrient consumption during the spring bloom.

Figure 10 shows an example of vertical distribution of the primary production rate. At Sta. 6, the production rate per day was high at the surface with a tendency to rapidly decrease in the depth of stratification and to gradually decrease in the depth of vertical mixing. Sta. 3 showed roughly the same verticality. Moreover, the production maximum layer was seen where the chlorophyll *a* maximum layer existed in the spring. In order to make a clear temporal and spatial difference in primary production, the values on each surface of the observation station are shown in Figure 11. In the summer, the primary production rates were very small in the offshore surface, ranging from 7 to 12 μg-C/L/day. In the other season, the values ranged from 15 to 100 μg-C/L/day. Values from 11 to 245 μg-C/L/day were obtained at the shoreline. This result can be called equal or a higher value compared with the value of the offshore station. However, there is no winter measurement yet.

Assimilation number (*μg-C/μg-chl.a*/h) was compared as an index of the photosynthetic activity. Assimilation number ranged from 0.4 to 5.0 in the offshore surface, and from 0.7 to 3.1 in the surf zone. Because vertical distribution of chlorophyll *a* was roughly the same except in May, vertical distribution of the assimilation number showed the same tendency as vertical distribution of production. A clear correlation was not obtained between assimilation number and water temperature.

In estimating primary production vertically for the whole water column, though the production per unit area at Sta. 6 was naturally large compared with the onshore area where the depth was shallower than the compensation depth, there was

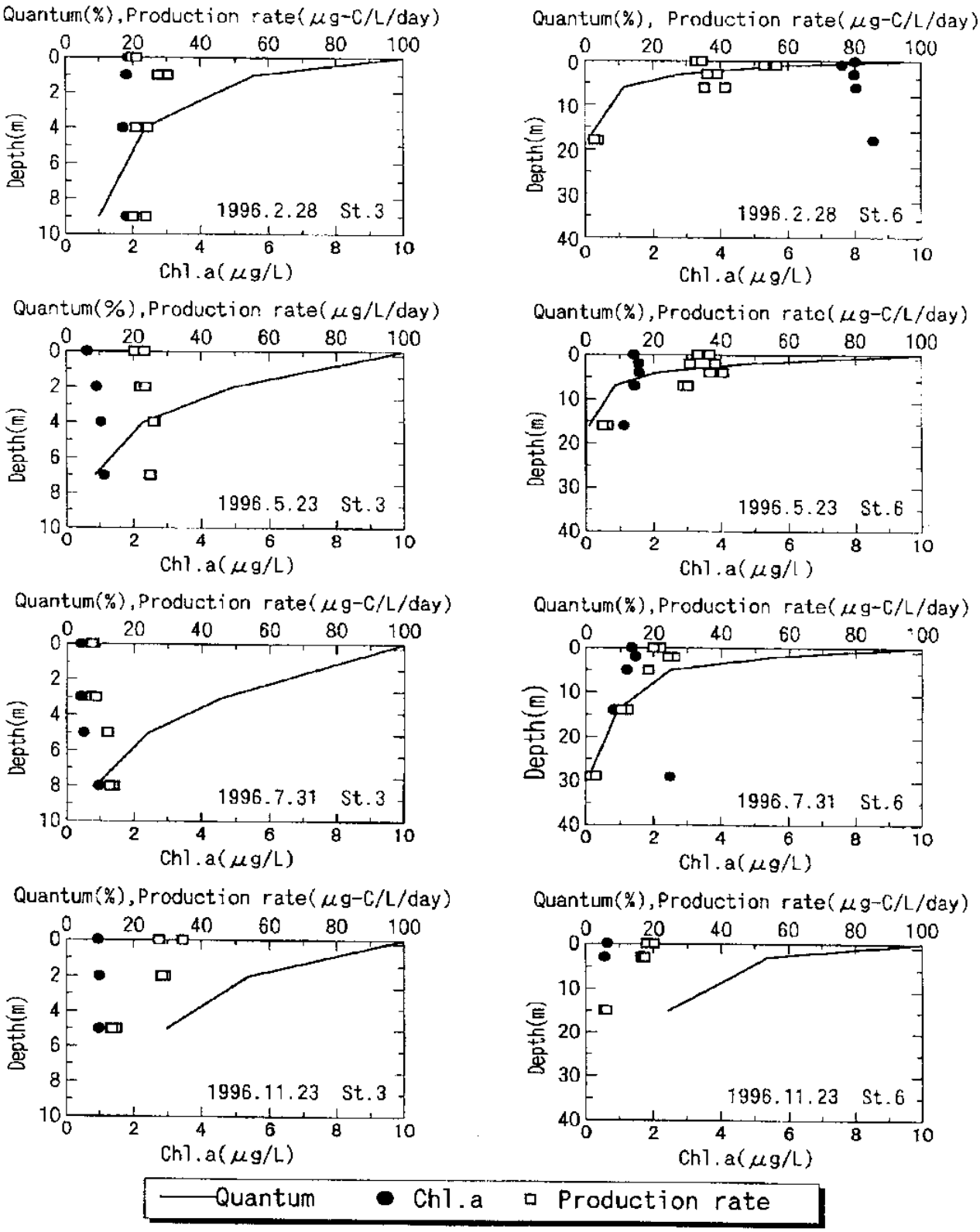


**Figure 10.** The vertical distribution of the primary production rate per day at Sta. 3 and Sta. 6 in 1996.

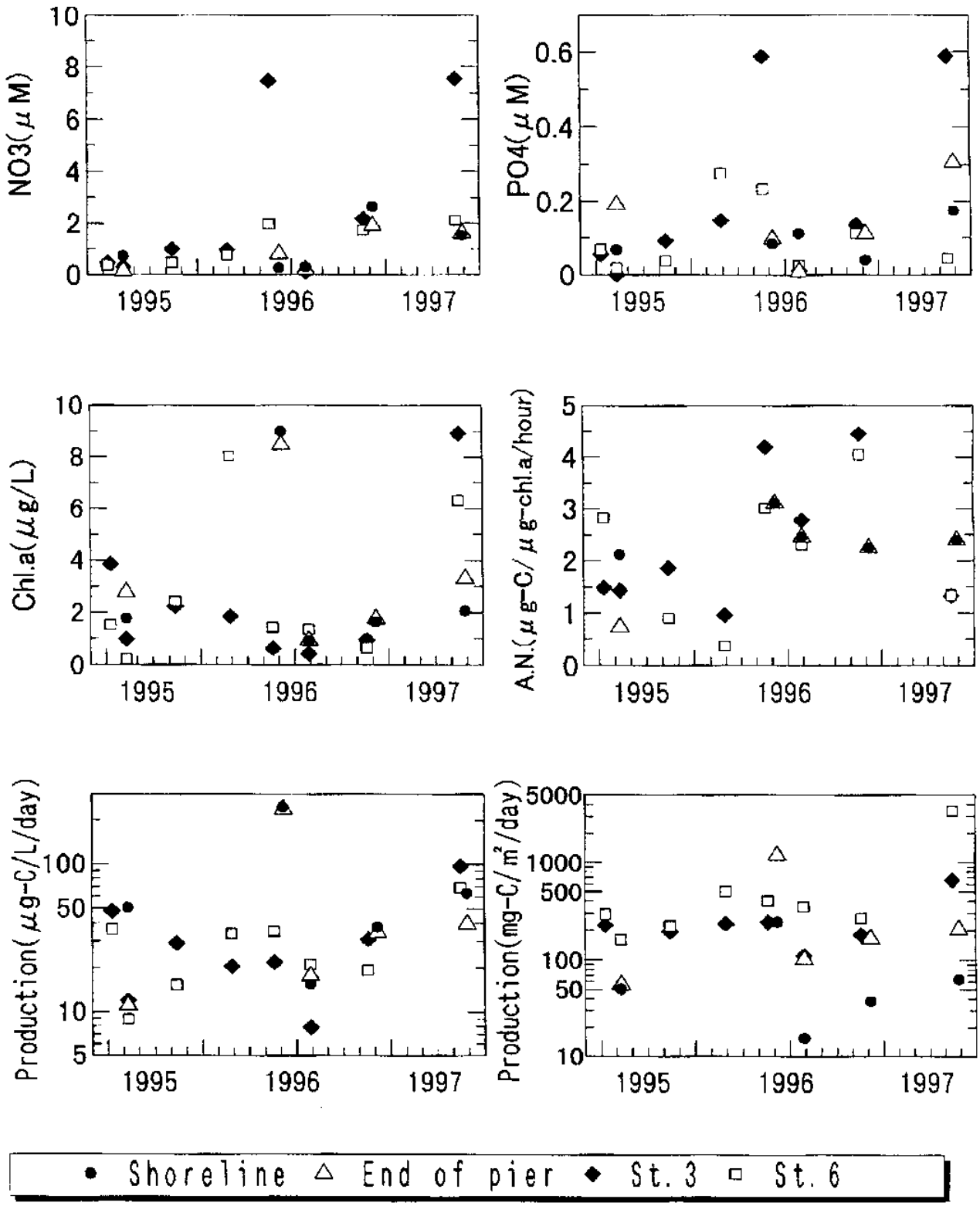


**Figure 11.** The values of nutrients, chlorophyll *a*, assimilation number and primary production at sea surface and production per each unit area of water column on the surf zone along the Hasaki Oceanographical Research Station and offshore area.

no marked difference as the depth changed. It was supposed that the reason for this was the tendency for a large chlorophyll *a* concentration in the surf zone compared to the offshore area. Results from Sta. 6 were about the same as reported as the mean value in the whole area of the Seto Inland Sea of Japan (0.38g-C/m$^2$/day) (Coastal Oceanography Research Committee 1985).

Primary production on the sandy shore in Kashima-nada was roughly the same as that on semi-sheltered areas observed in other research. Moreover, it was shown to be very high in very shallow areas such as near the surf zone. But chlorophyll *a* decreases in summer and so follows primary production. In general, the assimilation number in the ocean strongly depends on water temperature and light intensity under water (Harrison and Platt 1980). However, our research results on exposed sandy shores showed that the assimilation number was not so high in the summer although conditions of both light and temperature were ideal. This suggests that nutrient concentration is also an important factor in primary production.

Abundant bivalves inhabit the surf zone. Because bivalve ingestion rises with water temperature, estimates of primary production may be low in summer because phytoplankton is being consumed. We believe that nutrients supplied are larger than the quantity estimated, and are used promptly. It is important to understand the behavior and supply mechanism of nutrients in order to evaluate the biological productivity on exposed sandy shores more closely.

## ACKNOWLEDGMENT

We thank the staffs of the Port and Harbor Research Institute, the R/V *Taka-maru*, and the Marine Production Division of the National Research Institute of Fisheries Science for the field studies in Kashima-nada and carbon analysis.

## LITERATURE CITED

Brown, A. C. and A. McLachlan. 1990. Ecology of Sandy Shores. Elsevier, Amsterdam, Netherlands. 328 p.

Coastal Oceanography Research Committee, The Oceanographical Society of Japan, Editors. 1985. Coastal Oceanography of Japanese Islands. Tokai Univ. Press, Tokyo. 1106 p.

Harrison, W. G. and T. Platt. 1980. Variations in assimilation number of coastal marine phytoplankton: effects of environmental co-variates. J. Plankton Res. 2: 249-259.

Lorenzen, C. J. 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equation. Limnol. Oceanogr. 12. 343-346.

River Bureau, Ministry of Construction, Editors. 1992-1994. Annual Report of Discharge. Vol. 43-45. Japan River Association, Tokyo. [In Japanese].

# NUTRIENT CONCENTRATIONS IN GROUNDWATER THROUGH SANDY BEACHES

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## ABSTRACT

The ecological functions of sandy beaches are maintaining biological productivity and purifying coastal water quality. Quantitative estimation of these functions is necessary for conservation of sandy beaches and maintenance of biological productivity. We studied the function of maintaining biological productivity. Generally, groundwater which flows through sandy beaches to coastal waters is considered to be the source of nutrients that supports the biological productivity of coastal waters. Samples of groundwater were collected in an exposed sandy beach at Hasaki, Ibaraki Prefecture, Japan, and the concentrations of nitrate, nitrite, ammonium, phosphate, silicate, and salinity contained in the groundwater were analyzed. Eight sample pipes at different locations and at various depths from the shoreline to the backbeach were set. The sampling period was from July 1996 to January 1997. The quantity of nutrients into coastal water was estimated, considering the moving volume of water caused by the change of groundwater level following tidal change. From this experiment, it was found that the nutrients of freshwater in the backbeach flowed into coastal water mixing with seawater and decreasing the concentration.

## INTRODUCTION

In Japan, there used to be 10,000 km of sandy beaches out of 30,000 km of coastline. Presently, natural sandy beaches have been reduced to only 4000 km long due to various constructions for disaster prevention, ports, and amenity facilities. On the other hand, many researchers have reported the importance of the ecological functions of sandy beaches (Brown and McLachlan 1990, Morimoto 1993, Adachi et al. 1994). The ecological functions of sandy beaches are maintaining biological productivity and purifying coastal water quality. Quantitative estimation of these functions is necessary for conservation of sandy beaches and maintenance of biological productivity of coastal waters. Generally, biological productivity of the nearshore ocean is very high. This high productivity is supported by the high concentration of nutrients in coastal waters. The main source of nutrients is considered to be freshwater from rivers, upwelled waters from the deep sea, and groundwater through sandy beaches. Now, aquaculture without artificial feeding has been proposed to relieve the organic load to offshore, and the knowledge on the dynamics of nutrients in groundwater through sandy beaches will help develop the technology of aquaculture without artificial feedings.

In this paper, we investigated the function of maintaining biological productivity. We measured the nutrient concentrations in groundwater through

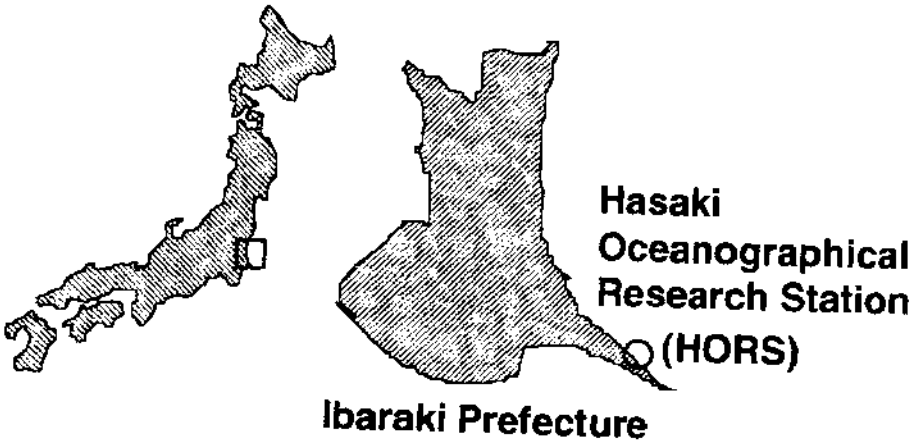


**Figure 1.** Sampling site.

sandy beaches to understand the role of nutrients in groundwater.

## MATERIALS AND METHODS

### SAMPLING SITE

We performed sampling in the sandy beach around the research pier at the Hasaki Oceanographical Research Station (HORS) of the Port and Harbor Research Institute, Ministry of Transport, Japan (Fig. 1). The length of the research pier is 427 m to offshore. This coastal area is an exposed sandy beach facing the Pacific Ocean. The mouth of Tone-gawa (gawa means river in Japan) which has the largest river basin in Japan is located 16 km south. The fish and the clams which live in this nearshore ocean are very valuable resources for fisheries.

### SAMPLING DESIGN

We sank eight sample pipes at different locations and at various depths from the shoreline to the backbeach. Sampling locations were 0 m (P1), 25 m (P2), and 65 m (P3) from the base of the pier to the backbeach (Fig. 2). Considering the influence of the drain to the ocean which is 200 m north of the pier, we established P4 at the north side of P2 (Fig. 2). At each point, we sank two or three pipes at various depths (Fig. 3). Furthermore, we collected seawater samples from the shoreline and surface area at 200 m (water depth of 2 m) and 380 m (water depth of 5 m) from the base of the pier (200-0, 380-0) to the

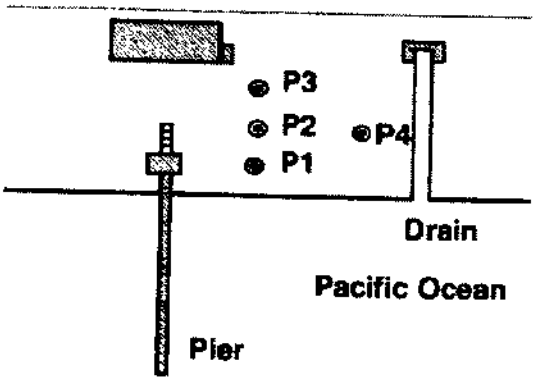


**Figure 2.** Hasaki Oceanographic Research Station (HORS).

offshore, and from the bottom layer at 380 m (380-B). Groundwater samples were collected through each pipe using a pump from July 1996 to January 1997. From 31 July to 1 August 1996, we sampled continually every 2 or 3 h.

Water samples were collected in 300-ml bottles. Subsamples for determining concentration of dissolved nutrients were filtered through 0.45-mm membrane filters to remove suspended solids and were kept frozen until analysis to avoid biological change of the nutrients. We used an autoanalyzer for analysis of nutrients. The remaining water samples were used to determine salinity with an inductively coupled salinometer. At HORS, the basic characteristics data of the coastal environment has been collected periodically. We used this data for analysis.

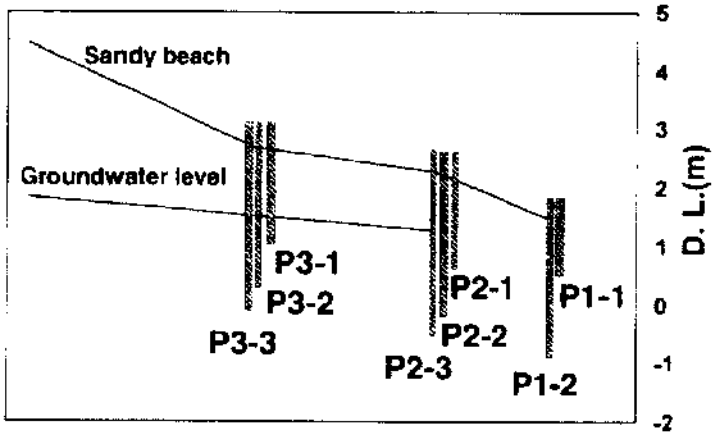


Figure 3. Sampling pipes. The term D.L denotes datum level.

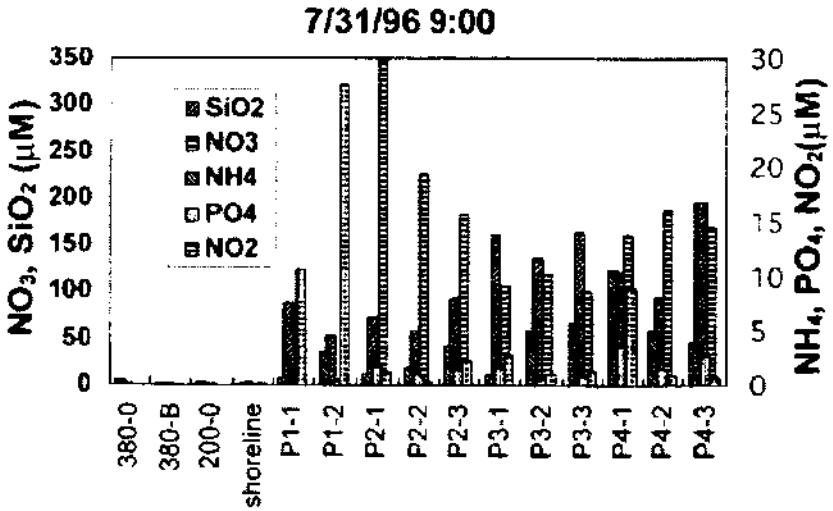


Figure 4. Nutrient concentrations in summer.

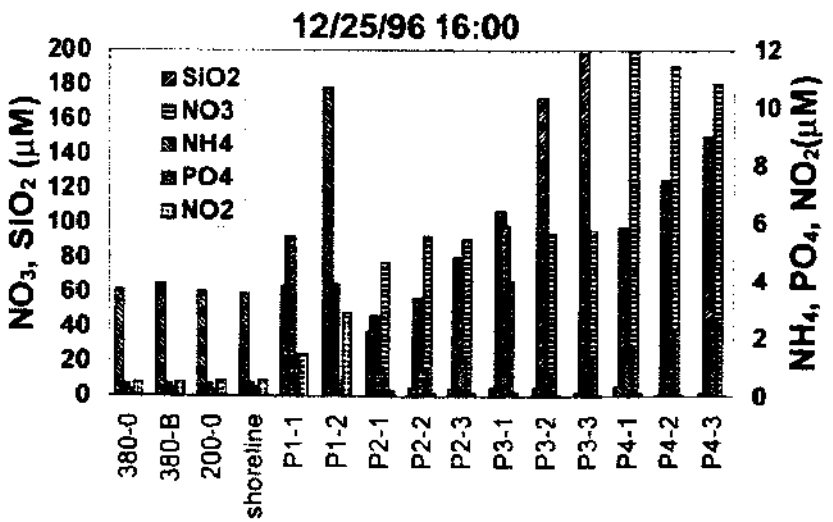


Figure 5. Nutrient concentrations in winter.

| | 7/31/96 9:00 | 12/25/96 16:00 |
|---|---|---|
| P1-1 | 24.5 | 21.8 |
| P1-2 | 21.1 | 31.7 |
| P2-1 | 6.3 | 26.1 |
| P2-2 | 4.3 | 25.8 |
| P2-3 | 3.7 | 23.4 |
| P3-1 | 1.1 | 0.5 |
| P3-2 | 0.6 | 0.8 |
| P3-3 | 0.5 | 0.5 |
| P4-1 | 2.6 | 6.5 |
| P4-2 | 1.9 | 5.8 |
| P4-3 | 1.2 | 6 |
| shoreline | 33.1 | 34.2 |
| 200-0 | 33.1 | 34.3 |
| 380-0 | 33.2 | 34.3 |
| 380-B | 33.2 | 34.3 |

**Table 1.** Salinity concentration (ppt).

## RESULTS AND DISCUSSIONS

The groundwater level at P2 varied with the influence of tide but at P3 it was uniform without any tidal influence. The salinity at P3 was usually less than 1 ppt, and is considered to be nearly freshwater. In the summer season, salinity at P2 was 2-5 and at P1 was 20-30 ppt, respectively. In the winter season, due to the increase of seawater surges inshore following decrease of ground elevation, salinity increased to approximately 25 ppt. We found that the salinity at shallow points was higher than at deep points (Table 1). Nutrient concentrations of coastal seawater except phosphate were low in summer and high in winter. However, this tendency was not distinctly recognized in the case of groundwater. Nitrate concentration of groundwater varied between 150 and 250 μM, and silicate concentration varied between 80 and 150 μM, which is very high compared with the nutrients of seawater (Figs. 4, 5). In most cases, the concentration of ammonium in groundwater was less than 2 μM, and the concentration of nitrite was less than 1 μM. However, the concentration at P3 and P4 increased occasionally. This phenomenon is thought to occur by the inflow of freshwater from the backbeach and from the drain.

At P4, nitrate concentration varied between 100 and 120 μM; silicate varied between 130 and 170 μM; and phosphate varied between 0.5 and 2 μM. Usually, the nutrient concentrations at the location of the upland side were higher than that of the sea side. This suggests that the nutrients were supplied by freshwater in the backbeach and flowed into coastal water mixing with seawater. There was no distinct feature for vertical distribution of nutrients.

The nutrient concentrations at shoreline became high when the discharge of groundwater increased, noticeable by observing tide and groundwater level fluctuations (Fig. 6). The groundwater level was consistently higher than seawater level. This suggests the contribution of groundwater to the nutrients of coastal water. However, this phenomenon was observed only in the summer season.

Mixing of groundwater with seawater creates gradients of nutrients and salinity. When salinity is considered as a conservative tracer of mixing, then it is possible to quantify the uptake and release of biologically and chemically-reactive compounds within the mixing zone. If the correlation between salinity and the nutrient

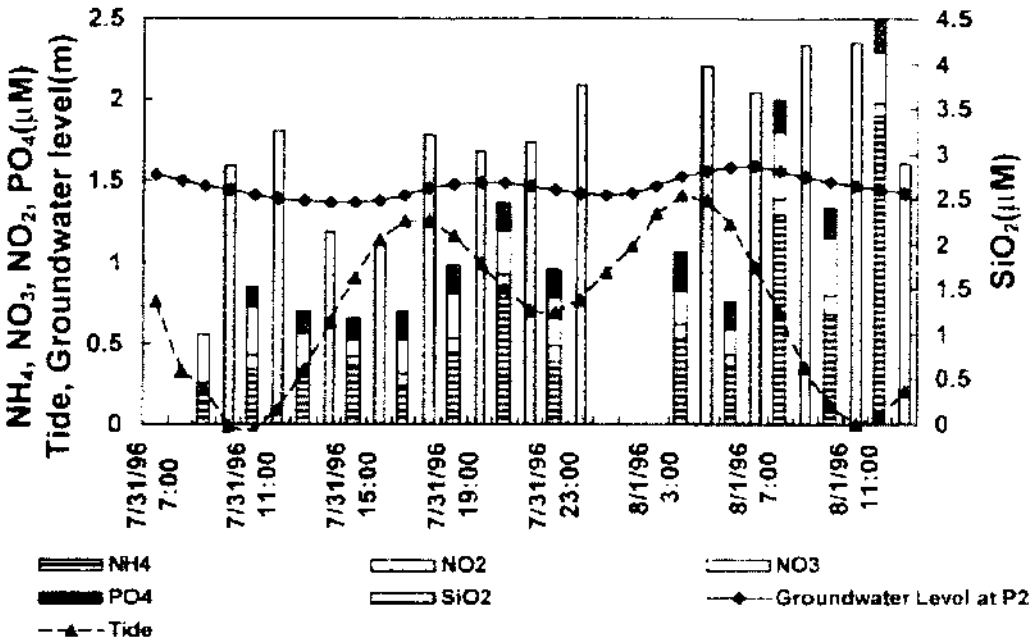


**Figure 6.** Time variation of nutrient concentration at shoreline.

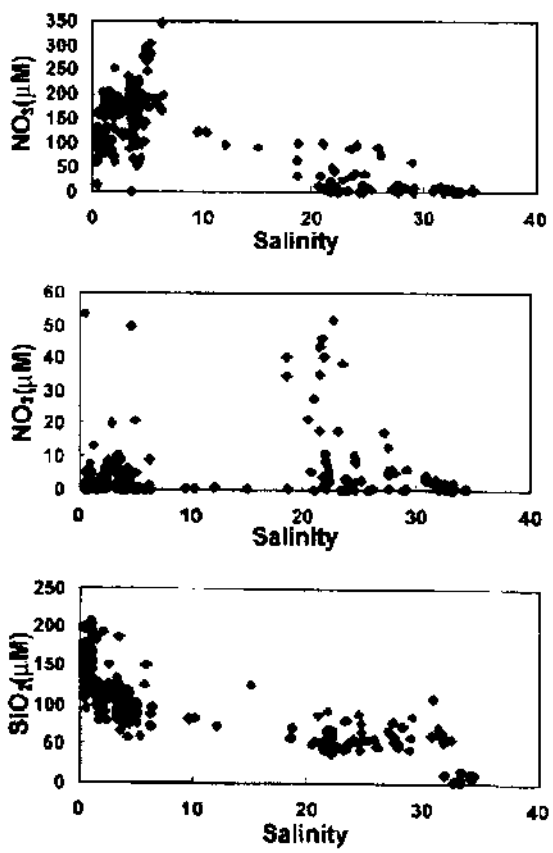


**Figure 7.** Mixing diagram.

concentration is linear, nutrients may be considered conservative. Therefore, we used the relation between salinity and nutrients (Fig. 7). This figure is called the 'mixing diagram.' Nitrate and nitrite concentrations became very high in the case of some concentrations of salinity. The nitrate concentration became especially high when the salinity was 3-5 ppt which mainly occurred at P2, and the nitrite concentration became high when the salinity was approximately 20 ppt which occurred mainly at P1. This phenomenon is considered to be nitrification by nitrifying bacteria. Reportedly, the activity of bacteria which oxidizes nitrite is maximum when the salinity concentration is 3-5 ppt (Kurihara 1988). Our results correspond to that report. Furthermore, from our results we can infer the presence of dissolved oxygen in the groundwater. From Figure 7(c), there was a linear relation between salinity and silicate. Therefore, silicate seemed to be supplied by freshwater in the backbeach and flowed into the coastal water mixing with seawater.

The fluctuation of the concentration of nitrate at P2 seemed to correlate with nitrite at P1, ammonium at P2, and nitrite at P2 (Fig. 8). We consider that it was the facilitation of nitrification due to the increase of ammonium.

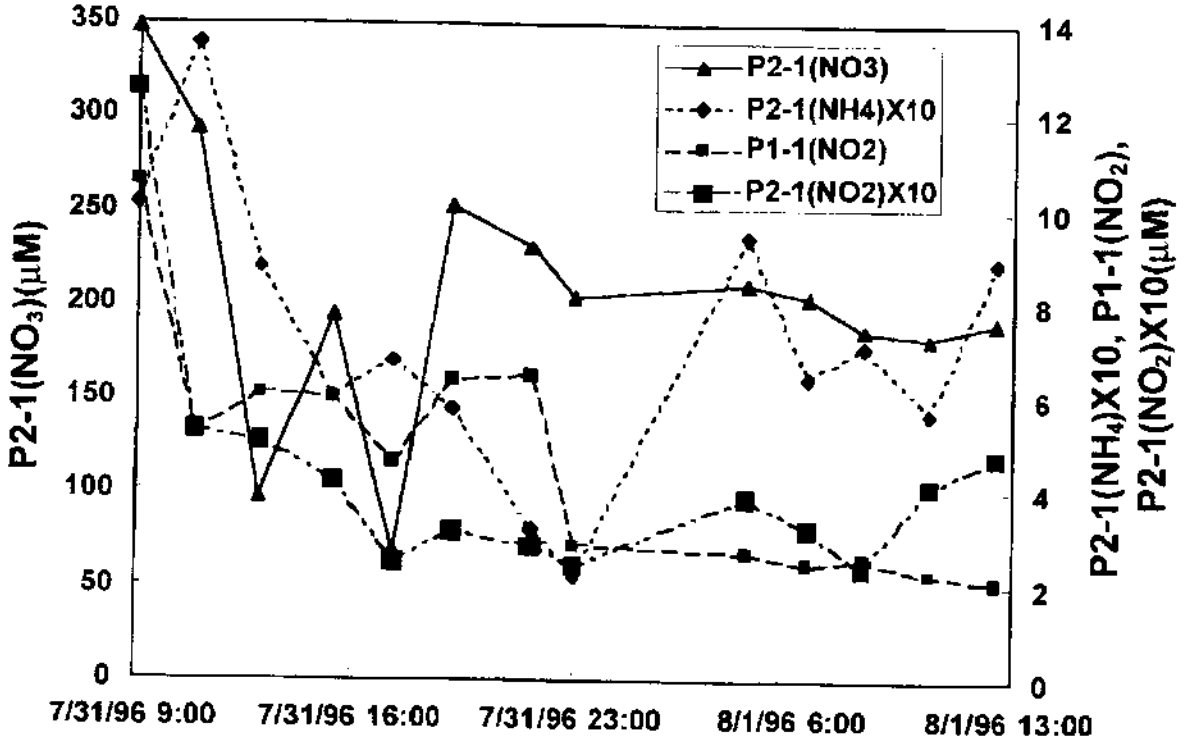


Figure 8. Time variation of nitrate, nitrite and ammonium.

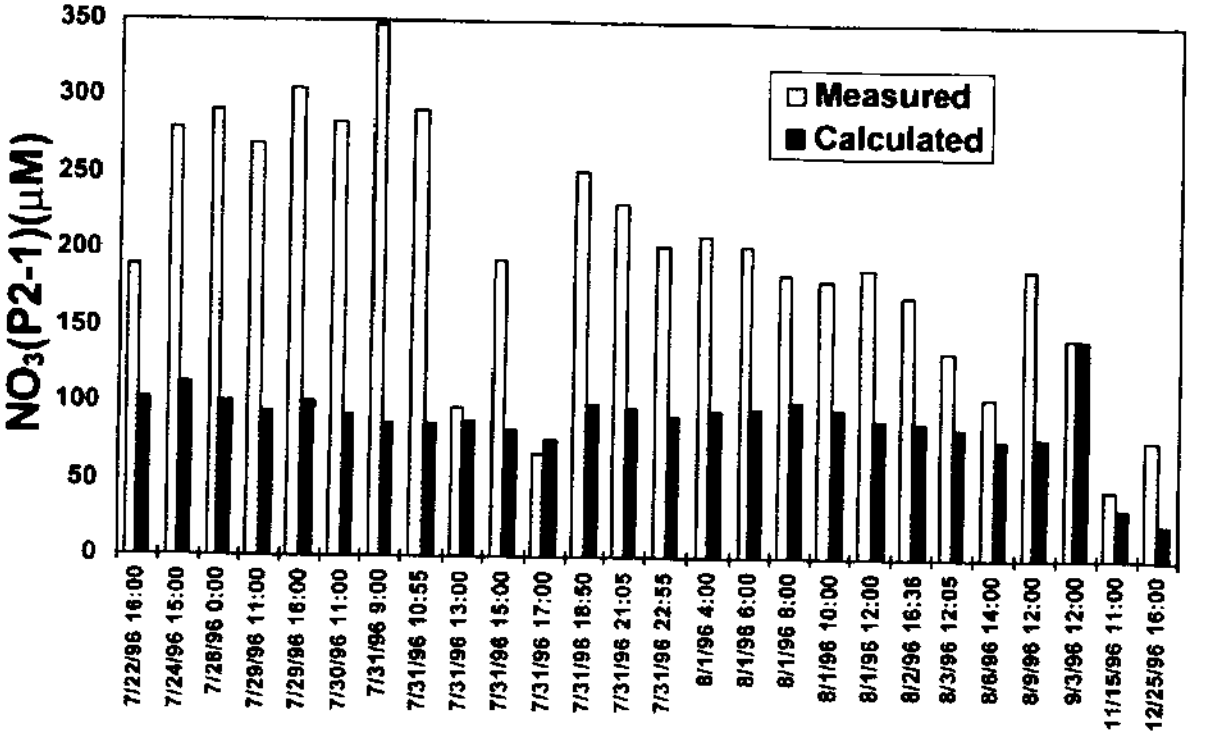


Figure 9. The variation of nitrate concentration.

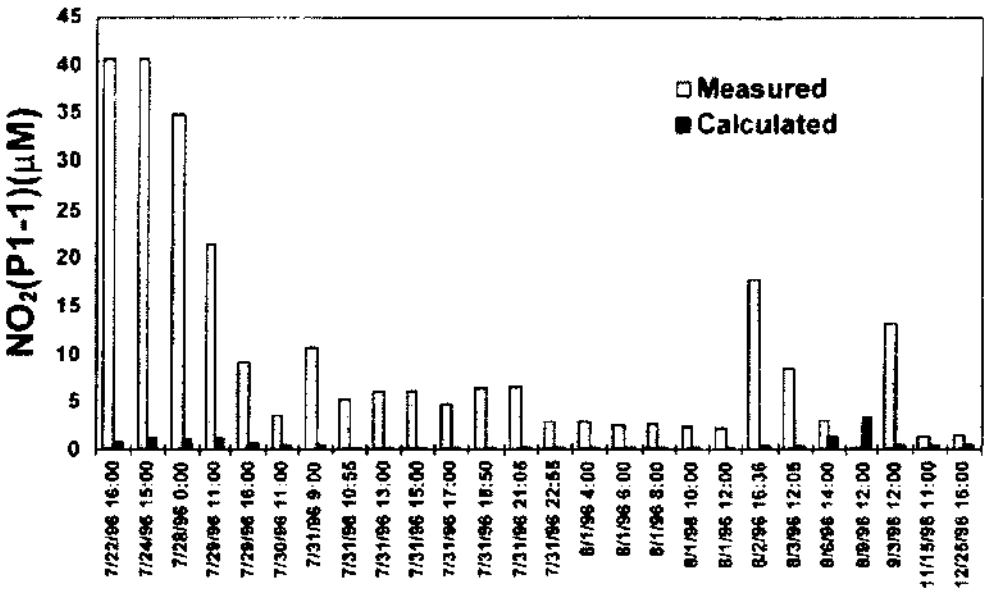


**Figure 10.** The variation of nitrite concentrate.

| Nutrient | Quantity (mol.) |
|---|---|
| Ammonium ($NH_4$) | 4.3 |
| Nitrite ($NO_2$) | 10 |
| Nitrate ($NO_3$) | 26 |
| Phosphate ($PO_4$) | 1 |
| Silicate ($SiO_2$) | 260 |

**Table 2.** Estimation of quantity of nutrients (for 1 km coastaline for a tidal cycle).

The concentrations of freshwater nutrients which were diluted by seawater were calculated by estimating the rate of mixing of freshwater and seawater. By these calculated values, we can estimate the quantity of nutrients which were biologically or chemically released or uptaken. The mixing rate was calculated from salinity concentration which was considered to be not reactive biologically or chemically. The measured concentration of nitrate was higher than the calculated concentration at P2 and the measured concentration of nitrite was higher than the calculated one at P1 (Figs. 9, 10). This indicates that nitrification occurred in the sandy beach. In the case of phosphate and silicate, the measured concentration corresponds to the calculated one, but there were a few cases which did not correspond (Figs. 11, 12). Noncorrespondence was considered to be the release following the decomposition of organic compounds by microorganisms or adsorption to sand particles or suspended solids (Sewell 1982, Johannes and Hearn 1985).

We estimated the quantity of nutrients which flow into the coastal sea. We calculated the discharge of groundwater into the coastal sea by the Sakamoto method (Sakamoto 1991). Sakamoto proposed that the discharge of

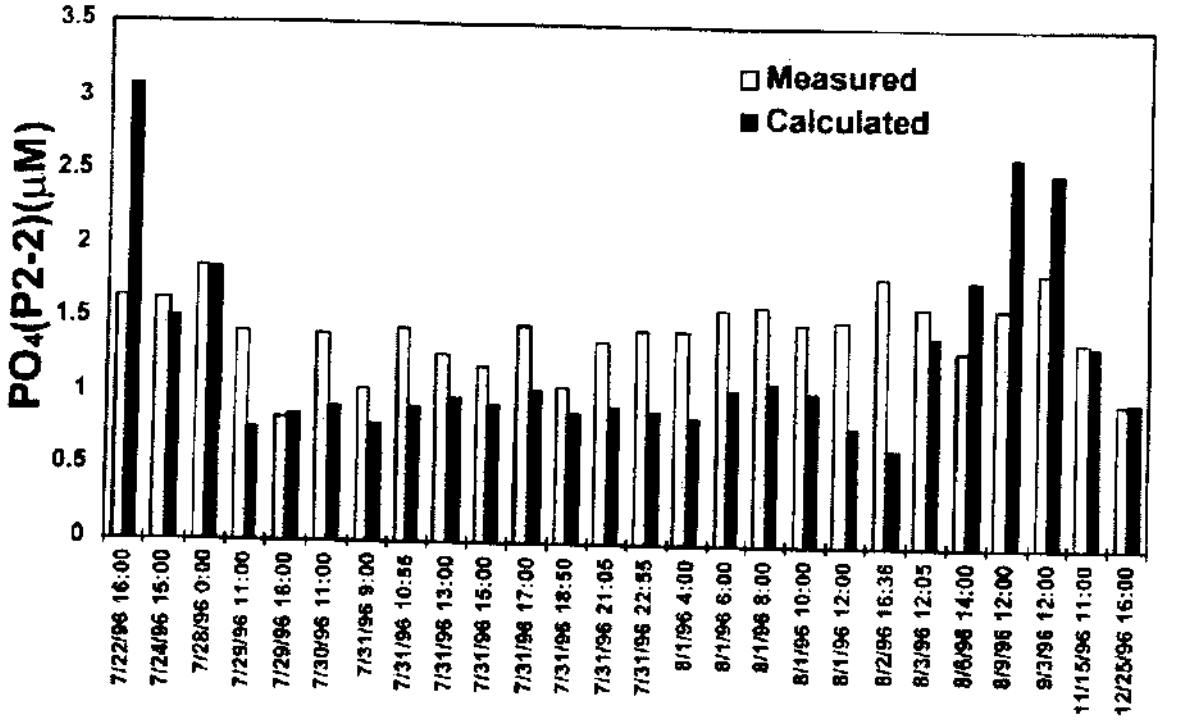


Figure 11. The variation of phosphate concentration.

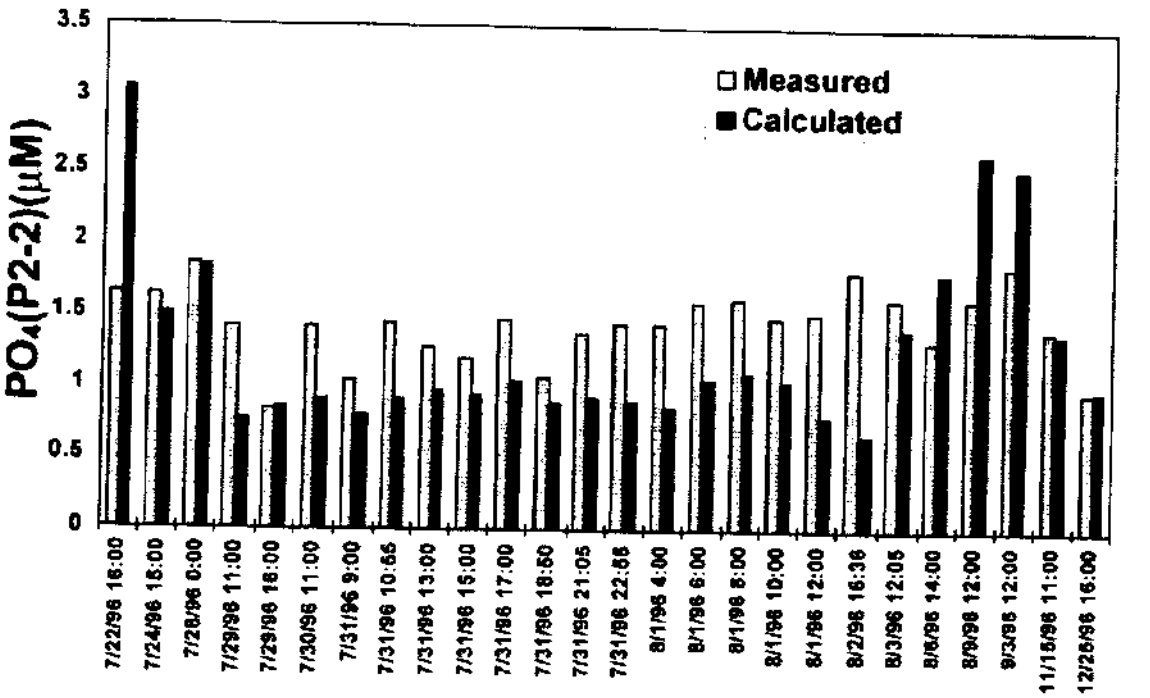


Figure 12. The variation of silicate concentration.

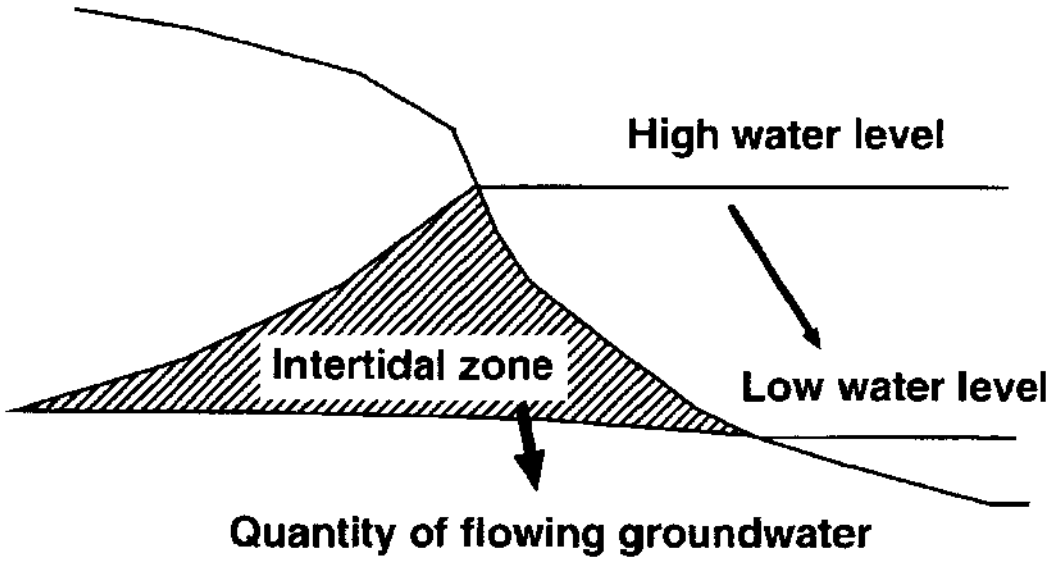


**Figure 13.** Moving volume of groundwater.

groundwater into the coastal sea was equivalent to the shift porosity of the intertidal zone (Fig. 13). We estimated the shift porosity from the median diameter of sand. Katoh et al. (1990) reported that the median diameter of sand around HORS was 0.18 mm. We used the data obtained from 31 July to 1 August 1996. The shift porosity was estimated to be about 0.16. The discharge of groundwater into the coastal sea in summer was estimated to be approximately 4000 $m^3$/km of coastline.

We used the value at P1 as the concentration of nutrients in groundwater which flow into the coastal water. The result appears in Table 2. These values were calculated only considering the moving volume of water caused by the change of groundwater level following tidal change, and not the influence of waves and inflow of freshwater from the backbeach. A more accurate value of the quantity of nutrients which flow into the coastal sea will be needed. That will be obtained by measuring the velocity of the moving groundwater. Furthermore, we need to understand the biological change of nutrients in sandy soil in detail.

## ACKNOWLEDGMENTS

We greatly appreciate the assistance of the staffs of HORS in providing us with the basic characteristics data of the coastal environment and in supporting field sampling.

## LITERATURE CITED

Adachi, K., J. Higano, and K. Kimoto. 1994. Primary productivity of an exposed sandy beach at Kashima-Nada. 1. Changes in phytoplankton biomass in 1992. Tech. Rep. Natl. Res. Inst. Fish. Eng. Aquacult. Fish. Port 16: 13-24. [In Japanese with English summary].

Brown, A. C. and A. McLachlan. 1990. Ecology of Sandy Shores. Elsevier Sci. Publ., Amsterdam. 328 p.

Johannes, R. E. and C. J. Hearn. 1985. The effect of submarine groundwater discharge on nutrient and salinity regimes in a coastal lagoon off Perth, Western Australia. Estuarine Coastal Shelf Sci. 21: 789-800.

Katoh, K., S. Yanagishima, Y. Kuriyama, T. Isogami, H. Murakami, and M. Fujita. 1990.

Changes of grain distribution of bed material in the surf zone - field observation at Hasaki Oceanographical Research Facility. Rep. Port Harbor Res. Inst. 29(2): 38-61. [In Japanese with English summary].

Kurihara, Y., Editor. 1988. Ecology and Ecotechnology in Estuarine-Coastal Area. Univ. Tokai Press. 335 p. [In Japanese].

Morimoto, K. 1993. Nutrients budget and water circulation at intertidal zone. Bull. Coastal Oceanogr. 30(2): 208-223. [In Japanese with English summary].

Sakamoto, I. 1991. Use of respiration in the sandy beach or on the tidal flat: 1. Permeable sandy beach. Mar. Pollut. Bull. 23: 123-130.

Sewell, P. L. 1982. Urban groundwater as a possible nutrient source for an estuarine benthic algal bloom. Estuarine Coastal Shelf Sci. 15: 569-576.

# CYSTEINE METABOLISM IN RAINBOW TROUT

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## ABSTRACT

A series of experiments were carried out to characterize sulfur amino acid metabolism in fish, in particular taurine formation from sulfur amino acids. Rainbow trout was used as the experimental fish. Results of both feeding and a tracer experiment indicated that cysteine is the main starting substance for taurine biosynthesis, and this is metabolized through cysteinesulfinate as an intermediate to hypotaurine, taurine, and sulfate in rainbow trout. Cysteine dioxygenase (CDO) which catalyzes the oxygenation of cysteine to form cysteinesulfinate was considered to play a regulative role of the cysteine metabolism in rainbow trout. On the other hand, a large dose of cysteine showed a serious toxicity to rainbow trout. Considering the rapid rise of the hepatic CDO activity after the cysteine intake, this enzyme presumably plays a role in detoxication by converting cysteine into non-toxic cysteinesulfinate.

## INTRODUCTION

Because of the limited global supply of fishmeal and its high price, many fish nutritionists have searched for suitable alternative sources of protein for fish feed (Watanabe 1994). Soybean meal may be utilized first, which affords a relatively large supply of cheaper protein. However, soybean protein is low in sulfur amino acids, especially methionine, which is known as one of the essential amino acids for fish (Nose and Murai 1990). Many studies, therefore, have been performed on sulfur amino acid requirements for several species of cultured fish (Ketola 1982, Moon and Gatlin 1991). In previous investigations on sulfur amino acid requirements for fish, little attention has been paid to sulfur amino acid metabolism including its regulatory mechanism.

There are numerous reports of analysis of free amino acids and amino acid-related substances in fish and shellfish, some of which are known as amino acid metabolites in mammals (Sakaguchi 1994). It has been frequently mentioned that some of these amino acids and amino acid-related substances vary greatly in content with species, organ, age of fish, seasons, and physiological condition of fish. In particular, taurine is present at a considerably high concentration in fish. Taurine was first discovered in bovine bile, and it is a generally accepted idea that taurine is one of the final metabolites of sulfur amino acids in mammals (Griffith 1987). This sulfur-containing substance is considered to have such physiological functions in mammals as membrane protection, detoxification, and antioxidation (Wright et al. 1986). Taurine also has attracted many fisheries biochemists with regard to its physiological function, particularly its participation in the osmotic regulation and its origin, because of its high concentration in fish tissues (Sakaguchi and Murata 1988). However, there has been little study on the metabolism of sulfur amino acid in fish, especially on that of cysteine; only a few studies suggested that taurine in fish body originated from dietary sulfur amino acids (Walton et al. 1982, Cowey et al. 1992). The metabolic pathway of sulfur amino acids in fish is still far from being understood. Investigation on sulfur amino acid metabolism in fish, of course, must be important in the improvement of the fish feed.

In this paper, the author describes the results of some experiments that were carried out to elucidate sulfur amino acid metabolism, especially cysteine metabolism, in fish from the point of view of sulfur amino acid nutrition and that of taurine formation as well. Rainbow trout was used

**Table 1** Composition of experimental diets (%) (Yokoyama and Nakazoe 1992).

| | Diet No. | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Ingredient | | | | | |
| Casein | 50 | 50 | 50 | 50 | 50 |
| L-Methionine | - | 1 | - | - | - |
| L-Cystine | - | - | 1 | 3 | - |
| Taurine | - | - | - | - | 1 |
| α-starch | 15 | 15 | 15 | 15 | 15 |
| Dextrin | 13 | 12 | 12 | 10 | 1 |
| Lipid* | 10 | 10 | 10 | 10 | 10 |
| Cellulose | 5 | 5 | 5 | 5 | 5 |
| Mineral mixture | 5 | 5 | 5 | 5 | 5 |
| Vitamin mixture | 2 | 2 | 2 | 2 | 2 |
| Sulfur amino acid content(%)† | | | | | |
| Methionine | 1.2 | 2.2 | 1.2 | 1.2 | 1.2 |
| Cystine | 0.2 | 0.2 | 1.2 | 3.2 | 0.2 |
| Taurine | - | - | - | - | 1.0 |

*Soybean oil: Pollock liver oil = 3 : 2
† The values were calculated from amino acid composition of casein and supplemented amino acids.

as the experimental fish. All of the rearing experiments were conducted at 15°C.

## METABOLIC FATE OF CYSTEINE

To confirm the metabolic fate of cysteine in rainbow trout, both tracer and feeding experiments focusing on the metabolic pathway from cysteine to taurine in rainbow trout were conducted.

### Accumulation and excretion of taurine in fish fed diets supplemented with methionine, cystine, and taurine

The effects of the amount of sulfur amino acids administered on the tissue levels of the corresponding amino acids and their metabolites, and on the amount of taurine excreted were examined as a preliminary study to look into the pathway from respective sulfur amino acids to taurine. Rainbow trout weighing about 10 g were fed with casein-based diets supplemented with methionine, cystine, and taurine for 15 days. The composition of experimental diets is given in Table 1.

In fish fed the methionine-supplemented diet (diet 2), methionine and cystathionine contents in the liver were observed to be about 25 and 2 times higher, respectively, than those in the liver of the fish in the other four dietary groups (Table 2).

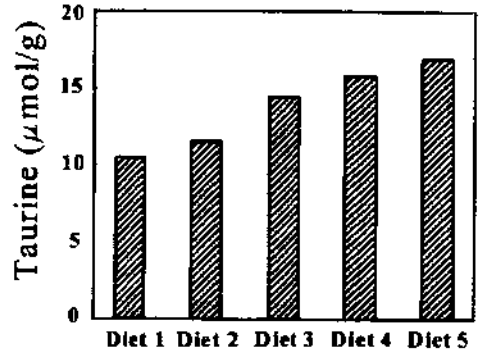


**Figure 1.** Taurine content of whole body of rainbow trout fed five different diets (Yokoyama and Nakazoe 1992). See Table 1 for the composition of five diets given in the figure.

However, the levels of cysteine and cystine showed only a slight change in all dietary groups. The taurine content in the liver increased to some extent in all dietary groups. In the whole fish body, there was little difference in taurine content between the control group (diet 1) and the 1% methionine group (diet 2) (Fig. 1). On the other hand, the taurine level in the fish fed either the 1% cystine diet (diet 3) or the 3% cystine diet (diet 4) was significantly higher than that of the control fish. Consequently, the fish fed cystine- or taurine-supplemented diets accumulated taurine at a high level in their bodies. Administration of sulfur amino acids and taurine

Table 2 The free amino acid and glutathione levels in the liver (μmol/g tissue) of rainbow trout fed diets differing in sulfur amino acid contents (Yokoyama and Nakazoe 1992).

| Amino acid | Diet No. 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Methionine | 0.05±0.01 | 1.21±0.86 | 0.05±0.01 | 0.05±0.01 | 0.05±0.01 |
| Cystathionine | 0.15±0.07 | 0.43±0.06 | 0.21±0.11 | 0.17±0.05 | 0.17±0.05 |
| Cysteine | 0.21±0.03 | 0.22±0.03 | 0.26±0.03 | 0.40±0.02 | 0.26±0.04 |
| Cystine | tr. | tr. | tr. | 0.01±0.00 | tr. |
| Taurine | 23.40±2.52 | 26.84±4.87 | 29.31±8.59 | 25.40±2.34 | 31.45±3.04 |
| Glutathione | 3.09±0.36 | 3.01±0.34 | 2.96±0.48 | 2.81±0.40 | 2.62±0.51 |
| Aspartic acid | 1.10±0.30 | 0.59±0.04 | 0.80±0.55 | 0.65±0.18 | 0.86±0.24 |
| Threonine | 0.48±0.12 | 0.33±0.10 | 0.45±0.10 | 0.55±0.48 | 0.53±0.07 |
| Serine | 0.38±0.11 | 0.07±0.05 | 0.32±0.07 | 0.39±0.13 | 0.38±0.10 |
| Glutamic acid | 3.78±1.14 | 2.73±0.42 | 3.90±2.52 | 3.77±1.89 | 3.07±0.42 |
| Glutamine | 0.63±0.54 | 0.43±0.32 | 0.56±0.54 | 0.71±0.53 | 0.58±0.17 |
| Proline | 2.51±1.00 | 1.62±0.67 | 3.21±1.57 | 1.94±0.55 | 2.49±0.85 |
| Glycine | 1.60±0.15 | 1.09±0.16 | 1.63±0.34 | 1.70±0.17 | 1.70±0.30 |
| Alanine | 5.72±0.83 | 4.78±0.65 | 5.86±1.15 | 5.53±1.01 | 5.55±0.87 |
| Valine | 1.17±0.23 | 1.03±0.16 | 1.31±0.38 | 1.34±0.36 | 1.18±0.15 |
| Isoleucine | 0.50±0.13 | 0.43±0.06 | 0.57±0.16 | 0.53±0.09 | 0.52±0.05 |
| Leucine | 0.96±0.25 | 0.78±0.11 | 1.07±0.30 | 0.99±0.12 | 0.97±0.10 |
| Tyrosine | 0.18±0.05 | 0.13±0.01 | 0.16±0.02 | 0.14±0.04 | 0.12±0.02 |
| Phenylalanine | 0.16±0.02 | 0.40±0.04 | 0.16±0.01 | 0.16±0.03 | 0.14±0.01 |
| Tryptophan | 0.05±0.00 | 0.05±0.01 | 0.05±0.01 | 0.04±0.01 | 0.04±0.02 |
| Lysine | 0.26±0.07 | 0.18±0.04 | 0.36±0.25 | 0.53±0.47 | 0.19±0.04 |
| Histidine | 1.37±0.14 | 1.37±0.08 | 1.46±0.12 | 1.25±0.20 | 1.34±0.09 |
| Arginine | 0.02±0.02 | 0.01±0.00 | 0.02±0.02 | 0.06±0.10 | 0.01±0.00 |
| Ammonia | 3.15±0.39 | 2.74±0.17 | 2.81±0.40 | 3.07±0.62 | 3.01±0.43 |

The values are means of five individual measurements ± standard deviation.
tr: trace amount.

showed no apparent effects on the levels of the non-sulfur amino acids in the liver and muscle except that of serine.

According to Finkelstein and Martin (1986), the methionine level in the rat liver was as low as 0.08 μmol/g, even when the rat was fed a diet containing 3% methionine for 7 days. In the present study, however, the methionine content in the liver of fish fed the 1% methionine diet was estimated to be as high as 1.2 μmol/g. Similar high methionine levels in the serum (Nose 1974) and the liver (Walton et al. 1982) of rainbow trout were reported for the fish fed a diet supplemented with excess methionine. The relatively high content of cystathionine in the liver observed in this study suggests that this amino acid was synthesized from the excess methionine *via* homocysteine. Since serine is one of the raw materials for cystathionine biosynthesis (Finkelstein and Martin 1986), the decrease in free serine content of the liver might be attributable to the consumption of free serine for the cystathionine biosynthesis, although no analysis was made in this study for homocysteine, which is another substitute for cystathionine formation. These facts also imply that cystathionine biosynthesis proceeds relatively rapidly, while cysteine biosynthesis from cystathionine proceeds very slowly. Therefore, both cystathionine and its precursor methionine might be accumulated in the tissues of rainbow trout when the fish is fed excess dietary methionine. In any event, it is evident that the excess methionine ingested was not used efficiently for cysteine biosynthesis in the liver, and did not lead to apparent accumulation of cysteine and taurine.

Hosokawa et al. (1988) observed for rats that taurine excretion into urine increased remarkably when a high level protein diet was administered. As can be seen in Table 3, the amount of ordinary amino acids excreted from the rainbow trout for 24 h after final feeding did not differ greatly between the control fish (diet 1) and

Table 3 Amino acid excretion of the rainbow trout fed diets supplemented with sulfur amino acids (μmol/g body weight/day) (Yokoyama and Nakazoe 1992).

| Amino acid | Diet No. 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Taurine | 0.23 | 0.35 | 1.31 | 1.31 | 2.37 |
| Aspartic acid | 0.08 | 0.09 | 0.10 | 0.07 | 0.11 |
| Threonine | 0.22 | 0.20 | 0.21 | 0.15 | 0.35 |
| Serine | 0.42 | 0.42 | 0.33 | 0.20 | 0.54 |
| Glutamic acid | 0.38 | 0.40 | 0.43 | 0.41 | 0.51 |
| Glycine | 0.25 | 0.20 | 0.43 | 0.27 | 0.42 |
| Alanine | 0.45 | 0.47 | 0.53 | 0.38 | 0.63 |
| Valine | 0.49 | 0.46 | 0.53 | 0.38 | 0.63 |
| Cystine | ND | ND | 0.04 | 0.18 | 0.01 |
| Methionine | 0.25 | 0.76 | 0.18 | 0.12 | 0.31 |
| Isoleucine | 0.38 | 0.39 | 0.26 | 0.25 | 0.53 |
| Leucine | 1.12 | 1.33 | 0.74 | 0.54 | 1.15 |
| Tyrosine | 0.69 | 0.85 | 0.32 | 0.20 | 0.56 |
| Phenylalanine | 0.52 | 0.71 | 0.6 | 0.15 | 0.44 |
| Lysine | 0.95 | 0.97 | 0.48 | 0.35 | 0.93 |
| Histidine | 0.17 | 0.16 | 0.10 | 0.08 | 0.23 |
| Arginine | 0.56 | 0.63 | 0.32 | 0.22 | 0.50 |
| Ammonia | 26.50 | 27.10 | 22.70 | 23.80 | 24.00 |

ND: not detected.

the fish administered methionine (diet 2), cystine (diet 3 and 4) or taurine (diet 5), with the exception of slightly increased methionine excretion from the methionine-administered fish. Fish fed the 1% methionine diet excreted almost the same levels of taurine as fish fed the control diet, whereas, fish fed the cystine- and taurine-supplemented diets excreted a large amount of taurine. Taurine excretion of the fish fed cystine-supplemented diets was four times higher than that of the fish fed methionine-supplemented diets. Clearly, the higher the taurine content of the whole body, the larger the excretion. This suggests that the taurine excretion becomes active when the net accumulation of taurine in the fish body exceeds a certain level.

Although Tateishi et al. (1977) reported for the rat that hepatic glutathione plays a role as a cysteine reservoir, there was no obvious change in the glutathione content of either liver or muscle in any experimental group. This agrees well with the results reported by Walton et al. (1982). At least for rainbow trout, glutathione does not seem to serve as a cysteine reservoir when fish are fed excess amounts of sulfur amino acids such as methionine or cystine.

**Metabolites derived from L-[$^{35}$S]cysteine injected into the peritoneal cavity**

When cystine was given to the fish, the content of taurine markedly increased; when methionine was given, no such increment was observed. This means that cysteine is the main starting material for the taurine biosynthesis in rainbow trout. Therefore, the fate of cysteine was traced by injecting radiochemically-labeled cysteine into the peritoneal cavity of rainbow trout. Metabolites derived from the radioactive cysteine were examined from whole fish body and the excreta. Each of the fish weighing about 10 g was kept in an Erlenmeyer flask containing 1 L of water for an of appropriate amount of L-[$^{35}$S]cysteine. The whole body of the frozen fish sample was cut into small pieces and homogenized with 4 volumes of distilled water. The homogenate was separated into a protein fraction and a soluble fraction. The soluble fraction was further fractionated into several metabolite fractions by the method of Yamaguchi and Ueda (1976) with minor modification. The water in which rainbow trout was reared was fractionated in a similar manner as above.

*In vivo* composition of radioactive substances derived from L-[$^{35}$S]cysteine injection

**Table 4** Distribution of $^{35}S$ substances in the whole body of rainbow trout which were injected with two different dose levels of L-[$^{35}S$]cysteine (Yokoyama et al. 1997)

| Dose of L-[$^{35}S$]cysteine ($\mu$mol/g body weight) | $^{35}S$ Substances ($\mu$mol/g body weight) | | | | | |
|---|---|---|---|---|---|---|
| | Total | Taurine | Hypotaurine | Sulfate | Protein | Others |
| 1.50 | 0.450 | 0.108 | 0.041 | 0.068 | 0.157 | 0.076 |
| 0.15 | 0.067 | 0.011 | 0.004 | 0.008 | 0.035 | 0.009 |

**Table 5** Excretion of $^{35}S$ substances into the water where the rainbow trout was kept for 24 h after the L-[$^{35}S$]cysteine injection (Yokoyama et al. 1997).

| Dose of L-[$^{35}S$]cysteine ($\mu$mol/g body weight) | $^{35}S$ Substances ($\mu$mol/g body weight/day) | | | | |
|---|---|---|---|---|---|
| | Total | Taurine | Hypotaurine | Sulfate | Others |
| 1.50 | 0.996 | 0.107 | 0.251 | 0.212 | 0.426 |
| 0.15 | 0.079 | 0.014 | 0.020 | 0.022 | 0.023 |

and that in the rearing water were tabulated in Tables 4 and 5, respectively. When the fish was injected with 1.5 µmol L-[$^{35}S$]cysteine/g fish body weight, one-third of the total radioactivity was retained, whereas one-half remained in the fish injected with 0.15 µmol/g. In both cases, the remainder of radioactivity was recovered from the rearing water. From the data in Table 4, taurine, hypotaurine, and sulfate can be said to be the major metabolites present in the soluble fraction of fish body regardless of the dose size, though a relatively large amount of radioactivity had been incorporated into the protein fraction. Even though it is not clear that the radioactive cysteine is incorporated into the protein molecule either as a disulfide linkage or as a constituent part, this fact indicates that the incorporation of cysteine into the protein molecule occurred very rapidly.

A considerably large amount of the metabolites such as taurine, hypotaurine, and sulfate was found to be excreted into the rearing water within the 24-h experimental rearing. However, in the case of 1.5 µmol/g body weight injection, the amount of radioactive substances corresponding to about one-third of the total activity could not be identified (Table 5). A part of the injected L-[$^{35}S$]cysteine is likely excreted directly into the water, because the proportion of the unidentified activity was much greater in the large dose than in the small dose. From the results given in Tables 4 and 5, the total amounts of taurine, hypotaurine, and sulfate formed from injected L-cysteine were calculated as 14, 19, and 19% of a dose of 1.5 µmol/g body, respectively. Regardless of the dose size, more than 50% of L-cysteine was metabolized within 24 h after injection. Evidently, a large portion of the cysteine administered was metabolized rapidly to taurine *via* hypotaurine, and the pathway

is accompanied by sulfate formation, although the details of pathway had not necessarily been clarified by this experiment.

Oxidation of cysteine to cysteinesulfinate is believed to be the major step of cysteine catabolism in mammals, particularly when cysteine availability is high (Wheldrake and Pasternak 1967, Yamaguchi et al. 1973, Stipanuk 1979). β-sulfinylpyruvate, a product of cysteinesulfinate transamination reaction, decomposes spontaneously into pyruvate and sulfite; sulfite is further oxidized by sulfite oxidase to sulfate (Griffith 1987). The formation of taurine, hypotaurine, and sulfate from L-cysteine suggests that rainbow trout has the L-cysteine-metabolic pathway similar to that of mammals where cysteinesulfinate plays a key role as the intermediate.

The mechanism of conversion of hypotaurine to taurine has not been elucidated yet. Both enzymatic (Oja and Kontro 1981, Kontro and Oja 1985) and non-enzymatic (Fellman and Roth 1985) reactions, however, have been considered to be involved in the taurine formation. On the other hand, as for rainbow trout, much of the hypotaurine was observed to be excreted into the rearing water, i.e., a considerable amount of hypotaurine is excreted without being converted into taurine.

**Changes in tissue level of the major cysteine metabolites by the continuation of oral administration of excess cystine**

An attempt was made to ascertain that taurine is originated from dietary cysteine by examining the effect of the oral administration of cystine on the tissue contents of the major metabolites. A feeding experiment was conducted using the 50% casein diet supplemented with 1% cystine. Contents of hypotaurine, taurine, and cysteinesulfinate in the tissues of rainbow trout were periodically measured throughout the 8-day feeding. The change in hypotaurine content in four different tissues is given in Figure 2. The hepatic hypotaurine level at the start of the experiment was about 1 μmol/g tissue. Excess dietary administration of cystine brought about the hypotaurine accumulation both in the liver and the kidney within the first 2 days. The accumulation in these tissues reached a maximum level (about 4 μmol/g) after 2 to 4 days. The hypotaurine content in the muscle tissue was almost zero at the very beginning, increasing gradually during feeding. The maximum level in the muscle, however, was only one-eighth of that in the liver.

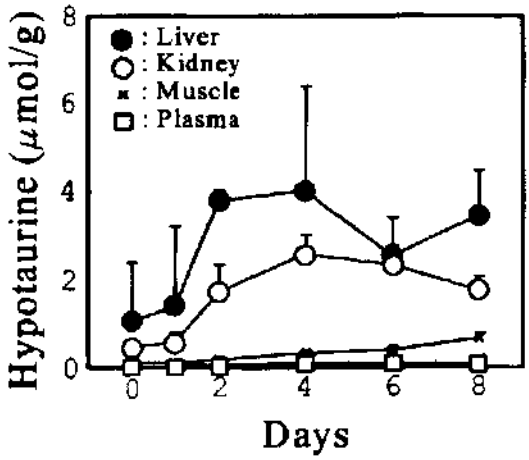


**Figure 2.** Changes in hypotaurine contents in several tissues of rainbow trout during feeding on cystine-supplemented diet (Yokoyama and Nakazoe 1998). Results are expressed as the mean ± standard deviation of five individuals.

Changes in taurine and cysteinesulfinate contents in several tissues are shown in Figures 3 and 4, respectively. A large amount of taurine existed in the kidney and the liver at a level of about 25 μmol/g tissue; the content was very low in the muscle and plasma. The taurine content appeared to be constant throughout the feeding period. The levels of cysteinesulfinate tended to increase in the liver and kidney by the cystine administration. However, these values were extremely low compared with those of taurine and hypotaurine; even at the maximum on the 4th day, cysteinesulfinate was only 0.015 μmol/g. In the muscle tissue and plasma, there was no change in the cysteinesulfinate content.

The cysteinesulfinate content remained low throughout the experimental period, although it was considered to be affected by the excess cystine administration. This fact suggests that in rainbow trout the cysteine metabolism is controlled mainly at the cysteine oxidation step rather than at the step of cysteinesulfinate breakdown. Therefore, the cysteine oxidation enzyme must

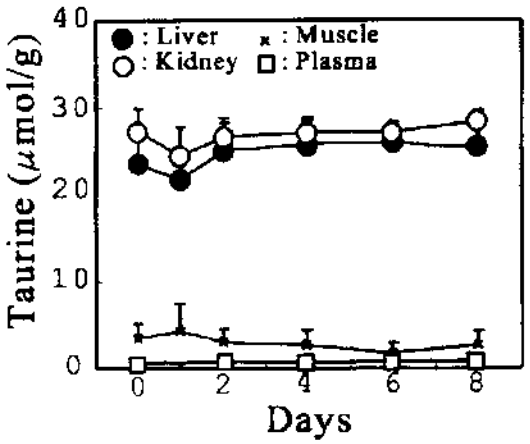


**Figure 3.** Changes in taurine contents in several tissues of rainbow trout during feeding on cystine-supplemented diet (Yokoyama and Nakazoe 1998). Results are expressed as the mean ± standard deviation of five individuals.

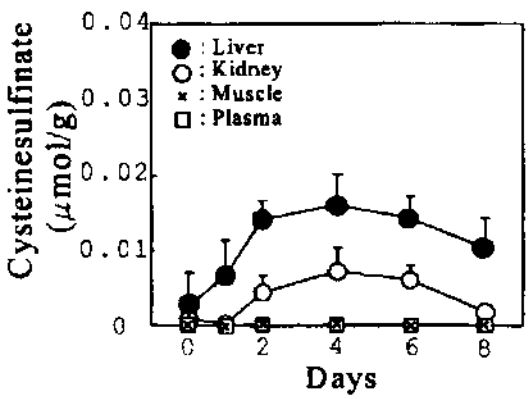


**Figure 4.** Changes in cysteinesulfinate contents in several tissues of rainbow trout during feeding on cystine-supplemented diet (Yokoyama and Nakazoe 1998). Results are expressed as the mean ± standard deviation of five individuals.

have fundamental significance in cysteine metabolism in the fish.

Hypotaurine is regarded as an antioxidant by scavenging highly reactive hydroxyl radicals, and to play an important role in preventing the attack by oxidants *in vivo* (Aruoma et al. 1988). Since taurine is the oxidative product of hypotaurine, the oxidation of hypotaurine to taurine in fish should prove to be interesting regarding physiological protection against the attack of radicals. The physiological role of hypotaurine should be investigated in the future in conjunction with the biological function of taurine.

## CYSTEINE DIOXYGENASE (CDO) ACTIVITY AS A DOMINANT FACTOR IN CYSTEINE METABOLISM

Cysteinesulfinate is a key intermediate both in the catabolic pathway to pyruvate and sulfate, and in the metabolic pathway to taurine in mammals (Griffith 1987). CDO [EC 1.13.11.20] catalyzing the oxygenation of L-cysteine to L-cysteinesulfinate plays an important role in mammalian cysteine metabolism (Yamaguchi and Hosokawa 1987, Kohashi et al. 1978).

It has become apparent that cysteine was metabolized into cysteinesulfinate as the very first step in rainbow trout, as in the case of mammals. Most probably, this enzyme participates in cysteine metabolism in rainbow trout as well. Since no information about CDO in fish has yet been available, response of CDO activity in the liver to the level of sulfur amino acid administrated was investigated to confirm the participation of the enzyme in this oxidation reaction.

### Enhancement of CDO activity by dietary supplementation of sulfur amino acids

Since CDO was considered to function in the initial step of cysteine metabolism, the tissue distribution of CDO activity and the effects of a large excess of sulfur amino acids in the diet on CDO activity in the tissue were examined. The fish were fed 1% sulfur amino acid-supplemented 40% casein diets for 10 days.

The activities in the liver of both the fish fed either methionine- or cysteine-supplemented diets were significantly higher than those of the

control group. This enhancement in the liver suggests that a high sulfur amino acid intake induced the hepatic CDO. On the other hand, no such remarkable rise in CDO activity was observed in other tissues (Fig. 5). This finding implies that a cysteine catabolic pathway to taurine *via* cysteinesulfinate exists in the liver, and the system works in concert with dietary sulfur amino acid levels as ascertained in mammals (Kohashi et al. 1978).

**Enhancement of hepatic CDO activity by intraperitoneal injection of sulfur amino acids**

It was observed that CDO occurs in the liver of rainbow trout, and its activity was enhanced by dietary supplementation of an excess amount of both methionine and cystine. These findings suggest that the sulfur amino acid metabolism is controlled by this enzyme in rainbow trout. In order to ascertain if the CDO activity is controlled by the sulfur amino acid level in the fish, and the enzyme is specific to cysteine, the effect of the dose size of cysteine and some different kinds of sulfur-containing compounds on hepatic CDO activity was examined by intraperitoneal injection.

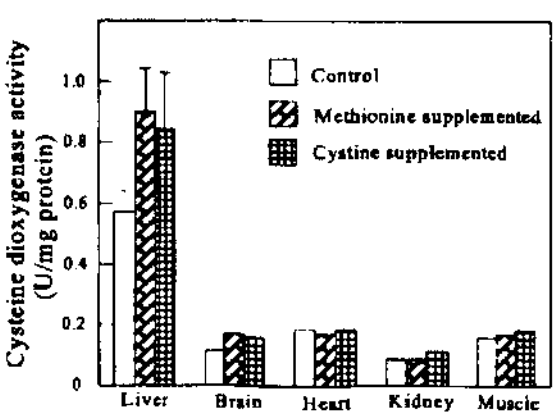


**Figure 5.** Cysteine dioxygenase activity in the tissue of rainbow trout fed experimental diets having different sulfur amino acid contents (Yokoyama and Nakazoe 1989). The activity of liver is expressed as mean ± SD of seven fish. Others are the measurements of the pooled sample of seven fish. One unit (U) of enzyme activity was defined as the amount of enzyme producing one μmole of cysteinesulfinate in 1 h at 37°C.

As a preliminary test, L-cysteine was injected intraperitoneally to rainbow trout in doses of 2.5, 5.0, and 10.0 μmol/g of fish body weight. Eighteen hours after injection, the hepatic CDO activity was measured. The hepatic CDO activity of the fish given by injection in a dose of 2.5 μmol/g of body weight rose as much as two times that of the control fish injected only with saline solution (results not shown). However, the fish given doses above 5.0 μmol/g of body weight died with heavy hemorrhage within 30 min after injection. Considering the toxicity of excess dosage of L-cysteine for rainbow trout, 2.5 μmol/g of body weight was employed as the dose level for the subsequent experiments on the response time. As shown in Figure 6, the activity of hepatic CDO of rainbow trout increased rapidly within the first 4 h after the injection. The activity reached a maximum level at 4 h, and the activity was about 2.5 times that at the beginning. It increased rapidly, passed through the maximum, and fell off gradually within the subsequent 18 h. Next, the experimental condition was re-designed to examine the dose-response. The activity was measured 4 h after injection of different doses. The results are shown in Figure 7. The activity of hepatic CDO increased in proportion to the increasing dose in the very limited dose range, i.e., below 1.5 μmol/g of body

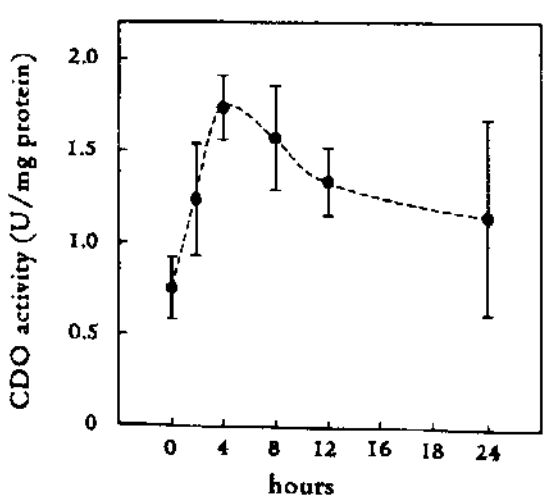


**Figure 6.** Effects of L-cysteine injection on hepatic cysteine dioxygenase activity in rainbow trout (Yokoyama and Nakazoe 1996). Curve was fitted to represent the mean values ± SD for the six sample fish.

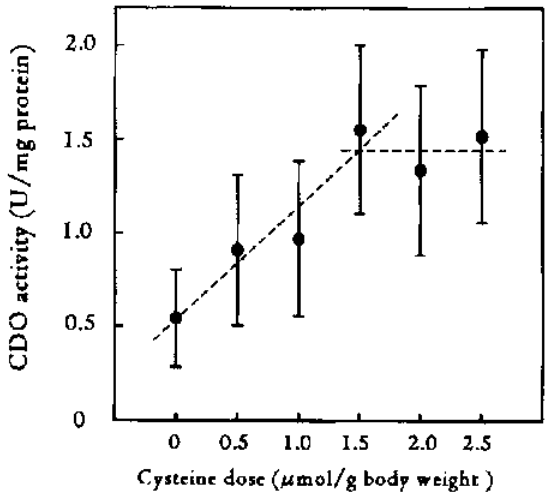


**Figure 7.** Effects of L-cysteine dose level on the hepatic cysteine dioxygenase activity in rainbow trout (Yokoyama and Nakazoe 1996). Values are means of measurements made on six fish per treatment.

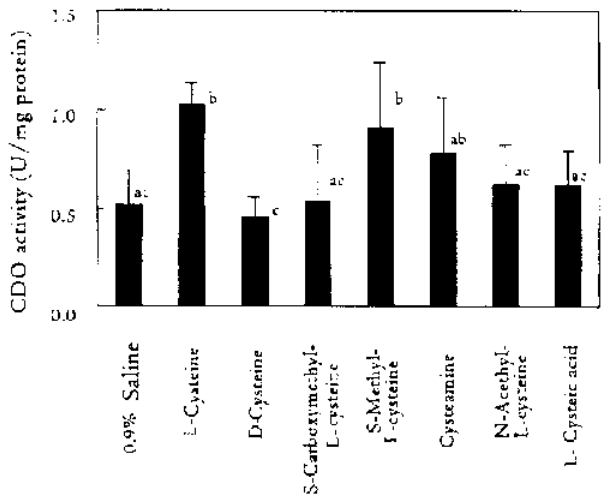


**Figure 8.** Effects of injection of L-cysteine and its derivatives on hepatic cysteine dioxygenase activity in rainbow trout (Yokoyama and Nakazoe 1996). Mean values not sharing a common letter are significantly different ($p<0.05$).

weight, and there was no additional increase in the activity above a dose of 1.5 μmol/g of body weight.

To elucidate how the hepatic CDO is induced and how its activity is controlled by sulfur amino acids *per se*, different forms of sulfur amino acids were injected into the peritoneal cavity of rainbow trout. The specificity for the induction of hepatic CDO was examined. Based on the above mentioned results of the dose-response experiments, the dose and the induction period were fixed as 1.5 μmol/g of body weight and 4 h, respectively. L-cysteine and its analogues which have a similar chemical structure to L-cysteine were selected as the substances. The relationship between inductive activity and molecular structure was determined. Results are shown in Figure 8. Among these substances, L-cysteine and *S*-methyl-L-cysteine showed the strongest induction of the enzyme activity, and other cysteine analogues such as D-cysteine, *S*-carboxymethyl-L-cysteine, cysteamine, *N*-acetyl-L-cysteine, and L-cysteic acid did not induce the activity. Further studies with other intermediates of sulfur amino acid metabolism and related compounds were performed. Results are shown in Figure 9. L-methionine did not affect the activity at all, and neither L-cysteinesulfinic acid produced from L-cysteine by CDO, nor taurine, the final substance of sulfur amino acid metabolic pathway, induced the activity. L-homocysteine which has the structure similar to that of L-cysteine and is an intermediate involved in methionine metabolism to cysteine (transsulfuration pathway) also showed negative effects.

The activity of hepatic CDO increased linearly with the increasing dose of L-cysteine, and the response was rapid and significantly specific to L-cysteine. These facts strongly indicate that the hepatic CDO activity, i.e., cysteine metabolism, might be controlled by the tissue concentration of sulfur amino acid, precisely of L-cysteine, in rainbow trout as in the case of rats (Kohashi et al. 1978). Excess intake of cysteine upon both oral administration and intraperitoneal injection brought about the induction of hepatic CDO activity, and cysteine might be metabolized to cysteinesulfinate as the intermediate product. This also suggests that the cysteine level might be kept at a low level in rainbow trout body. Therefore, there must be a regulative mechanism in the cysteine metabolism of rainbow trout similar to that of mammals.

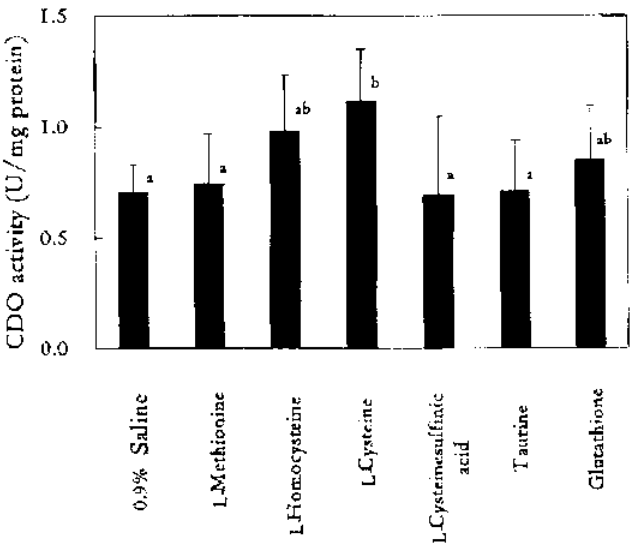


**Figure 9.** Effects of injection of sulfur amino acids involved in sulfur amino acid metabolism on hepatic cysteine dioxygenase activity in rainbow trout (Yokoyama and Nakazoe 1996). Mean values not sharing a common letter are significantly different ($P<0.05$).

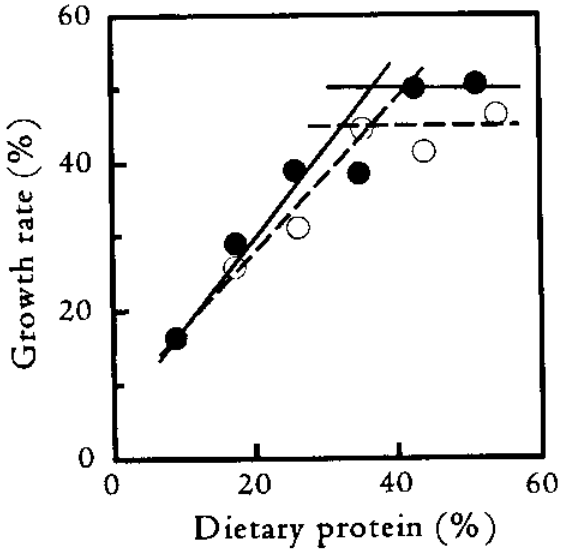


**Figure 10.** Effect of dietary protein levels on percent weight gain of rainbow trout (Yokoyama et al. 1994) ●: egg white albumin diets, 0: casein diets.

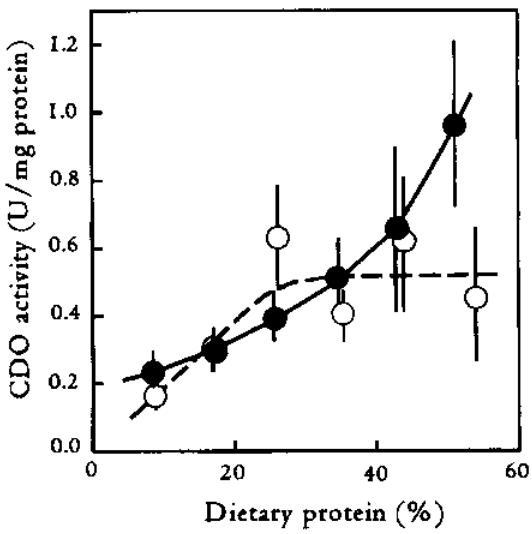


**Figure 11.** Effect of dietary protein levels on hepatic cysteine dioxygenase activity of rainbow trout (Yokoyama et al. 1994). Values are means ± SD of five fish. ●: egg white albumin diets, 0: casein diets.

## Influence of dietary protein levels on hepatic CDO activity

A feeding experiment of rainbow trout was conducted by use of either the diets containing some different levels of egg white albumin, or casein as a sole dietary protein source, because these proteins differ in amino acid composition. The relation between the hepatic CDO activity in rainbow trout and the dietary protein level was determined. Either egg white albumin denatured with hot ethanol under reflux for 6 h, or vitamin-free casein was employed as a sole dietary protein source.

Rainbow trout weighing about 17 g were divided into 12 experimental groups of 18 individuals. Body weight gain of rainbow trout fed the experimental diets for 10 days is shown in Figure 10. The maximum growth rate obtained by feeding casein diets was somewhat lower than the maximum growth rate observed in fish fed the albumin diets.

As shown in Figure 11, the activity of CDO in the liver of rainbow trout fed egg white albumin diets increased exponentially from 0.2 U/mg protein to 0.9 U/mg protein as dietary protein level increased up to 51%. The unit U denotes one unit of enzyme activity defined as the amount of enzyme producing one μmole of cysteinesulfinate in 1 h at 37°C. Also, the CDO activity in the dietary groups of casein increased with the increase of the dietary protein level up to 26%, while further increase in the casein level failed in enhancing the activity.

Hosokawa et al. (1988) observed that the hepatic CDO activity in rats, which were fed a

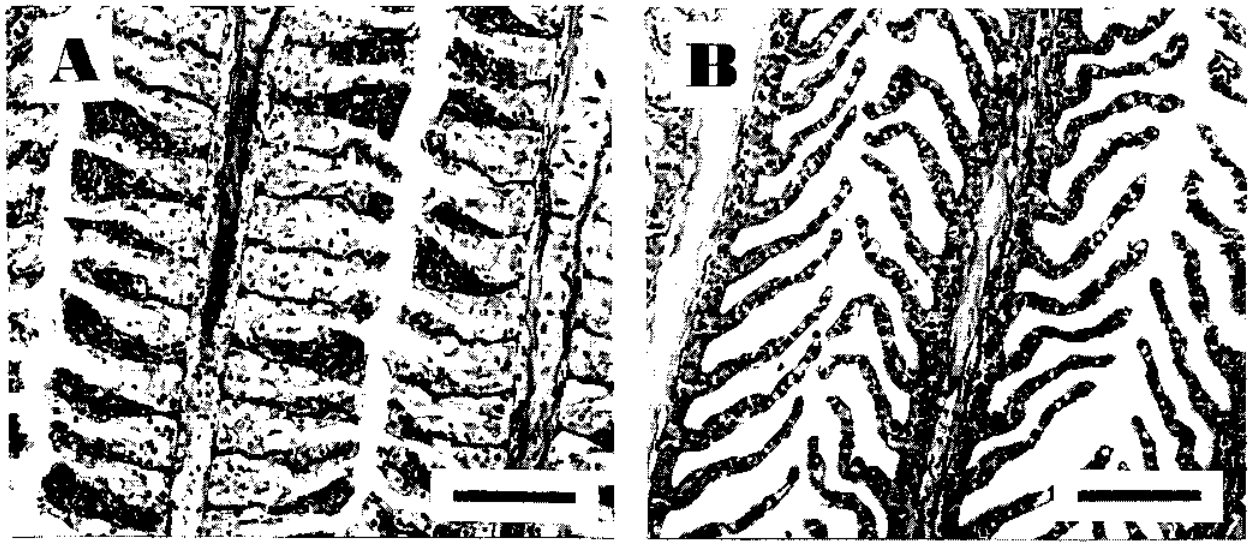


**Figure 12.** Optical microscopic images of the gill tissue of rainbow trout administrated with and without L-cysteine by injection (Yokoyama and Sakaguchi 1996). A, Fish injected with 10.0 $\mu$mol L-cysteine/g body weight; B, Control fish which were injected with only 0.9% saline solution. Bars indicate 100 $\mu$m. (Reproduction permitted from the Japanese Society of Fisheries Sciences).

casein diet or soybean protein diet, was boosted by supplementing methionine which is the first limiting amino acid of those proteins, but the activity was lowered by supplementing lysine, the first limiting amino acid to wheat gluten diet. In this experiment, the activity of CDO in the liver increased along with the increasing level of egg white albumin. However, the activity in the fish fed the casein diets remained low. Total sulfur amino acid content in egg albumin was almost twice as high as that in casein. The marked increase in activity of hepatic CDO observed in rainbow trout fed the egg albumin diets probably reflects the rise in dietary levels of sulfur amino acids, and the rise might depend on the cyst(e)ine level rather than the protein level *per se*. That is to say, the hepatic CDO may be involved in the regulation of sulfur amino acid metabolism, reflecting the sulfur amino acid balance in dietary protein in fish. Therefore, there is a possibility that the hepatic CDO activity is a useful index for evaluating the appropriate sulfur amino acid content in feed for rainbow trout.

## THE FUNCTIONAL SIGNIFICANCE OF THE CATABOLIC PATHWAY OF CYSTEINE WITH RESPECT TO DETOXICATION OF CYSTEINE: Acute toxicity of cysteine injected into peritoneal cavity

As mentioned before, cysteine administration by injection caused heavy toxicity to rainbow trout, although this amino acid is one of the physiologically important amino acids. There have been some papers that pointed out the toxicity of cysteine to animals (Anderson and Meister 1987, Griffith 1987, Olney et al. 1990); however, scarce information is available on the toxicity of cysteine for fish. The functional significance of the pathway of cysteine catabolism in rainbow trout is investigated in connection with its toxicity.

A dose of L-cysteine (2.5 to 10.0 µmol/g body weight) was injected to ten individuals each to estimate $LD_{50}$ by probit analysis. A large dose of cysteine led to mortality of rainbow trout with serious hemorrhage. $LD_{50}$ values within 2, 3, and 4 h were 7.5, 4.8, and 4.5 µmol/g body weight, respectively. A histological observation was made with several tissues of rainbow trout which died of an injection. No appreciable histological change was observed in the tissues examined except for the gill tissues. Figure 12-A is the photograph of the most typical change observed in the gill tissue (Figure 12-B is the photograph of the gill of control fish). The epithelia of secondary lamellae of the

**Table 6** Effect of the injection of sulfur amino acids on the degree of bleeding in the gills (Yokoyama and Sakaguchi, 1996).

| Amino acid | Dose† | $OD_{413}$‡×10 |
|---|---|---|
| L-Cysteine | 3.5 | 0.1±0.0 |
| | 5.0 | 0.8±0.2 |
| | 7.1 | 0.7±0.3 |
| | 10.0 | 2.2±0.5 |
| D-Cysteine | 5.0 | 0.6±0.2 |
| | 10.0 | 1.9±0.6 |
| *N*-Acetyl-L-cysteine | 10.0 | 0.0±0.0 |
| *S*-Methyl-L-cysteine | 10.0 | 0.3±0.1 |
| L-Cysteinesulfinic acid | 10.0 | 0.0±0.0 |
| L-Cysteic acid | 10.0 | 0.0±0.0 |
| Hypotaurine | 10.0 | 0.0±0.0 |
| Taurine | 10.0 | 0.0±0.0 |
| Glutathione | 4.0 | 0.2±0.0 |
| L-Methionine | 4.0 | 0.0±0.0 |

† μmol dose per kg body weight.
‡ The degree was expressed as the optical density of rearing water at 413 nm.
Values are means of eight individual measurements ± standard error.

gills swelled markedly. Bloodstains were also observed all over the gill. The control fish showed no sign of such histological change. This anomalous heavy hemorrhage might be accounted for by a functional disorder of cell membrane: the declined osmoregulation function of the epithelial membrane brings about the swelling of the cell, resulting in the destruction of the capillary vessel.

Next, the acute toxicity of several cysteine analogues was examined. For the sake of convenience, the toxicity was determined by the extent of bleeding, because this method is simpler and more reproducible than the method using mortality. The fish injected were kept in 1 L of water with aeration. Thirty minutes after the injection, the degree of bleeding was measured as the optical density of the water at 413 nm where hemoglobin shows its absorption maxima. The readings were corrected for the body weight of 30 g. Results are shown in Table 6. The degree of bleeding (OD at 413 nm) was almost proportional to the L-cysteine dose in the range of 3.5 to 10.0 μmol/g of body weight. Bleeding was observed in the gills within 30 min after injection even though the dose was only 3.5 μmol. This dosage is less than $LD_{50}$ for 4-h lethal time. D-cysteine showed similar toxic effect to that of L-cysteine. *N*-acetyl-L-cysteine showed hemorrhagic effect but *S*-methyl-L-cysteine did not. Other sulfur amino acids, L-cysteinesulfinic acid, hypotaurine, and taurine involved in the cysteine catabolic route also showed no hemorrhagic effect. Large doses of L-methionine and glutathione which is a tripeptide having a free SH group could not be administered to fish due to their poor solubility in water. However, glutathione showed a slight toxic effect even in a small dose. Neither L-methionine nor L-cysteic acid showed any effect. These findings indicate that the sulfhydryl group in the molecule might be involved in toxicity to rainbow trout.

## CONCLUSION

Results of both feeding experiments and a tracer experiment indicated that cysteine seemed to be the actual starting substance for taurine biosynthesis in rainbow trout. Cysteine was metabolized through cysteinesulfinate as an intermediate to hypotaurine, taurine and sulfate. Cysteine dioxygenase which catalyzes the oxygenation of cysteine to form cysteinesulfinate was considered to play a regulative role in cysteine metabolism. Thus, it was suggested that the enzyme activity in the liver can be used for evaluation of sulfur amino acid availability in diets for the fish. On the other hand, cysteine had serious

toxicity for rainbow trout. Considering both the rapid response of hepatic cysteine dioxygenase activity to cysteine and low toxicity of cysteinesulfinate, this enzyme presumably plays a role in detoxication by converting cysteine into non-toxic cysteinesulfinate.

## LITERATURE CITED


Anderson, M.E. and A. Meister. 1987. Intracellular delivery of cysteine, pp. 313-324. *In:* W.B. Jakoby and O.W. Griffith (eds.), Methods in Enzymology, Vol. 143. Academic Press, New York.

Aruoma, O.I., B. Halliwell, B.M. Hoey, and J. Butler. 1988. The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem. J. 256: 251-255.

Cowey, C.B., C.Y. Cho, J.G. Sivak, J.A. Weerheim, and D.D. Stuart. 1992. Methionine intake in rainbow trout (*Oncorhynchus mykiss*), relation to cataract formation and the metabolism of methionine. J. Nutr. 122: 1154-1163.

Fellman, J.H. and E.S. Roth. 1985. The biological oxidation of hypotaurine to taurine: Hypotaurine as a antioxidant, pp. 71-82. *In:* S.S. Oja, L. Ahtee, P. Kontro, and M.K. Paasonen (eds.), Taurine: Biological Actions and Clinical Perspectives. Alan R. Liss, Inc., New York.

Finkelstein, J.D. and J.J. Martin. 1986. Methionine metabolism in mammals: adaptation to methionine excess. J. Biol. Chem. 261: 1582-1587.

Griffith, O.W. 1987. Mammalian sulfur amino acid metabolism: an overview, pp. 366-376. *In:* W.B. Jakoby and O.W. Griffith (eds.), Methods in Enzymology, Vol. 143. Academic Press, New York.

Hosokawa, Y., S. Niizeki, H. Tojo, I. Sato, and K. Yamaguchi. 1988. Hepatic cysteine dioxygenase activity and sulfur amino acid metabolism in rat: possible indicators in the evaluation of protein quality. J. Nutr. 118: 456-461.

Ketola, H.G. 1982. Amino acid nutrition of fish: requirements and supplementation of diets. Comp. Biochem. Physiol. 73B: 17-24.

Kohashi, N., K. Yamaguchi, Y. Hosokawa, Y. Kori, O. Fujii, and I. Ueda. 1978. Dietary control of cysteine dioxygenase in rat liver. J. Biochem. 84: 159-168.

Kontro, P. and S.S. Oja. 1985. Hypotaurine oxidation by mouse liver tissue, pp. 83-90. *In:* S.S. Oja, L. Ahtee, P. Kontro, and M.K. Paasonen (eds.), Taurine: Biological Actions and Clinical Perspectives. Alan R. Liss, Inc., New York.

Moon, H.Y. and D.M. Gatlin III. 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95: 97-106.

Nose T. 1974. Effects of amino acids supplemented to petroleum yeast on growth of rainbow trout fingerlings-II. Methionine and cystine. Bull. Freshw. Fish. Res. Lab. 24: 101-109.

Oja, S.S. and P. Kontro. 1981. Oxidation of hypotaurine *in vitro* by mouse liver and brain tissues. Biochim. Biophys. Acta 677: 350-357.

Olney J.W., C. Zorumski, M.T. Price, and J. Labruyere. 1990. L-Cysteine, a bicarbonate-sensitive endogenous excitotoxin. Science 248: 596-599.

Sakaguchi M. 1994. Nitrogenous extractive components, pp. 287-292. In: The Japanese Society of Fisheries Science (eds.), Recent Advance in Fisheries Science. Kouseisha-Kouseikaku, Tokyo. [In Japanese].

Sakaguchi, M. and M. Murata 1988. Taurine, pp. 56-65. *In:* M. Sakaguchi (ed.), Extractive Components of Fish and Shellfish. Kouseisha-Kouseikaku, Tokyo. [In Japanese].

Stipanuk, M. 1979. Effect of excess dietary methionine on the catabolism of cysteine in rats. J. Nutr. 109: 2126-2139.

Tateishi, N., T. Higashi, A. Naruse, K. Nakashima, H. Shiozaki, and H. Sakamoto. 1977. Rat liver glutathione: possible role as a reservoir of cysteine. J. Nutr. 107: 51-60.

Walton, M.J., C.B. Cowey, and J.W. Adron. 1982. Methionine metabolism in rainbow trout fed diets of differing methionine and

cystine content. J. Nutr. 122: 1525-1535.

Watanabe, T., Editor. 1994. Use of alternative protein source in aquaculture. Kouseisha-Kouseikaku, Tokyo, 118 p. [In Japanese].

Wheldrake, J.F. and C.A. Pasternak. 1967. Oxidation of cyst(e)ine to sulfate in cultured neoplastic mast cells. Biochem. J. 102: 45p-46p.

Wright C.E., H.H. Tallan, Y.Y. Lin, and G.E. Gaull. 1986. Taurine: niological update. Annu. Rev. Biochem. 55: 427-453.

Yamaguchi, K. and Y. Hosokawa. 1987. Cysteine dioxygenase, pp. 395-403. *In:* W.B. Jakoby and O.W. Griffith (eds.), Methods in Enzymology, Vol. 143. Academic Press, New York.

Yamaguchi, K. and I. Ueda. 1976. Methods for quantitative analysis of cysteine and its sulfinic and sulfonic derivatives, pp. 451-453. *In:* The Japanese Biochemical Society (eds.), Vol. 11. Tokyo Kagaku Dojin, Tokyo. [In Japanese].

Yamaguchi, K., S. Sakakibara, J. Asamizu, and I. Ueda. 1973. Induction and activation of cysteine oxidase of rat liver II. The measurement of cysteine metabolism *in vivo* and the activation of *in vivo* activity of cysteine oxidase. Biochim. Biophys. Acta 297: 48-59.

Yokoyama, M. and J. Nakazoe. 1989. Induction of cysteine dioxygenase activity in rainbow trout liver by dietay sulfur amino acids, pp. 367-372. *In:* M. Takeda and T. Watanabe (eds.), Proceedings of the Third International Symposium on Feeding and Nutrition in Fish. eda M. and Watanabe T.). Japan Translation Center, Tokyo.

Yokoyama, M. And J. Nakazoe. 1992. Accumulation and excretion of taurine in rainbow trout fed diets supplemented with methionine, cystine, and Taurine. Comp. Biochem. Physiol. 102A: 565-568.

Yokoyama, M. and J. Nakazoe. 1996. Intraperitoneal injection of sulfur amino acids enhance the hepatic cysteine dioxygenase activity in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 15: 143-148.

Yokoyama, M. and J. Nakazoe. 1998. Effect of oral administration of L-cystine on hypotaurine level in rainbow trout. Fish. Sci. 64: 144-147.

Yokoyama, M. and M. Sakaguchi. 1996. Acute toxic effect of L-cysteine injected into the peritoneal cavity of the rainbow trout. Fish. Sci. 62: 660-661.

Yokoyama, M., M. Kaneniwa, and M. Sakaguchi. 1997. Metabolites of L-[$^{35}$S]cysteine injected into the peritoneal cavity of rainbow trout. Fish. Sci. 63: 799-801.

Yokoyama, M., M. Udagawa, and J. Nakazoe. 1994. Influence of dietary protein levels on hepatic cysteine dioxygenase activity in rainbow trout. Fish. Sci. 60: 229-233.

# THE EFFECT OF STOCKING DENSITY ON THE GROWTH OF JUVENILE SUMMER FLOUNDER *PARALICHTHYS DENTATUS*

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## ABSTRACT

The effect of stocking density on the growth of two size classes of juvenile summer flounder was studied in experiments which lasted for 40 and 58 days. In each experiment, density treatments of 100, 150, and 200% fish coverage of tank bottom surface area were tested. Fish were fed to satiation and randomly sampled for length, weight, and ventral surface area. Results from this study indicated that small (approx. 1 g, approx. 50 mm) juvenile summer flounder were unaffected by stocking density of at least 200% over 40 days. Larger (approx. 10 g, approx. 100 mm) juvenile summer flounder were affected by the nominal stocking densities, with fish initially stocked at 100% coverage growing to slightly larger sizes during the 58-day experiment.

## INTRODUCTION

With an increasing demand for high quality flatfish in domestic and overseas seafood markets, the summer flounder *Paralichthys dentatus* has become a new and promising candidate for worldwide fish farming. Its potential has been studied in the United States for several years, and commercial cultivation has been initiated in several locations along the east coast. Early research has focused on larval development and production (Bisbal and Bengtson 1991a,b, Malloy and Targett 1991, Bisbal and Bengtson 1993, Keefe and Able 1993, Bisbal and Bengtson 1995a,b,c), but less research has been directed towards issues associated with juvenile grow-out. The ability to raise the fingerlings at a relatively high density, thus maximizing water usage and fish production, is of particular importance to commercial operation. However, parameters which affect growth and survival, such as feeding efficiency, disease, and water quality, should be considered when determining an optimal stocking density for a particular system. Studies examining high rearing densities of several salmonid species attribute growth inhibition to reduced feed consumption, poor feed conversion, aggressive behavior, and oxygen depletion (Refstie and Kittelsen 1976, Refstie 1977, Vijayan and Leatherland 1988, Holm et al. 1990, Kindschi and Koby 1994). Similarly, for flatfish species like Japanese flounder *Paralichthys olivaceous*, turbot *Scophthalmus maximus*, and Atlantic halibut *Hippoglossus hippoglossus*, there appear to be some effects of higher stocking densities on growth rate and feed efficiency (Martinez-Tapia and Fernandez-Pato 1991, Jeon et al. 1993, Bjornsson 1994, Chang et al. 1995). To date, few studies have demonstrated optimal stocking density for juvenile flatfish under 50 g in weight, and none have examined juvenile summer flounder stocking density. This research was undertaken to estimate the optimal stocking density of early juvenile summer flounder in an experimental recirculating system. Two size groups, with initial weights of 0.7 and 7.8 g, were examined in two separate experiments.

## METHODS

### The recirculating system

The experimental recirculating system consisted of 14 round, 190-L, fiberglass tanks

associated with a 26-L biological filter. Water flowed (gravity) from the biological filter, which contained "Bio-Fill" media and nitrifying bacteria, through an ultraviolet light sterilizing unit to a distribution manifold above the tanks. Overflow from the tanks went into a central collection channel that was filled with a coarse polyester fiber mat for the removal of large particulate waste. This partially clarified water fell into a sump tank, and was then pumped through two cartridge filters (15 µm) back to the biofilter. Water flow to the tank was regulated using valves, and each tank was gently aerated. The system was inoculated with nitrifying bacteria and run for 6 weeks prior to the introduction of any fish. After the system was established, salinity, temperature, ammonia, and nitrite were measured daily, while dissolved oxygen and alkalinity were measured periodically.

**Relating total length to ventral surface area**

Fish density was measured as percent coverage of the tank bottom. Fish ventral surface area was estimated by tracing live, anesthetized specimens from several different size classes onto a 1 cm x 1 cm paper grid, and counting the number of $cm^2$ grids within each outline. Total length was also measured for each specimen. The curvilinear relationship between fish length and ventral surface area was determined using regression analysis. Because the relationship had a high coefficient of determination ($R^2 = 0.957$), it was possible to use each fish's total length (mm) to estimate its ventral surface area ($cm^2$).

**Experiment 1 - group 1 juveniles**

Newly weaned juveniles were stocked into white, plastic, 20-L aquaria, each with a bottom surface area of 506 $cm^2$. Each aquaria was set into the larger tanks of the recirculating system and supplied with seawater (18°C). Mean fish length, weight, and surface area was 43 mm, 0.7 g, and 6.35 $cm^2$, respectively (Table 1). The three density treatments of 100% (1.1 $kg/m^2$), 150% (1.7 $kg/m^2$), and 200% (2.2 $kg/m^2$) coverage of tank bottom were established by stocking 80, 120, and 160 individuals into each of the three replicates per treatment, respectively (Table 1). As mortality occurred through the course of the experiment, fish were replaced to maintain nominal stocking densities. All fish were fed to satiation twice a day using Moore-Clark® formulated feed. The experiment was terminated after 40 days, and 25 individuals from each replicate were weighed and measured. Final stocking density (percent cover) was determined for each replicate by multiplying the number of fish by the mean ventral surface area of the fish. This total fish surface area value was then expressed as a percentage of the surface

| | Day 0 | | | Day 40 | | |
|---|---|---|---|---|---|---|
| Nominal % cover | **100** | **150** | **200** | **100** | **150** | **200** |
| Mean length (mm) | 43 | 43 | 43 | 84 (+/- 16.7) | 84 (+/- 15.9) | 82 (+/- 19.4) |
| Mean weight (g) | 0.7 | 0.7 | 0.7 | 6.2 (+/- 3.45) | 6.3 (+/- 3.43) | 6.3 (+/- 4.58) |
| Mean surface area (cm2) | 6.35 | 6.35 | 6.35 | 15.38 | 15.38 | 14.94 |
| Observed mean % cover | 100 | 150 | 200 | 243 | 361 | 466 |
| Mean kg/m2 | 1.1 | 1.7 | 2.2 | 9.8 | 14.7 | 19.8 |

**Table 1.** Summary of data for experiment 1 (+/-) is standard deviation.

| | Day 0 | | | Day 27 | | | Day 58 | | |
|---|---|---|---|---|---|---|---|---|---|
| Nominal % cover | 100 | 150 | 200 | 100 | 150 | 200 | 100 | 150 | 200 |
| Mean length (mm) | 89 | 89 | 89 | 128(+/-2.52) | 122(+/-1.53) | 124(+/-1.15) | 155(+/-1.53) | 139(+/-4.04) | 148(+/-4.58) |
| Mean weight (g) | 7.8 | 7.8 | 7.8 | 24(+/-1.12) | 20(+/-.067) | 21(+/-1.42) | 46.5(+/-1.56) | 35.1(+/-1.65) | 41.1(+/-4.21) |
| Mean surface area (cm2) | 21.2 | 21.2 | 21.2 | 38.6 | 34.1 | 35.5 | 76 | 59 | 68.7 |
| Mean percent survival | 100 | 100 | 100 | 100 | 96(+/-4.6) | 78(+/-8.4) | 100 | 96(+/-7.5) | 77(+/-8.8) |
| Observed mean % cover | 100 | 150 | 200 | 180 | 223 | 258 | 355 | 399 | 493 |
| Mean kg/m2 | 3.7 | 5.5 | 7.3 | 11.2 | 13.1 | 15.3 | 21.8 | 23.7 | 29.5 |

**Table 2.** Summary of data for experiment 2 (+/-) is standard deviation.

area of the bottom of the experimental aquaria. Biomass per unit area (kg/m$^2$) was determined for each replicate by multiplying the mean fish weight by the number of fish. Mean total biomass per treatment was expressed as a proportion of the surface area (m$^2$) of the bottom of the experimental aquaria. Data were analyzed using one-way analysis of variance (ANOVA).

**Experiment 2 - group 2 juveniles**

Juveniles were stocked into gray, plastic, 20-L aquaria, each with a bottom surface area of 962 cm$^2$. Each aquaria was set into the larger tanks of the recirculating system and supplied with seawater (18°C). Initial mean fish length, weight, and surface area were 89 mm, 7.8 g, and 21.2 cm$^2$, respectively (Table 2). The three density treatments of 100% (3.7 kg/m$^2$), 150% (5.5 kg/m$^2$), and 200% (7.3 kg/m$^2$) coverage of tank bottom were established by stocking 45, 68, and 90 individuals into each of the three replicates per treatment, respectively (Table 2). Mortalities (probably due to handling and transfer stress) were replaced only during the first week of the experiment. The flounder were fed Moore-Clark® formulated feed twice daily to satiation. Random samples of fish were measured and weighed on day 27 and at the conclusion of the experiment on day 58. Stocking densities (percent cover) at days 27 and 58 were determined for each replicate by multiplying the number of fish by the mean ventral surface area of the fish. This total fish surface area value was then expressed as a percentage of the surface area of the bottom of the experimental aquaria. Biomass per unit area (kg/m$^2$) was determined for each replicate by multiplying the mean fish weight by the number of fish. Total treatment biomass was expressed as a proportion of the surface area (m$^2$) of the bottom of the experimental aquaria. Data were analyzed using ANOVA, followed by Tukey's multiple comparison tests when significant differences were found.

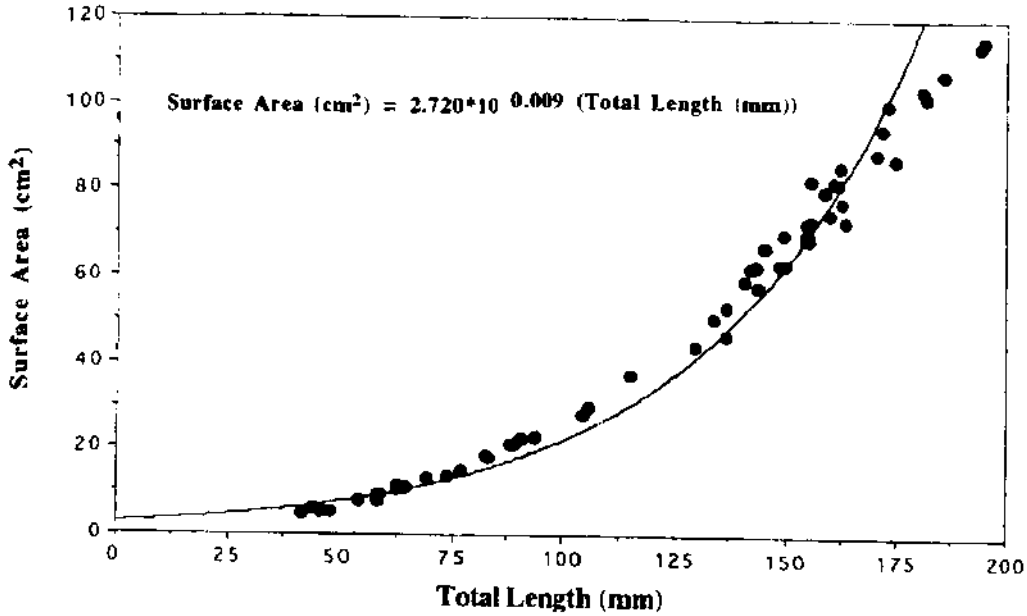


**Figure 1.** Relationship between total length and ventral surface area in juvenile summer flounder. Regression equation is based on 59 individuals. (The term * denotes multiply by.)

## RESULTS

### Relationship between fish length and surface area

Curvilinear regression analysis was used to relate total fish length to the ventral surface area of the fish (Fig. 1). The equation which describes this relationship, for summer flounder ranging from approximately 50-200 mm total length, is:

$$\text{Surface area (cm}^2\text{)} = 2.720 \times 10^{0.009\ (\text{total length (mm)})}$$

### Experiment 1 - group 1 juveniles

A total of 19 fish were replaced among replicates of the 100% treatment, and 17 fish among replicates in each of the 150 and 200% treatments. Additionally, a replicate was lost in the 200% coverage treatment due to accidental stoppage of water and subsequent depletion of oxygen. Mean lengths, weights, surface areas, and densities are reported in Table 1. Mean lengths and weights increased by approximately 40 mm and 5.5 g in each treatment. Fish density increased by about 140% in each treatment as juveniles grew both in length and weight over the 40-day experimental period. Final coverage of the tank bottom was 243, 361, and 466%. Estimates of biomass per unit area at the end of the experiment were 9.8, 14.7, and 19.8 kg/m$^2$ for stocking densities of 100, 150, and 200%, respectively. These represent 7 to 8 fold increases over the course of the experiment. Even at these high final densities, no significant differences ($P>0.05$) in total length or wet weight were found between juveniles in any of the three treatments.

### Experiment 2 - group 2 juveniles

Mean lengths, weights, surface areas, and densities are reported in Table 2. Following mortality replacement in the first week, final mean survivals were 100, 96, and 78% for treatments of 100, 150, and 200% coverage, respectively. At the end of this 58-day experiment, mean fish densities had reached 355, 399, and 493% bottom coverage. Final estimates of biomass per unit area were 21.8, 23.7, and 29.5 kg/m$^2$ for the 100, 150, and 200% treatments, respectively. At day 27, fish initially stocked at 100% coverage were significantly ($P<0.05$) larger, both in length and weight, than those initially stocked at 150 and 200% coverage (Table 3). There was no significant difference ($P>0.05$) in either length or weight

| | Day 27 | | Day 58 | |
|---|---|---|---|---|
| | Length | Weight | Length | Weight |
| 100 vs. 150% | * | * | * | * |
| 100 vs. 200% | * | * | ns | ns |
| 150 vs 200% | ns | ns | ns | ns |

**Table 3.** Results from one way analysis of variance (ANOVA) comparing mean lengths and weights from treatments in experiment 2. **ns** = not significant (P>0.05);* = (P<0.05).

between the 150 and 200% treatments at this time (Table 3). At the end of the experiment (day 58), fish initially stocked at 100% coverage were still significantly longer and heavier than those in the 150% coverage treatment (P<0.05), but not longer or heavier than those in the 200% coverage treatment (P>0.05). At day 58, there was no difference in the lengths and weights of fish in the 150 and 200% coverage treatments (P>0.05) (Table 3).

## DISCUSSION

Results from experiment 1 in this study indicate that small, recently weaned summer flounders (initial size of about 1 g and 40 mm) can be stocked at densities of at least 200%, and raised for at least 40 days without negatively affecting growth. In fact, we recorded densities greater than 400% coverage at the end of this experiment (Table 1) and saw no indication that growth was being impaired. Results from this experiment prevent us from suggesting an upper limit on stocking densities for fish of this size, but it is certainly greater than 200% coverage. We are unaware of any other density studies that have been done with flatfish of this size.

Results from experiment 2, however, which began with larger fish (initial size of about 8 g and 90 mm) and ran for a longer time, indicate that stocking density does affect the growth of these larger individuals. In this instance, fish stocked at the lowest density (100% coverage) grew faster than those in both of the other treatments from the start of the experiment until day 27. During this time, the mean length of fish in this treatment increased by about 44%, which was slightly higher than increases seen in the 150% (37% increase) and 200% (39% increase) treatments. Similarly, mean weight of fish in the 100% density treatment increased by about 208%, which was dramatically higher than the increases seen in the 150% (156% increase) and 200% (168% increase) treatments. It has been suggested that high stocking densities can lead to poor water quality (high ammonia, low oxygen) which in turn can lead to reduced growth performance (Brett 1979, Pickering and Pottinger 1987, Kebus et al. 1992, Kindschi and Koby 1994, Wagner et al. 1995). It is extremely unlikely that poor water quality was a factor in this study. First, because all treatments were associated with the same recirculating water and biological filter, water quality was probably identical in all treatments. Second, we found that nonionized ammonia never exceeded 0.05 ppm and dissolved oxygen never fell below saturation. Lastly, we observed no loss of appetite in any of the treatments that could

indicate poor water quality and/or stress. Thus, our recirculating system and biological filter were capable of maintaining ammonia below, and dissolved oxygen above, normally stressful levels. In effect, the system in which we conducted the experiment eliminated two of the variables (high ammonia, low oxygen) that are often associated with high stocking densities. Aside from water quality issues, food consumption and feeding behavior may also be affected by stocking density (Holm et al. 1990, Martinez-Tapia and Fernandez-Pato 1991). In such instances, "crowding" (high number of fish per unit area) can cause an increase in agonistic feeding behavior, which in turn increases stress and decreases growth. It is possible that these factors contributed to the results we found. Summer flounder are known to be aggressive feeders (Bigelow and Schroeder 1953), and we occasionally observed aggressive feeding behavior (e.g. chasing, tail biting) in this experiment. If such behavior increased with numerical density, it is possible that fish in the lowest stocking density (100%), which contained only 45 fish in each replicate, benefited from low numerical abundance. The fact that we saw no density effect in experiment 1, which was done with smaller fish, suggests that this mechanism, if applicable, may not operate until the fish are somewhat older and larger. Density-dependent behavioral changes are well documented (Fenderson and Carpenter 1971, Refstie and Kittelsen 1976), and Wagner et al. (1996) found that agonistic behavior in rainbow trout fry increased with age. These studies support our contention that behavioral mechanisms, which may change with fish size, were responsible for the differences we observed. At the end of experiment 2 (day 58), fish in the 100% density treatment were still larger than fish in the 150% treatment, but not the fish in the 200% treatment. The parity of fish in the 100 and 200% density treatments at the end of the experiment, but not at day 27, is an indication that a size convergence occurred between days 27 and 58. Thus, it appears that fish stocked at densities of 100 and 200% grow at different rates for a period of time (the first half of this experiment), but fish held at the higher density (200%) were able to "catch up" (compensatory growth) as time went on (the second half of this experiment). If, as speculated above, agonistic behavior increases with numerical density, which in turn increases stress and reduces growth rate, it is possible that a reduction in agonism over time explains the compensatory growth we observed. In the 200% density treatment, the mean number of fish per replicate decreased from 90 to 69, thus possibly reducing aggressive, agonistic interactions. Alternatively, the fish at the higher density (200%) simply could have become more "accustomed" to this density as time progressed, thereby reducing stress and resulting in compensatory growth. A third possible explanation is that summer flounder respond differently to stocking density as they increase in size. If, for example, larger fish are more tolerant of high density at larger sizes, then it would explain why fish at the higher density (200%) exhibited compensatory growth during the second part of this experiment.

Fish held at the intermediate density (150%) were significantly smaller than those at 100% on days 27 and 58, but not different from those at the 200% density on either day. These results partially support our hypothesis that numerical abundance and associated agonistic feeding behavior may be affecting the growth of juvenile summer flounders. Because of mortalities in replicates of the 200% treatment, mean numerical abundance in the 150% treatment (n=65) was nearly identical to that of the 200% treatment (n=69). These similarities would explain why growth was nearly identical in the 150 and 200% treatments, and why fish in both of these higher density treatments were smaller than those in the 100% density treatment which had a lower mean numerical abundance (n=45). The fact that fish in the 200% treatment demonstrated compensatory growth, while those in the 150% treatment did not, is difficult to explain. It is possible that the loss of fish in the 200% treatment triggered the compensatory growth we observed, and that this did not occur in the 150% treatment because numerical abundance was relatively stable throughout the experiment. This is very speculative, and additional research would be needed to address this issue.

Our results are similar to those of the few stocking density studies which have been done with other flatfish species. Jeon et al. (1993), who worked with young Japanese flounder

*Paralichthys olivaceous*, evaluated stocking densities of 33, 50, 100, 200, and 300% bottom coverage, and found that the highest feeding rate and growth occurred at 200% coverage. Similarly, Chang et al. (1995), who worked with larger (50-75 mm) Japanese flounder, in a semi-closed, recirculating seawater system, reported final densities as high as 260% (36.3 kg/m$^2$). Although our experimental fish were not grown to harvest size at our nominal stocking densities, results with both turbot *Scophthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus* suggest that larger sized flatfish can be grown at relatively high densities. Martinez-Tapia and Fernandez-Pato (1991) found no ill effects at a stocking density of 68 kg/m$^2$ for turbot, and suggested that specific growth and food conversion were greater at higher densities. Bjornsson (1994) conducted research with relatively large halibut (initial size 1.8-3.2 kg) at stocking densities of 50, 100 and 160% coverage. Although he observed a maximum coverage of 215% (95 kg/m$^2$), he indicated that growth rate was reduced in the highest coverage (160%) treatment, and that optimal stocking density was somewhere between one and two layers of fish on the tank bottom.

This study, as well as those with halibut, Japanese flounder, and turbot indicate that flatfish species are able to grow effectively at stocking densities of 100-200% (one to two layers thick on the tank bottom). Indeed, in this study we observed that fish, when not feeding, would crowd and overlap one another even when empty space was available, and that this occurred at all stocking densities. Similar "layering" behavior has been observed in halibut (Bjornsson 1994). Further, biomass densities can be relatively high. In this study, with relatively small fish, biomass densities reached only 29.5 kg/m$^2$, but work with Japanese flounder (Chang et al. 1995), turbot (Martinez-Tapia and Fernandez-Pato 1991), and halibut (Bjornsson 1994) suggest that biomass densities of 36.3, 68.0, and 95 kg/m2, respectively, were possible. The combination of layering, and tolerance of high biomass densities, suggest that flounders can be raised at high densities. This could be an enormous advantage to the grow-out farmer, provided that the recirculating system is capable of supporting these high biomasses. Results of this study suggest that recently weaned summer flounder can be stocked at densities of at least 200%, but that stocking density should be reduced to 100% for larger juveniles, at least for several weeks, and that densities could then be allowed to increase as the fish grow in size. Further observations and research, which develop with the commercial summer flounder industry, will undoubtedly refine our understanding of optimum stocking density.

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## LITERATURE CITED

Bigelow, H.B. and W.C. Schroeder. 1953. Fishes of the Gulf of Maine. Fish. Bull. U.S. 74: 1-577.

Bisbal, G.A. and D.A. Bengtson. 1991a. Characterization of starved versus fed summer flounder, *Paralichthys dentatus*, larvae and juveniles, pp. 216-218. *In:* P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevie (eds.), Larvi '91, European Aquaculture Society, Spec. Publ. No. 15, Gent, Belgium.

Bisbal, G.A. and D.A. Bengtson. 1991b. Effect of dietary (n-3) HUFA enrichment on survival and growth of summer flounder, *Paralichthys dentatus*, larvae, pp. 56-57. *In:* P. Lavens, P. Sorgeloos, E. Jaspers and F. Ollevie (eds.), Larvi '91, European Aquaculture Society, Spec. Publ. No. 15, Gent, Belgium.

Bisbal, G.A. and D.A. Bengtson. 1993. Reversed asymmetry in laboratory-reared summer flounder. Prog. Fish-Cult. 55: 106-108.

Bisbal, G.A. and D.A. Bengtson. 1995a. Description of the starving condition in summer flounder (*Paralichthys dentatus*) early life stages: morphometrics, histology, and biochemistry. Fish. Bull. 93: 217-230.

Bisbal, G.A. and D.A. Bengtson. 1995b. Development of the digestive tract in larval summer flounder, *Paralichthys dentatus*. J. Fish Biol. 47:277-291.

Bisbal, G.A. and D.A. Bengtson. 1995c. Effects of delayed feeding on survival and growth of summer flounder, *Paralichthys dentatus*, larvae. Mar. Ecol. Prog. Ser. 121: 301-306.

Bjornsson, B. 1994. Effects of stocking density on the growth rate of halibut (*Hippoglossus hippoglossus* L.) reared in large circular tanks for three years. Aquaculture 123: 259-270.

Brett, J.R. 1979. Environmental factors and growth, pp. 599-675. *In:* W.S. Hoar, D.J. Randall and J.R. Brett (eds.), Fish Physiology, Vol. VIII. Bioenergetics and Growth. Academic Press, New York.

Chang, Y.J., S.H. Kim, and H.S. Yang. 1995. Culture of the olive flounder (*Paralichthys olivaceous*) in a semi-closed recirculating seawater system. J. Korean Fish Soc. 28(4): 457-468.

Fenderson, O.C. and M.R. Carpenter. 1971. Effects of crowding on the behaviour of juvenile hatchery and wild landlocked Atlantic salmon (*Salmo salar* L.). Anim. Behav. 19: 439-447.

Holm, J.C., T. Refstie, and S. Bo. 1990. The effect of fish density and feeding regimes on individual growth rate and mortality in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 89: 225-232.

Jeon, I.G., K.S. Min, J.M. Lee, K.S. Kim, and M.H. Son. 1993. Optimal stocking density for olive flounder, *Paralichthys olivaceous*, rearing in tanks. Bull. Natl. Fish. Res. Dev. Agency Korea 48: 57-70.

Kebus, M.J., M.T. Collins, M.S. Brownfield, C.H. Amundson, T.B. Kayes, and J.A. Mallison. 1992. Effects of rearing density on the stress response and growth of rainbow trout. J. Aquat. Anim. Health 4: 1-6.

Keefe, M. and K.W. Able. 1993. Patterns of metamorphosis in summer flounder, *Paralichthys dentatus*. J. Fish Biol. 42: 713-728.

Kindschi, G.A. and R.F. Koby Jr. 1994. Performance and oxygen consumption of Snake River cutthroat trout reared at four densities with supplemental oxygen. Prog. Fish-Cult. 56: 13-18.

Malloy, K.D. and T.E. Targett. 1991. Feeding, growth and survival of juvenile flounder *Paralichthys dentatus*: experimental analysis of the effects of temperature and salinity. Mar. Ecol. Prog. Ser. 72: 213-223.

Martinez-Tapia, C. and C.A. Fernandez-Pato. 1991. Influence of stock density on turbot (*Scophthalamus maximus*) growth. Int. Counc. Explor. Sea C.M. 1991/F:20.

Pickering, A.D. and T.G. Pottinger. 1987. Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement. J. Fish Biol. 30: 363-374.

Refstie, T. 1977. Effect of density on growth and survival of rainbow trout. Aquaculture 11: 329-334.

Refstie, T. and A. Kittelsen. 1976. Effect of density on growth and survival of artificially reared Atlantic salmon. Aquaculture 8: 319-326.

Vijayan, M.M. and J.F. Leatherland. 1988. Effect of density on the growth and stress-response in brook charr, *Salvelinus fontinalis*. Aquaculture 75: 159-170.

Wagner, E.J., S.A. Miller, and T. Bosakowski. 1995. Ammonia excretion by rainbow trout over a 24-hour period at two densities during oxygen injection. Prog. Fish-Cult. 57: 199-205.

Wagner, E.J., S.S. Intelmann, and M.D. Routledge. 1996. The effects of fry rearing density on hatchery performance, fin condition, and agonistic behavior of rainbow trout *Oncorhynchus mykiss* fry. J. World Aquacult. Soc. 27: 264-274.

# IMPORTANCE OF DIETARY LIPIDS IN FLATFISH

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## ABSTRACT

Considerable attention has been focused on the n-3 polyunsaturated fatty acid (PUFA) requirements of marine fish larvae. To clarify the physiological role of dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the body of fish larvae, accumulation of dietary EPA and DHA in the eye including retina, brain, and liver of Japanese flounder was analyzed. Dietary DHA was rapidly incorporated into phospholipids of retina, brain, and liver suggesting that DHA may play an important role for fish larvae. On the albinism in the ocular side of flatfish, which results from the deficiency of pigments, and widely occurs during the process of seed production, I found that albinism results when 10-day-old larvae were fed with nutritionally deficient experimental microparticulate diets. I suggest that the rhodopsin formation of the eye retina was hindered when fat-soluble vitamin (vitamin A) and n-3 highly unsaturated fatty acid (DHA) were deficient in foods, resulting in the interruption of black pigment (melanin) formation. On the effect of dietary phospholipids on the stress tolerance of Japanese flounder investigated using feeding trials, I studied the tolerance of Japanese flounder to various stress factors such as changes in water temperature and salinity, and exposure to low dissolved oxygen, and noted that dietary soybean lecithin and krill phospholipid were effective in increasing the tolerance of flatfish to the various stress conditions.

## INTRODUCTION

Dietary lipids are important sources of energy and essential fatty acids for all animals. The n-3 fatty acid such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are highly unsaturated fatty acids (HUFA) that are commonly found in marine organisms. The useful roles and beneficial effects of these fatty acids have been recognized for marine animals and human health. It has been demonstrated that EPA is biosynthesized by phytoplankton and it then is assimilated by zooplankton of which a part of EPA is bioconverted into DHA. Both n-3 HUFA are deposited and accumulated in marine fish. Accumulation of dietary EPA and DHA in brain and retina of Japanese flounder, nutritional mechanisms involved in the occurrence of abnormal pigmentation in hatchery-reared Japanese flounder, and effect of phosholipids on stress tolerance of Japanese flounder were studied to illustrate the importance of dietary lipids in flatfish.

## ACCUMULATION OF DIETARY EPA AND DHA IN BRAIN AND RETINA OF JAPANESE FLOUNDER LARVAE

### Introduction

Considerable attention has been focused on the n-3 HUFA requirements of marine fish larvae (Kanazawa 1985). Studies on species such as *Scopthalmus maximus* (Witt et al. 1984), *Sparus aurata* (Koven et al. 1989), *Coryphaena hippurus* (Ostrowski and Divakaran 1990), and *Oplegnathus fasciatus* (Kanazawa 1993a) larvae have shown that DHA is strongly retained and is essential for these marine fish. When turbot *Scopthalmus maximus* larvae were fed on a pelleted diet containing 13-fold more DHA than *Artemia*, DHA is rapidly incorporated into the brain phospholipid, particularly in the phosphatidylcholine (Mourente and Tocher 1992).

### Materials and Methods

To clarify the physiological role of dietary EPA and DHA in the body of fish larvae, accumulation of dietary EPA and DHA in the brain,

eye including retina, and liver of Japanese flounder *Paralichthys olivaceus* was analyzed. A feeding experiment was carried out using semi-purified microparticulate diets containing 2% of either EPA or DHA. The protein sources in the diet were casein, white fish meal and squid meal, and gluten was used as the binder. The diet was a dry pellet type and the basal diet composition is given in Table 1. The ingredients were added in the following order and mixed well in every addition: (1) protein sources, water-soluble vitamins, minerals, activated gluten, etc.; (2) fat-soluble vitamins, soybean lecithin, EPA or DHA, oleic acid; (3) water at 30 ml/100 g diet. The well-mixed dough was pelletized three times by a meat mincer with 2.5 mm die. The pellets were then oven-dried at 40°C for 8 h, steamed for 90 sec, broken down into 1.9-mm particle sizes, and stored at -20°C. Pellets were cooled to room temperature before feeding. Twenty *P. olivaceus* larvae, 30 days after hatching (total length 35.32 ± 2.18 mm; weight 0.35 ± 0.05 g), were stocked in a 100-L tank. Seawater was allowed to flow at 2.4 L/min with temperature ranging from 15 to 18°C. Fish were fed three times a day.

### Results

After 30 days, EPA and DHA contents in brain, retina, and liver of flounder larvae fed with EPA or DHA diets were compared with those fed a HUFA-free diet. The brain and liver accumulated more EPA and DHA in the polar lipid than in the neutral lipid (Figs. 1, 2). In the retina, EPA was accumulated in both neutral and polar lipids; however, DHA was higher in polar than in neutral lipid fraction (Fig. 3). Dietary DHA was rapidly incorporated into phospholipids of brain, retina, and liver suggesting that DHA may play an important role for the larvae of this species.

## NUTRITIONAL MECHANISMS INVOLVED IN THE OCCURRENCE OF ABNORMAL PIGMENTATION IN HATCHERY-REARED JAPANESE FLOUNDER

### Introduction

The depigmentation (albinism) in the ocular side of flatfish, which resulted from the deficiency of pigments, has widely occurred during the process of seed production. Although many researchers

| Ingredient | g/100 g diet | | |
|---|---|---|---|
| | Free | DHA 2% | EPA 2% |
| DHA[1] | 0.0 | 2.3 | 0.0 |
| EPA[1] | 0.0 | 0.0 | 2.3 |
| Oleic acid[1] | 7.0 | 4.7 | 4.7 |
| Soybean lecithin | 6.0 | 6.0 | 6.0 |
| Basal ingredients[2] | 97.0 | 97.0 | 97.0 |

[1]Ethyl esters: purity 87%

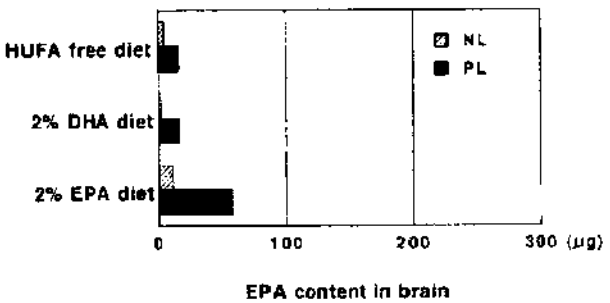
[2]Basal ingredients (g/100 g diet): casein, 20.0; white fish meal, 18.0; squid meal, 20.0; dextrin, 6.3; vitamin mixture,* 5.3; mineral mixture,** 5.0; activated gluten, 8.0; lysine HCl, 2.2; tryptophan, 0.7; attractants,*** 1.5.

*Vitamin mixture (mg/100 g diet): p-amino benzoic acid 144.48, biotin 2.18, inositol 1450.69, nicotinic acid 290.11, Ca-pantothenate 101.57, pyridoxine HCl 17.28, riboflavin 72.51, thiamin HCl 21.76, menadione 17.28, vitamin A palmitate 71.00, α-tocopherol 145.09, cyanocobalamine 0.03, calciferol 3.65, APM 25.31, folic acid 5.44, choline chloride 2965.31.

**Mineral mixture (mg/100 g diet): U.S.P. XII No. 2: NaCl 183.8, $MgSO_4 \cdot 7H_2O$ 685.0, $NaH_2PO_4 \cdot 2H_2O$ 436.0, $KH_2PO_4$ 1199.0, $Ca(H_2PO_4)_2 \cdot H_2O$ 679.0, Fe citrate 148.5, Ca lactate 1635.0; trace elements (J.E. Halver): $AlCl_3 \cdot 6H_2O$ 0.9, $ZnS_4O \cdot 7H_2O$ 17.9, CuCl 0.5, $MnSO_4 \cdot 4H_2O$ 4.0, KCl 0.8, CoCl 5.0.

***Attractants (g/100 g diet): alanine 0.3, glycine 0.3, taurine 0.3, proline 0.3, betaine 0.3.

**Table 1.** Composition of test diet containing eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) for Japanese flounder.



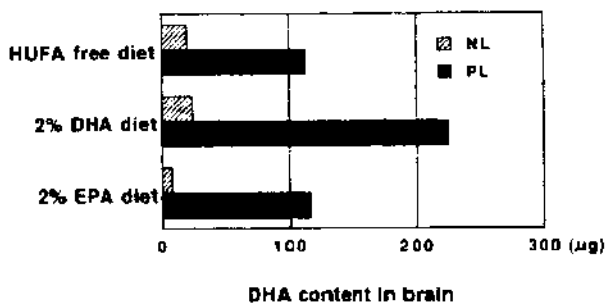
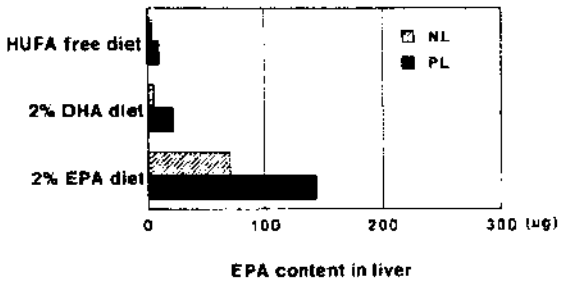




**Figure 1.** Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents (µg/mg) in polar (PL) and neutral lipids (NL) in the brain of Japanese flounder fed on EPA or DHA diet for 30 days. Data are the mean of three replicates.



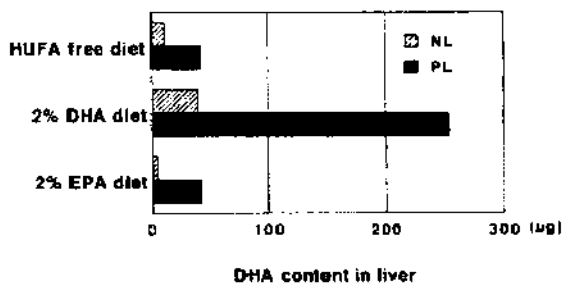




**Figure 2.** EPA and DHA contents (µg/mg) in polar (PL) and neutral lipids (NL) in the liver of Japanese flounder fed on EPA or DHA diet for 30 days. Data are the mean of three replicates.

| | |
|---|---|
| Fish used | Japanese flounder |
| Age | 4 days after hatching |
| Total length | 4.2 mm |
| Number of fish | 800/tank |
| Rearing and feeding methods | |
| Feeding period | 65 days |
| Tank | 100 L |
| Water temperature | 15.0-20.0°C |
| Flow rate | 0.2-1.0 L/min |
| Feeding frequency | 10 times/day |
| Type of diet | Microparticulate diet |

**Table 2.** Fish used and rearing methods for the abnormal pigmentation study

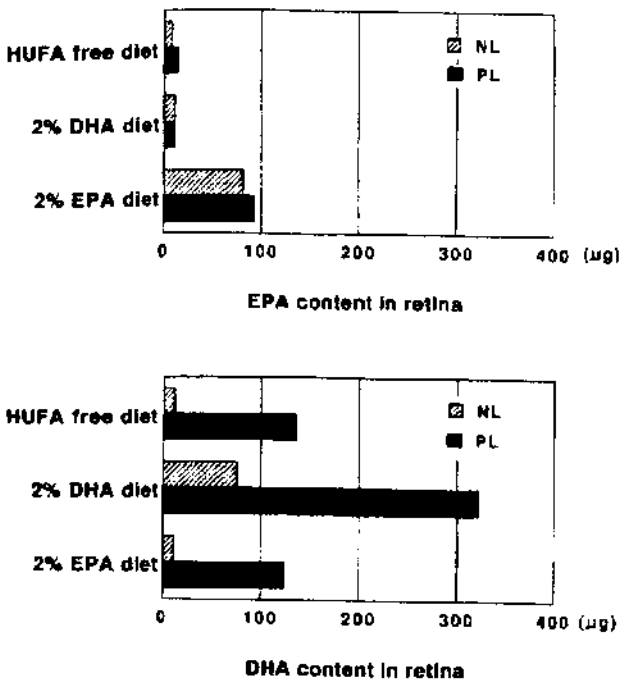



**Figure 3.** EPA and DHA contents (µg/mg) in polar (PL) and neutral lipids (NL) in the retina of Japanese flounder fed on EPA or DHA diet for 30 days. Data are the mean of three replicates.

have investigated the mechanisms of depigmentation, little is known in this field. The author found that depigmentation resulted when 10-day-old larvae were fed with nutritionally deficient experimental microparticulate diets. It was suggested that the rhodopsin formation of eye retina was hindered when vitamin A, DHA, and phospholipid were deficient in foods, resulting in the interruption of black pigment (melanin) formation (Kanazawa 1993b).

## Materials and Methods

Newly hatched Japanese flounder *P. olivaceus* larvae were fed with rotifers for 4 days. Thereafter, 800 fish were divided into experimental groups, and fed with microparticulate diets reared under conditions listed in Table 2. The microparticulate diets were mainly composed of vitamin-free casein, dextrin, lipids, mineral mixture, and vitamin mixture. As the binder, k-Carrageenan was used. The experimental treatments were: diet 1, complete diet; diet 2, n-3 HUFA- deficient diet; diet 3, fat-soluble vitamin deficient diet; diet 4, live food (rotifer and *Artemia*). The appearance of albinism in the ocular side of *P. olivaceus* was determined 65 days after feeding with the various test diets.

## Results and Discussion

Albinism (completely abnormal and partially abnormal) in flatfish was 23.1% in the group fed with the complete diet, but 83.4% in the

| Diet | Abnormal + partial abnormal (%) |
|---|---|
| Complete | 23.1[a*] |
| n-3 HUFA-deficient | 83.4[c] |
| Fat-soluble vitamin deficient | 43.2[ab] |
| Live food (rotifer and *Artemia*) | 53.3[b] |

* Values with the same superscripts are significantly different at 5% level ($p<0.05$). Each treatment was conducted in triplicate groups.

**Table 3.** Depigmentation of Japanese flounder fed with nutritionally deficient diets

n-3 HUFA-deficient diet and 43.2% in the fat-soluble vitamin deficient diets (Table 3). It has been suggested that DHA in the n-3 HUFA and vitamin A in the fat-soluble vitamins were essential in the reduction of albinism in hatchery-reared Japanese flounder (Kanazawa 1995). Rhodopsin in the rod cells conducting vision in the dark is composed of opsin (protein), retinal (vitamin A aldehyde), and phospholipid (phosphatidylcholine) including DHA.

| | |
|---|---|
| Fish used | Japanese flounder |
| Total length | 35.13 ± 0.36 mm |
| Body weight | 0.42 ± 0.12 g |
| Number of fish | 20 fish/tank |
| Rearing and feeding methods | |
| Feeding periods | 40 days |
| Tank | 100 L |
| Flow rate | 500-600 ml/min |
| Water temperature | 16.5 ± 0.3°C |
| Feeding frequency | 4 times/day |
| Feeding level | 5% of body weight |
| Type of diet | Dry pellet |
| Size of diet | Diameter 1.6 mm |

**Table 4.** Fish used and rearing methods for the stress study

The author assumed that in flatfish, the rhodopsin formation of the retina is interrupted when vitamin A, DHA, and phospholipid are deficient in initial foods after hatching of the eggs. For this reason, visual transmission from the retina is not transferred to the central nervous system, so that the melanophore-stimulating hormone from the endocrine organ does not secrete, resulting in the interruption of the black pigment formation. Therefore, when microparticulate diets or rotifers enriched with vitamin A, DHA, and phospholipid (soybean lecithin) are fed on flatfish 10 days after hatching, the prevention of albinism is possible.

## EFFECT OF PHOSPHOLIPIDS ON STRESS TOLERANCE OF JAPANESE FLOUNDER

### Introduction

Marine fish larvae were found to have a requirement for phospholipids on growth and survival (Kanazawa et al. 1985, Kanazawa 1993c, Kanazawa 1997). The present research was conducted to determine the effect of phospholipids on stress tolerance such as the changes in water temperature and salinity, and exposure to low dissolved oxygen (DO).

| | Phospholipid 0% | Phospholipid 1% (as soybean lecithin) | Phospholipid 1% (as krill phospholipid) |
|---|---|---|---|
| Survival (%) at temperature | | | |
| Rise from 16.5 to 22.0°C and kept at 22.0°C for 30 min | 100 | 100 | 100 |
| Rise from 22.0 to 27.0°C and kept at 27.0°C for 30 min | 100 | 100 | 100 |
| Rise from 27.0 to 33.0°C and kept at 33.0°C for 30 min | 20 | 60 | 60 |
| Rise from 33.0 to 34.0°C and kept at 34.0°C for 30 min | 0 | 0 | 0 |
| Low salinity (35 to 0 ppt) Time (min) when 50% of fish group laid down | 83.9 | 278.3 | 111.6 |
| Low dissolved oxygen (to 0.80 ml/L) Time (min) when 50% of fish group laid down | 7.7 | 15.6 | 12.1 |

Values are the mean of three replicates.

**Table 5.** Tolerance of Japanese flounder to stress due to increased temperature, reduced salinity, and dissolved oxygen

## Materials and Methods

Japanese flounder *P. olivaceus* were fed on diets containing soybean lecithin (1% as phosphatidylcholine) and krill phospholipid (1% as phosphatidylcholine) under the conditions listed in Table 4. The diet was mainly composed of vitamin-free casein, defatted squid meal and white fish meal, dextrin, lipids, mineral mixture, and vitamin mixture. Activated gluten was used as the binder (see Table 1).

## Results

After the feeding experiment, Japanese flounder were tested as to their response to stress due to low dissolved oxygen, low salinity, and increased water temperature (Table 5). Japanese flounder in increased water temperature (at 33.0°C) showed that those fed with the soybean lecithin and krill phospholipid diet had higher tolerance than the phospholipid-free diet. When Japanese flounder were exposed to low dissolved oxygen and low salinity, dietary soybean lecithin and krill phospholipid were effective in increasing the tolerance of fish. Phospholipids were not only indispensable nutrients for the growth of fish, but were effective in increasing their tolerance to the various stressful conditions.

## LITERATURE CITED


Kanazawa, A. 1985. Essential fatty acid and lipid requirement of fish, pp.281-298. *In:* C.B. Cowey, A.M. Mackie and J.G. Bell (eds.), Nutrition and Feeding in Fish. Academic Press, London.

Kanazawa, A. 1993a. Importance of dietary docosahexaenoic acid on growth and survival of fish larvae, pp.87-95. *In:* C.S. Lee, M.S. Su, and I.C. Liao (eds.), Proceedings of Finfish Hatchery in Asia '91. Tungkang Marine Laboratory, TFRI, Taiwan.

Kanazawa, A. 1993b. Nutritional mechanisms involved in the occurrence of abnormal pigmentation in hatchery-reared flatfish Heterosoma. J. World Aquacult. Soc. 24: 23-27.

Kanazawa, A. 1993c. Essential phospholipids of fish and crustaceans, pp.519-530. *In:* S.J.Kaushik and P. Luquet (eds.), Fish Nutrition in Practice. INRA Edition, Paris Les Colloques, No.61.

Kanazawa, A. 1995. Nutrition of larval fish, pp.50-59. *In:* C.E. Lim and D.J. Sessa (eds.), Nutrition and Utilization Technology in Aquaculture. AOCS Press, Champaign, IL.

Kanazawa, A. 1997. Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. Aquaculture 155: 129-134.

Kanazawa, A., S. Teshima, and M. Sakamoto. 1985. Effects of dietary bonito-egg phospholipids and some phospholipids on growth and survival of the larval ayu *Plecoglossus altivelis*. Z. Angew. Ichthyol. 4: 165-170.

Koven, W. M., G. M. Kissil, and A. Tandler. 1989. Lipid and n-3 requirement of *Sparus aurata* larvae during starvation and feeding. Aquaculture 79: 185-191.

Mourente, G. and D. R. Tocher. 1992. Effects of weaning onto a pelleted diet on docosahexaenoic acid (22:6*n*-3) levels in brain of developing turbot (*Scophthalmus maximus L.* ). Aquaculture 105: 363-377.

Ostrowski, A. C. and S. Divakaran. 1990. Survival and bioconversion of *n*-3 fatty acids during early development of dolphin (*Coryphaena hippurus*) larvae fed oil-enriched rotifers. Aquaculture 89: 273-285.

Witt, U., C. Quantz, D. Kuhlman, and G. Kattner. 1984. Survival and growth of turbot larvae *Scophthalmus maximus* L. reared on different food organisms with special regard to organisms polyunsaturated fatty acids. Aquacult. Eng. 3: 177-190.

# EFFECTS OF LOW SALINITY ON GROWTH AND SURVIVAL OF SOUTHERN FLOUNDER (*PARALICHTHYS LETHOSTIGMA*) LARVAE AND JUVENILES

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## ABSTRACT

The Southern flounder (*Paralichthys lethostigma*) is a euryhaline flatfish with a natural range that extends from North Carolina to Mexico. Since adult flounder are commonly found in freshwater sounds and rivers, it appears that there is potential for culture in fresh water. A series of studies was conducted to determine the effects of low salinity on growth and survival of flounder from metamorphosis through the advanced juvenile stage. Survival of larval flounder was significantly lower ($P<0.05$) when exposed to salinities below 20 ppt during metamorphosis, but postmetamorphic flounder were not adversely affected by salinities as low as 0 ppt. Two separate studies were conducted to determine the growth rate and survival of Southern flounder stocked in low salinity water and fed pelleted feed. In the first study, juvenile flounder with an average weight of approximately 7.0 g were grown in salinities of 0, 5, and 10 ppt water for 84 days. In the second study, advanced juvenile flounder with an average weight of approximately 32.0g were grown in salinities of 0, 5, 10, and 20 ppt for 58 days. The specific growth rate (SGR) of juvenile flounder ranged from 1.0 to 1.09%/day and was not significantly different among treatments. SGR of advanced juvenile flounder ranged from 1.6 to 1.71%/day and was not significantly different between treatments ($P>0.05$). Protein efficiency ratio, feed conversion efficiency (FCE) and daily feed consumption (DFC) values were not significantly different ($P>0.05$) between treatments. These results indicate that Southern flounder can be grown in salinities as low as 0 ppt within days after completing metamorphosis without affecting growth or survival.

## INTRODUCTION

The salinity of culture water is a critical parameter that directly affects fish growth. Fish that expend energy in osmoregulation to compensate for extremely high or low saline conditions have less energy available for growth (Grau et al. 1994). Defining the salinity range needed for optimum growth is important for achieving maximum performance of cultured fish.

The Southern flounder (*Paralichthys lethostigma*) is a euryhaline flatfish with a natural range that extends from North Carolina to northern Mexico. There is considerable interest in the potential culture of Southern flounder because of its high market value and apparent tolerance of low salinities. Postmetamorphic Southern flounder are commonly caught in freshwater sounds and rivers (Reagan and Wingo 1985). Premetamorphic Southern flounder have been found in salinities as low as 17 ppt (Burke et al. 1991), and juvenile Southern flounder appear to spend most of their time in water at less than 20 ppt salinity (Stokes 1977). Flounder only migrate out to the ocean to spawn once they reach sexual maturity. Culturing flounder in low salinity water offers several advantages for US mariculture; 1) the facilities for flounder culture can be located away from high-cost coastal land, 2) competition for limited coastal space and water resources is reduced thereby

lessening multi-user conflicts, and 3) disease causing parasites such as *Amylodinum* sp. and toxic algae such as *Pfisteria piscicida* can be avoided in water with less than 3 ppt salinity. However, little information is available on the growth performance of Southern flounder in low salinity water. This information would be useful for evaluating the potential for raising flounder in inland areas.

## MATERIALS AND METHODS

Four studies were conducted on different life stages of Southern flounder to determine the effects of low salinity on growth and survival.

Hatchery-reared larvae were obtained from strip-spawned broodstock (Berlinsky et al. 1996) and cultured according to methods described by Daniels et al. (1996). Larvae (day 25) were stocked into 20-L glass aquaria with water containing 30 ppt salinity at a density of 1 fish/L. The salinity of the water was gradually reduced over a 5-day period with fresh water from a well (200 mg/L total hardness and 350 mg/L total alkalinity) until target salinity levels of 0, 10, and 20 ppt were reached. Three replicates were used per treatment. Fish were harvested, measured to the nearest 0.5 mm and counted on day 60 posthatch.

Recently metamorphosed flounder (day 60 posthatch) were stocked at a density of 1/L into 20-L aquaria containing water with 30 ppt salinity. The salinity levels were abruptly reduced within a six hour period to 0 ppt by replacing saline water with fresh water. Aquaria were harvested after 5 days and fish were counted to determine survival.

Southern flounder juveniles weighing approximately 5 g were caught by trawl in the Pamlico Sound and weaned onto pelleted feed over a three-week period. Fish were then stocked into nine, 10-L, plastic tanks at a density of eight fish per tank containing water with 30 ppt salinity. Salinities were gradually lowered to 0, 5, and 10 ppt over a two-week period by replacing saline water with fresh water. Three replicates were used per treatment. Tanks at each salinity were in a separate closed recirculating system. Temperature was maintained at 20 C. Fish were fed a commercial extruded pelleted feed (42% protein; Southern States, Farmville, North Carolina, USA) twice daily at a total of 4% body weight. Fish were weighed weekly to the nearest 0.1 g then harvested after 84 days.

Advanced juvenile flounder weighing approximately 30 g each were stocked into nine 10-L plastic tanks containing water with 30 ppt salinity at a density of three fish per tank. Salinities were reduced to 0, 5, 10, and 20 ppt over a 1-week period by replacing seawater with fresh water. Each treatment had three replicates in separate closed recirculating systems. Water temperature was maintained at 25 C with heat pumps (Aquanetics model AHP-D). Fish were fed twice daily with a commercial pelleted feed (55% protein; Corey Feed Mill, New Brunswick, Canada) at a daily feed rate of 3% of body weight. One hour after each feeding uneaten pellets were counted and removed to estimate feed consumption. Fish were weighed weekly to the nearest 0.1 g and harvested after 58 days.

## RESULTS AND DISCUSSION

Tolerance to low salinity increased as soon as fish completed metamorphosis. Fish exposed to salinities of 0 to 10 ppt during metamorphosis had significantly lower survival than those in the 20 and 30 ppt treatments (Table 1). All the fish in the 0 ppt treatment died within 24 h of reaching this salinity. But postmetamorpic flounder at day 60 posthatch were able to withstand an abrupt drop in

| Salinity (ppt) | Survival (%) | Standard length (mm) |
|---|---|---|
| 0 | 0a | --- |
| 10 | 29b | 12.5a |
| 20 | 59c | 11.1a |
| 30 | 52c | 11.2a |

Means followed by different letters between groups or treatements are significantly different ($P<0.05$).

**Table 1.** Mean percent survival and final length of southern flounder *Paralichthys lethostigma* exposed to different salinities during metamorphosis.

| Initial Salinity (ppt) | Survival (%) |
|---|---|
| 10 | 96 |
| 20 | 100 |
| 30 | 80 |

**Table 2.** Mean percent survival of postmetamorphic southern flounder *Paralichthys lethostigma* exposed to 0 ppt salinity after a 6-h acclimation period.

salinity from 30 ppt to 0 ppt within a six-hour period without a significant ($P>0.05$) reduction in survival when compared to fish in the other treatments (Table 2). Fish in all treatments showed few signs of stress after the rapid acclimation; most fish were feeding and swimming actively within a few hours of reaching 0 ppt salinity. Burke et al. (1991) reported that premetamorphic Southern flounder were found in North Carolina sounds and estuaries in salinities as low as 17 ppt, hence the survival of some fish at 10 ppt is to be expected. Lasswell et al. (1977) reported survival rates of 100% for postmetamorphic flounder exposed to 0 ppt salinity after only a three hour acclimation period, so the high survival rates observed in this study are not surprising.

The results of the two growth trials at low salinities showed similar trends (Tables 3 and 4). Growth and feed conversion efficiency were not significantly different ($P>0.05$) between any of the salinities although total weight gain for juvenile and advanced juveniles was slightly higher in the 10 ppt treatment. Survival was not affected by the long-term exposure to low salinities in either of the studies. The mortality of advanced juvenile fish in the 5 ppt treatment was caused by a mechanical failure that resulted in a loss of water circulation to some of the test containers. With the exception of the loss of this one container of fish, there were no mortalities during either of these studies.

These results indicate that salinities as low as 0 ppt are as effective as higher salinities on growth and survival of postmetamorphic Southern

| | Salinity (ppt) | | |
|---|---|---|---|
| Variable | 0 | 5 | 10 |
| Initial wt (g) | 7.7 | 6.8 | 7.5 |
| Final wt (g) | 17.0 | 17.0 | 20.0 |
| Gain (g) | 10.0 | 10.2 | 12.5 |
| Survival (%) | 96 | 96 | 100 |
| Specific growth rate (%/day) | 1.1 | 1.1 | 1.2 |
| Protein efficiency ratio[1] | 1.6 | 2.2 | 2.0 |
| Feed conversion efficiency (%)[2] | 67 | 91 | 83 |
| Daily feed consumption (% bw/day) | 1.3 | 1.1 | 1.4 |

[1] Protein efficiency ration (weight gain/dietary protein intake)
[2] Feed conversion efficiency (weight gain/feed intake x 100)

**Table 3.** Production variable for juvenile southern flounder *Paralichthys lethostigma* grown at different salinities during an 84-day period.

| Variable | Salinity (ppt) 0 | 5 | 10 | 20 |
|---|---|---|---|---|
| Initial wt (g) | 33.4 | 32.2 | 32.8 | 30.6 |
| Final wt (g) | 71.6 | 70.4 | 78.5 | 67.2 |
| Gain (g) | 38.2 | 38.2 | 45.7 | 36.6 |
| Survival (%) | 1.6 | 1.6 | 1.7 | 1.6 |
| Specific growth rate (%/day) | 100 | 66 | 89 | 100 |
| Protein efficiency ratio[1] | 1.3 | 1.2 | 1.5 | 1.3 |
| Feed conversion efficiency (%)[2] | 61 | 58 | 70 | 63 |
| Daily feed consumption (% bw/day) | 2.0 | 2.2 | 1.9 | 2.0 |

[1] Protein efficiency ration (weight gain/dietary protein intake)
[2] Feed conversion efficiency (weight gain/feed intake x 100)

**Table 4.** Production variable for advanced juvenile southern flounder *Paralichthys lethostigma* grown at different salinities during a 58-day period.

flounder. We found considerable differences in growth of fish within each treatment which may reflect genetic variability inherent to wild populations. Further research is needed using a greater number of fish per treatment to determine if the slight differences in feed consumption and conversion efficiency at 0 ppt can be detected. Comparison of the growth/salinity relationship among wild-caught and domesticated fish should also be explored. Taken together, these investigations clearly indicate that Southern flounder larvae must be cultured in seawater but postmetamorphic flounder can be maintained in fresh water without hindering their rate of growth or feed conversion efficiency.

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## LITERATURE CITED

Arnold, C. R., W. H. Bailey, T. D. Williams, A. Johnson and J. L. Lasswell. 1977. Laboratory spawning and larval rearing of red drum and Southern flounder. Proceedings of the Annual Conference of Southeastern Association of Fish and Wildlife Agencies 31:437-440.

Berlinsky, D. L., W. King V, T. I. J. Smith, R. D. Hamilton II, J. Holloway, Jr. And C. V. Sullivan. 1996. Induced ovulation of Southern flounder *Paralichthys lethostigma* using gonadotropin releasing hormone analogue implants. Journal of the World Aquaculture Society 27 (2):143-152.

Burke, J. S., J. M. Miller and E. E. Hoss. 1991. Immigration and settlement pattern of *Paralichthys dentatus* and *P. lethostigma*

in an estuarine nursery ground, North Carolina, U.S.A. Netherlands Journal of Sea Research 27:393-405.

Daniels, H. V., D. L. Berlinsky, R. G. Hodson and C. V. Sullivan. 1996. Effects of stocking density, salinity, and light intensity on growth and survival of Southern flounder *Paralichthys lethostigma* larvae. Journal of the World Aquaculture Society 27(2):153-159.

Grau, E. G., N. H. Richmon, and R. J. Borski. 1994. Osmoreception and simple endocrine reflex of the prolactin cell of *Oreochromis mossambicus*. Proceedings XIII Intl. Cong. Comp. Endocrinal. In "Perspectives in comparative endocrinology". Eds. K. G. Davey, R. E. Peter, and S. S. Tobe, NRCC, Ottowa. pp 251-256.

Lasswell, J. L., G. Garza and W. H. Bailey. 1977. Status of marine fish introductions into freshwater of Texas. Texas Parks and Wildlife Department. PWD Report No. 3000-35.

Reagan, R. E., Jr. And W. M. Wingo. 1985. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico) - southern flounder. U.S. Fish and Wildlife Service Biological Report 82(11.30). U.S. Army Corps of Engineers TR EL-82-4.9.

Stokes, G. M. 1977. Life history studies of Southern flounder (*Paralichthys lethostigma*) and gulf flounder (*P. albigutta*) in the Aransas Bay area of Texas. Tx. Parks Wildl. Dept. Tech Ser. 25.

Zar, J. H. 1984. Biostatistical analysis, 2nd edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA.

# AN IMAGE ANALYSIS APPROACH TO DETERMINE MICROPARTICULATE FEED ACCEPTABILITY BY LARVAL FISH

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## ABSTRACT

Methods were developed to determine dietary preferences (acceptability) by first-feeding larval fish during a single feeding event. These methods involved: (1) determining the feeding incidence and (2) measuring the cross-sectional optical area of the bolus using image analysis. Both methods followed a short, defined feeding period. Both methods were used to determine spray-dried feedstuff preferences for larval zebrafish *Brachydanio rerio* and microparticulate diet formulation preferences for larval goldfish *Carassius auratus*. Feeding incidence was 100% for all diets with both species; however, diets differed significantly ($p<0.05$) in mean bolus size, indicating that larvae of these two species vary feeding rates with diet type even when diets are similar in formulation and manufacturing process.

## INTRODUCTION

A key impediment to intensive rearing of many altricial larvae of marine and freshwater fish is the lack of high quality microparticulate diets that are acceptable, digestible, and which meet the nutritional needs of the larvae. As aquaculture and fishery enhancement efforts grow, this impediment will become more acute, especially in the marine fish culture industry.

There are a variety of feeding strategies currently used for first-feeding larval and juvenile fish. These strategies include use of live diets, formulated diets, or a combination of live and formulated diets. Intensive systems often rely on cultured or wild caught live feeds for rearing fish larvae in tanks. Available live foods for intensive culture are limited to those which can be easily reared or captured from the wild. The most common live feed cultured for larval fishes is the brine shrimp *Artemia* (Lavens et al. 1986). Although *Artemia* is adequate for some fishes (Lavens et al. 1986), it is nutritionally deficient for many other species (Dendrinos and Thorpe 1987). Culture of zooplankton for feeding fish larvae is labor intensive, expensive, and prone to sudden "crashes." The uncertainty and expense of live diets provide motivation to develop formulated diets that are nutritionally complete, highly digestible, palatable, inexpensive, and easy to feed.

Microparticulate diets that are uniform both in size and nutritional quality can dramatically increase the rearing success of species such as lake whitefish, *Coregonous clupeaformis* (Zitzow and Millard 1988), carp, *Cyprinus carpio* (Lubzens et al. 1984), smallmouth bass, *Micropterus dolomieui* (Ehrlich

et al. 1989), and muskellunge, *Essox masquinongy* (Zitzow 1986). Survival in production hatcheries of larval walleye *Stizostedion vitreum* fed only microparticulate diets averaged approximately 60% after 30 days (Barrows and Ellis 1996). Striped bass, *Monone saxatilis*, has not been successfully reared on any formulated diet. In spite of success with some species fed exclusively microparticulate diets, survival during the early larval period generally has not been as good as when larvae are fed on live diets.

Our understanding of larval fish feeding is limited. Currently available techniques that have been used with larger fish to determine feeding responses are not appropriate for use with larvae. Elucidation of the developmental sequence of feeding response and the development of methods to assess the nutritional needs of larval fish are important to the scientific community, feed companies, hatcheries, fishery managers, and aquaculturists. The differences in success among species fed solely microparticulate diets, and between live and microparticulated diets for a given species, may be related to differences in diet acceptability, digestibility, or composition. Before microparticulate diet digestibility or nutrient composition studies can proceed, the diet must first be ingested by a high percentage of the larvae in the tank. There is a need for a method to distinguish among dietary treatments that is quick and not compromised by cannibalism and low survival rates common to larval feeding trials.

Effective microparticulate diets need to: (1) efficiently retain small, soluble nutrients after the particles are suspended in water; (2) possess physical and chemical characteristics that result in their ingestion by fish larvae; (3) be readily digested and assimilated by larvae; and (4) consist of an optimal nutrient composition for maximum larval survival, development, and growth. Before nutrient digestion can occur, microcapsules must first be ingested by the larval fish.

This research describes a method to differentiate among microparticulate diets based upon the amount of diet ingested (degree of fullness) by larval fish over a short time frame. This method will help to develop microparticulate diets that satisfy the second aspect of an effective microparticulate diet listed above. Although the method was developed with zebrafish *Brachydanio rerio* and goldfish *Carassius auratus*, methods are applicable to other species with transparent larvae. The method is illustrated with two experiments: the first to determine feedstuff preferences for larval zebrafish, and the second to define the optimal krill meal: fish meal ratio for larval goldfish.

## MATERIALS AND METHODS

Near first-feeding, 6-day post-hatch (3.5 mm) zebrafish or 9-day post-hatch (9.0 mm) goldfish larvae, both produced in our laboratory, were selected at random from holding tanks and stocked five larvae/tank into clear plastic tanks containing 100 ml of 5 µm filtered culture water. All tanks were then placed in a water bath held at 28°C. Larvae which had been feeding were left without food overnight to allow any residual feed to pass through the gut prior to the start of each trial.

Three tanks were randomly assigned to each dietary treatment. The zebrafish trial used a commercial diet (FFKB-250, Kyowa Hakko Kogyo Co., Ltd, Tokyo, Japan) known to produce good growth and survival as a positive control; unfed larvae served as a negative control. For the goldfish trial, live *Artemia* were used as the positive control and unfed larvae served as the negative control. Zebrafish test diets were spray-dried (approximately 100 µ average size) chicken meal, egg, or liver (American Dehydrated Foods, Inc., Springfield, MO). The experimental diets for goldfish varied in the krill meal and fish meal content (Table 1). Krill meal varied in 10% increments from 14% to 54%, while herring meal varied from 6% to 46% of the diet.

The diets fed to the goldfish were produced using the micro-extrusion/marumerization (MEM) method (Barrows et al. 1993). Marumerization is a process of shaping and smoothing an extrudate achieved by using a cylindrical machine in which the bottom of the cylinder rotates at very high speeds. The rotational forces within the machine result in a smoothing and densification of the surface of the extrudate. This process involves two pieces of equipment for the production of particles. An LCI, Inc. system (Charlotte, NC) included a radial discharge (EXDC(F)S-60)

extruder and a QJ-400 marumerizer. All ingredients were combined and mixed in a Marion ribbon mixer prior to addition of the fish oil. Thirty-two percent water was added to the mix before extrusion through a 500-μm screen. The mash was extruded at an auger speed of 19 rpm to form wet noodles. These noodles were then placed in the marumerizer which consists of a cylindrical chamber with a rotating plate on the bottom. The plate was grooved to impart energy from the marumerizer to the feed. This energy breaks the noodles, reshapes, and densifies the particles. The marumerizer is equipped with a variable speed motor to allow for a range from 300 to 1210 rpm. The noodles of all diets were first processed at 1060 rpm for 10 sec followed by about 90 sec at 500 rpm. The shaped particles were then placed in an ambient temperature (about 17°C) forced-air dryer until moisture levels were less than 10%. Moisture was determined using a 30-minute cycle of 125°C on an Ohaus MB 200 moisture analyzer. The feed was then sifted to the proper sizes and stored in nitrogen-flushed, vacuum-packed plastic bags and stored at room temperature until used.

Each tank was then fed 0.1 g of the appropriate feed. After 1.5 h, the larvae were anesthetized with MS-222 (Massee et al. 1995) and videotaped using a dissecting microscope with a video recording system. Larvae were oriented on their sides so that the bolus was visible through the transparent larvae as a cross-section. A stage micrometer was positioned so that a readable section of the micrometer was visible in each image.

Each image on the tape was then printed and the cross-sectional area of the bolus, and pre-flexion (standard) length was measured. Cross-sectional area of the bolus provides an index of the amount of feed ingested by each larva. Cross-sectional optical areas were determined using a planimeter. The appropriate conversion factor for each measurement was determined by measuring the image of a 1-mm$^2$ area on the micrometer coverslip which was in view in each of the printed images. Data was reported as the cross-sectional optical area of material in the gut (mm$^2$). Statistical significance was determined using analysis of variance and means separated using Fisher's Protected Least Difference Significant (PLSD) method (Zar 1984).

Feeding incidence was determined for each dietary treatment by counting the number of fish in each image with and without feed visible in the gut and expressing the ratio as a percentage feeding. No further analysis of feeding incidence data was undertaken as all larvae fed diets had visible feed in their guts after 1.5 h of feeding.

## RESULTS

For both species, feeding incidence was 100% for all treatments receiving diets, while feed consumption differed significantly among diets. In the zebrafish trial (Fig. 1), spray-dried egg (0.141 ± 0.016 mm$^2$, mean ± standard error) and chicken (0.138 ± 0.014 mm$^2$) were consumed at significantly higher rates ($p<0.05$) than the liver (0.104 ± 0.012 mm$^2$) or positive control diets (0.098 ± 0.007 mm$^2$). Significant differences ($p<0.028$) were found between all groups and the negative control group (0.002 ± 0.007 mm$^2$).

In the goldfish trial (Fig. 2), acceptability of live *Artemia* (0.81 ± 0.01 mm$^2$) was significantly greater ($p<0.05$) than all other diets. The diets containing 46% fish meal (0.59 ± 0.05 mm$^2$) were significantly more acceptable than diets containing less than 26% fish meal. The diet containing 36% fish meal (0.49 ± 0.04 mm$^2$) was not significantly different than any other fish meal-containing diet.

## DISCUSSION

Zebrafish larvae consumed all three spray-dried products at levels equal to or greater than the commercial diet, indicating potential for spray-dried products as feedstuffs for larval fish diets. Further trials using other species and other feedstuffs are needed to determine if these materials are widely acceptable. By testing the same diets with other species, it will be possible to determine if zebrafish are a suitable surrogate for other hard-to-obtain species. Once highly palatable feedstuffs are identified, then it will be easier to formulate diets that are highly acceptable.

Goldfish larvae preferred diets high in fish meal and low in krill meal. These results appear to be in contrast to survival data obtained using the same diet formulations with larval walleye (Barrows 1994). Larval walleye fed diets

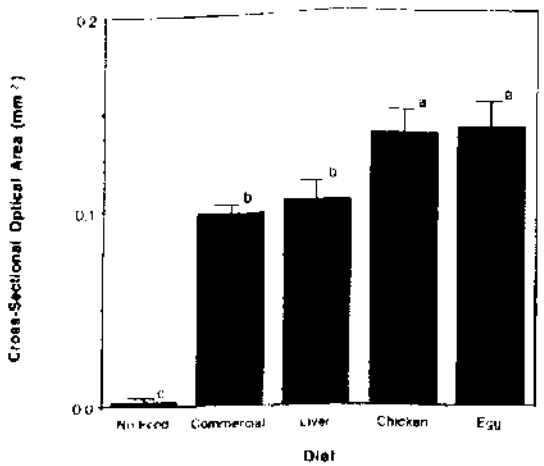


**Figure 1.** Mean cross-sectional area of the bolus in larval zebrafish fed spray-dried microparticulate diets over 1.5 h. Data are means ± standard error (n=3). Treatments with different subscripts are significantly different ($p<0.05$, Fisher PLSD method).

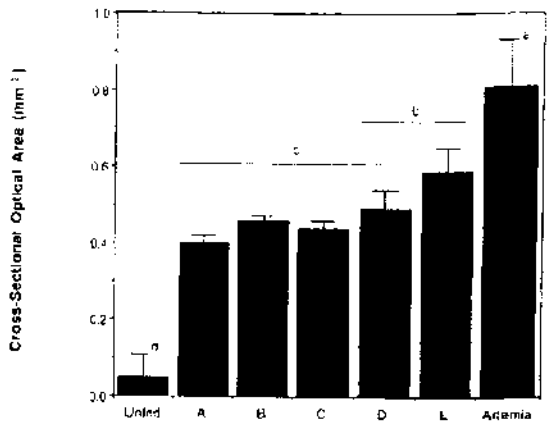


**Figure 2.** Mean cross-sectional area of the bolus in larval goldfish fed micro-extruded diets (see Table 1 for formulations) with varying levels of fish meal replacing krill meal fed over 1.5 h. Diet formulations (A,B,C,D,E) are given in Table 1 with diet A containing the most krill meal and diet E the least. Data are means ± standard error (n=3). Treatments with different subscripts are significantly different ($p<0.05$, Fisher PLSD method).

**Table 1.** Ingredient composition of experimental starter diets fed to goldfish.

| Ingredient | A | B | C | D | E |
|---|---|---|---|---|---|
| Krill meal[a] | 54.0 | 44.0 | 34.0 | 24.0 | 14.0 |
| Herring meal[a] | 6.0 | 16.0 | 26.0 | 36.0 | 46.0 |
| Egg solid[b] | 24.4 | 24.4 | 24.4 | 24.4 | 24.4 |
| Yeast extract[c] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Cod liver oil[c] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Binder, TIC 515[d] | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Vitamin Premix 9430[e] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Lecithin[c] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Liver extract[c] | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Betaine[c] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Ascorbic acid[c] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Trace Mineral premix[f] | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Inositol[c] | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |

[a]International Proteins Corporation, New York, NY
[b]International Ingredient Corporation, St. Louis, MO
[c]Amersham Life Sciences, Arlington Heights, IL
[d]TIC Gums Inc., Belcamp, MD
[e]Contributed per kg of diet: vit A, 1000 IU; vit D3, 720 IU; vit E, 530 IU; vit B12, 30 µg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; folacin, 13 mg; menadione sodium bisulfate, 25 mg; biotin, 1 mg; niacin, 330 mg.
[f]Contributed per mg/kg of diet: zinc, 100; manganese, 70; iron, 3; copper, 2; iodine, 1.

containing krill meal levels as low as 24% had survival rates equivalent to fish fed diets containing 54% krill. Reducing the krill content of the diet to 14%, with 46% herring resulted in a decrease in survival. Survival was greater for the fish fed the four high krill meal diets than fish fed a commercial larval diet. Barrows (1994) suggested a beneficial effect of including at least 24% krill in larval walleye diets. It could not be determined in that 30-day feeding study if the effect was nutritional or due to the acceptability of the diet. Combining feeding incidence and bolus measurement data with the survival data would have been beneficial and may have been able to pinpoint the reason for the differential survival.

Differences among dietary treatments could not be determined with feeding incidence data for either species (all except the unfed treatments were 100%). While the feeding incidence method provides a coarse evaluation of diet acceptability, it does not work when feeding incidence is uniformly high, such as was the case with zebrafish and goldfish. Measurement of the bolus provided an index which was more sensitive to smaller differences in diet acceptability. Conversely, under conditions where feeding incidence is low or variable, measurement of the bolus may not provide meaningful data. This is because samples consist only of feeding larvae and are not a good representation of all the larvae being fed.

Both methods are useful to determine diet acceptability over a very short time. Studies that determine differences over a short time are not compromised by high mortality rates common to larval feeding trials. Since diet composition and nutrient bioavailability are eliminated as potential causes for mortality or poor growth, acceptability trials using feeding incidence and/or bolus size as indices can yield meaningful data with species that cannot currently be cultured intensively.

The method for determining bolus size lends itself to computer-aided image analysis. We have successfully measured the bolus cross-sectional area in larvae using a Macintosh 8100AV (Apple Computers, Cupertino, CA) computer with NIH-image software. NIH-image is a free software package developed by the National Institutes of Health and is available at their web site ( http://rsb.info.nih.gov/nih-image/). Other programs and computer systems can also be used.

An additional benefit of using a computer-aided measurement system is that the cross-sectional area can be rotated in space to produce an estimate of volume. For a more accurate estimate of volume, a standard curve can be produced by intubating a known volume of colored liquid into the larval gut (Rust et al.1993a, b) and determining the cross-sectional area of the liquid droplet contained within the gut. The resulting regression will describe the relationship between cross-sectional area and bolus volume for a given species at a given developmental stage. This information may be useful for determining consumption and developing bioenergetic models.

No quantitative requirement for any nutrient has yet been determined for the larval stage of any species of fish. Diets are formulated based upon the composition of the fish larvae or the composition of zooplankton. Unfortunately, this approach assumes that the bioavailability of dietary nutrients are equal, an assumption that does not hold for altricial larvae (Rust et al. 1993c, Rust 1995). Quantitative and qualitative nutrient requirements for larval fish will be difficult to determine until a microparticulate test diet that is highly acceptable to larval fish is developed. The first step toward determining requirements may be to develop such a test diet using the methods outlined here.

Vision and chemoreception are the two most important sensory systems used by first-feeding larvae to locate and ingest food (Blaxter 1988, Noakes and Godin 1988). In order for microparticulate diets to be ingested, they must be attractive and visible to the larvae and must be presented under the proper environmental conditions. Fish larvae are primarily visual feeders, though taste buds and olfaction are often also functional at this time in most species (Noakes and Godin 1988). The microparticulate diet acceptability methods developed provide means to determine optimal environmental (light) and chemical (taste, olfaction) properties for successful larval feeding.

Once optimal feeding conditions are defined and a highly acceptable microparticulate test diet is available for the larvae of a species, then work can proceed more quickly on

determination of requirements. It is necessary to first (1) ensure that the feeds we are developing are being eaten by the larvae, and (2) understand the digestibility of those diets, before drawing conclusions as to larval nutrient requirements.

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## LITERATURE CITED

Barrows, F. T. 1994. Walleye research summary; 1993 season. Annual Progress Report: Fisheries and Federal Aid Project, USFWS, Denver, CO.

Barrows, F. T. and W. A. Ellis. 1996. Diet and nutrition, pp. 315-321. *In:* R. C. Summerfelt (ed.), Walleye Culture Manual. NCRAC Culture Series 101. North Central Regional Aquaculture Center Publications Office, Iowa State Univ., Ames.

Barrows, F.T., R.E. Zitzow, and G.A. Kindschi. 1993. Effects of surface water spray, diet and phase feeding on swim bladder inflation, survival and cost of production of intensively reared larval walleyes. Prog. Fish-Cult. 55: 224-228.

Blaxter, J. H. S. 1988. Pattern and variety in development, pp. 1-84. *In:* W. S. Hoar and D. J. Randall (eds.), Fish Physiology, Vol XI, A. Academic Press, San Diego.

Dendrinos, P. and J. P. Thorpe. 1987. Experiments on the artificial regulation of the amino acid and fatty acid contents of food organisms to meet the assessed nutritional requirements of larval, post-larval, and juvenile Dover sole (*Solea solea*). Aquaculture 61: 121-154.

Ehrlich, K. F., M. C. Cantin, M. B. Rust, and B. Grant. 1989. Growth and survival of larval and postlarval smallmouth bass fed a commercially prepared dry feed and/or *Artemia* nauplii. J. World Aquacult. Soc. 20: 1-6.

Lavens, P., P. Sorgeloos, and A. Flores Tom. 1986. Producion de biomasa, quistes y nauplios de *Artemia* en sistemas a flojo constante en alta densidad, pp. 229-247. *En:* Memorias del primer Curso sobre *Artemia* y su uso en camaronicultura. Instituto techológico del Mar, Guaymas, Son, Mexico.

Lubzens, E., G. Sagie, G. Minkoff, E. Meragelman, and A. Schneller. 1984. Rotifers (*Brachionus plicatilis*) improve growth rate of carp (*Cyprinus carpio*) larvae. Bamidgeh 36: 41-46.

Massee, K. C., M. B. Rust, R. W. Hardy, and R. R. Stickney. 1995. The effectiveness of tricaine, quinaldine sulfate and metomidate as anesthetics for larval fish. Aquaculture 134: 351-359.

Noakes, D. L. and J-G. J. Godin. 1988. Ontogeny of behavior and concurrent developmental changes in sensory systems in teleost fishes, pp. 345-384. *In:* W. S. Hoar and D. J. Randall (eds.), Fish Physiology, Vol XI, B. Academic Press, San Diego.

Rust, M. B., 1995. Quantitative aspects of nutrient assimilation in six species of fish larvae. Ph. D. dissertation, Univ. Washington, Seattle. 147 p.

Rust, M. B., R. W. Hardy, and R. R. Stickney. 1993a. How to force-feed fish larvae (video). Washington Sea Grant Publ. No. WSG-AV-93-01.

Rust, M. B., R. W. Hardy, and R. R. Stickney. 1993b. A new method for force-feeding larval fish. Aquaculture 116: 341-352.

Rust, M. B., R. W. Hardy, and R. R. Stickney. 1993c. Assimilation of three biochemical forms of radio-labeled methionine in four species of fish larvae, p. 261. (Abstract). *In:* M. Carrillo, L. Dahle, J. Morales, P. Sorgeloos, N. Svennevig, and J. Wyban (compilers), From Discovery to Commercialization. European Aquaculture Society Publ. No. 19.

Zar, J. H. 1984. Biostatistical Analysis. Prentice-Hall International, London, UK. 718 p.

Zitzow, R. E. 1986. Survival and growth of larval Muskellunge (*Esox Masquinongy* ) initially fed formulated diets. Information Leafl. No. 86-106, US Fish Wildl. Serv., Valley City, ND.

Zitzow, R. E. and J. L. Millard. 1988. Survival and growth of lake whitefish (*Coregonus clupeaformis*) larvae fed only formulated dry diets. Aquaculture 69: 105-113.

# FISH CAGE PHYSICAL MODELING FOR SOFTWARE DEVELOPMENT AND DESIGN APPLICATIONS

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## ABSTRACT

Fish cage response to waves was investigated using a physical model in the University of New Hampshire wave tank. The tank was built with below-waterline windows placed for convenient observation of moored cage models. Cage motion was measured using an optical system comprised of a high resolution video camera, a frame grabber, and a computer with expanded random access memory (RAM). Targets on the cage, consisting of two black painted dots on a white background, were tracked using image processing software. The system was, therefore, noninvasive to the fluid environment and did not alter the cage inertial characteristics. Specific experiments were done in support of a computer modeling effort which has resulted in a finite element program for fish cage dynamics. Experimental data was obtained using a physical model reduced in complexity in order to focus on basic parameters. Comparison of tank data with computer predictions indicated that the computer simulation reproduced the fundamental features of the observed cage motion.

## INTRODUCTION

Physical and computer models are essential tools for the design of offshore net pen systems. To avoid failure, net pens and their moorings must be engineered to withstand both severe storm events and the cumulative effects of long-term wave and current loading. We developed methods for testing physical models in the new University of New Hampshire (UNH) wave tank. The experimental methodology was then used to generate data for comparison with recently developed finite element computer models of fish cage response to waves and current.

The new UNH wave tank was designed and built with offshore aquaculture applications in mind. The tank itself and the building housing this and other facilities were constructed in 1994, while the wavemaking system was added in 1996 as described by Washburn (1996). The next step was to incorporate a measurement system for physical model motion response. In the study described here, this need was realized using an optical system. This strategy was chosen because it offered precision measurements without altering the fish cage dynamics.

The physical modeling approach complemented the UNH finite element computer programming effort which resulted in a net pen dynamics program. As demonstrated by Gosz et al. (1996), the program can be used to predict cage movement and structural loads for user-specified wave and current environments. To increase confidence in its predictions, however, it was determined that an experimental program should be set up to generate specialized, empirical cage

motion data for comparison with the finite element model predictions. The objectives of this work may, therefore, be summarized as:

- Development of test tank methods for fish cage experiments in the UNH wave tank;
- Implementation of an optical system for cage motion measurement;
- Obtaining specialized data in support of net pen computer modeling;
- Comparison with predictions from the existing UNH finite element program.

These objectives were addressed making use of unique features of the tank. The optical measurement system was positioned opposite built-in observation windows located halfway along the length of the tank. Fish cage physical models were then conveniently moored for clear viewing. For the software development application, the cage physical model was simplified to focus on major components and basic dynamic processes. The computer program was applied directly to the physical cage model at its actual size, with no potentially error-producing changes in scale. The evaluation was done by comparing displacement as a function of time for key points on the cage.

## UNH WAVE TANK

The wave tank is 36.6 m long, 3.66 m wide, and 3.05 m deep. It is usually filled with 2.44 m of water. A tow carriage is supported and cable-driven along a single main rail on one side (Darnell, 1996). A lightweight, protected outrigger supports the carriage on the opposite side which is reserved for observers. A hydraulically driven, computer controlled, flap-type wavemaker is at one end (Washburn, 1996). Software allows the user to run a regular wave of specified height and frequency or a random sea of specified spectrum. Waves are dissipated at the opposite end using a vertical "beach" consisting of vertical layers of geotechnical cloth suspended from an angled fiberglass frame.

Midway down the observer side, a pit allows access to two side windows in the wall—one covering the waterline and upper water column and the second placed just above the floor of the tank. A mid-width floor window can also be used from a tunnel beneath the tank which is entered

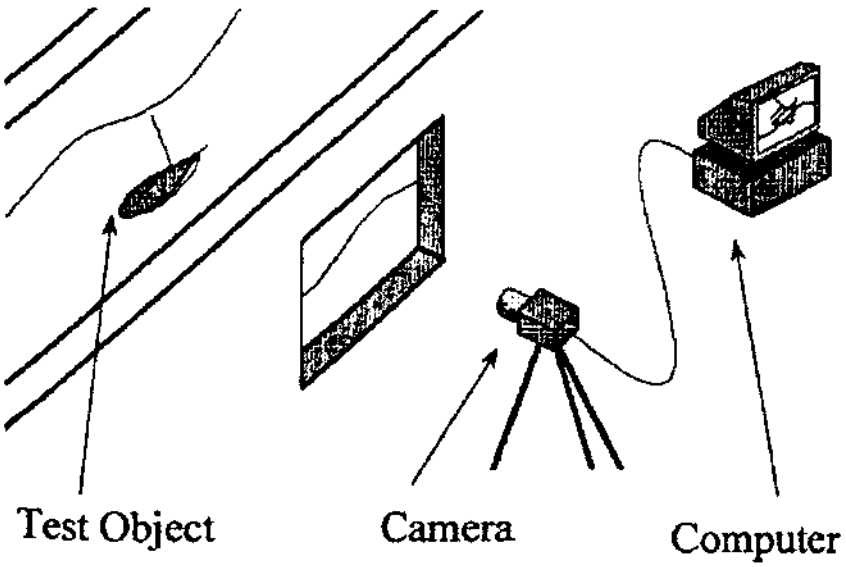


**Figure 1.** Schematic of optical measurement system.

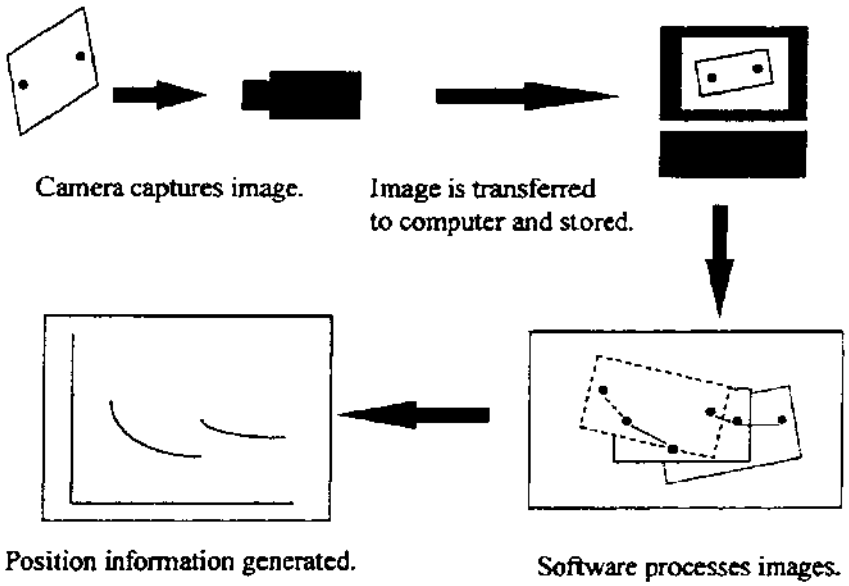


**Figure 2.** Information flow in the optical measurement system.

from the base of the pit. These windows are very convenient for viewing the net pen system experiments and allow optical monitoring of motion variables.

**Optical measurement system**

An optical measurement system for determining test object motions was developed to take advantage of the observation window opportunities. By using a noninvasive optical system, no dynamic altering sensors are attached to the test object. Figure 1 shows how images of the test object are captured by a camera and fed into a computer for analysis. The essential components of the present UNH system (see Michelin and Stott, 1996) include:

- A high resolution, black and white Pulnix video camera which can operate at 30 frames/second;
- A frame grabber to transfer the images to a computer;
- A personal computer with expanded random access memory (RAM);
- Software to analyze the stored sequence of images.

The steps involved in motion measurement begin, as indicated in Figure 2, with establishing a target on the test object. Two small black dots on a white background are painted on the test object. Horizontal, vertical, and angular changes (planar motion) can be inferred from the movement of the spots. The UNH system has been successful in resolving the gray-scale contrast between black dot and light background, so the use of potentially error-producing light sources on the test object is unnecessary. The camera captures a sequence of images and transfers each frame, via the frame grabber, to the computer for temporary storage. Later, specially written software is used to search each frame for the gray-scale difference indicating the presence of the target dots. Dot position as a function of frame number (converted to time) is then used to calculate test object linear and angular displacement components as a function of time.

**Model testing**

While the optical position measurement system is adaptable to any fish cage model, the present study made use of a special model to obtain data for comparison with finite element computer predictions. This skeleton model consisted only of a rectangular parallel-piped structural frame, a bridle, and a single mooring line (see Fig. 3). Thus, the comparison between empirical data and computer predictions represented a focused evaluation of basic fluid mechanic processes

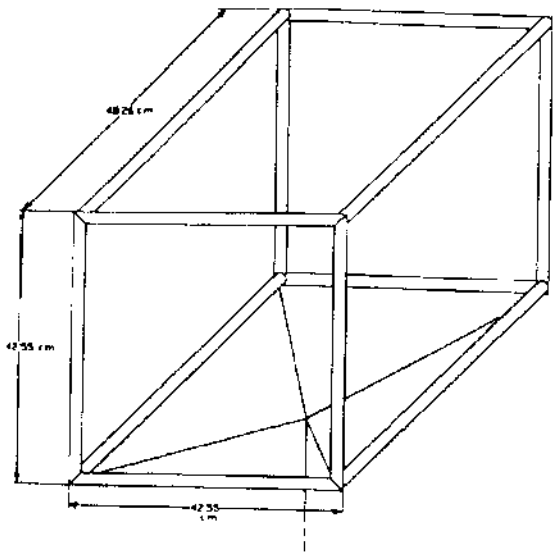

Figure 3. Skeleton fish cage physical model.

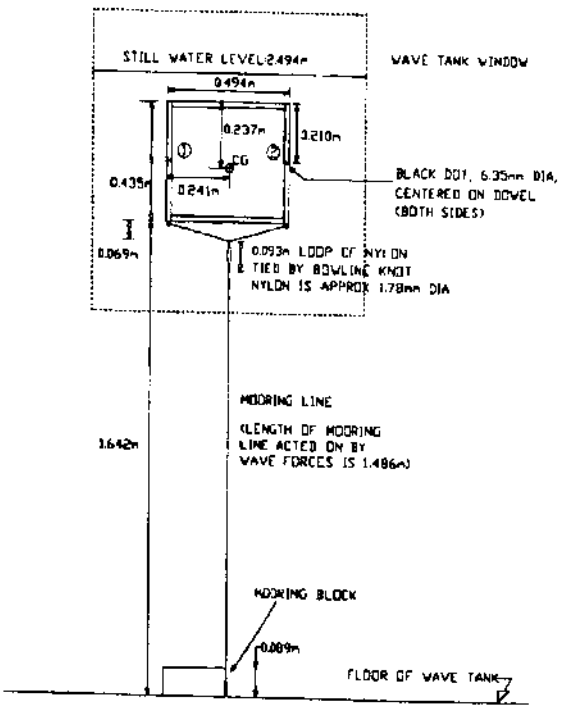


Figure 4. Experimental setup opposite upper window.

involving the main net pen components. Upon satisfactory validation, further complexities in the form of netting and small appendages will be added in future work.

Before wave tank testing, preliminary experiments on the physical model were carried out in the UNH recirculating flume. This 12.19 m long, 1.22 m wide, and 1.22 m high facility provided a steady current environment enabling the static drag characteristics of the model to be measured. After applying the finite element model to this case and obtaining a satisfactory comparison, the physical model was deployed for dynamic testing in the wave tank.

The model was moored, using the setup shown in Figure 4, so that the fully submerged cage was directly in front of the upper sidewall window. The high resolution camera was positioned to view the cage through the window over its full range of motion.

The model was allowed to come to vertical equilibrium and was then excited by regular, sinusoidal waves. Frequencies used ranged from 0.5 to 1.2 Hertz, and wave slopes were on the order of 1/15. After the leading edge of the wave train passed the model and the model appeared to be oscillating with the waves, position measurements were recorded over three wave cycles. The optical system software was then used to calculate time series of horizontal and vertical position of the two target points shown in Figure 4. Using relative height difference and the distance between target points, time series for the cage pitch angle were also calculated.

The finite element computer program was run for identical conditions. The finite element cage model, shown in Figure 5, used the exact dimensions and weights as the skeleton physical model. The excitation consisted of the same cases of regular wave forcing. It should be noted that the computer program input corresponded directly to the actual model dimensions. Thus, there was no need for either Froude or Reynolds number scale-up of results.

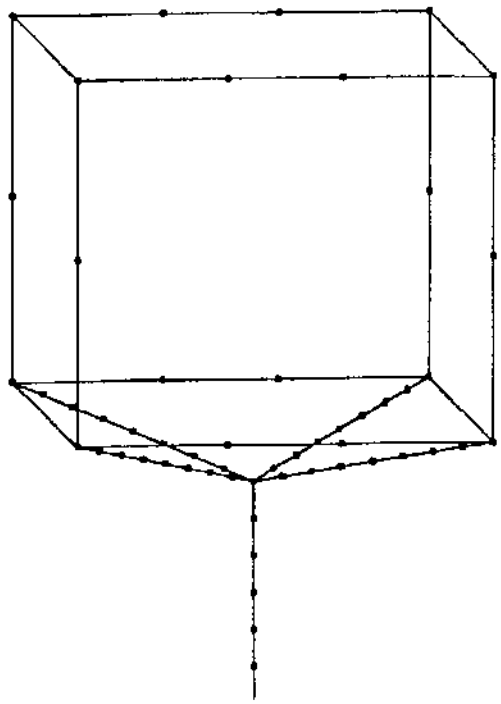

Figure 5. Finite element cage model.

## RESULTS

The wave tank, physical model response to regular waves is summarized in Table 1. For each separate test, wave period T, wave height H, and average amplitudes for pitch angle and horizontal (X) and vertical (Y) displacement of the two target points (see Fig. 4) are provided. Average amplitude is one-half the peak to trough difference averaged over the three waves measured.

Representative time series of horizontal and vertical displacement of the target points are plotted in Figures 6 and 7. The regular wave response is generally sinusoidal with the horizontal motion of the target points nearly equal and greater than the vertical motion. A drift can be seen which is due to a persistent transient initiated when the regular wave train encountered the upright cage/mooring system. Close examination of the time series reveals that the angular motion is opposite to that of an inverted pendulum. At the extremes of the horizontal displacement, the side of the cage

| Wave | | Average Amplitude | | | | |
|---|---|---|---|---|---|---|
| T(s) | H(m) | Angle(°) | X1(cm) | Y1(cm) | X2(cm) | Y2(cm) |
| 2 | 0.4 | 2.508 | 9.986 | 2.885 | 9.710 | 3.741 |
| 1.67 | 0.28 | 2.668 | 7.717 | 1.925 | 7.742 | 3.073 |
| 1.43 | 0.21 | 2.412 | 4.808 | 0.508 | 4.742 | 2.004 |
| 1.25 | 0.16 | 1.366 | 2.931 | 0.267 | 2.840 | 1.229 |
| 1.11 | 0.13 | 1.061 | 1.740 | 0.348 | 1.694 | 0.721 |
| 1 | 0.1 | 0.661 | 0.780 | 0.241 | 0.757 | 0.401 |
| 0.91 | 0.084 | 0.463 | 0.343 | 0.241 | 0.323 | 0.241 |
| 0.83 | 0.07 | 0.397 | 0.114 | 0.160 | 0.114 | 0.216 |

T = wave period; H = wave height; angle = pitch angle with respect to the horizontal; X1, X2 and Y1, Y2 are horizontal and vertical displacement components of the two target points 1, 2 shown in Figure 4.

Table 1. Measured cage response to regular waves.

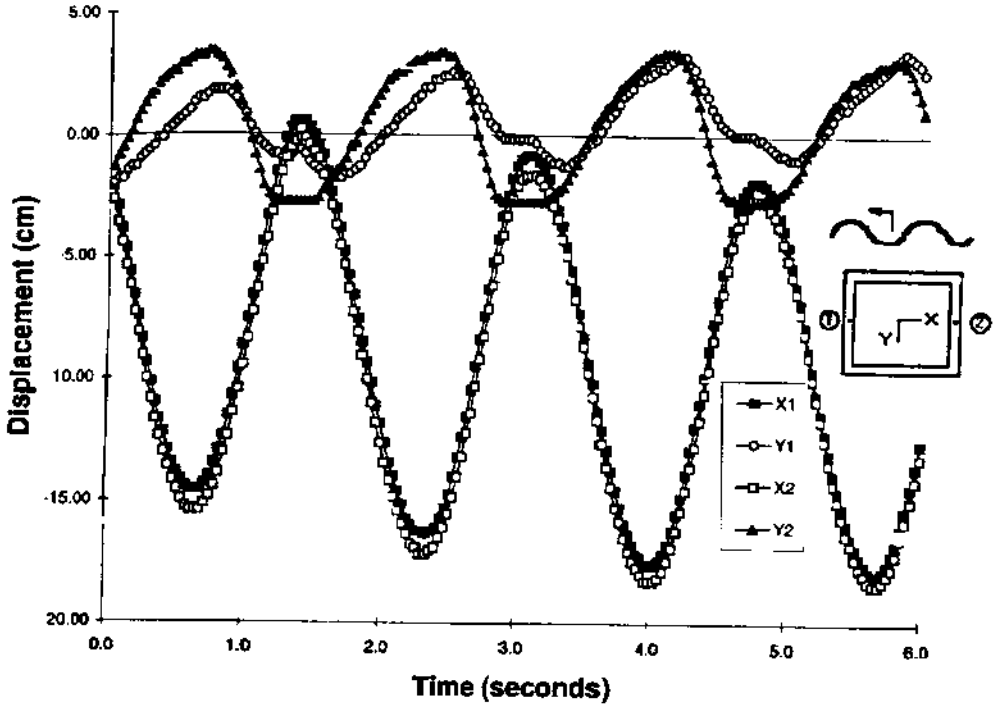


**Figure 6.** Measured cage response to a regular wave. Period = 1.67 seconds and wave height = 0.28 meters.

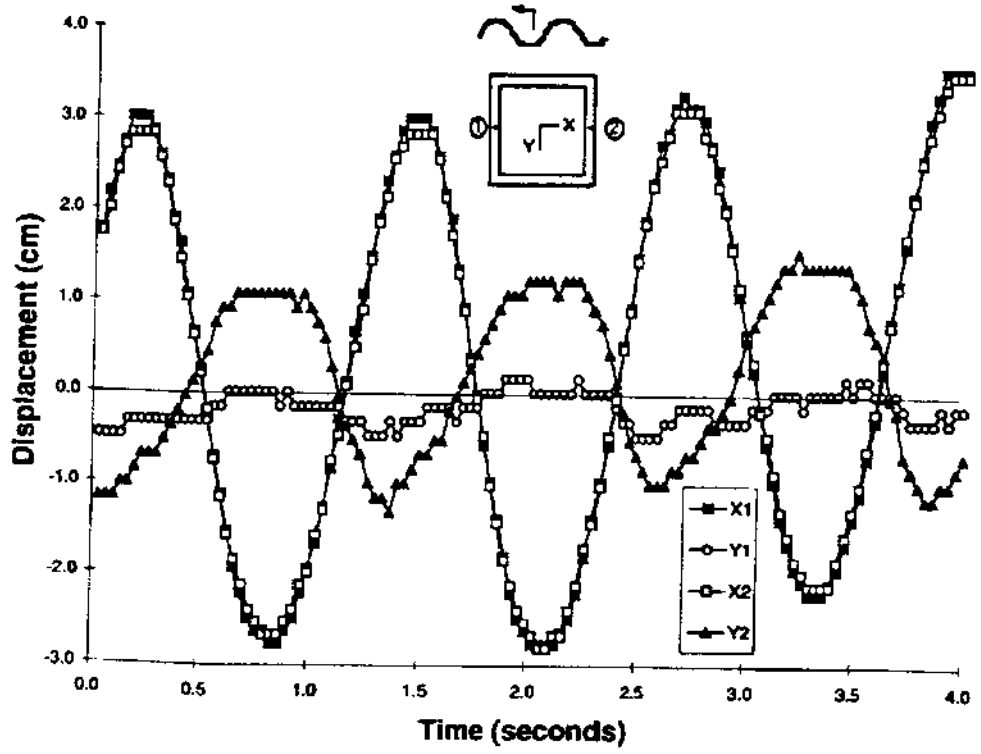


**Figure 7.** Measured cage response to a regular wave. Period = 1.25 seconds and wave height = 0.16 meters.

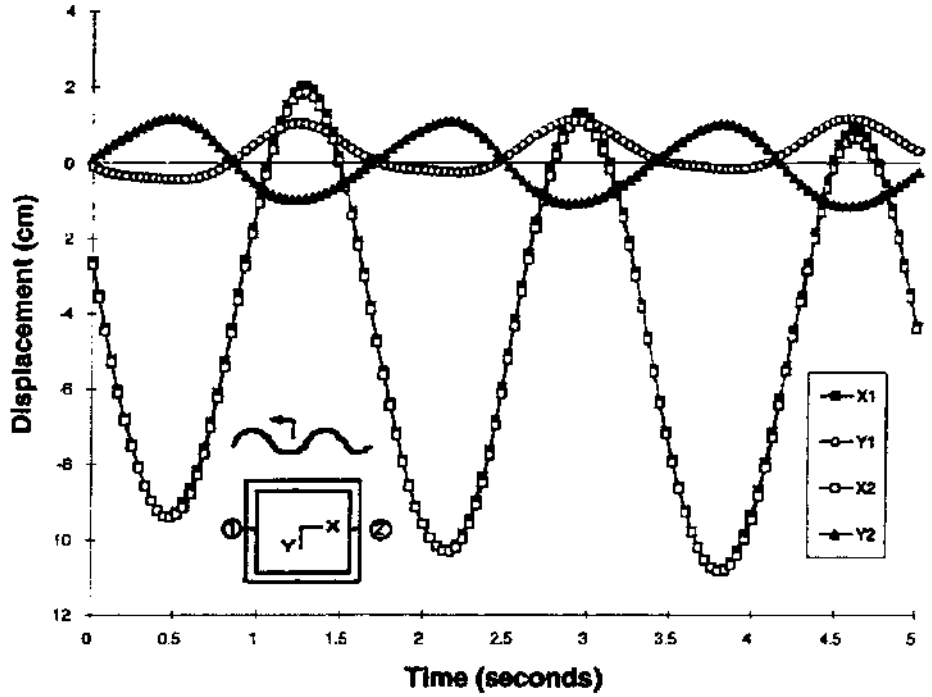


**Figure 8.** Finite element model predications of cage response. Period = 1.67 seconds and wave height = 0.28 meters.

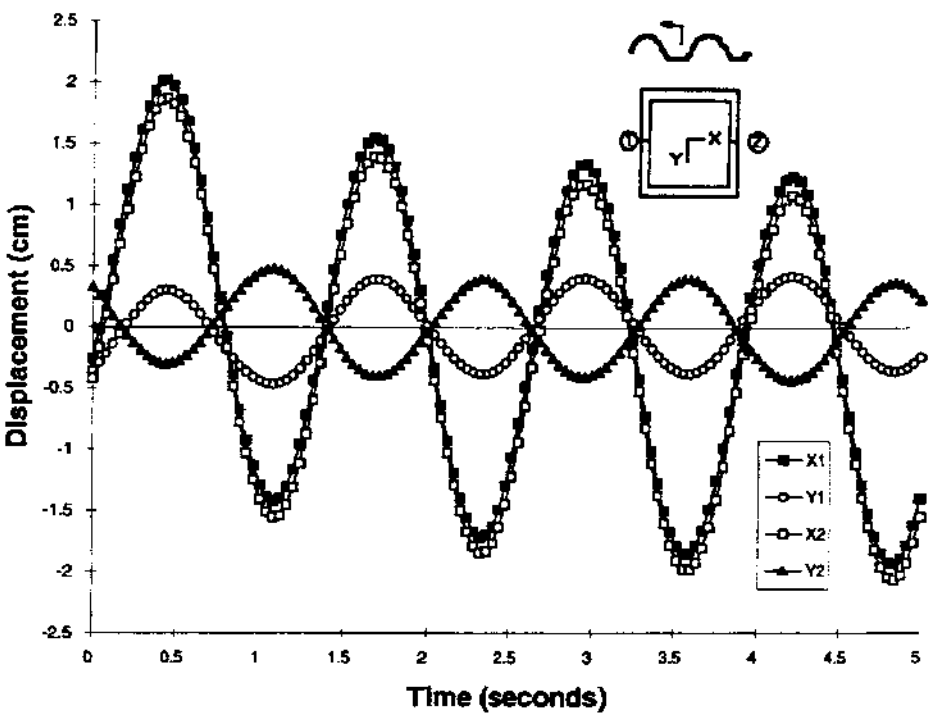


**Figure 9.** Finite element model predictions of cage response. Period = 1.25 seconds and wave height = 0.16 meters.

towards the mooring dips down. This is apparently due to wave action on the mooring/bridle system (having negligible inertia) kicking out the cage bottom.

Finite element model predictions corresponding to the Figures 6 and 7 experiment are shown in Figures 8 and 9, respectively. The same type of horizontal and vertical sinusoidal motion is seen and transient behavior is evident. The same bottom kick- out type angular motion is also evident, though vertical motion is more symmetric in the computer model output. Overall, the finite element model is seen to replicate the basic processes and motion response recorded in the empirical data. Direct comparison of the quantitative results, however, shows that the finite element model somewhat underpredicts the motion amplitudes.

It should be noted that coefficients in the Morison equation fluid forcing model were calculated using accepted theory (see Gosz et al., 1996) and were not tuned for this physical model application. Symmetry seen in the predicted vertical motion but not as evident in the physical model response may be due to using a linear wave theory in the program. The wave loading and other coding issues are currently under review in the ongoing model improvement effort.

## CONCLUSIONS

The UNH wave tank is ideally configured for testing offshore fish cage physical models. Conveniently placed observation windows allow precise, noninvasive measurement of cage motion using a passive optical technique.

The skeleton model approach reduces complexity allowing evaluation of how well computer programs simulate basic processes governing fish cage dynamics in waves. The Gosz et al. (1996) finite element program was found to replicate the fundamental characteristics of the physical model motion, but work is ongoing to obtain more exact numerical agreement.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

Darnell, L. 1996. A towing carriage for the University of New Hampshire towing and wavemaking basin. M.S. thesis, Univ. New Hampshire, Durham, NH. 178 p.

Gosz, M., K. Kestler, R. Swift, and B. Celikkol. 1996. Finite-element modeling of aquaculture net-pens, pp. 523-541. *In:* Open Ocean Aquaculture. Proceedings of an International Conference, Portland, Maine. New Hampshire/Maine Sea Grant College Program Report No. UNHMP-CP-SG-96-9.

Michelin, D. and S. Stott. 1996. Optical positioning intrumentation and evaluation. Ocean Projects Course final report, Univ. New Hampshire, Durham, NH. 85 p.

Washburn, S. 1996. A wave generator and wave absorber system for the University of New Hampshire wave/tow tank. M.S. thesis, Univ. New Hampshire, Durham, NH. 224 p.

# CREATON OF OFFSHORE AQUACULTURE GROUND BY FLOATING BREAKWATER

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## ABSTRACT

In Japan, cage culture has expanded mainly in calm water areas such as the Seto Inland Sea. The present culture condition can be described as overly intensive, and this has caused the deterioration of water quality, hindering the expansion of fish culture. In order to expand fish culture, it is necessary to enhance offshore culture. An important task is to develop a nursery system which can withstand the rough wave conditions of the ocean around Japan. Here, we introduce some offshore types of floating breakwater now in use and a floating breakwater equipped with aquaculture net cages which is under development, in order to realize offshore culture.

## INTRODUCTION

The coastal fisheries production is on a stable level. Coastal fisheries resources have been enlarged by the coastal fishing ground development, such as construction of artificial reefs and fishery nursery grounds for propagation. Only aquaculture has shown an upward tendency in area and production quantity.

Aquaculture was developed in the inland seas and calm bays because the wave conditions in the open sea were severe, thus detrimental to aquaculture facilities. The floating breakwater was developed in order to enlarge the aquaculture grounds, and at many locations in Japan has been constructed to create more suitable grounds for aquaculture.

It is necessary to maintain calm seas at the aquaculture grounds for safety and workability, and for an optimal environment for breeding fish. Therefore, the floating breakwater has been used to create aquaculture grounds because it has the following characteristics:

(1) Transmitted waves can be controlled by the scale and wave absorption principle of the floating breakwater;
(2) The floating breakwater does not obstruct the seawater exchange, mixing, and diffusion, so water quality is maintained;
(3) In the deep sea (>20m), the floating breakwater is more economical than the gravity type;
(4) The floating breakwater is convenient for planning and maintenance.

Reliability for safety of the floating breakwater has been established with actual results. In addition, aquaculture grounds have expanded from the bay and inland sea areas to the open sea because of environmental change and overcrowding. However, it is clear that the normal type of floating breakwater costs too much in order to achieve the required performance, and in the case of aquaculture grounds in the open sea, is very difficult to construct. A new type of floating breakwater which can absorb big and long waves effectively is warranted. Moreover, in the case of aquaculture grounds in the open sea far from the fishing port or fishing village, the floating breakwater must have additional functions, such as the cultivation of broodstock and nursery culture.

I would like to introduce two examples of the floating breakwater constructed in the open sea, and to describe the direction of its research in

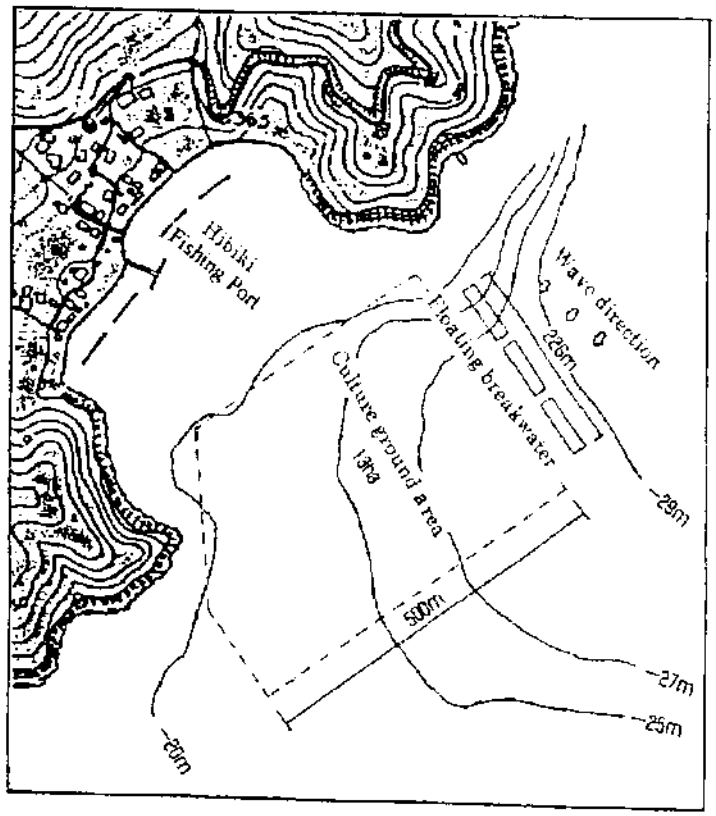



**Figure 1.** Project site and layout plan.

the future.

## Floating breakwater constructed in Takahama District, Fukui Prefecture

This floating breakwater was planned as part of the creation of new aquaculture grounds in Takahama District, Fukui Prefecture, located in the middle part of Japan along the Sea of Japan. This was the first one constructed along the Sea of Japan, which protects the aquaculture ground (13 ha) from big waves. Figures 1 and 2 show its layout and the aquaculture ground, and Figure 3 shows its structure.

Design Condition

(1) Natural condition

| | |
|---|---|
| Average water depth | : 28 m |
| Tidal range | : 1 m |
| Tidal current | : 0.5 m/sec |
| Wind velocity | : 28.0 m/sec |
| Sediment | : Silt mixed with fine sand |

(2) Wave condition

| | Design wave function | | Design wave for structural stability |
|---|---|---|---|
| Significant wave height | 1.3 m | 1.4 m | 3.9 m |
| Significant wave period | 7.5 sec | 6.0 sec | 11.7 sec |
| Wave length | 87.8 m | 56.2 m | 171.4 m |
| Wave direction | NNW | N | N |
| Transmission coefficient | 0.6 | 0.5 | - |

## The principle of floating breakwater

The floating breakwater shown in Figure 3 absorbs waves by interaction between the air flow and the internal water movement. It has chambers on both sides and air ducts connected to each chamber. Its scale is as follows: 1 unit length 68.0 m; width 14.5 m; height 8.7 m; total length 228 m (3 units).

## Characteristics of floating breakwater

Wave function design requires a relatively long period, so this floating breakwater is categorized as the open sea type. It is moored by

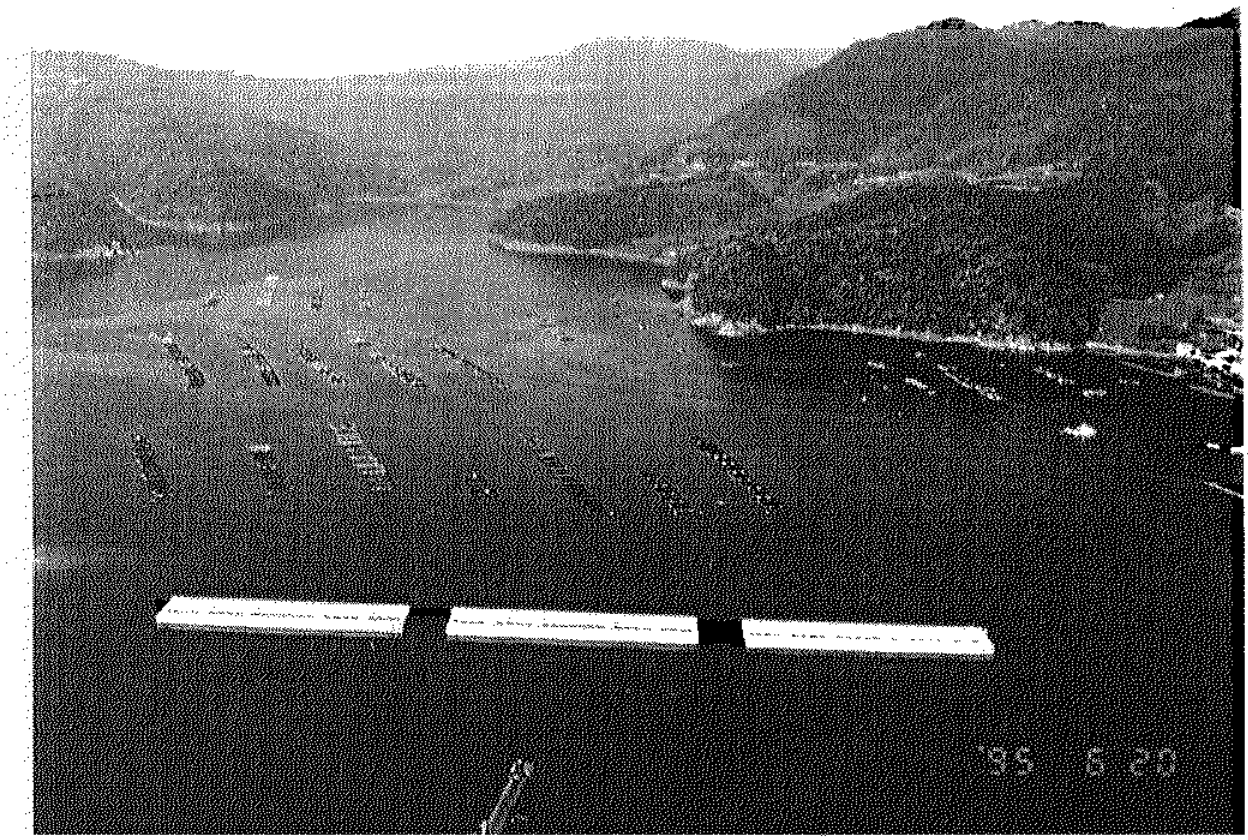



**Figure 2.** An airplane view of the floating breakwater.

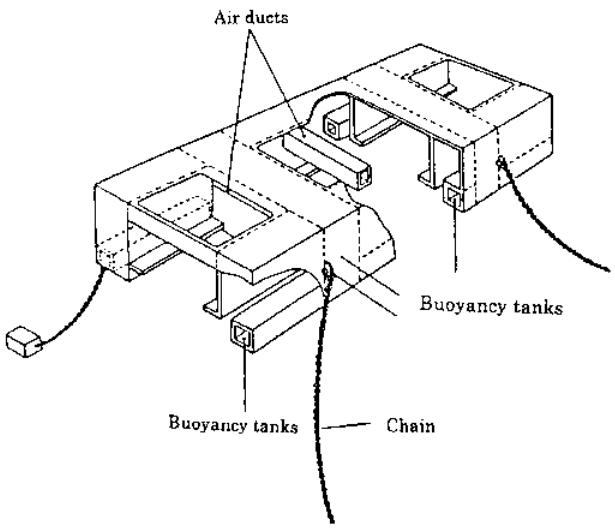



**Figure 3.** Structure of the floating breakwater.

six cross lines connected with anchors because wave direction is not normal to it. Moreover, a construction craft cannot be used because there is not one large enough in this region, and to move it from another place to the project site would be very expensive. The anchor is divided into three parts.

Figure 4 shows the three-part anchor. This anchor was used for the first time, and is applied to the open sea because of its size. In this case, its applicability to the oblique waves and problems in constructing in the open sea were made clear. The floating breakwater has performed very well 3 yr after installation, though high waves are frequent in the winter.

## Fishing ground constructed in Aba District, Nagasaki Prefecture

This fishing ground was planned as part of the creation for a multipurpose calm area, which enhances developing coastal fishing grounds. The

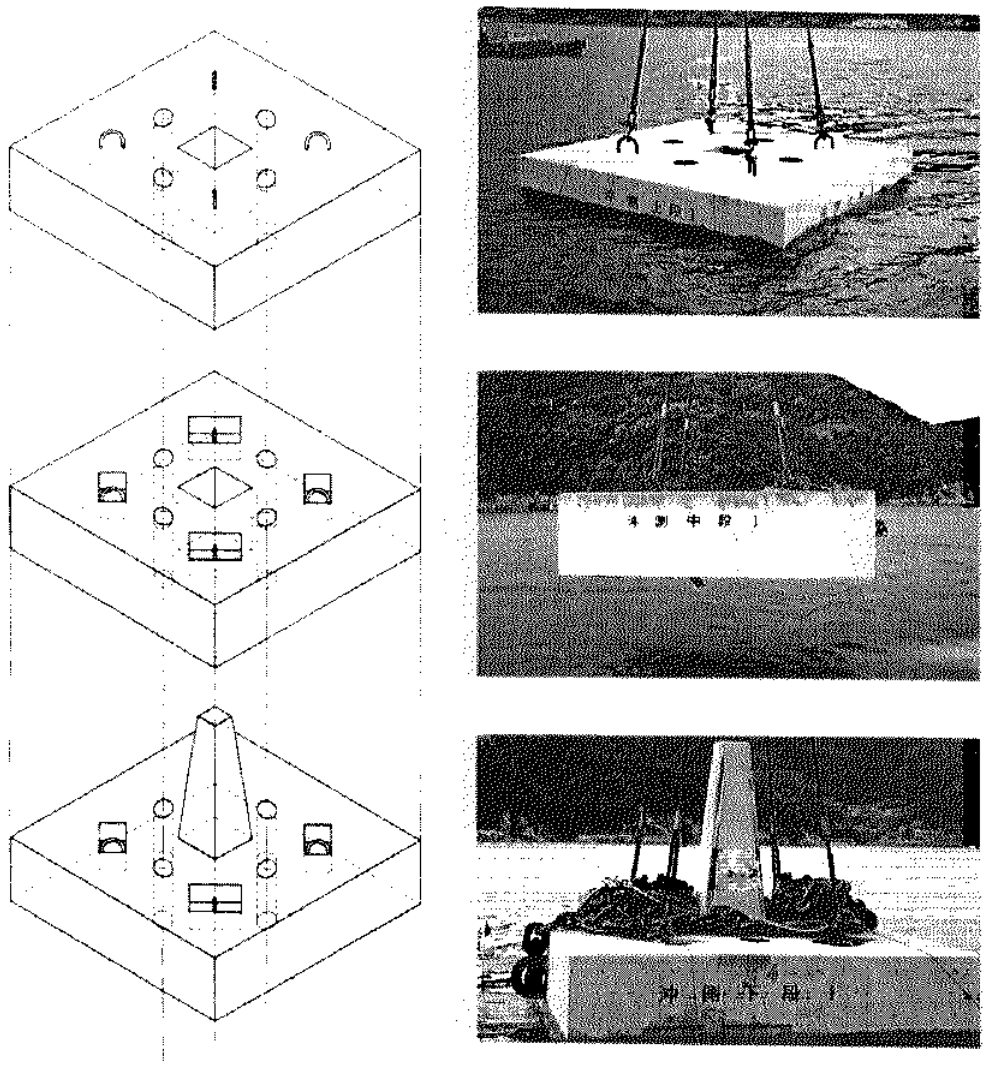


**Figure 4.** Parts of the new type of anchor.

plan included the gravity-type breakwater and the new floating type that applies the results of the Takahama case described earlier. The project site is located in the northern part of Kyushu Island along the east China Sea. Figure 5 shows the project site and the layout of facilities, and Figure 6 shows the new type of floating breakwater.

Design Condition

(1) Natural condition

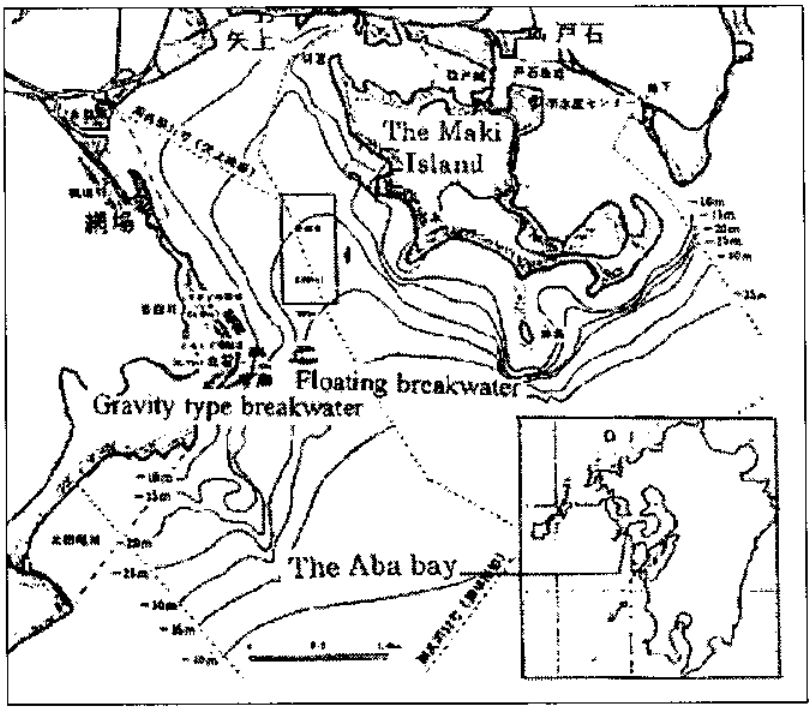
| | |
|---|---|
| Water depth | : 17-28 m |
| Tidal range | : 3.8 m |
| Tidal current | : 0.761 m/sec |
| Wind velocity | : 40.0 m/sec |
| Sediment | : clay |

(2) Wave condition

| | Design wave function | Design wave for structural stability |
|---|---|---|
| Significant wave height | 2.0 m | 3.9 m |
| Significant wave period | 6.7 sec | 13.3 sec |
| Wave length | 70.0 m | 276.0 m |
| Wave direction | SSW | SSW |
| Transmission coefficient | 0.5 | - |

## The principle of floating breakwater

Figure 5 shows the cross section of the new type of floating breakwater. The shape of its absorbing chambers is asymmetrical because three absorption principles are considered to work effectively: first is



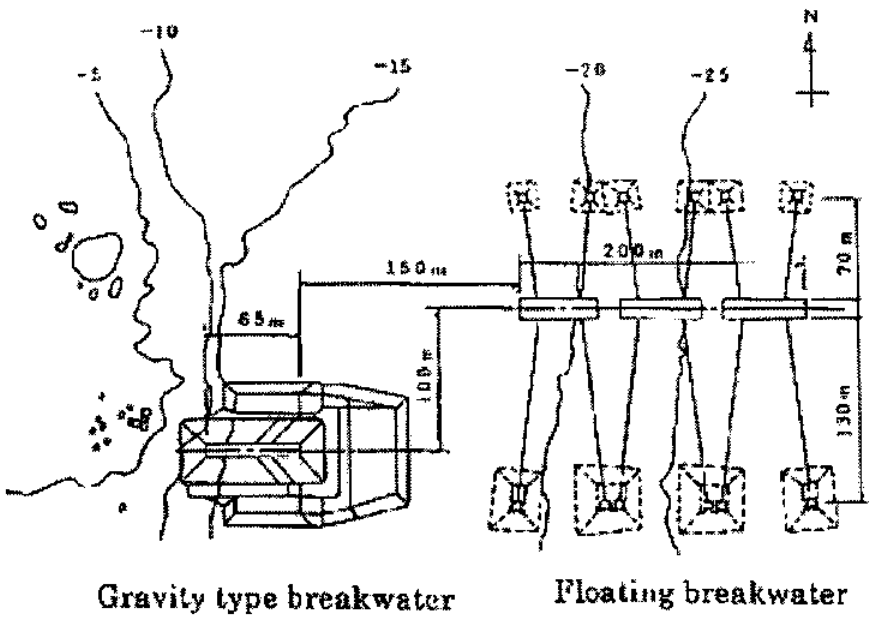




**Figure 5.** Project site and layout plan.

**Figure 6.** The floating breakwater

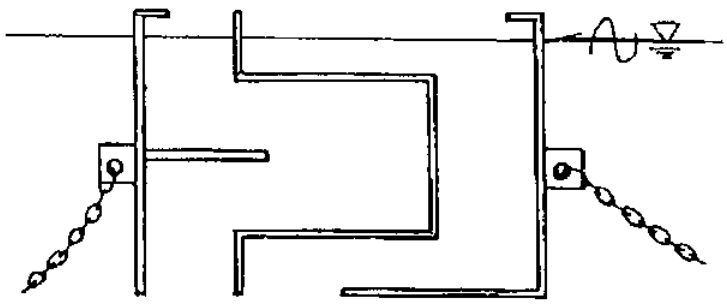
absorption by scattering waves due to the motion of the floating body; second is by interaction between the air motion and the internal water surface motion in the chambers; and third is by dissipation due to the water currents in the chambers. The floating body could be made smaller than the normal type by changing the length of the internal current in the chambers to achieve the required performance. Because its performance was also estimated by numerical simulation and the hydraulic model test, this floating breakwater was applied to the Aba aquaculture ground for the first time in Japan. Its scale is as follows: 1 unit length 57.0 m; width 11.0 m; height 8.3 m; total length 200 m (3 units).

## Characteristics of floating breakwater

The bottom at the project site is very soft and large waves are often generated, so it is difficult to moor the floating breakwater. This floating breakwater was very unique, which worked effectively in the open sea with its mooring system designed for ground stability. Its construction began in 1993 and was completed in 1995. It now works effectively although typhoons often attack this area. Its performance was also checked by the field survey.

## Development of new type of floating breakwater

Up to now, various types of floating breakwater have been proposed and applied in the creation of aquaculture grounds. Moreover, a new



Cross section of floating breakwater

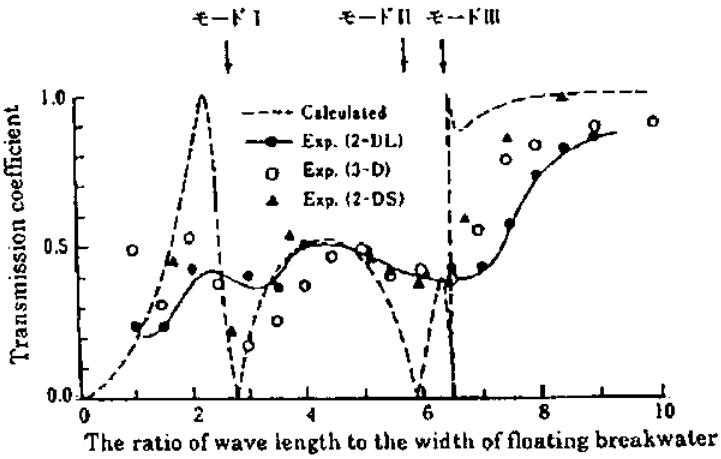



**Figure 7.** Cross section of the floating breakwater for the open sea and its performance.

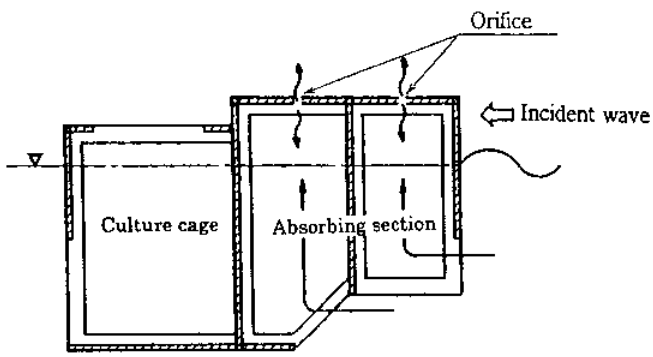



**Figure 8.** Cross section of the floating breakwater attached with culture cages.

type, one applicable to the open sea, was developed and constructed. Recently, for utilizing the floating breakwater in multiples, the one with culture cages has been proposed. By constructing this new type which has culture cages and the facilities for management, offshore aquaculture will become safer and more efficient.

## Current state of development

Figure 8 shows the cross section of the multipurpose breakwater. The front part absorbs waves by controlling air and water currents through orifices attached to the ceiling of each chamber. The back part is a culture cage. The main problem is reducing the wave motion which damages fish in the cage. The width and height of the absorption part must be considered for the safety of aquaculture and stability of the cage. Its

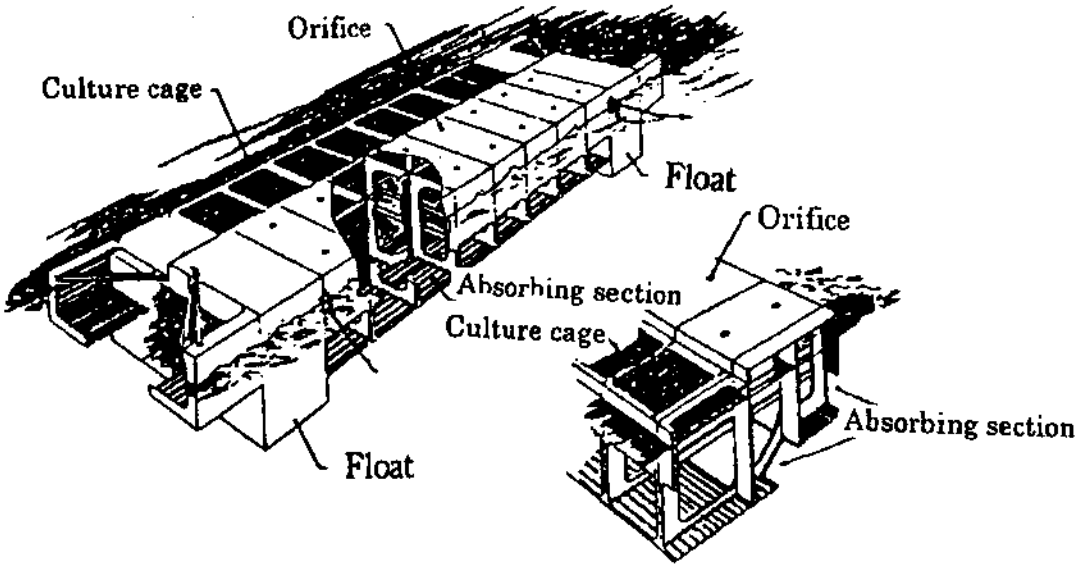



**Figure 9.** A new type of multipurpose breakwater.

performance and the water environment in the cage based on the image shown in Figure 9 are now being studied.

## EPILOGUE AND ACKNOWLEDGMENTS

These studies will serve as the basic technology for creating offshore aquaculture grounds and the offshore fisheries base. So we continue to study steadily. Finally, I express special thanks to the people who offered important photographs and data. In addition, development of the new type of floating breakwater was carried out in cooperation with the National Research Institute of Fisheries Engineering, Mitsubishi Heavy Industry Co., Ltd., Ishikawajima-Harima Heavy Industry Co., Ltd., and Hitachi Engineering Shipbuilding Co., Ltd.

## LITERATURE CITED

Takagi, N. and S. Akeda. 1992.. Hydraulic model test on formation of pisciculture ground at Takahama, Fukui Pref. Tech. Rep. Natl. Res. Inst. Fish. Eng., Aquacult. Fish. Port Eng. No.14, pp. 37-75.

Takagi, N., Y.Ohmura, M. Ozaki, Y. Isozaki, A. Arami, A. Kadono, and A. Nagano. 1995. Experimental study on application of new type floating breakwater for open sea area. Bull. Natl. Res. Inst. Fish. Eng. No.16, pp. 29-57.

Kagiyama, H. 1995. Development of new floating breakwater. Sci. Tech. J. Jpn. Int. Mar. Sci Technol. Fed. 8(4): 31-40.

Ohkusu, M., M. Kashiwagi, K. Ikegami, M. Ozaki, and Y. Isozaki. 1991. Study on performance of floating breakwater utilizing the relative motion of inside water. J. Soc. Nav. Archit. Jpn. 169: 215-222.

# AWATS: A NET-PEN AQUACULTURE WASTE TRANSPORT SIMULATOR FOR MANAGEMENT PURPOSES

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## ABSTRACT

An efficient mathematical modeling package called Aquaculture Waste Transport Simulator (AWATS) provides first-order estimates of the physical dispersion of finfish aquaculture wastes for regulatory purposes. The modeling strategy entails the utilization of a vertically averaged, two-dimensional flow model to produce flow-field information; this information is input to a particle tracking waste transport model to simulate the resulting transport of wastes. Since earlier studies have shown that the transport modeling results are sensitive to the threshold shear stress at which settled fish-pen wastes are resuspended, fieldwork was conducted to improve the parameterization of erodibility in the transport model. Application of AWATS to several aquaculture sites in coastal Maine (selected by the Maine Department of Environmental Protection) shows that it is a convenient tool in the regulatory process.

## INTRODUCTION

Due to high stocking densities and feed rates, net-pen aquaculture operations are regarded as potential polluters of the marine environment. Net-pen wastes, consisting primarily of fish feed and fecal pellets, can adversely impact the coastal environment through increased concentrations of ammonia, decreased dissolved oxygen, and the formation of bacterial mats at particularly problematic sites. While rates of deposition and accumulation of these wastes in the vicinity of net-pen operations depends on many factors (including stocking density, feeding rates and the amount of excess feed waste, settling rates of waste material, fish metabolism, grazing, bacterial decomposition, etc.), the degree of environmental deterioration depends ultimately on the hydrodynamic environment.

A considerable effort is put forth by regulators to monitor hydrodynamic, water quality, and benthic conditions and to evaluate environmental impacts of net-pen aquaculture operations. The efficiency of this work may be significantly enhanced through the use of mathematical models that give more complete information regarding the physical conditions in the domain. For example, Panchang et al. (1997) have shown that the use of blanket guidelines for minimum current speed and water depth do not automatically ensure favorable hydrodynamic conditions for a net-pen operation. The flow-fields seen in coastal Maine are complex and it is often difficult to discern prevailing current direction and overall flow-fields from discrete, site-specific measurements over limited time periods. Such

data fail to ascertain the spatial and temporal variations of the hydrodynamic environment (such as vorticity, wind, seasonal effects, etc.) within lease sites or the cumulative effects of several operations within a coastal embayment. The complex and restrictive regulatory environment is viewed as a limiting factor in the growth of the aquaculture industry in the United States (Schneider and Fridley 1993).

To resolve some of the above limitations, Panchang et al. (1997) developed a comprehensive modeling strategy involving an investigation of tidal and storm-induced currents, wave effects, and net-pen waste transport mechanisms such as settling, resuspension, and decay. This approach was shown to be successful in assessing the impact of aquaculture operations in Cobscook Bay and Toothacher Bay. First, a vertically averaged, two-dimensional flow model is constructed using appropriate field measurements, to simulate the currents induced by the tides and by storm winds. The resulting flow-fields were used as input to a particle tracking waste transport model. The results showed that at some sites, inferences drawn regarding the waste distribution using a combination of modeling methods and field data could be quite different from those drawn using isolated field measurements. The potential of the modeling methods for site selection and in deciding a priori which sites needed a greater level of monitoring was also demonstrated.

Before the modeling techniques can be adopted in regulatory practice, however, the work of Panchang et al. (1997) suggests that two problems need further attention. First, a more reliable description of the resuspension of settled wastes is needed. Since resuspension involves complex mechanisms that are not well-understood, it was modeled using a parameter $U_{crit}$, describing a threshold or critical current velocity at which settled waste material would be resuspended. Panchang et al. (1997) found that the waste dispersion and accumulation results were very sensitive to the threshold of shear stress at which settled fish-pen wastes are resuspended, thus limiting the usefulness of the models for site selection. Secondly, Panchang et al. (1997) were motivated more by a research perspective and did not offer tools readily available to regulators.

We describe efforts to improve estimates for the critical resuspension velocity of net-pen wastes, and to create a modeling package that could be routinely used to aid regulators with site evaluation and decision-making. Specifically, field measurements were made to estimate in situ erodibility of net-pen waste materials. A submarine annular flume called the Sea Carousel was used; this device was designed by the Geological Survey of Canada to study seabed instabilities and the mechanisms involved (Amos et al. 1992a). The Sea Carousel and the field measurement programs are described in section 1. In the interest of packaging the modeling technology for regulators, two reasonably well-known flow models were evaluated for accuracy and ease of use: a finite element model called RMA2 and a finite-difference model called DUCHESS. RMA2, developed through funding from the US Army Corps of Engineers and coupled with a sophisticated graphical interface, is a public-domain, two-dimensional hydrodynamic model. DUCHESS, which was developed at Delft University, Netherlands, is widely used for two-dimensional tidal and storm surge computations (e.g., Booij 1989, Jin and Kranenberg 1993). The transport model developed by Panchang et al. (1997) was enhanced and packaged with an interface used to extract flow solutions and graphically display flow and transport results. This work led to a package called AWATS (Aquaculture Waste Transport Simulator), described in section 2. It was applied to three sites selected by the Maine Department of Environmental Protection for testing and demonstration purposes as part of technology transfer efforts. Application of AWATS to modeling an aquaculture site in Maine is presented in section 3.

## 1. Fieldwork to estimate erodibility

In the initial development of the waste transport model, Panchang et al. (1997) found that the transport of net-pen aquaculture waste was sensitive to the ability of the currents to resuspend material once it had settled on the bottom. With settling rates of 4-10 cm/sec and typical depths

beneath pens of 15-25 m, net-pen wastes will settle in the vicinity of the pens in a matter of minutes. In constant low-velocity environments such as fjords, local settling can have adverse environmental impacts; in high-velocity environments the material may be resuspended and more effectively dispersed. Lacking applicable information regarding the complex process of resuspension in aquaculture environments, Panchang et al. (1997) used casual diver observations which suggested that net-pen wastes were eroded when the flow velocity exceeded approximately 30 cm/sec. In view of the uncertainty, however, Panchang et al. (1997) modeled multiple transport scenarios by varying the values of $U_{crit}$ over a range and found that the resulting waste dispersion was very sensitive to $U_{crit}$. For example, waste removal from the domain used to examine a commercial lease site in Deep Cove, Cobscook Bay, varied between 83% and 0% when $U_{crit}$ was varied between 10 cm/sec and 40 cm/sec. The area affected by the wastes also varied substantially.

Erosion of sediments is a function of bottom stress which is often expressed as shear velocity. In this sense, $U_{crit}$ is intended to be a measure of the threshold stress at which net-pen wastes would be eroded and resuspended. To obtain more reliable information regarding this mechanism, measurements were made under the direction of Dr. Carl Amos of the Bedford Institute of Oceanography (BIO) at the Connors Brothers Inc. commercial lease site at Deep Cove in Cobscook Bay near Eastport, Maine (Fig. 1). Figure 2 shows the locations of erodibility experiments in relation to the Deep Cove net-pen systems. A device called the Sea Carousel shown in Figure 3 was used to conduct the erosion experiments. The Sea Carousel is an annular flume designed by the Geological Survey of Canada to measure seabed erosion. Upon lowering it to the benthos from the side of a boat, a current was generated inside the flume and slowly increased in magnitude in a stepwise fashion. At each step over the course of the erosion program, a video of the erosion process was obtained in conjunction with water samples and turbidity measurements. The resulting turbidity measurements can be correlated with shear velocity to provide values of critical resuspension velocity (e.g., Amos et al. 1992b).

The Deep Cove site contains three pen systems (Fig. 2) consisting of net-covered cages arranged in rows of 10 cages, with two rows forming an independent floating pen system, each holding about 5,000 fish. At this site, we attempted to determine the erosion threshold and its variation in time and space. It was estimated that the greatest amount of sedimentation would be near the center of the three pen systems and would decrease outwards. Since it was possible that the erosion threshold varied with the amount of material already accumulated, the Sea Carousel was deployed at nine locations: three near the center of the site, four locations at different points on the sedimentation gradient, and two control locations closer to land deemed to be unaffected by the net-pen operation. Data were collected at two different times, one in April 1996 and one in

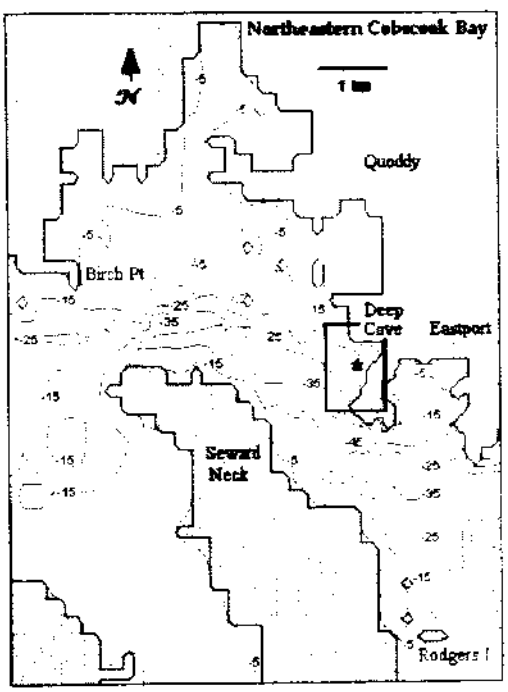


**Figure 1.** Northeastern Cobscook Bay illustrating the location of the Deep Cove field site. Bathymetry in meters.

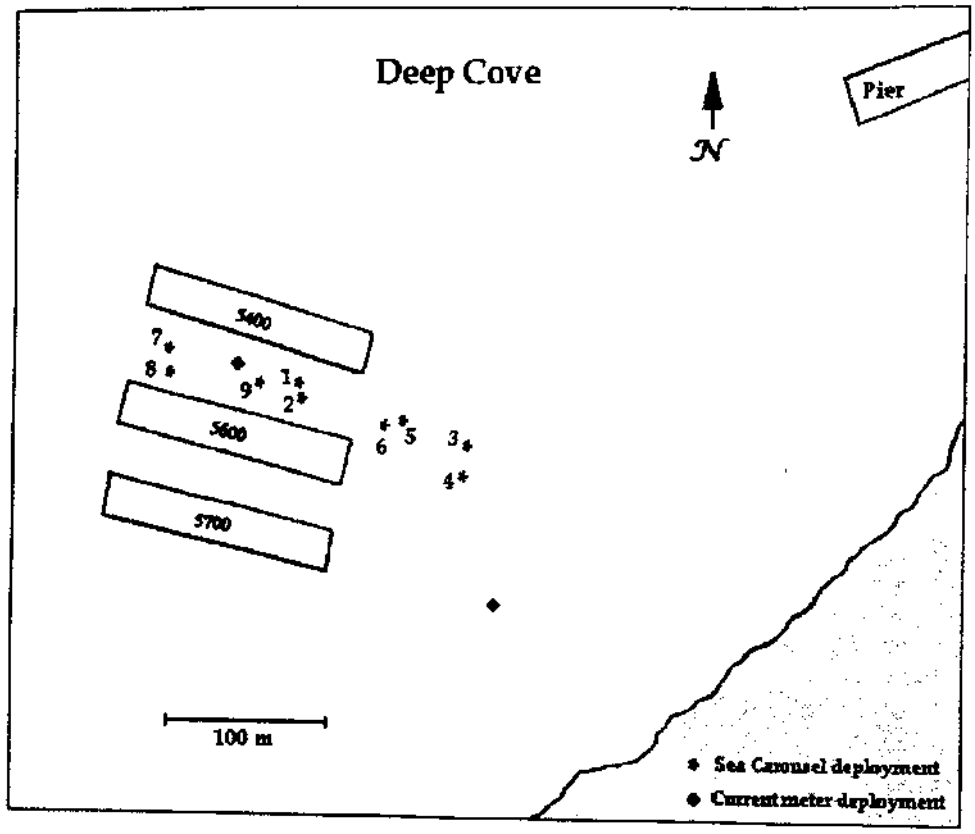


**Figure 2.** Deep Cove showing the locations of April and September 1996 Sea Carousel and current meter deployments in relation to Connors Brothers Inc. net-pen systems 5400, 5600, and 5700. Sea Carousel deployments are numbered 1-9

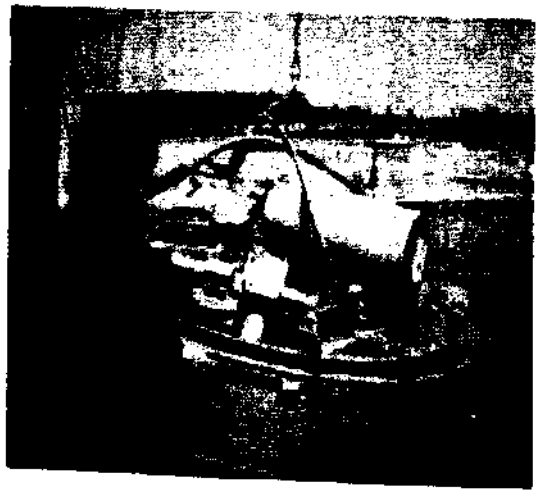

**Figure 3.** The Sea Carousel about to be lowered to the benthos at the Connors Brothers Inc. aquaculture site in Deep Cove near Eastport, Maine.

September 1996, since there is likely to be seasonal variation in the amounts of net-pen wastes present (due to higher feeding rates in the summer, and more frequent storm-induced erosional events in the winter).

During the fieldwork, locations of the net-pen sites, current gauges, and Sea Carousel deployments were determined via the Global Positioning System (GPS). The Sea Carousel work provided videos of the seabed erosion, samples of suspended sediments for each velocity step (Fig. 4), sediment core samples, water velocities, and turbidity data. The erodibility data from the Deep Cove aquaculture site were analyzed by Drs. Terri Sutherland and Carl Amos at BIO. A summary of the results is given in Table 1, in terms of $U_{(100)}$, the current velocity at 100 cm from the bottom. The $U_{(100)crit}$ values were determined from plots of suspended particulate

matter (SPM) against $U_{(100)}$ where SPM was observed to be significantly higher than the preceding ambient SPM concentrations (Fig. 5). The $U_{(100)crit}$ value was taken as the mean of the $U_{(100)}$ speed settings at that transition point.

Table 1 shows that, in general, the erosional velocity increases along a transect in the direction of the net-pen. Similarly, the values are higher in the summer than in the winter. This suggests that $U_{crit}$ is indeed affected by the amount of material present. The modeling strategy described later only allows for a constant $U_{crit}$. Average values of 0.40 m/sec for the winter/spring and 0.50 m/sec for the summer/fall are used. These numbers are close to anecdotal evidence provided by divers (Dr. R. Findlay, Department of Microbiology, Miami University, personal communication) that material seems to be resuspended when the flow speeds are greater than about 0.30 m/sec. It is important to note that the $U_{crit}$ values in Table 1 include values for all sediment types encountered in the field sessions from fine gel mud to coarse material and includes erosion of native material; research is currently being performed by Drs. Sutherland and Amos to estimate the erosion thresholds for strictly fish feed pellets.

## 2. Mathematical models

Modeling the physical transport of finfish aquaculture waste requires detailed knowledge of the spatial and temporal variations in tide and wind-induced currents in the particular region of interest. Hydrodynamic models, driven and validated with field data, simulate these currents and provide the necessary input information for transport models to compute the resulting waste dispersion. Previous modeling work conducted for Cobscook Bay indicated that a two-dimensional flow model based on the shallow water equations that yields depth-averaged velocity components is adequate for this task. This is fortunate, since three-dimensional schemes require intensive computer resources, particularly when large areas such as the coastal domains of Maine are to be modeled. In addition, data collected near aquaculture sites in Cobscook Bay indicate that the large tidal forcing leads to little vertical variation in the horizontal velocities in those areas (e.g., Panchang et al. 1993).

In the interest of assembling a user-friendly modeling software package to be used by regulators, we evaluated the ease of operation and accuracy of two-dimensional flow models. Both finite-element and finite-difference models were investigated. Finite elements usually afford greater flexibility in describing complex coastal boundaries and domains where aquaculture operations are carried out. As an example, we chose the model RMA2. This is a public domain model which is a part of the popular "Shallow Water Modeling System" developed by the U. S. Army Corps of Engineers and is hence readily available along with a sophisticated user interface. The finite-difference model DUCHESS was

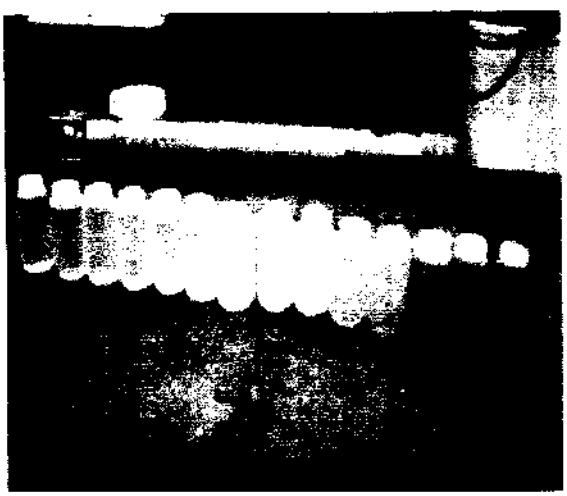

**Figure 4.** Water samples for suspended particulate matter analysis collected during a Sea Carousel erosion program conducted at the Connors Brothers Inc. aquaculture site in Deep Cove. From left to right, each bottle corresponds to a water sample taken 2 min after the onset of each step-wise velocity magnitude increment generated inside the annular flume.

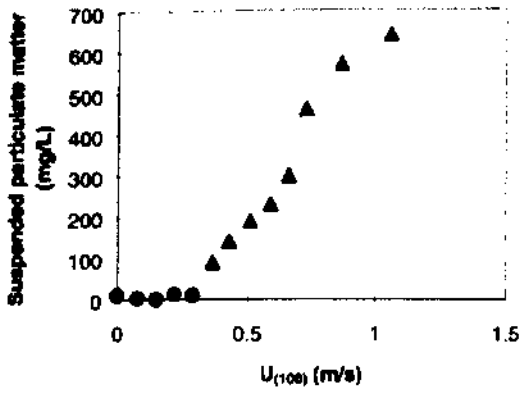


**Figure 5.** Estimate of the erosion threshold for an April 1996 Sea Carousel deployment at station 4 in Deep Cove, Maine. Ambient concentrations of suspended particulate matter concentration (SPM) are designated by round symbols, while the eroded concentrations of SPM are designated by triangular symbols. Based on the significant change in SPM illustrated above, $U_{100crit}$ for this particular experiment is estimated to be 0.33 m/sec.

chosen since the performance of DUCHESS had already been well established via prior modeling efforts (Panchang et al. 1997).

### a. Hydrodynamic models

The graphical user interface for RMA2 consists of a software package called Surface-water Modeling System (SMS) developed at the Brigham Young University Engineering Computer Graphics Laboratory (ECGL) in cooperation with the Army Corps of Engineers (Jones and Richards 1992, ECGL 1995). This enables users to graphically construct finite element meshes required as input to RMA2 and to display hydrodynamic solutions from RMA2. The SMS software provides the user with various tools and pull-down menus to facilitate digitizing scanned topography maps, constructing computational meshes, and displaying and animating solution data sets with color contouring and vectors.

A significant amount of modeling was pursued using RMA2 to assess its suitability for coastal modeling associated with aquaculture management. The evaluation of RMA2 included modeling of simple test cases as well as a systematic investigation of mesh construction and refinement, boundary conditions, time step size, and flooding and drying mechanisms for the coastal region of Cobscook Bay, Maine. The 15.5 x 13.7 km Cobscook Bay domain (Fig. 6) had been

| | $U_{100crit}$ (m/s) | | | | | |
|---|---|---|---|---|---|---|
| | April | | | September | | |
| Location. | Value | Mean | Std. Dev. | Value | Mean | Std. Dev. |
| 1 | 0.47 | 0.47 | 0.0 | 0.62 | 0.66 | 0.05 |
| 2 | 0.47 | | | 0.69 | | |
| 3 | 0.47 | 0.40 | 0.10 | 0.47 | 0.51 | 0.06 |
| 4 | 0.33 | | | 0.55 | | |
| 5 | 0.33 | 0.33 | 0.0 | 0.47 | 0.44 | 0.05 |
| 6 | 0.33 | | | 0.40 | | |
| 7 | 0.33 | 0.44 | 0.16 | 0.47 | 0.44 | 0.05 |
| 8 | 0.55 | | | 0.40 | | |
| 9 | 0.47 | | | 0.55 | | |
| | **all stations** | **0.42** | **0.09** | **all stations** | **0.51** | **0.10** |

**Table 1.** Summary of mean $U_{100crit}$ values from Sea Carousel data; deployments April and September 1996 at Connors Brothers Inc. aquaculture farm, Deep Cove, Maine.

already rigorously modeled and validated using DUCHESS. Initial RMA2 flow model runs using a 225-m resolution mesh of the Cobscook Bay domain resulted in problems with flooding and drying. If any node comprising an element met the drying criteria, the entire element to which it belonged became "dry" and was removed from computation. As a consequence, entire reaches of the bay would be shut off due to drying in shallow, narrow areas, resulting in a discontinuous domain and model failure.

Subsequent efforts, which involved refining the computational mesh and adjustments to various model parameters such as time step, eddy viscosity, and bottom friction, met with only moderate success. Due largely to the size and computational demands of the Cobscook Bay domain, the most successful model run using a relatively coarse mesh and 12-min time steps ran in near-real time on our 200 MHz PC. The mesh, at its finest, had a resolution of 75 m, the majority of which was much coarser, with a maximum of 225 m (see Fig. 6). The resulting simulations were not as satisfactory as those described by Panchang et al. (1997) using the finite-difference model DUCHESS. Moreover, DUCHESS could resolve two tidal cycles for the same domain with a constant 75-m resolution and a 40-sec time step, in about 40 min on the same PC.

In summary, though RMA2 has been used in other applications, its implementation was extremely time-consuming and problematic for this particular application. It also presented added complexity for regulators due to its sensitivity to grid sizes, requiring greater efforts in the construction and refinement of finite element meshes. Mesh construction and refinement is a complex problem requiring evaluation of domain geometry and bathymetry. While all modeling involves a certain level of trial-and-error before successful simulations are obtained, it was felt that working with finite element models would be too cumbersome from the point of view of routine management.

Most finite difference models, in comparison, require only a single resolution throughout, and entail a straightforward

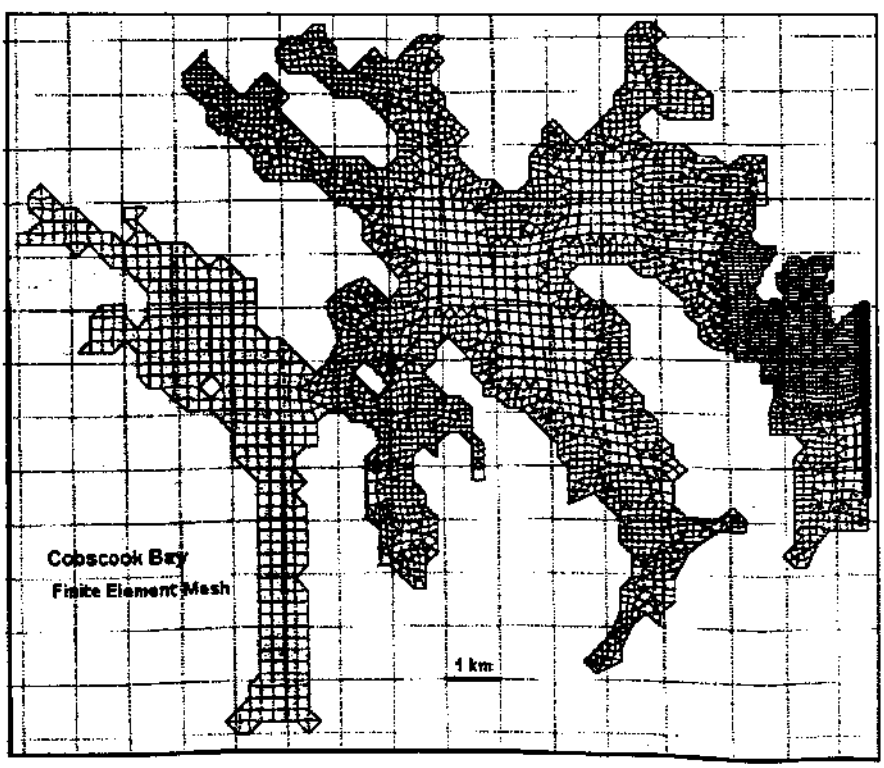


**Figure 6.** Summary illustration for the TRANS aquaculture net-pen waste transport simulator algorithm developed at the University of Maine.

relationship between the time step and the grid size. Although the flexibility of enhancing the resolution in specific parts of the domain is compromised, DUCHESS does allow options for subsequent simulations in "nested" domains. Flow modeling groundwork for computing aquaculture waste transport was performed by Panchang et al. (1997) for Cobscook Bay and Toothacher Bay using DUCHESS. The model has also been successfully applied to other fisheries-related problems (Newell 1991) and has been found to be generally robust.

One limitation of DUCHESS is that, unlike RMA2, it lacks a convenient graphical user interface to expedite the modeling process by aiding the user in model construction and viewing and interpreting model output. For this reason, we made efforts to interface DUCHESS with SMS to avail users of the graphical advantages of SMS. We developed a utility program called DUCHSMS which indirectly links the two programs. The program facilitates construction of the model domain using SMS, and graphical viewing of the flow model output. It enables bathymetry digitized with SMS to be exported in a form required by DUCHESS as input and also transforms DUCHESS output into a form readable by SMS. This allows easy graphical display and animation of flow solutions obtained from DUCHESS in SMS.

**b. Transport model**

A transport model called TRANS was developed at the University of Maine to simulate the advection and dispersion of finfish aquaculture wastes; it is included in the AWATS package, and models the mechanisms of settling, advection, and resuspension to describe the physical transport of fish-pen waste materials. To accomplish this, TRANS requires spatial and temporal flow-field information, bottom topography data, and properties describing the net-pen wastes such as resuspension threshold ($U_{crit}$), settling rates, and the location and the frequency of the introduction of wastes into the water. Parameters describing the aquaculture farm are input by the user providing coordinates of each net-pen in the domain coordinate system, as well as the size of each pen, its stocking density, and daily feed quantity. Other user-specified parameters in the model include: the simulation duration, begin and end times for food and fecal matter introduction each day, the uneaten food ratio as a percent of the daily food mass introduced, the daily fecal pellet production in g/kg of fish, percentage of organic carbon contained in the waste depending on the feed used, and first-order decay coefficient estimates for food and fecal matter.

The transport model computations involve breaking the daily feed and fecal introductions down into particles and tracking their dispersion throughout the model domain as they are advected by the currents computed by the hydrodynamic model (Fig. 7). Each particle represents a user-specified amount of mass representing a part of the total mass introduced over the course of the simulation. Each particle is tracked until it leaves the transport domain at which point it is considered to have been flushed away, and is not allowed to return. As the particles sink, they are advected by the flow-field until they reach the bottom. For modeling purposes, we chose sinking rates of 4 cm/sec for fecal particles and 10 cm/sec for feed particles (variable upon feed type) (Panchang et al. 1993). Once on the bottom, a check is made at each time step against the specified $U_{crit}$ to determine whether or not the particle is eroded from the bottom and resuspended in the water column to be further transported. Particles can decrease in mass over the course of a model run to first-order exponential decay. Values used for the decay coefficient depend upon the environment and oxygen availability. Values in fjords have been found to vary between 0.1 $yr^{-1}$ to 0.5 $yr^{-1}$ (Aure and Stigebrandt 1990, Hansen et al. 1991).

At the end of the simulation, TRANS outputs waste distribution snapshots at a user-specified time interval and a simulation summary. Particles remaining inside the model domain at the end of the simulation contribute to organic carbon loading to the benthos. The loading concentration, in $g/m^2$, is computed by dividing the total mass in each transport model grid by the area of the grid. TRANS will interpolate for transport model grid and time step sizes that are smaller than those of the flow model. A typical transport scenario is run for 15 days to approach

a steady-state loading pattern. The output snapshots represent the estimated concentration of net-pen wastes as a measure of organic carbon as it is distributed over time throughout the domain. These snapshots are output in a form readable by SMS for easy graphical display and animation. TRANS reports all model parameters as well as the amount of material flushed out of the domain, the residence time for material introduced on the first day of the simulation, and the maximum load rate and its location in the model domain in the summary file.

### c. The AWATS modeling package

We have constructed a package called AWATS that may be suitable for regulatory use. This package conveniently applies the hydrodynamic and transport models along with associated information regarding the net-pens and to obtain appropriate graphical displays. AWATS includes the waste transport program TRANS, the graphical interface SMS, and the flow model DUCHESS. (In the event the user does not have DUCHESS, output from another flow model may be used.) It also includes the utility program DUCHSMS which will extract flow and bathymetry data for the subdomain of interest (i.e., the general vicinity of the net-pen, specified by the user in the form of a rectangle) from the output files of the flow model (DUCHESS or alternative) and use this information to run TRANS.

It is helpful to describe the AWATS

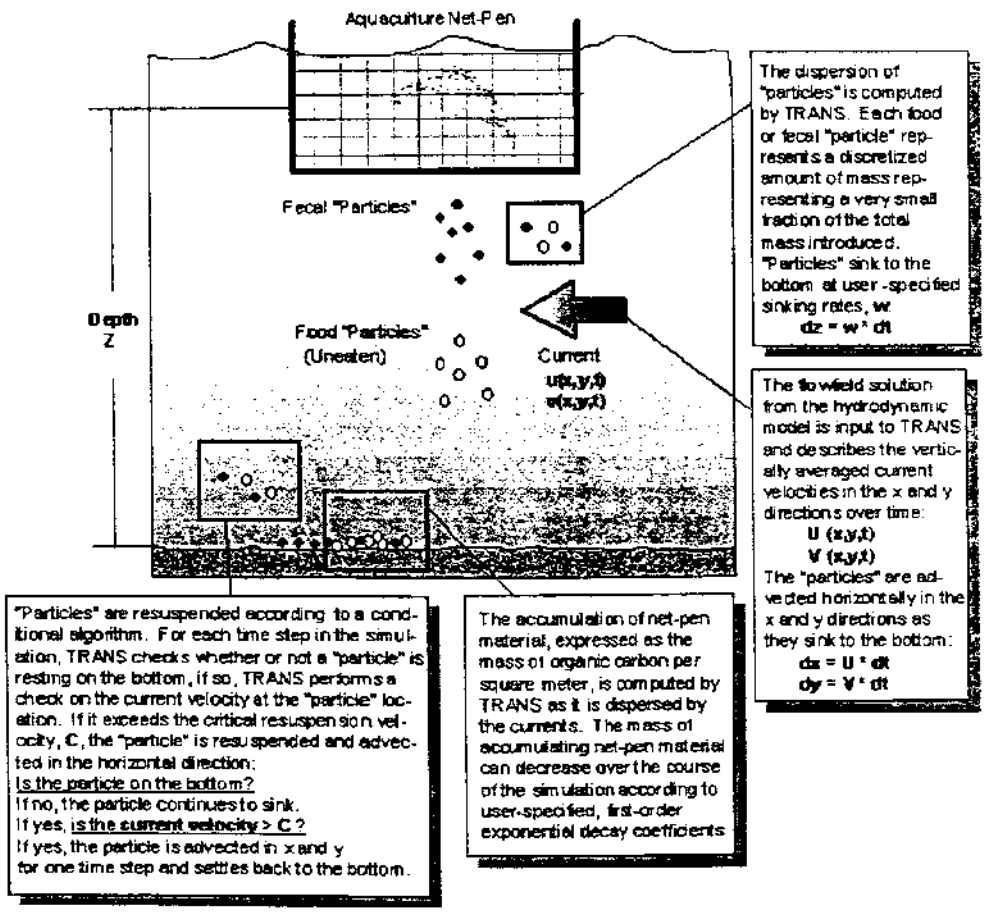


**Figure 7.** Summary illustration for the TRANS aquaculture net-pen waste transport simulator algorithm developed at the University of Maine.

modeling package in terms of its data file components:

1) DOMAIN.DOM, the overall domain descriptor file containing the size of the overall domain, grid spacing, number of time steps, and time step size in the flow solution file;

2) DOMAIN.TOP, the topography file, containing the depths at different grid points of the overall domain for which the flow model is run;

3) DOMAIN.FLW, the flow solution file, containing the x-directed and y-directed velocity fields at each time step;

4) DOMAIN.OZ, a one-zero file used by DUCHESS and AWATS to differentiate between "dry" (0) land points and "wet" (1) computational points in the overall domain;

5) SUBDOMAIN.BTH, containing depths of a subdomain in the vicinity of the net-pen (the subdomain in which waste transport simulations are to be made);

6) SUBDOMAIN.XYZ, a bathymetry file readable by SMS for use in constructing the domain geometry to graphically view flow and transport solutions;

7) SUBDOMAIN.UV, the flow solution file corresponding to the subdomain to be used by TRANS;

8) SUBDOMAIN.DAT, a second flow solution file readable by SMS that can be used to display/ animate the flow solution over the domain geometry;

9) SUBDOMAIN.FRM, containing user-defined net-pen parameters: coordinates of the center of each net-pen, and the volume, stocking density, and daily feed quantity of each individual pen;

10) TRANSIN.DAT, which contains the transport model grid spacing and time step, simulation duration, daily start and end times and frequency of food/fecal matter introduction, output requests, pen location coordinate adjustments, critical resuspension velocity ($U_{crit}$), settling velocities for food/ fecal material, the fraction of the introduced food and fecal matter that is organic carbon, the fraction of the daily feed quantity that is wasted, mass of fecal pellet production per unit mass of fish, and first-order exponential decay coefficients for food and fecal matter.

The first step in the modeling procedure (Fig. 8) consists of obtaining tidal and/or wind-induced velocities using a flow model solution. This involves the DOMAIN.* files. Since the overall model is often larger than the area of interest near the net-pen, a subdomain defined by the four corner points may be selected for further modeling. DUCHSMS uses the DOMAIN.* files as input to provide the necessary SUBDOMAIN. BTH and SUBDOMAIN. UV files, which are used along with the additional data contained in the SUBDOMAIN. FRM and TRANSIN.DAT files required to construct a transport simulation. The creation of the following output files using DUCHSMS functions requires the input of the DOMAIN.DOM file to coordinate the use of the other input files (*.TOP, *.FLW, *.OZ): SUBDOMAIN.BTH, the bathymetry file to be used as part of the farm description file; SUBDOMAIN.XYZ, a second bathymetry file readable by SMS for use in constructing the domain geometry to graphically view flow and

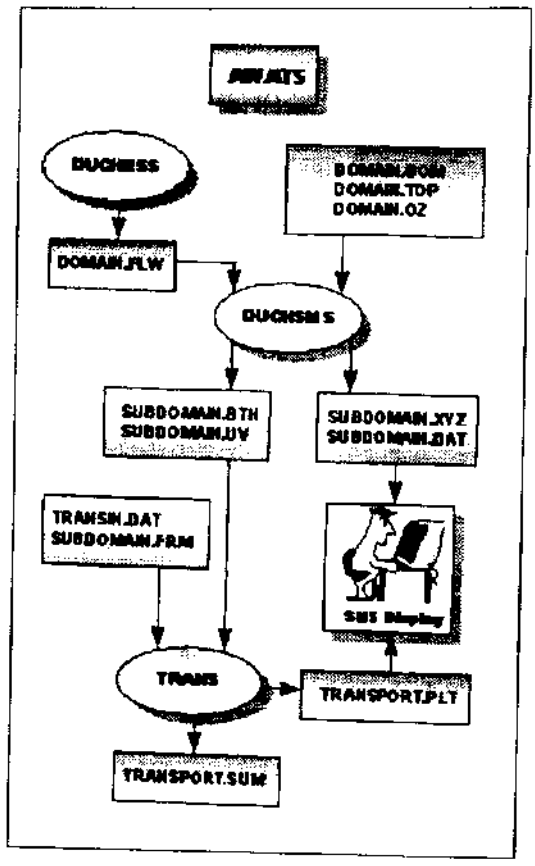


**Figure 8.** Operational chart for the AWATS package.

transport solutions; SUBDOMAIN.UV, the flow solution file corresponding to the subdomain to be used by TRANS; and SUBDOMAIN.DAT, a second flow solution file readable by SMS that can be used to display/animate the flow solution over the domain geometry.

Executing TRANS produces two forms of output. First, the TRANSPORT.SUM file is a simulation summary describing all user-defined parameters and also reports flushing efficiency of introduced particles from the domain, residence time, and the sedimentation rate and location of the point with greatest accumulation in the subdomain. The other file TRANSPORT.PLT is a data file that contains snapshots of the dispersion of net-pen wastes over the simulation, suitable for plotting in SMS for viewing/animation.

## 3. Simulation of net-pen waste distribution in Machias Bay, Maine

The AWATS modeling package was applied to six aquaculture sites in Maine: three in Cobscook Bay, and one each in Blue Hill Bay, Machias Bay, and Cutler Harbor. Here, space permits the description of our simulations in Machias Bay, which is located in Washington County (Fig. 9) in the Gulf of Maine. The typical tidal range for Machias Bay is about 4 m. The aquaculture site of interest for this domain is operated by Atlantic Salmon of Maine, Inc. (ASMI) located in Northwest Harbor off Cross Island. The island is situated in the mouth of Machias Bay close to the mainland where it forms the Cross Island Narrows to its northeast (Fig. 10).

Figure 10 shows the 13 x 14 km domain geometry representing the entire Machias Bay area. The domain coastline and bathymetry were digitized to 75 m resolution in SMS using a computer-scanned image of nautical chart 13326 of the National Oceanic and Atmospheric Administration (NOAA). These bathymetry data are stored in the topography file MACHIAS.TOP. The flow model DUCHESS was used with this bathymetry to simulate tidal currents. The model was forced with specified tidal amplitudes at the Gulf of Maine/Machias Bay boundary and the Cross Island Narrows boundary. Initial efforts in tuning the model yielded reasonable simulations which matched current data provided by the Maine

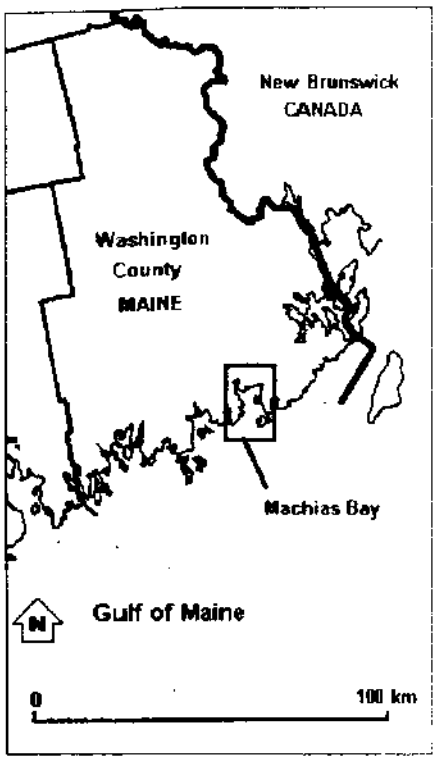


**Figure 9.** Location of Machias Bay in Washington County, Maine. The aquaculture operation at Cross Island in the mouth of Machias Bay is one of six sites modeled with the AWATS modeling package.

Department of Marine Resources (DMR) in the vicinity of the aquaculture lease area; however, the flow patterns in other areas of the model did not appear to be entirely realistic. For example, while the model produced high currents in the Cross Island Narrows (as related by anecdotal evidence) and varied over time, the direction of the current never reversed over the course of an entire tidal cycle. Additional current data were therefore collected in the Cross Island Narrows using an S4 current meter on 15 August 1997. These data allowed the adjustment of tidal amplitudes and phases at each open boundary, yielding greatly improved results not only near the Cross Island Narrows, but for the overall domain by providing a more complete picture of the tidal forcing at the boundaries of the model. A snapshot of current velocities just after high tide near Cross Island, taken from the model results

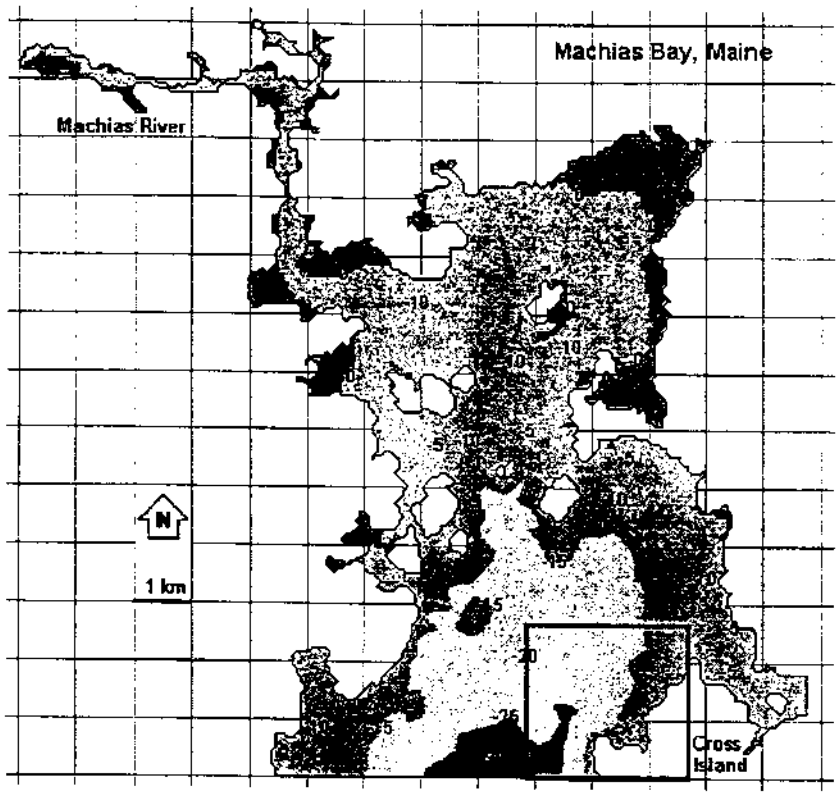


**Figure 10.** Machias Bay domain, bathymetry shown in gray scale. Location of the ASMI aquaculture site is denoted by an asterisk near Cross Island. The subdomain chosen for transport modeling is enclosed by a box. Grid squares are 1 km$^2$; depths are given in meters.

stored in MACHIAS.FLW, is shown in Figure 11.

For modeling net-pen waste transport at the ASMI aquaculture site, the subdomain outlined by the box in Figure 10 was chosen; it is defined by specifying the coordinates of the corners. DUCHSMS was used to extract the hydrodynamic solution and depths for this area of interest from the overall domain information contained in MACHIAS.FLW and MACHIAS.TOP. The resulting subdomain information is contained in ASMI.BTH and ASMI.UV. Another file called ASMI.XYZ is also obtained from DUCHSMS, which is read into SMS in order to construct the domain geometry for plotting and animating flow-field solutions and transport model output.

In addition to the hydrodynamic solution file, TRANS requires a farm description file defining the locations, volumes, stocking densities, and daily feed quantities for each pen. Eighty-six ASMI net-pens of various sizes and configurations were located with the aid of aerial photos from March 1996 provided by T. Riggens of the Maine DMR. Exact stocking and husbandry information for this site is confidential and so, for modeling purposes, general aquaculture husbandry data obtained for the previous modeling study in Cobscook Bay (courtesy of Connors Brothers Limited, Aquaculture Division) were used in conjunction with literature data (Laird and Needham 1988) to estimate pen stocking density, daily feed quantities per pen, and fecal production per unit mass of fish for the ASMI site. It is important to note that this nominal aquaculture husbandry information was used to simulate the dispersion and rates of sedimentation of waste effluent from this site in order to illustrate the application of AWATS.

Running a 15-day transport scenario for the Cross Island site produced the summary file ASMITRANS.SUM and the organic carbon

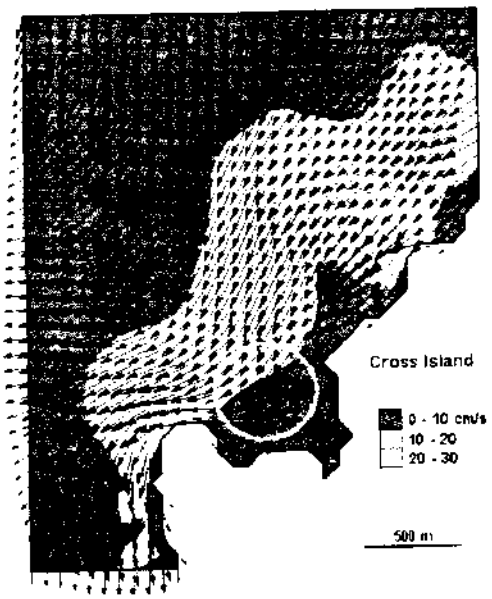


**Figure 11**. Tidal flow-field solution at the ASMI Cross Island aquaculture lease site immediately after high tide. Vectors denote magnitude and direction of current velocity. Gray-scale contours also represent velocity magnitude. The circle indicates the approximate location of the lease area.

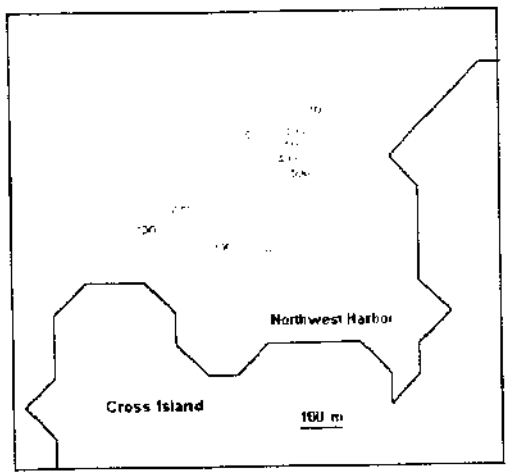


**Figure 12**. Contour plot of 15-day simulated net-pen aquaculture waste deposition at the Cross Island aquaculture site in Northwest Harbor. Contour intervals are 100 g/m$^2$ organic carbon.

concentrations file ASMITRANS.PLT which is read into SMS and plotted. Figure 12 shows a snapshot illustrating the loading pattern of finfish aquaculture waste deposition as g/m$^2$ organic carbon at the Cross Island aquaculture lease site at the end of the 15- day model run. For this simulation, the $U_{crit}$ value was set at 40 cm/sec. Since no waste material introduced on day 1 of the simulation was transported beyond the Cross Island domain bounds, average residence time was not computed for the summary. The eastern and southeastern portions of the lease area received the highest loading with one point receiving a maximum organic carbon loading rate (averaged over 15 days) of 38.9 g/m$^2$/per day. The mean and maximum velocities computed by the model for this particular area were 6.3 cm/sec and 10.0 cm/sec, respectively. Though not high enough to exceed the $U_{crit}$ criterion for resuspension, the currents in this area could supply sufficient oxygen to the benthos for adequate rates of decay of the effluent as well as high rates of water exchange in the embayment to prevent adverse impacts on the macrobenthos (Drake and Arias 1997). Findlay and Watling (1994) estimated that a constant 6 cm/sec current can deliver enough dissolved oxygen to sediments to support the theoretical maximum aerobic oxidation of nearly 50 g/m$^2$/day of organic carbon. The results demonstrate how AWATS can provide not only a picture of waste distribution, but information regarding spatial and temporal variations in current velocity that can be used in conjunction with benthic oxygen demand data to determine if organic enrichment in high-load regions has the potential to exceed the assimilative capacity of the environment.

## CONCLUSION

In situ measurements near the Deep Cove aquaculture site suggested that bottom sediments near net-pen aquaculture sites are eroded at $U_{100}$ velocities greater than about 40 cm/sec in the winter and about 50 cm/sec in the summer. These values are used in the development of the

modeling package AWATS which can be used for estimating the dispersal of net-pen wastes in a coastal environment with varying currents. Although not described here, the package may be used for storm-driven currents and wave-induced velocities as well. Application to the aquaculture site in Machias Bay and others in Maine suggest that AWATS is a convenient tool that can be used to aid with site evaluation and direction of field monitoring programs for areas of coastal Maine.

## ACKNOWLEDGMENTS

This work was supported in part by the National Marine Fisheries Service (NMFS)/ NOAA under the Saltonstall-Kennedy Program and by the University of Maine School of Marine Sciences. We are grateful to Dr. James Manning of NMFS, John Sowles of the Maine Department of Environmental Protection, Laurice Churchill and Tracey Riggens of the Maine Department of Marine Resources, and to Stephen Dickson of the Maine Geological Survey for their helpful assistance and advice over the course of this project.

## LITERATURE CITED

Amos, C. L., J. Grant, G. R. Daborn, and K. Black. 1992a. Sea Carousel - a benthic, annular flume. Estuarine Coastal Shelf Sci. 34: 557-577.

Amos, C. L., G. R. Daborn, H. A. Christian, A. Atkinson, and A. Robertson. 1992b. In situ erosion measurements on fine-grained sediments from the Bay of Fundy. Mar. Geol. 108: 175-196.

Aure, J. and A. Stigebrandt. 1990. Quantitative estimates of the eutrophication effects of fish farming on fjords. Aquaculture 90: 135-156.

Booij, N. 1989. User Manual for the program DUCHESS, Delft University computer program for 2D horizontal estuary and sea surges. Department of Civil Engineering, Delft University of Technology, Delft, The Netherlands.

Drake, P. and A. M. Arias. 1997. The Effect of Aquaculture Practices on the Benthic Macroinvertebrate Community of a Lagoon System in the Bay of Cadiz (Southwestern Spain). Estuaries 20(4): 677-688

Engineering Computer Graphics Laboratory. 1995. SMS Surface Water Modeling System Reference Manual. Brigham Young Univ., Provo, Utah.

Findlay, R. H. and L. Watling. 1994. Toward a process level model to predict the effects of salmon net-pen aquaculture on the benthos, pp. 47-78. *In:* B. T. Hargrave [ed.], Modeling Benthic Impacts of Organic Enrichment from Marine Aquaculture. Can. Tech. Rep. Fish. Aquat. Sci. 1949: xi + 125 p.

Hansen, P. K., K. Pittman, and A. Ervik. 1991. Organic waste from marine fish farms - effects on the sea bed, pp. 105-119. *In*: T. Makinen (ed.), Marine Aquaculture and Environment. Nord 1992: 22, Nordic Council of Ministers, Copenhagen, Denmark.

Jin, X. and C. Kranenberg. 1993. Quasi-3d numerical modeling of shallow-water circulation. J. Hydraulic Eng. 119: 458-472.

Jones, N. L. and D. R. Richards. 1992. Mesh generation for estuarine flow models. J. Waterway Port Coastal Ocean Eng. (ASCE) 118(6): 599-614

Laird, L. and T. Needham, Editors. 1988. Salmon and Trout Farming. Halsted Press, New York, 271 p.

Newell, C. R. 1991. Development of a model to seed mussel bottom leases to their carrying capacity. Phase 2 report, ISI8809760, National Science Foundation SBIR.

Panchang, V.G., G. Cheng, and C. Newell. 1993. Application of mathematical models in the environmental regulation of aquaculture. Final report to the National Marine Fisheries Service/NOAA.

Panchang, V. G., G. Cheng, and C. Newell. 1997. Modeling hydrodynamics and aquaculture waste transport in coastal Maine. Estuaries 20(1): 14-41.

Schneider, C. and R. Fridley. 1993. Aquaculture and the marine environment: the shaping of public policy. Working Paper for the Workshop, Marine Biological Laboratory, Woods Hole, MA. 10 p.

# ENGINEERING TECHNIQUES FOR ENHANCEMENT OF NEARSHORE ROCKY HABITATS FOR SEA URCHIN AND ABALONE AQUACULTURE

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## ABSTRACT

Commercial production of abalone and sea urchins has been markedly reduced by low availability of algal food they consume. There are two mechanisms responsible for the limited food: overgrazing by sea urchins, and a great loss of drift algae produced as they become dissipated by water movement. The wave-induced water motion may inhibit sea urchin grazing, and as such the importance of the wave action to protect kelp abundance from the destructive grazing leads us to an engineering possibility of developing kelp beds by increasing water velocity. In addition, a new device was developed to trap drift algae. The device is a box cage with a pendulum-like door and stoppers. The door is designed to be opened inwards by wave-induced oscillatory flow but the stoppers prevent its outward opening. Laboratory scale-model experiments on the trapping mechanism, effectiveness, and engineering design were carried out. Further field experiments demonstrated that the device could trap drift kelp and never lose them until they were consumed by aggregated sea urchins.

## INTRODUCTION

The commercial production of abalone and sea urchins is frequently limited by the availability of food. Two mechanisms are responsible for limited food in the habitats. First, kelp, Laminarian algae, are primary food resources necessary for growth of the animals but are frequently overgrazed, especially by sea urchins. In addition, most of the kelp production may be swept out of the shallow habitats as drift algae by coastal water motion. These benthic herbivores are of great importance to the nearshore fisheries in Japan. Artificial structures built to establish kelp beds and to trap drift algae have been incorporated in a long-term government subsidy program. The program was the Coastal Fishing Ground Improvement and Development Project, called the Ensei Project, initiated in 1976 to promote the enhancement of artificial habitats in Japanese coastal waters (Stone et al. 1991).

It is well known that new substrata may lead to an increased abundance of algae. This empirical knowledge has encouraged the operations of constructing artificial substrata, mainly with concrete blocks and quarry rocks to create kelp beds. However, the operations frequently failed to establish kelp forests as expected. Many of such failures seemed attributable to sea urchin grazing. Thus, a number of attempts were made to protect kelp plants from animal grazing with physical barriers, such as grid fences, plastic nettings and plastic-seaweed frills, as well as with chemical repellents (Kawamata 1994). Nevertheless, no technique is available for the artificial development project in sea urchin-dominated barren grounds. A recent study showed that wave-induced disturbance may restrict sea urchin feeding, thereby maintaining kelp forests adjacent to sea urchin-dominated areas (Kawamata in press). This view would be of great value to engineering practices.

On the other hand, the necessity of preserving drift algae in nearshore rocky habitats has been recognized by field researchers and fishermen involved in abalone and sea urchin aquaculture, but little has been known about their physical behavior in the field. A series of

engineering studies revealed the effectiveness of various types of structures of water motion in the laboratory and in the field (Kawamata 1987, 1988, 1991, Kawamata et al. 1993). The results were reflected in the publication of a guide book issued by the Japan Coastal Fisheries Promotion Association (1993) for design of artificial structures under the Ensei Project. The previous structures were "stable," or consisted of fixed materials. It is predicted from the guide that any of the stable structures could hardly trap and preserve drift algae in many of the shallow rocky habitats for a long enough period. To cope with the difficulty, recent studies (e.g., Kawamata and Suzuki 1995) developed a new trap with a pendulum-like door that is moved by wave-induced oscillatory flow.

This paper describes the importance of the wave-induced water motion to ecological balance between plants and herbivores, thereby showing the potential for establishment of kelp beds in engineering modifications. State-of-the-art technology of trapping drift algae is also described.

## ARTIFICIAL DEVELOPMENT OF KELP BEDS

It is well known that feeding by sea urchins may cause devastating effects on benthic marine plants (Lawrence 1975, Lubchenco and Gaines 1981). Unlike other benthic herbivores such as abalone, sea urchins have hard teeth and thereby easily feed on the stiff stipe and holdfast of macroalgae. Sea urchins may aggregate and denude the substratum of foliose algae, forming barren grounds that are covered solely by encrusting coralline algae. Sea urchin-dominated barren grounds are widely observed along the coasts of the Japanese archipelago. Such community types show long-term persistence because sea urchins can survive and reproduce in food-limited environments. However, preferred foliose algae are frequently abundant in shallow waters next to the sea urchin-dominated barren zones. The wave-induced benthic oscillating flow increases with decreasing depth (more precisely, up to a wave breaking point), so that the water motion in the shallow depth constantly prevents sea urchins from feeding on algae, even during calm sea periods. There is much evidence supporting the hypothesis that the absence of algal plants from deeper or sheltered sites results from herbivorous grazing but not from the shortage of light intensity or nutrients. First, experimental removal of sea urchins led to re-establishment of macroalgae (Iwate Prefectural Fisheries Experimental Station 1988, Agatsuma et al. 1997). In addition, it is frequently found that underwater floating objects such as mooring ropes, which benthic herbivores can scarcely climb, are overgrown by kelp, even immediately above barren beds.

## EFFECT OF WATER MOTION ON SEA URCHIN GRAZING

A previous study (Kawamata in press) evaluated the restrictive effect of the wave-induced oscillating flow on feeding by the sea urchin *Strongylocentrotus nudus*. The sea urchin is commercially important but is frequently a causal agent in clearance of macroalgae along the coast of northern Japan, from Hokkaido to central Honshu. The method of the study was briefly as follows. A kelp (*Laminaria* spp.) food with given dimensions was anchored to the bottom in an oscillating flow tank, where starved sea urchins were contained. A feeding experiment was then conducted for one or two days to examine the feeding rate under a periodic oscillating flow. The experiments showed that the restrictive effect of the oscillating flow on feeding rates somewhat varied with the animal size and food morphology, but indicated a mechanical constraint that strictly inhibited urchin feeding at a moderate water velocity, approximating 30-40 cm/sec. The sea urchin's mouth is at the center of its attachment base, so that it must mount a thallus by detaching more than half the number of tube feet used to cling to the substratum. Sea urchins were dislodged when they would try to eat at such moderately high velocities. These findings led to a conclusion that the urchin feeding on foliose algae is nearly impossible beyond 40 cm/sec.

Other sea urchins seem to show similar velocity limits for feeding. Kawamata (unpublished data) examined feeding rates of sea urchin *Hemicentrotus pulcherrimus* in the oscillating flow in the same method as described above. The sea urchins of 45-mm test diameter

(approximating the maximum size) showed higher feeding rates at the higher temperature over the peak velocity. However, the feeding rate under both temperatures began to cease at approximately 40 cm/sec.

The finding that sea urchins cannot feed on kelp at the peak velocity higher than 40 cm/sec might give quantitative estimates for understanding the spatial distributions of sea urchins and kelp. In shallow subtidal areas where waves are constantly broken, kelp are usually abundant. The wave-induced peak velocity $u_{max}$ is estimated from the equation (Denny 1988):

$$u_{max} = 0.3[g(h + H)]^{1/2}, \quad (1)$$

where $g$ is the gravitational acceleration (= 9.8 m/s$^2$), $h$ the water depth, and $H$ the local wave height. When the bottom of the area is horizontal, the wave height is solely related to the depth, approximating Denny (1988)

$$H = 0.78\, h \quad (2)$$

The wave height within the surf zone somewhat increases as the bottom slope is steeper. Hence the wave-induced benthic peak velocity in the surf zone is

$$u_{max} > 0.4(gh)^{1/2} \quad (3)$$

Eq. 3 indicates that the velocity almost everywhere in the surf zone exceeds the limit for sea urchin feeding. Two other typical examples might be explained by the spatial variation in wave-induced water velocity. First, the lower limit of kelp beds tends to be deeper with increasing degree of wave exposure. Second, kelp occur solely on the uppermost part of rock outcrops and artificial structures, where absence of kelp from the lower part is unlikely to be attributable to light intensity or drift sand (Terawaki et al. 1995).

In general, the peak velocity on the substratum produced by surface waves is estimated from wave data through numerical computation (e.g., Kawamata in press). Several problems remain in accurately predicting kelp abundance in the field, including engineering problems on predicting local water flow in the vicinity of microhabitat and biological ones on algal growth in nature. However, recent studies (Kuwahara et al. 1997, Kawamata in press) indicated that the velocity limit for feeding may give a reasonable estimate for the area with kelp plants exposed to intensive animal grazing.

## ENGINEERING TECHNIQUES FOR ARTIFICIAL KELP BEDS

Several observations indicate an engineering possibility of establishing kelp beds by increasing the water velocity: kelp overgrow the uppermost parts of concrete blocks immediately below low water level while kelp are absent from the lower parts (Terawaki et al. 1995); kelp are abundant on the onshore side of permeable breakwaters but absent from the onshore side of less permeable ones.

Although it is easy to increase the water velocity with conventional engineering structures, attention should be paid to other aspects of the wave effect on algal populations, such as breakage and dislodgment by waves. When an object is placed under waves, the water velocity is higher on the top of the object. The increased water velocity may lead to the higher maximum water velocity at severe waves, thereby increasing the risk of kelp breakage and dislodgment. No quantitative information is available for estimating the breakage and dislodgment of kelp. In addition to this biological problem, no practical method is available for estimating the local water velocity on the surface of structures under waves. Despite these problems, the velocity limit for sea urchin feeding will be undoubtedly an important criterion for deciding how to design or allocate artificial structures for kelp.

## DEVELOPMENT OF DRIFT-ALGAL TRAP

### BACKGROUND

Aimed at increasing food availability by trapping drift algae, various types of artificial structures have been constructed in nearshore rocky fishing grounds of Japan. In general, these structures may be divided into two types: block or grid. The block type settles drift algae on the upstream and downstream sides by controlling the surrounding fluid motion while the grid type obstructs drift algae by nettings with little variation of the flow. In practice, however, previous studies

suggested that the past attempts were too optimistic and that any of the conventional fixed structures can hardly control drift algae in wave-exposed shallow areas for a long enough period. Drift algae are considerably lightweight in seawater. For example, the ratio of weight in seawater to that in air approximates 0.025-0.04 for *Laminaria* spp. and 0.04-0.1 for *Eisenia bicyclis* (Kawamata 1991). In addition, the fall velocity, which is a measure of the difficulty in being raised or moved by turbulent water flow is approximately 2 to 4 cm/sec for *Laminaria* and 8 cm/sec for *E. bicyclis* (Kawamata et al. 1993). Therefore, drift algae are easily transported and raised up from the bottom by turbulent water flow. Occurrence of turbulent eddies is associated not only with rugged bottom and wave breaking but also with blockage effect of traps themselves. For example, let us consider that a very long impermeable concrete block with a height of 2 m is deployed parallel to the shore on a flat bottom at 6 m depth. It is predicted from Kawamata et al. (1993) that drift *Laminaria* may be raised up and spread out of it by wave-induced eddies at wave heights of only 0.4 m for a wave period of 10 sec. Further, a field study on 1.5-m hollow concrete cubes fitted with 10-cm mesh grids, specifically designed to trap drift algae, has demonstrated that *E. bicyclis* plants were raised out of the cubes by wave-induced turbulence, even at the peak bottom velocity of as large as 20 cm/sec (Kawamata et al. 1993). In addition, coastal water flow may transport drift algae in all directions. Consequently, drift algae may soon be driven away from a barrier which does not enclose trapped drift algae (Kawamata et al. 1993).

## INTRODUCTION

The newly developed drift-algal trap consists of a rectangular cage with a door and stopper (Fig. 1). The door is a plate as a whole, or a flow-shield and grating at the lower and upper parts respectively, which is suspended by hinges attached to the cage. The stopper consists of elastic bodies such as rubber and springs installed to stop the door from swinging outwards and lessening the shock of collision. By placing the trap on the seabed as the door confronts prevailing waves, the door is opened inwards by drag force exerted primarily on the flow-shield when the wave-induced water flow crosses the door inwards. Otherwise, the door is closed by the drag and gravitational forces.

As readily imagined, when drift algae are transported straight to the door by oscillatory flow, they may enter the trap with incoming water when the door is opened, and then are confined in the cage because the door is closed when the direction of flow is reversed. Furthermore, the trap may also capture drift algae passing by the trap. This process is slightly complicated and will be described later.

## MODEL FOR ESTIMATING THE IMPULSIVE FORCE

Because the door and the fluid near both sides of the flow-shield move freely and stop suddenly at the stopper, the consequent impulsive force may be considerably larger than the drag on the trap. The impulsive force $F_{max}$ is estimated from the equation (Kawamata and Suzuki 1995):

$$F_{max} = k^{1/2}\left[I + \frac{\pi}{4}C\rho b h_s^4\left(\frac{r^2}{h_s^2} - \frac{8}{3\pi}\frac{r}{h_s} + \frac{1}{4}\right)\right]^{1/2}\frac{u_c}{r - h_s/2}, \quad (4)$$

where $k$ is the modulus of the stopper, $I$ the moment of inertia of the door, $C$ the coefficient, $\rho$ the density of the fluid, $b$ the width of the flow-shield,

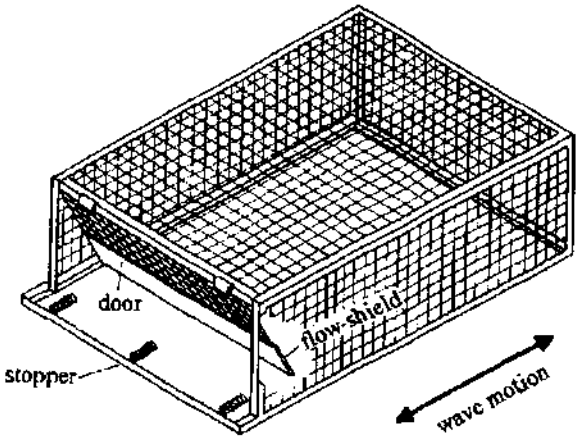


**Figure 1.** Drift-algal trap with a moveable door.

$r$ the height of the door, $h_s$ the height of the flow-shield, and $u_c$ the instantaneous water velocity at collision. The coefficient $C$ is empirically determined as 1 (Kawamata and Suzuki 1995).

## TRAPPING MECHANISMS

A laboratory scale-model experiment (Kawamata and Suzuki 1995) clarified the trapping mechanisms and efficiencies. Under progressive waves, drift algae are transported in the wave direction with oscillatory motion. When a trap is placed and oriented with the door facing the wave-coming direction, drift algae are trapped in three ways. First, drift algae straightly approaching the door are trapped as described earlier. Second, drift algae moving in the course outside of the trap are gradually drawn toward the side of the door, and then are moved back to the front of the door and enter the trap. Third, drift algae approaching the door from the front are once transported to the side of the door, and then are trapped in the same way as in the second process. The fact that drift algae are carried obliquely to the door and that drift algae once pass by the door and then are drawn back to the front of the door are explained by the crosswise flow produced as follows. When the direction of oscillatory flow turns from outwards to inwards of the door, the door turns back to the upright position and stops at the stopper. However, the water immediately outside of the flow-shield is entrained by the fluid passing over the flow-shield and then moves outwards, followed by the fluid from its sides. The crosswise flow carries drift algae from the sides immediately ahead of the door so that they are readily transported into the trap with the subsequent inward flow. When the moment of the hydrodynamic force on the door is large compared with that of the gravitational force on the door (i.e., the door readily follows the flow), drift algae coming from the front of the door are mostly trapped in the first process. Otherwise, they are trapped in the third process, because the slowly moving door produces a high pressure region (or a separated flow region) in front of the flow-shield when the water begins to move inwards of the door.

## TRAPPING EFFICIENCIES

Let us consider that drift algae are transported with progressive waves near the trap from the front to the back of the door, and denote the crosswise distance between the course of oncoming drift algae and the axis of the trap as $y$. A laboratory scale model experiment (Kawamata and Suzuki 1995) showed that a trap with the door properly designed has the following ability to trap drift algae. The probability that drift algae coming in the course of $y$ will be trapped can be high (more than approximately 90%) when the course is within the door (i.e., $y < b/2$) and then decreases as the course is more distant from the door. The approaching course at which the trapping probability begins to be zero may reach the door width away from the side of the door (i.e., $y = 1.5b$). The relationship between the trapping probability and the ratio $y/b$ seemed independent of the absolute value of the door width. Hence, the trapping efficiency defined as the integration of the trapping probability over $y$ divided by $b$ may reach almost 2. This suggests that the trap may have the equivalent of completely capturing drift algae passing through twice the width of the door. The trapping efficiency may remain at such a high level when the height of the flow-shield is greater than a limit, which is never lower than 0.25 m in full scale. The higher the door (or the flow-shield), the greater the impulsive force on the stopper. Thus, the optimal height of the flow-shield is the limit, probably approximating 0.4 to 0.5 m.

## FIELD TESTS

To verify the effectiveness of the trap, field experiments were conducted with a simple test device from August to December 1994 (Kawamata and Suzuki 1995) and were redone with a revised test model from August 1995. The devices were placed at 9-m depths on a relatively flat boulder area on the northeastern Pacific coast of Honshu, Japan (38°22'N,141°26'W). The site was immediately offshore of a steeply sloping bed that reached the shore. The shore was partly protected but constantly washed by waves. Kelp *E. bicyclis* were abundant immediately below low water level while the deeper area was barren with a high density of sea urchin *Strongylocentrotus nudus*. Abalone *Haliotis discus hannai* occurred mostly in the kelp bed but with lower density. Wave action seemed to prevent sea urchins from

**Figure 2.** The first test device placed on the experimental site

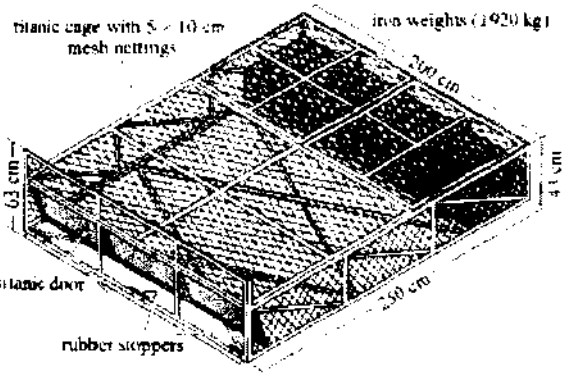


**Figure 3.** The titanium drift-algal trap test device.

invading the shallow kelp bed, as described by Kawamata (in press).

The first device was a stainless steel cage (2.4 x 2 x 0.6 m) with a door of 53 cm height, whose lower portion was covered with a 5-mm-thick fiber reinforced plastic board to create a 286-mm-high flow-shield. The mass and the moment of inertia of the door were 11.1 kg and 1.54 kg/m$^2$, respectively. The trap was firmly anchored to the bottom using underwater drilling equipment and oriented with the door facing the shore and perpendicular to the direction of oscillatory flow (Fig. 2). Three iron springs with 300-kg capacity mounted on iron plates (45 x 30 x 1.5 cm) were separately embedded in front of the door as the stopper. The device successfully trapped drift *Eisenia* nearly to the full. In addition, investigations suggested that the trap could hold drift algae until they were consumed by aggregated sea urchins. Despite such high effectiveness, engineering problems for practical application surfaced during the first test. First, stainless steel may be corroded in seawater, so that making a door of stainless steel might result in a heavy door with a lower trapping efficiency. Thus, it might be better to use more corrosion-resistant metal with a smaller specific mass density. Second, to facilitate the installation of traps, the stopper should be combined with the main cage and the trap should be simply fixed by weights in a general way. Finally, drift kelp caught in the cage accumulated from the innermost part of it but did not pile up approximately 0.4 m above the bottom, suggesting that the height of the cage could be lower without decreasing the effective capacity for drift algae.

Improved in these respects, the second device was made as a more practical model. The device was a 2.5 m long x 2.0 m wide x 43 cm -high titanium cage incorporated with a titanium door and rubber stoppers (Fig. 3). The door was a 196 cm wide x 40 cm -high titanium plate reinforced with thicker plates at the margin. The mass and the moment of inertia of the door was 6.37 kg and 0.361 kg/m$^2$, respectively. Iron weight amounting to 1920 kg was placed in the innermost part of the cage. The weight necessary to stabilize the trap was estimated from Eq. 4 with a design water velocity of 1.2 m/sec, which was determined from the velocity measurement conducted in 1994. The improved device also succeeded in trapping drift kelp up to the full (approximately 80 kg wet weight) (Fig. 4), and in holding drift algae until they are consumed by aggregated sea urchins (Fig. 5). Drift algae were almost absent around the device throughout the year except in early autumn, whereas drift algae frequently remained in the trap with intensive grazing by sea urchins. The door was amended because of slight damage due to its insufficient stiffness. With this amendment, the

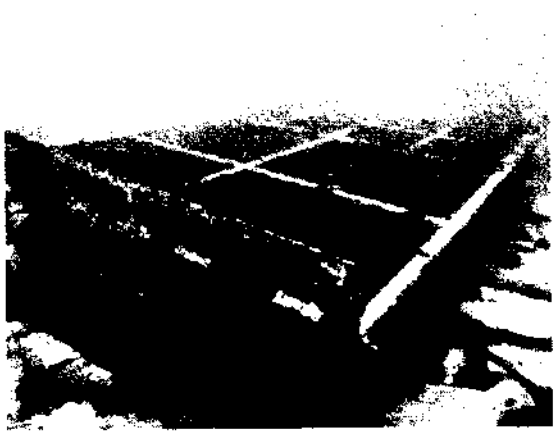

**Figure 4.** The improved test device trapping drift kelp to the full.

**Figure 5.** Sea urchins aggregated at the trapped drift kelp.

device has been functioning over 2 yr, showing promising results.

## DISCUSSION

Observations supported the hypothesis that most drift algae were swept out of shallow habitats before being consumed by animals under natural conditions. Abalone and sea urchins occasionally captured small pieces of drifting kelp, but never fed on entire detached kelp plants. It was observed that large amounts of drift kelp were accumulated in a crevice near the trap in early autumn. However, the drift kelp soon disappeared without aggregating animals. The drift kelp in the crevice occasionally oscillated in a "huge" body, with the result that small animals hardly grasped them. In contrast, the test devices frequently caught drift algae and maintained them until they were consumed by congregated sea urchins.

The observed variation in trapped drift algae suggested that drift algae sporadically occur at storms, especially during the first storm after summer, in which kelp biomass reaches maximum. Considering such sporadic occurrence of drift algae, an effective drift-algal trap is a device which can catch a large amount of drift algae occurring at rough seas and can reserve them under subsequently repeated severe waves. Although the capacity of the test devices was too small compared with a great consumptive capacity of aggregated sea urchins, it could be expected from the high trapping effectiveness that the device might be an effective technique for nearshore rocky aquaculture.

Only one or two abalone were found in the device, probably because of a low population density. However, observation made in late August 1997 recorded nine adult shells in the device.

Since the densities of drift algae and their herbivores also vary with the location, the trap should be placed at the path on a nearshore, deeper barren ground, through which plants detached from kelp beds may frequently pass. Like this field test, a small embayment with a relatively flat and depressed bottom near the opening is a potential appropriate site for application.

With high trapping efficiencies, the trap may also be used as an "automatic feeding system" for underwater cage culture, e.g., by stocking starving adult sea urchins or lean adult abalone in a cage with adequately small mesh grids placed closely behind the trap.

Finally, the durability required to resist the repeated collision should be examined by field tests. The present experiment will be continued to validate its practical applicability.

## CONCLUSION

In nearshore rocky fishing grounds with a high density of sea urchins, kelp beds may be confined to areas where the wave-induced oscillatory flow constantly prevents sea urchin grazing. The velocity limit for feeding by sea urchins is approximately 40 cm/sec. There is a possibility of developing artificial kelp beds in sea urchin-dominated areas by increasing the wave-induced water velocity at calm sea periods. In such areas, drift plants may be the primary food of sea urchins and abalone, but most of them may be swept out of the shallow habitats by water movement. The previous stable structures could not trap drift algae in most shallow habitats because of the high mobility of drift algae in water flow. The device developed by Kawamata in 1994 could catch drift algae effectively and hold them until consumed by animals.

## LITERATURE CITED

Agatsuma, Y., K. Matsuyama, A. Nakata, T. Kawai, and N. Nishikawa. 1997. Marine algal succession on coralline flats after removal of sea urchins in Suttsu Bay on the Japan Sea Coast of Hokkaido, Japan. Nippon Suisan Gakkaishi 63: 672-680. [In Japanese].

Denny, M.W. 1988. Biology and the Mechanics of the Wave-swept Environment. Princeton Univ. Press, Princeton, NJ. 329 p.

Iwate Prefectural Fisheries Experimental Station. 1988. Ganshou-kaitei ni okeru moba-zoseijouken ni kansuru kenkyu (study on the condition of kelp-bed development on the rocky bottom). Specific Research Grant Program Report, 1975-1977. Iwate Prefectural Fisheries Experimental Station, Kamaishi, Iwate. 15 p. [In Japanese].

Japan Coastal Fisheries Promotion Association. 1993. Structural design guide for the Coastal Fishing Ground Improvement and Development Project (Engan-Gyojo Seibi-Kaihatsu-Jigyo Kozobutsu Sekkei-Shishin). [In Japanese].

Kawamata, S. 1987. Drift alga traps to feed benthic algal feeders. Fish. Eng. 24(1): 53-60. (In Japanese).

Kawamata, S. 1988. Developmental studies on block-type drift alga traps with slit openings. Bull. Natl. Res. Inst. Fish. Eng. 9: 1-8. (In Japanese).

Kawamata, S. 1991. Physical considerations for the design of algal drift traps. U.S. Dept Commerce, NOAA Tech. Rep. NMFS 102: 171-180.

Kawamata, S. 1994. Importance of physical disturbance for artificial development of nearshore rocky fishing grounds. Fish. Eng. 31(2): 103-110. [In Japanese].

Kawamata, S. In press. Effect of wave-induced oscillatory flow on grazing by a subtidal sea urchin *Strongylocentrotus nudus* (A. Agassiz). J. Exp. Mar. Biol. Ecol.

Kawamata, S. and H. Suzuki. 1995. Development of a drift-algal trap for nearshore rocky aquaculture, pp. 628-633. *In*: Proceedings of the International Conference on Ecological System Enhancement Technology for Aquatic Environments (Sixth Int. Conf. Aquat. Habitat Enhancement). Japan International Marine Science and Technology Federation, Tokyo, Japan.

Kawamata, S., S. Hagino, and M. Yamamoto. 1993. Modome-shisetsu no kaiso-hosoku koka ni kansuru kenkyu (studies on the trapping effects of drift algal traps). Heisei 3-Nendo Engan-Gyojo Seibi-Kaihatsu-Jigyo ni Kansuru Suisancho-Kenkyusyo Kenkyu Hokoku (reports of the research institutes of Japan Fisheries Agency on the Coastal Fishing Ground Improvement and Development Project), pp.139-164. (In Japanese).

Kuwahara, H., S. Akaike, H. Hayashi, and Y. Yamashita. 1997. Mechanism of group seaweed formation in isoyake area. Jpn. Soc. Civil Eng. 44: 1181-1185. [In Japanese].

Lawrence, J.M. 1975. On the relationships between marine plants and sea urchins. Oceanogr. Mar. Biol. Ann. Rev. 13: 213-286.

Lubchenco, J. and S.D. Gaines. 1981. A unified approach to marine plant-herbivore

interactions. I. Populations and communities. Annu. Rev. Ecol. Syst. 12: 405-437.

Stone, R.B., J.M. McGurrin, L.M. Sprague, and W. Seaman, Jr. 1991. Artificial habitats of the world: synopsis and major trends, pp. 31-60. *In*: W. Seaman, Jr. and L.M. Spraque (eds.), Artificial Habitats for Marine and Freshwater Fisheries. Academic Press, San Diego, CA.

Terawaki, T., S. Arai, and Y. Kawasaki. 1995. Methods of submarine forest formation considering local limiting factors of distribution. Fish. Eng. 32(2): 145-154. (In Japanese).

# DESIGN CONCEPTS FOR INTEGRATION OF OPEN OCEAN AQUACULTURE AND OSPREY™ TECHNOLOGY

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## ABSTRACT

A unique opportunity exists to develop a multi-benefit, commercial ocean enterprise off the coast of New England, with potential sites reaching from Nantucket, Massachusetts, to Eastport, Maine. This enterprise could generate electric power, provide a base for installation of open ocean aquaculture facilities, help revitalize depleted fisheries, recycle discarded materials, and enhance economic development in several coastal industries, including shipbuilding and tourism. The concept is centered on the Ocean Swell Powered Renewable Energy (OSPREY) technology device developed by Applied Research & Technology (ART) of Inverness, Scotland. The OSPREY is a hybrid wind/wave energy generation system wherein wave energy is harnessed by means of an oscillating water column within a collector chamber anchored to the ocean floor by large, permanently installed ballast tanks. The OSPREY forms the central element in a multi-use ocean structure. Successful establishment of aquaculture facilities in the high energy environment off the New England coast will require solutions to a number of engineering challenges including structural integrity, security, operability, maintainability, and affordable design. The concept of design integration of aquaculture net pens into the OSPREY provides the opportunity to meet a number of these challenges in a cost-effective manner. Concepts for integration of submersible and floating net pen structures and their operation into the OSPREY technology must be advanced, potential sites must be studied, and benefits to the aquaculture industry determined.

## INTRODUCTION

The purpose for this paper is to present and discuss ideas for an existing technology which may provide a viable technical and commercial partner for offshore aquaculture facilities in the future. It is clear, from research conducted by the present authors and many others, that the establishment of a viable offshore aquaculture industry in the Northeast United States will require meeting numerous technical and economic challenges.

An offshore location for aquaculture facilities will require structural design for survivability in the severe weather, which is prevalent during the fall and winter seasons in these areas. Large, wind-driven waves and strong currents are common in these latitudes, even relatively close to the coastline. Permanently moored net pen structures must be designed to maintain structural integrity and net integrity in conditions approaching sea state seven (perhaps higher sea state during hurricane season), and currents up to 3 to 4 knots.

The offshore aquaculture facilities must be designed to provide for logistic support from shoreside facilities through the use of service vessels. The service vessels must be able to come alongside, dock, and offload personnel, supplies, and equipment. Furthermore, products will be

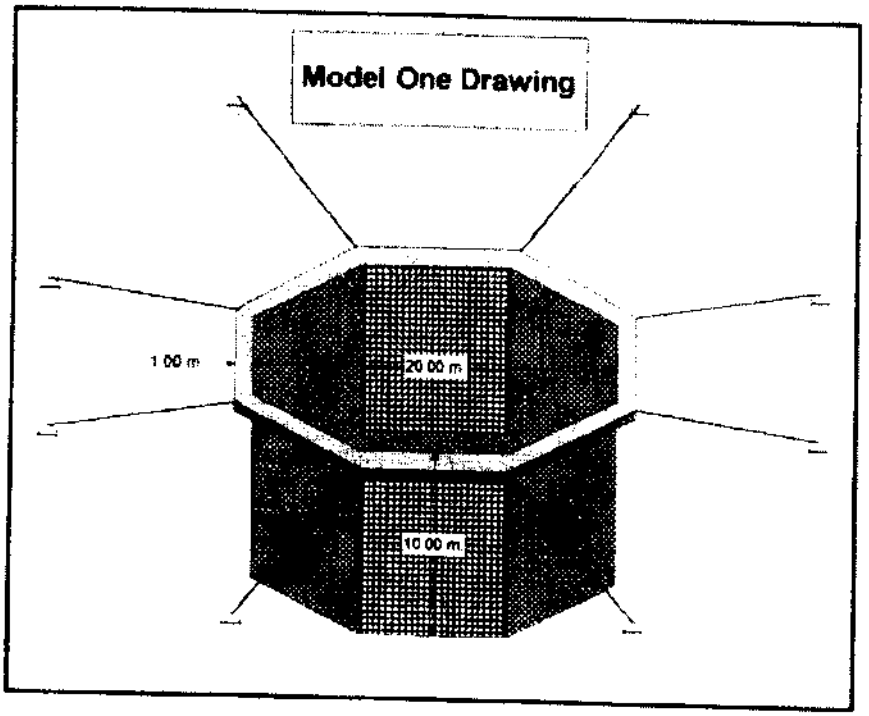


**Figure 1.** Modular floating net pen.

harvested and delivered to shoreside facilities for processing by the same service vessels. The aquaculture facility must provide for effective harvesting and net handling.

One of the major concerns voiced by the operator community has been associated with security of such offshore facilities. These structures will represent potentially large capital investment in net pen structures and finfish stock. Access to these facilities will be limited and/or impossible during extended periods of bad weather. This is in contrast to the existing operating conditions inshore, where daily access and visual inspection are possible during all weather conditions. Potential security concerns include accidental collisions by commercial shipping and offshore fishing vessels, intrusion by marine mammals, human intrusion, and detection of structural degradation.

The economic challenges associated with establishment of a viable offshore aquaculture industry are substantial. The market conditions for finfish products in the United States are extremely competitive today. As finfish aquaculture expands in developing countries, market competitiveness is expected to increase. The cost for purchase, maintenance, and operation of offshore facilities will be higher than such costs for today's inshore facilities. These trends are clearly in conflict. Future offshore aquaculture operators will be required to explore new and novel techniques for reducing cost and increasing productivity in order to remain competitive.

Several net pen architectures are currently in use or are under consideration for offshore facilities. Figures 1 through 4 illustrate floating, modular net pens, two types of individual floating net pens, and a submersible net pen concept advanced by the Ocean Engineering Department at the University of New Hampshire. Each of these architectures provides relative advantages and disadvantages. Each meets the projected requirements with varying degrees of success.

## Notional Technical Requirements

In order to visualize the potential advantages to integration of aquaculture net pens with OSPREY™ technology, it is useful to review specific notional engineering technical requirements for offshore aquaculture. It is expected that these

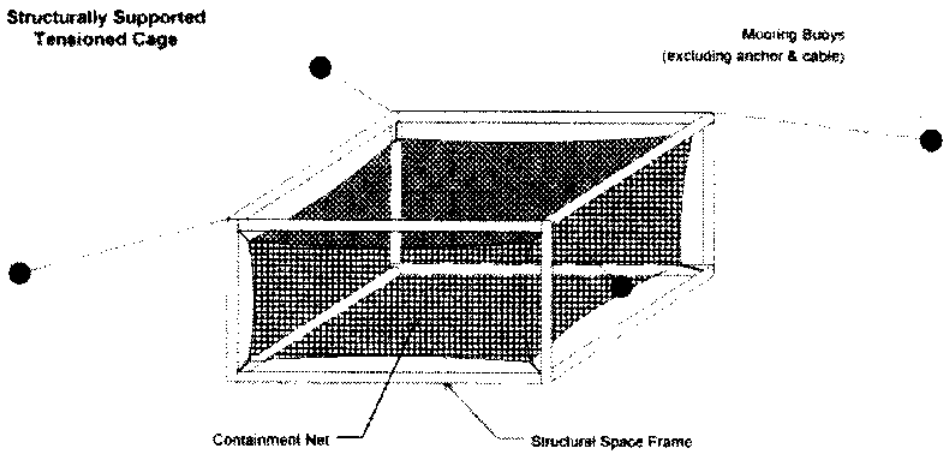


**Figure 2.** Individual rigid floating net pen.

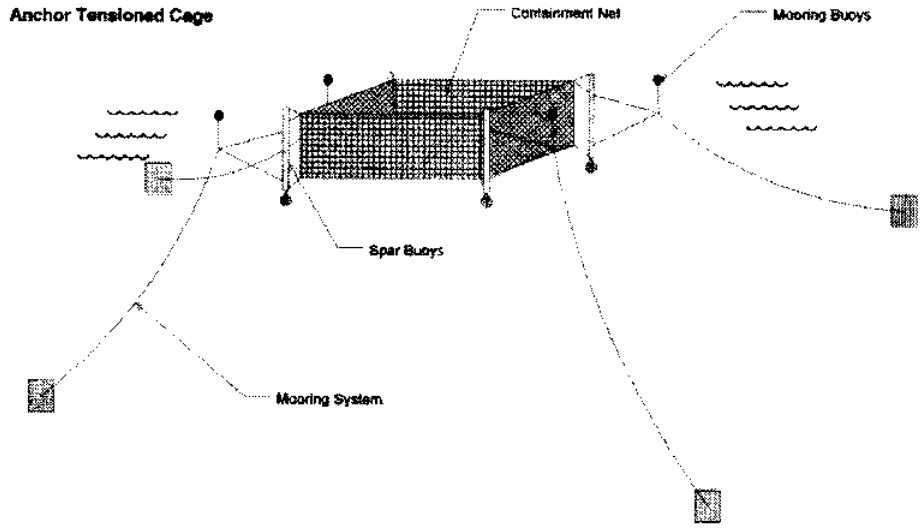


**Figure 3.** Individual flexible floating net pen.

facilities must meet the following operational and technical requirements:

- The facility must have the capability for independent operation without human intervention for extended periods. These periods would be primarily due to weather conditions. This operational need is distinct from today's practice of daily human intervention which is, in most cases, continuous during daylight hours.
- The structure must retain integrity during periods of severe weather. As described earlier, these periods would be primarily during the fall and winter seasons. However, brief periods of severe weather may occur during spring and summer seasons as well.
- The facility must provide a stable platform for personnel to perform operation and maintenance tasks during periods of occupation. Walkways and enclosures are

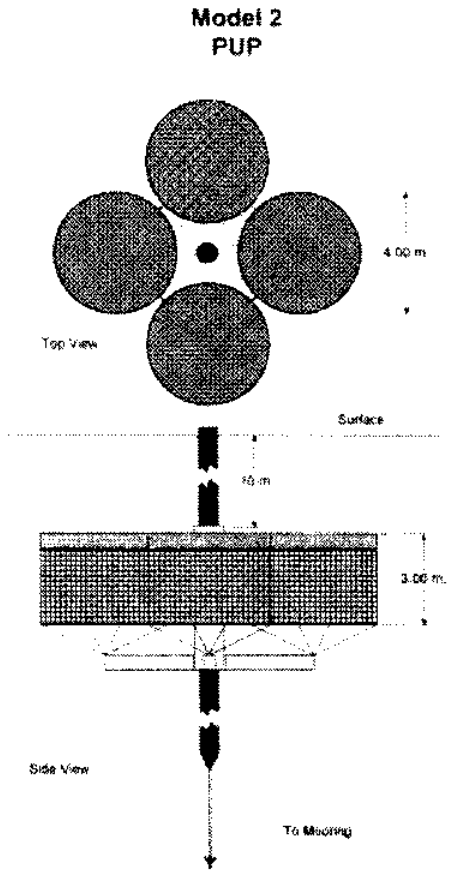


**Figure 4.** Pull-up pen (PUP), submersible net pen.

needed. Personnel safety must be ensured. Also, stability of the platform may be an issue for certain species of finfish envisioned for offshore culture.

- Provision for alongside operations by service vessels is required. This consists of features for tie-up, fending, loading and unloading of cargo and personnel, and conduct of net handling operations. Successful performance of these evolutions in open water will require careful engineering design and careful planning at time of execution.
- The facility must include sheltered storage space for feed, tools, spare parts, lines, nets, and other miscellaneous equipment.
- The facility should provide habitability space for short overnight visits by personnel during periods of settled weather and high activity aboard.
- The facility must provide sheltered spaces with suitable environment for electrical and electronic equipment. Such equipment may include sensors and monitoring equipment for intrusion detection and alarming, net containment breach, structural failure, weather conditions, automated feeding equipment, navigational aids, telemetry electronics, actuators for surfacing and submerging of submersible net pens, materials handling equipment such as cranes and hoists, electricity generation, and others.
- The facility must provide security monitoring and alarming. Security includes human intrusion, predation, structural integrity, and net containment integrity.
- The facility must provide an on-board electrical power source for sensors, monitoring equipment, processors, navigational aids, telemetry electronics, materials handling, automated feeding, and actuators.

Through consideration of the operation and maintenance of a future offshore aquaculture facility, it becomes clear that the design of such a facility must be very carefully considered, with participation by experienced operators along with mechanical, electrical, ocean engineers, and marine biologists. A successful design will require an interdisciplinary approach by a dedicated design team.

In considering a relatively mature technology for electrical power generation using wave energy, known commercially as **O**cean **S**well **P**owered **R**enewable **E**nerg**Y** (OSPREY™), it became clear to the authors that an opportunity for strong synergy between renewable power generation and aquaculture off the New England coast exists. Design of an aquaculture facility for integration with an OSPREY™ array offers the potential to satisfy many if not all of the engineering technical requirements for aquaculture described above. In addition, business arrangements may be feasible between the aquaculture operator and the power generation commercial entity (such as lease) which greatly reduce the cost of operation and maintenance of the aquaculture facility. In the next section, we provide a brief overview of the OSPREY™ system and notional concepts for

integration of net pen designs. The reader is encouraged to consider the potential advantages for structural integrity, protection from wave action, housing, storage, and electrical power which may accrue from such integrated design.

## OSPREY™ system description and concepts for integration with aquaculture net pen designs

The concept of utilizing ocean wave action as an oscillating water column for energy generation is an accepted technology and is utilized in numerous applications around the globe. Most of these installations are based upon coastal geology and therefore offer poor replicability on a general scale. The ability to fabricate such a device into a deployable configuration would have far-reaching impact on renewable ocean energy utilization and sustainable-yield aquaculture development. This proven technology was realized through the development of OSPREY by Applied Research &Technology of Inverness, Scotland. The OSPREY unit is fabricated of a closed rear bulkhead catamaran design. Each unit would consist of 800 tons of steel or concrete and is estimated to cost $5 million US to build, including an NEG Micon 1-Mw wind generator. Figures 5 through 7 depict OSPREY units in various settings.

Each unit will be constructed in a local shipyard and launched, towed or thrusted to the deployment site where it is scuttled and ballasted to the bottom, then interconnected to the grid via submarine cable. Each unit will be designed for local wave resource optimization. When deployed, each unit will displace in excess of 8000 tons. This formidable structure, when deployed in arrays of five to ten units, will be able to enhance the wave capture resource and the aquaculture resource yield can be increased substantially.

## LIMPET (Locally Installed Marine-Powered Energy Transformer) coastal harbor deployment unit

Where practicable, structures could be deployed along coastal harbors, improving harbor defense against ocean wave action while generating clean renewable energy. We have identified our oceans as an important new frontier; we will need a platform to develop our coastal marine technologies and OSPREY is ideal. An

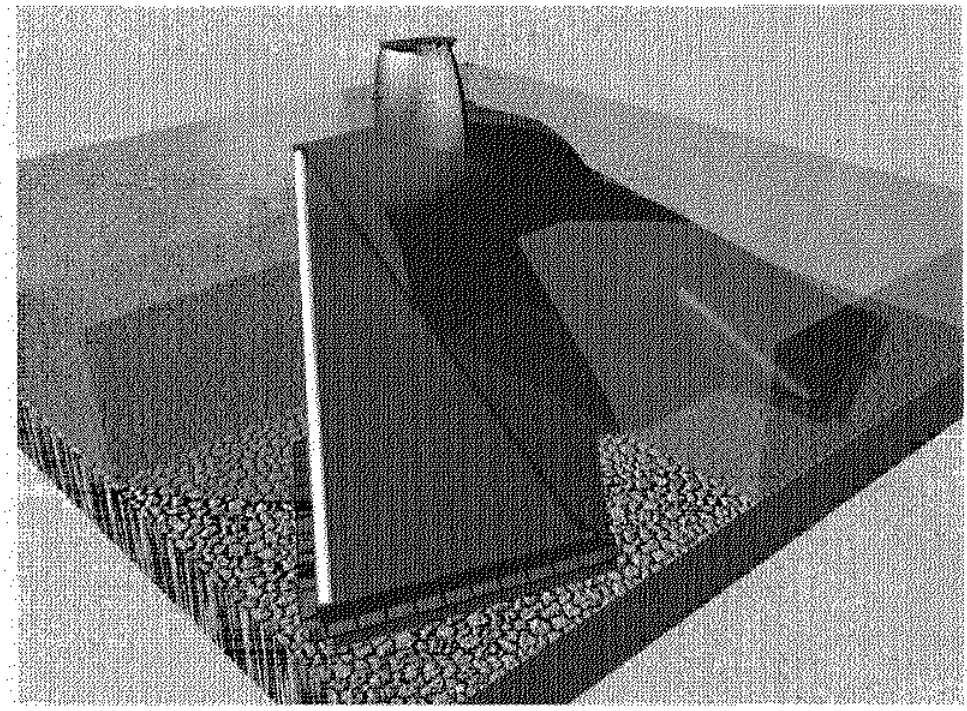

**Figure 5.** Dimensional 3-D drawing of OSPREY.

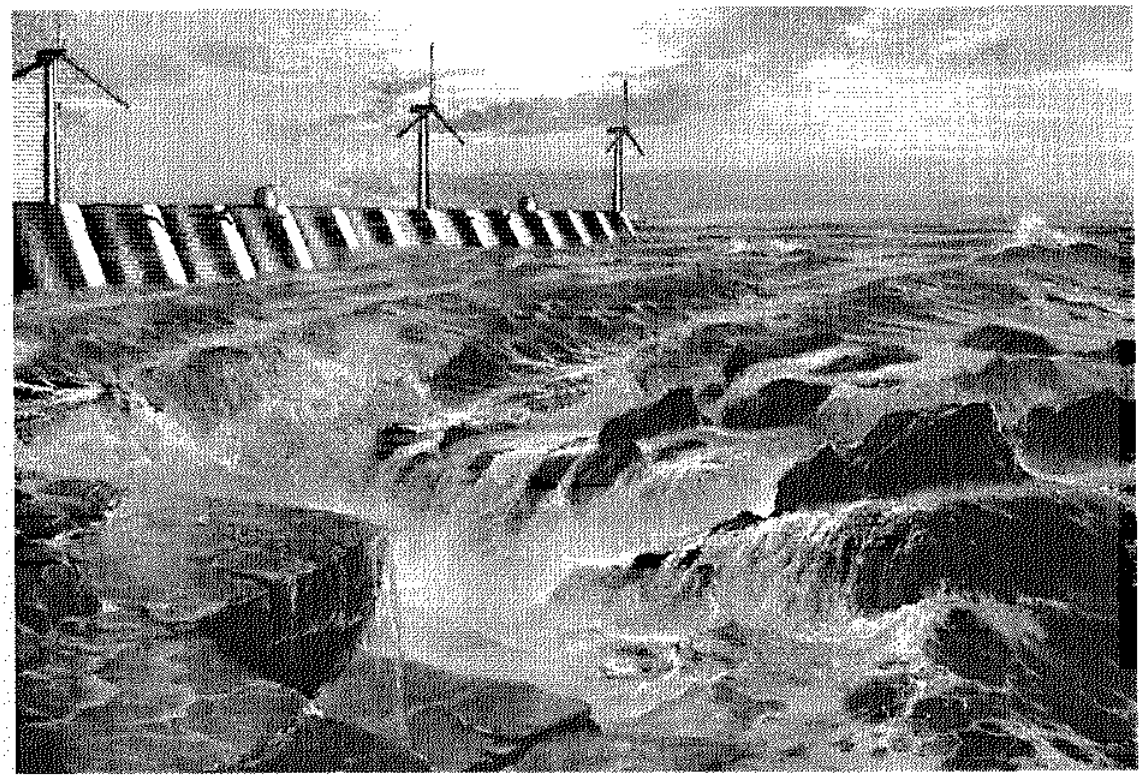

**Figure 6.** Rocky coastal harbor unit.

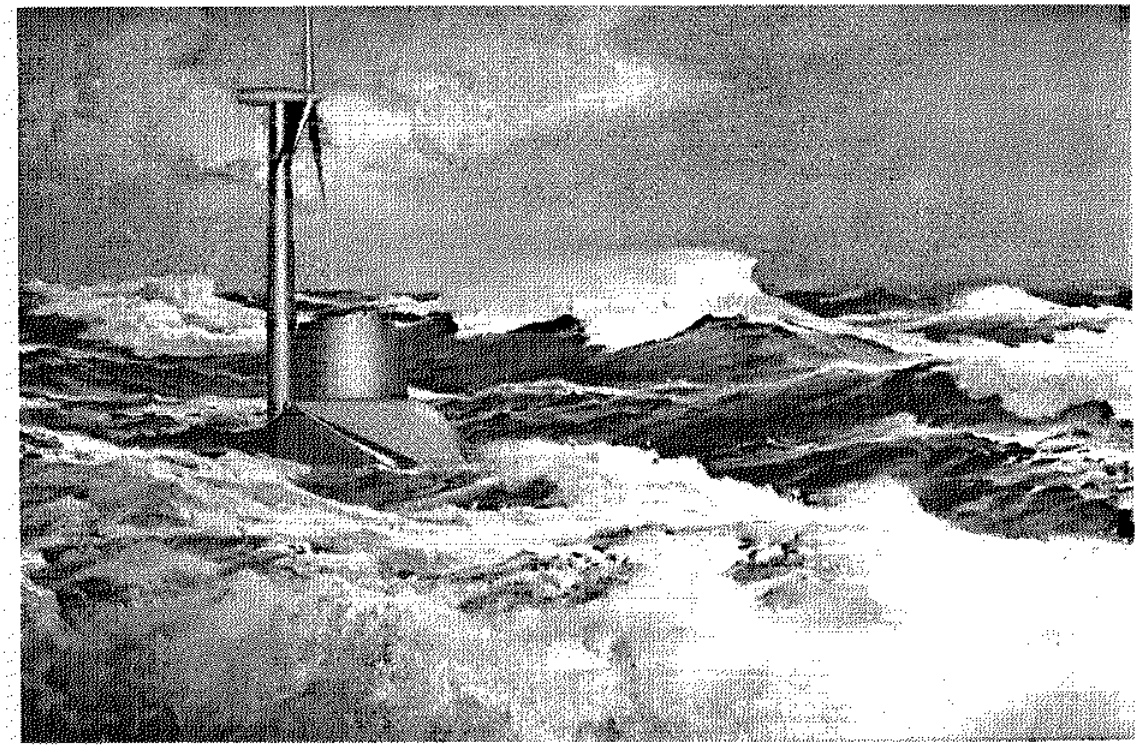

**Figure 7.** Ocean energy unit in storm conditions.

array of ten OSPREY could provide offshore research opportunities for a better understanding of our coastal ocean resources. The structure could provide sheltered facilities for the support of ocean ranching as suggested in the open ocean cage culture section of this paper. The structure would perform well as a structure for meteorological arrays, remotely operated vehicle (ROV) deployment, aquaculture, and cage culture as well as offshore laboratory deployment.

**Each site will determine structure design**

The variability of the ocean wave resource makes the specific design of the wave capture chamber and ancillary design criteria such as those outlined previously very site-specific. It is therefore necessary to understand the resource from a regional coastal perspective with energetic resource sites given a priority development focus. Coastal resources, demand characteristics, and demographics will determine the final economic potential of each deployment opportunity.

The authors believe that the overall economic resource represented in the development of regional OSPREY centers will be substantial. The aquaculture community is in general agreement that offshore cage culture will become an important component of the future for sustainable-yield aquaculture development. The OSPREY unit presents the opportunity for a myriad of marine technology applications as well. A detailed ocean energy resource assessment should be conducted. This study will determine the wave resource potential and unit design criteria for a pre-designated area, ideally, from Florida, USA, through Newfoundland, Canada.

**Recommendations for future work**

Future offshore aquaculture facilities must be designed to meet challenging engineering and technical requirements. Potential for meeting these requirements may be substantially improved through integration of these facilities into the design of OSPREY™ offshore power generation structures. The OSPREY™ technology is relatively mature and commercially available. Potential exists for attractive commercial arrangements between the aquaculture operators and the power generation business entity which may increase the cost competitiveness of finfish culture. Furthermore, the integration of aquaculture structures into the OSPREY™ design could probably be accomplished with minimal impact to the OSPREY™ mission.

The first step must be to fully evaluate the potential market demand for commercial power generation with the OSPREY™ system off the Northeast coast of the United States. It must be shown that OSPREY™ can generate power at significant levels at a competitive cost with fossil or nuclear sources. It should be noted that with the on-going deregulation of the power generation industry in the Northeast, several states are considering mandatory use of renewable energy sources at a percentage of the total energy usage. It must be shown that OSPREY™ can compete with wind and hydropower sources.

A systems engineering study is required to integrate net pen concepts with the OSPREY™ structure. Concepts for aquaculture mechanical and electrical systems, aquaculture primary structure for alternative architectures, and concepts for operation of both the OSPREY™ system and the associated aquaculture facility must be developed and evaluated. An important element of this systems engineering effort must be cost estimates for acquisition, construction, and operation of the integrated facility.

A final element of this early work must be the examination of the range of commercial arrangements which may be possible between the aquaculture operators and energy generation operator. The objective of this systems engineering effort will be to establish the technical and commercial feasibility of the integration of aquaculture with OSPREY™ power generation for sites off the Northeast coast of the United States.

# WATER QUALITY GUIDELINES FOR AQUACULTURE: AN EXAMPLE IN JAPAN

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## ABSTRACT

The basis for setting up water quality criteria (WQC) for the protection of aquatic living resources set by the Japan Fisheries Resource Conservation Association (JFRCA) is that good water quality is needed to provide for the healthy growth of fish and shellfish and to maintain their high economic values. Eleven parameters are established to maintain optimal conditions. The parameters are dissolved oxygen (DO), chemical oxygen demand (COD), pH, suspended particulate matter (SPM), total phosphorus (TP), total nitrogen (TN), amount of coliform bacteria, *Escherichia coli*, petroleum hydrocarbons, temperature, toxic chemicals, and sediments.

## INTRODUCTION

In the 1960s, water pollution was an epidemic in Japan and many fears concerning contamination of fish and shellfish were widespread. In response to those public concerns, in 1965, the Japan Fisheries Resource Conservation Association (JFRCA) had established Water Quality Criteria (WQC) for the Protection of Aquatic Living Resources. The WQC have been revised several times, and a major revision was made in 1995 (JFRCA 1995) reflecting the renewal of the Environmental Quality Standards for Water Pollution (EQSWP) set by the Environment Agency of Japan (EAJ). These WQC in the marine environment are presented and the strategy for determining WQC values is discussed in this report.

### Basis for Water Quality Criteria

The ideal situation in a good marine environment for fish and shellfish often is one without any anthropogenic perturbation. But, that is almost impossible and it is not practical to set WQC based on no human impact. Therefore, we need to seek a way to reconcile human activities with preservation of a good marine environment. Under these circumstances, good water quality is the water condition which produces normal and safe-to-eat fish. Harvested fish also need to be economically high in value so that fishermen can earn enough money for a living. These basic ideas should also be applicable to aquaculture. Human impacts are actually larger in aquaculture as it is normally operated in semi-enclosed waters where water quality tends to get worse even without anthropogenic perturbation.

### Items Related to Living Environment

Eleven items are listed in Table 1. These were made by reviewing existing data and also considering the environmental quality legislation (Environmental Quality Standards and its related legislation) issued by EAJ. Each item is discussed below.

### Dissolved oxygen (DO)

Normal environmental water contains oxygen close to its saturation depending on temperature and salinity. But the concentration may fluctuate significantly in a short period of time and it is difficult to maintain at a steady level. Only a few studies have been reported for DO requirements for saltwater fish. An excellent review by Davis (1975) indicates that an oxygen saturation level less than 60% may induce physiological changes in some marine species such as pile perch, dogfish, and dragonet. Saunders (1963) even reported that any reduction in ambient

**Table 1.** Water Quality Criteria for the protection of aquatic living resources: items related to living environment (by JFRCA).

| Items | Concentration |
|---|---|
| Dissolved Oxygen | 6 mg/L |
| COD, Open waters | 1 mg/L |
| Coastal waters | 2 mg/L |
| pH | 7.8 - 8.4 |
| SPM | 2 mg/L |
| TP, Open waters | 0.03 mg/L |
| Coastal waters | 0.05 mg/L |
| Nearshore waters | 0.09 mg/L |
| TN, Open waters | 0.3 mg/L |
| Coastal waters | 0.6 mg/L |
| Nearshore waters | 1.0 mg/L |
| *Escherichia coli* (MPN/100 mL) | 1000 |
| n-Hexane extracts | <D.L. |
| n-Hexane extracts in sediment | 0.1% (dry) |
| sediment COD | 20 mg/g (dry) |
| Sediment sulfide | 0.2 mg/g (dry) |

oxygen level produces a rise in ventilatory water flow in Atlantic cod.

For common Japanese commercial fish such as jack mackerel, sandfish, yellowtail, black seabream, red seabream, stingfish and puffer, the incipient lethal level of DO ranges from 0.2 to 1.5 ml/L (JFRCA 1989). Chiba (1983) reported, based on his laboratory studies, that the 60% oxygen saturation level is necessary for the healthy growth of silver bream. Field surveys of benthic organisms in the Seto Inland Sea by Imabayashi (1983) show that a decrease in DO causes a decrease in the species diversity. Moreover, the number of benthic organisms exponentially decreases with decreasing DO and below the DO concentration 2 ml/L their survival becomes very low. Similar studies in Omura Bay by Mori et al. (1973) indicate that benthic organisms such as goby, shrimp, and crab can apparently swim away from low oxygen waters (< 3 ml/L). By considering all these facts, JFRCA judged the critical concentration level to induce a physiological change in saltwater fish to be 3.0 ml/L (= 4.3 mg/L). Considered as a safe factor, JFRCA recommends 6 mg/L as WQC.

**Chemical oxygen demand (COD)**

The COD is an amount of oxygen molecules equivalent to organic matter consumed by oxidizing chemical reagents. There are several methods for the determination of COD. Japanese Industrial Standards (JIS) has listed three methods (Japanese Industrial Standard 1993). Among them, the dichromate reflex method is most widely used for COD ($COD_{Mn}$). But, this method is interfered by chloride ion and suffers reproducibility. Since the chloride ion is the major component of seawater, this method is not suited for seawater analysis. The recommended procedure is alkaline oxidation using potassium permanganate ($COD_{OH}$).

The COD is considered an indicator of eutrophication. A high load of nutrients and organic matter from land disrupts the material cyclings in coastal environments and produces nutrient-rich conditions and eutrophication (Fig. 1). The target value of $COD_{OH}$ is not an anthropogenic perturbation level. In the marine environment, reported values of COD during a pre-highly industrialized period in Japan between 1931 and 1940 are less than 1 mg/L. However, some coastal species such as mullet, sea bass, and sardine are known to be caught in higher COD waters (Satomi 1985). Therefore, JFRCA recommends 1 mg/L as WQC for open waters and 2 mg/L for coastal waters.

**pH**

An event of acid rain leads the public to an increasing awareness of a pH change in aquatic environments, especially in the freshwater system. A suitable pH range for organisms is 6.5 - 8.5. A pH change may induce a physiological disturbance. It may also promote a higher toxic effect of chemicals dissolved in water to aquatic organisms. However, in the marine environment, the effect of acid rain is minimal due to the buffering capacity of seawater. JFRCA recommends natural levels

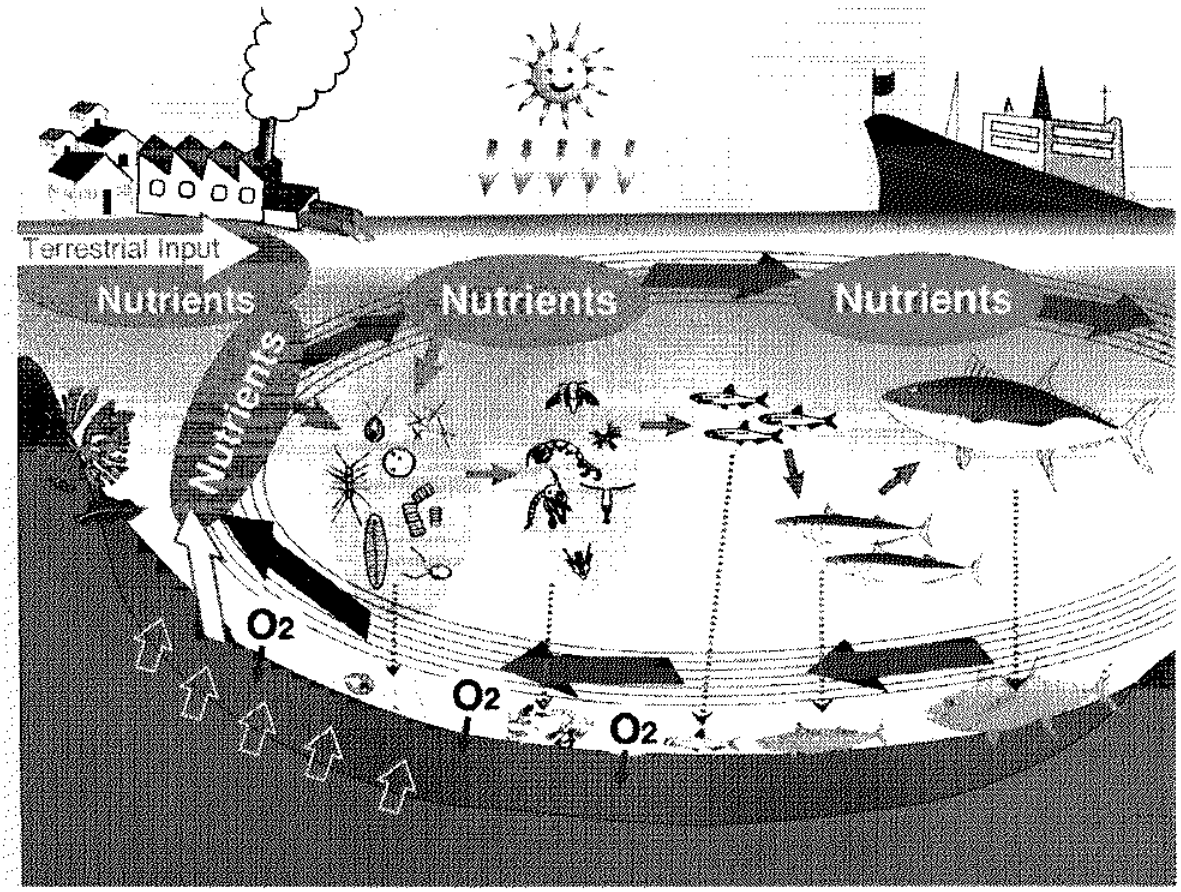


**Figure 1.** Material cycling in coastal environments (modified after JFRCA).

of pH, 7.8-8.4 for seawater, as WQC.

**Suspended particulate matter (SPM)**

In general, aquatic organisms, especially bottom-dwelling fish, can adapt to high concentrations of chemically non-reactive SPM. However, pelagic fish may show an escape movement from muddy waters. Furthermore, 5 mg/L of SPM can be fatal to juvenile striped knifejaw (*Oplegnathus fasciatus*). Natural population of phytoplankton can also be considered as SPM since SPM is defined as any substances trapped on a filter. Therefore, JFRCA recommends the man-induced SPM (such as from sewage and industrial effluents) of 2 mg/L for seawater as WQC.

**Total phosphorus (TP) and total nitrogen (TN)**

These are also used as indicators for eutrophication. However, it is difficult to determine the baseline concentration and to set a WQC, especially in coastal waters. Coastal waters are always influenced by land runoff and offshore currents. Nutrients are continuously supplied by lands and diluted with open-ocean seawater. Furthermore, natural causes such as upwelling can bring nutrient-rich waters to the surface.

The TP and TN are closely related to COD in seawater, and they are a sum of inorganic and organic forms of phosphorus and nitrogen, respectively. Living organisms such as phytoplankton are included in these fractions. By definition, these living organisms are also part of COD. Therefore, TN, TP, and COD are necessary for good environmental conditions for fisheries. There needs to be adequate levels of TP and TN to support good fisheries. After all, phosphorus and nitrogen are the nutrients for primary producers (Fig. 2).

Since the input of these nutrients and CODs

from lands is tightly regulated by the water pollution control legislation, TN, TP, and COD in seawater are mainly the results of organic production by primary producers, especially in nonpolluted areas. However, in a semi-enclosed water body where water circulation is restricted, TP and TN may be accumulated in the water column and they may trigger undesirable plankton bloom (red tide). An eventual collapse of the bloom can lead to the consumption of DO and higher COD values. Therefore, we need to seek desirable TP and TN levels to sustain primary producers, but not too much to produce high COD values (such as a production of red tide).

Satomi and Takayanagi (1987) reviewed the existing data of TN, TP, and COD in unpolluted coastal waters in Japan and recommended that 0.022 mg/L for TP and 0.32 mg/L for TN to meet the EQSWP COD value. Satomi (1983) also reported that higher populations of many commercially available marine species are found in the water having TP < 0.03 mg/L and TN < 0.3 mg/L. On the other hand, high populations of coastal fish such as mullet, sea bass, and sardine are found in the water having TP > 0.1 mg/L and TN > 1.0 mg/L (Satomi 1985).

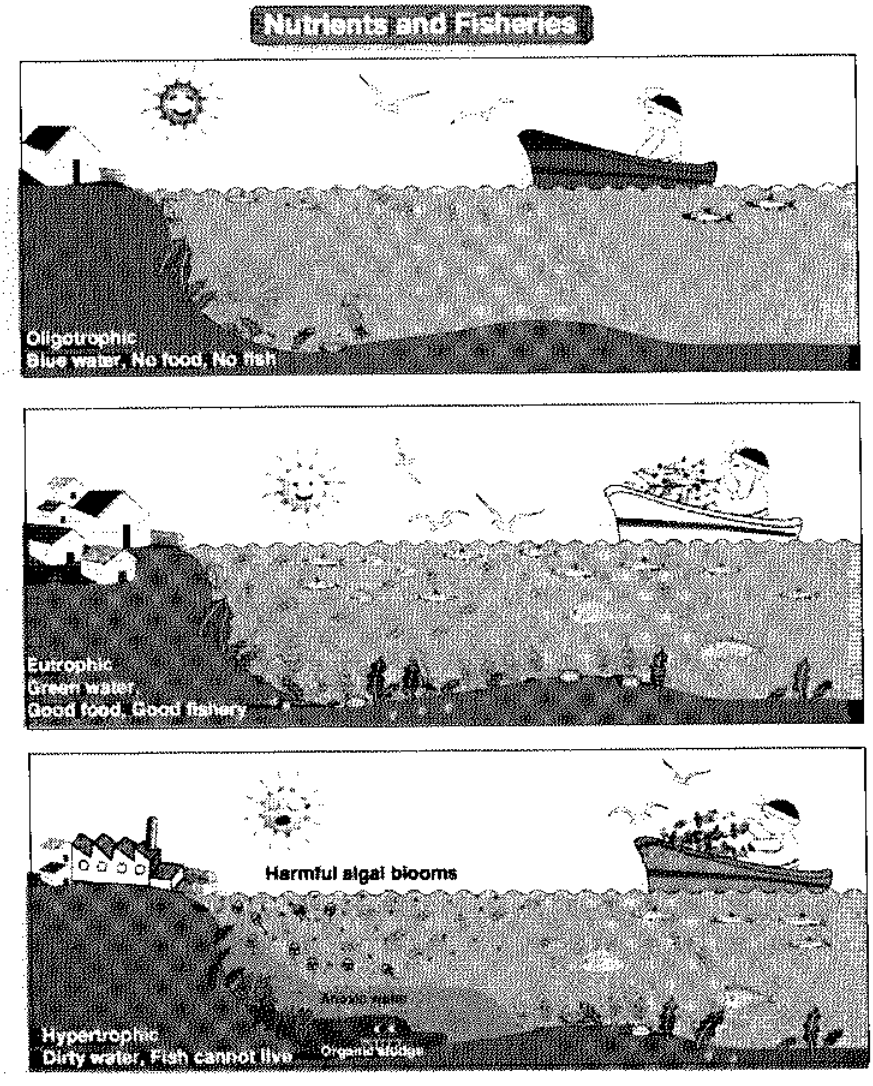


**Figure 2.** Nutrient levels and fisheries.

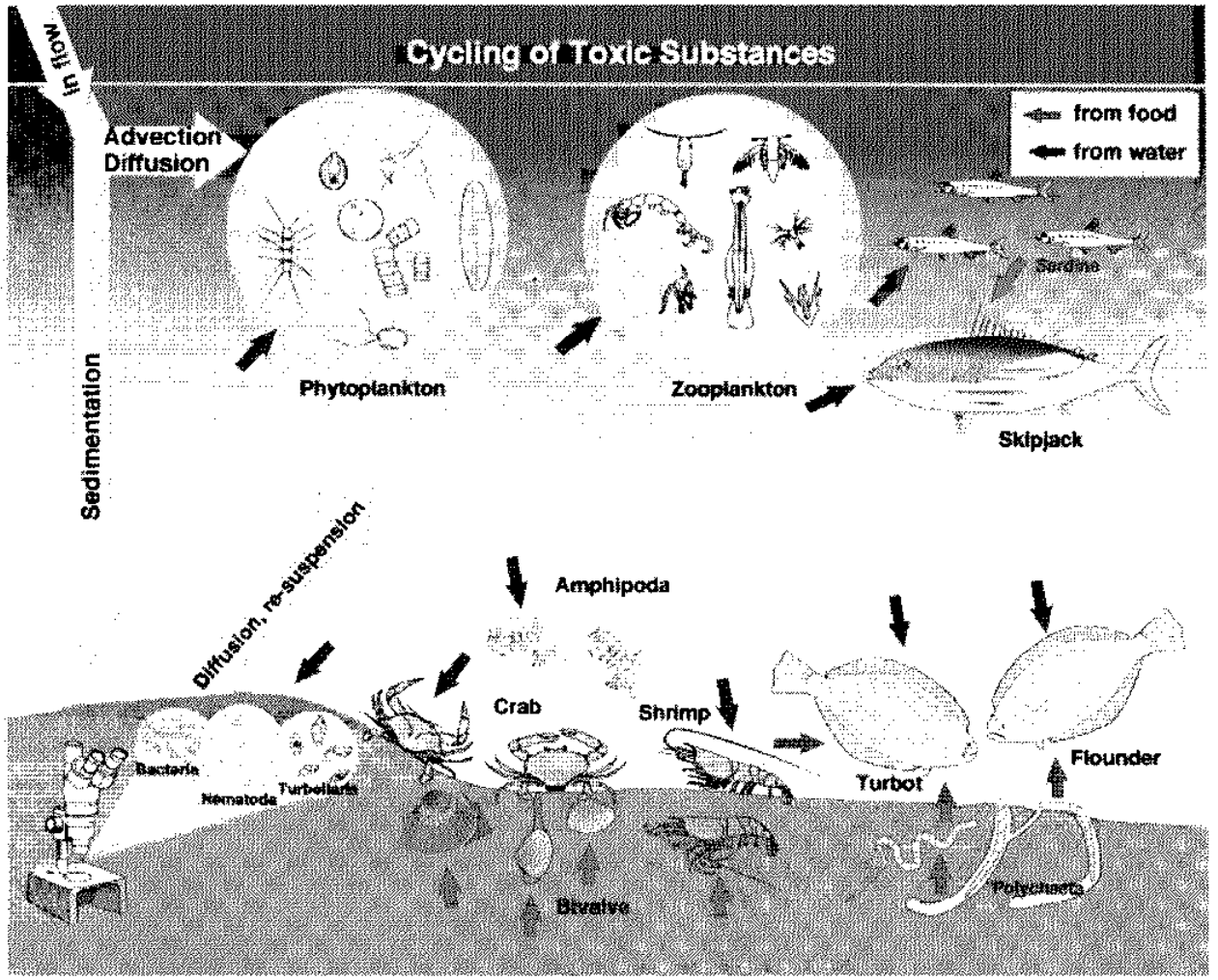


**Figure 3.** Cycling of toxic substances in coastal environments (modified after JFRCA).

By considering these reviews, JFRCA divides water bodies into three categories, and recommends TP values of 0.03, 0.05, and 0.09 mg/L and TN values of 0.3, 0.6, and 1.0 mg/L for open waters, coastal waters and nearshore waters, respectively.

### Coliform bacteria, *Escherichia coli*

The main concern is for oyster aquaculture. The WQC needs to be determined based on the safe eating of uncooked oysters. The value of 70/100 ml is set by the food hygiene legislation for oyster aquaculture. Therefore, JFRCA adapted this value for oyster aquaculture, and recommends 1000/100 ml.

### n-Hexane extracts

Remnants of oil spills and tar balls are included as n-Hexane extracts. Toxic effects of n-Hexane extracts may not be clear, but fish can accumulate odor-producing substances (Motohiro 1973). Bad smell certainly reduces the economic value. Recommended concentration is less than 0.001 mg/L, which is currently the detection limit.

### Sediment parameters

Biogeochemical processes occurring in the sediments can affect the water quality. Sediment may act as a secondary source of contamination and may worsen all the water quality parameters described above. Therefore, WQC is also considered for sediments. Sulfide, COD, and n-

Table 2. Water quality criteria for metals in seawater by JFRCA and EAJ.

| Metal | Concentration | |
|---|---|---|
| | JFRCA* | EAJ** |
| Al | 0.1ppm | |
| As | 0.01 ppm | 0.01 ppm |
| B | 4.5 ppm | |
| Br | 1.0 ppm | |
| Cd | 0.0001 ppm | 0.01 ppm |
| Cl (as free chlorine) | 0.02 ppm | |
| CN (as cyanide compounds) | <D.L. (0.001 ppm) | |
| Cr (as Cr + 6) | 0.01 ppm | 0.05 ppm |
| Cu | 0.005 ppm | |
| F (as flouride compounds) | 1.4 ppm | |
| Fe | 1.0 ppm | |
| Fe | 2 ppm | |
| Hg | 0.0001 ppm | 0.0005 ppm |
| Alkyl Hg | <D.L. (0.5 ppb) | <D.L. (0.5 ppb) |
| Mn | 0.6 ppm | |
| Mo | 0.07 ppm | |
| Ni | 0.01 ppm | |
| Pb | 0.003 ppm | 0.01 ppm |
| Sb | 0.002 ppm | |
| Se | 0.01 ppm | 0.01 ppm |
| Sn (as tributyl Sn compounds) | 0.002 ppb | |
| Zn | 0.01 ppm | |

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** Environment Agency of Japan

Table 3. Water Quality Criteria for synthetic organic substances in seawater by JFRCA and EAJ.

| Synthetic Organic Substances | Concentration | |
|---|---|---|
| | JFRCA* | EAJ** |
| 1,1,1-Trichloroethane | 1 mg/L | 1 mg/L |
| 1.1.2-Trichloroethane | 0.006 mg/L | 0.006 mg/L |
| 1,1-Dichloroethylene | 0.02 mg/L | 0.02 mg/L |
| 1.2-Dichloroethane | 0.004 mg/l | 0.004 mg/L |
| 1,2-Dichloropropane | 0.06 mg/L | |
| 1,3-Dichloripropene | 0.002 mg/L | 0.002 mg/L |
| Benzene | 0.01 mg/L | 0.01 mg/L |
| Carbon Tetrachloride | 0.002 mg/L | 0.002 mg/L |
| Chloroform | 0.01 mg/L | |
| cis-1,2-Dichloroethylene | 0.04 mg/L | 0.04 mg/L |
| CN (as cyanide compounds) | <D.L (0.001 ppm) | <D.L. (0.001 ppm) |
| Diazinon | 0.04 g/L | |
| Dichloromethane | 0.02 mg/L | 0.02 mg/L |
| Dichloropropane | 0.06 mg/L | |
| Linear Alkylbenzenesulfonate | 0.002 mg/L | |
| p-dichlorobenzene | 0.1 mg/L | |
| PCB | <D.L. (0.5 ppb) | |
| Simazine (2-Chloro-4,6-Bis(ethylamino)-1,3,5-Triazine) | 0.003 mg/L | 0.003 mg/L |
| Tetrachloroethylene | 0.01 mg/L | 0.01 mg/L |
| Thiodicarb | 0.02 mg/L | 0.02 mg/L |
| Thiuram | 0.006 mg/L | 0.006 mg/L |
| Toluene | 0.4 mg/L | |
| Trichloroethylene | 0.03 mg/L | 0.03 mg/L |
| Xylene | 0.4 mg/L | |

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Hexane extracts have been chosen as the parameters. By reviewing the existing data, JFRCA recommends 0.2 mg/g, 20 mg/g, and 0.1% for sulfide, COD, and n-Hexane extracts as WQC, respectively.

**Toxic chemicals**

Cycling of toxic chemicals entering the marine environment is shown in Figure 3. Since there are two passages of these chemicals to marine organisms, through water and through food, toxic effects by both waterborne and food contaminants need to be considered. The WQC for heavy metals and selected organic chemicals are listed in Tables 2 and 3, respectively. These WQC values are based on the no-effect concentration level. By comparing the reported toxicity values from chronic toxicity test, early-life stage toxicity test, and acute toxicity test, the lowest value was taken as WQC. Environmental Quality Standards for Water Pollution for health items are also listed in Tables 2 and 3 for comparison.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

Chiba, K. 1983. The effect of dissolved oxygen on the growth of young silver bream. Bull. Jpn. Soc. Sci. Fish. 49: 601-610.

Davis, J. D. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. J. Fish. Res. Board Can. 32: 2295-2332.

Japan Fisheries Resource Conservation Association. 1989. Final report of 5-year carrying capacity project. Japan Fisheries Resource Conservation Association, Tokyo. 1003 p. [in Japanese]

Japan Fisheries Resource Conservation Association. 1995. Water quality guidelines for living resources in aquatic environments. Japan Fisheries Resource Conservation Association, Tokyo. 68 p. [in Japanese]

Japanese Industrial Standard. 1993. Testing methods for industrial wastewater. JIS K 0102-1993. Japanese Standards Association, Tokyo. 327 p. [in Japanese]

Imabayashi, H. 1983. Effects of oxygen deficient water on the benthic communities. Bull. Jpn. Soc. Sci. Fish. 49: 7-15 [in Japanese].

Mori, I., T. Tokunaga, M. Kuwaoka, and T. Fujiki. 1973. Distribution of bottom fishes in relation to oxygen contents in the bottom water of Omura Bay. Bull. Jpn. Soc. Sci. Fish. 39 (7): 753-758 [in Japanese].

Motohiro, T. 1973. Water pollution by oil and some biological considerations. Japan Fisheries Resource Conservation Association, Tokyo. 82 p. [in Japanese]

Satomi, Y. 1985. Some problems on total nitrogen and phosphorus criteria. J. Water Wastewater Res. 27: 131-139 [in Japanese].

Satomi, Y. and k. Takayanagi. 1987. Water quality criteria for fisheries in Japan: total phosphorus and total nitrogen. Water Qual. Bull. 12(4): 163-165.

Saunders, R. L. 1963. Respiration in the Atlantic cod. J. Fish. Res. Board Can. 20: 373-386.

# MARINE MAMMAL – GEAR INTERACTIONS: PROBLEMS, ACOUSTIC MITIGATION STRATEGIES, OPEN OCEAN AQUACULTURE

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## ABSTRACT

Interactions between marine mammals and fishing gear have changed as fishing technology and activity have evolved. The interactions are wide ranging, and have included large seines for tuna with dolphins in the eastern tropical Pacific, cod traps and humpbacks in Newfoundland, harbor porpoise and gillnets all around the northern hemisphere, and seals and salmon aquaculture pens. Many of the mitigation strategies have been active acoustic devices targeted at a single marine mammal species. Moving aquaculture offshore to the open ocean presents a situation where a variety of interactions could occur due to the many marine mammal species present. Resolving the interactions will require new approaches to the multiple species situation. Further, because offshore aquaculture may be moving into significant marine mammal areas, special precautions must be taken to ensure that any conflict mitigation does not lead to excluding a species from habitat critical for its survival.

## INTRODUCTION

To meet the demand for future grow-out space for finfish, aquaculture activities will have to move offshore, especially in New England. As offshore aquaculture activity begins, there is a need to consider all the potential situations that can develop with regard to marine mammals, and to address them from the beginning. This new level of aquaculture activity will require placing large, fixed net-pen structures at offshore sites. It is essential that these structures be designed to resist the weather and seas, to accommodate the fish adequately, and to avoid negative interactions with marine mammals. This new initiative in aquaculture should not be the victim of shortsighted design that results in conflicts with marine mammals, and hence a difficult start.

### HISTORICAL OVERVIEW

As the demand for fish increased, the fishing industry expanded to meet this demand. This required more gear in the water. Technology evolved to optimize the ability of a single boat to haul more nets. For example, net twine which was traditionally a hemp or cotton-based material became monofilament nylon. This enhanced the fishing operation, but created problems for marine mammals. Nylon monofilament is virtually acoustically transparent (Vicedomine 1991), and its use led to marine mammal entanglements, damaging fishing gear and killing marine mammals.

In Newfoundland, Canada, this scenario existed in the inshore cod fish traps. Collisions with humpbacks always occurred according to the older fishermen. The collisions resulted in gear damage to the cotton and hemp lines. With the advent of stronger lines, more collisions happened with more whale entanglements. Over the period from 1979-1990, Lien (1994) reported that 30% of the marine mammals entangled in fishing gear in Newfoundland were dead. The response to this situation was to begin a program focused on developing an acoustic alarm to warn marine mammals about the presence of the fishing gear. A 4-kHz acoustic alarm resulted and was effective in reducing the marine mammal-fishing gear conflict.

A similar scenario exists with the gillnet fishery in the northern hemisphere with the entanglement of harbor porpoise. The old twine nets had minimal problems with entanglements, but the nets when soaked were difficult to haul due to the weight. The movement to synthetic monofilament nets improved the hauling efficiency, but rendered the nets virtually acoustically transparent. Entanglements of harbor porpoise became relatively commonplace. The mitigation strategy was to use an acoustic pinger to warn the harbor porpoise about the existence of the gillnets. This acoustic pinger has a fundamental frequency of 10 kHz with harmonics up to the 100-kHz range with a source level of approximately 135 dB re 1 μPa. The initial testing of this pinger, in experiments with a valid statistical design, indicates that it is very effective at deterring harbor porpoises from gillnets (Kraus et al., 1997).

Presently, the primary conflict between marine mammals and aquaculture gear is with the salmon net pens. Seals are predators to the fish in the net pens and the industry has developed the practice of using acoustic harassment devices (AHD) to scare the seals away from the pens. A commonly used AHD broadcasts a 10-kHz signal at 210 dB re 1 μPa with a 10% duty cycle every 4 sec. This is an effective deterrent for the seals, but the sound propagates a long distance, and there is some evidence that harbor porpoise in British Columbia, Canada, have been displaced due to the sound (Olesiuk et al. 1995).

In the examples stated above, there were a variety of interactions with marine mammals and fishing gear. The mitigation strategies have all been acoustic, and have been a reactive fix to the problem. The mitigation strategy for one species interaction can potentially lead to displacement or habitat exclusion of another species. To effectively address the potential for marine mammal interactions and acoustic deterrents in open ocean aquaculture, it is necessary to formulate a basic model of the acoustic propagation and how marine mammals fit into this environment and interact acoustically. This acoustic interaction scenario will provide a basis for understanding mitigation strategies.

## ACOUSTIC PROPAGATION MODEL

The objective to developing a propagation model is to have an understanding of how a sound that is generated at a source travels a path and is received. Models can be simple and qualitative in which case they identify the salient features such as source, path, receiver. Or they can be a very quantitative sonar model with all the complexities one desires. The goal here is to present a simple model that explains qualitatively the propagation environment for marine mammals and fishing gear.

The basic propagation model has a source, path, and a receiver. This generic scenario is depicted in Figure 1. The source in the aquaculture case could be the sound from a deterrent device or sounds associated with the aquaculture activity. The former is meant to keep marine mammals away, but the latter could be a curiosity enhancer.

The path is the ocean between the source and receiver. This path is most likely short when considering the size of the oceans, but still it can be complicated due to local propagation effects. Topography, temperature profiles, and ambient noise are three factors that can readily effect the path. Figure 2 is a plot of the various components of ambient noise. The spectral

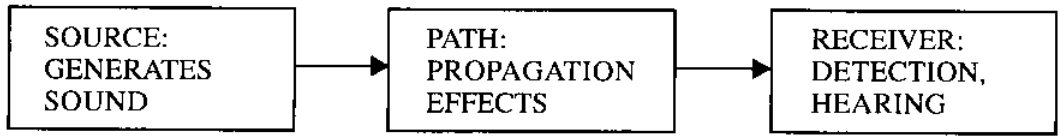


**Figure 1.** Source - Path – Receiver model for defining basic acoustic parameters.

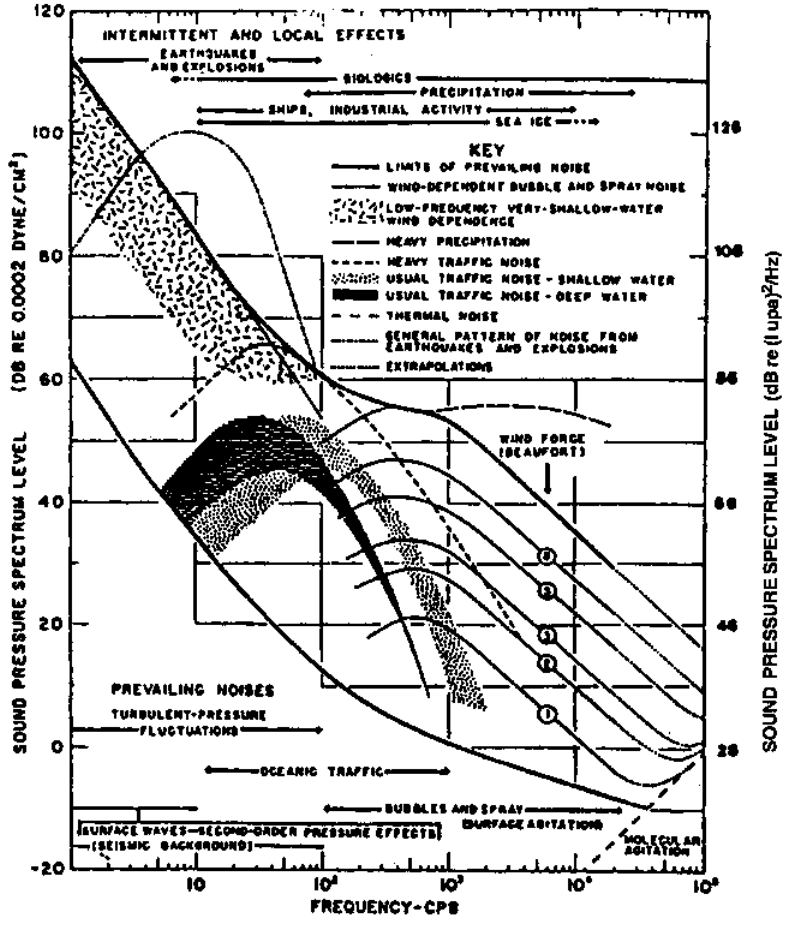


**Figure 2.** Ambient noise spectrum (Richardson et al. 1989)

content is presented as sound pressure level or sound pressure spectrum level. The latter presentation is more readily accepted in practice as the units $(\mu P)^2/Hz$ infer frequency dependence. The basic trends show that there is a higher level of ambient noise at lower frequencies with a steady decrease in level as frequency increases. Shipping and industrial activity typically have a frequency range from 10 Hz to 10 kHz. Biological sounds cover a wider range of the spectrum.

This basic model can provide insight into how a sound is propagated and its sound pressure level, how loud it is, at range from the source. There are two basic concepts to understand here: there is a zone of audibility and a zone of influence (Richardson et al. 1989).

The zone of audibility is determined by the ambient noise level. As sound propagates out from a source, its level is diminished due to spreading losses (geometric) and attenuation (true energy loss). These two loss mechanisms

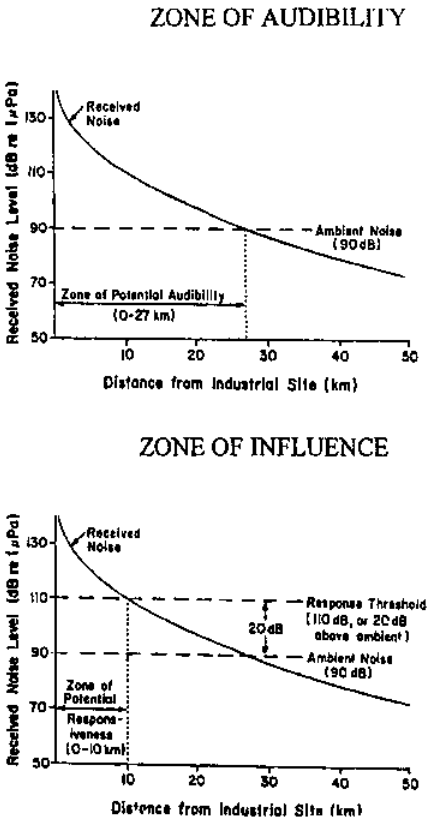


**Figure 3.** Graphical definitions of Zone of Audibility (A) and Zone of Influence (B) (Richardson et al. 1989)

are defined as transmission loss in sonar modeling. On a plot of sound pressure level vs. range, and a constant ambient noise level vs. range, the intersection of these two lines defines the zone of potential audibility. When the received sound pressure level is greater than the constant ambient noise level, then the sound can be heard. This occurs at shorter ranges. At longer ranges, the ambient noise is greater than the received sound pressure level; the sound is effectively inaudible. This concept is shown in Figure 3A.

The zone of influence is a modification to the above as it incorporates a response threshold. The response threshold is a sound pressure level above the ambient noise that is required for a marine mammal to determine that a specific sound is present. The effect of this response threshold is to reduce the range at which the two plots intersect. Hence, the zone of potential influence is a shorter range from the source than the zone of potential audibility. This is shown in Figure 3B.

This basic model is useful in defining the

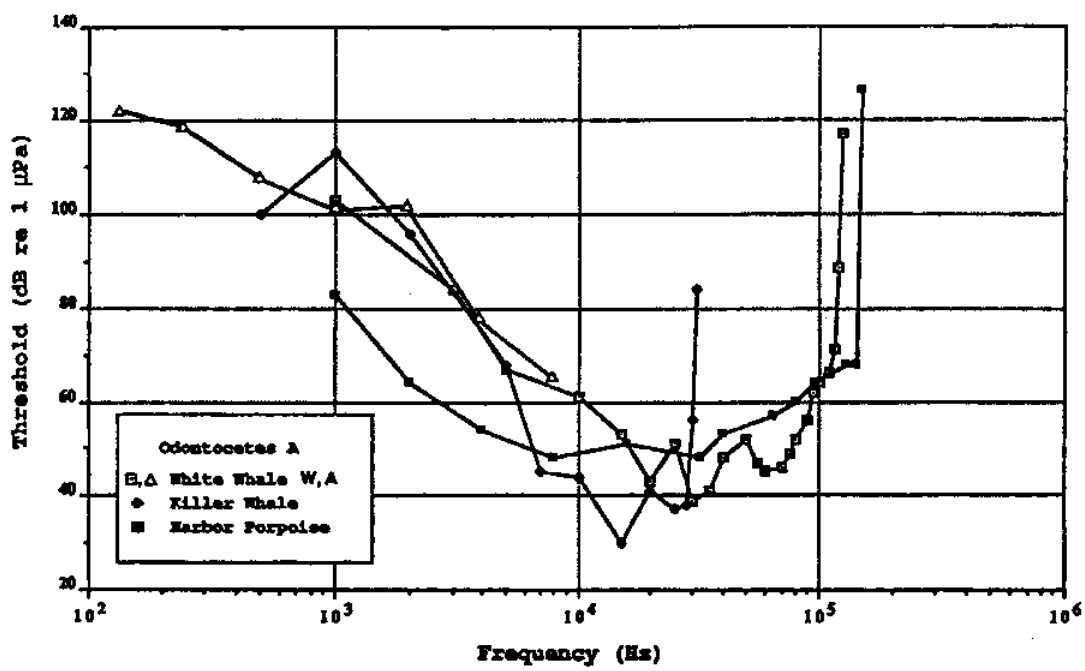


**Figure 4.** Underwater audiograms of several odontocetes: (A) white whale (White et al. 1978; Awbry et al. 1988); killer whale (Hall and Johnson 1972); harbor porpoise (anderson 1970a); (B) bottlenose dolphin (Johnson 1968a; Ljunblad et al. 1982c); Amozon river dolphin or boutu (Jacobs and Hall 1972); false killer whale (Thomas et al. 1978). (Richardson et al. 1989)

above concepts. The limitation is the use of a single number, constant level, for the ambient noise and the response threshold. As shown earlier, ambient noise has definite spectral characteristics. Upon close examination of marine mammal acoustics, it is apparent that they too have spectral characteristics to their acoustic behavior.

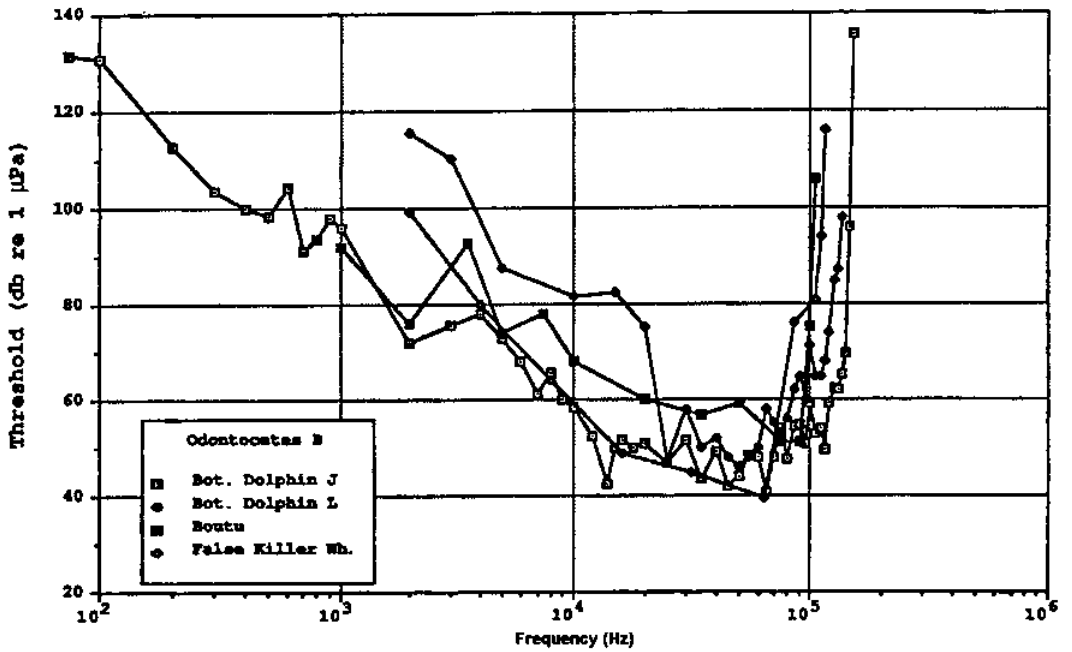


**Figure 5.** Underwater audiograms of several odontocetes: (A) white whale (White et al. 1978; Awbrey et al. 1988); killer whale (Hall and Johnson 1972); harbor porpoise (Andersen 1970a); (B) bottlenose dolphin (Johnson 1968a; Ljungblad et al. 1982c); Amazon river dolphin or boutu (Jacobs and Hall 1972); false killer whale (Thomas et al. 1978). (Richardon et al. 1989)

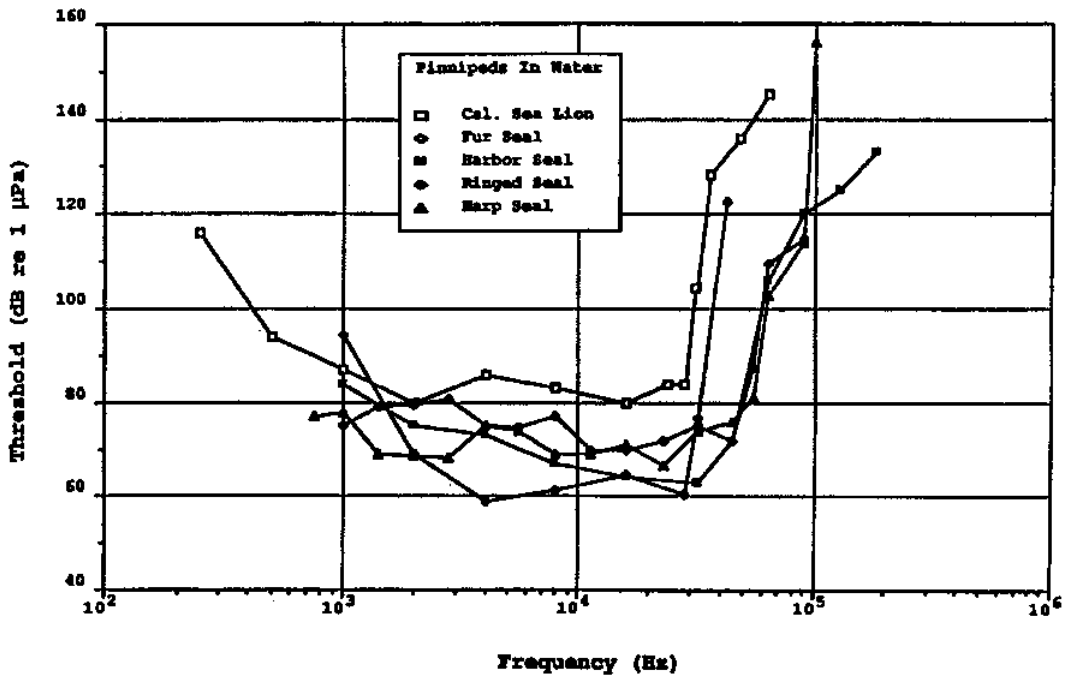


**Figure 6.** Underwater audiograms of several pinnipeds: California sea lion (Schusterman et al. 1972); average of two fur seals (Moore and Schusterman 1987); harbor seal (Møhl 1968a); average of two ringed seals (Terhune and Ronald 1975a); harp seal (Tehrune and Ronald 1972). (Richardson et al. 1989)

## MARINE MAMMAL ACOUSTICS

Marine mammals are the receiver in the previous model. They have spectral characteristics associated with their receiving mechanisms, and also they have spectral content associated with various vocalization used for communication, navigating, and foraging. The effect of ambient noise and noise generated from aquaculture activities can potentially affect the intent of the marine mammal vocalizations.

Audiograms are the method of defining the spectral characteristics of hearing. Most audiograms have a band of frequencies over which the hearing is most sensitive. This band varies for different marine mammals, as well as the threshold or hearing sensitivity. The audiograms in Figures 4, 5, and 6 show typical results for a variety of marine mammals, both odontocetes and pinnipeds. There is a difference in the basic structure of these audiograms. The odontocetes are typically more sensitive between 10 and 108 kHz, while pinnipeds are more sensitive between 5 and 40 kHz. Also, the actual threshold for the odontocetes is in the 40-60 db area while the threshold for the pinnipeds is in the 60-80 db area.

The other aspect of marine mammal acoustics is their vocalizations. This activity has both spectral and source level characteristics. From Table 1, it is seen that a variety of type of sounds are produced. These sounds have different dominant frequencies, bandwidths, and source levels. The functions of the sounds are short and long distance communication, echolocation, and reproductive displays.

Introducing other sounds into the water column, on a continual basis, that can interfere with these behaviors can have adverse effects on the species. These "introduced sounds" can impact the ability of an animal to hear the

| Species | Type of Vocalization | Frequency Range of Vocalizations (kHz) | Dominant Frequencies (kHz) | Source Level (dB re 1 µPa at 1 m) | References |
|---|---|---|---|---|---|
| White whale | whistles | 0.26-20 | 2-5.9 | - | Sjare and Smith 1986a,b |
| | pulsed tones | 0.4-12 | 1-6 | - | Sjare and Smith 1986a,b |
| | noisy vocalizations | 0.5-16 | 4.2-8.3 | - | Sjare and Smith 1986a,b |
| | echolocation clicks | 40-120 | variable | 160-222 | Au et al. 1985, 1987 |
| Killer whale | whistles | 1.5-18 | 6-12 | - | Steiner et al. 1979; Ford and Fisher 1983; |
| | pulsed tones | 0.5-25 | 1-6 | 160 | Awbrey et al. 1982; Ford and Fisher 1983; Schevill and Watkins 1966 |
| | echolocation clicks | 0.1-35 | 12-25 | 180 | Wood and Evans 1980 |
| Northern right-whale dolphin | clicks | 1-40+ | 40+? | 180 | Fish and Turl 1976 |
| | whistles | ?-16+ | - | - | Leatherwood and Walker 1979 |
| | tones | 1-4 | 1.8, 3 | - | Leatherwood and Walker 1979 |
| Pacific white-sided dolphin | whistles | 2-20+ | 4-12 | - | Evans 1973; Caldwell and Caldwell 1971 |
| | echolocation clicks | 0.2-150 | 60-80 | 170 | Evans 1973 |
| Dall's porpoise | clicks | 0.04-12, 125-135 | - | 120-168 | Evans 1973; Evans and Awbrey 1984 |
| Harbor porpoise | clicks | 100-160 | 130 | 132-149 | Möhl and Andersen 1973 |
| | clicks | 2 | - | 100 | Busnel and Dziedzic 1966a; Schevill et al. 1969 |
| Rough-toothed dolphin | clicks | 16-100+ | - | - | Norris and Evans 1967 |
| | whistles | - | 4-7 | - | Busnel and Dziedzic 1966b |
| Short-finned pilot whale | whistles | 0.5-20+ | 2-14 | 180 | Fish and Turl 1976; Caldwell and Caldwell 1969 |
| | echolocation clicks | 0.1-100 | - | 180 | Evans 1973 |
| Sperm whale | clicks | 0.1-30 | 2-4, 10-16 | 160-180 | Backus and Schevill 1966; Levenson 1974; Watkins 1980a |
| Beaked Whale | whistles | 3-16 | - | - | Winn et al. 1970a |
| | clicks | 0.5-26+ | - | - | Winn et al. 1970a |

**Table 1.** Characteristics of underwater sounds produced by Alaskan odontocetes whales. (Richardson et al., 1989)

behavioral sounds, thus potentially driving a species from a site. This is obviously the goal of acoustic deterrent devices. The key to effective design of these devices is to target a single species that is problematic to the aquaculture operation and minimize the effect on other species.

## DISCUSSION AND SUMMARY

Historically, most fishing interactions have been single species and a reactive fix has been found. The key to an effective acoustic fix is to assume that the initialization of one species interaction is not exclusive for another. Alternatively, what deters one species could easily be the dinner-bell for another. A deterrent for one species could effectively impact the vocalization responses for another. Long term studies on marine mammal exclusion due to deterrent sounds have not been done yet. When considering an acoustic deterrent for aquaculture sites, specific local information about the ambient noise, propagation path and marine mammals needs to be assimilated for an objective, focused approach for solving the problem.

To facilitate objective approaches to marine mammal-aquaculture acoustic interactions, the following points need consideration. The database for auditory response of marine mammals needs to be enhanced. Threshold levels where sounds produce detrimental effects need to be clearly defined. Developing a better understanding between species vocalizations, environmental noise, and deterrent sounds is critical to effective use of sound as a warning/deterrent. These efforts will lead to a rational context for designing species specific acoustic alarms for use in offshore aquaculture.

## LITERATURE CITED

Kraus, S., A.D. Read., A. Solow , K. Baldwin, T. Spradlin, E. Anderson, and J. Williamson. 1997. Acoustic alarms reduce porpoise mortality. Nature 388: 525.

Lien, J. 1994. Entrapments of large cetaceans in passive inshore fishing gear in Newfoundland and Labrador (1979-1990). Report of the International Whaling Commission, Special Issue 15.

Olesiuk, P. F., L. M. Nichol, P. J. Sowden, and J. K. B. Ford. 1995. Effects of sounds generated by an acoustic deterrent on the abundance of harbor porpoise (*Phocoena phocoena*) in Retreat Passage, British Columbia. Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B. C.

Richardson, W. John, J. P. Hickie, R. A. Davis, D. H. Thomson, and C. R. Greene, 1989. Effects of offshore petroleum operations on cold water marine mammals: a literature review. API Publ. No. 4485, American Petroleum Institute, Washington, DC.

Vicedomine, J. 1991. Investigation of the acoustic interaction between harbor porpoise (*Phocoena phocoena*) and Gulf of Maine commercial gillnet fishing. MS thesis, Ocean Engineering Program, University of New Hampshire, Durham, NH.

# NORTH AMERICAN LOBSTER CULTURE (*HOMARUS AMERICANUS*), HATCHERY METHODS, AND TECHNIQUES: A TOOL FOR MARINE STOCK ENHANCEMENT?

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## ABSTRACT

The North American lobster (*Homarus americanus* Milne-Edwards) is fished intensively throughout its 11,000-km range resulting in only a fraction of these animals surviving long enough to reproduce. Success in marine stock enhancement programs suggest that enhancement may be possible for lobsters as well. Personnel at the lobster rearing facility of the New England Aquarium are studying the methodologies used in the culture of larval and juvenile lobsters, as well as explore the major considerations involved for initiating and carrying out a productive and effective lobster stock enhancement program.

## INTRODUCTION

The American lobster is found naturally along the east coast of North America, from North Carolina to Labrador, being most abundant from Nova Scotia to New York (Herrick 1895). The major population centers, and therefore inshore fisheries, are located within the Gulf of Maine and in the New Brunswick and Nova Scotian coastal waters, where over 90% of the inshore landings are made (Cobb and Phillips 1980). During the last decade, areal expansion of the lobster fishery and the continued intense inshore fishery have focused attention on the relationship between animals in inshore and offshore areas. If consistent recruitment in coastal areas depends on egg production from offshore, heavy exploitation of offshore populations could impact all fisheries. The New England Fishery Management Council (NEFMC) currently considers the American lobster resource overexploited. There is currently no commercial culture for the North American lobster; however, these animals have long been reared in pilot scale projects, and have received a considerable amount of attention from aquaculturists. The reason for the attention is clear: lobster is a very popular seafood with a high market value. Lobster stock enhancement is not a new concept. Efforts to rear and culture lobsters have existed since the early 1880s (Addison and Bannister 1994). Lobster hatcheries originated in France and Norway over 130 yr ago, hatching and raising the European lobster *Homarus gammarus* (Herrick 1909). The first successful attempt at hatching *H. americanus* larvae was in 1885 at the newly established laboratory of the U.S. Fish Commission in Woods Hole, Massachusetts (Rathbun 1886). The world's first lobster culture facility was completed at St. Andrews, New Brunswick, Canada, in 1974. The work done at St. Andrews closed the lobster cycle from egg to broodstock. Because the private sector was not prepared for the direct transfer of this technology, potential governmental and academic research programs stalled. The popular perception of lobster aquaculture is that it is analogous to farming, in which lobsters produce eggs that are hatched, reared, and then sent to market. The other potential application however, is in marine stock or resource enhancement, whereby young of year lobsters are cultured and released to enhance natural stocks. To be able to move forward in this area of research however, it is important to disseminate the details of culturing such animals for these types of studies,

and relate what others have done to contribute to lobster stock enhancement.

## MATERIALS AND METHODS

A year-round culturing facility for the American lobster is located in the Harold E. Edgerton Research Laboratory of the New England Aquarium. The culturing facility incorporates two separate systems: a cold seawater system (8-10°C) for holding gravid female lobsters, and a warm seawater system (18°C) for the production and growout of larvae and juvenile lobsters.

### Broodstock system:

Female lobsters obtained through the Marine Research Station (Vineyard Haven, Massachusetts), New England Aquarium staff divers, or other similar sources are put through a strict quarantine process before they are integrated into the primary lobster recirculating system. When obtained, wild-caught females are first given a physical checkup in which they are visually inspected for lesions, infections, abnormalities, or anything else which looks or appears suspicious. In most cases, this procedure is often done off-site prior to bringing animals into the facility so as not to introduce diseased or abnormal animals. Animals then undergo a series of dips, swabs, and brushes. They are sprayed lightly with distilled water and large portions of the shell and joint areas are swabbed and/or brushed with a 10% solution of betadine. A small sample of eggs are removed and inspected microscopically for any severe cases of fungal or bacterial growth and are also analyzed for their current embryonic developmental stage by applying the Perkins eye index formula (Perkins 1972). In some cases, new lobsters are weighed, measured, and tagged as well. These lobsters remain in quarantine for up to 3 wk at which point they can be integrated into the rest of the system.

### Larval system:

The larval rearing system consists of eight circular tanks or "kreisels" with cross-section conical bases (Figs. 1 and 2) each of 40-L capacity.

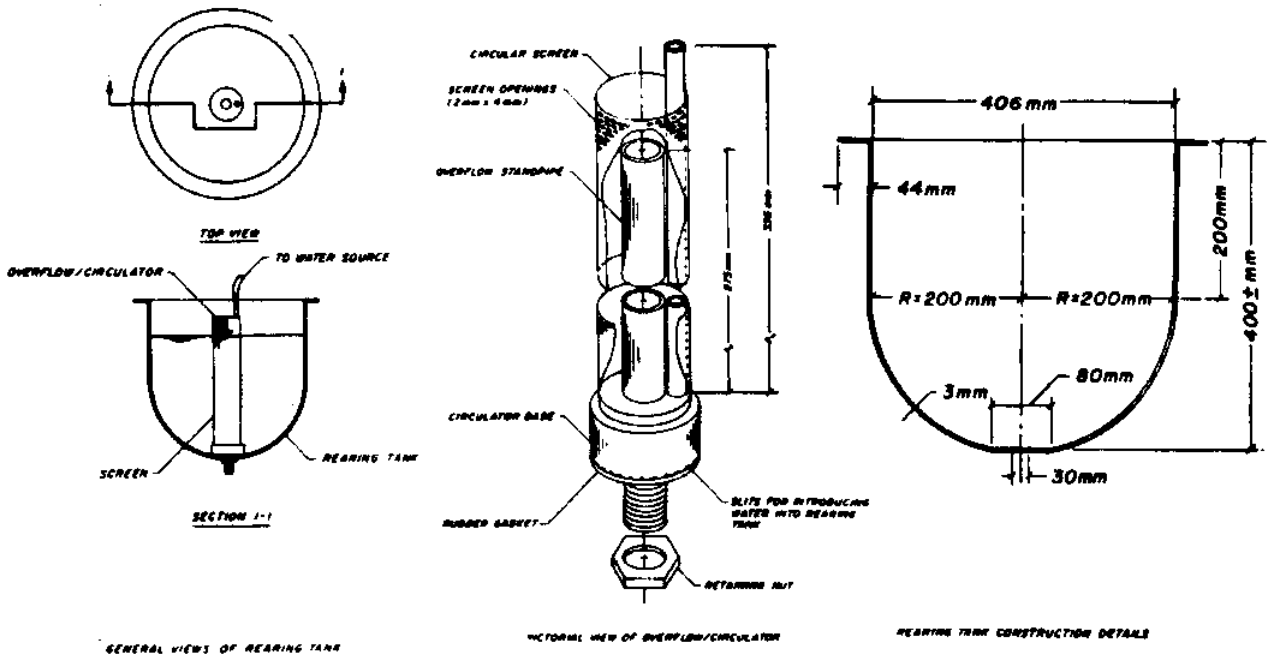


**Figure 1.** The kreisel or circulating rearing tank. (Modified from Schuur et al., 1976. With permission.)

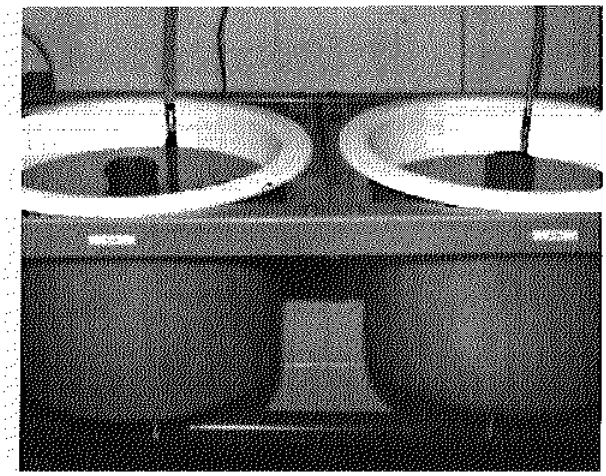

**Figure 2.** Planktonic kreisel. These 40-L tanks are ideal for larval culture. Cannibalism can be avoided by keeping the water moving and intensively feeding.

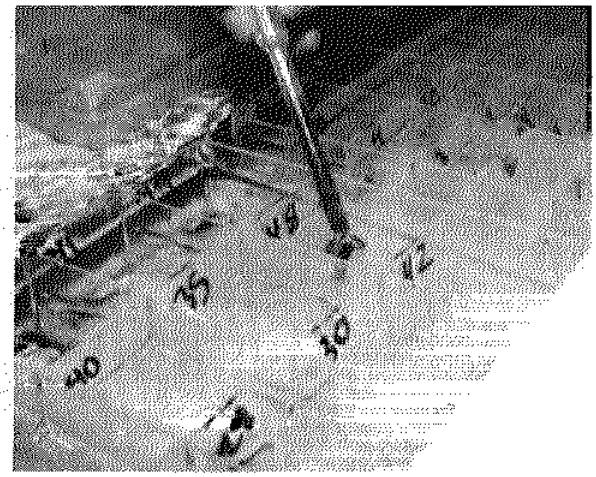

**Figure 3.** Juvenile holding tank tray. Probably one of the most unique attributes to lobster lab culture is the use of individual rearing compartments. Elevated and seated in shallow recirculating seawater trays, to flush out organics and keep water circulating, compartmentalizing animals allows for careful identification and cataloging of lobsters, as well as a tool for easy sorting and grading.

Water is pumped into the base of the tanks through a manifold with a series of offset holes producing a cyclonic upwelling water circulation. This is necessary to keep larvae dispersed and hence reduce losses by cannibalism. Kreisels are typically stocked with 2000 larvae each. Larvae are fed frozen brine shrimp *Artemia salina*, frozen mysids, and live amphipods. Larvae are typically held for 4 wk at 18-20°C during which they molt three times. Survival to the post-larval stage is variable, with 10%-15% typically surviving.

### Juvenile system:

Once larvae have molted to the post-larval stage (stage IV), when they normally would be ready to assume a benthic existence in the wild, they are removed from the larval system and placed in individual holding trays or "condo trays" (Fig. 3). These shallow seawater trays, perforated with holes, allow them to be flushed of uneaten food and wastes and also allow for identification and cataloguing of lobsters. Water exchange in the cubicles is achieved by continuous circulation of water along the trays. These animals are then weaned off of the brine shrimp diet and introduced to a gelatin-bound diet called "Supergel" (Figs. 4 and 5).

## Life Support: The Key to a Successful Hatchery

To efficiently culture and produce animals for potential resource enhancement, a sound and well-designed system should be used. Recirculating aquaculture systems (RAS) offer complete control over environmental growing conditions such as temperature, salinity, and water quality, while eliminating conventional concerns about weather and climatic conditions. Additionally, stock management and inventory control are greatly simplified. A properly designed RAS can be placed almost anywhere and used to produce a wide variety of aquatic animals. The following three components are excellent additions to any lobster hatchery setup.

### Chiller units:

Continuous seed (larval) supply requires manipulation of either or both the spawning or hatching cycles (Waddy and Aiken 1992). Temperature is the dominant regulator for the continuance of year-round larval lobsters. To assist in this challenge, having one or more chiller units

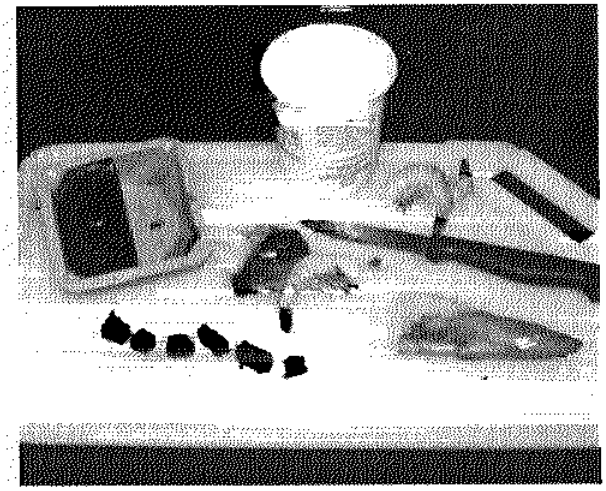

**Figure 4.** Aside from temperature, nutrition is the most important parameter governing the survival and growth of cultured lobsters. Adult brine shrimp is the staple food used here as well as enriched brine shrimp nauplii. Juvenile lobsters receive a gelatin-bound diet of brine shrimp, krill, kelp, spirulina, soy lecithin, bone meal, and crabmeat all pictured here.

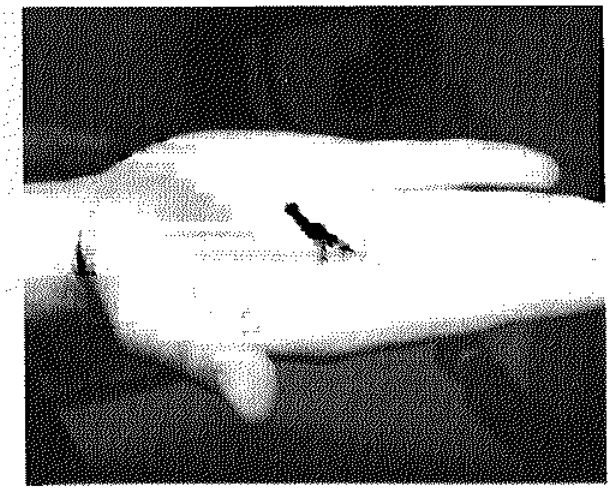

**Figure 5.** This 1-month-old, 9-mm *H americanus* is ready for transfer into the seawater condo tray system.

allows incubation periods which result in staggered hatch-out periods. This application of temperature greatly increases the chances to regulate and sustain a year-round population of larval lobsters.

**Ultroviolet disinfection:**

Ultraviolet radiation in the 200-300 nm range is extremely effective in killing most microorganisms.

**Protein skimmer (foam fractionator):**

Foam fractionation is a cost-effective and efficient means of removing fine suspended solids (<30 μm in size) and dissolved organic matter. Foam fractionation is accomplished by bubbling air through water to trap fine solids and organics which then generates a foam (Weeks et al. 1991).

## DISCUSSION

A lobster does not achieve its benthic existence until some point in the fourth stage of development (Botero and Atema, 1987, Govind and Pearce 1989). Releasing these animals during fourth stage still exposes them to heavy predation. One management practice used in Europe is to rear juvenile lobsters for up to 1 yr or more before stocking them in appropriate benthic substrates which offer the proper habitat release component.

One excellent case study on lobster stock enhancement involves a comprehensive 6-yr study in Cardigan Bay in the Northwestern Wales district, UK (Cook 1995). The stock enhancement experiment carried out at Aberystwyth has demonstrated that releases of hatchery-reared juveniles onto carefully selected substrate can result in a good rate of recapture by the commercial fishery, with peak returns occurring between 4-6 yr after release. Differences are apparent between the inshore and offshore release sites. The Aberystwyth experiment failed to establish the optimum release size for juvenile lobsters. Carapace lengths as low as 14 mm produced very good returns, but the recapture rate of small lobsters (9 mm carapace length) was extremely poor (Cook 1995). This is an important aspect of lobster restocking because post-larval lobsters would be very inexpensive to produce in large numbers by mass rearing. It still seems inevitable that to rear juveniles through several molts with acceptably low mortality, individual compartments will be necessary to avoid cannibalism. The consequent

increase in space required, and particularly in husbandry and heating costs, means that the unit production cost of lobsters rises steeply the longer they are kept. Advantages to later release means a greatly increased survival; the disadvantage is at a much greater cost. The long period of intensive cultivation prior to release raises the potential stock enhancement cost considerably (Factor 1995). Any future lobster stock enhancement program should consider the following factors: (1) more detailed and extensive surveys of potential areas of suitable substrate within the particular test site or area, (2) an assessment of the natural recruitment patterns and population structure of lobsters within these areas with a view to assessing their suitability for enhancement, (3) a more rigorous investigation of the optimum release size for lobsters, (4) development of improved hatchery techniques in order to reduce the unit cost of the juvenile lobsters, and (5) a detailed appraisal of the economics of lobster stock enhancement.

## ACKNOWLEDGMENTS

I am grateful to the New England Aquarium and the Edgerton Research Laboratory for providing the support and expertise of so many talented staff members, in particular Marianne Farrington and Albert Barker. Also my gratitute to W. Cook of the University of Lancaster, UK. My thanks also go to continuous and past funding agencies, the National Science Foundation, Grass Foundation, and the National Institutes of Health.

## LITERATURE CITED

Addison, J.T. and R.C.A. Bannister. 1994. Re-stocking and enhancement of clawed lobster stocks: a review. Crustaceana 67(2): 131-155.

Botero, L. and J. Atema. 1987. Behavior and substrate selection during larval settling in the lobster, *Homarus americanus*. J. Crust. Biol. 2: 59-69.

Cobb, J.S. and B.F. Phillips, Editors. 1980. The Biology and Management of Lobsters, Vol. I. Academic Press, New York. 463 p.

Cook, W. 1995. A lobster stock enhancement experiment in Cardigan Bay. North Western and North Wales-Sea Fisheries Committee Report, Univ. Lancaster, 32 p.

Factor, J.R., (Editor). 1995. Biology of the Lobster *Homarus americanus*. Academic Press, New York. 528 p.

Govind, C.K. and J. Pearce. 1989. Critical period for determining claw asymmetry in developing lobsters. J. Exp. Zool. 249: 31-35.

Herrick, F.H. 1895. The American lobster: a study of its habits and development. Bull. U.S. Fish Comm. 15: 1-252.

Herrick, F.H. 1909. Natural history of the American lobster. Bull. U.S. Bur. Fish. 29: 148-408 +20 plates.

Perkins, H.C. 1972. Developmental rates at various temperatures of embryos of the northern lobster (*Homarus americanus* Milne-Edwards). Fish. Bull. 70: 95-99.

Rathbun, R. 1886. Notes on lobster culture. Bull. U.S. Fish Comm. 6: 17-32.

Schuur, A., W.S. Fisher, J.C. Van Olst, J.M. Carlberg, J.T. Hughes, R.A. Shleser, and R.F. Ford. 1976. Hatchery methods for the production of juvenile lobsters *Homarus americanus*. Sea Grant Publ. No. 48, Univ. California, Inst. Mar. Resour., La Jolla

Waddy, S.L. and D.E. Aiken. 1992. Environmental intervention in the reproductive process of the American lobster, *Homarus americanus*. Invertebr. Reprod. Dev. 22: 1-3, 245-252.

Weeks, N.C., M.B. Timmons, and S. Chen. 1991. Feasibility of using foam fractionation for the removal of dissolved and suspended solids for fish culture. Aquaculture (57): 101-106.

# BLUE MUSSELS IN THE DIET OF JUVENILE JAPANESE FLOUNDER

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## ABSTRACT

Blue mussels *Mytilus galloprovincialis* were used in the diet of juvenile Japanese flounder. The control diet mainly consisted of white fish meal, potato starch, and pollack liver oil, and part of the control diet was exchanged with freeze-dried meat, fresh meat, or fresh whole blue mussel in experimental diets. Fish of about 5 g in initial body weight were fed each diet to satiation, twice daily, 6 days\wk for 8 wk at 20°C. The final body weight and weight gain of fish fed diets containing freeze-dried and fresh blue mussel meat were higher than those fed the control diet ($p < 0.05$). These parameters in the whole blue mussel diet were comparable to the control. Addition of freeze-dried and fresh blue mussel meat to the diet did not affect the feed efficiency, while the feed efficiency of fish fed the whole blue mussel diet was lower than that of fish in the other treatments. Protein efficiency ratio was in the same range among all dietary groups tested. There were small differences in body composition, or hematological and hematochemical parameters of the cultured fish.

## INTRODUCTION

Blue mussels are a nuisance organism for electric power plants located along the coast of Japan. Excessive mussel growth along water intake pipes constricts and impedes the inflow of cooling water. Generally, they are collected once or twice a year and buried in the landfill of the plant after incineration of organic matter. The removal and disposal require considerable cost; moreover, landfill space rapidly fills.

Several studies on the utilization of collected mussels have been conducted to work out the disposal problems; however, no effective ways but as a source of fertilizer have been developed. We previously reported that the freeze-dried meat of blue mussels *Mytilus galloprovincialis*, predominant in power plants of Japan, can effectively replace fish meal as a main ingredient in the diet of juvenile Japanese flounder *Paralichthys olivaceus*. In addition, blue mussel meat appears to stimulate the feeding behavior of the flounder (Kikuchi and Sakaguchi 1997). In this study, freeze-dried blue mussel meat, fresh mussel meat, and fresh whole mussel were used, and effects on the growth of juvenile flounder were examined to develop techniques for practical use of the blue mussel in the diet of fish.

## MATERIALS AND METHODS

### EXPERIMENTAL DIET

The formulation and composition of the experimental diets are shown in Table 1. The control diet basically consisted of 81.7% white fish meal, 10.8% potato starch, 3.0% mineral mixture, and 4.5% vitamin mixture. The basic ingredients of the control diet were replaced with fresh blue mussel meat at a rate of 17 and 33% (weight/weight) in diets 2 and 3, respectively. Five percent of the basic ingredients in the control diet were replaced by freeze-dried mussel meat in diet 4, and 20 and 30% with fresh whole mussel in diets 5 and 6, respectively. Live blue mussels obtained from a fish market were used. In diets 2, 3, 5, and 6, fresh blue mussel meat and whole blue mussels were minced and ground into a liquid (mostly water), and used as an ingredient in the diet. Freeze-dried mussel meat was prepared as described previously (Kikuchi and Sakaguchi 1997). Blue mussels in diets 2 through 6 replaced fish meal such that the ratio of white fish meal protein to

| | Diet | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Ingredient (%) | | | | | | |
| White fish meal | 81.7 | | | | | |
| Potato starch | 10.8 | 83.0 | 67.0 | 95.0 | 80.0 | 70.0 |
| Mineral mixture*1 | 3.0 | | | | | |
| Vitamin mixture*1 | 4.5 | | | | | |
| Blue mussel meat-fresh | | 17.0 | 33.0 | | | |
| Blue mussel meat-dried | | | | 5.0 | | |
| Whole blue mussel-fresh | | | | | 20.0 | 30.0 |
| Pollack liver oil*2 | 7.0*2 | | | | | |
| Proximate composition (%) | | | | | | |
| Moisture | 4.9 | 4.7 | 4.4 | 5.2 | 3.9 | 4.5 |
| Crude protein | 45.1 | 45.4 | 45.5 | 44.8 | 44.9 | 42.6 |
| Carbohydrate | 20.7 | 20.5 | 21.1 | 21.0 | 19.8 | 19.8 |
| Crude lipid | 13.9 | 14.3 | 14.3 | 13.9 | 14.4 | 14.1 |
| Crude ash | 15.4 | 15.1 | 14.7 | 15.1 | 17.0 | 19.0 |

*1 Nippon Formula Feed Mfg. Co., Ltd.

*2 Riken Vitamin Co., Ltd. (Feed oil Ω);
Pollack liver oil was added to be 7.0 % in the diet after fomulation and drying.

**Table 1.** Composition of six dietary experiments containing whole, fresh meat, or freeze-dried meat of blue mussel for Japanese flounder in an 8-wk growth trial.

blue mussel protein was 98.7:1.3, 97.5:2.5, 97.5:2.5, 99.3:0.7, and 98.5:1.5, respectively. All feedstuffs, except the pollack liver oil, were ground, minced, and formed into spheres of about 2 and 4 mm in diameter using a twin extruder with additions of tap water. The formulated diets were dried in an air dryer at 20°C, and an equal amount of pollack liver oil was added to each diet. Diets were dried again and stored at -35°C until used.

As shown in Table 1, the crude lipid contents of all diets were similar to each other; however, the crude protein of diet 6 was lower than that of the others.

## EXPERIMENTAL PROCEDURE

In July 1996, juvenile flounder of about 1 g in body weight were transported from the Chiba Prefectural Fisheries Experimental Station to our laboratory in Chiba Prefecture. Fish were reared in 2000-L tanks at 20°C with a commercial diet used for Japanese flounder (Higashimaru Foods Inc.), until the start of the feeding experiment. The feeding experiments were conducted for 8 wk beginning in August 1996 in two 2000-L tanks equipped with a closed recirculating seawater system. The tanks were placed in a room under natural light conditions and the water temperature was kept at 20 ± 1°C. At the start of the feeding experiment, the fish were transferred into floating net cages (25 x 35 x 25 cm - W x L x H) within the aquarium, 20 fish/cage, with three replications per dietary treatment. Fish were fed to satiation twice daily for 6 days/wk with each experimental diet. The body weight of each fish was measured at the beginning and at the end of the study after the fish were starved for 36 h. At the end of experiment, analyses of proximate composition of the whole body and of hematological and hematochemical parameters were conducted by the methods described previously (Kikuchi et al. 1994a, b).

Differences in proximate composition of the whole body, and the hematological and hematochemical parameters among treatments were tested for significance using the Mann-Whitney test (Campbell 1983). Data on final body weight, weight gain, feed efficiency, and protein efficiency ratio were analyzed for significance by Duncan's multiple range test (Duncan 1955).

## RESULTS

The growth and feed performance data are shown in Table 2. All fish soon accepted the experimental diets and fed actively for the duration of the experiment. Survival rates were high in all dietary groups and most of the mortality was from

| Diet | Average body weight(g) | | Weight gain | FE*2 | PER*3 | DFC*4 | Survival |
|---|---|---|---|---|---|---|---|
| | Initial | Final | (%) | | | | (%) |
| 1 | 4.6 | $28.2^{cd*5}$ | $513^{b}$ | $144^{a}$ | $3.2^{ab}$ | 2.1 | 100 |
| 2 | 4.6 | $29.7^{abc}$ | $525^{b}$ | $149^{a}$ | $3.3^{a}$ | 2.0 | 97 |
| 3 | 4.5 | $30.8^{ab}$ | $569^{a}$ | $147^{a}$ | $3.2^{ab}$ | 2.1 | 97 |
| 4 | 4.6 | $31.3^{a}$ | $573^{a}$ | $148^{a}$ | $3.3^{a}$ | 2.1 | 98 |
| 5 | 4.5 | $27.4^{d}$ | $498^{b}$ | $137^{b}$ | $3.0^{c}$ | 2.2 | 98 |
| 6 | 4.6 | $28.8^{bcd}$ | $520^{b}$ | $133^{b}$ | $3.1^{bc}$ | 2.3 | 98 |

*1 Average value of triplicate for each dietary group.

*2 Feed efficiency (%, weight gain/feed intake).

*3 Protein efficiency ratio (weight gain/dietary protein intake).

*4 Daily feed consumption (% body weight).

*5 Values in the same column having same superscript are not significantly different ($P>0.05$

**Table 2.** Growth data of Japanese flounder fed six experimental diets for 8wk*1

| Diet | Hemoglobin (g/100ml) | Hematocrit (%) | Red blood cell (x10⁴/ml) | Protein (g/100ml) | Triglyceride (mg/100ml) | Glucose (mg/100ml) | Phosphate (mg/100ml) | Calcium (mg/100ml) | Chloride (mEq/L) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 27.1±3.1$^{abc*2}$ | 5.1±0.9$^{ab}$ | 3.1±0.3$^{a}$ | 4.0±0.2$^{a}$ | 702±262$^{a}$ | 24.3±5.0$^{a}$ | 7.6±0.7$^{a}$ | 11.5±0.5$^{a}$ | 136±7.7$^{a}$ |
| 2 | 24.6±3.8$^{bc}$ | 5.2±0.5$^{ab}$ | 3.1±0.5$^{a}$ | 4.5±1.1$^{a}$ | 570±286$^{ab}$ | 21.7±4.3$^{a}$ | 7.7±0.6$^{a}$ | 11.5±0.6$^{a}$ | 134±2.3$^{a}$ |
| 3 | 27.4±2.4$^{ab}$ | 5.7±0.3$^{a}$ | 3.1±0.1$^{a}$ | 3.5±0.1$^{b}$ | 624±235$^{ab}$ | 22.0±6.6$^{a}$ | 6.8±0.2$^{a}$ | 11.4±0.4$^{a}$ | 138±5.4$^{a}$ |
| 4 | 23.0±2.4$^{c}$ | 5.2±0.7$^{ab}$ | 3.0±0.5$^{a}$ | 3.5±0.1$^{b}$ | 341±117$^{b}$ | 21.2±3.3$^{a}$ | 6.8±0.6$^{a}$ | 11.3±0.3$^{a}$ | 139±3.8$^{a}$ |
| 5 | 25.1±4.8$^{abc}$ | 4.9±0.4$^{b}$ | 3.0±0.1$^{a}$ | 4.2±1.4$^{ab}$ | 738±279$^{a}$ | 21.8±1.6$^{a}$ | 6.9±0.3$^{a}$ | 13.2±3.5$^{a}$ | 131±4.9$^{a}$ |
| 6 | 31.0±2.8$^{a}$ | 5.5±0.2$^{a}$ | 3.1±0.5$^{a}$ | 3.5±0.8$^{ab}$ | 744±219$^{a}$ | 28.2±6.9$^{a}$ | 6.8±0.2$^{a}$ | 11.4±0.1$^{a}$ | 137±5.8$^{a}$ |

*1 Means and standard deviations for five fish.

*2 See the footnote of Table 2.

**Table 3.** Hematological characteristics and contents of some plasma constituents of Japanese flounder fed six experimental diets for 8 wk*1

fish that jumped out of the net cages. Final body weight and weight gain of fish fed diets 3 and 4 were significantly higher than those in the control ($p$ <0.05). These parameters in the other experimental groups were comparable to the control. Feed efficiency was similar for diets 1 to 4, and significantly higher than that of diets 5 and 6 ($p$ <0.05). Some differences were observed in protein efficiency ratio; however, a significantly lower value vs. the control was obtained only in diet 5 ($p$ <0.05). Final body weights and weight gain of fish increased as blue mussel in the diet increased (diets 1 to 3).

The hematological characteristics and the plasma constituents are shown in Table 3. All dietary groups were identical in terms of red blood cell counts. Although there were some fluctuations in hemoglobin and hematocrit values, no experimental dietary groups were significantly different from those of the control. Plasma protein in diets 3 and 4, and triglyceride in diet 4 were significantly lower than those in the control; however, there were not any differences in the other parameters.

The whole body composition of the cultured fish is shown in Table 4. No significant differences were observed among dietary groups on the basis of crude protein, crude lipid, and crude ash contents. The moisture content of fish fed diet 6 was significantly higher than that of fish fed diets 1 and 3 ($p$ <0.05).

## DISCUSSION

Similar trends as shown in the previous report (Kikuchi and Sakaguchi 1997)—superior growth, comparable feed efficiency, and protein efficiency ratio to the control—were observed in dietary groups which contained blue mussel meat (diets 2 to 4) in this study. Therefore, it is clear that the inclusion of blue mussel meat in the diet promotes feeding and growth of the flounder, even if it is a very small amount (5% on a dry basis). Furthermore, the positive effect on growth was not different whether the mussel was fresh or freeze-dried according to the results of this study (diets 3 and 4, Table 2).

Many studies have been conducted on stimulating the feeding behavior of fish with various kinds of organisms. Some amino acids such as glycine, alanine, and valine showed activity to fish, especially when two or three of them form complexes (e.g., The Japanese Society of Fisheries Science 1981). Ina and Higashi (1978) and Ina (1986) reported that amino acid fractions of blue mussels *Mytilus edulis* stimulated feeding activity of red sea bream *Chrysophrys major*. There is no useful information on feeding attractants for Japanese flounder; however, it is considered that amino acids in the mussel may contribute to the promotion of feeding activity of the flounder.

The final body weight and weight gain of fish fed diets containing whole blue mussel were similar to those in the control, although the feed

| Diet | Moisture | Crude protein | Crude lipid | Crude ash |
|---|---|---|---|---|
| 1 | 74.4±0.2[b*] | 17.4±1.4[a] | 4.2±0.6[a] | 3.4±0.2[a] |
| 2 | 74.5±0.9[ab] | 18.2±1.9[a] | 4.3±0.5[a] | 3.5±0.1[a] |
| 3 | 74.4±0.5[b] | 17.3±2.6[a] | 4.8±0.6[a] | 3.5±0.1[a] |
| 4 | 74.3±0.8[ab] | 16.5±1.1[a] | 4.7±0.3[a] | 3.4±0.1[a] |
| 5 | 75.1±1.1[ab] | 17.2±1.5[a] | 4.6±0.3[a] | 3.4±0.1[a] |
| 6 | 75.2±0.4[a] | 17.7±2.1[a] | 4.5±0.7[a] | 3.3±0.1[a] |

* See the footnote of Table 3.

**Table 4.** Proximate composition of the whole body of Japanese flounder fed six experimental diets for 8 wk (%)*.

efficiency was lower due to higher crude ash content in the diet. This means that even whole mussel can be used as an ingredient in the diet for the flounder if the inclusion level is low (up to 30% as fresh whole blue mussel), because fish feed actively and compensate for the high ash content of the diet as shown in increasing daily feed consumption (Table 2). Whole blue mussels *M. edulis* were used by Berge and Austreng (1989) as substitute for fish (argentine *Argentinus silus*) in a moist pellet for rainbow trout *Salmo gairdneri*. They showed that there was a tendency toward poorer growth and feed efficiency with increasing level of the blue mussel in the diet; however, statistical differences were not observed among dietary groups as shown in this study. They also stated that the mussel meat may be a good protein source and has a favorable fatty acid composition.

On the other hand, Grave et al. (1979) reported that rainbow trout fed frozen blue mussel *M. edulis* meat showed much higher growth, feed efficiency, and protein efficiency than those fed commercial trout pellets, when the daily ration levels were in the same range. Kitamura et al. (1981) reported that the growth of red sea bream *Pagrus major* fed moist pellets containing a powder of freeze-dried blue mussel *M. galloprovincialis* meat as a main ingredient was almost equal to that of fish fed commercial pelleted diets when the moist pellets were supplemented with Vitamin B1.

These studies and our previous work show that blue mussel meat can be considered as a replacement for fish meal in the diet for many kinds of fish. However, considering the high market price of blue mussels, it is hard to say that mussels can be used as an economically attractive ingredient for fish meal in the diet. Moreover, because many of the mussels obtained from power plants are shell and sometimes contain sludges and other contaminants, acquiring a large amount of meat is not considered to be cost effective. Therefore, from a practical point of view, it would be better to utilize its effect on feeding stimulation as an additive in the diet than as a main ingredient, at least for juvenile Japanese flounder.

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## LITERATURE CITED

Berge, G.M. and E. Austreng. 1989. Blue mussel in feed for rainbow trout. Aquaculture 81: 79-90.

Campbell, R.C. 1983. Mann-Whitney test, pp. 55-59. *In:* Statistics for Biologists, 2nd ed. Baifukan, Tokyo. [In Japanese]

Duncan, D.B. 1955. Multiple range and F tests. Biometrics 11: 1-42.

Grave, H., A. Schultz, and R. Van Thielen. 1979. The influence of blue mussel, *Mytilus edulis*, and krill, *Euphausia superba*, on growth and proximate composition of rainbow trout, *Salmo gairdneri*, pp. 575-586. *In:* J.E. Halver and K. Tiews (eds.), Proceedings of the World Symposium on Finfish Nutrition and Fishfeed Technology, Vol.1. Heenemann, Berlin.

Ina, K. 1986. Artificial diet with plant protein for fish. Suisan no Kenkyu 5: 85-91. [In Japanese]

Ina, K. and K. Higashi. 1978. Surveys of feeding stimulants of the sea bream (*Chrysophrys major*). Nippon Nogeikagaku Kaishi 52: 7-12. [In Japanese]

Kikuchi, K. and I. Sakaguchi.1997. Blue mussel as an ingredient in the diet of juvenile Japanese flounder. Fish. Sci. 63: 837-838.

Kikuchi, K., T. Furuta, and H. Honda. 1994a. Utilization of feather meal as a protein source in the diet of juvenile Japanese flounder. Fish. Sci. 60: 203-206.

Kikuchi, K., T. Furuta, and H. Honda. 1994b. Utilization of soybean meal as a protein source in the diet of juvenile Japanese flounder, *Paralichthys olivaceus*. Suisanzoshoku 42: 601-604.

Kitamura, H., H. Mizutani, and Y. Dotsu. 1981. Rearing experiment on young of some fishes and decapodid crustaceans fed on the meat of the common mussel, *Mytilus edulis galloprovincialis*. Mar. Foul. 3: 23-27. [In Japanese]

The Japanese Society of Fisheries Science. 1981. Chemical sense of fish and feeding stimulants. Koseisha Koseikaku, Tokyo. 128 p. [In Japanese].

# SUMMARY OF THE PANEL DISCUSSION ON CULTURE HELD AT THE CONCLUSION OF THE UJNR AQUACULTURE PANEL'S SCIENTIFIC SYMPOSIUM IN DURHAM, NEW HAMPSHIRE, USA, 18 SEPTEMBER 1997

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The conveners of the 1997 UJNR Aquaculture Scientific Symposium included in the final day's program an afternoon session during which Japanese and U.S. scientists could discuss their most important research needs and try to identify areas and strategies for collaboration. The discussion on culture was facilitated by Drs. D. Bengtson, K. Fukusho, and M. Wilder and about 30 scientists participated (other panels on stock enhancement and offshore aquaculture were held concurrently in separate rooms).

The primary research needs are presented here in outline form:

I. A definition of "egg quality." We all talk about the importance of quality of gametes to the growth and survival of offspring and we are interested in the parental contribution to gamete quality, but we do not have a standard definition of "quality."

II. Natural spawning. The need here is for information transfer from Japanese scientists to U.S. scientists.

III. Broodstock nutrition. Collaborative research would aid the understanding of the contribution of nutrition of the parents to the quality of their gametes, however we define that quality.

IV. Cryopreservation of eggs. This is something that industry wants, but the panel identified it as high-risk research because of lack of previous success in this area.

V. Selective breeding. Especially in the areas of disease resistance and reducing problems of inbreeding.

VI. Larval rearing. The main areas of focus should be understanding the microbial ecology of rearing tanks and the replacement of live foods with formulated diets.

VII. Disease.

- A. Investigation of the potential for spread of diseases from aquaculture facilities to natural populations.
- B. Work is specifically needed on diseases of cultured abalone and flounder and on mechanisms for better import controls on foreign seed.
- C. Research and development of new diagnostic technology.
- D. Research and development of new vaccine technology.
- E. Research on disease prevention and treatment.

VIII. Recirculation systems.

A. Engineering of more efficient systems.

B. Biology and physiology of the organisms in the systems.

C. Nutrition of the organisms in the system, especially to reduce the ammonia and solid waste outputs.

D. Economics related to A-C above.

IX. Nutritional requirements of "new" species. We still lack detailed knowledge of the nutritional requirements of many species that are in commercial culture.

X. Development of culture techniques for new species, especially fry production technology. The list identified by the panel included: grouper, Seriola, tuna, true cod (Pacific), Sebastes, tautog, sea bass, haddock, and northern species of flatfish (sole, Pacific halibut, Atlantic halibut).

While the identification of research needs was relatively easy for this group, the identification of strategies and funding for collaboration was not. The UJNR meetings are an excellent way for scientists from the two countries to meet each other, but there should be more funding available on both sides for attendance at UJNR meetings. This panel discussion was a good way for the scientists to think about, and agree on, research needs and it would make sense for the UJNR Panel on Aquaculture to continue as a focal point for exchanges. Flounder research is a good example of a joint research project. Interested scientists can use existing programs for exchange (e.g., STA and JSPS post-doctoral fellowships, NSF and USDA programs, and Fulbright exchanges), but the group felt that the existing programs were insufficient for the kind of long-term collaboration that would be necessary to solve many of the problems identified above. New ideas and programs, probably involving additional government funding, will be required if significant collaborative efforts are to take place in addressing the above research priorities.

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