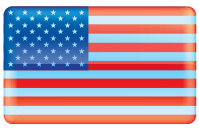

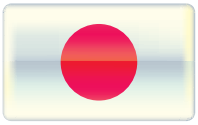

Aquaculture Technologies for Invertebrates

Proceedings of the Thirty-sixth U.S.-Japan Aquaculture Panel Symposium

Durham, New Hampshire
October 29-30, 2007
and
Milford, Connecticut
November 2, 2007

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

NOAA Technical Memorandum NMFS-F/SPO-99

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Robert Stickney*, Robert Iwamoto, and Michael Rust, editors

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U.S. Department of Commerce
Gary Locke, Secretary

National Oceanic and Atmospheric Administration
Dr. Jane Lubchenco, Under Secretary for Oceans and Atmosphere

National Marine Fisheries Service
Dr. James W. Balsiger, Assistant Administrator for Fisheries

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Milford Laboratory Minisymposium Presentations

Introduction

The U.S.-Japan Cooperative Program in Natural Resources (UJNR) was established on January 29, 1964. Since its creation, the UJNR has evolved to become one of the oldest and most effective cooperative agreements between Japan and the United States. In 1969, the UJNR Aquaculture Panel was created as a vehicle for scientists of both countries to meet and discuss aquaculture research accomplishments, needs, and priorities, as well as provide opportunities for cooperative research and scientific exchange.

Under UJNR Aquaculture Panel auspices, U.S. and Japanese scientists have met annually since 1971 without interruption. The venue for these meetings has alternated between the two countries. The 36th annual meeting and symposium were held in October 2007 at the University of New Hampshire, Durham, and a minisymposium was held in November at the Northeast Fisheries Science Center's Milford Laboratory, Milford, Connecticut. By prior agreement of the U.S.-Japan panels, contributors to the proceedings were requested to submit manuscripts in the form of brief papers or abstracts.

Hiroshi Nakano, Japan Panel Chairman
Robert Iwamoto, United States Panel Chairman

36th UJNR Science Symposium
October 29-30, 2007 | Durham, New Hampshire USA

The Basic Plan for Fisheries and Current Status of Aquaculture Research in Japan

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Keywords: aquaculture research, sustainable production

Abstract

Alterations to the structure of the fisheries industry have become necessary in Japan due to low levels of capture fisheries production, an aging population, environmental concerns, and increased worldwide demand for marine food products. In accordance with these developments, the Fisheries Agency, Ministry of Agriculture, Forestry, and Fisheries, established the new Basic Plan for Fisheries in 2007.

Under this plan, measures to realize the improvement of food self-sufficiency and global competitiveness of the industry are given priority, such as the promotion of stock management in Japan's exclusive economic zone and on the high seas, development of ecosystem-based sustainable aquaculture, establishment of new distribution systems, promotion of the export of marine products to expanding international markets, and utilization of multiple functions of fisheries communities.

Concerning aquaculture technology, the plan calls for the promotion of sustainable production based on the responsible use of aquaculture grounds and development of multiaquaculture technology employing low environmental-loading feed. Development of large-scale aquaculture systems and offshore aquaculture technology should also be important in achieving more efficient usage of sea areas. Innovation of alternative protein sources for feed, that is, plants or agricultural waste products, is urgently required to deal with the shortage of fish meal. The Fisheries Research Agency (FRA) has recently initiated projects on blue-fin tuna and kanpachi (amber jack) aquaculture in accordance with these research schemes. In this paper, current aquaculture research in the FRA was introduced.

Overview of NOAA's Aquaculture Program

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Keywords: sustainable aquaculture, fisheries management

Abstract

As a federal agency under the U.S. Department of Commerce, the National Oceanic and Atmospheric Administration (NOAA) is focused on creating domestic seafood supplies to meet the growing demand for all seafood products and on working with international partners to foster development of sustainable aquaculture. Currently, 80% of the seafood that Americans consume is imported, and at least half of those imports are farmed seafood.

Spurred on by the growth of aquaculture worldwide, the role of aquaculture in meeting consumer seafood demand, and the enhancement needs of commercial and recreational fisheries, aquaculture continues to attract international attention from researchers, fisheries managers, policy makers, and the public.

Over the past three years, the NOAA Aquaculture Program has successfully focused U.S. attention on marine aquaculture as a vital tool for fisheries management and additional domestic seafood production. NOAA is also interested in continuing to advance an international dialogue on the role of aquaculture in seafood supply—a dialogue that includes a science-based examination of the benefits and challenges of aquaculture production.

This presentation provided an overview of NOAA's rich tradition of marine aquaculture research and the importance of nurturing important international partnerships, such as the U.S.-Japan Natural Resources Panel on Aquaculture.

Application of Pedigree and Relatedness Analyses Based on Microsatellite DNA and Mitochondrial DNA Markers to the Stock Enhancement and Population Studies on the Kuruma Prawn (*Marsupenaeus japonicus*)

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Keywords: kinship, Penaeidae, genetic diversity, stock effectiveness, resource management

Abstract

The availabilities of pedigree and relatedness analyses with microsatellite DNA (MS-DNA) and mitochondrial DNA markers for stock enhancement and population studies on the kuruma prawn (*Marsupenaeus japonicus*) are discussed based on our current research. Among experimentally reared full-sib groups, kinships were completely described on an unweighted pair group method with arithmetic mean (UPGMA)–based dendrogram constructed from the MS-DNA analysis. Released juveniles were successfully distinguished by pedigree analysis in a mark-recapture experiment, showing the usefulness of the DNA markers as genetic tags. Moreover, relatedness analysis revealed the existence of closely related groups in wild populations of the shrimp, showing that the analysis can provide detailed information about their genetic variability.

Introduction

The kuruma prawn *(Marsupenaeus japonicus)* is a marine shrimp that is widely distributed in the temperate and tropical zones of the world (Dall et al. 1990). While this shrimp is one of the most famous fishery animals in Japan, the yield rapidly declined during the late 1960s. That led to the establishment of stock enhancement programs resulting in the annual release of approximately 200 million hatchery-reared individuals for about 30 years, mainly in southern Japan (Hamasaki and Kitada 2006). However, it has been difficult to assess the precise effectiveness of the program because of the lack of suitable tags to distinguish introduced shrimp from wild ones at all life history stages. In addition, the massive releases led to a concern about the conservation of genetic variability in the wild population.

Microsatellite DNA (MS-DNA) is made up of tandem repeats of very short nucleotides. These repetitive regions of nuclear DNA exhibit high variability due to length differences (Tauz 1989). Mitochondrial DNA (mtDNA) also shows high variability because of its nucleotide substitutions (Brown et al. 1979). In particular, the control region that does not code for proteins or RNA shows high variability with regard to relaxed functional constraints (Brown 1985). Those markers can provide sufficiently rare genotypes that can not only identify individuals taken randomly from wild populations, but also estimate the genetic relatedness among individuals with unknown pedigree (Finch et al. 1996, Cronin et al. 1999). Therefore, DNA markers should be useful for the assessments of the effectiveness and the genetic influences of stock enhancement of kuruma prawns.

Recently, we have tried to apply the pedigree and relatedness analyses with MS-DNA and mtDNA markers to the stock enhancement and population studies. In this paper, we discuss the availability of the analyses in the stock enhancement program of kuruma prawn based on our current studies.

Pedigree Analysis of Kuruma Prawns by MS-DNA Markers

The feasibility of MS-DNA markers for pedigree analysis of kuruma prawns was tested on seven experimentally reared full-sib groups (Sugaya et al. 2002a). The genetic distances among individuals were estimated by the inverse of genetic identities among individuals (Nei 1987), which were calcu-

Figure 1. Genetic relationship among seven full-sib groups: Group 1 = ●, Group 2 = ▲, Group 3 = ◍, Group 4 = △, Group 5 = ○, Group 6 = □, and Group 7 = ■.

lated from multilocus genotypes of five MS-DNA loci. The averages of the distances were 0.392 ±0.161 within siblings and 0.783 ±0.138 between siblings. Moreover, each sibling was clearly distinct on an unweighted pair group method with arithmetic mean (UPGMA)–based dendrogram constructed from the identities among individuals (Figure 1). These results demonstrate the availability of the MS-DNA markers for pedigree analysis estimating genetic relatedness among individuals in shrimp populations of unknown pedigree.

The Detection of Released Shrimp in a Stocking Area

The feasibility of the DNA markers as tags for released shrimp was examined with a mark-recapture experiment in Saiki Bay along the eastern coast of Kyusyu island in southern Japan (Figure 2). Two hundred thousand juveniles of 5 cm total length were produced from 6 wild females. They were released in late July and early August in the bay. From August to November, 539 prawns were sampled by experimental trawling and gill net fishing by commercial fishermen. The pedigree analysis by the MS-DNA and mtDNA markers detected 51 offspring from the six females in the samples. The contributions of the offspring ranged from 2 to 17.2% on a monthly basis (Figure 3). From that data we can conclude that the microsatellite and mitochondrial DNA markers are useful as tags for released kuruma prawns in stocking programs. In addition, the relatedness analysis of the samples revealed the existence of many related shrimp in the bay. Although mean genetic relatedness (r_{xy}) of Queller and Goodnight (1989) estimated by the five MS-DNA markers was almost zero, the relatedness among individuals sharing common haplotypes of mtDNA markers were from 0.126 to 0.458 in each month (Figure 4).

In the bay, approximately 500,000 hatchery-reared juveniles, derived from 100 to 200 wild females caught near the stocking area, were released annually by the local government and a fishery union. The relatedness analysis is considered to have detected those released prawns. This may show the possibility of the analysis of large-scale stocking effectiveness estimation.

Analysis of Genetic Relatedness Structure of Wild Kuruma Prawns

The genetic relatedness (r_{xy}) among wild shrimp individuals was examined in four localities around Japan (Figure 5) with MS-DNA and mtDNA markers (Sugaya et al. 2002b). Average r_{xy} among 310 individuals within each locality calculated from the information of the five MS-DNA loci ranged from 0.059 to 0.063. The values from Kagoshima and Kumamoto were significantly higher than simulated values estimated on the assumption of random mating (permutation test, $p < 0.05$). The frequencies of r_{xy} in Kumamoto and Kagoshima were high (permutation test, $p < 0.05$, Figure 6). Furthermore, the relationship between r_{xy} and the distribution of the haplotypes of mtDNA markers examined by an UPGMA-based dendrogram showed that individuals sharing a common haplotype tended to be closely related in Kagoshima (Figure 7).

The results showed the existence of full-sib level kinships in the localities because the theoretical values of r_{xy} were approximately 0.5 among the full sibs. In the East China Sea, including Kumamoto and Kagoshima, 40–90 million kuruma prawns have been released annually. Since those shrimp are generally reared from a limited number of parents, the release might explain the closely related individuals detected in those two localities. However, these results do not necessarily show the gene flow among wild and released populations, as we cannot determine whether the closely related individuals were born in the wild or in a hatchery. Hence the magnitude of gene flow should be examined to reveal the genetic effects of release in the future.

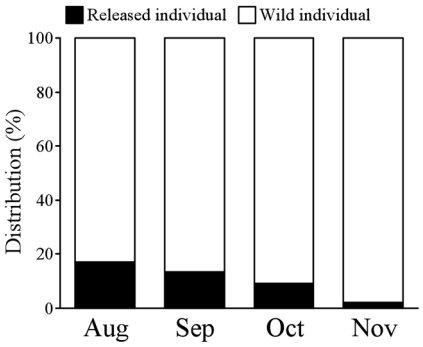

Figure 3. Distribution of released prawns detected by pedigree analysis in each month.

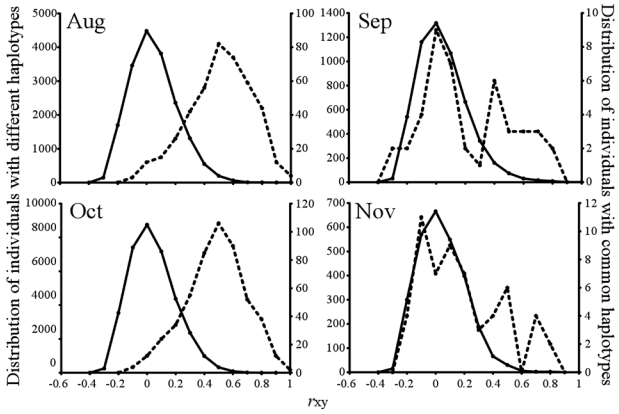

Figure 4. Relatedness distributions among the individuals with common haplotypes (dotted line) and among those with different haplotypes (solid line) each month.

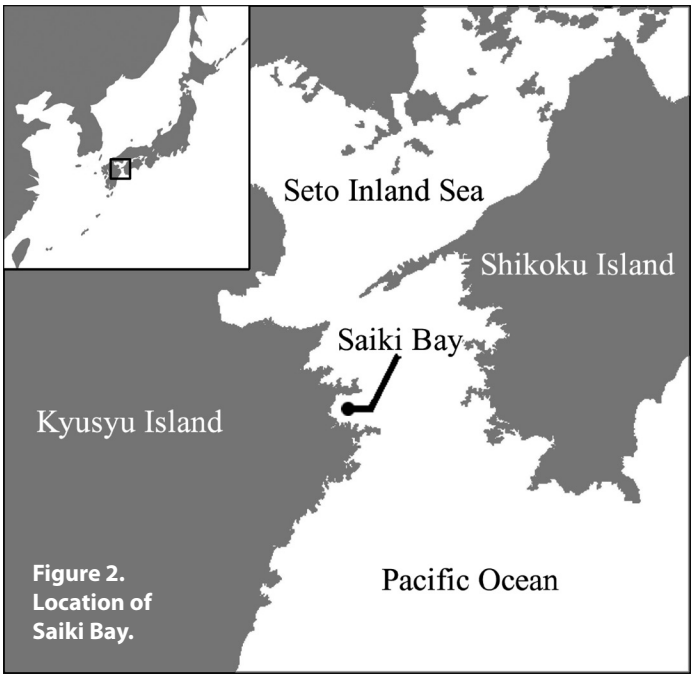

Figure 2. Location of Saiki Bay.

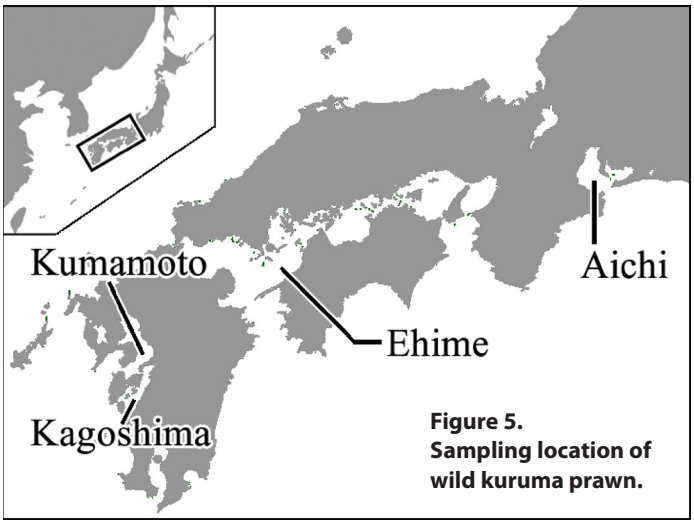

Figure 5. Sampling location of wild kuruma prawn.

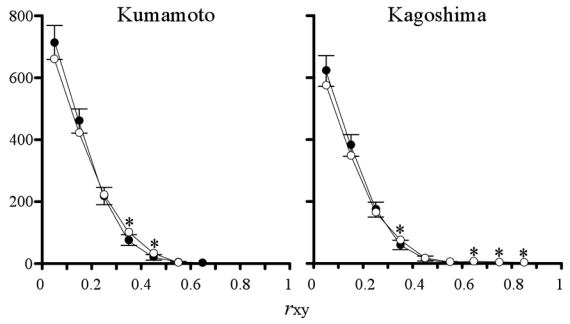

Figure 6. Distribution of relatedness among individuals calculated from the data of MS-DNA in localities of Kumamoto and Kagoshima. Relatedness less than zero was regarded as zero. Observed value = ○, simulated value ±95% confidence interval (CI) = ●, and significant difference = *.

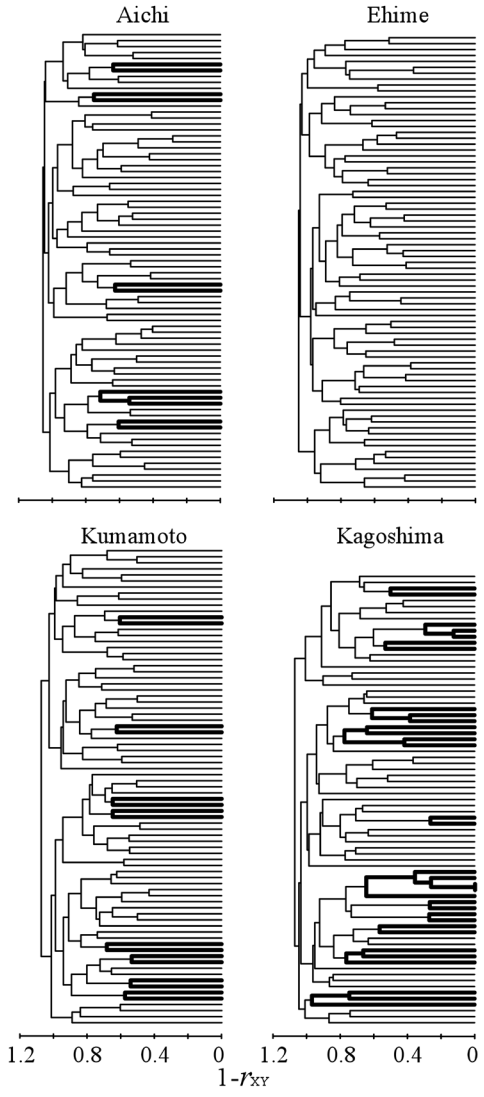

Figure 7. A UPGMA-based dendrogram created from the genetic relatedness among individuals calculated from the data of MS-DNA. Bold lines show the branches that join common haplotypes 1 to 64.

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Marine Ornamental Aquaculture

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Keywords: aquarium trade, *Lysmata*, seahorses, *Hippocampus*

Abstract

Many species of marine animals belonging to different taxonomic groups are collected from the wild for the aquarium trade. Direct and indirect impacts of the collection, especially the use of explosives and toxins (e.g., cyanides), have caused grave concerns. In recent years, efforts have been made to understand the biology of some of the species and develop cultivation technology to reduce wild collection while sustaining the aquarium industry trade. My laboratory has worked on developing aquaculture protocols for marine ornamental crustaceans (primarily shrimp, but also crabs and lobsters) and, in more recent years, seahorse species.

Our focus has been on several *Lysmata* spp., popular in the aquarium industry. The caridean shrimp species have a unique (among decapod crustaceans) reproductive system, protandric simultaneous hermaphrodite. The shrimp, which first matures as a male, may change through several transitional stages to simultaneous hermaphrodite that can function as both a male (during intermolt) and female (during postmolt).

Spawning of these shrimp in captivity is relatively easy, especially for the *Lysmata* spp., as they are simultaneous hermaphrodites. A female can produce several hundred to several thousand eggs during each spawning and carries the embryos under her abdomen until the larvae hatch 10 to 20 days later. Within several to 48 hours after hatching, the female molts, is receptive to mating, and spawns (with or without mating) again. The larvae are composed of many (9–13) zoea stages. *Artemia* nauplii can be used as the sole food for all life stages of *Lysmata* spp.

The biggest challenge for commercial culture of marine ornamental shrimp is the long and variable larval durations, due to mark time molting. Shortening larval cycle through nutrition and culture system improvement is the key for successful commercial production of these ornamental shrimp species. Improving diets of broodstock, and especially of larvae, may also accelerate the rates of larval development and increase the potential of commercial aquaculture.

Seahorses (genus *Hippocampus*) are a group of fish that have fascinated people for centuries for their unusual upright body form, unique reproductive system (males give birth and are the major caretakers of the offspring) and healthcare value. More than 20 million individuals are collected annually for use in traditional Chinese medicine and aquarium trade. Overfishing has led to placement of all 34 seahorse species under Convention on International Trade in Endangered Species protection since 2004. Unlike for Chinese medicine, tank-raised seahorses are preferred in the marine aquarium market (and demand higher prices). In recent years, efforts have been made to develop protocols for culturing seahorse species that are popular in aquarium trade.

We are in the process of developing aquaculture protocols for the lined seahorse (*H. erectus*), one of the most popular aquarium species and native along the Atlantic coast of North America from Nova Scotia to Florida and throughout the Caribbean. Pairing, mating, and copulation behavior were observed. Gestation time and brood size were 17.33 ±2.94 days and 272.33 ±66.45 juveniles/brood, respectively. The highest growth rate and survivorship of the juveniles during the first 9 weeks occurred at 28–29°C among the temperatures tested (24–33°C).

Nutritional Significance of n-3 Highly Unsaturated Fatty Acids for Larval Survival and Development in Mass Production of Brachyuran Crab Larvae

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Keywords: brachyuran crab, seed production, n-3 HUFA, survival rate, larval morphogenesis

Abstract

To evaluate the n-3 highly unsaturated fatty acid (HUFA) requirement by larvae of the swimming crab (*Portunus trituberculatus*), two mud crab species (*Scylla serrata* and *S. paramamosain*), snow crab (*Chionoecetes opilio*), and horsehair crab (*Erimacrus isenbeckii*), we conducted two types of larval rearing experiments using small vessels and large larval production tanks. In small rearing vessels, n-3 HUFA in live foods improved larval survival of the swimming crab and mud crabs. On the other hand, in large tanks, larval mass mortality of *S. serrata* frequently occurred during the metamorphosis to megalops due to abnormal molting by morphologically advanced last stage zoea. These last stage zoea have morphological features similar to those of megalops, such as large chelipeds.

The excess dietary n-3 HUFA is concluded to cause this hypermorphogenesis of larval *S. serrata*. The phenomenon was also observed for the swimming crab and the mud crab *S. paramamosain*. Horsehair crab larvae fed unenriched *Artemia* showed high survival rates to the first crab stage in small rearing vessels. In contrast, enrichment with n-3 HUFA, especially docosahexaenoic acid (DHA) in live food, improved the survival of snow crab larvae in small rearing vessels. However in large tanks, DHA con-

tent of snow crab larvae largely decreased because of the low content of DHA in live food. We concluded strategies for optimal n-3 HUFA enrichment in live food are specific for each species, requiring development for each crab species.

Introduction

Hatchery technologies of commercially important brachyuran species such as the swimming crab (*Portunus trituberculatus*), mud crabs (*Scylla serrata* and *S. paramamosain*), snow crab (*Chionocetes opilio*), and horsehair crab (*Erimacrus isenbeckii*) have been studied for stock enhancement programs by the Fisheries Research Agency (FRA) of Japan (Hamasaki 2003, Hamasaki et al. 2002a, 2002b, 2006, 2007, Suprayudi et al. 2002, 2004, Arai et al. 2004, 2007, Jinbo et al. 2005, Kogane et al. 2005, 2007a, 2007b). While the swimming crab and two mud crabs are warm water species, snow crab and horsehair crab inhabit cold water.

Between 1993 and 2005, approximately 50 million juveniles and several million juveniles of the first crab stage for swimming crab and mud crabs have been produced annually for restocking purposes (Fisheries Agency et al. 1995–2007). Accordingly, hatchery technologies have been developed for those warm water species. However, larval mass mortality has frequently occurred during larval production (Hamasaki et al. 2002a, Arai et al. 2004). Reliable techniques are needed for the efficient mass production of large numbers of juveniles. On the other hand, large-scale larval production techniques remain undeveloped for snow crab and horsehair crab (Hamasaki et al. 2006, 2007, Kogane et al. 2007a, 2007b).

The n-3 highly unsaturated fatty acids (n-3 HUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential fatty acids (EFA) for marine fishes (Watanabe et al. 1989a, 1989b, Izquierdo et al. 1989, Takeuchi et al. 1990, 1994, 1998, Furuita et al. 1996, 1998). It is well known that n-3 HUFA affect larval survival, growth, and stress tolerance of marine fish larvae and juveniles. However, the effectiveness of n-3 HUFA for larval brachyuran crabs as EFAs has not been studied in detail. Since the early 1990s, we have investigated this issue to improve and develop mass production technologies for brachyuran crabs.

We conducted larval rearing experiments for swimming crab, mud crabs, horsehair crab, and snow crab using small vessels and large larval production tanks with live foods containing various levels of n-3 HUFA and examined the significance of dietary n-3 HUFA for larval development and survival of the species of interest. In this paper, we summarize the results of our experiments and discuss future prospects for the development of mass seed production techniques of brachyuran species from the point of view of n-3 HUFA requirement by larvae.

Materials and Methods

Rearing experiments in small vessels. Larvae of each species were reared in 1–2 L plastic beakers with gentle aeration (Anger 2001). The beakers were set in shallow water baths in which temperatures were regulated by heaters, chillers, or both. Thirty newly hatched larvae were stocked in each beaker with seawater and prey (rotifers, *Brachionus plicatilis* sp. complex, or *Artemia* sp.). The larval developmental stages were determined when they were transferred daily using a largemouth pipette into new beakers with new seawater and prey. Rotifers and *Artemia* nauplii were enriched with emulsified oils containing different levels of n-3 HUFA (mainly EPA and DHA).

Rearing experiments in large production tanks. Larvae of the mud crab *S. serrata* were reared in 200,000 L tanks and snow crab in 20,000 L tanks. Newly hatched larvae were stocked in the flow-through tanks with aeration at a density of 10,000–40,000 individuals/1,000 L. Rotifers were supplied daily to the tanks to maintain the designated concentrations in the larval rearing water. *Artemia* nauplii were used as prey from the third zoeal and first zoeal stages for mud and snow crabs, respectively. Phytoplankton (*Chlorella vulgaris, Nannochloropsis* sp.) were also added to the tanks as food for the rotifers and *Artemia*. The rotifers were enriched with brewer's yeast or phytoplankton containing cellular n-3 HUFA before being fed to the larvae. Larval survival rates were estimated by the volumetric method. The length of chelae and carapace of fifth stage zoea of mud crabs were measured under a microscope and the percent ratio of relative chela length to carapace length (termed the relative chela length in this study) was calculated as an index of the degree of morphogenesis in fifth stage zoea.

Fatty acid analysis. The n-3 HUFA contents of larvae and prey used in each experiment were determined. Lipids were extracted by the chloroform-methanol (2:1, v/v) method (Folch et al. 1957). Total lipids were saponified with KOH. The saponifable matter was then esterified by BF3 in methanol. Fatty acid methyl esters were diluted in hexane and analyzed with a gas chromatograph equipped with a silica capillary column. Fatty acid methyl esters were identified by comparing retention times against standards.

Results and Discussion

Effect of dietary n-3 HUFA on larvae of swimming and mud crabs. Larvae of the swimming crab and the two mud crab species fed rotifers and *Artemia* enriched with n-3 HUFA showed higher survival rates than larvae fed unenriched live foods in the rearing experiments using small vessels (Takeuchi et al. 1999, Takeuchi et al. 2000, Suprayudi et al. 2004) (Figure 1). Thus it was determined that dietary n-3 HUFA greatly improves the survival rates of larvae of the swimming crab and mud crabs.

Based on the results of the rearing experiments using small vessels, we enriched the rotifers and *Artemia* with n-3 HUFA for feeding larvae of the same species in large production tanks. Larval mass mortality of the mud crab *S. serrata* has been frequently observed during metamorphosis to megalops due to abnormal molting of morphologically advanced last stage zoea (Hamasaki et al. 2002a, 2002b). The last stage zoea have morphological features similar to those of megalops, including large chelipeds compared with normal last stage zoea (Figure 2). Morphologically advanced last stage zoea often fail to molt to normal megalops. The abnormal molting patterns by morphologically advanced last stage zoea were classified by Hamasaki et al. (2002a) as follows (Figure 3): type A, serious abnormality (larva has not shed its exuvia completely and died during molting); type B, partial abnormality (larva has shed its exuvia but was unable to shed the integument of the chelipeds, walking legs, or both); and type C, slight abnormality (larva has shed its exuvia except for the integument of one cheliped).

To elucidate the relationship between morphogenesis of zoea and abnormal molting, percentages of larvae showing abnormal molting were plotted against the relative chelae lengths as an index of the degree of morphogenesis of fifth stage zoea (Figure 4). Almost all larvae with a relative chela length exceeding 50% failed to molt successfully and the frequency of serious abnormal molting (type A) linearly increased against the relative chela length. As can been seen in Figure 4, the upper limit of relative chela length should be less than 50% for normal molting of last stage zoea into megalops (Hamasaki et al. 2002a).

To examine what factors affect larval morphogenesis in terms of larval n-3 HUFA requirement, the relationship was determined between n-3 HUFA content in rotifers sampled from the larval rearing

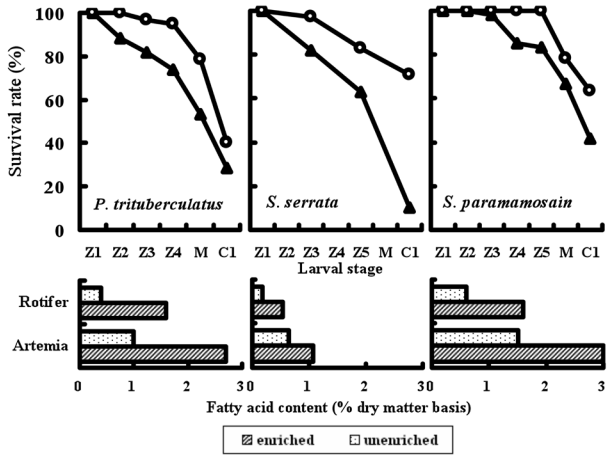

Figure 1. Survival rates to each larval stage of *Portunus trituberculatus*, *Scylla serrata*, and *S. paramamosain* reared in 1-L beakers with rotifers and *Artemia* nauplii that were either unenriched (▲) or enriched with n-3 HUFA (○), and n-3 HUFA contents in rotifers and *Artemia* used in the experiments. In the all experiments, rotifers were used as prey from first zoeal and second zoeal stages, and *Artemia* were used from third zoeal to megalopal stage. (Takeuchi et al. 1999, Takeuchi et al. 2000, Suprayudi et al. 2004.)

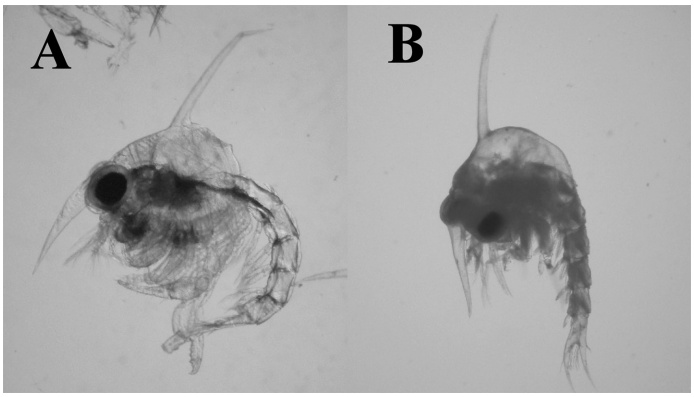

Figure 2. Photographs show the fifth stage zoea in the production of *S. serrata*. Photo A, the fifth stage zoea which have morphological features similar to those of megalops. Photo B, a normal megalopa. (Modified with permission from Hamasaki et al. 2002a.)

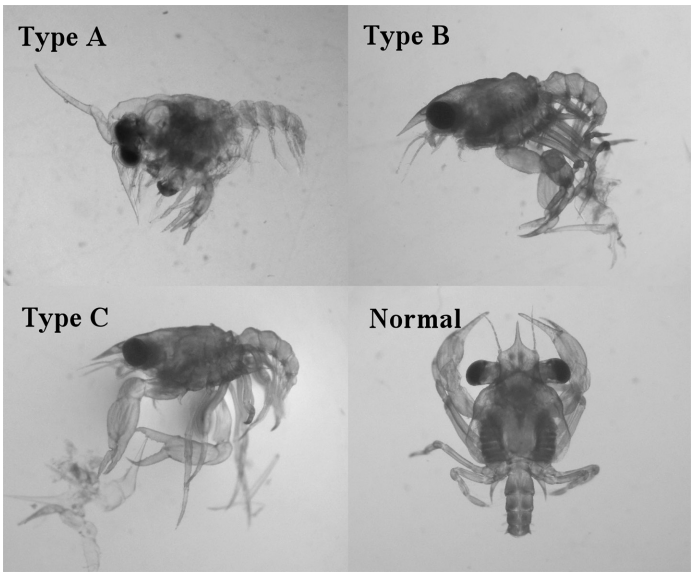

Figure 3. Photographs show the molting patterns during the metamorphosis to megalops in the production of *S. serrata*. Type A, serious abnormality (larva has not shed its exuvia completely and died during molting); Type B, partial abnormality (larva has shed its exuvia but was unable to shed the integument of the chelipeds or walking legs or both); Type C, slight abnormality (larva has shed its exuvia except for the integument of one cheliped). (Modified with permission from Hamasaki et al. 2002a.)

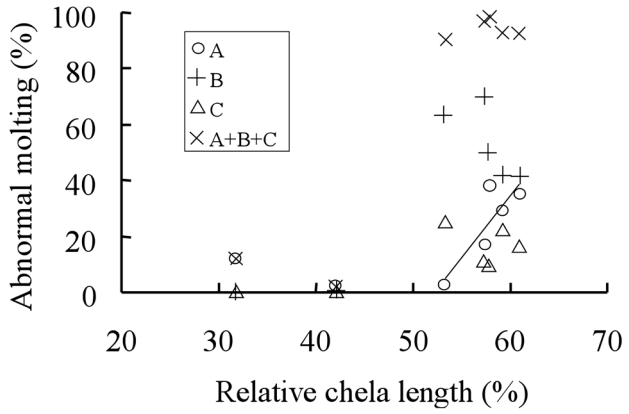

Figure 4. Relationships between the relative chela length of fifth stage zoeas to the carapace length and the percentage showing abnormal molting during the metamorphosis to megalops in the production of *S. serrata*. Symbols indicate the types of abnormal molting as shown in Figure 3. The line indicates linear approximation of type A. (Modified with permission from Hamasaki et al. 2002a.)

tanks with phytoplankton that contained different supplementary levels n-3 HUFA in their cells and relative chela length of last stage zoeas (Figure 5). The relative chela lengths increased with increasing n-3 HUFA content in rotifers and the upper limit content of n-3 HUFA in rotifers in larval rearing tanks of *S. serrata* that prevented larval mass mortality during the metamorphosis to megalops was around 0.6% (Hamasaki et al. 2002b). That upper limit in rotifers was similar to the n-3 HUFA content of enriched rotifers but less than that of enriched *Artemia* that were provided in the rearing experiments using beakers by Suprayudi et al. (2004) (see Figure 1). The optimum level of n-3 HUFA content in *Artemia* should be determined in the large production tanks.

Larval mass mortality of morphologically advanced last stage zoeas has also been observed in the production of the swimming crab and the mud crab *S. paramamosain* (Arai et al. 2004, 2007). However, the upper limit content of n-3 HUFA in rotifers has yet to be determined.

We found that dietary n-3 HUFA markedly affects the survival and morphogenesis of swimming crabs and mud crabs. We concluded that the relative chela lengths of last stage zoeas reflect the nutritional condition of larvae based on the positive correlation between the n-3 HUFA content of prey fed to *S. serrata* larvae and relative chela length of the larvae. Furthermore, this relationship can be used as a useful index to evaluate the effects of environmental conditions on larval development and survival as found in our rearing experiments of *S. serrata* larvae (Dan and Hamasaki unpubl. data).

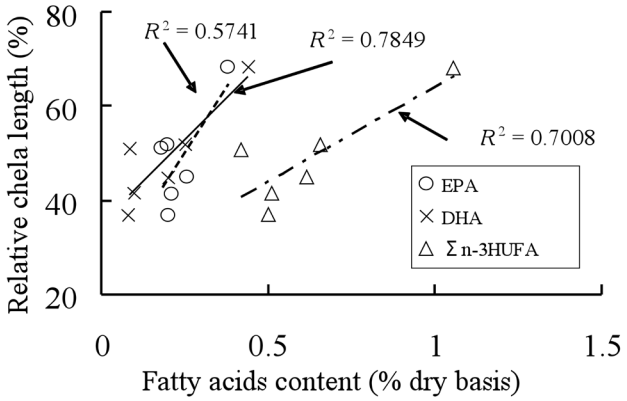

Figure 5. Relationships between the fatty acid contents in the rotifers and the relative chela length of fifth stage zoea to the carapace length of *S. serrata*. (Modified with permission from Hamasaki et al. 2002b.)

Survival rates of *S. serrata* larvae reared in 1 L beakers at different salinity levels between 10 and 35 ppt with an interval of 5 ppt were low at 10, 15, 30, and 35 ppt conditions. Based on that result, it might be natural to conclude that the optimal salinity for survival and development of *S. serrata* larvae was 20–25 ppt. However, the relative chela lengths of last stage zoeas increased with increasing salinity and they exceeded the critical level of 50% at 30 and 35 ppt, so the larvae failed to undergo normal metamorphosis to megalops at those salinity levels. Based on these observations, it is clear that hypermorphogenesis caused the larval mass mortality at 30–35 ppt. Therefore, we recommend that larval morphogenesis should be examined to evaluate the rearing experiments with swimming crab and mud crabs.

Effect of dietary n-3 HUFA on larvae of horsehair and snow crabs. Horsehair crab larvae were reared in 2 L beakers with *Artemia* unenriched and enriched with n-3 HUFA (Jinbo unpubl. data). Horsehair crab larvae showed high survival rates to the first crab stage even when fed unenriched *Artemia*. On the other hand, the snow crab larvae that were fed rotifers enriched with n-3 HUFA showed higher survival rates than larvae fed unenriched rotifers. In addition, larvae that were fed rotifers containing mainly DHA showed the highest survival rate (Kogane unpubl. data). We also analyzed the n-3 HUFA compositions in rotifers and Artemia supplied to the larval rearing tanks and snow crab larvae reared in large production tanks. Rotifers and *Artemia* contained minimal DHA. On the other hand, first stage zoea contained the highest amount of n-3 HUFA, mainly composed of EPA and DHA (Figure 6).

The DHA content of larvae decreased with growth through second stage zoea to megalops stages and larval mass mortality occurred (Figure 7) during metamorphosis to megalops (Kogane et al. 2007b). This was probably due to the low DHA content in the live food. Consequently, we conclude that improving the DHA content in live food is one of the primary ways to improve the mass production of snow crab.

Our recent research demonstrated that dietary n-3 HUFA is essential to improve larval survival and development in the production of swimming crab, mud crabs, and snow crab. However, the requirement by larvae of brachyuran crabs for n-3 HUFA varied with species. So, strategies for n-3HUFA enrichment in live food require individual verification for each species in future research.

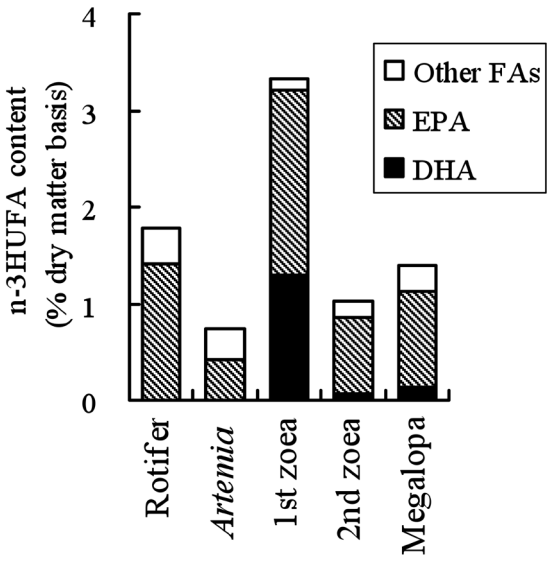

Figure 6. The n-3 HUFA content in rotifers and *Artemia* nauplii supplied to the larval rearing tanks and larvae of snow crab (*Chionocetes opilio*) reared in large production tanks. (Based on data from Kogane et al. 2007b.)

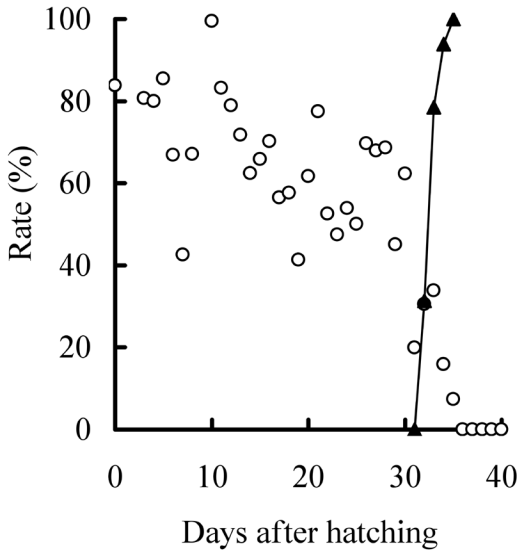

Figure 7. Survival rates of larvae (○) and percentage composition of megalops (▲) of the snow crab (*Chionocetes opilio*) reared in large seed production tanks. (Modified with permission from Kogane et al. 2007b.)

Acknowledgments

The authors thank the staff of the Akkeshi, Obama, Tamano, and Yaeyama stations of the FRA National Center for Stock Enhancement, especially K. Murakami and M. Ashidate for their kind hospitality and cooperation throughout the experiment.

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The Chesapeake Bay Blue Crab (*Callinectes sapidus*): A Multidisciplinary Approach to Responsible Stock Enhancement

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Keywords: blue crab, Chesapeake Bay, stock enhancement, juvenile

Abstract

Blue crab (*Callinectes sapidus*) catches, the major remaining harvest of the Chesapeake Bay (more than $100 million in value), dropped over 70% from record highs in the early 1990s. Overfishing and environmental degradation led to a sustained decline of 84% in the blue crab breeding stocks, which in turn resulted in historically low levels of juvenile recruitment and in nursery habitats being under carrying capacity. This situation makes the Chesapeake Bay blue crab an excellent candidate for stock enhancement efforts that target replenishment of the declining breeding stocks. A multidisciplinary program was therefore developed to 1) study the basic biology and life cycle of the blue crab, 2) develop hatchery and nursery technologies for the mass production of blue crab juveniles, and 3) assess the potential of using hatchery juveniles to enhance blue crab breeding stocks and, in turn, bay-wide abundance and harvests.

Understanding the environmental regulation of the reproductive cycle led to full photothermal control of the timing of ovulation and hatching of wild-caught inseminated females. Intensive larval rearing (60–140 larvae/liter), utilizing microalgae species of high nutritional value and omega-3 enriched rotifers and brine shrimp larvae (*Artemia* nauplii), resulted in 30–80% survival from hatch to megalopae in 3 to 4 weeks. Megalopae were reared to 20 mm juveniles (mean carapace width) at lower densities (5–20/liter) in four weeks at survival rates ranging from 10 to 30%, depending on rearing density.

During 2002 to mid-2007, in excess of 400,000 hatchery-reared juveniles were experimentally released into nursery habitats of the Chesapeake, both in upper and lower bay waters. All released juveniles were individually tagged with coded microwire, elastomer tags, or both and monitored to study survival, growth, migration patterns, field performance, and enhancement. Simultaneous releases and monitoring of hatchery and wild crabs demonstrated that performance of hatchery-reared juveniles did not differ from wild juveniles in most variables, including survival, growth, feeding, and habitat use, despite some minor differences in morphology and behavior.

Enhancement doubled to tripled the wild population in release sites, and survival from release to sexual maturity averaged 15% (range 6–26%). Survival varied among years, depending on environmental conditions such as salinity and temperature, and was inversely dependent on stocking density. Optimal juvenile release size was found to be 20 mm and above, which corresponds with change in dispersal behavior of smaller juveniles.

We also examined optimal release sites, habitats, density, and timing. Hatchery crabs grew rapidly to maturity, and were observed mating in as few as two months after release. Inseminated female crabs migrated in the fall along the bay's main deepwater channel from nursery and mating habitats to the spawning sanctuary in the lower bay, suggesting that hatchery crabs can contribute to the spawning

stock as soon as several months postrelease. This, together with evidence showing the effectiveness of the spawning sanctuary, suggests the importance of implementing protected migratory corridors linking nursery habitats with spawning grounds.

In summary, using a multifaceted approach, we demonstrated the feasibility of releasing hatchery-produced juvenile crabs to restore the dwindling blue crab breeding stocks. Working with watermen, the production of juvenile crabs is now being scaled up to allow for larger releases and to optimize release strategies, which will be ultimately recommended to and implemented by the Chesapeake crabbing industry.

An Outline of the Research Project, Development of Seed Production Technology in Japanese Spiny Lobster

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Keywords: spiny lobster, seed production, diet

Abstract

The Japanese spiny lobster (*Panurilus japonicus*) is a commercially important crustacean in Japan. Since the lobster fishery is fully exploited, development of the seed production and rearing techniques of the lobster has been eagerly desired. However, specific biological characteristics of phyllosoma, such as their peculiar body form, protracted lifespan (about one year), and pelagic open ocean life, have hindered significant progress in culture. To overcome these problems and produce large numbers of juveniles through larval culture, a research program for development of seed production technology in the Japanese spiny lobster was started in 2005. The project team consists of two subteams, one to improve and develop diets and the other to improve and develop rearing methods of phyllosoma.

In order to improve and develop diets, we are investigating natural diets of lobster phyllosoma by molecular methods. Improvement of dietary value of brine shrimp (*Artemia*) and mussel gonads, which are known to be effective as foods for phyllosoma, and development of artificial feed are in progress. We are also attempting to develop methods to evaluate and control conditions of phyllosoma in the rearing tank by monitoring expression of DNA responsible for biodefense, molting, and digestion. The survival rate of phyllosoma has been gradually increasing year by year by incorporating these results into culturing methods.

Shellfish Aquaculture Research on Oysters and Clams at Haskin Shellfish Research Laboratory

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Keywords: aquaculture, oysters, clams, genetic structure

Abstract

Haskin Shellfish Research Laboratory has been working on various aspects of shellfish aquaculture for more than 100 years. Most current genetic work is mapping genes for disease resistance in eastern oysters (*Crassostrea virginica*) using our 50-year breeding program that has developed disease resistant lines. These studies augment our development of tetraploid stocks of *C. virginica*, and continued development of strains of eastern oyster resistant to diseases caused by MSX (*Haplosporidium nelsoni*) and Dermo (*Perkinsus marinus*). Information from long-term studies suggests that native stocks have developed some resistance to MSX disease. This data has encouraged a program of large-scale restoration of oysters in Delaware Bay using classic shelling techniques. This restoration program is being coupled with a study to develop an understanding of disease transmission in these populations using field studies and modeling.

We are involved in efforts to identify species of oysters along the coast of China. To date approximately 16 distinct genetic entities have been identified. While this is not direct aquaculture research, it has shown that oyster culturists in China prefer to use Hong Kong oysters (*C. hongkongensis*) rather than Suminoe oyster (*C. ariakensis*). The reason for this decision is not clear.

We are working on a model of the genetic structure of oysters. The model is at the level of the allele, but tracks a representative selection of chromosomes each with a selection of alleles. When completed this could be utilized to model such things as the probability of generating a reduced genetic complement through inbreeding, how difficult it might be to increase growth by selective breeding, or how aquaculture populations might affect the genetics of nearby natural populations.

In addition, our hatchery has been providing disease resistant lines of oysters to growers in a number of states, and assisting in developing rack and bag oyster culture in New Jersey. Clam investigations include identifying the effects of the disease QPX on aquaculture of five strains of the hard clam (*Mercenaria mercenaria*). Work to date has shown that there are interactions between strains and the disease and latitude. Strains of southern origin (Florida and South Carolina) were more susceptible to the disease than those from Virginia, and the New Jersey and Massachusetts strains were the least susceptible when planted in either Virginia or New Jersey.

We are also evaluating the effects of the alga that causes brown tide (*Aureococcus anophagefferens*) on hard clam larval and juvenile growth and survival, and adult feeding. Laboratory and field experiments have shown that blooms of this alga reduce or stop feeding in clams and scallops. We have been able to model the reduced growth of seed and adults and are currently adding a larval model based on lipid, protein, and carbohydrate levels in the food. Ultimately, the larval model will be added to our existing hard clam numerical population model. Additional work on clams focuses on Manila clam (*Ruditapes philippinarum*) and modeling the combined effects of the dinoflagellate *Alexandrium* and brown ring disease on growth and reproduction in France.

In other studies, we have just begun a small project investigating the potential to use aquaculture to enhance the population of horseshoe crabs (*Limulus polyphemus*) in Delaware Bay, and an industry sponsored effort on the potential for the use of triploid bay scallops (*Argopecten irradians irradians*).

Improvements in Yield of the Pacific Oyster (*Crassostrea gigas*) by Selective Breeding

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Keywords: broodstock, Pacific oyster, yield

Abstract

The Molluscan Broodstock Program (MBP), funded by the U.S. Department of Agriculture, was initiated in 1995. Six cohorts of 50 to 60 families were initially produced from about 600 founder broodstock oysters collected from several naturalized populations on the U.S. West Coast and Canada. Cohorts were evaluated at commercial test sites and top-yielding families from each cohort used as broodstock to produce the next generation.

Two complete selection cycles were completed in 2003 and an average 16.7% improvement in yield was obtained per generation of selection. Improvements in yield were mainly a result of increased survival. Most of the highest yielding families were derived from founder broodstock obtained from Pipestem Inlet, Vancouver Island, Canada. Yields of the highest yielding families were more than three times greater than those of families from nonselected broodstock.

MBP will continue the selection program using a rotational breeding scheme among all lines as well as more focused selection on the Pipestem Inlet line. We will also include shell shape and color in the selection program.

MBP works closely with the West Coast oyster industry in order to provide commercial hatcheries with improved broodstock. We have increased production of improved broodstock oysters by creating inbred lines. These inbred lines can be crossed in hatcheries to produce high-yielding families.

Synthesis of Vitellogenin in the Pacific Oyster (*Crassostrea gigas*)

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Keywords: estrogen receptor, bivalve, oyster, vitellogenin, follicle cells, immunolocalization

Abstract

Vitellogenins are precursors of the major yolk protein (vitellin) in oviparous vertebrates and invertebrates. In mollusks, a full-length cDNA encoding vitellogenin was cloned from the Pacific oyster (*Crassostrea gigas*) and its amino acid sequence was deduced. The deduced primary structure of vitellogenin in the Pacific oyster was shown to be similar to vitellogenins of fish, crustacean, and nematode species, especially in the N-terminal region. Vitellogenin mRNA expression was detected only in the ovary. To determine the distribution of oyster vitellogenin mRNA expression in the ovary, we performed in situ hybridization using DIG-labeled RNA probes. A strong signal was detected in follicle cells. It is concluded that follicle cells are the site of vitellogenin synthesis. In all vertebrate species, estradiol-17ß (E2) is involved in many regulatory steps controlling reproduction. In the Pacific oyster, E2 is detected in the ovary and its content shows a synchronous profile with gonadal maturity.

To investigate estrogen signaling in the vitellogenesis, a cDNA encoding the Pacific oyster estrogen receptor (cgER) was cloned. The phylogenetic analysis indicated that the cgER is an ortholog of the other mollusk estrogen receptors (ERs). Reporter gene assay revealed that cgER is unresponsive to estrogen. This result is similar to those of other mollusk ERs. We examined the localization of cgER in the oyster ovary at the vitellogenic stage using anti cgER peptide antiserum. The immunohistochemical study indicated that cgER was mainly localized in the nuclei of follicle cells—the site of vitellogenin synthesis—in the oyster ovary. This result suggests that cgER could work as a nuclear receptor. Our results will facilitate further research to understand the vitellogenesis in the oyster.

Introduction

In marine bivalves as in most oviparous animals, a large amount of yolk protein (vitellin) is accumulated in oocytes during ovarian maturation. In vertebrates such as fish, amphibians, and birds, yolk protein is synthesized from a precursor, vitellogenin (Vg) produced by the liver, and is transported to the oocytes via the blood circulation system. In bivalve mollusks, a few biochemical studies of yolk proteins have been carried out (Osada et al. 1992, Suzuki et al. 1992). In the oyster, vitellin has been isolated and characterized (Suzuki et al. 1992, Li et al. 1998), but the characterization of Vg has not been reported, and information on bivalve vitellogenesis remains limited. To identify the genes associated with vitellogenesis, we cloned and sequenced more than 100 cDNA fragments using mRNA differential display and found that one of the isolated genes was similar to Vg. We cloned the full-length of the Pacific oyster (*Crassostrea gigas*) Vg cDNA and examined the expression of its mRNA.

Estrogen receptors (ERs) are members of the nuclear receptor superfamily, which have a number of common features, and their proteins can be divided into several distinct domains (Krust et al. 1986). The DNA-binding domain (C domain) and the ligand-binding domain (E domain) are highly conserved between species (Mangelsdorf et al. 1995). The ER is a transcription factor that directly binds to a specific DNA sequence, the estrogen responsive element (ERE, consensus = 5'-AGGTCAXXXT-GACCT-3', Umesono and Evans 1989) and regulates the transactivation of estrogen target genes (Schwabe et al. 1993) such as Vg.

In fish, as in other oviparous vertebrates, it is clearly established that the Vg gene expression is regulated by estradiol-17ß (E2) via ER (Pakdel et al. 1991). In marine bivalves, the presence of steroids was reported in the 1960s (Botticelli et al. 1961).

Estrogens have been detected in blue mussels (*Mytilus edulis*) and softshell clams *(Mya arenaria*) and are considered to have various physiological functions in bivalves (Reis-Henriques et al. 1990, Stefano et al. 2003, Gauthier-Clerc et al. 2006). In the oyster and scallop, E2 has been detected in the ovary, and its contents show a synchronous profile with gonadal maturity on the basis of HPLC, suggesting that E2 plays a role in reproductive events (Matsumoto et al. 1997). In addition, E2 treatment in vivo and in vitro stimulated vitellogenesis in oysters (Li et al. 1998) and scallops (Osada et al. 2003, 2004). These findings suggest that E2 may be involved in the control of vitellogenesis mediated by ER.

In invertebrates, ER has been cloned from mollusks, the California sea slug (*Aplysia californica*) (Thornton et al. 2003), intertidal snail (*Thais clavigera*) (Kajiwara et al. 2006), and common octopus (*Octopus vulgaris*) (Keay et al. 2006), but no ER or other steroid hormone receptors have yet been cloned in bivalves. We isolated the oyster ER homologue, which is highly similar to ERs of other species, and detected the presence of an ER in the nuclei of follicle cells using an antibody against synthetic oyster ER peptide.

Oyster Vg

In order to identify the expressed genes in the ovary, transcripts derived from the ovaries during the spawning season and the spent gonads containing no oocytes were examined by mRNA differential display. During this procedure, we identified a cDNA of approximately 1.2 kb in length. Sequence analysis of the cDNA fragment followed by a database search revealed a similarity to Vg. Subsequently the 5' and 3' ends of this cDNA were obtained by 5' and 3' RACE. The sequence of oyster Vg cDNA is submitted to DDBJ/EMBL/GenBank with the accession number AB084783 (Matsumoto et al. 2003). The cDNA consisted of 5,023 bp and had an ORF (open reading frame) that encoded 1,583 amino acid residues with a predicted molecular mass of 179,191 Da. The deduced amino acid sequence contained a consensus cleavage site, R-X-R-R (Arg^{828} to Arg^{831}), capable of undergoing processing by endoproteases of the subtilisin family (Barr 1991) that have also been reported in Vgs of insects and crustaceans (Chen et al. 1994, Yano et al. 1994, Tsutsui et al. 2000, Okuno et al. 2002).

The overall serine content of the oyster Vg (11.9%) was higher than in the mosquito (10.1%) and the silkworm Vg (9.8%), which possess polyserine clusters. The deduced amino acid sequence contained 56.2% of serine residues in the region between Ser1149 and Ser1180, and this region may be the polyserine domain in Pacific oyster Vg. This protein possessed six potential N-linked glycosylation sites, which is conserved in vertebrate and invertebrate Vgs. The deduced amino acid sequence has 19–35% identity with scallop, nematode, fish, and kuruma prawn Vgs in the N-terminal and the central region. The C-terminal region showed no significant similarity to any of the other reported species. Thus this region appears to possess unique properties compared with Vg of the other known species.

The levels of Vg mRNA in various tissues from female oysters were measured by reverse transcription-mediated PCR. Vg mRNA expression was detected only in the ovary. To determine the distribution of oyster Vg mRNA expression in ovary, we performed in situ hybridization using DIG-labeled RNA probes. A strong signal was detected in the follicle cells using an antisense RNA probe (Figure 1a). In contrast, hybridization with a sense control probe produced no significant signal (Figure 1b). In marine bivalves, autosynthesis of yolk proteins in the ovary has been postulated, based on the morphological evidence (Pipe 1987, Dorange and Le Pennec 1989, Eckelbarger and Davis 1996). In oyster and other bivalve species, the ovarian acinus is a simple

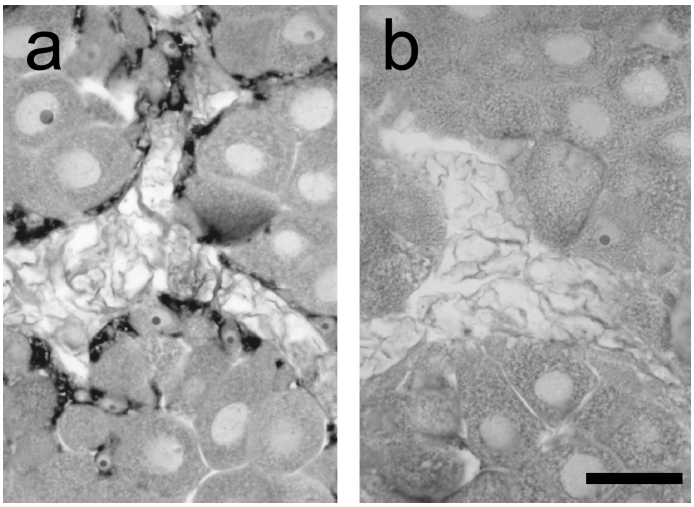

Figure 1. Localization of putative Vg mRNA in the ovary by in situ hybridization. A) Strong expression of putative Vg was seen in the follicle cells with an antisense probe in the ovary. B) Hybridization with a sense probe produced no significant signal in the ovary. Scale bar = 50 µm.

structure containing only developing oocytes and associated follicle cells within a thin germinal epithelium. The function(s) of follicle cells in the bivalve ovary are not well understood, although they are suspected of playing some role in oocyte nutrition. In the present study, Vg mRNA expression was in the follicle cells in the ovary of Pacific oysters. This suggests that yolk synthesis in Pacific oysters may take place in the follicle cells.

Oyster Estrogen Receptor (cgER)

Using the degenerate primers derived from conserved regions within human ERs, we cloned a PCR fragment from oyster ovary. The amino acid sequence of the fragment showed high similarity with those of human ERs. Subsequently the 5' and 3' ends of this cDNA were obtained by 5' and 3' RACE. The cDNA sequence of cgER is deposited as Accession number AB259818 (Matsumoto et al. 2007). It contained an ORF of 1,455 bp encoding a polypeptide of 485 amino acids. The molecular mass of the mature receptor protein, based on its deduced amino acid sequence, is 53.6 kDa. The deduced protein contains eight cysteine residues that constitute two zinc finger structures and P-box (CEGCKA) in the C domain. The position of cysteine residues and other residues around them are conserved in mollusk ERs including cgER.

The amino acid sequence of cgER protein revealed a high identity to mollusk ERs. The phylogenetic analysis indicated that there are three groups of ER: ER-α, ER-β, and mollusk ERs and cgER is most closely related to the other mollusk ERs (Figure 2). This indicates that the cgER we isolated is an ortholog of Aplysia ER, tcER1, and octopus ER. We showed that cgER protein did not activate luciferase expression in the presence of E2 in the mammalian cell-culture system. This is consistent with the results on other mollusk ERs (Thornton et al. 2003, Kajiwara et al. 2006, Keay et al. 2006). The cgER's insensitivity to E2 is unlikely to be due to a lack of ER protein, because the expressions of receptor proteins in U2OS cells were confirmed by immunoblotting analyses.

The cgER that we have isolated was not active in mammalian cells in our reporter assay. However, the cgER mRNA was detected in all tissues tested, with the higher expression in the ovary. In addition, The immunohistochemical study indicated that cgER was mainly localized in the nuclei of follicle cells in the oyster ovary (Figure 3a). The negative control using preimmune rabbit serum instead of antiserum

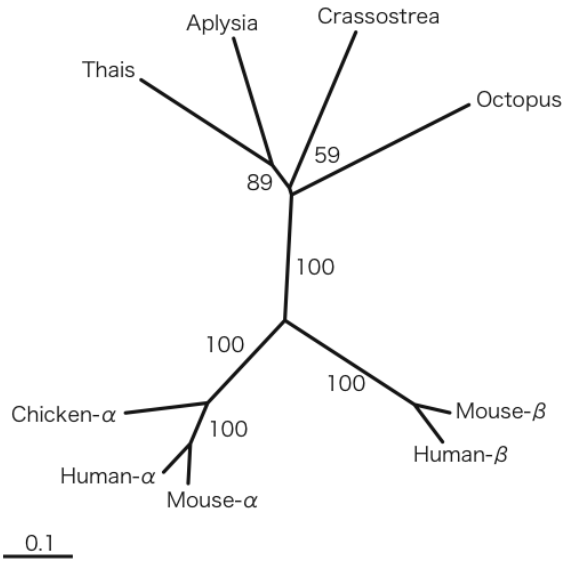

Figure 2. Phylogenetic tree based on amino acid sequences of ERs. The unrooted phylogenetic tree was constructed by the neighbor-joining method after alignment. The sequence data have been submitted to the GenBank database under the following accession numbers: Octopus (DQ533956), Thais (AB077032), Aplysia (AY327135), Human-α (M12674), Chicken-α (X03805), Mouse-α (M38651), Human-ß (AB006590), and Mouse-ß (U81451). Bootstrap values are calculated from 1,000 replicates and expressed as percentages. Scale bar = 0.1 substitutions per site.

showed no immunoreactivity (Figure 3b). In oviparous species, E2 mainly controls vitellogenesis mediated by ER (Wallace 1985). These results imply that cgER could regulate the transactivation of the Vg gene in the nuclei of follicle cells. Moreover, cgER immunoreactivity was also detected in the nuclei of oocytes (Figure 3a), suggesting that estrogens play various roles in regulating reproductive events. Our results will facilitate further functional research to understand the roles of estrogens in the oyster.

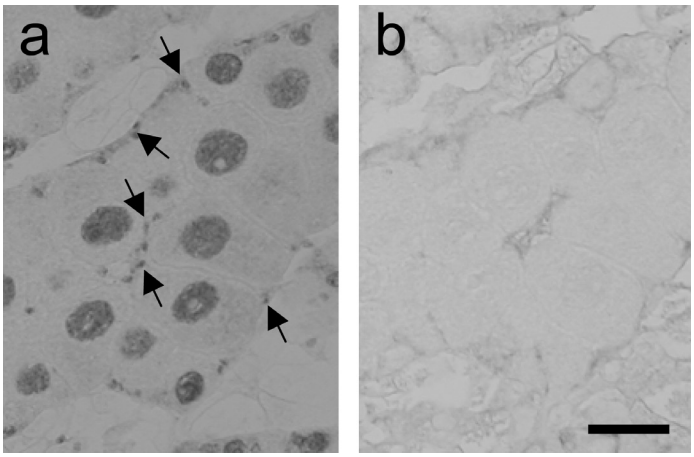

Figure 3. Immunohistochemical staining for ER in ovary of oyster. A) The cgER immunoreactivity was visualized using diaminobenzidine. The cgER immunostaining was detected in the nuclei of follicle cells (arrows) and oocytes (o). B) Preimmune rabbit serum was used as a negative control. Scale bar = 25 µm.

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Disease Diagnostics and Treatment of Hemic Neoplasia in the Softshell Clam (*Mya arenaria*)

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Keywords: hemic neoplasia, leukemia, softshell clam

Abstract

The softshell clam (*Mya arenaria*) has been harvested commercially in the United States and was a reliable source of income for local fisherman. Since the 1980s, the annual harvest has drastically declined, and the loss of seasonal and full-time jobs has been significant in New England and the Chesapeake Bay area. The situation is particularly severe in New Hampshire, where commercial clam digging has been banned since 1951, though recreational harvesting still occurs.

Softshell clam hemic neoplasia (leukemia), one of the six most devastating bivalve diseases, is one of very few marine diseases that has been characterized at the molecular level. Yet almost nothing is known about the environmental triggers of this disease. Leukemic clam hemocytes express a highly conserved homolog for human p53 protein that is rendered nonfunctional by sequestration in the cytoplasm by mortalin when the latter protein is overexpressed.

Treatment of leukemic clam hemocytes with etoposide overcomes mortalin-based cytoplasmic sequestration and promotes translocation of clam p53 protein from the cytoplasm to the nucleus. Cytotoxicity, DNA damage, and apoptosis of leukemic clam hemocytes follow. Since disease diagnosis, treatment, and prevention are among the most significant variables in aquaculture, these results will aid in generating and distributing information and finally developing treatments to potentially cure hemic neoplasia.

The Japanese Monitoring System on Shellfish Toxins and Development of Liquid Chromatography–Mass Spectrometry of Lipophilic Toxins Associated with Diarrhetic Shellfish Poisoning for Improvement of the Monitoring System

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Keywords: shellfish, diarrhetic shellfish poisoning, toxins

Abstract

Diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP) cause serious quality assurance problems for bivalve industries in Japan. The toxicities of cultured bivalves are periodically monitored by mouse bioassay (MBA) at selected monitoring stations. When the toxicity of the bivalves exceeds the quarantine levels (0.05 MU/g wet weight for DSP, 4.0 MU/g wet weight for PSP), harvesting ceases. Shellfish harvesting is not resumed until testing indicates that the toxicity of the bivalves is below the quarantine levels on three successive weeks. Since establishment of the monitoring system for shellfish toxins, no human poisonings due to contaminated bivalves distributed on commercial markets have been reported. In addition to the direct monitoring of bivalve toxicity, the occurrence and abundance of the planktonic dinoflagellates which cause DSP (*Dinophysis* spp.) and PSP (*Alexandrium* spp.) are also monitored. This provides an early warning and risk assessment of the chances of toxicity developing in the bivalves.

Recently, we developed liquid chromatography–mass spectrometry (LC-MS) of lipophilic toxins in bivalves associated with DSP. Using a C8-silica reversed phase column and a mobile phase of aqueous acetonitrile containing 2 mM ammonium formate and 50 mM formic acid, okadaic acid (OA), dinophysistoxin-1 (DTX1), 7-O-palmitoyldinophysistoxin-1 (DTX3), pectenotoxin-1 (PTX1), pectenotoxin-2 (PTX2), pectenotoxin-6 (PTX6), pectenotoxin-2 seco-acid (PTX2sa), yessotoxin (YTX), and 45-hydroxyyessotoxin (45-OHYTX) in bivalves were quantified by LC-MS. Approximately 200 bivalve samples collected from various production areas in Japan were analyzed by LC-MS. Comparison of the quantitative results obtained for bivalve samples between LC-MS and MBA showed that many samples assayed as being below the quarantine level (0.05 MU/g whole tissues) by MBA were quantified as exceeding the quarantine level by LC-MS. This difference was due to YTXs, which are poorly detected by MBA. The results indicate that LC-MS is a better method than MBA in terms of sensitivity and accuracy to quantify known lipophilic toxins including YTXs.

Introduction

Diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP) cause serious quality assurance problems for the bivalve industries in Japan; however, no human poisonings due to contaminated bivalves with DSP or PSP toxins distributed on commercial markets have been reported since establishment of the monitoring system for shellfish toxins in 1979. This indicates that the monitoring system is successful. The present Japanese shellfish toxin monitoring system is described in this report.

DSP is a condition resulting from ingestion of shellfish contaminated with lipophilic toxins (Yasumoto et al. 1978). Three lipophilic toxin groups (Figure 1) differing in basic structures have been associated with DSP (Yasumoto et al 1985, Yasumoto and Murata 1993). The most important toxins responsible for diarrhea are okadaic acid (OA) analogues. These toxins have been shown to be potent phosphatase inhibitors (Bialojan and Takai 1988), a property which can cause inflammation of the intestinal tract and diarrhea (Terao et al. 1986). OA analogues have also been shown to have tumor-promoting activity

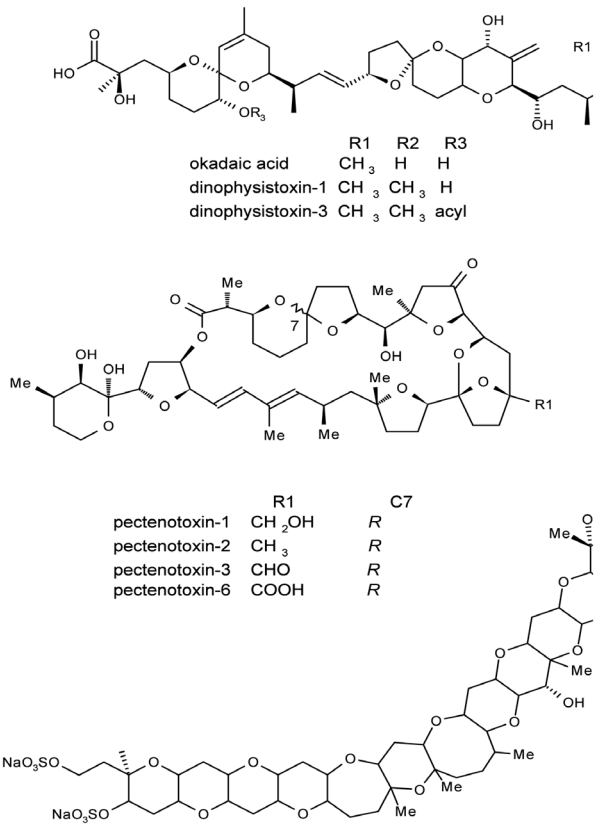

Figure 1. Structure of typical lipophilic toxins found in Japanese bivalves. Okadaic acid is a rare toxin in Japanese bivalves.

(Fujiki et al. 1988). In contrast to OA analogues, it has been suggested that the risk assessment of the other two toxin groups for mammals may be relatively low compared to OA analogues when they are injected orally (Ogino et al. 1997, Aune et al. 2002, Miles et al. 2004).

European authorities have set a higher control limit for the yessotoxins (YTXs) than OA analogues. The different functional toxicities and control limits for the three toxin groups require a method for shellfish testing that can quantify each toxin group separately. Recently, we developed liquid chromatography–mass spectrometry (LC-MS) of lipophilic toxins in bivalves associated with DSP (Suzuki et al. 2005).

In 2003 in Japan, harvesting of scallops (*Patinopecten yessoensis*) and mussels (*Mytilus galloprovincialis* and *M. coruscus*) ceased due to contamination with lipophilic toxins. During that event, approximately 200 bivalve samples were collected from production areas and lipophilic toxins in the bivalves were analyzed by our recently developed LC-MS method (Suzuki et al. 2005). A comparison of the quantitative results obtained for these bivalve samples between LC-MS and mouse bioassay (MBA) was carried out to investigate the applicability of this LC-MS method for routine monitoring of lipophilic toxins in Japanese bivalves.

Materials and Methods

Samples. Approximately 200 bivalve samples were collected from six production areas during a DSP outbreak from May to September 2003 (Figure 2). Scallops were collected in several production sites in Hokkaido (5 sites), Aomori (10 sites), Iwate (8 sites), and Miyagi (12 sites). Mediterranean mussels (*M. galloprovincialis*) were collected in Aomori (2 sites), Iwate (1 site), and Miyagi (3 sites). Mussels (*M. coruscus*) were collected in Akita (1 site), Yamagata (2 sites), and Niigata (1 site).

Hepatopancreas (HP) samples from more than 10 individuals collected from the same monitoring site on the same date were combined and homogenized. An aliquot of the homogenate was immediately analyzed by the official Japanese testing method for DSP by MBA (Japanese Ministry of Health and Welfare 1981). The remainder of the homogenate was kept frozen at –30°C until used for extraction of toxins for LC-MS.

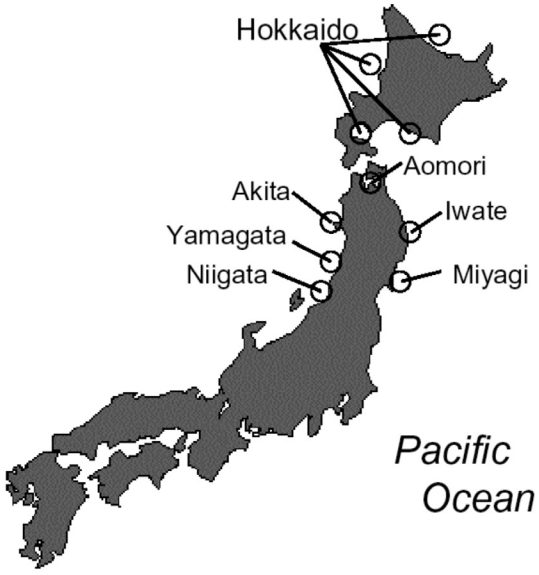

Figure 2. Production area of bivalves where harvesting was prohibited due to DSP events in 2003.

Extraction of toxins for LC-MS. The remainder (ca. 5 g) of the homogenates tested by MBA was extracted with nine times volume of 90% methanol and the extracts were centrifuged at 3,000 rpm for 5 min. An aliquot of each supernatant was passed through a 0.5 μm filter (Millipore Corp., Billerica, Massachusetts) for direct injection into the LC-MS.

LC-MS. A Hewlett-Packard Model 1050 series liquid chromatograph was coupled to a Finnigan MAT SSQ-7000 instrument (San Jose, California) equipped with an atmospheric pressure electrospray ionization (ESI) interface. Separations were performed on Quicksilver cartridge columns (50 mm x 2.1 mm internal diameter) packed with 3 μm Hypersil-BDS-C8 (Keystone Scientific, Bellefonte, Pennsylvania) maintained at 35°C. Eluent A was water and B was acetonitrile/water (95:5), both containing 2 mM ammonium formate and 50 mM formic acid (Suzuki et al. 2005). Gradient elution from 20% to 100% B was performed over 10 min, then held at 100% B for 15 min for LC separation. Re-equilibration was taken for 13 min with 20% B. The flow rate was 0.2 mL/min and the injection volume was 5 μL.

Using a divert valve, LC eluent between 9 and 15 min and between 19 and 22 min were introduced into an ESI interface. The other eluent fractions were discarded to reduce contamination of the ion source and MS detector by bivalve matrices. Toxins were detected using selected ion monitoring (SIM) of negatively charged ions for [M-H]- of OA (m/z 803.5), DTX1 (m/z 817.5), DTX3 (m/z 1055.7), PTX2 seco acid (m/z 875.5), and for [M+HCOOH-H]- of PTX1 (m/z 919.5), PTX2 (m/z 903.5), PTX6 (m/z 933.5), and for [M-2Na+H]- of YTX (m/z 1141.5), 45-OHYTX (m/z 1157.5). The SIM scan width for each toxin was 0.3 U (unit mass). The voltage on the ESI interface was maintained at approximately 4.5 kV. The temperature of the heated capillary was 200°C. High-purity nitrogen was used as a sheath gas (nebulizer gas) at an operating pressure of 480 kPa and auxiliary gas flow at 5 units.

MBA. MBA was carried out according to the official testing method in Japan (Japanese Ministry of Health and Welfare 1981). Whole tissues and separated HP of each sample were weighed and the weight percentage (w/w%) of the HP was calculated. The homogenate of the HP was extracted with acetone three times. After removal of the acetone by evaporation, the aqueous suspension was extracted with diethyl ether. The ether solution was back-washed with distilled water and evaporated. The residue was suspended in 1.0 mL of 1% Tween-60 saline solution for each 20 g whole tissue equivalent. An aliquot (1.0 mL) of this solution corresponding to 20 g of the whole tissues was injected intraperitoneally into each of three mice. When more than two mice die within the 24 hour observation period, the toxicity of the extracts is defined as greater than 1 mouse unit (MU) and the whole contents are defined as more than 0.05 MU/g whole tissues, the current Japanese quarantine level.

Comparison of mouse toxicities obtained by LC-MS and MBA. Total mouse toxicities of samples quantified by LC-MS were compared to the MBA results. The conversion factors to the mouse toxicities of OA, DTX1, DTX3 (7-O-palDTX1), PTX1, PTX2, PTX6, YTX, and 45-OHYTX are 4.0, 3.2, 5.0, 5.0, 4.6, 10, 2.0, and 10 μg/MU, respectively (Yasumoto et al. 1995, Satyake et al. 1996). Mouse toxicities obtained by MBA are shown between minimum and maximum toxicities according to the dilution factors of bivalve extracts (e.g., 0.05–0.1 MU/g). Average values obtained by the MBA were used for the comparison to the LC-MS results.

Results and Discussion

Figure 3 shows the SIM LC-MS chromatogram obtained from a mixture of standard toxins. A clean chromatogram was obtained, showing discrete peaks for all toxins. Although YTX, PTX1, and PTX6 coeluted, this was not problematic for the quantification of these toxins due to the SIM separation.

Figure 4 shows a comparison of mouse toxicities quantified by LC-MS and MBA for the 196 scallop and mussel samples. Ninety-six samples were quantified by both LC-MS and MBA as being below the quarantine level. Thirty-three samples were above the quarantine level by both LC-MS and MBA. Six samples tested as exceeding the quarantine level by MBA were quantified by LC-MS as being below the quarantine level. As the toxicities of these six samples were between 0.05 and 0.1 MU/g by MBA, which is the lowest level measurable by the MBA, false positives due to free fatty acids in the samples are suspected (Tagaki et al. 1984, Lawrence et al. 1994, Suzuki et al. 1996). Sixty-one samples as exceeding the quarantine level by LC-MS were quantified by MBA as being below the quarantine level.

The Japanese MBA protocol includes a partition of bivalve extracts between water and diethyl ether to remove water soluble saxitoxin analogues.

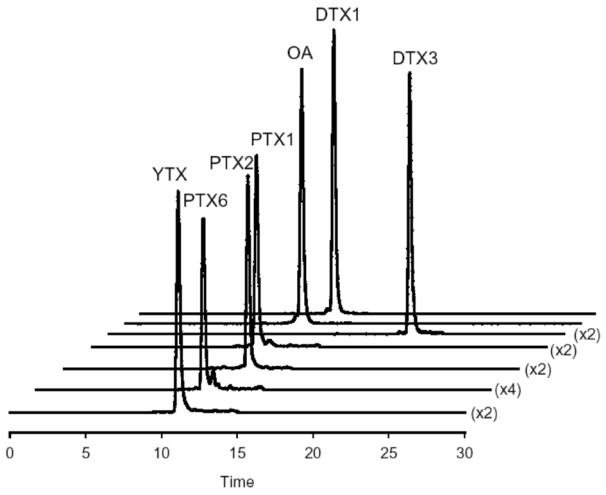

Figure 3. SIM LC-MS chromatogram for a standard mixture (100 ng/mL) of lipophilic toxins. Toxins were detected using ESI- and SIM for [M-H]- of OA (m/z 803.5), DTX1 (m/z 817.5), DTX3 (m/z 1055.7), and for [M+HCOOH-H]- of PTX1 (m/z 919.5), PTX2 (m/z 903.5), PTX6 (m/z 933.5), and for [M-2Na+H]- of YTX (m/z 1141.5).

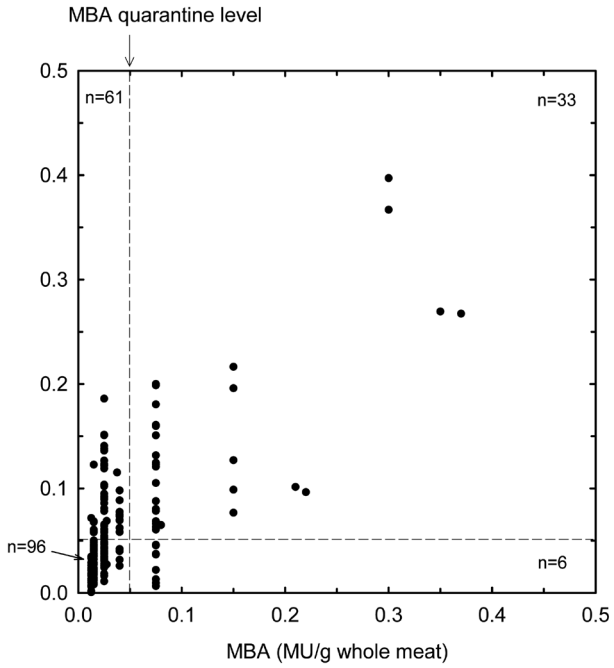

Figure 4. Comparison of total mouse toxicities obtained by LC-MS and MBA for the same scallop and mussel samples (n = sample numbers in the square).

It is reported that a major portion of the YTXs are partitioned into the aqueous fraction (Ramstad et al. 2001) and are therefore not measured by MBA. The discrepancies are probably caused by YTXs that are poorly detected by MBA. These results indicate that the LC-MS is definitely a better method than MBA, in terms of the sensitivity and the accuracy, to quantify known lipophilic toxins including YTX. It is noteworthy that the numbers of samples exceeding the quarantine level increase if MBA is replaced with LC-MS in routine monitoring of toxins in Japanese bivalves (Figure 4). This impact will be huge to scallop industries because a dominant toxin in scallops is YTX.

The risk assessment of YTX to mammals is thought to be lower than the other toxin groups (Ogino et al. 1997, Aune et al. 2002), and the European Union (EU) recently set a higher control limit for the YTXs than OA analogues. When the LC-MS is applied to the toxin monitoring in Japanese bivalves, an adequate control limit for the YTXs as proposed by the EU should be considered to decrease economic impacts to scallop industries.

A simple and rapid LC-MS method without time-consuming cleanup of samples for the quantification of lipophilic toxins in Japanese bivalves was proposed in the present study. Approximately 200 bivalve samples collected in various production areas in Japan have been tested by the method. Comparisons between LC-MS and MBA results for these samples demonstrate that the present LC-MS method is applicable to routine monitoring of lipophilic toxins in Japanese bivalves. When the different control limit for each toxin group is required as proposed by EU, the LC-MS method is a suitable alternative to MBA.

Acknowledgments

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Bivalves as Biofilter: Efficient, Profitable, and Tasty as Well!

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Keywords: bivalves, commercial culture, polytrophic

Abstract

Bivalve production has increased dramatically worldwide in the last three decades. According to the Food and Agriculture Organization of the United Nations, fishery and aquaculture production increased fourfold, reaching almost 12 million metric tons in 2000.

The choice of a particular bivalve species for commercial culture involves considerations such as fast growth rate, low food conversion ratio, resistance to pests, and tolerance to a wide range of environmental conditions. The technology for its reproduction and culture should be straightforward and user-friendly for the growers, and the mollusk should meet market demands with respect to appearance, taste, smell, texture, processing considerations, and market behavior. Profitability depends on yield per unit of area, grow-out time, harvest frequency, farm-gate price, and the cost of waste treatment.

Bivalves can be cultured by sea ranching or in land-based facilities. Sea ranching usually involves bottom culture, rack culture, or suspended culture. In the open sea, the bivalves are vulnerable to weather conditions, predation, red tide, and poaching. Because they are generally cultured close to shore, they are also subjected to urban pollution. As filter feeders, bivalves can accumulate high concentrations of toxic and pathogenic material, which can affect the economics of bivalve culture. Land-based facilities, wherein bivalves are cultured in ponds, tanks, or indoor hatcheries and nurseries, are safer because the quality of the incoming water can be controlled. Bivalve monoculture in such systems is of doubtful profitability due to the high costs of construction, the need for highly trained technicians, water pumping, food (microalgae) supply, and waste control.

Polytrophic culture in integrated systems holds much greater economic promise because it saves resources such as feed and water purification, diversifies the farm's market products, allows intensification and optimization, and is environmentally friendly. In this system, fishpond effluent, rich in dissolved nutrients, drains through an earthen sedimentation pond. The dissolved nutrients, coupled with the high incidence of solar radiation, generate an extremely high phytoplankton production, mainly of diatoms, that supports the growth of bivalves on the bottom of the sedimentation pond.

This presentation summarized state-of-the-art research and development in the use of bivalves as biofilters and as safe, valuable by-products in land-based integrated aquaculture systems, and thus as a valid alternative to open sea monoculture.

Considerations on the Fine-scale Topography in Sand Flats, Habitat Heterogeneity, and Refugia for Clams

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Keywords: clams, topography, habitat, refugia

Abstract

We discuss the use of the fine-scale topography on sand flats as an explanatory variable for spatial patterns of clams. Topography on a sand flat is a fingerprint of sediment-water interactions, although its interpretation has not yet been systematically confirmed by fluid mechanics. We attempted to derive some implications about the effect of hydrodynamics on clam populations from the correspondence between topography and spatial patterns of clams. Individual numbers of two common clam species, Manila clams (*Ruditapes philippinarum*) and surf clams (*Mactra veneriformis*) were compared. For the mean log transformed individual numbers of Manila clams, effects of position between sandbars and within a sandbar were significant, while the effect of ripples and the interaction term were not. Within the middle sandbar, the mean individual number in the trough was greater than in other positions. Between three crests of sandbars, the mean decreased seaward.

Individual numbers of surf clams did not show significant variations within the middle sandbar. In the test for the effects of position between sandbars and ripples, the interaction term was significant. Vertical profiles of ripples showed that ripples on the trough of the middle sandbar were concave upwards having rounded tops. Ripples on other positions in the same sandbar were convex and slightly skewed seaward. On crests of sandbars, ripples in the landward sandbar were concave upwards while ripples in other sandbars were convex and slightly skewed. The small abundance of Manila clams corresponded with occurrence of convex ripples that suggest unidirectional currents mixed with wave actions.

Introduction

Any element of habitat structure that diminishes the likelihood that an organism on or near it will die is a spatial refuge for that organism, and is crucial to understanding the interactions between the mortality source and the distribution and abundance of refugia (Woodin 1978). Spatial patterns of benthic organisms on sand flats are a result of physical and biotic processes. Recruitment of benthic organisms and their larvae through bedload transport has crucial roles in recovery processes of disturbed communities. Hydrodynamic processes have a significant effect on larval settlement (Jonsson et al. 1991, Butman and Grassle 1992, Grassle et al. 1992a, 1992b, Snelgrove et al. 1993, Roegner et al. 1995, Snelgrove et al. 1998). Adults and juveniles are also moved through passive transport and active drifting (Sigurdsson et al. 1976, Beukema and Devlas 1989, Hiddink et al. 2002).

To establish sound strategies for management of aquaculture and fishing grounds on tidal flats, it is important to understand the relationship between spatial heterogeneity of hydrodynamic conditions and the distribution of refugia that have a significant effect on growth and degradation of clam aggregations. In some cases, refugia emerge after alternation of the sediment surface structure. For example, it was suggested that shell debris has a role in increasing and maintaining biodiversity by reducing disturbance regimes (Hewitt et al. 2005). Dense tube beds of polychaetes persist for several years and offer a refuge wherein infaunal diversities are greater than those of more hydrodynamically rigorous areas (Bolam and Fernandes 2002). Local aggregations of Manila clams (*Ruditapes philippinarum*) were formed on a tidal flat in Kumamoto, Japan, after artificial capping with coarse sand.

Indices that identify location and scale of refugia on sand flats, if developed, would be helpful in the management of fishing grounds on tidal flats. Grain size distribution can be used as a marker for changes in the physical sedimentary conditions for benthic organisms on the sand flat because grain size is an important factor influencing benthic communities and is often correlated with other sediment properties (Zajac and Whitlatch 2003). On the other hand, there are diverse topographic patterns with various spatial scales on sand flats. Roughness of the bottom produced by grain size distribution of the substrate is the smallest structure, on the order of 0.1–1 mm. Ripples and dunes on the order of 1–10 cm are often seen on the sandy bottom. Multiple sandbars, ridges, and runnels are large structures on the order of 10 m developing on sand flats exposed to strong wave forces.

Besides spatial structures formed by physical processes, biological and human activities modify the surface structure of sand flats (e.g., mounds, tubes, mats, pits by predation, digging, and human footprints). These structures produce shaded and exposed sides to wave and current forces, and this differentiation in the strength of physical disturbance may be an important factor that creates habitat heterogeneity on sand flats. Multiple sandbars are apparent and their shapes are easily captured using topographical survey techniques.

Roughness of the sand surface can be estimated from the grain size distribution of the sediment. It is difficult to capture the real shape of ripples in the field. Wave length and amplitude of ripples can be obtained with replicate measurements in the field, but their detailed shapes cannot be included in the data.

Sand flat topography provides a fingerprint of sediment-water interactions, although its interpretation has not yet been systematically confirmed by fluid mechanics. In tentative bed state definitions, wave ripples have concave-upwards two-sided (or more) slip faces, and there is vortex shedding from the tops when sharp crested with top angles approximating the angle of repose, whereas current dunes have approximately triangular cross sections but often convex-upwards sides, lee sides commonly at the angle of repose and vortex shedding (Kleinhans 2005). There are also mixed-flow ripples; bedforms with characteristics of both wave and current bedforms. Thus bedforms provide important information on the physical state in the benthic boundary layer, relative contribution of waves and currents, and disturbance for the benthic community.

In this study, we discuss the use of the fine-scale topography on sand flats as an explanatory variable for spatial patterns of clams. Previous literature has correlated benthic assemblages with physicochemical factors. Many of them included sediment characteristics and water qualities, but less attention has been paid to hydrodynamics, whereas they are critical in the settlement and survival of young benthic organisms. We tried to derive some implications about the effect of hydrodynamics on clam populations from the correspondence between the topography and the spatial pattern of two common clams, Manila clams and surf clams (*Mactra veneriformis*).

Materials and methods

Matsunase Beach is an intertidal sand flat at the eastern margin in the mouth of the Kushida River (lat 34°36′N, long 13°35′E), Matsusaka City, Japan (Figure 1). The catchment area of the river is 436 km^2. The sand flat is covered with various sizes of substrate material from fine sand to cobbles. In winter, the shallow subtidal area is filled with thousands of bamboo rods with nets extended between them for mariculture. Seaweeds grow densely in the subtidal zone and in deep troughs of sandbars that emerge at low water during spring tides. Bivalves predominate the macrofauna on the sand flat. Other taxonomic groups are less abundant than bivalves. Major bivalve species are Manila clams, surf clams, and horse mussel (*Musculista senhousia*).

We established sampling stations along a line transect from the coastal sand dune to the point of low water during spring tide. Location of the

line transect was determined to make elevational changes over sandbars along it as contrasting as possible. On 6 September 2006, land elevations at haphazardly chosen points scattered along the line transect were measured with a VRS-RTK receiver (Trimble 5800, Trimble Navigation Ltd., Sunnyvale, California). From the results of this survey, the land shape of the sand flat was described in detail and it was shown that the upper intertidal zone was a gradual slope while multiple sandbars developed in the lower intertidal zone (Figure 2). Wave length of the sandbar was equal to 30–40 m, and amplitude was from 20 to 60 cm. Sampling was conducted on 7 and 8 September 2006 at stations established on the crests of three discrete sandbars (landward, middle, and seaward sandbar) and three other locations (trough, onshore side slope, and offshore side slope) on the middle sandbar. Two additional stations (5 and 6) were established on the foreshore gradual slope (Figure 2).

At each station, photographs of the sand surface enclosed by a 60x60 cm^2 unit made from plastic pipes placed on the sand surface were taken using two digital cameras (Coolpix 7900, Nikon Co.). At the same time, a compass and six circular markers were included in the pictures to be used in the calibration process. The cameras were mounted in parallel and looked downward under a tripod. The distance between the centers of the camera lenses was 23 cm. The height of the lenses from the sand surface was approximately 85 cm. After taking pictures, 6 sediment samples were collected using 96x46x46 mm rectangular stainless quadrats haphazardly placed in the square unit. Three replicates were obtained from the crests of sand ripples and another three replicates from troughs. Sediment samples were sieved through a 1.0 mm mesh screen, and materials retained on it were fixed in a 5% formaldehyde solution.

Fixed specimens were rinsed with water in the laboratory and organisms were sorted under a binocular microscope. Shell lengths of specimens of Manila clams and surf clams were determined, and the individuals were placed in 0.25 mm intervals and counted. Shell length distribution was evaluated using the Solver function in Microsoft Excel 2000 to fit the summation of normal distribution curves using a published cohort-analysis program (Aizawa and Takiguchi 1999). In this program, fitness of the model was evaluated employing the maximum likelihood method. Intersections of normal distribution curves were recognized as the border of different

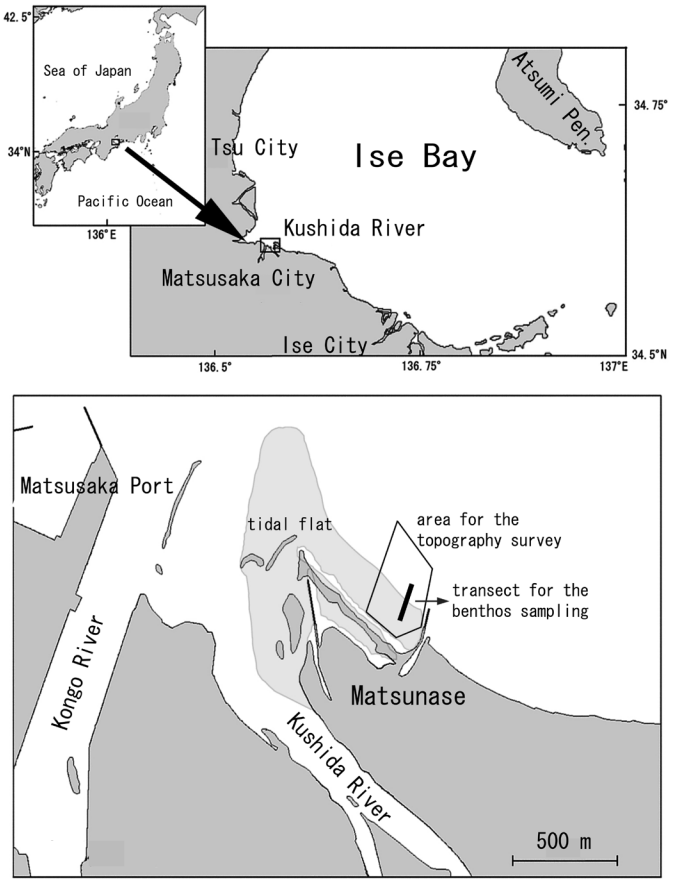

Figure 1. Locality of Matsunase Beach tidal flat and the position of the line transect for this study.

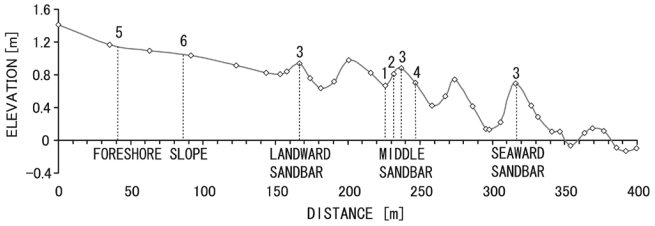

Figure 2. Vertical profile of the line transect and positions of sampling stations.

size groups, and the individual number of separated size groups was counted. In order to focus on the relationship between the spatial pattern of young clams and the latest topography, older individuals were eliminated from further analyses.

The numbers of clams in each replicate unit were log-transformed. Two-way analysis of variance (ANOVA) was calculated using the "aov" function in R, a high level language and an environment for data analysis and graphics distributed by the Comprehensive R Archive Network. The null hypotheses tested were:

- There was no difference in individual numbers between troughs and crests of sand ripples
- There was no variation in individual numbers between locations of sandbars including the two extra stations in the foreshore slope
- There was no variation in individual numbers between positions in the middle sandbar.

To test these hypotheses, we employed several two-way ANOVA models. One model was composed of two orthogonal fixed factors, the effect of ripple (R, n = 2) crossed with the effect of location of sandbars (B, n = 5). The other model was composed of two orthogonal fixed factors, the effect of ripple (R, n = 2) crossed with the effect of position within the middle sandbar (P, n = 4). Homogeneity of variance between sampling stations was tested with Cochran's test for variance outliers (Kanji 2006). Post hoc multiple comparisons were performed to detect subgroups between sampling stations using Tukey HSD.

The three-dimensional shape of the sand surface enclosed with the square unit in the field was visualized from several stereo pictures (a stereo pair) with image analysis software (PI-3000 version 3.0, Topcon Co., Tokyo, Japan). This procedure involved selecting six circular targets manually in each of the images to determine the initial estimates of locations, and the process was then completed by the automatic estimation of all remaining locations. Locations were estimated from the similarity of pixel patterns, parallax in a stereo pair, and the calibration file for adjusting the lens distortion. The calibration file was prepared using PI-Calib software (Topcon Co.). The shape of the sand surface was visualized as a triangulated irregular network (TIN) produced in 5 mm grids. Inclination of the sand surface against the camera angle was removed by subtracting the height of the regressed plain from the corresponding apex of TIN. Then the vertical profile of ripples was sectioned along an assigned line rectangular to the crest line of ripples.

Results

The cohort analyses revealed that there were several size groups in Manila clams and surf clams (Figure 3). Individual numbers of Manila clams smaller than 17 mm and surf clams smaller than 5 mm in shell length were enumerated. The border for Manila clams, 17 mm in shell length, was adopted to eliminate a few large individuals. Although it is likely that there are two cohorts in Manila clams smaller than 17 mm in shell length (shown as S and M in Figure 3), they were pooled in further analyses because the border between them was very narrow and the distinction was not clear. The distinction between the smaller two cohorts of surf clam was not clear (shown as S and M in Figure 3), but we estimated that there were at least two cohorts because the size distribution would be strongly skewed to

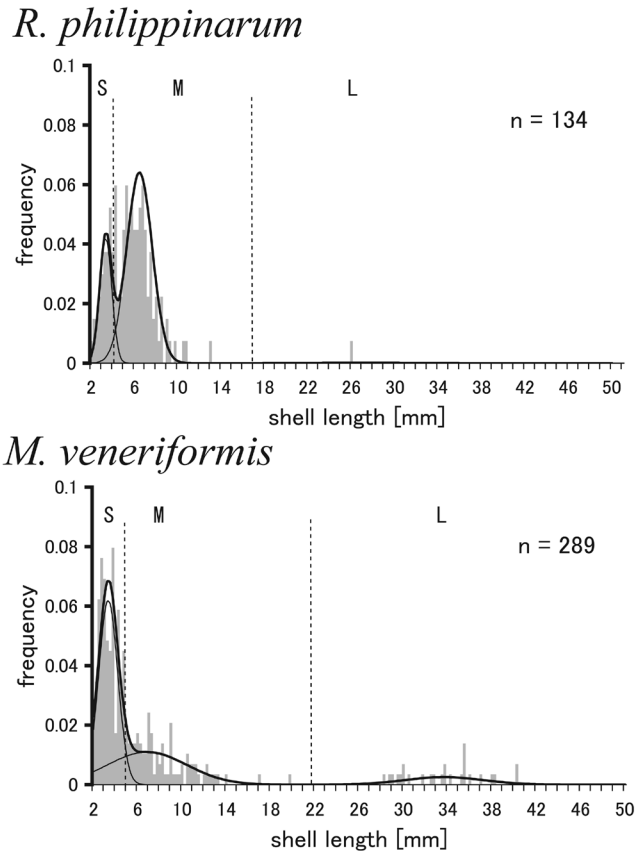

Figure 3. Shell length distributions of Manila clams (*Ruditapes philippinarum*), above, and surf clams (*Mactra veneriformis*), below. Curves fitted to the shell length histograms are the results of cohort analyses.

Table 1. Results of two-way ANOVAs on log-transformed individual numbers of Manila clams (*Ruditapes philippinarum*), shell length < 17 mm, and surf clams (*Mactra veneriformis*), shell length < 5 mm. Effects of spatial positions within the middle sandbar (P), locations between crests of three discrete sandbars and two stations in the foreshore slope (B) and the difference between troughs and crest of sand ripples (R) were included in the model as fixed factors.

	Df	Sum sq	Mean sq	F value	Pr(>F)	Significance
R. Philippinarum						
P	3	3.188	1.063	23.479	0.000	***
R	1	0.003	0.003	0.071	0.793	ns
P x R	3	0.016	0.005	0.120	0.947	ns
Residuals	16	0.724	0.045			
M. veneriformis						
P	3	0.186	0.062	1.298	0.309	ns
R	1	0.016	0.016	0.332	0.572	ns
P x R	3	0.078	0.026	0.542	0.660	ns
Residuals	16	0.764	0.048			
R. philippinarum						
B	4	3.328	0.832	34.208	0.000	***
R	1	0.000	0.000	0.004	0.949	ns
B x R	4	0.016	0.020	0.822	0.527	ns
Residuals	20	0.486	0.024			
M. veneriformis						
B	4	1.327	0.332	7.855	0.001	***
R	1	0.000	0.000	0.000	0.991	ns
B x R	4	0.562	0.141	3.327	0.030	*
Residuals	20	0.845	0.042			

the right if regarded as single cohort. In surf clams, there was another size group of large individuals, larger than 22 mm in shell length, that was eliminated in the following analyses.

Two-way ANOVA to test the effects of ripples (R) and positions within the middle sandbar (P) showed that, for Manila clams, the effect of positions was strongly significant ($F = 23.479$, $p < 0.001$) whereas the effect of ripples was not significant (Table 1). Neither effect was significant in the case of surf clams. Tests on the effects of ripples and sandbars (including the two extra stations in the foreshore slope) revealed that the effect of sandbars was strongly significant for Manila clams ($F = 34.208$, $p < 0.001$) and surf clams ($F = 7.855$, $p < 0.001$), and interaction between ripples and sandbars was significant in the case of surf clams ($F = 3.327$, $p < 0.05$). Cochran's test for variance outliers found that the null hypothesis about homogeneity of variance presupposed in ANOVA was retained in all tests above ($p > 0.05$).

In multiple comparisons, replicate samples from troughs and crests of ripples were not separated if the effect of ripples and the interaction term were not significant in ANOVAs. Tukey HSD revealed that the mean log-transformed individual number of Manila clams in the trough of the middle sandbar was significantly greater than those of other positions (Figure 4). Subgroups detected were shown alphabetically. Surf clams did not show significant variations in the mean log-transformed individual numbers among four positions in the middle sandbar. Multiple comparisons between sandbar crests, including the two extra stations in the foreshore slope, revealed that the mean log-transformed individual number of Manila clams in the landward

sandbar was prominently greater than those in other stations. Mean log-transformed individual numbers of surf clams were overlapped among five stations.

The interaction term B×R was significant in ANOVA though the difference between troughs and crests of ripples in the same unit was not significant for all stations. As an example, a three-dimensional image and the contour plot of sand ripples on the crest of the middle sandbar were superimposed in Figure 5. Vertical profiles of ripples showed that ripples on the trough of the middle sandbar were concave upward having rounded tops (Figure 6). Ripples of this type have wide and relatively flat troughs. Ripples at other positions on the same sandbar were convex upward and slightly skewed seaward having flat tops. Those had narrow troughs compared to ripples that concave upward. Referring to the tentative bed state definition in Kleinhans (2005), the former ripples were classified as wave ripples while the latter were classified as mixed-flow ripples.

Wave length of ripples in the trough, onshore slope, crest, and offshore slope of the middle sandbar were approximately 12, 10, 8, and 8 cm, respectively, and heights of the ripples were approximately 2.0, 1.0, 1.0, and 0.7 cm, respectively. There were overlaps of ripples having different phases on the onshore and the offshore slopes. Along the crests of sandbars, ripples on the landward sandbar were rather concave upward while ripples on other sandbars were convex upward (Figure 7). Ripples were slightly skewed seaward.

Wave lengths of ripples on the foreshore slope and the crest of the landward sandbar were approximately 11, 12, and 9 cm, respectively, and heights of ripples were approximately 1.0, 1.3, and 1.3, respectively. Ripples on the crest of the seaward sandbar had very flat shapes with height of only about 0.7 cm, and wave lengths were irregular, ranging from 7 to 9 cm. From these data, steepness of ripples as defined by height/wave length could be calculated. Steepness of ripples in the trough, onshore slope, crest, and offshore slope of the middle sandbar were 0.17, 0.10, 0.13, and 0.09, respectively. Steepness of ripples in the foreshore slope and the crest of the landward sandbar were 0.09, 0.11 and 0.14, respectively. Steepness of ripples on the crest of the seaward sandbar ranged from 0.08 to 0.10.

Discussion

In bottom culture systems, large clams survive better than small clams (Gosling 2003). Retention

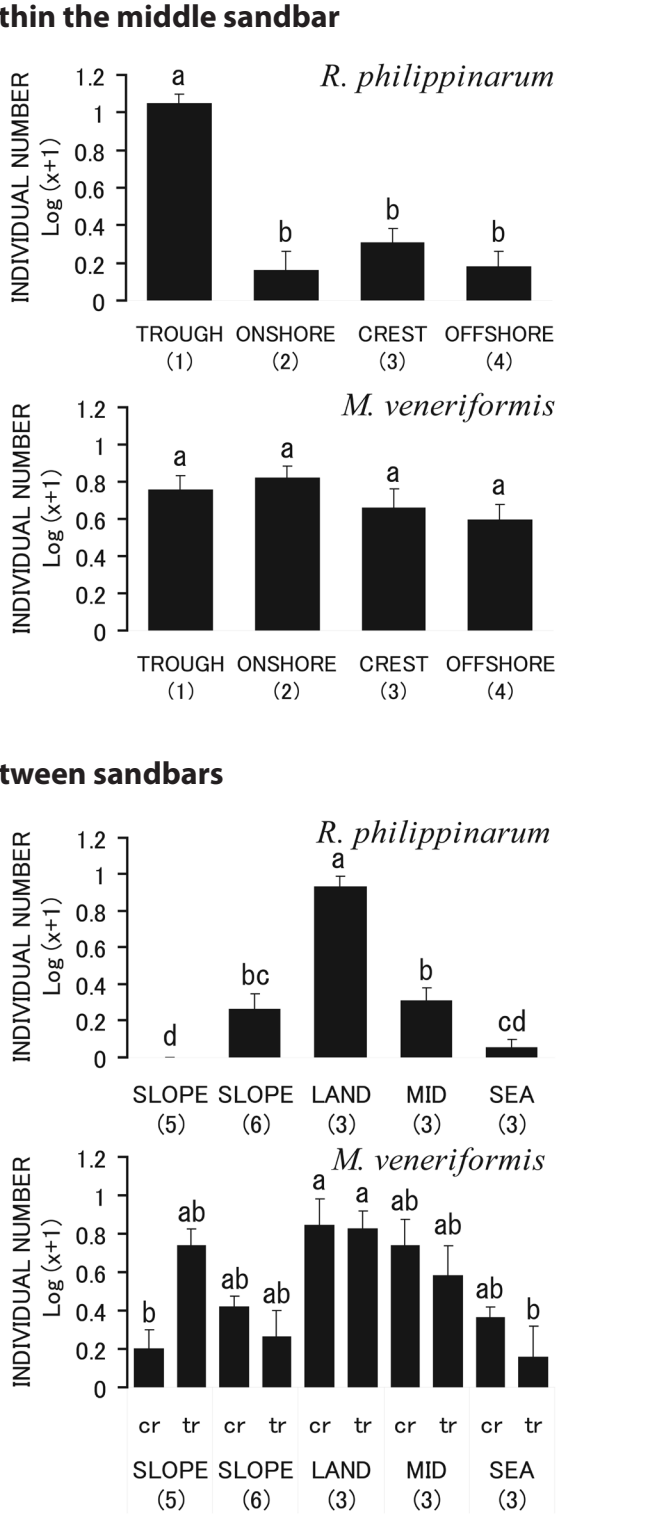

Figure 4. Mean log-transformed individual numbers of Manila clams (*Ruditapes philippinarum*), shell length < 17 mm, above, and surf clams (*Mactra veneriformis*), shell length < 5 mm, below. Individual numbers were compared at four positions in the middle sandbar (left), and the crests of three sandbars and two stations on the foreshore slope. Troughs and crests of ripples were compared separately for surf clams because the interaction term was significant in ANOVA.

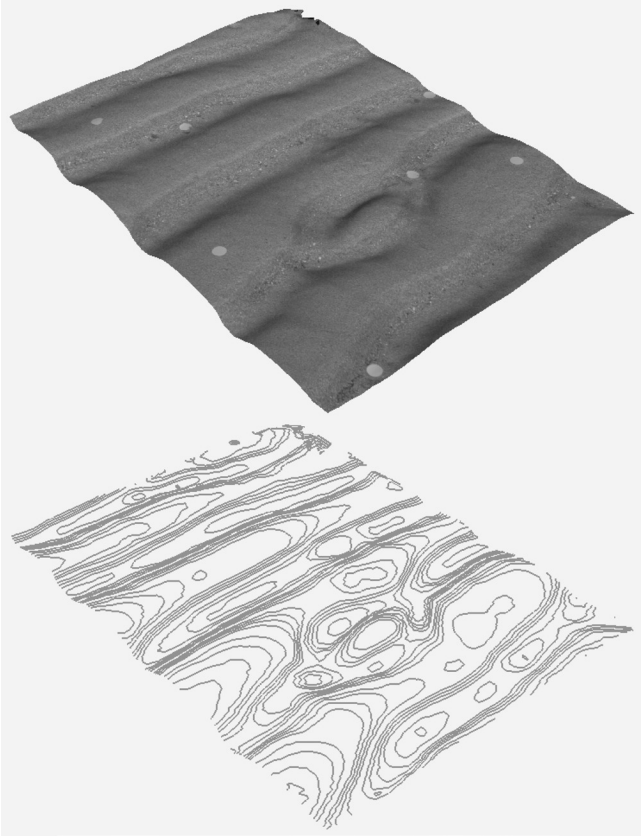

Figure 5. An example of three-dimensional analysis on the fine-scale topography of the sand surface. A photograph of the sand surface was pasted on the TIN, above. A contour plot in 1 mm intervals was automatically drawn, below.

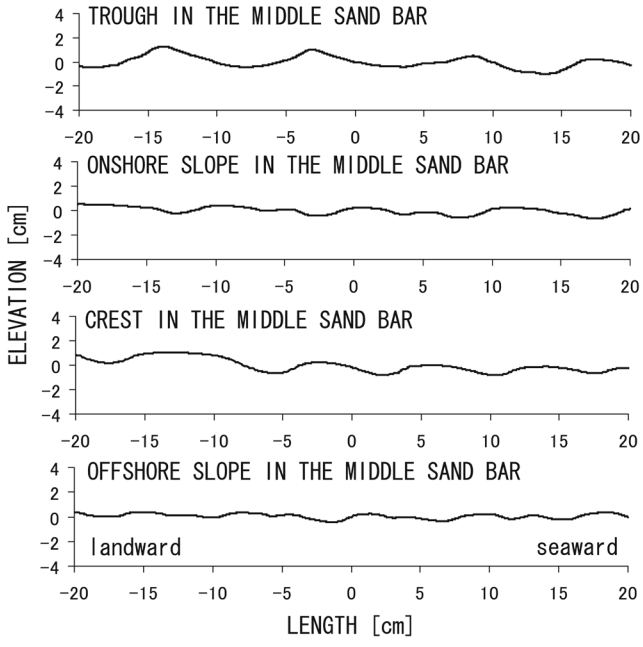

Figure 6. Vertical profiles of sand ripples in four stations established in the middle sandbar.

rate of Manila clams of 26–28 mm shell length in the field experiment decreased when bottom friction velocity was greater than 4 cm/s (Kakino 2000). It is likely that disturbance caused by waves and currents has critical effects on clam survival. Thread secretion by young Manila clams to attach to large objects such as stones and shells is an adaptation to turbulent conditions on the bottom. However, clams attached to the objects would be passively transported under strong wave conditions that remove the objects or break the threads.

Recovery rates of benthic communities from impacts of disturbances on sand flats are dependent on spatial scales of disturbed sites (Thrush et al. 1996). Generally, a larger disturbed site needs a longer period to recover its community structure than ambient areas. Community recovery occurred after 4.5 months in 1 m^2 disturbed plots on a sand flat (Zajac and Whitlatch 2003). Recovery times for disturbances of larger areas, 100 m^2 to 1 km^2 in size, ranged from about 1 to 2 years (Zajac 1999). Wave length of multiple sandbars in this study was 30–40 m, and crests of sandbars may be exposed to strong

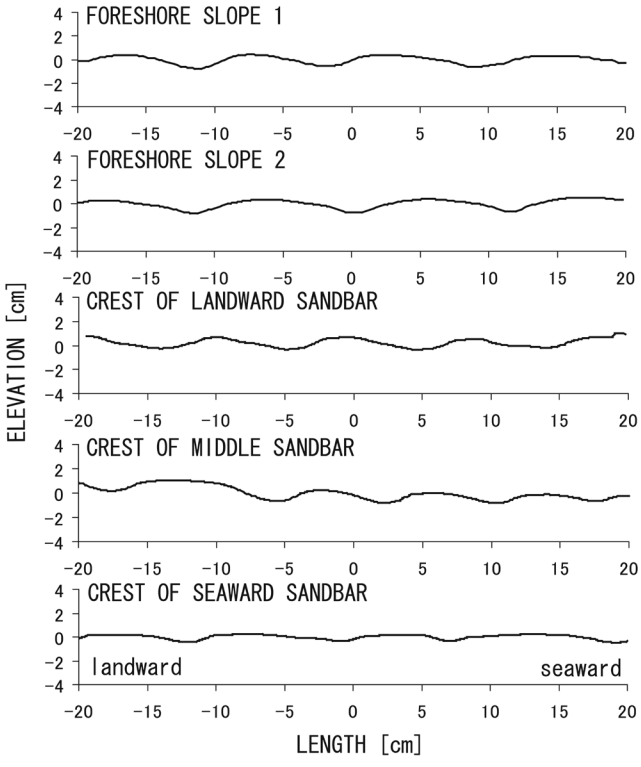

Figure 7. Vertical profiles of sand ripples at two stations established in the foreshore slope and on the crests of three discrete sandbars.

wave and current forces. If sandbar crests were exposed to yearly or more frequent strong wave actions, the benthic community on them would be severely disturbed before it recovers from the previous disturbance. In such situations, a sandbar trough, shaded by a sandbar crest, would work as a refuge against wave exposure for many days until extreme waves change the morphology and location of the sandbars.

In a study on the morphology of intertidal multiple sandbars of Okoshiki Beach in Ariake Bay, Japan, the sand bar morphology appears to be a permanent feature in both form and position (Yamada and Kobayashi 2007). On the other hand, it was reported that the morphology of multiple sandbars, referred as ridges and runnels, on Mablethorpe Beach, North Lincolnshire, England, responded to changing wave conditions (Masselink 2004).

In this study, abundance of Manila clams in the trough on the middle sandbar was greater than at other positions on the same sandbar. When the crests of three sandbars were compared, the abundance of this clam was greatest on the landward sandbar where the ripple shape was rather concave upward and slightly steep, suggesting that the effect of unidirectional current was milder than those at the other two stations. On the onshore parts of the sandbar zone, the bottom surface may be more protected from exposure than that on the offshore part through a shading effect by offshore sandbars. The abundance of Manila clams on the foreshore slope was small, especially at the station located at higher part of the slope (Figure 4).

Steepness of ripples on the foreshore slope was small, and the ripple shape there was similar to those on the crest of the middle and seaward sandbar where the clam was not abundant. This implied that the hydrodynamic condition on the bottom of the foreshore slope was different from that on the crest of the landward sandbar where the clam was abundant. Further, extremely shallow parts may be harsh environments for the clam because the submerged period is limited. The low abundance of Manila clams corresponded with the occurrence of convex ripples that suggested the effect of unidirectional currents mixed with wave actions. In contrast, this clam was abundant in the trough of the middle sand bar where the ripple shape was concave upward. This type of ripples—wave ripples—is seen where unidirectional currents are weak relative to oscillatory waves. It has been reported that Manila clam abundance was greater in the trough of the sandbar than in other positions (Kakino 1996).

Abundance of Manila clams was not significantly different between troughs and crests of sand ripples in this study. However, a contrasting phenomenon was that the difference in the community structure between troughs and crests of ripples was observed in the subtidal soft bottom (Barros et al. 2004) though the height of the ripples was approximately 5–10 cm, which is much greater than observed in this study. A technical problem in sampling may be another reason why the difference was not significant in the present study. The width of quadrats used in this study, 5 cm, was not small enough to separate troughs and crests of ripples when the wave length of the ripples was less than 10 cm. The wave length of ripples observed in this study was sometimes smaller than 10 cm. In this case, troughs and crests could not be clearly separated because the area sampled partly overlapped.

The spatial pattern of surf clams was different from that of Manila clams, which were evenly distributed within the middle sandbar (Figure 4). Comparing the abundances on three sandbar crests, surf clams increased landward. That pattern was similar to that of Manila clams, however the difference in abundance between stations was more contrasting in Manila clams. To explain the contrast between the two species, behavioral responses to wave exposure should be compared in future studies. From the skew of sand ripples, it was estimated that landward motion is stronger than seaward motion. Small clams may experience a high risk to be passively transported landward.

In studies on benthic communities, the literature referring to ripple shapes on sand flats is limited (Shull 1997, Barros et al. 2004). However, from the patterns of ripples, we may be able to deduce the similarity or the dissimilarity in hydrodynamic conditions on the sea bottom among sites and localities. Hydrodynamic processes on the sea bottom are crucial environmental factors that control movement and settlement of young benthic organisms. However, generalization and theorization of nonsteady hydrodynamics in turbulent conditions is difficult. A modular approach characterizing the ecological role of benthos in large coastal seas and estuaries identified types of modules that comprise coastal systems and depends on the overall complexity of geomorphology and coastlines (Tenore et al. 2006). The approach aimed at providing insights as to how the ecology of the benthos may be related to physical variables that are used in classification schemes for

coastal systems. Fine-scale topographies like sand ripples may be used as indices of physical conditions in the modular approach.

Three-dimensional analyses on ripple shape under water were developed in a previous study using video cameras in waterproof housings, though measurement of ripple profiles was not successful because it was not possible to identify distinct points on the ripple profiles in each of the images comprising the stereo pairs (Doucette et al. 2002). Our application method is able to obtain stereo pairs using portable cameras, although the process is limited to intertidal zones. Not being used underwater, our method was successful, although improvement of the process so it can be used under submerged conditions should be a future task. A detailed explanation of our method and examination of its ability is in preparation.

In previous literature on movable bed roughness in unsteady oscillatory flow that employed a model wherein the roughness was divided into contributions due to the form drag over wave-formed ripples and the near-bed sediment transport, authors demonstrated that when ripples having steepness greater than 0.1 are present, they determine the majority of the magnitude of the roughness under the waves, while the roughness depends to a large extent on the intensity of the near-bed sediment transport for ripple steepness less than about 0.1 under high sediment transport conditions (Grant and Madsen 1982). In another study conducted on reef-associated sediments of an exposed coast that used the mean ripple wave length as an index of exposure to wave energy, significant negative relationships were detected between relative wave exposure (as estimated from ripple wave length) and taxonomic richness, number of individuals, and diversity (DeFelice and Parrish 2001).

In the present study, ripple steepness less than 0.1 was observed on the offshore slope on the middle sandbar, the crest of seaward sandbar, and the upper part of the foreshore slope. At those stations, the abundance of Manila clams was small compared to that in other stations (Figure 4). On the other hand, ripple steepness was no less than 0.14 in the trough on the middle sandbar and the crest of the landward sandbar. The abundance of Manila clams there was much greater than at other stations. From this, it was suggested that juvenile Manila clams survived better in places where most of the roughness magnitude is determined by the presence of steep ripples. Such places may serve as spatial refugia against wave and current exposure. Contrasting with the result of Manila clams, abundance to surf clams did not show any clear correspondence with ripple steepness.

In conclusion, common clams in Matsunase Beach, especially Manila clams, showed a spatial pattern related to the sandbar topography. This spatial pattern corresponded to some extent with ripple shapes on the sand surface. Presence of steep ripples concave upward may be used as an index of refugia against wave and current exposure, although susceptibility to the exposure would be different among species. TIN images and vertical profiles of ripples made from stereo pictures taken in the field will help us share information on the fine-scale topography of sand surfaces that is difficult to describe and needs to be quantified in the field. Statistical tests on clam abundance performed here are not unambiguous. Random spatial variations within each sampling station were not included in the tests because the scale of square units equivalent to 60x60 cm adjusted to the size of stereo pictures was too small to represent the whole area considered. To keep the robustness of the tests, the square units should have been replicated within each station. An extensive sampling design is necessary to solve this problem.

Acknowledgments

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Methods, Economics, and Commercialization of Open Ocean Mussel Culture in the Northeast United States

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Keywords: open ocean, culture, mussel, protocols

Abstract

Constraints on expansion of culture operations in protected, nearshore embayments where sea conditions are favorable for raft and surface-referenced longline culture are forcing mussel industries to explore the potential for developing farms in the open ocean.

A project at the University of New Hampshire has taken this exploratory approach and established an open ocean aquaculture demonstration site located 10 km from shore in the open waters of the U.S. Gulf of Maine. Water depth is 52 m at the site, which is fully exposed to wind and waves from all directions and can experience significant wave heights of 9 m during severe storms. Two longlines, each approximately 120 m in length and submerged 12 m below the surface, were installed in 1999.

The project was designed to identify and demonstrate offshore commercial aquaculture opportunities for local and regional capture fishing communities; therefore, a fishing vessel typical of those used in nearshore ocean fisheries was equipped to tend submerged longlines. Gear and technology used in surface referenced longline culture was modified for use in the open ocean environment, and several different types of buoys, growout ropes, and socking materials and methods were evaluated to determine optimal materials and practices for use in offshore environments.

The project has been successful in developing operational protocols and production strategies and has demonstrated that excellent growth and production can be achieved in the open sea. Since 1999 eight seed cohorts of blue mussels (*Mytilus edulis*) have been grown to market size with an average production cycle of 13 months from spat settlement to 55 mm shell height. Yield at market size has ranged from 7.5 to 12 kg/m of mussel rope, depending on the initial seeding density. The product quality and meat yield has been consistently excellent, with meat yields ranging from 42% to greater than 55% depending on density and season.

An economic analysis that examined optimal farm size, ownership options, and capital costs concluded that high quality mussels could be produced at a cost of $0.53 USD per kilogram, indicating excellent potential for profitability. Project personnel have worked with the regional fishing industry in New England to transfer technology and a commercial farm was established in 2005. Project personnel continue to provide technical support for commercial start-ups. This presentation highlighted system design, production strategies, economics, and the process of moving from applied research to commercialization.

Bivalve Mollusk Culture on the U.S. West Coast

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Keywords: oysters, clams, mussels, shellfish farms

Abstract

Shellfish farms on the U.S. West Coast annually produce roughly 48,000 mt of oysters, clams, and mussels valued at approximately $111 million. Oysters dominate production with an estimated 42,731 mt valued at $84.8 million. The bulk of the oyster production is Pacific oysters (*Crassostrea gigas*) with edible oyster (*Ostrea edulis*), Olympia oyster (*O. lurida*), Kumamoto oyster (*C. sikamea*), and eastern oyster (*C. virginica*) being produced in smaller quantities for the live half shell oyster market.

Manila clams (*Venerupis philippinarum*) are the next most significant species farmed with an estimated 3,880 mt produced annually valued at $17 million. Mediterranean mussels (*Mytilus galoprovincialis*) and foolish mussels (*M. trossulus*) are two species of mussels farmed on the U.S. West Coast with an estimated annual production of 1,238 mt valued at $3.5 million. Pacific geoduck clams (*Panopea abrupta*) are relatively new to the suite of bivalves cultured. With an estimated 850,000 pounds annual production valued at more than $5 million, they are the most valuable species per pound.

Washington dominates West Coast production. This is largely attributable to laws passed in the late 1800s allowing the sale of tidelands into private ownership specifically for the purpose of culturing shellfish. Subsequently, Washington has encouraged the development of a robust shellfish industry. Pacific and Grays Harbor counties in southwest Washington rely on the shellfish culture industry to support a significant portion of their economy.

Oysters are cultured predominantly in the intertidal zone, planted directly on the bottom. The market in recent years has shifted from fresh shucked oyster meats to live oysters on the half shell trade. With these shifting markets have come improvements in nursery systems for rearing single oyster seed and culture and processing systems for producing quality single oysters. In recent years, there have also been advancements in the mechanization of Manila clam culture and harvest.

Relationship Between Gametogenesis and Food Quality in Sea Urchin Gonads

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Keywords: aquaculture, food quality, gametogenesis, ovary, testis, sea urchin

Abstract

The sea urchin gonad contains two main types of the cells: germinal cells (GCs) and somatic nutrient storage cells called nutritive phagocytes (NPs). The proportion of GCs and NPs varies during the year. Before gametogenesis, NPs fill the gonadal lumina and increase in size by accumulating nutrients derived from food. As gametogenesis proceeds, NPs decrease in size as nutrients are mobilized and transferred to GCs. In fully mature gonads, ova or spermatozoa fill the gonadal lumina and NPs shrink to their smallest size. The best season for eating gonads is restricted to a few months around the initiation of gametogenesis when NPs begin to mobilize their nutrients. Before that period, the size of the gonad is too small. After that period, as gametogenesis proceeds, the quality of gonads as food products gradually decreases. The tissues of the gonads become fragile as they mature. After the ripe gonads are removed from the testis, ova or spermatozoa ooze from the gonoduct, reducing their commercial value. In some species, strong bitterness develops in the ovary as oogenesis proceeds. To prolong the period during which commercially valuable sea urchin gonads can be harvested and to improve the quality of the gonad, two strategies are proposed. One is to accelerate the growth of NPs by feeding sea urchins with an artificial feed instead of macroalgae. The other is to suppress gametogenesis by manipulating environmental cues that trigger gametogenesis or by generating infertile, triploid sea urchins.

Introduction

The sea urchin is a popular seafood in Japan, where market demand for this delicacy has been growing for decades. Japan is annually importing more than 6,000 tons of sea urchin gonads (FAO 1996), which means that the Japanese consume about 80% of the total world catch. As wild stocks of sea urchins gradually decline as a result of overfishing, aquaculturists have become interested in culturing several species (Keesing and Hall 1998). However, sea urchin aquaculture is still in its infancy in Japan, the United States, and some other countries. As only the gonad is edible in sea urchins, knowledge of unique characteristics of the sea urchin gonad as food products would be useful for successful aquaculture. Here we describe the features of gametogenesis, describe the relationship between gametogenesis and quality of gonads as food products, and propose some possible strategies to improve the food quality. We use the northern green sea urchin (*Strongylocentrotus droebachiensis*) and red sea urchin (*Pseudocentrotus depressus*) as models, as they are popular edible sea urchins in the northeastern United States and in the southern part of Japan, respectively.

Structure of the Gonad

The sea urchin has five gonads, each of which is attached internally to the test (shell) and can release gametes to the outside world by a separate gonoduct. Each gonad consists of hundreds of acini and resembles a bunch of grapes. Two sacs (Figure 1b) of tissues (outer and inner) compose the wall of each

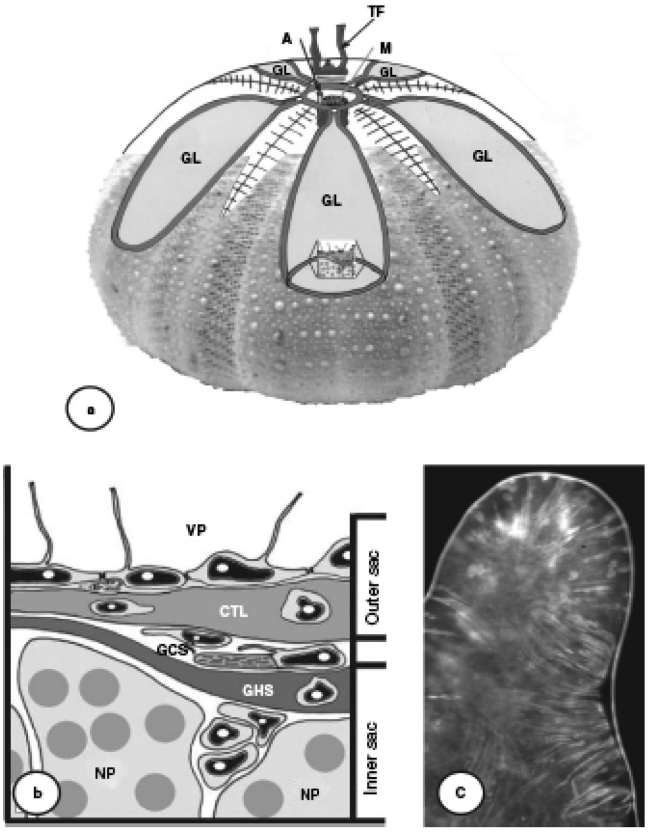

Figure 1. a) Diagrammatic representation of the sea urchin reproductive system in aboral side view, b) Diagrammatic representation of the tissues in the sea urchin gonadal wall, after Strenger (1973), c) Lobe of a sea urchin gonad stained with phalloidin to show muscles on the exterior surface of the GHS of the inner sac (white strips). A = anus, CTL = connective tissue layer, GL = gonadal lumen, M = madreporite, NP = nutritive phagocytes, GCS = genital coelomic sinus, GHS = genital hemal sinus, TF = tube feet, and VP = visceral peritoneum. Reprinted with permission from Walker et al. 2006.

acinus and each sac consists of several characteristic layers (Figure 1a) (Walker et al. 2006). Throughout the gonad, the genital coelomic sinus (GCS) separates the outer sac from the inner sac. The outer sac includes a visceral peritoneum that faces the perivisceral coelom and is attached to a connective tissue layer (CTL). Nonmuscular epithelial cells also line the CTL on its opposite surface toward the GCS. The inner sac is a genital hemal sinus (GHS) that bears ciliated myoepithelial cells on its outer face (Figure 1b,c,) (Walker 1982). These muscles contract rhythmically during gamete release (Okada et al. 1984). On its luminal face, the GHS supports the germinal epithelium. The principal functions of the inner sac are gametogenesis and nutrient storage.

The germinal epithelium consists of the two major populations of cells: germinal cells (GCs, ranging from oogonia to ova in the ovary and from spermatogonia to fully differentiated spermatozoa in the testis) and somatic cells called nutritive phagocytes (NPs) that are present in both sexes (Walker 1982). NPs are versatile cells that provide a structural and nutritional microenvironment for GCs throughout gametogenesis (Figure 2) (Walker et al. 2005). NPs have multiple functions. NPs may contain amitotic oogonia or spermatogonia and growing primary oocytes or spermatogenic cells basally and simultaneously phagocytize residual ova and spermatozoa luminally (Walker et al. 1998, 2005). At full maturity, each ovarian NP encloses a single, growing vitellogenic oocyte in a basal incubation chamber where it may be enveloped by dissolved or particulate nutrients (Figure 2a) (Walker et al. 2005). The same NPs may also simultaneously contain additional GCs at several earlier stages of oogenesis within smaller, discrete basal incubation chambers. Testes also contain numerous NPs that cooperate to provide large basal incubation chambers that ultimately become continuous and together supply nutrients to enormous numbers of spermatogenic cells at diverse stages (Figure 2b) (Walker et al. 2005).

Stages in Gametogenesis

During the annual reproductive cycle, gonads of both sea urchin sexes pass through a predictable series of structural changes. Nutrient accumulation in NPs and its use for gametogenesis are linked processes in sea urchin reproduction (Walker et al. 2006). The proportion of GCs and NPs in the gonadal acini varies with the progress of gametogenesis. Figure 3 shows structural changes inside the gonad of red sea urchins during gametogenesis as classified into five stages by Fuji (1960) with modifications for that species (Unuma et al. 1996, Unuma 2002).

Stage 1. Before gametogenesis: In both sexes, each acinus is filled with NPs (eosinophilic cells). In ovaries, a few young oocytes are present at the periphery of the acini. Hematoxylin-stained round spots, residue from phagocytized ova (Masuda and Dan 1977, Tominaga and Takashima 1987), are occasionally present centrally in the ovarian lumen. In testes, detection of spermatogenic cells is difficult at this stage in paraffin sections. Instead, many hematoxylin-stained speckles, residue from phagocytized spermatozoa (Kato and Ishikawa 1982, Reunov et al. 2004), may be present in NPs. Unlike the round spots observed in the immature ovary, these speckles are amorphous, and are a

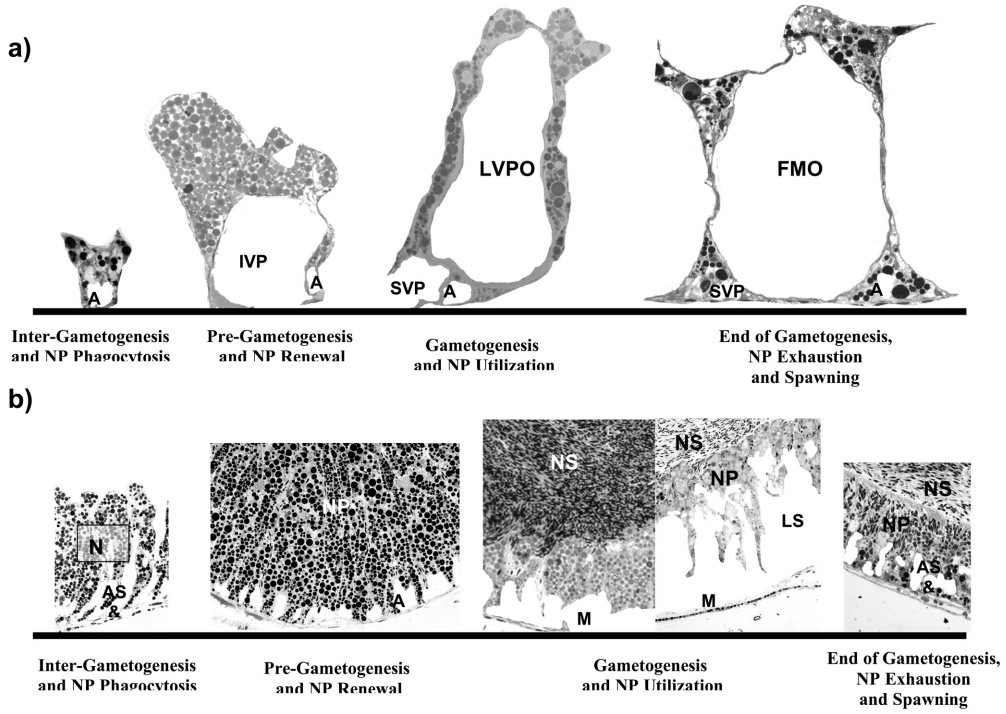

Figure 2. Nutritive phagocytes (NPs) in a) ovaries and b) testes of northern green sea urchins at each gametogenic stage with germ cells removed from NP incubation chambers (not to scale). For ovaries, the shapes and dimensions of representative, individual NPs are shown, as well as the positions of various germ cell types within discrete NP incubation chambers. For testes, the positions of germ cells relative to groups of NPs are shown, especially the continuous incubation chamber that eventually forms. AO = amitotic oogonia, AS = amitotic spermatogonia, NVPO = new vitellogenic primary oocyte, O = ovum, LSS = later spermatogenic stages, RVPO = residual vitellogenic primary oocyte, NP = nutritive phagocyte, NS = new spermatozoa, and MS = mitotic spermatogonia. Modified with permission from Walker et al. 2005.

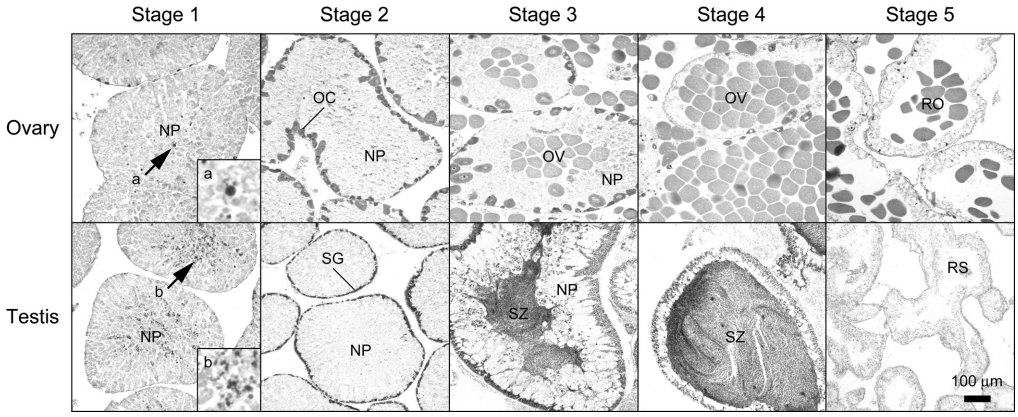

Figure 3. Representative structure of the ovary (upper panels) and testis (lower panels) of red sea urchins at different gametogenic stages. Paraffin-embedded sections are stained with hematoxylin and eosin. At stage 1, the gonadal lumina are filled with nutritive phagocytes. At stage 2, many developing oocytes or clusters of spermatogonia or spermatocytes are present at the periphery. At stage 3, nutritive phagocytes are replaced with ripe ova or spermatozoa in the center of the lumina. At stage 4, the lumina are filled with ripe ova or spermatozoa. At stage 5, the lumina contain a few residual ova or spermatozoa. NP = nutritive phagocyte, OC = oocyte, OV = ripe ovum, SG = spermatogonium, SC = spermatocyte, and SZ = spermatozoon. Inset a, round spot representing a phagocytized residual ovum. Inset b, amorphous speckles representing phagocytized residual spermatozoa. Scale bar represents 100 µm. Modified with permission from Unuma 2002.

useful feature to distinguish testes from ovaries.

Stage 2. Beginning of gametogenesis: Many developing oocytes or clusters of spermatogonia are present at the periphery of the acini, and the gonadal lumina are still filled with NPs.

Stage 3. Middle of gametogenesis: NPs are replaced with ripe ova or spermatozoa in the center of the gonadal lumina. Numerous developing oocytes or clusters of spermatogonia and spermatocytes (Ward and Nishioka 1993, Walker et al. 2005) are present at the periphery of the acini. NPs are gradually decreasing in size and are present between the GCs.

Stage 4. End of gametogenesis: The gonadal lumina are filled with ova or spermatozoa. Shrunken NPs, which have already lost nutrients, are present only at the periphery of the acini.

Stage 5. After spawning: The gonadal lumina have numerous empty spaces and a few residual ova or spermatozoa. NPs gradually phagocytize residual gametes and begin to grow as they store nutrients. After this stage, gonads return to Stage 1 and a new cycle starts.

Seasonal Changes in Gonadal Size and GCs/ NPs Proportion

Sea urchin gonads grow in size not only because gametogenesis increases the size or numbers of GCs but also because NPs store extensive nutrient reserves before gametogenesis (Walker et al. 2006). Figure 4 shows the seasonal changes in the gonad index and in the proportion of GCs and NPs in red sea urchins (Unuma 2002). This species spawns around November and gonad indices rapidly decrease. After spawning, the gonad index gradually increases until the next spawning. The increase before gametogenesis is attributable to the growth of NPs. NPs accumulate various nutrients, such as proteins, lipids, and carbohydrates, derived from ingested food and increase in size. About 80% of the total protein contained in NPs is a glycoprotein with a molecular mass of about 170 kDa (Unuma et al. 2003). This protein was originally identified as the predominant component of yolk granules in sea urchin eggs and termed the major yolk protein (MYP) (Yokota and Sappington 2002). Unlike other oviparous animals where the yolk protein is female-specific, both male and female sea urchins produce MYP, store it in NPs prior to gametogenesis (Unuma et al. 1998), and utilize it as nutrient source for gametogenesis (Unuma et al. 2003, Unuma and Walker in press).

Proliferation and development of GCs begin two months before spawning in red sea urchins (Unuma 2002). After gametogenesis begins, the size of the gonad continues to increase but the proportion of NPs in the gonad rapidly decreases. The period of gametogenesis of red sea urchins is shorter than those of other species, where gametogenesis usually takes more than three months.

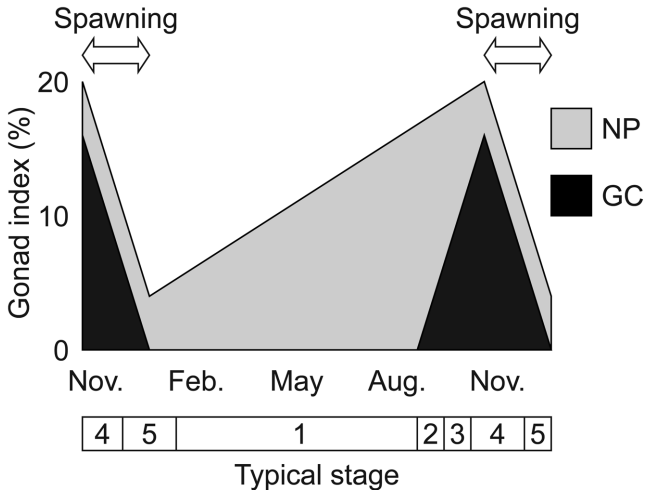

Figure 4. Diagrammatic representation of the seasonal changes in the gonad index and in the proportion of nutritive phagocytes (NPs) and germ cells (GCs) in red sea urchins. Stages of gametogenesis are indicated below the graph. Spawning occurs around November. The long-term increase in the gonad index before gametogenesis is mostly attributable to the growth of NPs. Modified with permission from Unuma 2002.

Relationship Between Gametogenesis and Food Quality

In most countries that consume sea urchins, gonads containing fewer GCs than NPs (unripe) are preferred as food, although there may be some exceptions (Walker et al. 2006). In red sea urchins, the best season to eat gonads is from June to August, when NPs have grown sufficiently but gametogenesis has not yet begun (Unuma 2002). Before that period, the size of the gonad is too small and the color of the gonad is not attractive (brownish). After the period, as gametogenesis proceeds, the quality of gonads as food products gradually deteriorates. This is due to adverse events, the most serious of which is the oozing of gametes (Unuma 2002). The tissues of the gonads become fragile as gametogenesis proceeds.

After ripe gonads are removed from the testis, they appear to be melting because of the oozing of gametes (Figure 5). Eggs or sperm ooze from the gonoduct, and the gonads cannot maintain their consistency. This phenomenon, common among all the edible sea urchins, considerably reduces the commercial value of the gonads. To suppress this gamete ooze, alum, an aluminum compound, is usually used in Japan. After removing the gonads from the testis, they are lightly washed in salt water and then immersed in a 0.5–1.5% alum solution for 5–20 minutes. In this way, the mature gonads can maintain their shape for a longer time. The alum cannot, however, prevent gamete ooze completely; besides, it alters the taste of gonads, which become astringent and bitter.

Another reduction in quality results from the bitterness of mature ovaries in some species. In the red sea urchin and the green sea urchin (*Hemicentrotus pulcherrimus*, another popular species in the southern part of Japan), mature ovaries have an extremely bitter taste, unlike the mature testis. Recently, pulcherrimine, a novel sulfur-containing amino acid, has been isolated as the bitter component from the mature ovary of the green sea urchin (Murata and Sata 2000, Murata et al. 2001, 2002). The bitter component in the mature ovary of red sea urchins has not been identified. The bitterness of mature

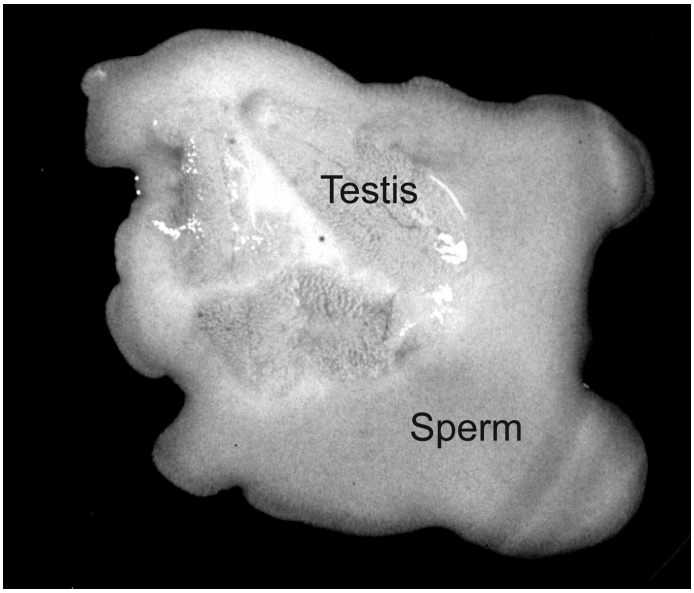

Figure 5. Deterioration of commercial quality in the gonads of red sea urchins. Mature testes were taken out of the testis and placed in a petri dish for 30 minutes. The testes appeared to be melting because of oozing sperm. The same process also occurs in the mature ovaries. Reprinted with permission from Unuma 2002.

ovaries in these two species results in a shortened commercial season compared to that of other species where ovaries at the end of gametogenesis are not bitter. Usually, red sea urchins and green sea urchins are harvested only before gametogenesis begins (Stage 1), while other species in which the mature ovary has no bitterness have commercial value until mid gametogenesis (Stage 3).

In addition to the deterioration in quality with the advance of gametogenesis, subsequent spawning causes a large year-to-year decrease in the size of the gonad (Unuma 2002). Following spawning, it takes time for the gonads to grow to marketable size again. The best season for harvesting sea urchin gonads is rather short because of these problems. Low-quality or small gonads can be harvested for a longer period of time, but large gonads of commercial quality can be harvested for only about one or two months. Therefore, gametogenesis and subsequent spawning are disadvantageous for culturing sea urchins.

Methods to Improve the Food Quality

For successful sea urchin aquaculture, it is important to produce large gonads that contain few GCs. If the gonad can be enlarged without increase in GCs/NPs proportions, the food quality of the gonad could be improved and the season during which commercial quality gonads can be harvested would be prolonged. For this purpose, acceleration of NP growth or suppression of gametogenesis should be effective as shown in Figure 6 (Unuma 2002). Both methods prolong the season during which large gonads that contain fewer GCs can be harvested. However, the acceleration of NP growth is easier than the suppression of gametogenesis and is now being put to practical use. In contrast, the suppression of gametogenesis, which is more effective at yielding large gonads with fewer GCs, is technically difficult and requires much additional research before it can be put to practical use.

Use of Formulated Feed to Accelerate NP Growth

Most edible sea urchins are herbivorous and eat macroalgae (seaweed or kelp). However, food that contains higher amounts of protein than macroalgae can enhance the gonadal growth more efficiently than diets of algae, for example, formulated feeds (de Jong-Westman et al. 1995, Lawrence et al. 1997, Barker et al. 1998, Walker and Lesser 1998, Akiyama

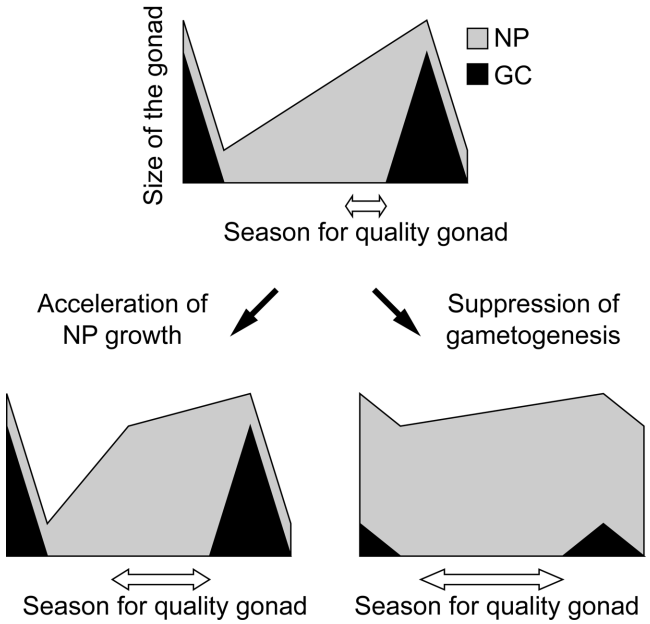

Figure 6. Two strategies for extending the season of harvesting high-quality gonads. If growth of NPs is accelerated (left) or gametogenesis is suppressed (right), the season during which quality gonads containing fewer GC can be harvested is dramatically prolonged. Modified with permission from Unuma 2002.

et al. 2001, Pearce et al. 2002, Hammer et al. 2006) and fish meat (Agatsuma and Nishikiori 1991). This implies that protein ingestion and assimilation are limiting factors for gonadal growth. Figure 7 shows the gonad indices of young red sea urchins fed formulated feeds of various protein levels and brown alga *Eisenia bicyclis* for 8 weeks, one of the main sources of food for natural populations of red sea urchins (Akiyama et al. 2001). The gonad indices of the sea urchins fed the formulated feeds are higher than those fed the brown alga and highest in feeds containing 20% and 30% protein, suggesting that the optimum protein level for gonadal growth of young red sea urchins is about 25%.

The protein level of formulated feeds is correlated to the taste of gonads, which becomes increasingly bitter in sea urchins fed higher protein feeds (Hoshikawa 1993). Considering the taste, the appropriate protein level for red sea urchins should be lower than 25%. Besides the bitter taste, formulated feeds have some disadvantages compared to algae: the color of the gonad becomes unattractive (whitish), formulated feeds are often more costly, and they decay more quickly than algae (Unuma 2002). These negative aspects must be overcome before formulated feeds will be practical in sea urchin aquaculture. Indeed, the performance of formulated feeds has improved dramatically year by year.

Control of Environmental Conditions to Suppress Gametogenesis

Land-based aquaculture systems for sea urchin are rapidly developing (Devin 2002, Böttger et al. 2004). In such systems, suppression of gametogenesis is advantageous for more efficient production of commercial quality gonads (Unuma 2002). Control of environmental conditions can be utilized to suppress gametogenesis in land-based systems (Böttger et al. 2006, Walker et al 2006). In purple sea urchins (*Strongylocentrotus purpuratus*), northern green sea urchins, and pencil urchins (*Euchidaris tribuloides*), the environmental cue to initiating gametogenesis is suggested to be the photoperiod (Pearse et al. 1986, Bay-Schmith and Pearse 1987, Walker and Lesser 1998, Böttger et al. 2006, Dumont et al. 2006), whereas in red sea urchins, green sea urchins, and violet sea urchins (*Anthocidaris crassispina*), it is suggested to be the water temperature (Yamamoto et al. 1988, Sakairi et al. 1989).

In some hatcheries in Japan, which produce seedlings for restocking of fish and shellfish including sea urchins, out-of-season maturation of brood stock of red sea urchins and green sea urchins is induced by manipulating water temperature (Ito et al. 1989, Masaki and Kawahara 1995, Noguchi et al. 1995). Alternatively, for culturing adult sea urchins, suppression of gametogenesis is required. In order to suppress gametogenesis by manipulating photoperiod or water temperature, the control regime needs to be further investigated. However, controlling water temperature by use of cooling or heating may not be financially practical for commercial-scale aquaculture because of high energy costs. Economical methods, such as use of warm effluent from an electric power station or cold seawater from the deep sea, are essential to control water temperature in aquaculture facilities.

Böttger et al. (2006) investigated the effects of invariant summer photoperiod and progressing ambient (fall/winter) photoperiod on the gonads of northern green sea urchins. Maintenance under invariant photoperiod yielded gonads containing fewer GCs than NPs (unripe), while maintenance under ambient photoperiod yielded ripe gonads (Figure 8). There was no significant difference in the gonad size between the two photoperiod regimes. These results imply that rearing northern green sea urchins under invariant summer photoperiod may improve the food quality and prolong the harvest season of the species in a land-based aquaculture system.

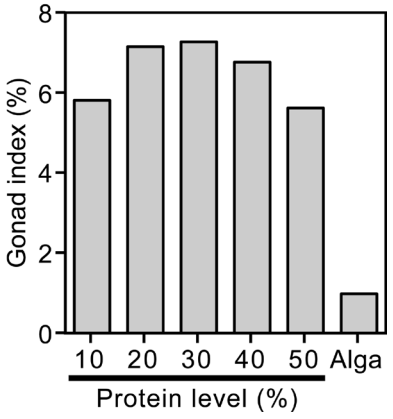

Figure 7. Gonad indices of red sea urchins fed different diets. Formulated feeds of 10–50% protein level and the brown alga *Eisenia bicyclis* were provided for 8 weeks to the young red sea urchins of 15 mm testis diameter. Casein was used as the protein source for the formulated feed. Based on data from Akiyama et al. 2001.

Production of Infertile Sea Urchins

Production of infertile sea urchins may also be utilized to suppress gametogenesis. To generate infertile adults, triploidy is often induced in other organisms, such as fish and bivalves (Arai 2001, Nell 2002). In sea urchins, however, there are very few reports concerning triploidy. One of the difficulties

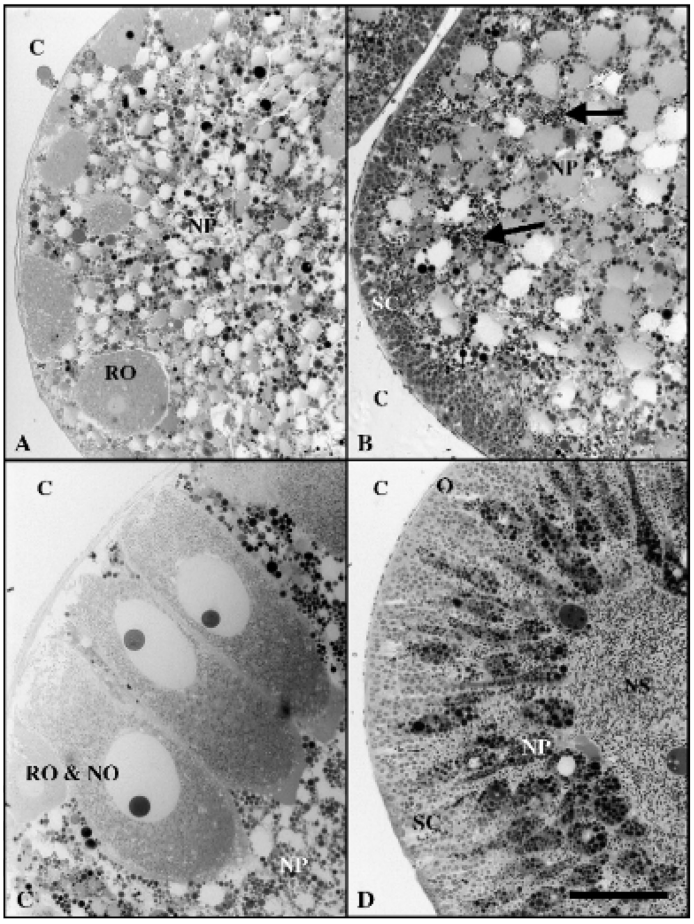

Figure 8. Plastic sections of A) a representative northern green sea urchin ovary and B) testis maintained for 5 months under invariant photoperiod showing predominance of NPs and few gametes. Limited numbers of residual (RO) primary oocytes (no new ones) are present near the ovarian wall and limited numbers of new spermatozoa (arrows) are evident between the expanded NPs as are spermatogonial mitoses among the spermatogenic cells (SC). C) Plastic section of a representative northern sea urchin ovary and D) testis maintained for 5 months under ambient photoperiod showing growing residual (RO) and new (NO) primary oocytes in NP incubation chambers. The testicular lumen is filled with new spermatozoa and the NPs (dark granule containing cells) are reduced in size with only a slender strand of cytoplasm connecting them to the testicular wall; circle points out a spermatogonial mitosis (SC); C = coelom. Scale bar represents 50 µm. Reprinted with permission from Böttger et al. 2006.

in generating triploid sea urchins is that sea urchin eggs complete both meiotic divisions before spawning (Walker et al. 2006). Therefore, retention of the polar body, a popular method of generating triploid fish and shellfish, cannot be applied to sea urchins. Production of a tetraploid sea urchin by suppressing the first cleavage after fertilization and its subsequent crossing with a normal sea urchin may be an efficient method to generate triploid sea urchins (Unuma 2002).

Recently, Böttger et al. (in press) succeeded in generating triploid embryos of northern green sea urchins by fusing two eggs followed by fertilization with normal sperm (Figure 9), although development of the triploid embryos was not observed past the gastrula stage. If this method can be improved, it may become a useful technique to generate triploid urchins.

If production of infertile sea urchins is accomplished, it would solve all the drawbacks associated with gametogenesis, loss of gonad size resulting from spawning, oozing of gametes, bitterness caused by oogenesis, and astringency caused by the use of alum. As a result, production of infertile sea urchins can dramatically improve the food quality and prolong the season during which commercial quality gonads can be harvested. Needless to say, it is crucial to confirm that infertile triploid sea urchins accumulate nutrients in NPs as normal diploid sea urchins do.

Conclusions

Intragonadal nutrient storage and its utilization for gametogenesis are linked processes in sea urchin reproduction. This situation, which is not found in other echinoderms including sea stars and sea cucumbers, is the basis for using sea urchin gonads as food products. A thorough understanding of sea urchin reproduction should permit some methods to improve gonad quality and prolong the season during which quality gonads can be harvested in sea urchin aquaculture.

Acknowledgments

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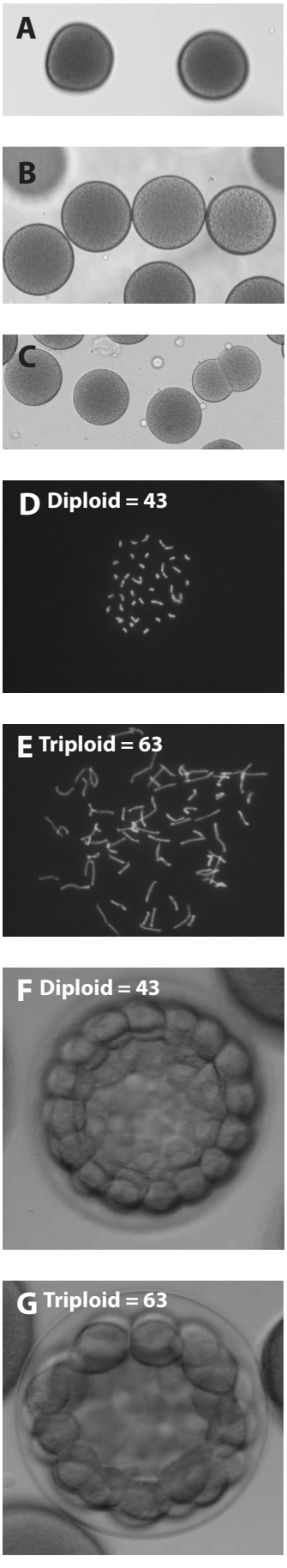

Figure 9. Triploidy in northern green sea urchins. A) two ova with the jelly coat and vitelline membranes removed, B) several ova just prior to fusion, C) three successful fusions with two other ova beginning to fuse, D) normal diploid chromosomal spread, E) triploid chromosomal spread, F) normal diploid blastula, and G) triploid blastula (notice larger blastomeres). Courtesy of S. A. Böttger et al., from Böttger et al. in press.

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An Overview of the Culture of Marine Invertebrates in Maine

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Keywords: Maine, aquaculture, invertebrates, polyculture

Abstract

Maine's aquaculture industry encompasses a growing variety of producer sectors and an increasingly differentiated set of products and culture methods. Once solely recognized as a salmon-producing state, Maine enjoys a growing awareness for the quality and value of its other products and the care with which producers grow their crops. Marine invertebrates are principal examples, with the premier species being eastern oyster (*Crassostrea virginica*) and blue mussel (*Mytilus edulis*). Other marine invertebrates raised in Maine include hard clam (*Mercenaria mercenaria*), softshell clam (*Mya arenaria*), sand worm (*Nereis virens*), sea scallop (*Placopecten magellanicus*), and green sea urchin (*Strongylocentrotus droebachiensis*). Species are raised for the food market, for stock enhancement, and for other uses, employing a broad variety of culture techniques. Macroalgae are also being investigated as companion species in polyculture arrangements.

The annual value of marine invertebrates raised in Maine is approximately $10 million, though precise figures are difficult to obtain. Approximately 50 producing companies are distributed through every coastal county, with many industry members having history in traditional capture fisheries. Several academic institutions are engaged in education and applied research, most frequently in collaboration with industry partners, and both organizational and physical infrastructure in the state is improving. Funding for research and development within the state is nonetheless fairly limited, though applicants have been successful at attracting outside funding. The industry association is strongly engaged in a variety of issues.

A review of the species and methods of production was given, along with details about regulatory structure, market conditions, and current opportunities and challenges.

Polyculture of Red Abalone (*Haliotis rufescens*) and Pacific Dulse (*Palmaria mollis*) in a Land-based Recirculation System

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Keywords: polyculture, red abalone, Pacific dulse, protein

Abstract

Integrated, intensive polyculture usually depends on maintaining a balanced system made up of different, complementary biological units that share a common water source. Exchanges of water, nutrients, and organic material among the linked components are often designed to enhance production, reduce nutrient loss, and enhance economic gains.

We studied a simple marine integrated polyculture system in which red macroalga Pacific dulse (*Palmaria mollis*) was cocultured with red abalone (*Haliotis rufescens*) in the same culture system. Dulse provided abalone with a high quality diet. Ammonia and carbon dioxide produced by abalone were efficiently removed by dulse and converted into feed for abalone. Light, nutrients, and inorganic carbon were added to the system.

Under optimal summer conditions in Oregon, (lat 44°37′N, long 124°02′W), we measured dulse specific growth rates (SGR, % increase in dry weight d^{-1}) of 17%, dulse productivities of 67 g dry weight m^{-2} d^{-1} (equivalent to 413 g wet weight m^{-2} d^{-1}), and light utilization efficiencies as high as 7.2% for dulse. These production rates and efficiencies were comparable to those reported for high-yielding terrestrial agricultural crops, such as maize, rice, and sugar cane.

Dulse was an excellent food for abalone and had a high average protein content of 28% dry weight. Juvenile red abalone (shell length 25 mm) fed on dulse at 18°C showed growth rates as high as 198 μm shell length increase d^{-1}, exceeding previously reported growth rates for this species. Food conversion efficiencies were as high as 20%, resulting in an overall energy conversion efficiency of light energy into abalone biomass of up to 1.41% for this system.

In this integrated polyculture system, abalone production was much more limited by dulse production rates than the capacity of dulse to maintain water quality by removing excreted ammonia. Dulse production rates and efficiencies may be improved by optimizing conditions for gas exchange, light availability, and by selecting fast-growing dulse strains suited to intensive culture conditions. However, the economic benefits of these improvements should be balanced against costs in a commercial facility.

Wave Induced Flow in Seawater Exchange Structures for Improving Seawater Quality

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Keywords: seawater exchange, coastal structures, physical model, theoretical considerations

Abstract

In order to improve and preserve the quality of seawater and the seabed in dead water regions such as ports and harbors—including aquaculture grounds—seawater circulation and exchange needs to be enhanced. It may be possible to use a seawater exchange structure driven by wave motion. Among the many kinds of seawater exchange structures that have been developed, seawater exchange structures with blockwork mounds may be one of the most effective. This paper presents the results obtained from physical model tests and theoretical considerations performed on modified structures. Two models aimed at flexible applicability were used in the experiment; both of the modified structures proved effective. The research affirms that the volume rate of discharge for inlet flow through a conduit can be estimated quantitatively by the present discharge model.

Introduction

Seawater circulation and exchange in ports and harbors are inevitably restrained by surrounding facilities. If effective countermeasures against stagnation are not adopted, there may be pollution problems in ports and harbors, including aquaculture grounds, which can sometimes result in ecological damage. Enhanced seawater circulation and exchange are needed, in order to improve and preserve the quality of the seawater and seabed in such regions.

A large amount of work has been conducted to develop countermeasures against seawater pollution and various systems have been adopted in stagnant regions (Table 1). Some designs enhance the advection-diffusion of pollutant loads through seawater exchange, while others directly improve seawater and sediment conditions. Use of tidal and ocean currents or wave induced currents may be one of the possible solutions to the problem. However, the driving forces of such currents are generally weak in ports and harbors and consequently ineffective. It may be possible to use a seawater exchange structure driven by wave motions.

In Japan, many kinds of seawater exchange breakwaters that conduct seawater into basins through the use of wave power have been constructed for fisheries in ports and harbors. We have developed a seawater exchange breakwater in which the unidirectional current is excited by vortex flows (Ohmura et al. 2005). I have also developed another seawater exchange structure, in which one-way flow is excited by mass transport due to waves (Ohmura 2002).

Among the many kinds of seawater exchange breakwaters, seawater exchange structures with blockwork mounds may be one of the most effective (Figure 1). The structure was originally developed by Yamamoto et al. (1992) as a breakwater in which one-way flow is excited in a wave chamber due to wave breaking or wave overtopping. When water level exceeds the crest of mound, wave setup occurs by wave breaking. When water level is below the crest, wave setup occurs by wave overtopping. The structures not only have an ability to conduct seawater seaward through a conduit into the basin, but

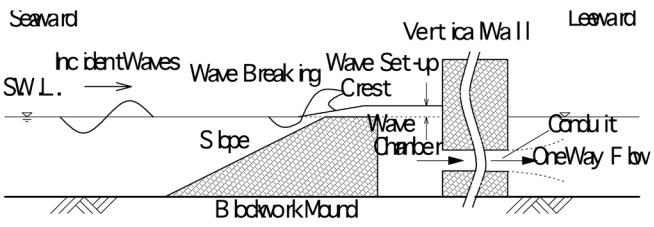

Figure 1. Mechanism of wave setup induced flow in seawater exchange structures with blockwork mounds.

Table 1. Representative works for improvement and preservation of seawater and sediment conditions.

Function	Works (energy)
Enhancement of seawater exchange	Seawater exchange structure (wave, tide, current) Waterway (tide, current, internal tide) Current training wall (tide, current, internal tide) Bay mouth improvement (tide, current, internal tide) Water gate (tide, electric power) Seawater training pump (wave, electric power) Air bubble curtain (electric power)
Supply oxygen to seawater	Seawater exchange structure (wave) Tideland (wave, tide) Sand beach (wave, tide) Seaweed (solar) Armor blocks (wave) Rubble mound type gentle sloping breakwater (wave) Revetment with aeration function (wave) Air bubble curtain (electric power)
Organic digestion by benthos and plankton	Tideland (solar, wave, tide) Sand beach (solar, wave, tide) Facilities for oxidation digestion by rocks (wave, tide, current, electric power) Rubble mound type gentle sloping breakwater (wave, tide, current)
Keeping nutrient salts	Tideland (solar, wave, tide) Sand Beach (solar, wave, tide) Seaweed (solar)
Preventing nutrient salts from sediment	Sediment dredge Sand capping Supply oxygen

also help supply oxygen to the seawater. It is known that when the distance from seawater level to the top of the crest becomes more than the values of incident wave height, the structures do not produce one way flow at all.

This paper presents information on the effectiveness of modified seawater exchange structures with blockwork mounds for a wide range of applicability such as wide tidal change and long conduits. Physical model tests and theoretical considerations were employed to investigate hydraulic characteristics of the modified structures (Ohmura 2007).

Physical Model Tests

Experimental setup. Experiments were conducted in a wave basin at the National Research Institute of Fisheries Engineering as shown in Figure 2. The wave basin was 40 m long, 22 m wide, and 0.8 m deep. It was equipped with a 10-m wide piston-type wave generator. Two modified models not only for wide tidal exchange but also for long conduits were used in the experiment. Model A had different blockwork mounds consisting of low crest, high crest, and intermediate crest as shown in Figure 3. We theorized that different crest heights would play an important role to induce wave setup in wave chambers over a wide range of water levels. The

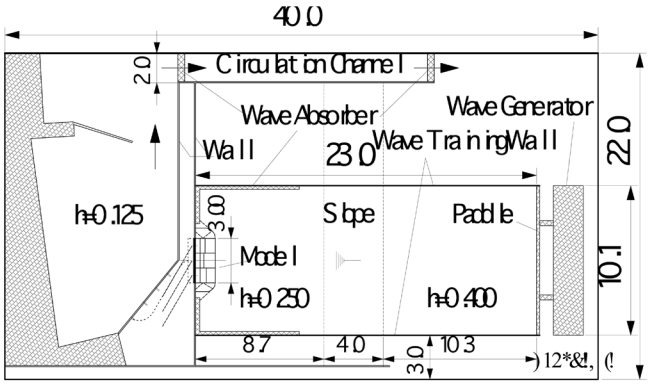

Figure 2. Plane view of a wave basin.

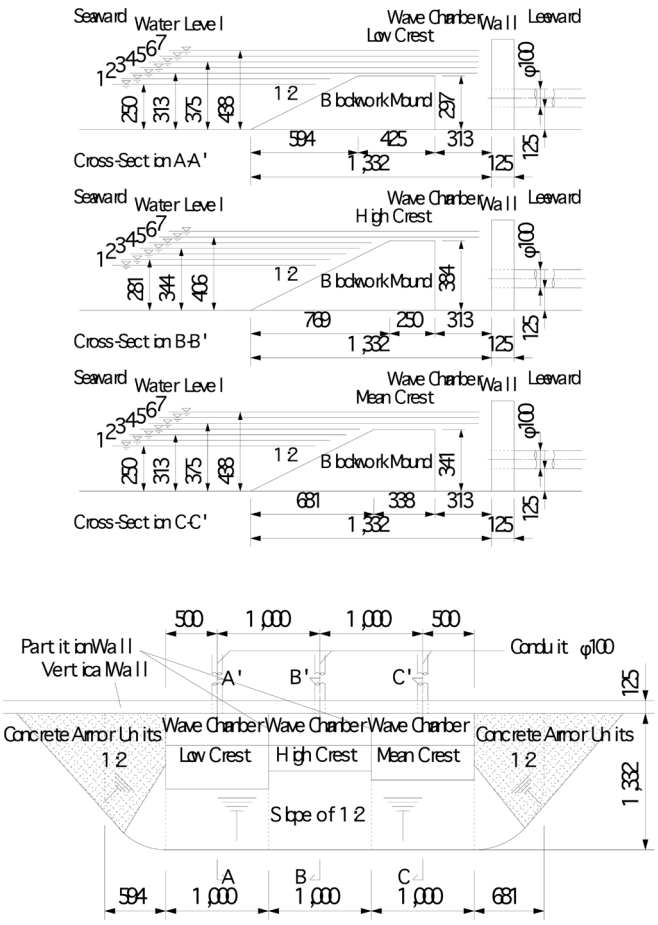

Figure 3. Plane view and cross section of model A with three different crests.

outer face of each mound had a 1:2 slope in order to enhance more wave setup in the wave chamber. Each conduit was made of PVC pipe with a diameter of 0.1 m and a length of 4.3 m. Model B used a rear chamber as shown in Figure 4. The specifications of model B were the same as model A except for the rear chamber. The conduit was made of PVC pipe with a diameter of 0.1 m and a length of 6.7 m. Figure 5 is a photograph of model B used in the experiment.

Waves acting on the models were the regular waves under the following test conditions:

- Deepwater wave height: H_0 = 2, 4, 6, 8 cm
- Wave period: T = 1.0, 1.5 s
- Water depth: h = 25.0, 28.1, 31.3, 34.4, 37.5, 40.6, 43.8 cm

The relationships among seven water levels and each crest height are shown in Figure 3 and Figure 4. The geometric scale was assumed about 1/10 to 1/20 according to Froude similitude. Measurements taken were surface elevation in the wave chambers in front of the wave paddle and at the exit of the pipe by capacitance-type wave gauges, and velocity in the center of the pipe at the exit by electromagnetic velocity meters.

Calculation methods of spatially averaged velocity

In order to calculate the volume rate of discharge through the pipe, adequate methods should be employed to estimate the spatially averaged velocity from the center velocity at the exit. The procedures described below were adopted.

Log-law equation for turbulent flow in pipes may be expressed as:

$$\frac{u(y)}{u_*} = \frac{1}{\kappa}\log_e \frac{y}{y_0} \tag{1}$$

where y is the polar coordinate, y_0 is the length representative, $u(y)$ is the velocity at y, u_* is the shear velocity, and $\kappa = 0.4$ is the von Karman constant.

Integrating with respect to r, the relationship between the spatially averaged velocity U and the center velocity ,u_c is given by:

$$U = \frac{Q}{A} = \frac{1}{\pi r^2}\int_0^r u(y)2\pi(r-y)dy = u_c - \frac{1.5u_*}{\kappa} \tag{2}$$

where Q is the volume rate of discharge, A is the area of pipe, and r is the radius of pipe.

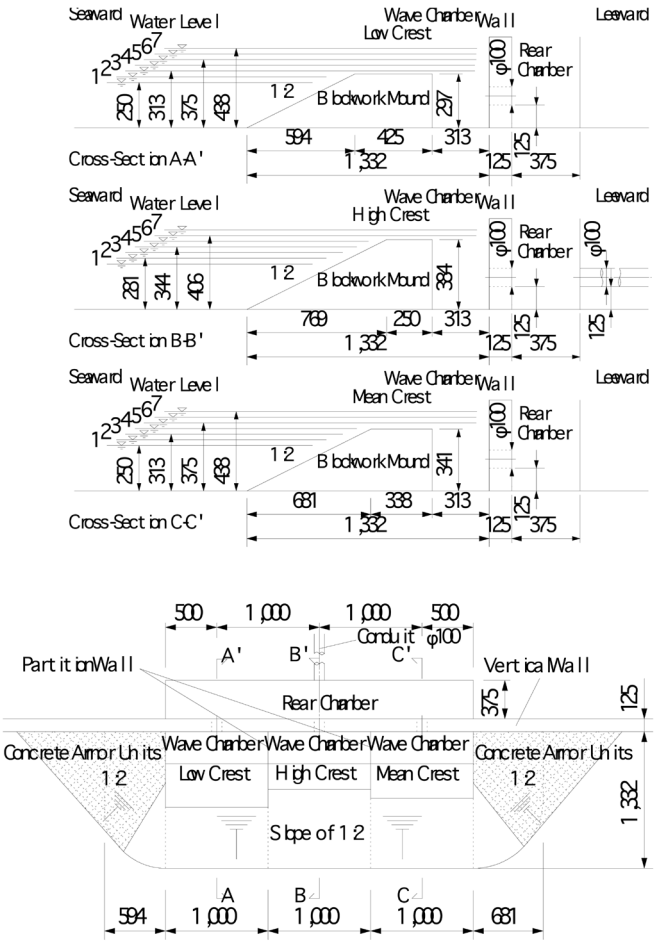

Figure 4. Plane view and cross section of model B with three different crests and rear chamber.

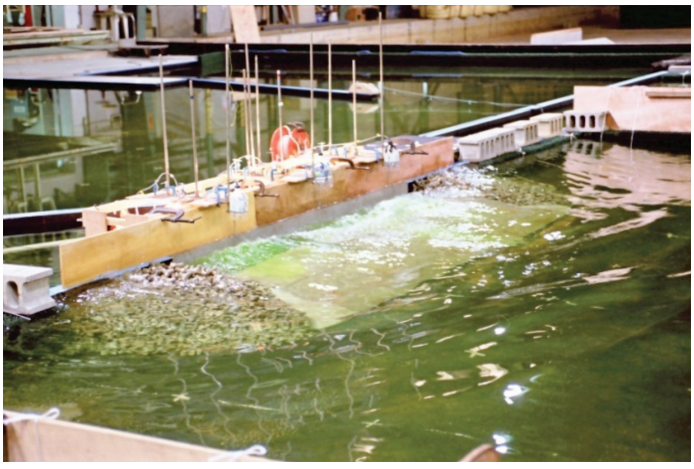

Figure 5. Photograph of seawater exchange structures with blockwork mounds in the experiment.

Shear velocity u_* and the friction loss factor f are defined as:

$$u_* = \sqrt{\frac{\tau_0}{\rho}} = \sqrt{\frac{f}{8}}U \tag{3}$$

$$f = \frac{8gn^2}{(D/4)^{1/3}} \tag{4}$$

where τ_0 is the shear stress, ρ is the water density, g is the gravitational acceleration, D is the diameter of pipe, and n is the Manning's roughness coefficient.

Consequently, relation between the spatially averaged velocity U and the center velocity u_c is given by:

$$U = \frac{u_c}{1 + \dfrac{3.75ng^{1/2}}{(D/4)^{1/6}}} \tag{5}$$

In the experiments, D, n, and g in Equation 5 should be taken as 0.10 m, 0.010 $m^{-1/3}s$, and 9.8 m/s^2, respectively, and finally $U=0.82u_c$ is obtained.

Methodology for Prediction of Discharge

Volume rate of discharge from the mound. The discharge model may not only be necessary for predicting the volume rate of discharge, but is also helpful to investigate hydraulic characteristics on the seawater exchange structures with blockwork mounds. The discharge model on the structures for regular waves were newly developed and calculated.

The relationship between the time-averaged surface elevation in the wave chamber without discharge and the deepwater wave height for regular waves may be given by:

$$\begin{aligned}
&\frac{\eta_c}{H_0} = 0.49\left(1 - \frac{h_c}{1.2H_0}\right) && for\ -0.2 \le \frac{h_c}{H_0} \le 1.2 \\
&\frac{\eta_c}{H_0} = 0.49\,\frac{7}{6}\left(1.2 + \frac{h_c}{H_0}\right) && for\ -1.2 \le \frac{h_c}{H_0} \le -0.2 \\
&\eta_c = 0 && for\ \frac{h_c}{H_0} < -1.2,\quad 1.2 < \frac{h_c}{H_0}
\end{aligned} \tag{6}$$

where $\overline{\eta}_c$ is the time-averaged surface elevation in wave chamber without discharge, h_c is the crest elevation from still water level, and H_0 is the deepwater wave height.

Considering both of the past experimental results on blockwork mounds and the results from this study, formulas in Equation 6 are determined to be a concise expression excluding the effect of wave period. Yamamoto et al. (1988) examined the relationship between the time-averaged surface eleva-

tion in the wave chamber without discharge and the deepwater wave height for regular waves, and determined that $\overline{\eta} = 0.49H_0$ when $h_c = 0$ was obtained.

We assumed that the phenomena in the wave chamber are steady as shown in Figure 6. Based on Bernoulli's theorem, the volume rate of discharge for incident wave Q_1 and the discharge for return flow from wave chamber Q_R can be expressed as:

$$Q_I = C_I \sqrt{2g\eta_c}\ (\eta_c + h_c)\, B \tag{7}$$

$$Q_R = C_R \sqrt{2g\eta}\ (\eta + h_c)\, B \tag{8}$$

where C_1 is the coefficient of discharge for incident wave, C_R is the coefficient of discharge for return flow, $\overline{\eta}$ is the time-averaged surface elevation in wave chamber with discharge, and B is the crest length.

The volume rate of discharge for inlet flow Q_T may be written as:

$$Q_T = C_{in}\, A \sqrt{2g(\eta - \eta_{out})} \tag{9}$$

$$C_{in} = \sqrt{\frac{1}{f_e + f_o + f_b + \dfrac{f\,l}{D}}} \tag{10}$$

where $\overline{\eta}_{out}$ is the time averaged surface elevation at exit, C_{in} is the coefficient of discharge for inlet flow, f_e is the entrance loss factor, f_o is the outlet loss factor, f_b is the bending loss factor, and l is the pipe length.

The continuity of the volume rate of discharge in the wave chamber gives:

$$Q_I = Q_R + Q_T \tag{11}$$

Surface elevation with discharge $\overline{\eta}$ can be calculated easily from these equations mentioned above with the Newton-Raphson method. It should consider the conditions which are $0 \le \overline{\eta} \le \overline{\eta}_c$ and $h_c = 0$ for $h_c < 0$.

The volume rate of discharge for inlet flow Q_T can be calculated by the present discharge model.

Volume rate of discharge from rear chamber. The volume rate of discharge for inlet flow from each mound can be calculated by the discharge model described above. The volume rate of discharge from the rear water chamber may also be expressed as given by Equation 9 and Equation 10 by substituting the time-averaged surface elevation at the rear chamber $\overline{\eta}_{wc}$ into $\overline{\eta}$. It is also necessary to take into account the continuity of the volume rate of discharge in the rear chamber. The equation of continuity is written as:

$$\sum_{k=1}^{3} Q_{T,k} = Q_{T,4} \tag{12}$$

where $Q_{T,\kappa}$ is the volume rate of discharge for the inlet flow from each mound and $Q_{T,4}$ is the volume rate of discharge from the rear water chamber.

Equation 12 can be arranged as:

$$\sum_{k=1}^{3} C_{in,k}\, A_k\ sign(\overline{\eta}_k - \overline{\eta}_{wc}) \sqrt{2g|\overline{\eta}_k - \overline{\eta}_{wc}|} = C_{in,wc}\, A_{wc}\ sign(\overline{\eta}_{wc} - \overline{\eta}_{out}) \sqrt{2g|\overline{\eta}_{wc} - \overline{\eta}_{out}|} \tag{13}$$

Then $\overline{\eta}_{wc}$ can be calculated by solving Equation 13. Thus $Q_{T,k}$ and $Q_{T,4}$ can be obtained. The conditions are $0 \le \overline{\eta}_{wc} \le \max(\overline{\eta}_1, \overline{\eta}_2, \overline{\eta}_3)$, $\overline{\eta}_k = \overline{\eta}_{wc}$ for $\overline{\eta}_\kappa + h_{c,\kappa} < 0$ and $\overline{\eta}_{wc} + h_{c,k} < 0$, $\overline{\eta}_\kappa = h_{c,k}$ for $\overline{\eta}_\kappa + h_{c,k} < 0$, and $\overline{\eta}_{wc} + h_{c,\kappa} \ge 0$.

Results and Discussion

Figure 7 shows the results on the mass of water transport from each mound Q for model A. From the figures, it is easily seen that the discharge from each mound appears effective at a different water level. When water level is comparatively low, the discharge from low crest mounds shows high performance. On the other hand, when water level is comparatively high, the discharge from high crest mounds shows high ability. The reason is that the wave setup in the wave chamber that induces the discharge for inlet flow from each mound is influenced by the crest elevation from the still water level. The volume rate of discharge becomes gradually larger for longer and larger waves.

Figure 8 shows the dimensionless volume rate of discharge from each mound Q^* for model A as a function of crest elevation by deepwater wave height h_c/H_0. The volume of water transport for unit width Q/B is normalized by the amount $HL/2\pi$, which is equivalent to the water volume set in motion during a half wave period where H is the incident wave height and L is the wave length. From the figure, it can be seen that the value of the dimensionless volume rate of discharge becomes maximum, say about 0.25 when h_c/H_0 is around –0.2 for each mound. It affirms that the water transport function is effective when $-1.2 < h_c/H_0 < 1.2$.

Figure 9 shows the time-averaged surface elevation in wave chamber for model A as a function of

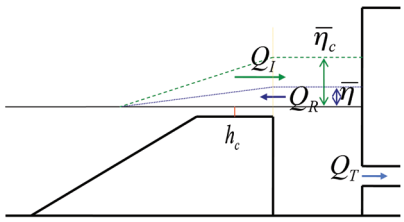

Figure 6. Time-averaged phenomena in wave chamber.

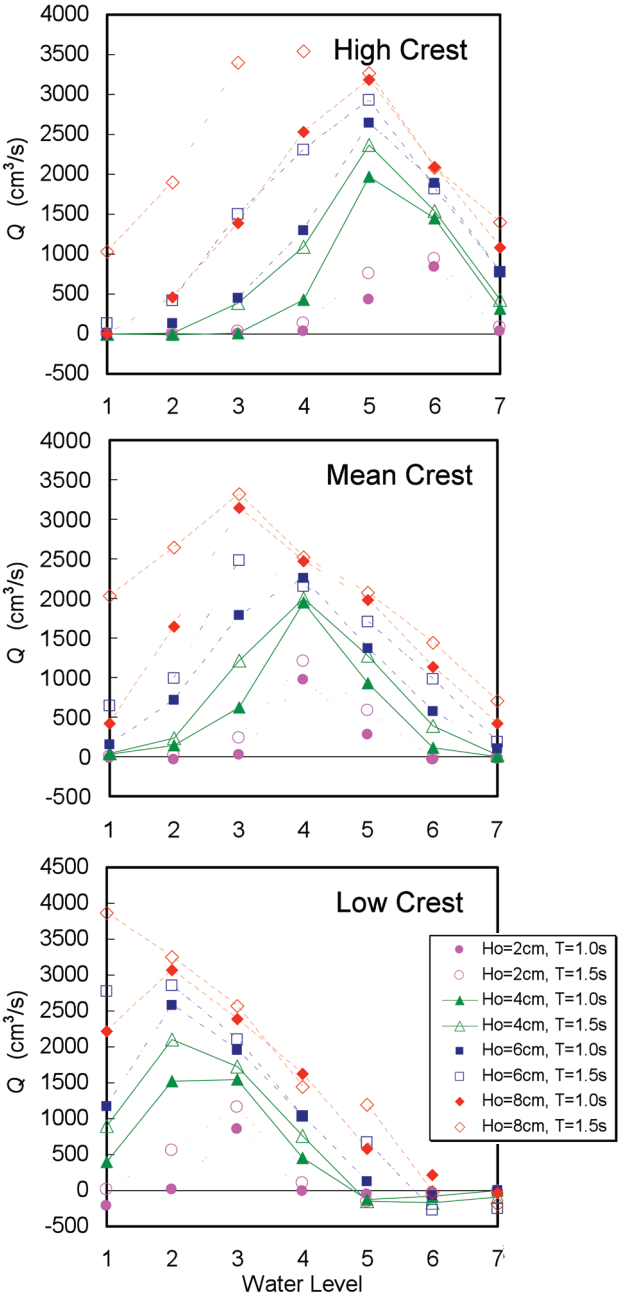

Figure 7. Volume rate of discharge for inlet flow from each mound for model A.

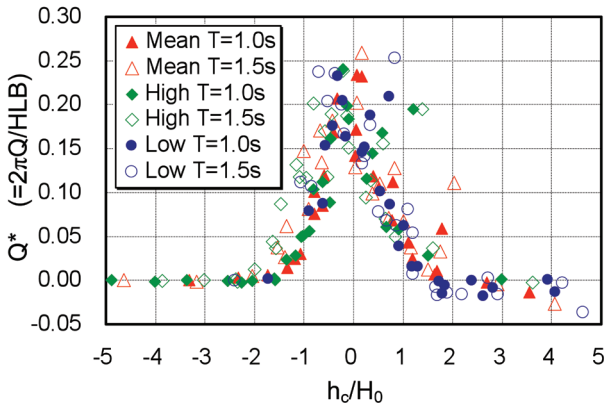

Figure 8. Dimensionless volume rate of discharge for inlet flow from each mound for model A.

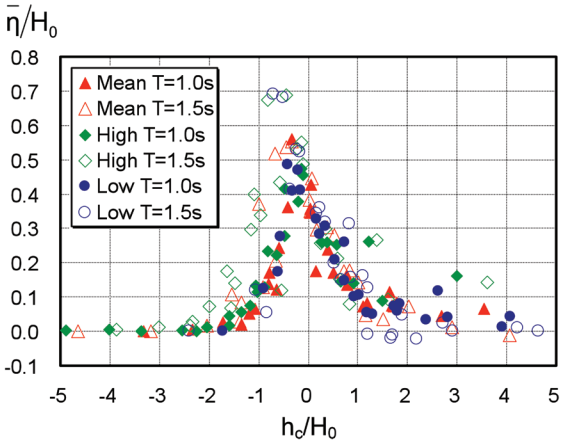

Figure 9. Time-averaged surface elevation in wave chamber for model A.

h_c/H_0. From the figure, it can be seen that the value of the dimensionless surface elevation becomes maximum at about 0.5 when h_c/H_0 is around –0.2 for each mound. It is also confirmed that the surface elevation occurs when $-1.2 < h_c/H_0 < 1.2$. The relationship between surface elevation and h_c/H_0 is linear over the range of $-1.2 < h_c/H_0 < 1.2$, which is the same tendency as shown in Figure 8.

Figure 10 shows the comparison of measured and calculated time-averaged surface elevation in wave chamber for model A. In the calculation, C_I, C_R, f_{fe}, f_{fo}, f_{fb}, and $\overline{\eta}_{out}$ were chosen as 1.0, 1.0, 1.0, 1.0, 0.29, and 0.0, respectively. The calculated surface elevations show fairly good agreement.

Figure 11 shows the comparison of measured and calculated volume rates of discharge for inlet flow from each mound for model A. The calculated

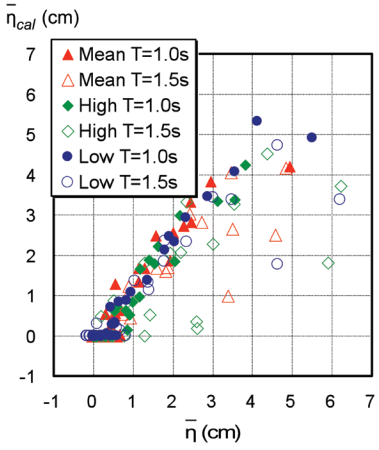

Figure 10 (at right). Comparison of measured and calculated time-averaged surface elevation in wave chamber for model A.

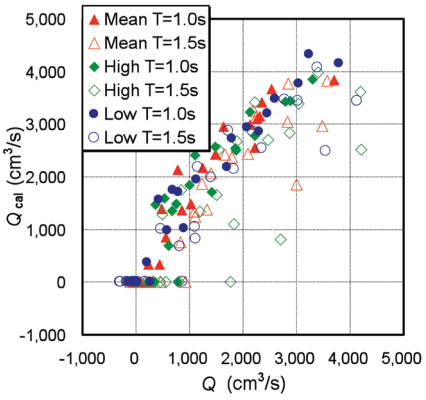

Figure 11 (at right). Comparison of measured and calculated volume rate of discharge for inlet flow from each mound for model A.

values also show fairly good agreement. The data show that the volume rate of discharge for inlet flow through the conduit can be estimated quantitatively by the present discharge model. However, the calculated values are overestimates compared with the value from the experiment, so there needs to be a correction to increase accuracy.

Figure 12 shows the comparison of measured and calculated volume rates of discharge from the rear chamber for model B. In the calculation, f_e, f_o, f_b, and $\overline{\eta}_{out}$ for pipes from the rear chamber were chosen as 1.0, 1.0, 0.256, 0.0, respectively, and C_I, C_R, f_{fe}, f_{fo}, and f_{fb} for pipes from each mound were 1.0, 1.0, 1.0, 1.0, and 0.0, respectively. From the figure, we see that both measured and calculated volume rates of discharge from the rear chamber appear reasonable over a range of water levels. However, when the water level is relatively high, say levels 6 and 7, the volume rate of discharge decreases. It should be noted that the calculated values show fairly good agreement

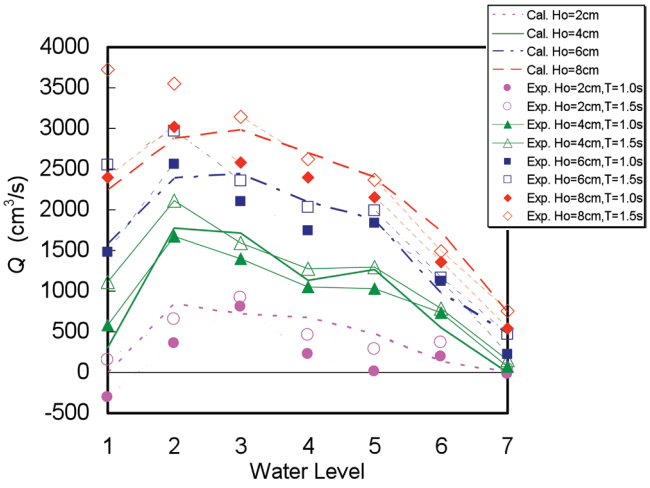

Figure 12. Comparison of measured and calculated volume rate of discharge from rear chamber for model B.

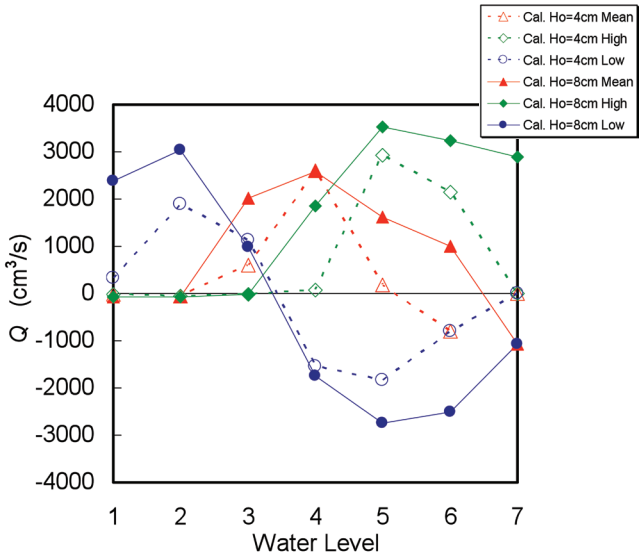

Figure 13. Calculated volume rate of discharge for inlet flow from each mound for model B.

compared to the values from the experiment.

Figure 13 shows the calculated volume rate of discharge for inlet flow from each mound in model B. We see that the volume rate of discharge for inlet flow from low mounds show negative values when the water level is relatively high. It may be necessary to change the specifications of the structures in order to prevent return flow from the rear water chamber. The present discharge model may be useful in predicting the performance and specifications of the structures.

Conclusions

Our results confirm that both of the models are effective not only for wide tidal change but also for long conduits because the different crest heights play an important role to induce the wave setup in wave chambers over a wide range of water levels. We also affirmed that the volume rate of discharge for inlet flow through conduits can be estimated quantitatively by the present discharge model, although correction may be needed for more accuracy. In addition, the present discharge model may be helpful in predicting the effective specifications for modified seawater exchange structures using blockwork mounds. It is hoped that the technology of seawater exchange structures can play a significant role on aquaculture grounds in the near future.

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Novel Culture Feed for Short-neck Clam Using Single-cell Material from Porphyra

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Keywords: short-neck clam, Porphyra, food supplement

Abstract

The short-neck clam (Manila clam [*Ruditapes philippinarum*]) is an important Japanese seafood and supports profitable coastal fishing. Short-neck clam culture is a key component and rapidly increasing area of Japanese aquatic production. Mainstream production is from natural populations while, increasingly, stocks are close to or have exceeded utmost sustainable yields. Stock improvement through the capture and imparting of natural seed in both extensive and intensive forms of culture is a frequent practice.

Nevertheless, the consistency of natural recruitment can never be guaranteed. A method to understand how to meet the seed requirements of the short-neck clam is the hatchery culture trial. The production of seed through hatchery propagation accounts at present for only a small percentage and may be attributed to the unavailability of artificial feeds. Although shortneck clam larvae and spats have been reared successfully for algal foods (i.e., mainly diatoms), these are expensive to produce and do not always coordinate with producer's requirements; researchers have paid insufficient attention to investigating substitutes for algal foods. Expensive algal concentrates, universally used throughout the world, have overcome some constraints associated with shelf life of algal concentrate, including storage of algal foods.

Red algae (*Porphyra* spp.) are widely cultured on the coastal lines of Japan, are rich in nutrients, and are known as functional seafoods. Generally, Porphyra has a high percentage of protein and also contains an outstandingly high amount of taurine, which is an important amino acid for larval marine animals in addition to various kinds of minerals and vitamins.

Recent innovations in biotechnology including protoplast/spheroplast isolation techniques using polysaccharide-degrading enzymes have shown some promise to use these nutritious algae as a food supplement without cell walls, because these single-cell materials are easily digestible when ingested. We therefore attempted to develop a novel feed for culturing shellfish by using single-cell materials obtained by enzymatic means and to determine the dietary effect of this feed for growth and production of short-neck clam. Many successful results have been obtained in the series of experiments so far.

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Culture Systems and Habitat Evaluation Branch Overview– Molluscan Aquaculture

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Keywords: aquaculture, bivalves, bay scallops

Abstract

The Culture Systems and Habitat Evaluation Branch of the Aquaculture and Enhancement Division can trace its scientific roots to the history of Milford Laboratory. Early research led by Dr. Victor Loosanoff succeeded in devising techniques for larval rearing of bivalves, enabling the development of the aquaculture industry as we know it today. Milford research was extended to nutrition, immunology, disease control, field grow out, and predator control.

While early Milford research focused on the eastern oyster (*Crassostrea virginica*), rearing methods were applied to many bivalve species. Research during the 1970s developed culture methods for the bay scallop (*Argopecten irradians irradians*), the Atlantic surf clam (*Spisula solidissima*), and the hard clam (*Mercenaria mercenaria*).

Current branch research has concentrated on investigating recirculating aquaculture seawater systems to grow large (25 mm) bay scallop seed under controlled conditions. The ideal system would be highly automated, economic to operate, and generate little or no waste discharge. Producing large seed bay scallops allows for single season grow out to market size in the northeastern United States and also provides release animals that would likely spawn naturally in stock enhancement efforts. Research topics include understanding the chemical ecology within closed systems, measuring the physiological responses of scallops to different culture parameters, overcoming unexplained winter mortalities, and defining the ecology of natural populations.

The branch has recently incorporated research themes that reflect the National Oceanic and Atmospheric Administration's priorities to further expand the U.S. aquaculture industry. These include refining sustainable high-volume seed production methods, determining interactions between aquaculture operations and the environment, and exploring stock enhancement strategies for shellfish. Research on aquaculture and stock enhancement of bay scallops is particularly pertinent because of marked declines in natural bay scallop populations over the past decades.

Bay Scallop (*Argopecten irradians irradians*) Husbandry Program

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Keywords: bay scallop, husbandry, adductor muscle, seed

Abstract

The bay scallop (*Argopecten irradians irradians*) is a recreationally and commercially harvested bivalve in the eastern United States. Imported to China in the early 1980s, it has become a major part of that country's aquaculture production. It has many characteristics that make it a prime candidate for aquaculture: rapid growth rate, achieving market size in less than 18 months, well-known husbandry methods, amenability to culture in nets or pens, high consumer acceptance, and a primary consumer.

Unfortunately, the bay scallop has variable survival throughout the winter in New England, which currently restricts bay scallop aquaculture to a single-season endeavor. When scallops were grown in lantern nets in a single season in Long Island Sound, Connecticut, they reached a mean shell height of 50 mm. In the United States, only the scallops' adductor muscle is consumed. Raising scallops to 50 mm yields a three gram adductor muscle, which is not economically profitable in the United States at this time.

It is possible to produce a larger adductor muscle in a single season by relying on shellfish hatcheries to produce scallop seed early in the season. If a 25 mm scallop could be deployed by May in the northeast United States, it should grow to a minimum size of 62 mm, which would double the adductor muscle yield to six grams per scallop.

Any method to produce larger seed in the northeast United States must rely on heated sea water and economically producing large amounts of phytoplankton. One method to reduce seawater heating demands would be to rely on partially recirculated bay scallop culture of post-set (dissoconchs). In recirculating shellfish culture systems at the Milford laboratory, we need to monitor the accumulation of toxic nitrogenous compounds. Recently we found that scallops were most susceptible to ammonia in comparison to nitrite or nitrate. We have developed automated demand feeding systems to minimize the nitrogen load, in particular ammonia, in recirculating scallop culture systems.

Our current research indicates that low cell concentrations, 1,000 cells/ml of the phytoplankton *Tetraselmis chuii*, yields faster growth of scallops than those cultured at higher cell concentrations. Research is now focusing on augmenting the single-algal species to produce a superior diet that will yield faster growth rates.

Growth and Stress Response of Bivalve Mollusks in Response to Different Microalgal Diets and Arachidonic Acid Supplementation

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Keywords: scallop, diets, fatty acids

Abstract

The bay scallop (*Argopecten irradians irradians*) and sea scallop (*Placopecten magellanicus*) are two commercially important scallop species in the United States. Few data exist concerning dietary requirements during the early postlarval stages of these species, and therefore costly multispecies algal diets are often used in hatcheries to ensure high growth and survival. Thus there is an interest in identifying cost-effective, high-performance algal diets for implementation in commercial hatcheries, and in identifying specific dietary compounds which will not only enhance growth but also reduce stress response, thereby improving health and survival.

Bay and sea scallops were grown in recirculating systems during five separate trials, each lasting 3–4 weeks. Scallops were offered unialgal and binary diets consisting of one of three diatoms and one of five flagellates. Two binary diet combinations, CHGRA (*Chaetoceros muelleri*) in combination with either *Pavlova* spp. (CCMP strain 459, Pav 459) or *P. pinguis*, consistently ranked highest among the diets tested for both scallop species.

While previous work has established a requirement for n-3 fatty acids in bivalves, our work strongly suggests that scallop growth rate is influenced by two n-6 polyunsaturated fatty acids (PUFA): arachidonic acid (AA) found in CHGRA and docosapentaenoic acid provided by Pav 459 and *P. pinguis*. Enrichment in tissues (relative to diet) of these individual fatty acids, as well as total n-6 fatty acids, were observed in tissues of both scallop species regardless of dietary treatment, suggesting a requirement for n-6 fatty acids in pectinids that has been largely overlooked.

The specific role of AA was further examined by offering AA supplemented algal diets to bay scallop larvae and juveniles. Changes in hemocyte morphology were associated with AA supplementation as well as with stress, imposed by centrifugation. Future work will determine whether cortisol concentrations can be used as a measure of stress in bivalves, as previously shown in vertebrate systems. To this end, an enzyme-linked immunosorbent assay, also known as an ELISA, is currently under development to measure cortisol concentrations in bivalve hemolymph.

Biotechnology Branch Overview on Probiotic Bacteria and Harmful Algal Bloom Research

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Keywords: biotechnology, HAB, probiotic bacteria, bivalves

Abstract

The research of the Northeast Fisheries Science Center's Biotechnology Branch applies the contemporary tools of several scientific disciplines, including genetics, proteomics, microbiology, immunology, chemistry, and ecology to research relevant to marine aquaculture and its ecosystem interactions. Examples of several specific projects are presented by other branch personnel; this presentation focuses on two additional areas of research: probiotic bacteria for use in bivalve hatcheries and harmful algal bloom (HAB) interactions with aquacultured mollusks.

The project on probiotic bacteria was established by a postdoctorate from the National Fisheries Research and Development Institute in Korea, Dr. Hyun-Jeong Lim. Working with branch staff, she isolated a number of bacterial strains from within the shells of healthy mollusks and screened these for biological effects on oyster larvae and on bacterial pathogens of oyster larvae. Promising strains have been used in challenge experiments with oyster larvae. General findings are that the probiotic strains can support improved survival and growth of larval oysters, alone and in the presence of pathogens, but that the effectiveness depends on the dose administered. Experiments are continuing to refine effective administration of probiotic strains.

The branch's HAB research has focused primarily on trophic interactions between bivalves and HAB taxa, including dinoflagellates, prymnesiophytes, and raphidophytes. Our main accomplishments in this area are: 1) demonstrated variable expression of toxic effects by the dinoflagellate *Prorocentrum minimum* on several shellfish species, including pathologies, immunomodulation, and mortality; 2) developed a new method to produce large numbers of *Alexandrium* resting cysts, allowing experiments demonstrating accumulation of saxitoxins in oysters feeding on the resting cysts; 3) revealed a widespread risk that HABs can be introduced into receiving waters when bivalves are transplanted, but 4) found a cost-effective means to mitigate this risk by holding shellfish 24 hours out of water between harvest and transplant; and 5) showed that bivalves infested with parasitic diseases are more susceptible to immunomodulation and development of pathologies by HABs than are nonparasitized individuals.

In addition to research, the branch provides direct support to the shellfish industry by convening the Milford Aquaculture Seminar and Milford Microalgal Culture Workshop, by providing microalgal seed cultures and advice on their use in commercial hatcheries, and by troubleshooting microalgal culture and disease problems in hatcheries, either on-site or remotely. These activities serve industry throughout the United States, not just in the northeast region.

The Importance of Nutrients, Light Duration, Light Intensity, and pH when Growing Large Volumes of Phytoplankton

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Keywords: mass cultures, microalgae

Abstract

A number of variables, including pH, light intensity, day length, nutrient availability, and temperature, are important in mass culturing microalgae. In aquaculture, large volumes of phytoplankton food are often grown outside to reduce culturing costs. However, mass cultures of phytoplankton outdoors are complex because light intensity, day length, and temperature are not as easy to control as in the laboratory. Furthermore, outdoor cultures often become contaminated with bacteria and other algal species. This presentation focused on how different light intensity, day length, pH, and contaminates that are often seen when growing cultures outdoors can affect the growth of a phytoplankton feed.

Phytoplankton Ecology and Aquaculture

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Keywords: phytoplankton, shellfish, toxicity, scallops

Abstract

Phytoplankton provide not only food for shellfish, but also can include species harmful or toxic to shellfish. Shellfish, on the other hand, as one major group of consumers of phytoplankton in some coastal marine environments, do not affect phytoplankton by simply reducing their biomass, but also by selectively consuming some groups of phytoplankton and by providing recycled nutrients. Thus the interactions between phytoplankton and shellfish can be complex. My research is aimed at studying such interactions in laboratory, seminatural, and in the near future, natural aquaculture settings.

In the laboratory, we examined the possible relationship between the toxicity of the dinoflagellate *Prorocentrum minimum* to bay scallops (*Argopecten irradians irradians*) and proteins that this harmful alga excretes into the culture medium. Although *P. minimum* is generally considered a harmful species, its toxicity varies between strains and according to different physiological status.

In this study, a gradient of toxicity of this particular strain was achieved by reducing the supply of phosphate or carbon. Using state-of-the-art protein-profiling technology—the ProteinChip and surface enhanced laser disorption ionization, time of flight mass spectrometry (SELDI-TOF-MS)—a number of proteins were detected and some patterns of protein expression associated with toxicity were revealed. This study can help with the identification of the toxin or toxins responsible and may lead to the development of tools for detecting *P. minimum* toxicity and providing an early warning in a natural environment.

The impact of scallops on their surroundings was examined in a close-to-natural, yet controlled environment by using 10 m–long raceway tanks with constant flow of seawater. In this seminatural setting, scallops were exposed to natural seawater with mixed particles, and the net uptake of particles was quantified by analyzing inflows, outflows, and settling. The removal of phytoplankton by scallops was quantified, but the other effects of scallops upon phytoplankton community structure, such as those attributable to the change in nutrient supply, could not be examined as the residence time in the tank was too short.

Thus our next step will be examining the interaction of shellfish with the environment in a natural aquaculture setting.

Cross-field Technologies in Aquaculture

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Keywords: software, cross applications, aquaculture

Abstract

When determining how to do something new, it makes sense to consider whether there are tools already available that can be used. Often tools and techniques, collectively referred to as technologies, are developed within specific fields or industries for specific purposes. These techniques often can be transferred, with or without modification, to other seemingly unrelated applications.

Adaptation, scale-up, and scale-down of existing technologies for aquaculture applications will have special considerations and conditions under any given circumstance. Requirements for precision may constrain the level of technology to apply. For example, there may be no reason to invest in industrial process control software and programming if a light timer can accomplish the task needed. However, process control software and requisite interfaces are desirable for research and development applications where requirements evolve and change. One of the most important aspects of these considerations is a thorough knowledge of not only the engineering aspects of a project but also how an aquacultured organism can perform in the given conditions.

Hollow fiber filters, as well as tangential flow and membrane plate filters, were developed for the food processing and blood dialysis industries. These filters are readily employable in the aquaculture industry. However, prefiltration is absolutely essential in aquaculture applications. Industrial process control software and circuit systems are steadily being applied to aquaculture processes as the aquaculture industry grows and evolves. Cross applications such as these are only a few examples of what has been accomplished and what is still to come as aquaculture evolves.

An Overview of Genetic Studies on Commercial Species of Bivalves

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Keywords: genetics, inbreeding depression, markers

Abstract

With significant advances in the culture of marine organisms has come increased interest in improving genetic traits—especially those of economic importance, such as growth, survival, and disease resistance—for increased productivity.

The overall goal of breeding or management of a species is to maximize productivity. An understanding of what it takes to maximize production enables the breeder or culturist to recognize signs of inbreeding depression which could be manifested as slower growth, decreased viability, disease susceptibility, or overall decreased production. An example of inadequate attention to genetic consequences of culture with bay scallops (*Argopecten irradians irradians*) occurred when some of the industry collapsed which was attributed to inbreeding depression from a narrow gene pool.

Genetics of commercial oysters, clams, and scallops were reviewed from such perspectives encompassing three aspects: breeding or quantitative genetics, chromosomal or cytogenetics, and molecular (DNA) genetics. These areas of genetics have been applied to commercial bivalves separately and in combination with varying degrees of success as measured by different responses for hatchery culture and in field programs.

Conventional approaches consist of breeding methodology similar to that applied historically in agricultural genetics with the domestication of farm animals and crops. Selective breeding and heritability values have indicated positive responses for growth in bivalves. Chromosome manipulation to induce polyploidy and cloning also has produced some favorable results. Alternatively, biotechnological techniques can be employed to facilitate progress in improvements.

Various types of molecular markers are being used to supplement conventional approaches of breeding to improve characteristics with quantitative trait loci in marker-assisted selection. In addition, genetic markers are being used to identify stocks and estimate genetic diversity of wild populations. DNA markers have been observed to result in variation with many alleles that could be useful for applications such as species, stock, and population identification. If environmental and habitat qualities are not suitable, however, genetic improvement in desired traits may not find expression, an important consideration for future developments in increasing the commercial production of bivalves.