# UJNR Aquaculture Panel

# 36th Scientific Symposium

# Aquaculture Technologies for Invertebrates

**October 29th and 30th at University of New Hampshire and**

**November 2nd at Northeast Fisheries Science Center, Milford Aquaculture Lab**

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## Contents

**The Seventh Three-year Plan of the UJNR Aquaculture Panel, 2007-2009**

This plan represents a change from five year planning to three year planning in order to be more responsive to rapid changes in quickly emerging issues related to aquaculture. The future success of seafood production must integrate aquaculture into coastal communities and benefit from the synergies of having both traditional capture fisheries and aquaculture, to maximize the economic value and societal benefits. Advanced technologies are being applied with great success and benefits in all areas of our lives. Aquaculture is no exception. Advances in biotechnology applied to aquaculture affects feeds, reproduction, organism health, product quality, human health, breeding, and ultimately the economics and environmental impacts of aquaculture. Further, advanced technology in culture systems, such as open-ocean, multi-tropic level, and recirculation systems, are rapidly evolving and promise to produce more product at less economic and environmental cost. The social and economic foundations of aquaculture in the US and Japan greatly impact the development and growth of aquaculture industries in both countries and will continue to be important in the future. In the present three-year plan we will address these issues to enhance the management and development of aquaculture in our countries including the implications for industry, consumer health and environmental impacts. Specifically we will focus on how advanced technologies, social and economic structure, and interactions of aquaculture and the environment impact future feeds development and invertebrate culture. The last meeting of this plan will integrate the multi-faceted aspects of fisheries and fishing communities relating to aquaculture.

1) Aquaculture Technologies for Invertebrates. (Durham, New Hampshire, 2007)

Culture systems for mollusks, crustaceans, echinoderms, and other invertebrates with commercial importance will be considered.

2) The Future of Aquaculture feeds. (Yokohama, November, 2008)

Sources for essential nutrients for aquatic animals, the tailoring of feeds for special life stages (larvae or broodstock), special situations (low pollution, off-shore, recirculation

systems, animal health) or for enhanced product quality (human health, product stability/quality) will be investigated.

3) Interactions of fisheries and fishing communities related to aquaculture (USA TBD, 2009)

The interrelated roles of fisheries and aquaculture in managing the coastal environment, economics of fishing communities, resource allocation, management costs, and how advanced technologies impact these issues will proved the focus for this last symposium of the three year plan.

Approved at the 35th meeting of UJNR**,** Mie, Japan 2006

**International Symposium on Current Status and Future Development of Tuna Aquaculture**

**- A Satellite Symposium of 5th World Fisheries Congress at Yokohama -**

**http://www.5thwfc2008.com/index.html -**

Global tuna consumption is increasing with worldwide expansion of eating fish promoted by the health oriented people and it makes some tuna stocks being decreasing. Recent increasing trend of capturing small size bluefin tuna for aquaculture also makes a new concern on stock management. It is necessary to develop urgently the tuna aquaculture using artificial produced juvenile. In this symposium, participants will discuss how we grow the tuna aquaculture industry and supply safe and reliable tunas continuously to the market.

**Date:**

October 25-26, 2008

**Venue:**

Yokohama Port Opening Memorial Hall
1-6, Hon-cho, Naka-ku, Yokohama-shi, 231-0005 Japan
TEL□+81-45-201-0708
□A ten-minute walk from Kannai Station (JR Line, Yokohama Municipal Subway)
□A three-minute walk from Nihon Odori Station (Minato Mirai Line)

**Keynote Speakers:**

Invited speakers will present current and future Bluefin Tuna farming in Japan, North America, and Europe.

**Official language:**

English (Japanese simultaneous interpretation will be prepared.)

**Organizing Committee:**

Chair: Tokio Wada, Fisheries Research Agency
Committee secretary: Misao Arimoto, Fisheries Research Agency

**Social event:**

The field excursion will be held at 25th October morning, visiting MISAKI tuna landing port and processing factory.

**Inquiries:** Dr. Misao Arimoto (arimoto3@affrc.go.jp)
Fisheries Research Agency
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**The First International Symposium on Asari Clam**

**-Stock enhancement and management-**

October 25-26, 2008

***National Research Institute of Fisheries Science, Fisheries Research Agency***

***Yokohama, Japan***

The production of Asari clam (Manila clam, *Ruditapes philippinarum*) has heavily declined in Japan and Korea since 1986 and 1992, respectively. The Japanese National Committee of Manila Clam Stock management has been taking various measures to recover the production in Japan; however, because of the complex nature of causes of the stock decline, more diversified information sources are required. Although more than 80% of Manila clam used to be produced in eastern Asian countries, it is now also produced in many countries in North America and Europe. Different countries have different problems in the clam production and therefore different information to cope with. Information exchanges are in the mutual interest of scientists and civil servants in many countries.

The First International Symposium on Manila clam provides an opportunity for scientists from many countries to get together and share invaluable information about Manila clam stock enhancement and management.

**Topics to be covered:**

Scientific stock management of the Manila clam

Biological analyses of the physiological conditions of the Manila clam

Diseases and pathogen of the Manila clam

Environmental conditions affecting the standing stock of the Manila clam

Reclamation techniques of Manila clam fishing ground

**Participants:**

Scientists, fisheries managers, and others with a specific interest in the Manila clam fishing are welcome to attend.

**Symposium Organization Committee:**

Dr. Masami Hamaguchi

National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency

Dr. Junya Higano

National Research Institute of Aquaculture, Fisheries Research Agency

Dr. Satoshi Watanabe
National Research Institute of Fisheries Science, Fisheries Research Agency
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**UJNR Scientific Symposium Program**

October 29-30, 2007

New England Center, University of New Hampshire

Day 1- October 29, 2007

Afternoon session 13:30 –17:00

13:30- 13:45 Welcome, logistics and opening remarks- Richard Langan, University of New Hampshire

13:45-13:55 Opening remarks - Hiroshi Nakano, Japan Panel Chairman

13:55-14:20 The Basic Plan for Fisheries and current status of aquaculture research in Japan- Kazumasa Ikuta, Aquaculture Systems Division, National Research Institute of Aquaculture

14:20-14:45 Overview of the National Oceanic and Atmospheric Administration's Aquaculture Program- Michael Rubino, NOAA Aquaculture Program Manager

Crustacean Culture

14:45-15:05 Assessment of the Genetic Diversity of Wild Kuruma Prawn Based on the Relatedness Analysis Using Microsatellites DNA and Mitochondrial DNA Markers-Takuma Sugaya, [*], Kazuhisa Teruya[1], Masahiro Kato and Keiichi Mushiake, National Research Institute of Aquaculture

15:05-15:25 Marine Ornamental Aquaculture- Junda Lin, Florida Institute of Technology

15:25-15:45 Refreshment break

15:45-16:05 Nutritional Significance of n-3 Highly Unsaturated Fatty Acids to the Larval Survival and Development in Mass Seed Production of Brachyuran Crabs- Shigeki Dan, Katsuyuki Hamasaki, Takayuki Kogane, Tadao Jinbo, Takashi Ichikawa, Seikai National Fisheries Research Institute

16:05-16:25 Hatchery production and stock enhancement of the Chesapeake Bay blue crab- Odi Zmora and Yonathan Zohar, Center of Marine Biotechnology, University of Maryland Biotechnology Institute

16:25-16:45 An Outline of the Research Project, Development of Seed Production Technology in Japanese Spiny Lobster- Hideaki Aono, Keisuke Murakami, Masahiko Awaji, National Center for Stock Enhancement

16:45-17:00 Questions and Discussion

Day 2- Tuesday, October 30

Mollusc Culture

8:30-8:50 Shellfish aquaculture research on oysters and clams at Haskin Shellfish Research Laboratory- John Kraeuter, Associate Director

8:50-9:10 Improvement in yields of the Pacific oyster *Crassostrea gigas* by selective breeding- Chris Langdon, Ford Evans and Alan Barton
Oregon State University

9:10-9:30 Synthesis of Vitellogenin in the Pacific Oyster, *Crassostrea gigas*
- Toshie Matsumoto, National Research Institute of Aquaculture

9:30- 9:50 Disease diagnostics and treatment of hemic neoplasia in the soft-shelled clam *Mya arenaria* - Stephanie Boettger, Department of Zoology, University of New Hampshire

9:50-10:10 Diarrheic Shellfish Toxin and Lipophilic Toxin Profiles in Japanese Bivalves and an Effective Monitoring System by Using a Rapid Assay Kit- Toshiyuki Suzuki, Reiji Sekiguchi , Taketo Jin , Yuri Shirota , Motohisa Honma , Yutaka Okumura , Takashi Kamiyama , Tohoku National Fisheries Research Institute

10:10-10:30 Bivalves as Biofilter: Efficient, Profitable, and Tasty As Well!- Muki Shpigel, Israel Oceanographic and Limnological Research, National Center for Mariculture

10:30-10:50 Refreshment Break

10:50-11:10 Geospatial tools for site selection and production modeling for blue mussels- Carter Newell, Great Eastern Mussel Farms, and John Richardson

11:10-11:30 Considerations on the Fine Scale Topography in Sand Flats, Habitat Heterogeneity and Refuges for Clams- Hajime Saito, Hideyuki Takahashi, Akihiko Matsuda, Yuka Ishihi, Tomoko Sakami, Junya Higano and Hisami Kuwahara, National Research Institute of Fisheries Engineering

11:30-11:50 Methods, Economics and Commercialization of Open Ocean Mussel Culture in The Northeast U.S.- Richard Langan, University of New Hampshire

11:50-12:10 Mollusc culture in the US Pacific Northwest- Bill Dewey, Taylor Shellfish, Inc.

12:10- 12:30 Questions and Discussion

12:30-12:45 Group photo session

12:45-14:00 Lunch

Echinoderms and other species

14:00-14:40 Relationship between gametogenesis and food quality in sea sea urchin gonads - Tatsuya Unuma, Japan Sea National Fisheries Research Institute and Charles Walker, University of New Hampshire

14:40-15:00 An Overview of the Culture of Marine Invertebrates in Maine, USA- Dana L. Morse, Extension Associate, Maine Sea Grant College Program, and University of Maine Cooperative Extension

15:00-15:20 Polyculture of red abalone *Haliotis rufescens* and dulse *Palmaria mollis* in a land-based, recirculation system- Chris Langdon and Ford Evans, Oregon State University

15:20-15:40 Wave Induced Flow in Seawater Exchange Structures for Improving Seawater Quality- Tomohiro Ohmura, National Research Institute of Fisheries Engineering

15:40-16:00 Refreshment Break

16:00-16:20 Novel Culture Feed for Short-neck Clam Using Single-cell Material from *Porphyra*- Takao Yoshimatsu, Alok Kalla, Nakib Dad Khan, Toshiyoshi Araki and Shuichi Sakamoto, National Research Institute of Aquaculture

16:20-16:40 Questions and Discussion

16:40-17:00 Closing Remarks - Bob Iwamoto, USA and Hiroshi Nakano, Japan

# THE BASIC PLAN FOR FISHERIES AND CURRENT STATUS OF AQUACULTURE RESEARCH IN JAPAN

Kazumasa Ikuta

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Alterations to the structure of the fisheries industry has become recently necessitated in Japan due to low levels of capture fisheries production, an increasingly aged population, environmental concerns, and increased world-wide demand for marine food products. In accordance with these developments, the new Basic Plan for Fisheries was established by the Fisheries Agency, Ministry of Agriculture, Forestry and 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 the EEZ 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 multi-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 the development of multi-aquaculture 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, i.e., plants or agricultural waste products, is urgently required in order 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 the present paper, current aquaculture research in the FRA will be introduced.

# OVERVIEW OF NOAA'S AQUACULTURE RESEARCH PLAN

Michael Rubino

NOAA Aquaculture Program

1315 East-West Highway, SSMC3-Route: F-AQ Room 13117,

Silver Spring, Maryland 20910;

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As a federal agency under the U.S. Department of Commerce, the National Oceanic and Atmospheric Administration (NOAA) is focused on creating domestic seafood supply 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 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 will provide 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*.

# ASSESSMENT OF THE GENETIC DIVERSITY OF WILD KURUMA PRAWN BASED ON THE RELATEDNESS ANALYSIS USING MICROSATELLITES DNA AND MITOCHONDRIAL DNA MARKERS

Takuma Sugaya[1*], Kazuhisa Teruya[1], Masahiro Kato[1] and Keiichi Mushiake[1]

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Kuruma prawn *Marsupenaeus japonicus* is a marine shrimp widely distributed from temperate to tropical zones of the world. While this prawn is one of the most famous fishery animals in Japan, the fishery yield has rapidly declined during late 1960's. From such a situation, stock enhancement programs with annual release of approximately 200 million hatchery-reared individuals have been promoted mainly in southern Japan for about 30 years. However, the genetic influences of such the massive stockings are not fully understood because of the lack of the information about the genetic diversity of wild kuruma prawn.

Microsatellites (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. Mitochondrial (mt) DNA also shows high variability because of its nucleotide substitutions. In particular, the control region that does not code for proteins or RNAs shows high variability with regard to relaxed functional constrains. Recently, those markers have been employed not only to estimate population genetic parameters, but also for refined estimates of genetic relatedness between individuals of unknown pedigree from a natural population in many organisms. Then, population genetic structure or level of genetic variability was examined with pedigree and kinship structure analysis. Therefore, we have tried to examine the genetic diversity of wild kuruma prawn using MS-DNA and mtDNA markers and to estimate the genetic structure of relatedness among individuals.

The wild kuruma prawns were obtained from the coasts of Aichi, Ehime, Kagoshima and Kumamoto prefectures in Japan. The relatedness estimated by five MS-DNA markers showed that individuals were related significantly in Kumamoto and Kagoshima. In Kagoshima, some individuals showed full-sib level of relatedness. Besides, the analysis of

mtDNA PCR-RFLP showed that the closely related individuals in Kagoshima tended to share a common haplotype. It is, therefore, supposed that there are many kins in Kumamoto and Kagoshima. However, the heterozygosities and allelic and genotypic frequencies in MS-DNA analysis were not significantly different among the localities. Moreover, the haplotype distributions of mtDNA in Kumamoto and Kagoshima were significantly different from other localities. Thus, it is suggested that no spatial differentiations occurred due to the geographic or historical effects between the localities and that there is the possibility of a mixture of hatchery populations in Kumamoto and Kagoshima.

Furthermore, the possibility of the relatedness analysis for the detection of the stocked hatchery prawns was examined in Saiki Bay where approximately 500,000 hatchery-reared juveniles were released annually. The released prawns were derived from 100 to 200 of wild copulated females caught near the stocking area. Samples were collected by the experimental catch carried out for about 3 month after the release by both the trawl and the gill nets. Although mean relatedness in the samples estimated by the five MS-DNA markers were almost zero, the relatedness among the individuals sharing common haplotypes in nucleotides sequences analysis of mtDNA control region were from 0.126 to 0.458, suggesting the existence of the hatchery-reared juveniles around the stocking area.

**MARINE ORNAMENTAL AQUACULTURE**

Junda Lin

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Many species of marine animals, belonging to different taxonomic groups, are collected from 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 trade industry. My laboratory has worked on developing aquaculture protocols for marine ornamental crustaceans (mostly shrimp, but also crabs and lobsters) and, in more recent years, seahorse species.

Our focus has been on several *Lysmata* species, popular in the aquarium industry. The caridean shrimp species have a unique (among decapod crustaceans) reproductive system, protandric simultaneous hermaphrodite. The shrimp first matures as a male, may change through several transitional stages to simultaneous hermaphrodite that can function as both a male (during inter-molt) and female (during post-molt). Spawning of *Lysmata* shrimp in captivity is relatively easy, especially for the *Lysmata* species, as they are simultaneous hermaphrodites, a unique reproductive system among the decapod crustaceans. A female can produce several hundred to a couple of 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, be receptive to mating, and spawn (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* species. 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 be the major care taker of the offspring) and healthcare value. Over 20 million individuals are collected annually for use in traditional Chinese medicine and aquarium trade. Over-fishing has led to the placement of all 34 seahorse species under CITES II 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*, native along the Atlantic coast of N. America from Nova Scotia to Florida and throughout the Caribbean and one of the most popular aquarium species. Pairing, mating and copulation behavior were observed. Gestation time and brood size were found to be 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 TO THE LARVAL SURVIVAL AND DEVELOPMENT IN MASS SEED PRODUCTION OF BRACHYURAN CRABS

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Brachyuran crabs such as the swimming crab *Portunus trituberculatus*, mud crabs *Scylla serrata* and *S. paramamosain*, snow crab *Chionoecetes opilio*, and horsehair crab *Erimacrus isenbeckii* have been targeted for stock enhancement programs in Japan. Hatchery technologies have been developed for the swimming crab and mud crabs; however, larval mass mortality has frequently occurred during the seed production. On the other hand, large-scale seed production techniques have not been developed for snow crab and horsehair crab. Therefore, to improve and develop the mass seed production technologies for these brachyuran crabs, we studied the effect of n-3 highly unsaturated fatty acids (n-3HUFA) in food organisms, which are essential fatty acids (EFA) for marine fishes, on survival and development of larvae of our target species. We conducted two types of rearing experiments using small vessels and large tanks during this research.

Firstly, in small rearing vessels, we demonstrated the effectiveness of n-3HUFA in live foods (rotifers and *Artemia*) as EFA for larval swimming crab and mud crabs, i.e., n-3HUFA improved the larval survival and development and the survival rate to the first crab

stage reached ca. 75%. On the other hand, in large tanks, we have frequently observed larval mass mortality of *S. serrata* during the metamorphosis to megalops due to abnormal molting by morphologically advanced last stage zoeas. These last stage zoeas have morphological features similar to those of megalops such as large chelipeds. We found that the larval morphogenesis was accelerated in relation to the n-3HUFA content in live foods. This phenomenon was also observed in the seed production of swimming crab and the other mud crab *S. paramamosain*. From these results, we suggest that the development of megalopal features in the zoeal stage can be used as an index of the nutritional condition of larvae. In addition, the effect of environmental conditions on larval development could be evaluated by using the degree of megalopal features in the zoeal stage. For example, we conducted rearing experiments of *S. serrata* larvae at various salinity levels (10-35 ppt) using small vessels and showed that megalopal features in the last zoeal stage represented by the ratio of chela length to carapace length tended to be more advanced with increasing salinity.

The horsehair crab larvae showed high survival rates to the first crab stage in small rearing vessels even though the live food (*Artemia*) were not enriched with n-3HUFA. On the other hand, enrichment with n-3HUFA, especially docosahexaenoic acid (DHA) in live foods improved the survival and development of snow crab larvae in small rearing vessels. However in large tanks, the content of DHA in snow crab larvae largely decreased because of the low content of DHA in the live foods. We consider that improving the DHA content in food organisms of snow crab is one of the main subjects in the future research for mass seed production techniques.

# THE CHESAPEAKE BAY BLUE CRAB (*CALLINECTES SAPIDUS*): A MULTIDISCIPLINARY APPROACH TO RESPONSIBLE STOCK ENHANCEMENT

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Blue crab catches, the major remaining harvest of the Chesapeake Bay (over $100 M in value), dropped over 70% from record highs in the early 1990s. Over-fishing 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 the blue crab breeding stocks and, in turn, Bay-wide abundance and harvests

Understanding the environmental regulation of the reproductive cycle led to full photo-thermal 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 *Artemia* nauplii, resulted in 30-80% survival from hatch to megalopae in three to four 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 10-30%, 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 micro-wire and/or elastomer tags 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 amongst 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. Optimal release sites, habitats, density, and timing were also examined. 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 deep-water channel from nursery/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 post-release. This, together with evidence showing the effectiveness of the spawning sanctuary, suggests the importance of implementing protected migratory corridors linking nursery habitats with the spawning grounds.

In summary, using a multifaceted approach, the feasibility of releasing hatchery-produced juvenile crabs to restore the dwindling blue crab breeding stocks has been demonstrated. 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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The Japanese spiny lobster, *Panurilus japonicus*, is 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 desired eagerly. 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, the research program, Development of Seed Production Technology in Japanese Spiny Lobster, was started in 2005. The project team consists of two sub-teams, sub-team to improve and develop diets, and sub-team to improve and develop rearing methods of phyllosoma.

In order to improve and develop diets, we are trying to investigate natural diets of lobster phyllosoma by molecular methods. Improvement of dietary value of *Artemia* and gonad of mussel, which are known to be effective as foods for phyllosoma, and development of artificial feed are in progress. We are also trying 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, NEW JERSEY**

John Kraeuter, Associate Director,

Haskin Shellfish Research Laboratory,

Institute of Marine and Coastal Science, Rutgers University, USA

Haskin Shellfish Research Laboratory has been working on various aspects of shellfish aquaculture for over 100 years. Most current genetic work is mapping genes for disease resistance in *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 *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo). 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 *Crassostrea hongkongensis* rather than *Crassostrea 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.

Lastly 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 5 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*).

# IMPROVEMENT IN YIELDS OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS* BY SELECTIVE BREEDING

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The USDA-funded Molluscan Broodstock Program (MBP) 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 West coast, US 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 non-selected broodstock.

MBP will continue the selection program using both 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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Vitellogenins are the precursors of the major yolk protein (vitellin) s in oviparous vertebrates and invertebrates. In invertebrates, many studies on cDNAs encoding vitellogenin have been reported in crustaceans. 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 *C. gigas* was shown to be similar to vitellogenins of fish, crustacean and nematode species, especially in the N-terminal region.

The levels of vitellogenin mRNA in various tissues from female oyster and stage-specific expression were measured by reverse transcription-mediated PCR. Vitellogenin mRNA expression was detected only in the ovary, and indicated maximum level in March (the early stage of maturation). To determine the distribution of oyster vitellogenin mRNA expression in ovary, we performed *in situ* hybridization using DIG-labeled RNA probes. A strong signal was detected in the follicle cells. It is concluded that the follicle cells are the site of vitellogenin synthesis.

The synthesis, secretion and processing of vitellogenins differ among phyla. Vitellogenins are synthesized by extraovarian tissues such as the liver in vertebrates and the fat body in insects, secreted into the circulatory system, and transported into the ovary. In teleosts, as in other oviparous vertebrates, it is clearly established that the vitellogenin gene expression is regulated by estradiol-17ß (E2) via estrogen receptor (ER). In the Pacific oyster, E2 is detected in the ovary, and its content shows a synchronous profile with gonadal maturity.

To investigate the estrogen signaling in the vitellogenesis, a cDNA encoding the Pacific oyster, *Crassostrea gigas*, estrogen receptor (cgER) was cloned. Comparisons of the amino acid sequence of cgER with other mollusk ERs show high similarities of the C domain (95-

97%), and the E domain (56-66%). The phylogenetic analysis indicated that the cgER is an ortholog of the other mollusk 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.

**DISEASE DIAGNOSTICS AND TREATMENT OF HEMIC NEOPLASIA IN THE SOFT-SHELLED CLAM *MYA ARENARIA*.**

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The soft-shell clam *Mya arenaria* has been harvested commercially in the US and was a reliable source of income for local fisherman. Since 1980's, the annual harvest has drastically declined and 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. Soft-shell 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 non-functional by sequestration in the cytoplasm by mortalin when the latter protein is over-expressed. 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.

# DIARRHETIC SHELLFISH TOXIN AND LIPOPHILIC TOXIN PROFILES IN JAPANESE BIVALVES AND AN EFFECTIVE MONITORING SYSTEM BY USING A RAPID ASSAY KIT

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Diarrheic shellfish poisoning (DSP) 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 whole tissues), 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.

We developed liquid chromatography-mass spectrometry (LC-MS) of lipophilic toxins in bivalves associated with diarrheic shellfish poisoning (DSP). Using a C8-silica reversed phase column and a mobile phase of aqueous acetonitrile containing 2mM ammonium formate and 50mM 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 collected in Japan in 2003, 2004 and 2005 were quantified by LC-MS. PTX6 and DTX1 are the most dominant toxins in scallops and mussels respectively, whereas YTX is a dominant toxin in both scallops and mussels. Although the toxin profiles were quite different between scallops and mussels, DTX1 and DTX3 were detected in almost all of scallop and mussel samples.

Recently, a rapid assay for OA and DTX1 analogues based on enzyme inhibition was developed by our research project. A good correlation was found between the enzyme inhibition assay and LC-MS results in our previous study. To reduce numbers of MBA and establish an effective monitoring system in Japan, an applicability of the enzyme inhibition assay as an initial screening test in MBA was investigated by using the LC-MS results for more than 800 bivalve samples collected in Japan in 2003, 2004 and 2005. Almost all of the samples quantified as exceeding the quarantine level (0.05 MU/g whole tissues) by MBA contained a level exceeding 0.016 mg/kg as the total amounts of OA, DTX1 and DTX3 in whole tissues of bivalves. This level (0.016 mg/kg) is 1/10 of the EU regulatory level for OA analogues. More than 60 % of the numbers of MBA were reduced when this level was applied to the initial screening test in MBA. The results indicate that 0.016 mg/kg whole tissues is a practical screening level to reduce the number of MBA when the hydrolyzed samples are analyzed by an enzyme inhibition assay.

# BIVALVES AS BIOFILTER: EFFICIENT, PROFITABLE, AND TASTY AS WELL!

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Bivalve production has increased dramatically worldwide in the last three decades. According to FAO, fishery and aquaculture production increased four-fold, reaching almost 12 million MT in year 2000.

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 mollusc should meet market demands with respect to appearance, taste, smell, texture, processing considerations and market behaviour. 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. Due to the high costs of construction, need for highly trained technicians, water pumping, food (microalgae) supply and waste control, bivalve monoculture in such systems is of doubtful profitability.

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

# GEOSPATIAL TOOLS FOR SITE SELECTION AND PRODUCTION MODELING FOR BLUE MUSSELS

Carter Newell, Great Eastern Mussel Farms

# CONSIDERATIONS ON THE FINE SCALE TOPOGRAPHY IN SAND FLATS, HABITAT HETEROGENEITY AND REFUGES FOR CLAMS

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In this presentation, we discuss the use of the fine scale topography on sand flats as an explanatory variable for spatial patterns of clams. Previous literatures have 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 settlement and survival processes of young benthos. Topography on the sand flat is a fingerprint of sediment-water interactions although its interpretation has not been systematically confirmed by fluid mechanics yet. We tried to derive some implications about the effect of hydrodynamics on clam populations from the correspondence between the topography and spatial patterns of clams.

As a preliminary, we compared individual numbers of 2 common clam species, *Ruditapes philippinarum* and *Mactra veneriformis*, in 8 stations across multiple sandbars in Matsunase Beach, Mie, Japan. To capture the fine scale topography in each station, a couple of stereo pictures covering a 60 x 60 cm unit were taken. Vertical profiles of ripples were analyzed with a 3-D image software. Three stations were established on the crests of discrete sandbars. Two stations were on the foreshore slope. Other 3 stations were on the trough, the onshore- and the offshore-side slopes in the middle sandbar. In each station, 6 sediment samples were obtained using 10×5×5 cm quadrats haphazardly placed in the unit. Quadrats were evenly allocated to crests and troughs of sand ripples. Sediments were sieved through a 1 mm mesh screen. Clam species mentioned above were measured their

shell length, and individual numbers of the smallest size group were enumerated. Two-way ANOVA were performed for (log-transformed) individual numbers to test the effects of positions between sandbars (3 crests and 2 stations on the foreshore slope) and within a sandbar (tough/onshore slope/crest/offshore slope) orthogonally crossed with the effect of ripples (crest/trough). Multiple comparisons were conducted using Tukey HSD.

For the mean individual numbers of *R. philippinarum*, effects of positions between sandbars and within a sandbar were significant while the effect of ripples and the interaction term were not significant. Within the middle sandbar, the mean individual number in the trough was greater than other positions. Between 3 crests of sandbars, the mean decreased seaward. Individual numbers of *M. veneriformis* did not show significant variations within the middle sandbar. In the test for the effects of positions 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 but rounded. Ripples on other positions in the same sandbar were convex and slightly skewed. On crests of sandbars, ripples in the landward sandbar were concave upwards while ripples in other sandbars were convex and slightly skewed. Small abundance of *R. philippinarum* corresponded with occurrence of convex ripples that suggest unidirectional currents mixed with wave actions. Unfortunately, statistical tests here are confounded with random spatial variations around each sampling station because sampling units adjusted to stereo pictures were too small to represent the whole area considered. An extensive sampling design is necessary to solve this problem.

This study was supported by the Special Coordination Funds for Promoting Science and Technology, the Ministry of Education, Culture, Sports, Science and Technology of Japan.

# METHODS, ECONOMICS AND COMMERCIALIZATION OF OPEN OCEAN MUSSEL CULTURE IN THE NORTHEAST U.S.

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Constraints on expansion of culture operations in protected, near shore 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 ten kilometers from shore in the open waters of the Gulf of Maine, USA. Water depth is 52m at the site, which is fully exposed to wind and waves from all directions and can experience significant wave heights of 9m during severe storms. Two longlines, each approximately 120m in length and submerged 12m 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 near shore 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 have been grown to market size with an average production cycle of thirteen months from spat settlement to 55 mm shell

height. Yield at market size has ranged from 7.5-12 kg/meter 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 will highlight system design, production strategies, economics, and the process of moving from applied research to commercialization.

**BIVALVE MOLLUSC CULTURE ON THE UNITED STATES WEST COAST**

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Shellfish farms on the West Coast of the United States produce roughly 48,000 M/T of oysters, clams and mussels annually valued at approximately $111,000,000. Oysters dominate production with an estimated 42,731 M/T valued at $84.8 million. The bulk of the oyster production is Pacific oysters (*Crassostrea gigas*) with *Ostrea edulis, Ostrea lurida, Crassostrea sikamea* and *Crassostrea 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 M/Ts produced annually valued at $17 million. *Mytilus galoprovincialis* and *Mytilus trossulus* are two species of mussels farmed on the U.S. West Coast with an estimated annual production of 1,238 M/Ts valued at $3.5 million. Geoduck clams are relatively new to the suite of bivalves cultured. With an estimated 850,000 pounds annual production valued at over $5 million they are the most valuable species per pound.

Washington State dominates West Coast production. This is largely attributable to laws passed in the late 1800s allowing for 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 County 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 of 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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Sea urchin aquaculture is now in its infancy in Japan, USA, 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 a successful sea urchin aquaculture. Here we describe the relationship between gametogenesis and quality of the gonad as food products and propose some possible strategies to improve the quality by manipulating gametogenesis.

The sea urchin gonad contains two main types of the cells: somatic nutrient storage cells called nutritive phagocytes (NPs) and germ cells (GCs). The proportion of NPs and GCs 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, a number of 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 this period, the size of the gonad is too small. After this 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 test, eggs or sperm ooze from the gonoduct, making the gonads less desirable. In some species, strong bitterness develops in the ovary as oogenesis proceeds. For these reasons, gonads containing predominantly NPs are more desirable as food than those containing predominantly GCs. In addition to deterioration in quality, spawning causes a large

decrease in the gonad size every year. Gametogenesis and subsequent spawning are disadvantageous for sea urchin aquaculture.

To prolong the period during which commercially valuable sea urchin gonads can be harvested and to improve the quality of the gonad, two strategies can be proposed. One is the acceleration of the growth of NPs. By feeding sea urchins with formulated feeds or fish instead of macroalgae, nutrient accumulation into NPs is accelerated and the gonad reaches the marketable size earlier than in nature. The other is the suppression of gametogenesis. If gametogenesis is effectively suppressed, most of the drawbacks associated with gametogenesis and spawning are moot. Production of triploids will be the most useful to suppress gametogenesis, though the methods generating triploid sea urchins have not yet been established. Control of environmental conditions, such as temperature or photoperiod, can also be applied to the suppression of gametogenesis.

Grant support from Sea Grant, USDA and NRAC to CWW.

# AN OVERVIEW OF THE CULTURE OF MARINE INVERTEBRATES IN MAINE, USA

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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, using a broad variety of culture techniques. Macroalgae are also being investigated as companion species in polyculture arrangements.

The value of marine invertebrates raised in Maine is approximately $10 million, though precise figures are difficult to obtain. Approximately 50 producing companies are geographically 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 will be given, along with details about regulatory structure, market conditions, and current opportunities and challenges.

# POLYCULTURE OF RED ABALONE *HALIOTIS RUFESCENS* AND DULSE *PALMARIA MOLLIS* IN A LAND-BASED, RECIRCULATION SYSTEM

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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 co-cultured 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, U.S. (lat. 44$^{o}$ 37$^{'}$ N; long. 124$^{o}$ 02$^{'}$ W), we measured dulse specific growth rates (SGR; % increase in dry weight $d^{-1}$) of 17%, dulse productivities of 67 g dry weight $m^{-2}$ $d^{-1}$ ($\doteq$ 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 a 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 $^{o}$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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Harbor tranquility is basically required for berthing ships to piers and for loading and unloading cargos at piers safely. In order to facilitate the calm water basin in ports and harbors, it often needs to set the breakwater layout in almost closed shape. On the contrary to the sufficient harbor tranquility, seawater circulations in a harbor and seawater exchange between inside and outside harbor are inevitably restrained by the surrounding breakwater facilities. If any effective countermeasures against stagnation have not adopted, it may cause seawater pollution problems in a harbor and may sometimes result in ecological damages.

A large number of works for countermeasures against seawater pollution have been proposed and have been adopted in stagnation regions. Some works enhance an advection-diffusion of pollutant loads by seawater exchange; others directly improve seawater and sediment conditions. Usage of tidal and ocean currents and wave induced currents may be one of possible solutions for the problem. However, the driving forces of such currents are generally weak in a harbor and consequently ineffective. It may be more possible solution to use a breakwater with an ability of seawater exchange driven by wave motions.

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

Among many kinds of seawater exchange breakwaters, seawater exchange structures with blockwork mounds may be one of the most effective works. This structure was originally developed by Yamamoto *et al*. (1992) as a breakwater, in which one-way flow is excited by wave set-up due to wave breaking and wave overtopping. The structures not only have an ability of conducting seaward seawater through conduit into inside harbor, but also help to supply oxygen to seawater. It is known that if the distance from sea water level to the crest becomes more than incident wave height, the structures don't make one-way flow at all.

I have proposed the modified structures with blockwork mounds and have examined its effectiveness for the wide range of applicability such as wide tidal change and long conduit. Both of physical model tests and theoretical considerations were employed in order to investigate hydraulic characteristics on the modified structures. It is confirmed that the modified models have proved to be effective not only for wide tidal change but also for long conduit because the different crest heights play an important role to induce the wave set-up in wave chambers for the wide range of water level. It can be affirmed that the volume rate of discharge for inlet flow through conduit can be estimated quantitatively by the present discharge model. It is also pointed out that the present discharge model may be helpful to consider the effective specification of the modified seawater exchange structures with blockwork mounds.

# NOVEL CULTURE FEED FOR SHORT-NECK CLAM USING SINGLE-CELL MATERIAL FROM *PORPHYRA*

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The short-neck clam (Manila clam), *Ruditapes philippinarum*, is among the Japanese important seafood and supports some of the profitable coastal fishing. The short-neck clam culture is a key and rapidly escalating area of Japanese aquatic production. The mainstream of production is from natural populations while increasingly stocks are imminent 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 frequent practice. Nevertheless the consistency of natural recruitment can never be guaranteed. An elucidation to meet the seed requirements of the short-neck clam is hatchery culture trial. The production of seed through hatchery propagation accounts at the present for only a small percentage may only be attributed to unavailability of artificial feeds. Although short-neck clam larvae and spats have been reared successfully on algal foods (i.e. mainly diatoms), these are expensive to produce and do not always coordinate with producer's requirements; insignificant attention paid by researchers to investigate substitute to algal foods. Expensive algal concentrates, universally used throughout the world, have overcome some constrain associated with self-life of algal concentrate, besides over and above storage of algal foods.

Red algae, *Porphyra* spp., are widely cultured on the coastal lines of Japan and are rich in nutrients and known as functional seafood. Generally *Porphyra* has high percentage of protein and also contains outstanding 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 on biotechnology including protoplast/spheroplast isolation technique by using polysaccharide- degrading enzymes have shown some promise to use these nutritious algae as a food supplement without cell wall, because these single-cell materials are easily digestible when ingested by animals. We, therefore, tried to develop a novel feed for culturing shellfish by using single-cell materials obtained by enzymatic means and determine the dietary effect of this feed for growth and production of short-neck clam, and many of successful results were obtained in the series of experiments so far. This research was supported by a grant from the Agriculture, Forestry and Fisheries Research Council in Japan (Research Project for Utilizing Advanced Technologies in Agriculture, Forestry, and Fisheries. No.1681, 2004-2006).

# UJNR Mini Science Symposium Program and Abstracts

November 2, 2007

Northeast Fisheries Science Center, Milford Aquaculture Lab

**UJNR Milford Min-Symposium**

**Friday 2 November 2007**

| | | |
|---|---|---|
| 8:15 | Christopher L. Brown | Welcome & overview |
| 8:30 | Ronald Goldberg | Culture/Habitat Branch overview |
| 8:45 | James Widman | Bay scallop husbandry program |
| 9:00 | Lisa Milke | Bay scallop physiology |
| 9:15 | Coffee Break | |
| 9:45 | Gary Wikfors | Biotechnology Branch & HAB program |
| 10:00 | Shannon Meseck | Chemical ecology of aquaculture systems |
| 10:15 | Yaqin Li | Phytoplankton ecology and aquaculture |
| 10:30 | Barry Smith | Cross-field technologies in aquaculture |
| 10:45 | Sheila Stiles | Overview of shellfish genetics and breeding |
| 11:00 | Lab tour | |
| 12:00 | Lunch | |

# CULTURE SYSTEMS AND HABITAT EVALUATION BRANCH OVERVIEW - MOLLUSCAN AQUACULTURE

Ronald Goldberg

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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*, the surfclam, *Spisula solidissima*, and the hard clam, *Mercenaria mercenaria*. Current Culture Systems and Habitat Evaluation 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 US 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 NOAA's national priorities to expand further 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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The bay scallop, *Argopecten irradians irradians*. is a recreationally and commercially harvested bivalve in the Eastern United States, and was imported to China in the early 1980s where it has become a major part of their 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, amenable to culture in nets/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 USA, they reached a mean shell height of 50mm. 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 producing large amounts of phytoplankton economically. 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 the most susceptible to ammonia in comparision to nitrite or nitrate (Widman et al. in press). 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 *Tetraselmis chui* 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 MOLLUSCS IN RESPONSE TO DIFFERENT MICROALGAL DIETS AND ARACHIDONIC ACID SUPPLEMENTATION

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The bay scallop, *Argopecten irradians*, and the 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 multi-species 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 reduce stress response, therefore improving health and survival. To this end, 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, *Chaetoceros muelleri* (CHGRA) in combination with either *Pavlova* spp. (CCMP strain 459; Pav 459) or *Pavlova 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 (AA) found in CHGRA and docosapentaenoic acid (DPA) 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 (ELISA) is currently under development to measure cortisol concentrations in bivalve hemolymph.

**BIOTECHNOLOGY BRANCH OVERVIEW AND HAB RESEARCH**

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The research of the 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 will be presented by other Branch personnel; this presentation will focus on two additional areas of research: probiotic bacteria for use in bivalve hatcheries, and harmful-algal bloom (HAB) interactions with aquacultured molluscs.

The project on probiotic bacteria was established by a post-doc from NFRDI in Korea, Dr. Hyun-Jeong Lim. Working with Branch staff, she isolated a number of bacterial strains from within the shells of healthy molluscs and screened these for biological effects upon oyster larvae and upon 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, both alone and in the presence of pathogens, but that the effectiveness is dependent upon dose administered. Experiments are continuing to refine effective administration of probiotic strains.

The Branch's HAB research has focused mainly upon 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*, upon 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 24hr out of water

between harvest and transplant; 5) showed that bivalves infested with parasitic diseases are more susceptible to immunomodulation and development of pathologies by HABs than are non-parisitized 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 trouble-shooting microalgal-culture and disease problems in hatcheries, either on-site or remotely. These activities serve industry throughout the US, 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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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 will focus 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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The phytoplankton provides 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, does 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, semi-natural, and in the near future, natural aquaculture settings.

In the laboratory, we examined the possible relationship between the toxicity of *Prorocentrum minimum* to bay scallops and proteins that this harmful alga excretes into the culture medium. Although *Prorocentrum 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 toxin/s 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 10m-long raceway tanks with constant flow of seawater. In this semi-natural setting, scallops were exposed to the 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, shellfish-aquaculture setting.

# CROSS-FIELD TECHNOLOGIES IN AQUACULTURE

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When determining how to do something new, it makes sense to consider if 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. 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, as well as tangential flow and membrane plate, filters were developed for food processing and blood dialysis industries. These filters are readily employable in the aquaculture industry. However pre-filtration 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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With significant advances made in the culture of marine organisms has come increased interest in improving genetic traits, especially 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 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 will be 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 (QTLs) in marker-assisted selection (MAS). In addition, genetic markers are being used to identify stocks and to 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.