Marine Aquaculture: Emerging Technologies and Global Opportunities

Workshop Abstracts

26-27 June 1998
University of Connecticut, Stamford

Co-Sponsors:

Connecticut Sea Grant College Program

Biotechnology Center
University of Connecticut

Nancy C. Balcom, Editor

1998

Publication No. CTSG-98-05

Connecticut Sea Grant College Program
University of Connecticut
1084 Shennecossett Road
Groton CT 06340

This workshop would not have been possible without the additional sponsorship provided by Aquarium Systems, Kent SeaFarms Corporation, Coastal Plantations International, Inc., and the University of Connecticut's Marine Sciences and Technology Center.

Connecticut Sea Grant Publication No. CTSG-98-05

The Connecticut Sea Grant College Program is part of a national network of 29 university-based programs in coastal and Great Lakes states, established by Congress in 1966 and modeled after the Land Grant colleges, administered through the National Oceanic and Atmospheric Administration (NOAA), and supported by federal and state funds. Connecticut Sea Grant, a partnership between NOAA and the University of Connecticut, fosters the wise use of marine and coastal resources through research, outreach and education.

The views expressed in this publication are those of the authors, and do not necessarily reflect those of NOAA or any of its sub-agencies. Any typographical errors or omissions are the responsibility of the editor.

Table of Contents

Table of Contents *(continued)*

Foreword

As we are about to enter the third millennium of the modern era, we find the population of our earth is increasing at a rate approaching one billion additional men and women per decade. At the same time we find that the various fisheries of the world, long thought to be a potential source of the additional protein for the expanding population, are in fact now typically being exploited to a point at or beyond their levels of maximum sustainable yield.

Clearly there is a need to increase the wild stocks of finfish and shellfish, by introducing comprehensive fisheries management and ecosystem preservation approaches, but equally obvious is the need to increase the global harvest of seafood, including edible seaweeds, by fostering advances in aquaculture. Biotechnology has great potential, in areas such as stock enhancement and disease reduction, as regards the wild fisheries, but the gains accruing from the application of the tools of biotechnology, such as genetic alteration and hormonal manipulation, are most immediate and striking when it comes to aquaculture.

In light of the foregoing, it is apparent why the University of Connecticut's Biotechnology Center, and the Connecticut Sea Grant College Program, whose mandate, along with its sister Sea Grant Programs, is to foster the wise use and conservation of our marine resources, have undertaken to co-sponsor this workshop on "Marine Aquaculture: Emerging Technologies and Global Opportunities", at the University of Connecticut at Stamford on June 26-27, 1998.

Aquaculture not only has the clear potential of helping feed the people of the world, it also has the potential for creating employment opportunities for those with the appropriate training.

It is estimated that 180,000 people currently work in the aquaculture sector in the U.S. alone, and a stipulated objective of the U.S. Department of Commerce Aquaculture Policy is to increase the number of jobs in U.S. aquaculture to 600,000 by the year 2025. The U.S. Department of Commerce has also adopted the objective of increasing the value of domestic aquaculture production by 2025 to $5 billion per annum, from the current figure of $800 million, thereby eliminating the current $3 billion annual U.S. seafood trade deficit.

Connecticut, in common with most of its sister New England states, is already the site of significant aquaculture activity. The aquaculture industry in this state is currently valued at approximately $65 million per annum, thanks in the most part to long-established oyster farming. A variety of aquaculture initiatives are presently being developed in Connecticut, many of them focused on Long Island Sound.

Given that our workshop is being held in Stamford, it is appropriate to acknowledge that we share, along with many of the community leaders in our older coastal cities at the west end of Long Island Sound, a conviction that intensive, environmentally responsible, high-density, shore-based aquaculture can be conducted in these urban centers. We envision the accommodation in the near future of closed, or semi-closed, recirculating systems in currently unutilized factories, that would incorporate polyculture systems and/or non-biological filtration units, and would be devoted to the rearing of high-unit-value fish or shellfish, often for specialized niche markets.

To this end, both the UConn Biotech Center and Connecticut Sea Grant are actively collaborating with the Regional Vocational Aquaculture High Schools located in two such urban centers in this state.

(continued)

We are pleased to note that Dr. Philip E. Austin, the President of the University of Connecticut, stated in his keynote address to the attendees at this workshop, "As a land-grant and sea-grant institution, the University of Connecticut is critical to Connecticut's technology-intensive economy. As an emerging industry, aquaculture represents a prime example of our ability to make a major contribution to one specific field that will come to play an important role in this state's economic growth. UConn researchers are currently active in a range of technical pursuits central to the development of aquaculture—the application of transgenic biotechnology to promote rapid growth of fish and shellfish, the improvement of cultivated algae, the provision of molecular probes for finfish and shellfish identification—and this is just a partial list. We are proud to be a collaborator with other state and federal agencies and with the private sector in this important field."

In addition to engaging in aquaculture research collaborations with industrial partners in our state, collaborations on practical and conceptual topics facilitated by the Biotechnology Center, the Marine Sciences and Technology Center, the College of Agriculture and Natural Resources, and by numerous academic departments throughout the University, UConn looks forward to training future aquaculturists and new generations of scientists devoted to research on topics vital to the aquaculture industry.

The UConn Biotech Center and Connecticut Sea Grant are pleased to have been able to co-sponsor this workshop on "Marine Aquaculture: Emerging Technologies and Global Opportunities", and wish to acknowledge that this workshop would not have been possible without the additional sponsorship provided by Aquarium Systems, Kent SeaFarms Corporation, Coastal Plantations International, Inc. and the University of Connecticut's Marine Sciences and Technology Center.

Likewise, this workshop would not have become a reality without the devoted work of the workshop coordinator, Ms. Nancy C. Balcom, Connecticut Sea Grant Extension Program.

We both wish to thank her, and all who assisted in the preparation and conduct of this workshop, for their efforts.

Edward C. Monahan, Ph.D., D.Sc.
Director, CT Sea Grant College Program

Thomas T. Chen, Ph.D.
Director, UConn Biotechnology Center

Production of Algae and Algal Products for Aquaculture

Paul W. Behrens
Martek Biosciences Corporation
6480 Dobbin Road
Columbia, Maryland 21045 USA

Aquaculture animals must obtain all of their nutrients (except minerals) through the food chain, and algae form the basis of that food chain. Therefore algae are critical to successful aquaculture, and often a substantial amount of resources are devoted to the production of algae to support this food chain. Loss of algal cultures due to "crashes" or poor growth can seriously limit aquaculture production and adversely affect the economics of an aquaculture facility. As world population increases and natural catches of fish decrease, the aquaculture industry is expected to grow significantly in the coming years. Efficient production of algae will be vital to support this anticipated growth.

Two major challenges in a commercial aquaculture facility are to (1) reduce the cost (space, time, labor) and increase the reliability of the algae that support the food chain, and (2) improve the survival and growth of fish larvae. Although algae are an important part of any aquaculture facility, the reliability of algal supply is a major factor in attaining a profitable operation. If there is an interruption in the supply of algae, the entire food chain could be broken, resulting in loss of fish larvae and eventually decreased production of adult fish. Various systems have been proposed and constructed to increase the efficiency and reliability of algal growth, including sophisticated photobioreactors. Also, there is growing emphasis on producing algae and zooplankton with enhanced nutritional quality, since the algae and zooplankton form the basis of the aquaculture food chain. Martek has worked to develop algal growth systems that are both low in cost and high in reliability, and recently we have begun formulating algae and algal co-products to enhance the nutritional quality of *Artemia* and rotifers.

Algal Growth Systems

Photosynthetic systems

The two major types of photosynthetic algal growth systems are those that are outdoor and powered by natural sunlight, and those that are indoors and powered by electric lights. Each type of system has advantages and disadvantages and the choice of system should depend on the objectives and requirements of the end-user. For reliability and consistency of algal biomass, indoor systems that are illuminated with electric lights are the preferred choice of growth system. These systems can be as simple as large tanks with overhead illumination or as complicated as completely closed systems with elaborate control strategies that are, in essence, light-driven fermentors.

Photobioreactors are highly controlled systems, and this control contributes to the high reliability of such systems. These systems can provide monitoring and control of many important culturing parameters including temperature, pH, O_2, CO_2 and nutrients. These systems usually have a higher capital cost than large tanks or outdoor ponds, but they provide several advantages that can justify their use. For example, the high parameter control on photobioreactors enables algal culturing to be very reproducible and this in turn leads to consistent algal biomass for feeds. Photobioreactors also enable high density cultures to be grown. This reduces the volume of culture that is needed to produce a given amount of biomass and also reduces the amount of space required to grow the algae. Closed photobioreactors also minimize contamination from other algae or other organisms. This contributes to the consistency and reliability of the algal biomass

which, in turn, provides a more consistent and reliable food chain for the aquaculture operation. Finally, because a photobioreactor has high parameter control it is suitable for the growth of many different types of algae, and the growth conditions can be optimized for any particular species.

Martek has photobioreactors that range in size from 12 liters to 500 liters. The vessel design is modular, and can easily be modified to produce a photobioreactor of any desired volume. The vessel is composed of clear acrylic, and is illuminated by fluorescent lamps. The lamps are integrated into the vessel to maximize the capture of light by the algal culture. Since the energy to power the electric lights is a very substantial part of the cost of growing algae, it is critical that the light be produced with energy efficient lamps and that the light be used efficiently. This lamp arrangement results in the near complete absorption of all of the photons from the lamps to provide maximal utilization of the photons. The photobioreactors are nearly completely closed systems. The only exceptions are that CO_2 is admitted to the vessel, O_2 is removed from the system, and acid and base are added as needed to control pH. The system allows for continual monitoring and control of temperature, pH, CO_2 concentration and O_2 concentration.

These vessels have been used to culture several dozen different types of algae ranging from small, unicellular species to large multicellular algae, covering every major algal group. Culture dry weights in excess of 10 g/l are easily attainable, and the culture conditions can be optimized to achieve efficient growth of any alga. The relatively high dry weight simplifies the harvesting effort required to obtain a given amount of biomass, and this in turn, reduces the labor costs associated with producing the algae.

Although sophisticated photobioreactors have been built and operated with very high efficiency, there is still a fundamental problem of the cost of electricity needed to operate lights. Even with complete utilization of photons to produce biomass, the cost of electricity is a substantial portion of the cost of growing algae (even when compared to capital costs) and this cost results in algal biomass being a rather expensive material.

Heterotrophic Systems

A less expensive and more efficient means for producing algal biomass is to grow the alga heterotrophically. In the heterotrophic mode the algae use an organic carbon source (i.e. glucose) as both the source of carbon and energy for the growth of culture. In this growth mode electric lights are not needed, allowing an expensive energy source to be replaced by an inexpensive energy source (glucose).

The heterotrophic growth mode has several distinct advantages over the phototrophic mode that make it the preferred mode of growth for the rapid and high density accumulation of algal biomass. The major advantage is that electric lights are not needed and therefore the cost of lights is eliminated. Other advantages of heterotrophic growth include high cell density which reduces harvesting effort, readily available largescale growth vessels, and the use of existing and standardized technology. Collectively these features bestow a substantial advantage on heterotrophic growth as the means to produce algal biomass.

Although heterotrophic growth can produce algal biomass less expensively than phototrophic growth, there are not a large number of algae that are capable of growing heterotrophically. Martek has conducted a screening program to identify heterotrophs within our culture collection, and we have found that a significant number of algae can grow heterotrophically. We employ 96 well plates in which each well has a different carbon source. Growth can be determined using an automated plate reader, and this system is able to screen a large number of carbon sources in a short period of time. The initial screening is followed by more detailed characterization in both test tubes and shake flasks to confirm and optimize the heterotorphic growth. This technique has enabled the rapid and efficient screening of our culture collection for heterotrophs.

We have pursued the heterotrophic growth of several algae as aquaculture feeds, including *Chlorella* species, *Tetraselmis* species, diatoms and dinoflagellates. The maximal growth rate attainable with heterotrophically-grown *Chlorella* (5 hour doubling time) is comparable to or exceeds the best growth rate attainable when the organism is grown phototrophically, but the final dry weight of the culture is several fold higher (exceeding 40 g/l), and the time required to achieve that high density is less than with the phototroph. Analysis of the input costs indicates that *Chlorella* is significantly less expensive to produce heterotrophically than phototrophically.

Improvement of Larval Nutrition

Among the algae that we have grown heterotrophically are several that are abundant producers of docosahexaenoic acid (DHA). One species in particular, *Crypthecodinium cohnii*, produces about 20% fatty acid in the biomass and DHA accounts for over 50% of that fatty acid. This organism has been scaled up to the 100,000 liter scale and a scheme has been devised for the extraction and purification of the DHA-rich oil.

As the oil is purified, various co-product fractions are generated. One of these fractions (the DHA phospholipid fraction) has a total fatty acid content of over 50%. DHA (the only polyunsaturated fatty acid in the material) constitutes about one-third of the total fatty acids. In addition, this material is high in phospholipids, free fatty acids and it contains significant quantities of protein and carbohydrate.

The DHA phospholipid material has been spray dried and tested as an enrichment product for *Artemia* and rotifers. This material is well-ingested by both *Artemia* and rotifers, and results show that the DHA levels of both the *Artemia* and rotifers are significantly increased. Since the DHA phospholipid does not contain any other polyunsaturated fatty acids, the ratio of DHA/AA is significantly increased in these organisms. Experiments are in progress to determine the effects of the DHA-enriched *Artemia* and rotifers on larval growth and development. The DHA phospholipids are being formulated with other nutrients to further enhance the nutritional properties of the enrichment material.

Hybrid Striped Bass Culture Industry: A Case Study

James M. Carlberg, Michael J. Massingill, Jon C. Van Olst,
Stephen J. Mitchell, Rodney J. Chamberlain, and David A. Dove
Kent SeaFarms Corporation
11125 Flintkote Avenue, Ste. J
San Diego, California 92121

Brief History of Striped Bass Culture

The first successful artificial culture of striped bass occurred at the Weldon Hatchery in North Carolina in 1874. However, it was not until after the establishment of a reproducing population of striped bass in Santee Cooper Reservoir in South Carolina in 1941 that additional hatcheries were constructed. Striped bass fingerlings were stocked in landlocked lakes, and the success of these programs increased the demand for hatchery-reared fry and fingerlings. Traditionally, this demand has been filled by the culture of striped bass fingerlings in extensive pond culture methods. Most of the enhancement efforts have been conducted by state and federal hatchery programs, which have successfully stocked striped bass in more than 37 states. In the early 1960's, pond rearing methods were developed at the Edenton National Fish Hatchery in North Carolina, the Moncks Corner Hatchery in South Carolina, and the Richloam Hatchery in Florida. Within ten years, there were 57 hatcheries in the southeastern U. S. involved with broodstock collection, egg incubation, and fingerling culture. Refinement of controlled spawning techniques soon followed, using intraovarian catheterization and hormonally induced ovulation. In the mid 1960's, striped bass were successfully crossed with white bass to produce a hardy and faster growing hybrid strain. Subsequently, significant progress was made in the refinement of fry rearing techniques and considerable preliminary research was conducted on methods to improve fingerling production. Much of this research was conducted in the early 1970's at Auburn University, Virginia Institute of Marine Sciences, University of Rhode Island, Southern Illinois University, Tennessee Valley Authority, and at U.S. Fish and Wildlife Laboratories in Alabama, Arkansas and Oklahoma.

Several private fingerling operations were developed in the 1980's to supply fingerlings to the rapidly evolving commercial food fish culture industry. Today, there are at least twenty private fingerling producers that produce over 200 million fry annually, resulting in about 25 million fingerlings. About 15 million fingerlings are sold to domestic food fish grow-out operations in the U.S., and the remaining 10 million fingerlings are exported to Taiwan, Israel, and other locations in Asia, Latin America, and Europe. Keo Fish Farms, Jackson Currie, and Farm Cat in Arkansas, Florida Fish Farms and Chivington Fish Farm in Florida, American Aquaculture International in Mississippi, American Sport Fish in Alabama, Southland Fisheries in South Carolina, Carolina Fisheries in North Carolina, and Delmarva Aquatics in Delaware, are a few examples of private fingerling suppliers.

Many of the original hatchery operations, along with university and private research groups, have continued to refine hatchery technologies. Work on domestic broodstock development has been centered at the Wadell Center in South Carolina, at the Aurora Hatchery of North Carolina State University, at the University of Maryland, Virginia Tech, and Southern Illinois University, to mention a few. In recent years, several private producers have invested considerable effort to develop methods to intensively rear fingerlings in tanks. Some of the principal groups involved are Kent SeaFarms, AquaFuture, and Southland Fisheries.

Recent work has focused on the development of techniques to spawn fish year-round by phase-shifting adults, using environmental cues of photoperiod and water temperature, along with the use of hormones. Since zooplankton cannot be cultured in open ponds in the cold winter months, it was necessary to develop methods to intensively rear fry to fingerling stages in controlled environments in tanks. The two greatest constraints to the perfection of this technology have been the low incidence of swim bladder inflation and the lack of a suitable complete diet for the fry, with its associated cannibalism. Early work on the development of intensive culture methods for production of fingerlings was conducted at the Gulf Coast Research Laboratory in Mississippi. Subsequent studies were conducted during 1970-80 at the Verplanck and Hudson River Hatchery of Consolidated Edison Company, to mitigate for power plant entrainment of eggs and larvae. Research by several private groups, including a major effort by Kent SeaFarms, has overcome many of the constraints to the development of economically viable, intensive fingerling production. Due to these considerable efforts over the past five years, some success has been achieved in the development of reliable intensive rearing methods for production of hybrid striped bass fingerlings. This technology is still limited by the lack of knowledge of dietary requirements and availability of adequate diets for fry and early fingerlings, control of disease, and the prevention of cannibalism.

Development of the Commercial Industry

The first major private effort to grow striped bass commercially was conducted by Marine Protein in Homestead, Florida in 1974. Due to the high energy cost required for pumping water during the energy crisis, this facility quickly closed. The second attempt was conducted in 1978 by Multi-Aquaculture Systems on Long Island, New York, where fish were raised in tanks in a greenhouse during the winter and subsequently grown to market-size of 0.5 lb in floating cages in the summer. Due to economies-of-scale and environmental constraints, this project was terminated. It was not until the early 1980's that Kent SeaFarms Corporation, formerly Aquatic Systems Incorporated, succeeded by developing intensive tank culture technologies utilizing geothermal water and oxygen injection. The first commercial quantities of farm-raised product were marketed in 1986 and the facility was quickly expanded to 96 large circular concrete tanks. Production volumes have been steadily increased to over three million pounds per year. Other intensive flow-through or semi-recirculated systems were soon constructed. This was facilitated by the availability of hybrid striped bass fingerlings from private pond producers that evolved in the mid to late 1980's. In 1988, Sea Chick in Mississippi constructed a 48 tank flow-through facility utilizing geothermal water. Other existing flow-through sites, including Susquehana Aquaculture in Pennsylvania and South Florida Aquaculture, switched production to hybrid striped bass. Some closed system operations and totally recirculating system facilities also switched to striped bass culture, including Anguilla Fish Farm in Florida and Integrated Food Technologies in Pennsylvania, managed by Fresh Culture Systems. Two large-scale closed system operations, Aquafarms Associates of Colorado and Brown Forman in Kentucky, were constructed, but both closed due to high costs of operation and technical difficulties. Two large-scale, closed system facilities were developed in the 1990's, AquaFuture in Massachusetts and Advanced Aquaculture Technologies in Indiana. Both of these operations have made considerable advancements toward the development of closed system technologies.

Concurrent to the development of tank culture systems in the late 1980's, several facilities began to use traditional extensive open pond culture methods for rearing hybrid striped bass. The first successful pond production was accomplished by Ekstrom Enterprises in Texas and Carolina Fisheries in North Carolina. Progress over the past ten years has resulted in production volumes in excess of 4,000 pounds per acre per year (4,500 kg/ha/yr). Soon after their success, several other pond production facilities were constructed in North Carolina, including Castle Hanes Fisheries, Aquafoods, North State Fisheries, and TCA International, to mention a few. Similar operations

were constructed in several states along the mid-Atlantic, including HyRock in Maryland, Shenandoah Waters in Virginia, Edisto Farms and Swimming Rockfish in South Carolina, Twin Oaks in Georgia, Westover Farms in Louisiana, Harvest Fresh in Texas, and Colorado Catch. One of the largest pond operations is Nature's Catch in Mississippi, formerly owned by Brown Forman. Two recent entries into the pond culture arena are Scotland Fisheries in Mississippi, which also raises catfish, and Southern Star in Texas, which also raises marine shrimp. There are numerous small pond producers in several other states. There are a few pond growers of striped bass in California who raise yearlings for mitigation and stock enhancement. Very little production occurs in cage culture systems. The initial cage culture efforts were made by Domsea Farms in 1978 in South Carolina and most recently by La Fourche Mariculture in Louisiana.

Experience at Kent SeaFarms

The principals of Kent SeaFarms Corporation (KSF) began their research on striped bass culture in 1976 while directing aquaculture research projects at San Diego State University and Scripps Institution of Oceanography. Some of the initial research was funded by grants from the electric utility industry and from the National Science Foundation. This work involved studies on the use of thermal effluent from power plants to provide a source of water at a constant elevated temperature. KSF demonstrated that striped bass could be grown at high stocking densities in tanks, and with the use of warm water, growth rates were accelerated and fish attained market-size of over one pound in less than a year. From 1980 to 1983, KSF's research focused on refinement of hatchery techniques and on development of methods to grow hybrid striped bass at high densities in tanks supplied with supplemental oxygen. This also involved changing regulations for procurement of public resource broodstock from the wild and modification of regulations for the capture and sale of wild striped bass affecting the transport and sale of the cultured product.

In the early 1980's, KSF developed the first commercially successful striped bass culture facility in the world. This facility, located near Palm Springs, California, utilizes a unique geothermal ground water resource to provide warm water to optimize the growth rate of striped bass. Following the initial success of this project, the size of the facility was doubled in 1988, to a production level of one million pounds per year, and then tripled in 1992, to a capacity of over three million pounds annually. KSF continues to be the leading producer, supplying over one-third of the U.S. production. Current annual production is nearly equal to the entire U.S. commercial fishery production of striped bass from the Chesapeake Bay and ocean fishery off New England. In total, KSF has now produced and sold over 18 million pounds of striped bass since 1987. The intensive culture methods which KSF uses have proven to work extremely well in full-scale practice.

To effectively utilize the geothermal heat available year-round, KSF has constructed 96 high-density fish culture production tanks, measuring 8 to 12 meters in diameter. A liquid oxygen injection system has been developed to maintain dissolved oxygen levels at saturation concentrations, to meet the respiratory needs of the fish and to allow them to be held at densities approaching 85 kg/m^3. A central venturi, outside standpipe drain system has been designed to facilitate water drainage and recirculation. The water quality in the tanks is maintained with an innovative water quality monitoring and control system, consisting of multiplexers, chemical sensing electrodes, and computerized automated control and emergency alarm systems. High-quality, formulated feeds are delivered automatically to each tank, allowing different diets and feed sizes to be dispensed in precise quantities at controlled frequencies. Fish health is examined daily and protective measures taken to control disease and parasitic infections. The fish are inventoried monthly and results compared to sophisticated mathematical predictive models. Each production tank contains up to 180,000 liters of water and has the capacity of holding a standing biomass of up to 18,000 kg. Other provisions of this sophisticated tank culture system include devices for automatic feed delivery, chemical control, self-cleaning, and ease of harvest. Over the past three years, KSF has

developed innovative nitrifying reactor systems and constructed wetlands to treat and recycle wastewater. The effluent water is now integrated with agricultural irrigation of row crop vegetables. These additions have resulted in significant water conservation measures and reduced over-all production costs. Fish are harvested daily and packaged and delivered under HACCP regulations to major seafood distributors throughout the U.S., Canada, Europe, and Asia.

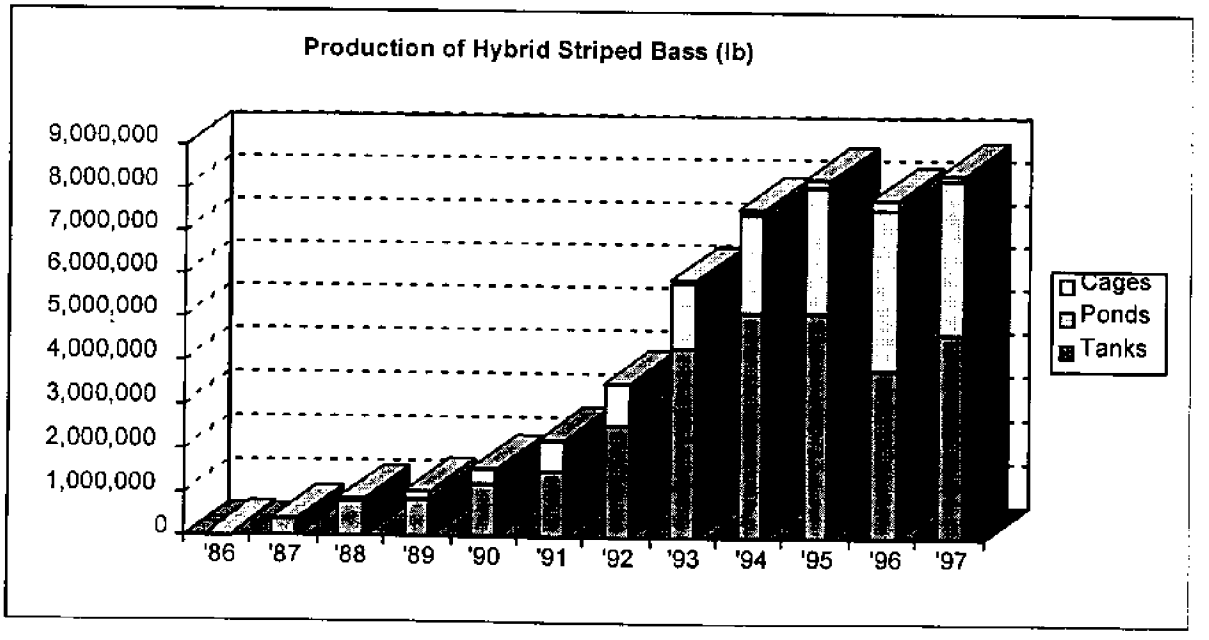
In addition to the modern fish production facility in Palm Spring, KSF operates a 10,000 ft² research laboratory in San Diego, California. This facility has several environmental control rooms used to shift reproductive cycles of domesticated broodstock to provide eligible fish for spawning year-round. There are systems to grow algae, rotifers, and brine shrimp needed to feed fry. There are five large systems used for indoor tank rearing of fry to fingerlings, prior to transferring fingerlings to the grow-out facility. In addition to the development of intensive hatchery methods, research is being conducted on nutrition and disease. Preliminary work has been conducted on genetic improvement and on the potential for use of transgenic technologies to accelerate growth, increase disease resistance, and increase tolerance to low oxygen levels.

Production and Sales Statistics

Production statistics will be presented, based on a survey of the leading 22 producers and information from state extension agents and aquaculture coordinators. Production volumes in 1997 from 95 registered hybrid striped bass producers was estimated to be 8.4 million pounds (Figure 1). Only 31 farms produce over 10,000 pounds each and five of the largest producers supplied nearly 6 million pounds, or 71% of the production. A majority of the product was grown in tanks (56%) and ponds (43%), with the balance from cages (1%). Production by major geographical region was estimated to be 47% in the West, 24% in the Southeast, 15% in the Mid-Atlantic, and 14% in the Northeast. Sales of live product increased to over one million pounds (12%) of the total 1997 production. Over half (53%) of the production from the Northeast was sold live, while 99% of the production from the Southeast was sold fresh. Most of the live product was sold into the ethnic Asian markets in New York and Toronto.

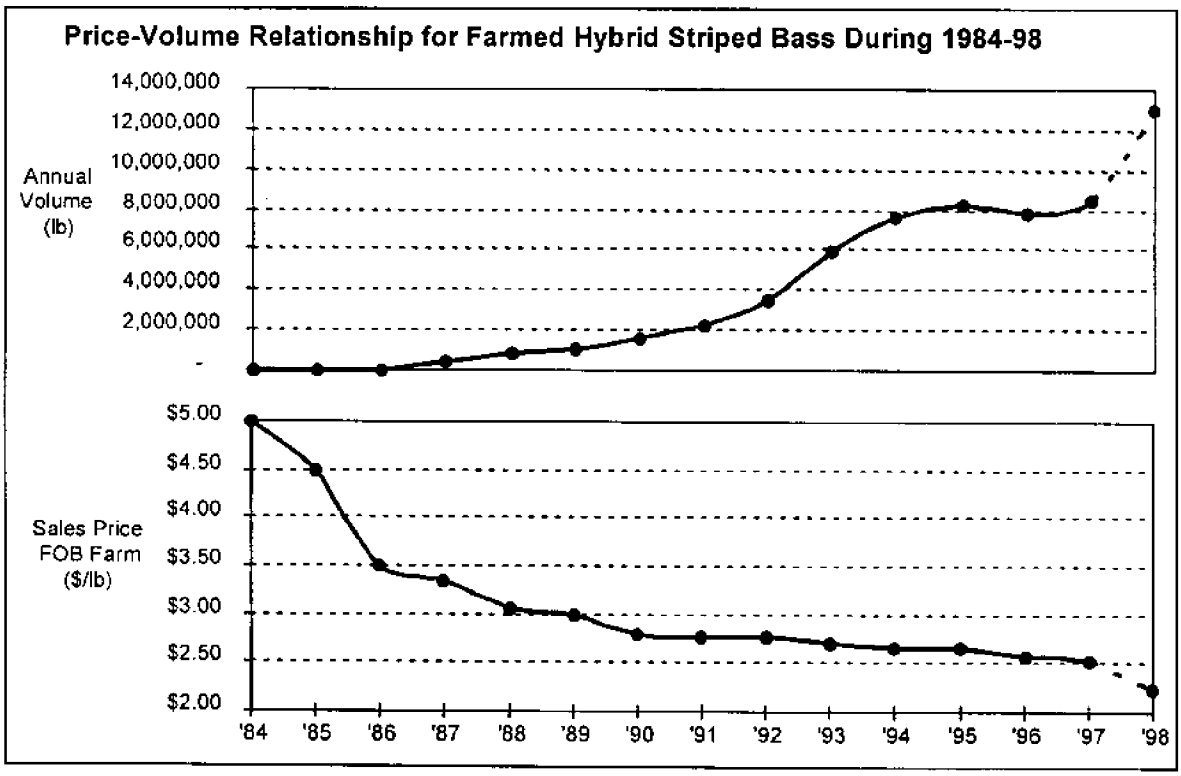
The average FOB farm price for fresh product was estimated to be $2.53 per pound in 1997 and $3.06 per pound for live fish (Figure 2). The price for fresh product was down 28% from original prices obtained from the first sales ten years ago in 1986. Projections for 1998 indicate that production may

Figure 1.



increase to 12.9 million pounds and prices could fall below $2.25 per pound. Some of the competition last year came from commercially landed striped bass from the Chesapeake Bay and coastal fisheries of New England (4.7 million in 1996) and from the white bass commercial fishery in the Great Lakes (2.3 million in 1996). Based on the Atlantic States Fisheries Commission's Virtual Population Analysis, the total allowable catch for striped bass is predicted to be 20 million pounds annually, with 15 million pounds allocated to the recreational fishery and 5 million pounds to the commercial fishery. Production of farm-raised hybrid striped bass for foreign markets is principally from four Kibbutz farms in Israel that produced approximately 600,000 pounds in 1997, which was sold in Western Europe, and about 6 millions pounds from Taiwan and China, which was sold in Asia. The projected increase in production volumes and intensification of competition suggests a further decline in sales price. To counter this trend, it is imperative that the industry expand marketing efforts to increase awareness of the product and to develop new markets.

Figure 2.



Development of a Recombinant Cell Line and Transgenic Fish for Bioassays of Metal Contamination

Maria Chen[1], Carlos Jurado[1], Ka-Man Shiu[2], King Ming Chan[2], and Thomas T. Chen[1]
[1]Biotechnology Center and Department of Molecular and Cellular Biology
University of Connecticut
Storrs, Connecticut 06269
[2]Department of Biochemistry
The Chinese University of Hong Kong
Shatin, Hong Kong

We are interested in developing a sensitive and standardized reporter gene system to monitor metal contamination in the aquatic environment. Previously, we have reported the cloning and characterization of a common carp metallothionien (ccMT) gene promoter when fused upstream of the coding regionfor green fluorescent protein (EGFP) as a reporter gene. the ccMT gene promoter of 650 bp contains five metal-regulatory elements (MREs), two SP1 sites, one AP1 site and a TATA box. We demonstrated that this ccMT-EGFP reporter gene system is responsive to metal administration in primary cultures of common carp hepatocytes. The ccMT-EGFP gene construct has now been transformed into a rainbow trout liver cell line (RTL-W1) via electroporation and a trasnofrmed cell line has been isolated. The expresison of EGFP in the transformed cell line is found to be responsive to metal administration. We have also introduced the ccMT-EGFP construct into the embryos of Japanese Medaka (*Orizyas latipes*) via electroporation. The P1 and F1 transgenic individuals are found to carry the reporter gene constructs and the characterization of different transgenic lines is underway. Furthermore, the expression of EGFP in the transgenic medaka following exposures to various metal ions is being investigated.

Transgenic Fish Technology and Aquaculture: An Overview

Thomas T. Chen
Biotechnology Center, U-149
University of Connecticut
Storrs, Connecticut 06269

Traditionally the worldwide supply of fishery products depends upon the natural population of finfish, shellfish and macroalgae of freshwater and marine sources. In 1992, the worldwide catch of fisheries products was approximately 98.1 million metric tons (~2.2 trillion pounds) which has surpassed the maximal sustainable production from natural sources. The Food and Agriculture Organization (FAO) of the United Nations projected that while the worldwide demand for fishery products will increase to 120 million tons by year 2000, the wild catch will decrease to the level of about 85 million metric tons. As a result, aquaculture (fish farming) will have to increase from the 1994 level of 13.9 million metric tons to approximately 35 million metric tons in only six years, in order to make up the difference. Projections show that aquaculture production will have to further increase to 52 million metric tons by 2010 and 77 million metric tons by 2025.

In the United States, per capita annual consumption of fishery products is expected to increase from the current level of about 15 pounds to 20 pounds in year 2000. As a result, the projected year 2000 U.S. population of 268 million people will require 2.7 million metric tons of fisheries products, a 64% increase over the 1994 consumption of 1.6 million metric tons. In 1994, the U.S. ran a trade deficit of fishery products on the order of $6 billion, and this deficit will continue to increase as consumption of fishery products by the general public continues to increase. To reverse this trend, aquaculture will be the only solution.

Success in aquaculture depends upon six factors: (a) complete control of the reproductive cycle of the fish species in culture; (b) excellent genetic background of the broodstocks; (c) efficient detection and prevention of diseases; (d) thorough understanding of the optimal physiological, environmental and nutritional conditions for growth and development; (e) sufficient supply of excellent quality water; and (f) application of innovative management techniques. By improving these factors, the aquaculture industry has performed remarkably well during the last two decades. To sustain this growth, however, newly developed technologies of molecular biology and genetic engineering will have to be increasingly applied in aquaculture. These technologies can be used to enhance growth rates, control reproductive cycles, improve feed compositions, produce new vaccines, and develop disease resistant and hardier genetic stocks.

Introduction of foreign DNA into developing embryos by microinjection or electroporation has been used to produce a wide range of transgenic animal species, including fish. Besides conducting basic research, this technology offers an exciting opportunity for improving the genetic background of aquaculture important finfish, shellfish and crustaceans (Chen *et al.*, 1996). Studies conducted in our laboratory showed that application of recombinant fish growth hormone to juvenile rainbow trout (Figure1) or oyster spat resulted in a significant growth enhancement (Agellon *et al.*, 1988; Paynter and Chen, 1991). The food conversion efficiencies of the hormone-treated animals are also increased substantially. These results point to the possibility of utilizing recombinant fish growth hormone to improve the somatic growth of finfish and shellfish in aquaculture.

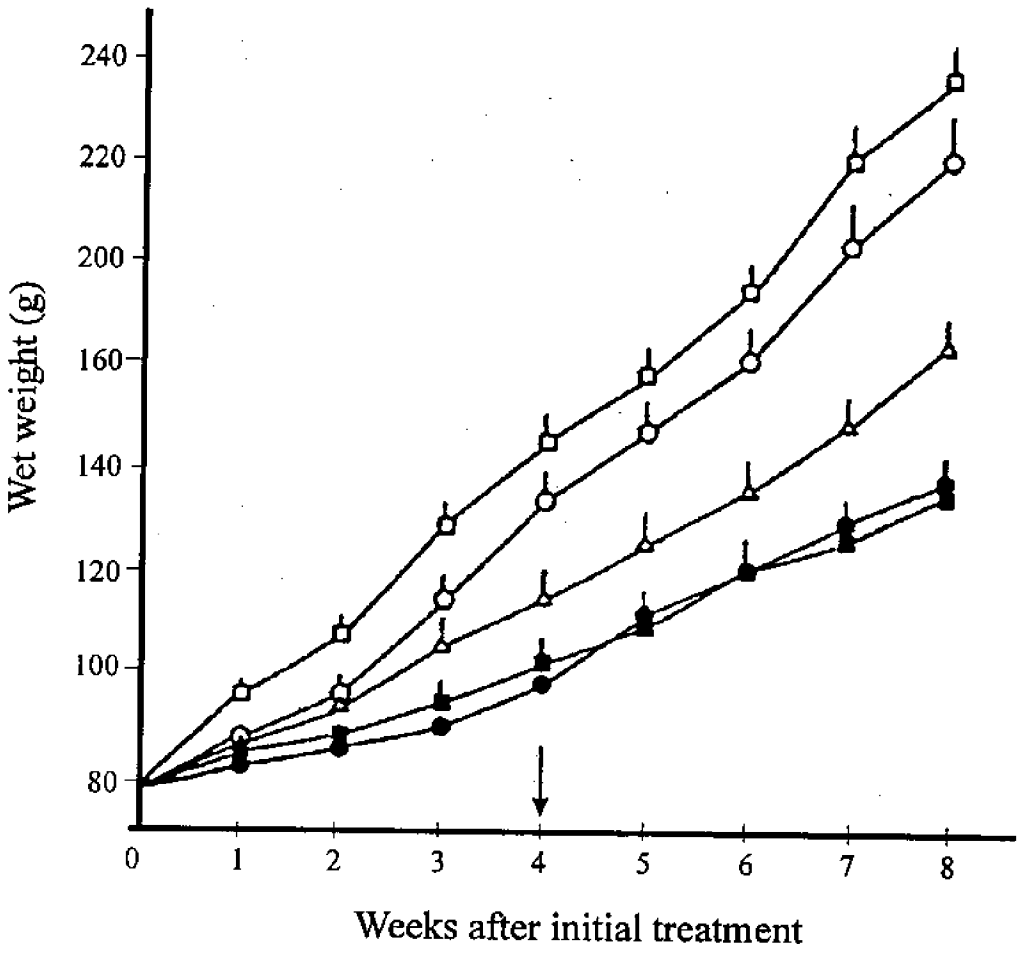
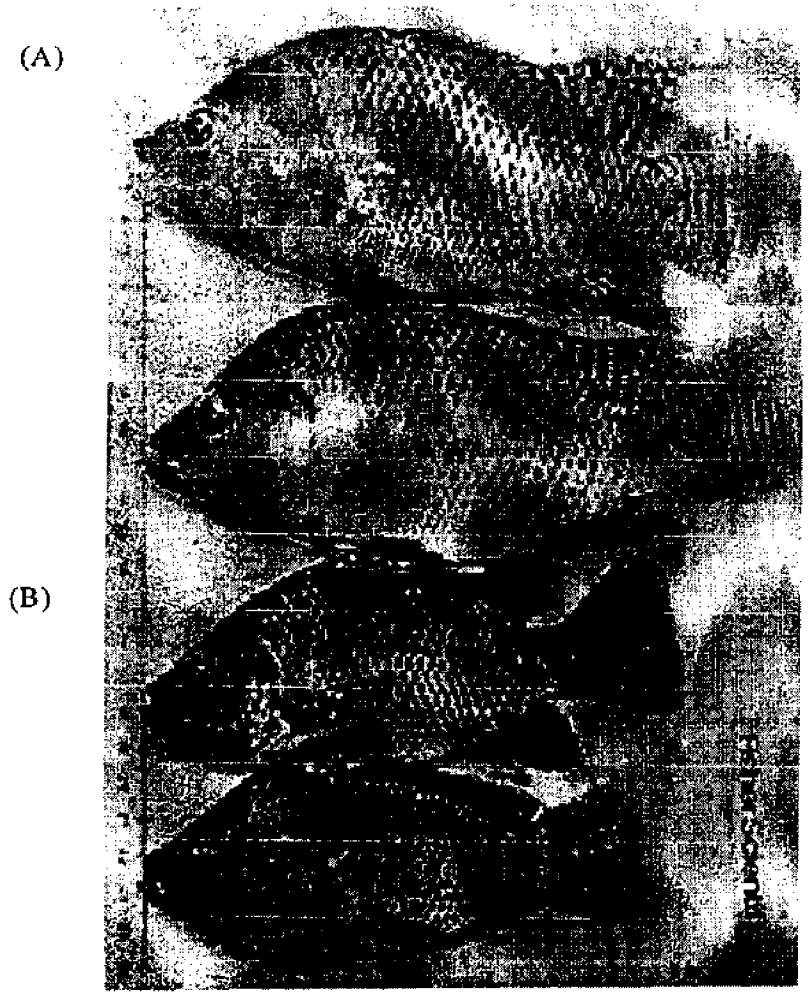


Figure 1. Effect of recombinant trout GH on growth of yearling rainbow trout. Groups of yearling rainbow trout received intraperitoneal injection of recombinant GH or control extract for 5 weeks. Wet weights of GH-treated and control fish are shown (mean + SE). Open symbols, GH-treated fish: ○, 0.2 ug/g body weight; □, 1.0 ug/g body weight; △, 2 ug/g body weight. Closed symbols, Control fish: ●, mock-treated fish; ■, untreated fish. The arrow indicates the time of the last hormone treatment. (From Agellon *et al.*, 1988)

Although administration of an appropriate amount of recombinant fish growth hormone to fish will result in enhancement of somatic growth rate, it may not be cause effective. Alternatively, one may produce fish strains able to secrete higher levels of growth hormone by manipulating growth hormone genes by the transfer technology. To test this possibility, we have produced transgenic common carp, channel catfish, medaka and tilapia by microinjecting or electroporating gene constructs containing the long terminal repeat (LTR) sequence of Rouse sarcoma virus (RSV) or the common carp β-actin gene promoter fused to rainbow trout GH or insulin-like growth factor-I (IGF-I) cDNA. These transgenic fish not only transmit the transgenes into subsequent generations but also grow substantially (from 60% to 800%) faster than their non-transgenic siblings (Figure 2). These results point to the potential of improving the growth rate of aquaculture fish by the gene transfer technology involving fish GH or IGF genes. Improving growth rate of fish by



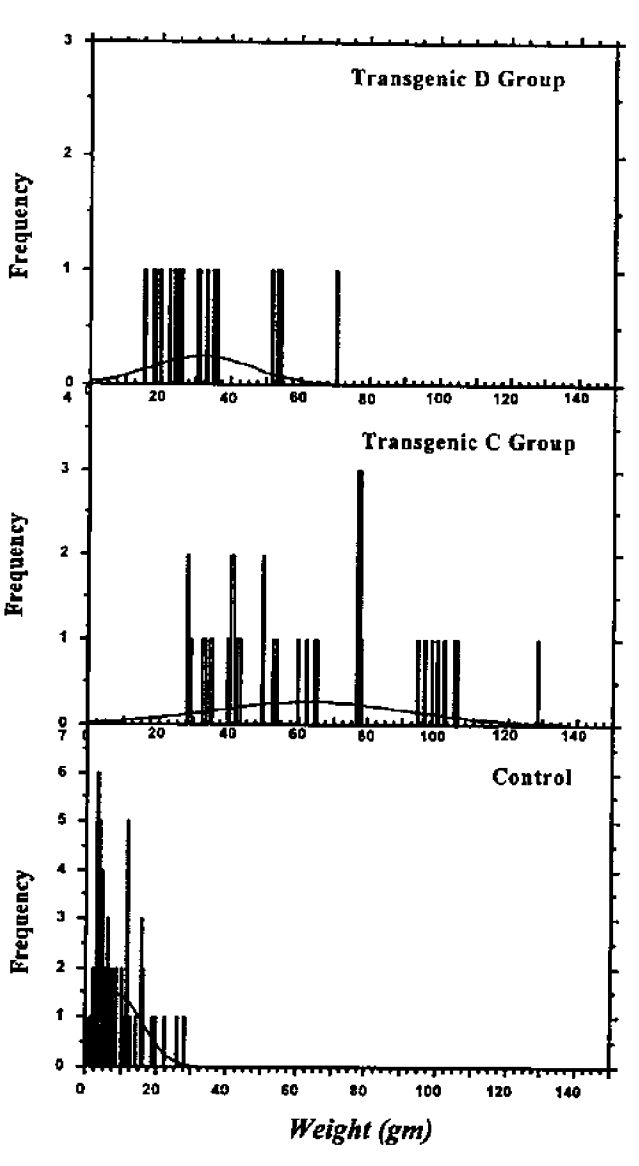


Figure 2. Weight distribution of P_1 transgenic and nontransgenic tilapia. (A) P_1 transgenic, (B) P_1 non- transgenic, and (C) Body weight. D group, embryos electroporated about 2 hours after fertilization and C group, embryos electroporated 30 minutes after fertilization.

manipulating GH or IGF-I gene is one of the examples of application of the transgenesis technology. This technology can also be used to improve traits of aquaculture important finfish; shellfish and crustaceans for disease-resistant, value-added property and others.

Although transgenic finfish can be routinely produced by microinjecting or electroporating transgenes into developing embryos, it is still very difficult to produce transgenics in life-bearing fish and crustaceans. Very recently, we have succeeded in introducing foreign genes into *Peociliopsis* (desert guppy), dwarf surf clams and crayfish by involving pantropic defective retroviral vectors (Lu *et al.*, 1996; Sarmasik, Zoon and Chen, unpublished results). These results clearly suggest that transgenic shellfish or crustaceans can also be routinely produced by the method involving pantropic defective retroviral vectors. (Research supported by grants from NSF, NIH, Connecticut Sea Grant College Program and USDA to T.T.C.)

References

Agellon, L.B., Emery, C.J., Jones, J., Davies, S.L., Dingle, A.D., and Chen, T.T. 1988. Growth hormone enhancement by genetically-engineered rainbow trout growth hormone. *Can. J. Fish. Aqua. Sci.* 45: 146-151.

Chen, T.T., Powers, D.A., Lin, C.M., Kight, K., Hayat, M., Chatakondi, N., Ramboux, A.C., Duncan, P.L., and Dunham, R.A. 1993. Expression and inheritance of RSVLTR-rtGH1 cDNA in common carp, *Cyprinus carpio*. *Mol. Marine Biol. Biotechnol.* 2: 88-95.

Chen, T.T., Vrolijk, N.H., Lu, J.K., Lin, C.M., Reinschuessel, R. and Dunhasm, R.A. 1996. Transgenic fish and its application in basic and applied research. *Biotechnol. Ann. Rev.* 2: 205-236.

Chen, T.T., J.-K. Lu and Fahs, R. 1997. Transgenic fish technology and its application in fish production. *Agricultural Biotechnology.* Marcel Dekker, Inc. New York, N.Y.

Dunham, R.A., Ramboux, A.C., Duncan, P.L., Hayat, M., Lin, C.M., Gonzales-Villasenor, L.I., Kight, K., Chen, T.T., and Powers, D.A. 1992. Transfer, expression and inheritance of salmonid growth hormone genes in channel catfish, *Ictalurus punctatus*, and effects on performance traits. *Mol. Marine Biol. Biotechnol.* 1:380-389.

Lu. J.K., Chrisman, C.L., Andrisani, O.M., Dixon, J.E., and Chen, T.T. 1992. Integration expression and germ-line transmission of foreign growth hormone genes in medaka, *Oryzias latipes*. *Mol. Marine Biol. Biotechnol.* 1: 366-375.

Lu, J.-K., Chen, T.T., Allen, S.K., Matsubara, T. and Burns, J.C. 1996. Production of transgenic dwarf surfclam, *Mulinia lateralis*, with pantropic retroviral vectors. *Proc.Natl. Acad. Sci. U.S.A.* 93: 3482-3486.

Paynter, K.T. and Chen, T.T. 1991. The regulation of bivalve growth: effects of exogenously applied vertebrate growth hormone. *Biol. Bull.* 181: 459-462.

Zhang, P., Hayat, M., Joyce, C., Gonzales-Villasenor, L.I., Lin, C.-M., Dunham, R., Chen, T.T. and Powers, D.A. 1990. Gene transfer, expression and inheritance of pRSV-Rainbow Trout-GHc-DNA in the carp, *Cyprinus carpio* (Linnaeus). *Mol. Reproduc. Develop.* 25: 3-13.

Perspectives in the Development of Irish Moss Cultivation

James S. Craigie
Institute for Marine Biosciences
National Research Council
1411 Oxford Street
Halifax, N. S. B3H 3Z1 Canada

The seminal event that stimulated research on seaweed aquaculture in Canada was the highly successful Fifth International Seaweed Symposium held in Halifax in 1965. The red seaweed, *Chondrus crispus* (Irish moss), was selected as the target species owing to its complement of valuable carrageenans. It was also becoming evident at the time that the rapidly increasing global demand for these hydrocolloids could not be sustained by the natural harvest of Irish moss. The late Arthur C. Neish, then Director of our laboratory, initiated research aimed at determining whether *C. crispus* was susceptible to cultivation as a new marine crop. It was believed that land-based systems rather than ocean farming would be more suited to the climatic and jurisdictional realities of the Canadian Maritimes. The intent was to develop a simple cultivation system that individuals in coastal communities could use to augment their incomes and create employment, thus serving both economic and sociological goals.

The first step was to domesticate *C. crispus* by selecting strains that could survive and even flourish in the environment created by enclosed tanks or ponds of seawater. The first success was achieved in early 1970 with the selection of clone T3 after screening some 500 plants taken near Sambro, Nova Scotia. The T3 clone grew well, produced high quality kappa carrageenan and, for several years, stood as the gold standard for measuring success in red seaweed aquaculture. This alga, later shown to be a functional male gametophyte, has been vegetatively propagated to the present day. A decade later, a cohort of 5,000 individual *C. crispus* fronds from sites ranging from Rhode Island to Prince Edward Island were evaluated in my laboratory. Rigorous culling was carried out, conserving only individuals having superior growth rates, minimal sporulation, and resistance to disease and to thallus fouling by weed algae. A clone named H3, originating near Avery Point (Groton), Connecticut, was chosen and is now in commercial cultivation.

By 1972, two competitors, Marine Colloids Inc., and Genu Canada, had established facilities at Meteghan, Nova Scotia and Point Sapin, New Brunswick, respectively, to develop the technology and to try to commercialize the cultivation of Irish moss. Both discovered that their sites were unsuitable for the original purpose, although for quite different reasons. The former moved to Charlesville, Nova Scotia in 1978, and the latter company established a new site at Hay Point, near Halifax. The Charlesville site was acquired and developed by Acadian Seaplants Ltd., while the Hay Point operation was abandoned.

The market for Irish moss suffered major setbacks during the mid 1970's due to the rapid development of *Kappaphycus* (*Eucheuma*) cultivation in the Philippines. The availability of a new, relatively inexpensive source of kappa carrageenan ensured that Irish moss would not be cultivated for this purpose. Accordingly, Marine Colloids Inc., and later Acadian Seaplants Ltd., focused on cultivating sporophyte (diploid) clones of *C. crispus* to produce lambda carrageenan. This highly sulfated, non-gelling hydrocolloid was known to the industry, but no abundant natural

source of good quality polymer had been found. Selection of several clones of diploid *C. crispus* was carried out and their cultivation formed the commercial basis for Irish moss aquaculture.

Experimental studies were conducted on physiological and environmental factors affecting growth and composition of *C. crispus*. Aspects of carbon supply and fertilizer nutrients, including nitrogen, phosphorus and trace metal requirements, were investigated in long-term experiments under conditions of high productivity. Since the uptake of carbon by the alga was found to be virtually stoichiometric, daily productivity measurements can be calculated from a measure of the carbon consumed. Recovery of fertilizer nitrogen as plant biomass was approximately 75% in long-term trials. Growth of *C. crispus* was shown to be a linear function of solar irradiance under optimal culture conditions in outdoor systems.

It was recognized very early that opportunistic Chlorophyceae such as *Cladophora* spp., *Enteromorpha* spp., and *Ulva* spp., and various filamentous brown algae could seriously foul cultures of Irish moss. In addition, certain red algae including *Ceramium* spp., *Cystoclonium purpureum* and the *Trailliella* phase of *Bonnemaisonia intricata* can be troublesome weeds if they become established in culture tanks. Biological methods of weed control have been employed with some success using isopod and gammarid grazers. These Crustacea exhibit a marked feeding preference for filamentous brown and green algae relative to *C. crispus*. To utilize these animals effectively to control algal weeds in large-scale systems, it is necessary to be able regulate the grazer populations in culture tanks, otherwise they will ravage the Irish moss crop when their preferred food species are depleted. Both *Mytilis* spp. and *Lacuna* sp. larvae also enter culture tanks via the incoming seawater. The former can settle directly on the alga causing fouling. The latter reproduces in the tanks and can establish sizable populations by the end of the cultivation season. When Irish moss is destined for hydrocolloid extraction, small numbers of animals are of little consequence; however, their presence cannot be tolerated when the alga is marketed for more direct human consumption.

Both bacterial and fungal diseases have been encountered. Bacterial rotting of *C. crispus* occurs when the fronds are not agitated properly in the tanks, or if they are mechanically damaged. This is a serious hazard in *C. crispus* sporophytes infected with the green algal endophyte, *Acrochaete operculata*. When the endophyte sporulates, the cuticlar and cortical layers of the host are ruptured, thus creating entry ports for bacteria. The presence of ammonium in the seawater can greatly exacerbate the rotting by activating the bacteria. The problem can be avoided by proper management of the culture systems. The most serious pathogen to date has been the unicellular fungus, *Petersenia pollagaster*, which was first encountered in *C. crispus* in 1982. Its zoospores penetrate the growing apices of *C. cripus* and, under optimal conditions, will sporulate within 72 h. When the fungal discharge tube emerges, the surrounding cortical cells of the host die. The outcome of even moderate infections by this fungus is the complete destruction of the meristematic tissues of *C. crispus* with the total loss of all production for the remainder of the growing season. Effective control of the infection was obtained using sodium dodecyl sulfate and later by non-chemical means. Infectious zoospores of *P. pollagaster* have been detected in the incoming seawater at Charlesville for 16 consecutive years.

When the lambda carrageenan market failed to develop as rapidly as anticipated, Acadian Seaplants Ltd. undertook to find new outlets for cultivated Irish moss. It was determined that a niche was open in the human food market, provided that certain criteria for color and texture could be met. Research in my laboratory and by the company resulted in practical processes that gave an acceptable product. This radical departure from the traditional uses of Irish moss in the hydrocolloid industry dramatically raised the requirements for high quality plants. New culture strategies and management practices were developed and implemented to meet this challenge. Scale-up and evaluation of the processing steps

were also completed and quality assurance protocols applicable to food grade products were incorporated.

In summary, the aquaculture of Irish moss has evolved in a way that none could have predicted 30 years ago. Instead of a cottage industry providing supplemental income for relatively unskilled workers, we have a highly sophisticated, vertically integrated operation. *Chondrus crispus* clone H3 now is completely managed from the milligram-scale library cultures through a seedstock nursery to the multi-ton grow-out facilities. The harvest is processed in value-added steps to yield the final food-grade product, with rigorous quality control being maintained throughout. The successful outcome can be attributed to the sustained, close interaction of managerial, scientific and operational personnel coupled with innovation and real time introduction of new knowledge as it became available.

Aquaculture of Marine Ornamentals

Thomas Frakes
Aquarium Systems
7344 Demshar Drive
Mentor Ohio 44060

Raising marine ornamental fish and invertebrates for the hobby aquarium trade may appear to be an extremely lucrative business for an experienced mariculturist to pursue. To breeders of food fish that wholesale for a few dollars per pound, the lure of raising a product that sells for $500 to $1000 per pound is almost overwhelming. It is a mirage. Despite numerous attempts by both small entrepreneurs and large well-financed corporations over the last 25 years, none has been a financial success.

The bulk of the work has focused on the anemonefish, an extremely popular group of aquarium fish that readily spawn in captivity, produce large benthic eggs, and hatch larvae ready to accept rotifers. Despite the relative successes of several large farms in rearing commercial quantities, profits have been elusive, with most companies eventually hoping to just break even on a monthly basis. Early facilities included Aqualife Research, Instant Ocean Hatcheries, and SeaWorld Aquaculture of San Diego, all started in the 1970's. Eventually they all closed. Price competition from wild-caught fish restricted sales, and the production costs of handling large numbers of small fish in small batches have remained high. Efforts to shift to more profitable species have only recently begun to show promise. C-Quest, Inc. in Puerto Rico has introduced several new groups of fish in the last couple of years, such as the Pseudochromids which have a much higher market value than the clownfish. They also have a number of gobies and miscellaneous species that provide consumers with a broader range of fish species to order at one time. Table 1 lists the species this facility has been able to produce.

Many of the desirable aquarium fish, such as angelfish, require extremely small food organisms for the new hatched larvae. This remains the primary obstacle to raising these fish successfully. Many organisms have been tried, as with food fish culture, but none have been found to be suitable.

In addition to fish, aquaculture of ornamental crustaceans has been attempted. The extremely long and complicated larval period has restricted success with the banded coral shrimp (*Stenopus hispidus*). However many of the cleaner shrimp of the genus *Lysmata* have been reared, including the peppermint shrimp (*L. wurdemanni*) and the scarlet-striped cleaner (*L. grabhami*). A long and delicate larval period has also frustrated culturists working on the scarlet cleaner (*L. debelius*). Because of their advanced size at hatch, they are raised using techniques similar to fish, starting with live zooplankton such as *Artemia* nauplii and dry foods like flake or granular foods.

During this time period the miniature reef aquarium emerged as a popular segment of the hobby. With it has come a demand for invertebrates and live rock. Harvest of live rock escalated during the late 1980's but environmental concerns led to the banning of rock collection. Ocean ranching of live rock on state leases and permitted federal sites in Florida has attempted to meet this demand, but much of the rock in the trade is still imported from the South Pacific. Cultured rock is being produced by placing upland rocks on sand bottom in permitted areas, creating artificial reefs. The rocks are colonized by benthic organisms over several years, and then harvested by SCUBA divers at depths ranging from several feet to more than 100 feet. Acceptance by the trade has been slow, although the appearance of corals on the rock this past year is helping and more decorative shaped rocks are now being deposited.

Cultured giant clams (*Tridacna* spp.) have been sold into the pet trade for a number of years as a

side product from South Pacific hatcheries originally set up either for re-stocking purposes or to supply restaurants. Wholesale prices to shops run from $20 to $50 or more for prime specimens 1 to 2 years old. Many countries prohibit or severely restrict the harvest of these over-fished clams. The main sources are Palau, the Marshall Islands and the Solomon Islands. The focus has been on producing brightly-colored clams, especially the blue varieties, but consistent production of blue clams has not been achieved. It is felt that consistent production is dependent on both genetics and environment. Several of these facilities have ceased operation as government support dried up. Attempts to set up production of these clams in the United States have all failed so far. Gerald Heslinga, former director of the highly successful MMDC in Palau, has run into numerous unexpected obstacles at his new facility in Hawaii. Broodstock manipulation has been one of the biggest problems. Maintaining and conditioning large numbers of adult (5-7 year old) clams in captivity while avoiding unplanned mass spawning events has not been easy.

Culture of hard and soft corals is a thriving home-based activity. Many advanced aquarists are setting up miniature farms to produce corals for the pet trade. Since hard corals are listed on CITES II, importation is restricted. Small colonies can retail for $20 to $50, so hobbyists encouraged by the rapid growth achieved in their reef tanks are setting up culture systems in back yard greenhouses, basements or garages. Most of this work is based on fragmentation methods utilizing mother colonies that are periodically pruned. Fragments are attached to base supports such as plastic bud holders, concrete plugs or natural rocks, using either epoxy or "super glue". These hobbyists will be competing with South Pacific farms, such as the new facility in the Solomon Islands that is producing small colorful colonies selected from the back-reef rubble zone, attached to new rocks and grown in a protected lagoon setting until ready for sale. American farmers will have to rely on low shipping costs and direct sales to retailers to compete with the ocean-ranched coral products. Soft corals are also being produced. Some are hardy but many are difficult to ship such as the delicate *Xenia* spp. This group grows like a weed for many hobbyists, so local markets can be saturated by a few hobby breeders. Commercial breeders will have to stay ahead of the pack with new and more colorful varieties to remain successful.

Culture Facilities List

Fish

C-Quest, Inc.
P.O. Box 1163,
Salinas, PR 00751
(787) 845-2160; FAX (787) 845-3929

Oceans, Reefs & Aquariums, Inc.
5600 US 1 North, A.C.T.E.D. Building
Fort Pierce, FL 34946
(561) 468-7008; FAX (561) 468-7353

Reef Propagations
705 Randi Lane
Hoffman Estates, IL 60194
(847) 885-7338

Live Rock

Tampa Bay Saltwater
1720 Eldred Dr.
Tampa, FL 33603
(813) 875-3574; FAX (813) 875-850;
email: www.tbsaltwater.com

Seacritters
13005 Seacritter Lane
Dover, FL 33527
(813) 986-6521; FAX (813) 986-0191

Corals

Geothermal Aquaculture Research Foundation (GARF)
1321 Warms Springs Ave.
Boise, ID 83712
(208) 344- 6163
email: www. garf.com

Tropicorium
20080 Inkster Rd.
Romulus, MI 48174
(313) 782-2622

Waikiki Aquarium
2777 Kalakaua Ave.
Honolulu, HI 96815
(808) 923-9741 (research only)

Books

Heslinga, G., T. Watson and T. Isamu. 1990. *Giant Clam Farming.* The MMDC Giant Clam Hatchery, Koror,Palau. 180 pp.

Hoff, F. H. 1996. *Conditioning, Spawning and Rearing of Fish with Emphasis on Marine Clownfish.* Aquaculture Consultants Inc., Dade City, FL. 212pp.

Knop, D. 1996. *Giant Clams, a comprehensive guide to the identification and care of Tridacnid clams.* Dahne Verlog GmbH, Ettlingen, Germany. 255 pp.

Moe, M. 1997. *Breeding the Orchid Dottyback.* Green Turtle Publications, Plantation, FL. 285pp.

Wilkerson, J. 1998. *Clownfishes, a guide to their captive care, breeding and natural history.* Microcosm Ltd., Shelburne, VT. 240pp.

Other Information Sources

Breeders Registry and *Journal of MaquaCulture*
P.O. Box 255373
Sacramento, CA 95865-5373

SeaScope
Aquarium Systems, Inc.
8141 Tyler Blvd.
Mentor, OH 44060
(800) 822-1100

Table 1. Fish Commercially Reared by C-Quest

Amphiprion akallopisos	Skunk clownfish
A.akindynos	Barrier reef clownfish
A. bicinctus	Two banded clownfish
A. clarkii	Clarke's clownfish
A. ephippium	Red saddleback clownfish
A. frenatus	Tomato clownfish
A. melanopus	Cinnamon clownfish
A. ocellaris	False clownfish
A. percula	Percula clownfish
A. perideraion	Pink clownfish
A. sandaracinos	Orange skunk clownfish
Premnas biaculeatus	Maroon clownfish
Coryphopterus personatus	Masked goby
Gobiosoma citrinus	Citron goby
G. genie	Genie goby
G. multifasciatum	Greenbanded goby
G. oceanops	Neon goby
G. okinawae	Yellow goby
G. prochilus	West Indian cleaner goby
Ogilbyina novaehollandiae	Australian dottyback
Pseudochromis aldabrensis	Neon dottyback
P. flavivertex	Sunrise dottyback
P. fridmani	Orchid dottyback
P. fuscus	Yellow dottyback
P. olivaceous	Olive dottyback
P. sankeyi	Striped dottyback

Other Species Reared

Calloplesiops altivelis	Comet
Gramma loreto	Royal Gramma
G. melcara	Blackcap basslet
Opistognathus gilberti	Yellowhead jawfish
Pterapogon kauderni	Bangai cardinalfish
Synchiropus splendens	Mandarin

Commercial Operation of Large-Scale Recirculating Aquaculture Systems

Joshua N. Goldman
AquaFuture, Inc.
15 Industrial Road
Turners Falls, Massachusetts 01376

Recirculating aquaculture systems (RAS) offer many advantages, including reduced water consumption and environmental impacts, and greater siting flexibility. Additionally, RAS offer the ability to cost-effectively control water temperature and other parameters which can beneficially affect key production variables, including (1) fish growth rate, (2) feed conversion efficiency, and (3) crop mortality.

Despite these important advantages, RAS have historically had limited commercial success due to their high capital costs and operational complexity. Major pitfalls in the design and operation of high-density RAS are discussed. Strategies which are improving the prospects for commercial food fish production are described. The economics of RAS are influenced by the scale of the life support system, with the most economical operation generally being achieved at the largest practical scale. Management procedures must be designed to meet the dual objectives of optimized feeding regimes while maintaining system stability and suitable water quality.

Commercial operation of RAS requires careful selection and integration of unit processes. Solids removal, biofiltration, carbon dioxide removal and oxygenation are the most common unit processes used in highly intensive RAS. These processes should be configured to achieve simplified operation, efficient utilization of inputs, and be highly robust to meet the requirements of commercial production. High biofilter oxidation rates which have the capability to respond to changes in water quality resulting from the dynamics of pulsed feed input is an important consideration, which has not been widely described in the literature. Environmental benefits, including the potential for reducing the escape of genetically-modified or non-native species and the ability to treat system effluent and re-use nutrients are also disucssed.

AquaFuture, Inc. of Turners Falls, Massachusetts, has over a decade of large-scale commercial experience in the design and operation of RAS. The company's facilities currently recirculate over 150,000 m^3 of water / day and produce hybrid striped bass, *Morone saxatilis* x *Morone chrysops*, and summer flounder, *Paralichthys dentatus*, for U.S. and international seafood markets. The Company's patented technology requires less than 10% of the water and 1% of the land of conventional ponds, and fish achieve market weight in approximately half the time of traditional production methods. Proprietary computerized management and control systems, genetically-improved hybrid fish stocks, and thoroughly tested production controls combine to reduce production risk, enhance efficiency, and ensure year-round supply.

Mariculture Development in Israel - Present and Future

Hillel Gordin
Mariculture Department, Fisheries and Aquaculture Division
Ministry of Agriculture and Rural Development
National Center for Mariculture (NCM)
Israel Oceanographic and Limnological Research (IOLR)
P.O. Box 1212, Eilat 88112 Israel

Israeli aquaculture started in the mid 1930's with the introduction of European fish (common carp) and central European technology. In the 1960's, tilapia species and hybrids were introduced. Most of the production was carried out in extensive, stagnant water fish ponds, with average yields of less than five tons per hectare. Presently, most of the freshwater fish culture occurs in reservoirs which hold rain water for summer irrigation. Fish are cultured in these reservoirs and are harvested towards the end of summer and fall. Almost half (> 8,000 metric tons) of the total freshwater fish production in Israel is carried out in the reservoirs. Some of the reservoirs are serving as "green lungs" for intensive-satellite fish ponds: water is circulated between the reservoirs and the intensive ponds to clean it time and again.

Chronic water shortage characterizes the State of Israel. The water situation is not likely to improve in the future, due to population increases and the ensuing Peace Agreements, which call for Israel to yield some of its water to past rivals and future friends. The fresh water situation underscores the need for freshwater aquaculture fish farming practices to shift toward intensive systems. The motto is "Raise fish with as little water as possible". Closed systems are designed with different modes of biofiltration. New species have recently been introduced to the Israeli fish farming, such as gray mullet, rainbow trout, hybrid bass, and lately, red drum and Australian silver perch.

However, due to the constraints stemming from the shortage of fresh water, mariculture development is a natural and welcome development. It took more than 20 years for the government to recognize the potential embedded in sea water to produce food; fish, mollusks, crustaceans and algae (micro and macro). At present, the Ministry of Agriculture and Rural Development's official policy is to increase mariculture production to more than 10,000 tons annually, within the next decade.

The commercial development commenced in the late 1980's around the Gulf of Aqaba/Eilat, with the active participation of the National Center for Mariculture (NCM). In 1997 total production was 1,550 tons, of which 1,300 tons were gilthead seabream and the remainder striped bass, red drum, sea bass and tilapia. Most of this production (1,220 tons) is carried out in the Gulf of Aqaba/Eilat However, the farms in the Gulf are under pressure by different environmental agencies in the country to halt their development, due to the sensitive oligotrophic and coral reef marine systems.

Lately, mariculture activity has begun in the Mediterranean region—in landbased seawater ponds and in offshore fish cages. The Eastern Mediterranean is a cruel sea; winter storms generate waves of 7-10 meters. However, moving offshore will free farmers from environmental impact problems, due to the large dispersion factors. The sea state, especially the winter storms, calls for specially-designed systems which can withstand/avoid the high energy events. A prototype, which descends and ascends by surface control upon an approaching storm or a fading one, respectively, will be discussed. Most of the current effort is establishing offshore cage farms.

There are two commercial hatcheries in Israel, one with a production capacity of 5-7 million fry; the other produces 2-3 million fry per annum. A third one is in the planning stages. Most of the fry production is gilthead seabream.

Mariculture activities started in the early 1970's as research and development (R&D) in the Eilat area, which is situated at the southern tip of the State on the shores of the Red Sea. Most of the marine aquaculture R&D is carried out at the National Center for Mariculture, situated in the City of Eilat.

The Center is built as an interdisciplinary institute operating 11 inter-related departments: fish reproduction physiology; fish larval physiology and mass production; live food chains, fish nurseries and physiology; mollusk reproduction, nutrition and culturing; fish nutrition; pathobiology; fish genetics; water quality and macroalgae; mariculture environmental impacts; and mariculture engineering.

The NCM operates according to the following concepts:

1. *Mariculture is an interdisciplinary field, requiring the interaction and integration of many experts to solve a problem.*

2. *Though by definition mariculture is an applied scientific field, basic research is also necessary in order to understand and solve practical matters (such as fish reproduction control).*

3. *Since mariculture is a relatively new field and a budding industry, the research and development (R&D) work at the NCM has to cover all technological developments, from the test tube to the commercial scale whenever possible.*

4. *Mariculture interacts very strongly with the environment. At the NCM, minimal environmental impact technologies are developed.*

5. *Graduate students from all universities in Israel are integrated into the research process.*

NCM Objectives

1. *Development of food production technologies based on sea water;*

2. *Development and adaptation of mariculture technologies;*

3. *Development of environmentally-friendly mariculture technologies; and*

4. *Development of the R&D infrastructure for mariculture in the State of Israel.*

Research and Development Goals 1998-2002

1. *Domestication of new marine species for mariculture;*

2. *Preventive and curing treatments for fish and mollusk diseases;*

3. *Preparing the integrated culture system for commercial practice;*

4. *Genetic selection and genetic engineering for the development of fish strains that perform better under culture conditions; and*

5. *Development of efficient fish feeds for mariculture with emphasis on fish meal substitutes.*

A landbased, integrated pond system (IPS) was developed in which fish are reared in intensive seawater ponds (25 kg of fish per m^2 per year). The pond effluent is directed to a sedimentation pond where solid particles settle out. Microalgae blooms are developed in this pond and bivalves are grown at very high densities (yields of clams 5-10 kg per m^2 per year). From the sedimentation pond, the water is pumped to a macroalgae (*Ulva* sp. and *Gracilaria* sp.) pond. Yields of *Ulva* reach 50 kg per m^2 per year. The macroalgae strip the dissolved nutrients from the water, thus cleaning it to a degree which allows sending it back to sea (without the danger of polluting it), or back to the fish pond. The IPS can be implemented worldwide at any place where the ocean meets the desert.

The employment of molecular biology-based technologies is crucial to the advancement of mariculture, if it is to achieve the production goal of about 60 million tons of fish in the year 2020. A few examples which were developed at the NCM in recent years:

1. A slow hormone release implant for the reproductive control of fish known as the ReproBoost;

2. Diagnostics of viral and bacterial diseases; and

3. Fingerprinting of genetic strains using the genomic DNA.

It is our intention, in the foreseeable future, to employ genetic engineering for improving the performance of cultured fish, to raise macroalgae to produce valuable compounds, and to commercially protect genetically-improved organisms. It is also our intention to develop fermentation technologies that will convert mariculture wastes to valuable, recycled protein.

Last, but not least, we intend to participate in an international effort to domesticate large pelagic fish such as the bluefin tuna, for farming purposes as well as for the preservation of the species.

Cloning and Characterization of Teleost Insulin Receptor Family Members: Expression of Insulin and IGF Type I Receptor Messenger RNAs

Michael W. Greene and Thomas T. Chen
Department of Molecular and Cell Biology and Biotechnology Center
University of Connecticut
Storrs, Connecticut 06269

Mammalian and fish ligands have been used to determine insulin and insulin-like growth factor (IGF) binding affinities and receptor number with remarkable similarities between these two vertebrate classes, suggesting functional conservation. Yet, the nature and structure of teleost insulin receptor family members is not known. Therefore, the cloning and mRNA characterization of rainbow trout insulin and IGF type I receptors was undertaken. Three insulin and two IGF type I receptor cDNAs were isolated by screening a cDNA library and 5' rapid amplification of cDNA ends (RACE) respectively, and confirmed as separate genes by separate genomic Southern hybridization. A high degree of amino acid identity was observed between rainbow trout insulin receptors (rtIRs) and rainbow trout IGF type I receptors (rtIGFRs) and their human homologs, confirming the structural similarities between mammalian and fish insulin receptor family members.

Polyadenylation status of rtIR and rtIGFR mRNA during embryonic development in rainbow trout was investigated by reverse transcription (RT)-PCR from total RNA using either oligo(dT) or random hexamer primers for reverse transcription. A quantitative RT-PCR assay was used to determine the relative levels of rtIR and rtIGFR mRNA in various rainbow trout tissues at two developmental stages. Results from these studies suggest a complex expression pattern of insulin and IGF type I receptor mRNAs in partial tetraploid fish.

Cell and Tissue Culture of *Porphyra pseudolinearis* Ueda (Rhodophyta)

Hyung Geun Kim
College of LIfe Science
Kangnung National University
Kangnung 210-702, Korea

Studies on protoplast and tissue culture were carried out to develop a new technique for seed production of *Porphyra pseudolinearis* Ueda (Bangiales, Rhodophyta). Selected vegetative pieces were incubated overnight in an antibiotic solution and used for protoplast and tissue culture. Protoplasts were prepared from fresh vegetative tissue of the foliose thallus. The vegetative tissue was pretreated, cut into small pieces and soaked in 2% papain solution, and then the small pieces of tissue were incubated in a tube containing 5 ml of alkaline hemicellulase solution (MES buffer and pH 6.0). The cell wall of the protoplasts regenerated after 2-3 days, and the proptoplasts germinated after 5-10 days in culture at 17°C and 10 μmol/m²/s (10L:14D). After 10 weeks, the protoplast germlings developed into big foliose thalli about 10 cm long. Two types of foliose thalli different from the original one were observed: one was wider and the other slender. In tissue culture, excised discoidal pieces (1.5 mm in diameter) from young thalli were cultured at various temperatures (5~30°C) and under various light intensities (10-80 μmol/m²/s) with a 14L:10D photoperiod. Fairly good conditions for the growth and reproduction of excised pieces were observed at 10 and 15°C, 40 and 80 μmol/m²/s. Compared with the developmental pattern of protoplast, small pieces of tissue were characterized by irregular cell division and disorganized cell growth. Protoplasts regenerated new thalli more easily than tissue. This study reveals aspects of the potential of vegetative propagation through protoplasts and tissue for *Porphyra*.

Traits We Select For: Has Temperature Been Forgotten?

George P. Kraemer
Division of Natural Sciences
Purchase College
735 Anderson Hill Road
Purchase, New York 10577

Biologists traditionally think of irradiance and nutrient availability when asked what factors control production by macrophytes. Consequently, mariculturists may base phenotypic selection upon photosynthesis-irradiance or nutrient uptake kinetics. However, temperature may also play an important role. I present research examining the correspondence between the potential environmental controls of production (irradiance, nutrient availability, temperature) and the growth and enzyme activity (glutamine synthetase) of three Mediterranean seagrasses. These results, coupled with those of other researchers, suggest that, while light and nutrients may determine the potential productivity, temperature may actually limit actual rates. Temperature constraints may act either via low temperature limitation of cellular enzymatic activity or high temperature respiratory consumption of production.

Hormones Affecting Reproduction and Morphogenesis in Crustaceans

Hans Laufer
Department of Molecular and Cell Biology
U-125, University of Connecticut
Storrs, Connecticut 06269

Crustacean reproduction, growth and morphogenesis are controlled by hormones. We have discovered that methyl farnesoate (MF), an unepoxidated form of the insect juvenile hormone (JH III) is found in all species of crustacea examined to date and includes, by now, more than thirty species. Furthermore, extensive data are available that indicate the synthesis and secretion of MF was not only correlated with reproduction but several lines of research suggest that reproduction appears to be dependent on the production of MF. We, along with others, have shown that there are binding proteins for MF in the blood and receptors for MF in target tissues. From metabolic studies, we concluded that MF is not metabolized to higher or more complex forms, such as JH III, but is degraded by specific esterases. The most critical evidence that MF functions as a reproductive hormone was provided by improving shrimp reproduction and larva production by as much as 80% with MF treatments. These experiments were carried out in a commercial hatchery in a blind study (Laufer, 1992, Laufer *et al.*, 1997). Shrimp maturation and reproduction are otherwise difficult to achieve reliably in captivity.

Many of our experiments used *Libinia emarginata,* a local spider crab, as a good example of a model crustacean. The spider crab was used to gain a better understanding of hormonal controls of reproduction, hormonal controls and morphogenesis, relevant to commercially significant species such as shrimp, crabs and lobster.

One of our recent, important findings was isolating and identifying the mandibular organ inhibiting hormone (MOIH) of *L. emarginata,* a new class of regulatory hormone. This hormone is a neuropeptide from the eyestalk X-organ-sinus gland complex and it inhibits the secretion of MF by the mandibular organ (MO), thus inhibiting reproduction (Liu *et al.*, 1997). This hormone has been sequenced and has recently been cloned (Liu *et al.*, 1998). The sequence indicates that it is a member of an important family of crustacean hormones, the hyperglycemic hormones (CHH); hormones that appear to have functions which include carbohydrate metabolism, molt inhibition, reproductive control, and morphogenesis.

In our investigations we have determined the MF concentrations in close relatives of *L. emarginata* that are commercially important. In the snow crab, *Chinoecetes bairdi,* actively reproductive females possess close to sixty times more MF in their blood than reproductively inactive females. Males have nearly ten times more MF while mating than non-mating males. Animals such as *L. emarginata* that are well past their maturational molt have significantly larger reproductive tracts (reproductive indices), with males and females having at least twice the MF concentration in their blood and three times larger reproductive indices (Laufer *et al.*, 1996). These results taken together strongly support the conclusion of an active role for MF in crustacean reproduction.

We have shown that male and female *L. emarginata* have terminal molts. Females are capable of reproducing promptly after molting while males have a significantly longer delay before they are sexually active. This delay was discovered to be almost a full year in *Libinia.* Similar events seem to apply to *Chionoecetes*

bairdi. These findings may have important implications for management of the population of spider crabs for the fishing industry.

Another important finding correlates with the morphogenetic functions of MF. We have shown, conclusively, that a function of MF suppresses maturation in juvenile crabs, while in the absence of MF females undergo their maturational molt (Laufer *et al.*, 1997A). Large claws on male spider crabs are produced in the absence of MF at the final critical pre-molt period (Ahl and Laufer, 1996). This process is called the "terminal differential" molt. Experiments on larval *Macrobraclium rosenbergil* also show that MF functions as an inhibitor of metamorphosis.

Recently we have shown that MF regulates itself in feed back control. While in low concentrations in vitro, MF achieves positive feedback by turning on the mandibular organs to synthesize MF. While at higher concentrations, MF appears to bring about a negative feedback by reducing MF synthesis in actively synthesizing glands. Such a control may be important for crustacean egg production and may function in vivo.

When applied to economically valuable species such as shrimp, our discoveries relating to MF and its control may prove to be ultimately extremely useful both for growth and reproduction. Viral diseases which are reintroduced when broodstock or larvae are introduced from the wild are the most important problem facing the shrimp industry today. Our research will permit the closing of the life cycle in captivity and the selection and production of superior domestic broodstocks and elimination of the need to reintroduce wild organisms. We predict that with the knowledge of how MF functions in crustacea, and with MOIH genes, the critical genes for control of MF synthesis, now in hand, it should be possible to control the expression of these genes and regulate both reproduction and growth of economically important crustaceans.

Acknowledgments

The research reported here was funded with support from the Connecticut Sea Grant College Program, NOAA. Without this support, this research would not have been possible.

References

Ahl, J.S.B. and Laufer, H., 1996. The pubertal molt revisited. *Invert. Reprod. and Develop.* 30: 177-180.

Laufer, H., 1992. Method for increasing crustacean larval production. United States Patent & Trademark Office, Patent Number 5,161,481 (Nov. 10, 1992).

Laufer, H., Ahl, J.S.B., Takac, P., Scott, G., Stevens, B., Munk, J.E., and Payne, S.A., 1996. Hormone and reproductive strategies in spider crabs with emphasis on commercially important species., *High Latitude Crabs: Biology, Management, and Economics.* Alaska Sea Grant College Program. 02:383-387.

Laufer, H., Takac, P., Ahl, J.S.B., and Laufer, M.R., 1997. Methyl Farnesoate and the effect of eyestalk ablation on the morphogenesis of the juvenile female spider crab, *Libinia emarginata..*, *Invert. Reprod. Develop.* 31, 63-68 with Erratum in *Invert. Reprod. Develop.* 32:187.

Laufer, H., Paddon, J., and Paddon, M., 1997. A hormone enhancing laeva production in the pacific white shrimp *Panaeus vannamei.* IN: *IV Symposium on Aquaculture in Central America: Focusing on shrimp and tilapia.* D.E. Alson, B.W. Green and H.C. Clifford, ed., Asociacion National de Acuocultura de Honrurus and The Aquaculture Society, pp.161-162.

Liu, L., Laufer, H., Wang, Y., and Hayes, T., 1997. A neurohormone regulating both methyl farnesoate synthesis and glucose metabolism in a crustacean. *Biochem. Biophys. Research Communic.* 237: 694-701.

Liu, L., Laufer, H., Gogarten, P.J., and Wang, Y., 1998. CDNA cloning of a mandibular organ inhibiting hormone from the spider crab *Libinia emarginata. Invert. Neuroscience* (In Press).

Aquaculture: Feed Management, Feeds, and Environmental Quality

Addison L. Lawrence[1], M. Velasco[1], R. Montoya[1] and T. M. Samocha[2]
Shrimp Mariculture Project
Texas A&M University
[1]1300 Port Street
Port Aransas, Texas 78373
[2]4301 Waldron Road
Corpus Christi, Texas 78418

There is an increase in environmental discharge regulations by governments. This has been in response to an environmental impact to natural estuaries and bay systems from aquaculture. Profit margins of existing farms and expansion of the aquaculture industy are being constrained by the need to maintain adequate water quality parameters and acceptable levels of pollutants in effluent discharge. Effluent discharge has been associated with degradation of the quality of the receiving water, which is causing environmental concern. Thus, there is a need to minimize the pollutants in the effluent discharge for the benefit of the local ecosystem quality and productivity. Feeds represent a major contribution of pollutants in the discharge system. This is particularly true as the number of hectares in production in an estuary or bay system and/or intensification of production systems occur. This paper summarizes information concerned with decreasing the contribution of pollutants from aquaculture feeds using laboratory studies and the shrimp, *Penaeus vannamei,* as the experimental system.

Results indicated that by: (1) optimizing feed amino acid profile, feed protein availability in terms of apparent digestibility, feed management in terms of number of feedings per day, and ingestion rates by adding attractants; the feed protein requirement decreased from 30% to 15%; and (2) optimizing Ca and PO_4 feed levels and PO_4 ingredient source (e.g. $CaHPO_4$, Na_2HPO_4, and NaH_2PO_4) the feed PO_4 requirement decreased from 1% to 0.5%. Data also showed that the amount of PO_4 contributed to ambient water depends upon feed rate, feed level of NaH_2PO_4, and PO_4 source in terms of $CaHPO_4$, Na_2HPO_4, and NaH_2PO_4, and the amount of NH_3 contributed to ambient water depends upon feed rate and feed level of protein and energy.

These data show that NH_3 and PO_4 pollution to water effluent can be potentially reduced by 60% and 50%, respectively. Minimization of pollution from dry feeds and development of "environmentally-friendly feeds" or "low pollution feeds" is possible. For the future, because of environmental requirements with increasing aquaculture production, feeds may be formulated on an "environmental impact" basis and not "least cost" basis.

Rational Immunotherapy For Fish Vaccines

Jo-Ann C. Leong, John Drennan, Carol H. Kim,
Marc Johnson, Ben Simon, and Estelle Thoman
Department of Microbiology and Center for Salmon Disease Research
Oregon State University
Corvallis, Oregon 97331-3804

Disease outbreaks at aquaculture facilities have devastating economic impacts on an important U.S. industry and a severe ecological impact on endangered anadromous fish populations that require enhancement for continued reproduction. For example, the sale of trout products rose in the United States from $72 million in 1996 to $78 million in 1997 (Harvey, 1997). However, recorded trout losses were 6.79 million pounds and 74% of those losses were due to disease (*Aquaculture Magazine*, 1998). The larger U.S. catfish industry with production reaching 520 million pounds (worth approximately $427 million) in 1997 was also hit with disease problems. Clearly, disease remains a primary concern for the aquaculture industry. Since the use of antibiotics and chemotherapeutic treatments in aquaculture has been restricted, vaccines are needed to control disease outbreaks. The problem is that there are very few vaccines available in the marketplace. The process for developing vaccines, evaluating their efficacy, and licensing them is too lengthy and sometimes too expensive for the aquaculture vaccine industry.

Conventional methods for evaluating vaccines take large numbers of animals, specialized tank facilities, and highly trained personnel. They involve protocols requiring fish immunization by injection or immersion followed after 30 days by lethal challenge with the pathogen. The efficacy of the vaccine is measured by percentage of animals that survive pathogen exposure as compared to percentage of survivors among the control, unvaccinated fish. This process takes a minimum of 60 days and in most cases, more time is required for sample processing and evaluation. In the meantime, the test animals have to be maintained at significant expense to the researcher. To further complicate matters, there are many viral and bacterial systems, namely infectious pancreatic necrosis virus (IPNV) and bacterial kidney disease (BKD), where symptomatic or lethal infection is consistently observed. With BKD, the in vivo challenge assays can take as long as 90 days. For IPNV, producing a lethal challenge in control animals is not always possible. Although the immune response may be assessed by measuring antibody titer, the development of humoral immunity has not always been a good indicator of vaccine efficacy in fish. These difficulties have led us to ask whether there are other methods for evaluating vaccine efficacy and other methods for immunizing fish so that the immune response is predictable and efficacious.

In mammalian systems, vaccine efficacy is estimated by the survival of vaccinated animals after challenge with a lethal dose of the pathogen and by the immune response to the vaccine. The immune response is measured by in vitro assays for antibody and with an assortment of assays for cell-mediated immunity (CMI), cytokine production, and cytokine receptor expression. For fish, CMI assays are difficult and reagents to detect cytokines or their receptors are not available; researchers at this time have no other choice than to rely on lethal challenge to evaluate the efficacy of all vaccines. This is a major constraint to progress in vaccine development.

As part of our continuing effort to develop effective vaccines for fish, our laboratory has identified several markers for immune function in sahnonid fish. The Mx gene, a type I interferon (IFNα/β)-inducible gene, has been cloned,

characterized, and a rabbit anti-Mx polyclonal antibody reagent has been produced (Trobridge and Leong, 1995). The Mx promoter has also been cloned and inserted upstream from a luciferase reporter gene to produce a quick detection system for the IFN α/β in fish sera. Since IFN α/β is induced by viral infection in leukocytes and fibroblasts, the assay provides a quick assessment of the non-specific immune response to viral infection in fish. In addition, the promoter for the interferon regulatory factor (IRF) has been cloned and inserted upstream of a luciferase (luc) reporter gene. This IRF-luc plasmid was constructed to monitor the production of interferon gamma (IFN-γ), which is induced by mitogenic or antigenic stimulation of T lymphocytes and natural killer (NK) cells. These clones provide us with tools that might be used to monitor the immune response to vaccination and may lead to the development of efficient, cost effective methods of testing vaccines in fish.

Preliminary results indicate that when fish are vaccinated with formalin-killed virus or with an *E. coli* producted subunit vaccine to infectious hematopoietic necrosis virus (IHNV), no Mx induction until challenge with live IHNV was observed. These fish were moderately protected against virus challenge, i.e. 50% of the fish died after challenge. However, when fish were vaccinated with the DNA vaccine, pCMVG, Mx was found prominently in extracts of liver and kidney tissues of the vaccinated fish early after injection. Upon virus challenge, Mx disappeared from these tissues. The genetically immunized fish were well protected against the lethal effects of virus challenge with 98% survival. This results has been observed in several different experiments and led us to predict that Mx and other similar markers might be used to assess vaccine efficacy.

References

Anon. 1998. *Aquaculture Magazine Buyer's Guide*. p.24.

Harvey, D. 1997. *Aquaculture Outlook*, Nov. 1.

Trobridge and Leong. 1995.

Bioremediation to Sushi: New England Marine Agronomy, from Farming to Biotechnology

Ira A. Levine
Coastal Plantations International, Inc.
4 Moulton Street, Suite 440
Portland, Maine 04101

The red alga *Porphyra* (nori) is utilized by humans as a food source throughout the world. In 1996, approximately 14 billion sheets (approximately 44,000 dry metric tons) of *Porphyra* were produced with an annual value of $ 1.6 billion. The consumption of nori, long prized as a complement to rice, sushi, soups and salads, is also a source of phycoerythrin, a potential source of pharmaceuticals, nutraceuticals and cosmaceuticals. The source for U.S. nori was previously dependent upon the importation from Japan, Korea and China prior to Coastal Plantations International (CPI) entering the market. CPI is trying to expand the uses of nori in America through alternative food and industrial uses.

Commercial cultivation of the red alga *Porphyra* (nori) was initiated in Cobscook Bay, Maine in 1991 by CPI. The transfer and modification of Japanese, Korean, Chinese and Washington State's nori cultivation and processing technologies to Maine has resulted in the world's first internationally- certified, organically-cultivated and processed nori. Additionally, a pilot bioremediation project was initiated with the establishment of a nori-salmon integrated polyculture system.

The current American nori industry, by nature of CPI's sole participation, is vertically integrated as compared to their Asian counterparts. The company has had to develop corporate, financial, scientific, regulatory, political, engineering, marketing, distribution, and socioeconomic expertise to evolve the emerging industry to its current status. CPI has authored farming manuals, offered single day seminars and a six month long nori farming training course in hopes of recruiting the next generation of independent nori farmers. The company desires to support independent nori farming and polyculture concerns from Long Island Sound to Newfoundland.

The success of nori cultivation is a function of matching a cultivar to local environmental conditions. The company's original cultivar, *Porphyra yezoensis*, imported from Japan, is not an ideal species for Maine's waters. Therefore hybrids were developed utilizing genetic modification and transformation techniques. CPI set out to develop new growing strains of *Porphyra* for farming along the New England coast and the Western Pacific Ocean. The use of CPI's licensed technologies has resulted in the development of "Super Nori", a faster growing strain. The next cultivar enhancement effort will concentrate on developing a disease-resistant strain to offset the industry's annual 30% crop loss due to fungal and bacterial infections.

CPI's progress has resulted in the formation of two divisions. The Maine Nori Company represents CPI's effort to produce and market organic nori and develop new independent nori farming and polyculture operations. PhycoGen, a marine biotechnology research and product development effort, is focusing on producing new industrial products and pharmaceuticals through natural product discovery, site-directed mutagenesis, phycoremediation, and genetic modifications and transformations.

Integrated Cold Water Aquaculture in the Tropics

George Lockwood
P.O. Box 169
Carmel Valley, California 93924

Abalone, kelp (to feed abalone), salmon, oysters, and microalgae (to feed oysters) have been successfully grown on a commercial scale in an integrated system with cold, pure water pumped from the deep ocean into on-shore ponds and tanks in Hawaii at a location with high levels of solar radiation. In this system, the salmon and kelp grew together in large ponds. The salmon were fed pelleted rations and their metabolites of ammonia, carbon dioxide, nitrate and phosphate were nutrients for kelp. The photosynthesizing kelp returned oxygen to the pond. In addition, microalgae grew in the rich pond environment and oysters feed on the microalgae. Abalone grew in separate tanks and were fed kelp grown in the pond.

The cold water from a depth of approximately 650 meters is virtually free of biological life and contains no organisms harmful to either the species grown or to humans who consume them. This water is also rich in nitrate, phosphate, silica, and dissolved carbon dioxide. High levels of solar radiation in this environment support growth of marine algae. Deep ocean water entered the ponds at 6°C to 7°C and was warmed by solar radiation and contact with the air. The pond was held at 15°C which is a good temperature for all of the species grown.

In each case, rapid growth was experienced with a lack of diseases. Constant temperatures with the absence of disease organisms provided good animal health conditions. Survival rates were high and feed conversion rates low. Production costs for each commercial species were low. The system was very stable.

This system is the forerunner of a planned integrated facility in Jamaica that, in addition to growing salmon, kelp, abalone, microalgae and oysters, will include tilapia and *Gracilaria*, an agar-producing marine macroalgae. Cold seawater will be pumped from the deep ocean into ponds with salmon and kelp. As in Hawaii, abalone in separate tanks will be fed kelp. Solids will be separated from the water flowing out of the salmon-kelp ponds and abalone tanks. The clarified water will then flow into algae ponds. The microalgae-rich water will flow into oyster ponds, and from there into ponds with tilapia. Saltwater tilapia will feed upon fecal matter from the salmon, oysters and abalone, microalgae, and a relatively small amount of pelleted rations. Finally, the now-warmed water will flow into ponds of *Gracilaria* before being discharged into the ocean.

During this process the nitrogen, phosphorus and carbon inputs are taken up by algae at various stages to produce protein, carbohydrates and lipids consumed by the fish and shellfish. The final stage of *Gracilaria* removes most of the remaining nutrients. A balance is maintained throughout the system with the addition of inorganic nutrients as required. Unlike any other form of agriculture or aquaculture, this multi-stage integrated system provides high levels of environmental sustainability.

Recirculating Aquaculture Technology for Finfish Production: A Review of Considerations and Components for Solids Removal and Biological Nitrification Options

Thomas M. Losordo
Departments of Zoology and Biological & Agricultural Engineering
College of Agriculture and Life Sciences
North Carolina State University
Raleigh, North Carolina 27695

Fish culture in many parts of the world is traditionally done in earthen ponds or in large tanks with flowing water. Pond culture requires large areas of flat land and significant quantities of clean groundwater. Tank culture requires less land, but needs more water per kg of fish produced to maintain good growing conditions within the tank. In many areas of the world, aquaculture is not possible in ponds or flow-through tanks because of limited water supplies or an absence of suitable land.

Recirculating aquaculture systems re-use water over and over again, cleaning the waste from the water and providing oxygen to the fish. Because water is reused, recirculating fish production systems utilize only a fraction of the water required by traditional fish production techniques. A small domestic well producing 10 to 20 liters per minute, when coupled with the proper recirculating technology, can be used in the production of hundreds of metric tons of fish annually. Recirculating aquaculture production systems have created a great deal of interest worldwide. There is no doubt that fish can be reared in great quantities and at high densities in recirculating systems. However, the economic viability of growing fish in recirculating systems is not as certain. Many businesses with production systems based on water re-use technology have failed in the past five years.

Before getting involved in raising fish with recirculating technology, you should understand the important principles at work within the technology that you are using. A number of "unit operations" are required to renovate water such that it can be used over and over again in an aquaculture production system. These unit operations, graphically listed in Figure 1, include: 1) Waste solids removal 2) Nitrification 3) Oxygenation 4) Carbon dioxide removal 5) pH and alkalinity control and 6) Sterilization and disease control.

This presentation is meant only to be an introduction to the subject and to review two unit operations that are critical to the success of any recirculating fish production system; waste solids removal and nitrification.

Waste Solids Removal

Fish are usually fed commercial pelleted diets. These diets are made of protein, carbohydrates, fat, ash and moisture. Large quantities of these feeds are not assimilated by the fish and produce an organic waste excreted as faecal solids. These faecal solids and uneaten feed, when digested by bacteria in a recirculating system, use oxygen and produce dissolved ammonia (NH_3 + NH_4, also referred to as total ammonia nitrogen (TAN)). The un-ionized portion of TAN, NH_3, is extremely toxic to fish. The amount of TAN in the un-ionized form is dependent to a large extent upon the pH of the water. At a pH of 7.0, less than 1% of the TAN is in the toxic form, while at a pH of 9.0 over 40 % is in the un-ionized (toxic) form. Waste solids need to be removed from the tank and waste

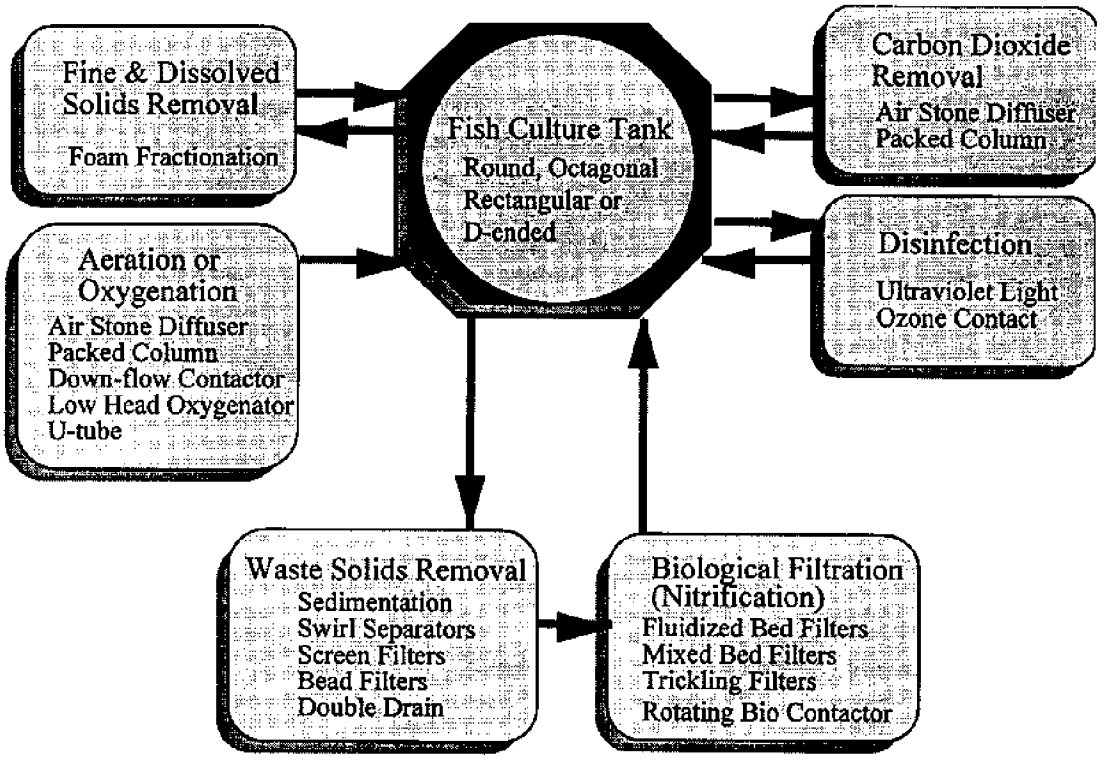


Figure 1. Required unit processes and some typical components used in recirculating aquaculture production systems.

treatment system quickly. Components that are being used in today's recirculating systems to remove waste solids include particle traps, sedimentation basins, swirl separators, screen filters and bead filters.

Biological Nitrification

Recirculating systems generally replace less than 10% of the total system volume per day with new water. As such, not enough new water can be flushed through the tank to control the build-up of harmful ammonia-nitrogen. To counteract this, the water treatment system of a recirculating production system must have a unit process designed to either remove or convert ammonia-nitrogen to a less toxic nitrogen compound. The process most often used is a biological one and it is referred to as biological filtration or biofiltration. In biofiltration, a process called nitrification occurs where toxic ammonia-nitrogen is converted to non-toxic nitrate nitrogen by naturally-occuring nitrifying bacteria. In biofiltration a "media" with a large surface area is provided for nitrifying bacteria to attach and multiply. Sand, plastic balls, plastic beads, plastic rings, and plastic plates are commonly used as biofiltration media. How the media is set-up within the filter and the conditions in which the bacteria come in contact with the water affects the efficiency of the process. Biofilters are very diverse in design. Some contain media (sand or plastic) that are continuously mixed within a column of water (fluidized bed and mixed bed bead filters). Some submerge the media in water in a fixed position and flow the water up or down through the media. Still others, referred to as trickling filters, hold the media out of the water and pass water over the media as in a shower, such that the water trickles over the media and attached bacteria. This presentation will review the most popular components and processes being used in the aquaculture industry today. The positive and negative aspects of each component will be highlighted.

European Aquaculture: Its Bright Future and the Technology We Use

Joseph McElwee
Turbard Iathar Chonamara Teo.
57 Drour Oir
Clybaum Road
Knocknacarra, Galway, Ireland

Without a doubt in the last 10 years throughout Europe, there has been a huge upsurge in the demand for seafood. This has been attributed to social and economic changes, but more importantly, due to the availability of a wide range of new species or a wider range of second value-added products, as processors become more adventuresome and knowledgeable.

So, exactly what is happening, and who is responsible? Put plainly, the consumer market is more educated, health-conscious, and has more disposable income than 10 years ago. The producers or aquaculturists have learned new methods of farming fish, better husbandry practices, and national governments are supporting the industry in a much more progressive manner. It must also be added that research institutions are now concentrating on practical research issues driven by industry, rather than pie-in-the-sky projects that will never see commercial realization. Make no mistake, we are all in this for money, either to support ourselves or a furthering of the industry. An added complication in Europe at the moment is the imposition of quotas on nearly all wild-caught stocks in European waters. This in turn is resulting in new species being caught and marketed. Education of the consumer market is now reaching new heights in commerciality, so as to ensure sales of the products.

So, how is it developing, and where is it going? Culture of salmonids has been pioneered by the Norwegians, followed by the Scots and Irish, with other smaller countries following. Shellfish culture has been traditional in most coastal regions of all European countries, but is now really economic in only certain areas, such as northern France and Spain in real quantities.

Ireland has pioneered the culture of salmon offshore in sites up to nine miles offshore, in completely exposed sea areas. The development of this equipment, the culture methods, and the technology required has been developed from scratch, and has cost the industry millions of dollars. However, the rewards are now being reaped, and the technology adapted for other species such as the farming of sea bass and bream in the Mediterrean in large flexible square cages. In Israel at present, there are also trials in exposed areas, so as to assess suitability for alternative species. Current marine farming in Europe is restricted to a few species due to the relatively tight temperature range, so for the more advantageous culture species such as turbot, halibut, eels, Artic char, abalone, seaweeds and seamosses, to name but a few, the design of indoor or land-based pump systems with high tech approaches is being taken. These are very expensive, and although they require a quick return

on investment, there appears to be investors out there, and it is these individuals who are driving the industry forward. The high tech equipment in use in recirculation facilities is constantly being adapted for both warmwater species such as eels, and colder water species such as cod or wolffish.

The forward development in Europe is being driven by consumer demand at present, due to the better education of people (or more efficient forms of advertising), a better economic climate, and a more health-conscious public. We are also preparing for a huge market that is about to open with the harmonisation or creation of the Federal Europe, which will mean a potential market of 400 million people! The products coming out of Irish farms or indeed European countries find a ready home in Eurpe, and little needs to be exported to the United States or Australia. This is negative thinking as there is a huge untapped market in the U.S. and not even they have realized this, as is evident by the slow development of aquaculture in the U.S. itself. This is not intended as an insult, but in reality with a potential market of 250 million people, the consumption per capita is extremely low compared to that of the Europeans. This needs to be addressed by producers and of course the legislative bodies who can help get the industry moving forward.

Europe is moving forward with a numberof incentives on the financial side, including tax incentives for investors in foreign countries, a number of technological and biological exchanges between countries and staff, the opening of trading borders between countries, and tariffs for those not adhering to the rules.

Genetic Engineering for the Improvement of Marine Macroalgae

Subhash C. Minocha
Department of Plant Biology
University of New Hampshire
Durham, New Hampshire 03824

Marine macroalgae (seaweeds), in addition to their food value (particularly in the Orient), are the primary source of a variety of polysaccharides (agars, algins, agarose and carrageenans) of commercial use (For review see Noda, 1993; Jensen, 1993; Renn, 1997). These polysaccharides are used as emulsifiers, gelling agents, food additives, and pharmaceuticals. Agar and agarose (and also the phycobiliproteins) are important products for biotechnology research and growth media for microorganisms. In addition, there is a tremendous potential to use seaweeds as future sources of new pharmaceuticals, nutraceuticals, and for biomonitors of organic pollutants and heavy metals (Jensen, 1993; Renn, 1997). Most of the seaweed is presently obtained either from harvesting of natural populations or shore-line culture of some selected varieties. Aquaculture of genetically improved genotypes has been variously discussed but not much practiced (Cheney, 1990; Renn, 1997).

Over the past two centuries, genetic improvements in plants have been achieved largely through breeding programs involving hybridization, selection and mutagenesis. More recently, somaclonal variation and somatic hybridization have also been attempted but with limited success. By far the most effective method for genetic improvement of specific traits that are controlled by single genes seems to be genetic engineering, i.e. the transfer and expression of cloned genes into plant cells followed by regeneration of the transformed plants.

Some species of seaweeds, such as *Porphyra yezoensis* (nori), have been subjected to considerable amounts of selection for adaptation to local growing conditions, optimization of growth on nets, taste, color, and texture, etc. (Shin and Miura, 1995). In other cases, limited laboratory attempts at selection of mutants, induced mutagenesis and sexual hybridization have been described but few, if any, strains have been brought to the field for commercial scale production. Still, the worldwide cultivation of nori is still dominated by a single species, which is grown largely in Japan, China, and Korea. Recently, attempts have been made to adapt this species in the waters off the coast of Maine in the northeastern United States (Sperr *et al.*, 1996; Yarish *et al.*, 1996, 1997). While some commercial success has been reported (Levine, 1998), much remains to be done for large-scale commercial success. Attempts are also being made to improve native species of *Porphyra* (e.g. *P. umbilicalis, P. linearis, P. purpurea,* and *P. amplissima)* for commercial cultivation for both human consumption as well as a source of pigments and other industrial products. While well adapted to growth in their native environments and otherwise highly suitable for commercialization, the quality of the product from these species requires much improvement (Charles Yarish, personal communication). Selection, somatic hybridization, and mutagenesis are all being attempted for genetic improvement of these species (Cheney *et al.*, 1998). While all these approaches will likely aid in the production of

improved strains, the use of molecular and genetic engineering techniques should have a profound impact on the rate of genetic improvement of these species as well as further improvement of desirable traits that can be targeted for such improvements.

Genetic engineering generally involves the transfer of the coding sequence of a homologous or a heterologous gene under the control of a constitutive, tissue specific, or an inducible promoter to modulate one or more physiological or biochemical traits. Thus, in contrast to conventional and somatic hybridization, where whole genomes are often altered, genetic engineering is very precise in targeting specific characteristics or pathways. In the case of edible seaweeds (e.g. nori), these could include texture, color, taste, flavor, nutritional value and quality, reproductive cycle, and virtually any other genetically regulated characteristic for which gene cloning is feasible. In other species, modification of cell wall polysaccharides (particularly their sulfur content), production of pigments and pharmaceuticals, and their ability to metabolize pollutants and sequester heavy metals from contaminated waters could be easily targeted.

In addition to having the appropriate gene for transfer, the primary requirements for genetic engineering are: (1) a reliable means of transferring the gene to a competent recipient cell, (2) selection of transformed cells from a large population of untransformed cells and their subsequent development into whole plants, (3) the ability of transformed plants to reproduce for mass propagation, and (4) the ability to regulate the expression of the transgene in desirable target cells and at specific stages of development of the plant. Whereas impressive advances have been made in producing transgenic varieties of commercial importance in dozens of higher plant species (Dale *et al.*, 1993; Kahl & Winter, 1995), the techniques of genetic engineering have not been attempted much with algae. Other than some examples of foreign gene transfer in a few microalgae (for review see Stevens and Purton, 1997), there are only scant reports on preliminary work with the macroalgae (Stevens and Purton, 1997). Our laboratory reported on the transient expression of foreign genes in a red macroalga *Porphyra miniata* (Kubler *et al.*, 1994) and a green macroalga *Ulva lactuca* (Huang *et al.*, 1996). Thus, the field of genetic engineering of macroalgae, with its vast potential for the improvement of many species, still remains wide open.

A number of approaches have been used to transfer genes into plant cells (Jones, 1995; Christou, 1996). A variety of plant promoters which show varying degrees of constitutive or inducible expression have been used to drive the expression of the transgenes. The selection of promoter is guided by the desired expression in different plant tissues depending upon the application. Recombinant plasmids carrying the desired genes are transferred to plant cells, either passively (direct uptake, with or without polyethylene glycol) or mechanically (microinjection) or with the aid of electric pulses (electroporation) or biolistic bombardment (gene gun) or fusion of liposomes. Viral or bacterial vectors are also often used to transfer the genes. With the exception of biolistic bombardment and viral/bacterial vectors, which can use intact plant cells and tissues, all other methods generally utilize protoplasts. The latter, because of their lack of cell walls are easy to transform but, in many cases, difficult to regenerate into whole plants. The selection of the transformed cells is either based upon visual aids using reporter genes (e.g. luciferase or green fluorescent protein - GFP) or their ability to grow on a cytotoxic compound (e.g. antibiotics, herbicides, etc.). A critical prerequisite for successful genetic engineering is the regeneration of transformed cells into whole plants. Several decades of work on the regulation of morphogenesis and somatic embryogenesis in plant cell cultures has resulted in hundreds of higher plant species being regenerated from protoplasts and callus. Although vegetative propagation by fragmentation and the formation of a variety of spores in algae is common, controlled sporulation and mass propagation of few species can be done on a commercial scale.

In comparison with land plants, the background information on all of the steps needed for genetic manipulation of macroalgae is scant. While a

number of seaweed species have been known to regenerate from excised tissues and protoplasts, the sensitivity of these cells/plants to cytotoxic compounds commonly used for selection of transformed cells has not been tested. Perhaps, the biggest limitation for genetic engineering in seaweeds is that no macroalgal promoters are yet available for testing with reporter or selection marker genes, and it is not established whether the higher plant promoters will or will not work for expression of transgenes in the algal cells. Since the life cycles of most macroalgae involve the production of several different types of spores which, in many cases, possess a relatively thin cell wall and show a very high frequency of regeneration, it should be a significant advantage for the use of gene transfer techniques. Furthermore, the presence of independently existing gametophytic (haploid) and sporophytic (diploid) generations, and the ability of clonal multiplication of either generation through fragmentation and/or specialized spores, should allow a rapid propagation of the transgenic plants in culture and in the field.

During the past few years, research in our laboratory has been aimed at developing reliable protocols for transfer of a reporter gene into protoplasts of *Porphyra* and *Ulva*. Significant progress has been made in several steps to achieve transformation. The three steps that were targeted were: (1) the regeneration from protoplasts; (2) the effects of several antibiotics on protoplast growth and development; and (3) transient expression of the β-glucuronidase (GUS) gene following transfer of DNA by electroporation.

While transient expression of the GUS gene was observed in several experiments, no multicellular thalli showing GUS expression were obtained. We believe that this is due to the problem with the 35S CaMV promoter that we used rather than a problem with the transfer and integration of DNA. It is conceivable that integration of the foreign DNA into the genome of these algae is followed by silencing of the promoter activity. Based upon this assumption, our present efforts are directed at the isolation and characterization of different types of promoters (constitutive, inducible and developmentally regulated) in these algae. The availability of these promoters will not only aid in the development of reliable transformation protocols, but also provide means of regulating the expression of commercially useful genes in macroalgae.

In conclusion, most, but not all, steps towards genetic transformation of seaweeds have been realized. Probably the most significant advance would be the cloning of homologous algal promoters for use with selection and reporter genes and, in future, in regulating the expression of commercially useful genes in macroalgae. Other areas of molecular biology of seaweeds that need to be worked on to take advantage of the genetic engineering tools include the identification of commercially useful genes to alter metabolic pathways of polysaccharide biosynthesis, genes controlling growth and development rates, genes controlling the biosynthesis of phytochelatins and metallothioneins, and genes controlling sex determination and gametogenesis. The latter should aid us in producing strains that are incapable of producing gametes and thus unable to spread the transgene from the transgenic populations to native populations.

Ultimately, the success of genetic manipulation techniques with seaweeds will depend upon the economic gains to the aquafarmers, who thus far have depended entirely on the empirical selection of naturally occurring genotypes and improvements in the mechanical aspects of aquaculture technologies. The precise genetic improvement of selected strains will require long-term investments into continued research, development and evaluation of genetic manipulation technologies specifically suited for the seaweeds. At present, the best that can be said is that we have started to think and plan about using the gene transfer technology and the associated molecular techniques for genetic improvement of seaweeds and also the microalgae, with the sporadic demonstration of success in the use of presently available techniques. Soon we must demonstrate and document improvement of the products at the field level and demonstrate its ultimate gains to the aquafarmers. In my view,

this is likely to come first with species that have industrial and pharmaceutical uses rather than those used directly as food. Some of these include: (1) improved quality and yield of carrageenans, agar, agarose, algin, pigments, and pharmaceuticals, (2) an increased ability to metabolize marine pollutants, (3) increased ability to sequester heavy metals, and (4) improvement in the traits that aid in the mechanization of aquaculture processes (e.g. seeding of nets and growth on shell-free surfaces for nori). Recent advances with land plants in the transfer of multigene constructs (Lough *et al.*, 1997), the ability to transfer large (hundreds of kb) fragments of DNA molecules (Hamilton *et al.*, 1996), targeting of the transgene to specific sites in the genome, manipulation of the 5' and 3' ends of the gene to optimize gene expression (Koziel *et al.*, 1996), site-directed mutagenesis, gene disruption and replacement through homologous recombination (Miao and Lam, 1995; Kempin *et al.*, 1997), and a multitude of other molecular approaches to regulate the transfer and expression of foreign genes into recipient cell should provide exciting new approaches to produce highly desirable strains of commercially useful seaweeds. Ultimately, the selection of the gene and the recipient plant will be determined by a balance of economic gains and potential risks of environmental damage or purterbance in biodiversity of natural populations of seaweeds.

The farming of seaweeds in open bodies of water and the ability of the reproductive spores of algae to travel considerable distances, could potentially pose environmental and ecological problems of an unwanted spread of the genetically modified organisms or the transfer of the transgene to natural populations of related seaweeds. Innovative approaches to limit both sexual and asexual reproduction through spores may have to be incorporated into the strategies for aquaculture of genetically engineered seaweeds. Some leads into these areas are being developed for land plants (e.g. those involving male sterility and embryo abortion through gene transfer), and these could be equally suitable for the seaweeds.

References

Cheney, D.P. 1990. Genetic improvement of seaweeds through protoplast fusion. In: C. Yarish, C.A. Penniman, and P. Van Patten (Eds.). *Economically Important Marine Plants of the Atlantic: Their Biology and Cultivation.* Connecticut Sea Grant College Program Vol. 1:15-25.

Cheney, D.P., B. Metz, I. Levine, and B. Rudolph. 1998. Genetic manipulation and strain improvement of seaweeds for aquaculture. *Proc. World Aquaculture '98.* Abs. p. 105.

Christou, P. 1996. Transformation technology. *Trends Plant Sci.* 1: 423-431.

Dale, P.J., J.A. Irwin and J.A. Scheffler. 1993. The experimental and commercial release of transgenic crop plants. *Plant Breed.* 111: 1-22.

Hamilton, C.M., A. Frary, C. Lewis and S.D. Tanksley. 1996. Stable transfer of intact high molecular weight DNA into plant chromosomes. *Proc. Natl. Acad. Sci.* USA 93: 9975-9979.

Huang, X., J.C. Weber, T.K. Hinson, A.C. Mathieson and S.C. Minocha. 1996. Transient expression of the GUS reporter gene in the protoplasts and partially digested cells of *Ulva lactuca* L. (Chlorophyta). *Bot. Mar.* 39:467-474.

Jensen, A. 1993. Present and future needs for algae and algal products. In: A.R.O. Chapman, M.T. Brown and M. Lahaye (eds.), Proc. 14th. Intern. Seaweed Symp., Brest, Fr., *Hydrobiologia.* 260/261:15-23.

Jones, H. (Ed.). 1995. *Plant Gene Transfer and Expression Protocols.* Humana
Press. Totowa, NJ. Pp. 1-466.

Kahl, G. and P. Winter. 1995. Plant genetic engineering for crop improvement. World J. *Microbiol. Biotech.* 11: 449-460.

Kempin, S.A., S.J. Liljegren, L.M. Block, S.D. Rounsley, E. Lam and M.F. Yanofsky. 1997. Targeted disruption in *Arabidopsis. Nature* 389: 802-803.

Koziel, M.G., N.B. Carozzi and N. Desai. 1996. Optimizing expression of transgenes with an emphasis on post-transcriptional events. *Plant Mol. Biol.* 32: 393-405.

Knbler, J.E., S.C. Minocha and A.C. Mathieson. 1994. Transient expression of the GUS reporter gene in protoplasts of *Porphyra miniata* (Rhodophyta). *J. Mar. Biotechnol.* 1:165-169.

Levine, I.A. 1998. Commercial cultivation of *Porphyra* (nori) in the United States. *Proc. World Aquaculture '98.* Abs. p. 326.

Lough, T., C. Tourneur, J. Masson and C. Robaglia. 1997. Expression of genes in transgenic plants from bicistronic transcriptional units. *Plant Sci.* 129: 91-99.

Miao, A.-H. and E. Lam. 1995. Targeted disruption of the TGA3 locus in *Arabidopsis thaliana*. *Plant J.* 7: 359-365.

Noda, H. 1993. Health Benefits and nutritional properties of nori. *J. Applied Phycol.* 5:255-258.

Renn, D. 1997. Biotechnology and the red seaweed polysaccharide industry: status, needs and prospects.TIBTECH. 15: 9-14.

Shin, J.A., and A Miura. 1995. Genetic Improvement of Eating Quality of Dried Sheets of *Porphyra* by Using the Recombinant Wild-Type in *P. yezoensis* (Bangiales, Rhodophyta). *Korean J. Phycol.* 10:109-115.

Sperr, A.E., C. Yarish, X.G. Fei, A.C. Mathieson, and I. Levine. 1996. Field and culture studies of several *Porphyra* species for the development of a commercially viable nori aquaculture industry in New England. *J. Phycol.* (Suppl). 32 (3): 45.

Stevens, D.R. and S. Purton. 1997. Genetic engineering of eukaryotic algae; progress and prospects. *J. Phycol.* 33:713-722.

Yarish, C., A.E. Sperr, X.G. Fei, A.C. Mathieson, and I. Levine. 1996. Field and culture studies of several New England *Porphyra* species in order to characterize the distributional ecology and develop strains. *J. Phycol. Suppl.* 32(3): 52.

Yarish, C., A.E. Sperr, X.G. Fei, A.C. Mathieson, and I. Levine. 1997. Preliminary field and culture studies for the development of a commercially viable nori aquaculture industry in New England. In Van Patten (ed). *The Proceedings of the Long Island Sound Research Conference* (Oct. 17-18, 1996). Conn. Sea Grant Publication CT-SG-97. Pp.512-517.

Environmentally-Clean Integrated Mariculture in Israel

Muki Shpigel, A. Neori, D. Popper, N. Mozes. O. Zmora and H. Gordin
National Center for Mariculture (NCM)
Israel Oceanographic and Limnological Research
P.O. Box 1212
Eilat 88112, Israel

Israel already utilizes all of its freshwater resources, and the pressure to transfer water from agriculture to urban and industrial use is mounting. Readily available seawater and the fact that 60% of Israel's land is a non-arable desert, make desert mariculture a promising industry which is expected to provide food, employment and income.

The National Center for Mariculture (NCM) has been developing mariculture technologies suitable to the specific environmental conditions in Israel. As a result, fish-cage mariculture is now practiced on a commercial scale in the Red and Mediterranean Seas, while the land-based culture of abalone and clams has begun on a semi-commercial scale by two enterprises near the Red Sea.

Mitigating the negative environmental impact of mariculture has always been a primary consideration in the NCM's R&D process. Protein is the most expensive component in fish feed. It is also the principal source of nitrogenous pollution in fish culture, either from excess feed or fish excretions. The discharge of nutrients and low-quality water is a major concern everywhere, but becomes of particular importance in oligotrophic seas, such as the Red Sea and the Mediterranean. Commercially-cultured fish, such as the gilthead seabream (*Sparus aurata*), assimilate only 20-30% of the feed nitrogen; the rest reaches the water, mainly as dissolved ammonia. It is therefore desirable both economically and environmentally to maximize the conversion of feed protein into a valuable biomass instead of pollution. Unlike with fish cages, the removal of this pollution from fish ponds is possible.

Several approaches have been developed at the NCM to reach this goal in a sustainable way. These approaches are based on the use of algae and invertebrates as biofilters, which remove the dissolved and particulate nutrients from fish pond effluents. Sunlight-dependent assimilation turns excess nutrients into algal (microalgae or seaweed) biomass, which is then consumed with other organic particulate matter by the invertebrates.

In the first approach (Figure 1A), fish pond effluents are recirculated through biofilters, stocked with *Ulva lactuca*. Ammonia is stripped from the water by this seaweed at a high efficiency. More than half of the nitrogen not utilized by the fish was converted to seaweed protein by these biofilters in a two-year pilot study. *Ulva lactuca*, however, has been an efficient biofilter and its commercial value has been low relative to its operational expenses. This situation changed, once we found that this seaweed can be an excellent food for lucrative marine macroalgivores, abalone and sea urchin. Culture of these organisms in integrated systems with the fish and the seaweed biofilters has therefore become an attractive option to increase the overall profit of land-based, environmentally- clean mariculture.

In the second approach (Figure 1B), abalone are integrated into the fish-seaweed system described above. The system consists of three units: a) an abalone culture unit with the Japanese abalone *Haliotis discus hannai*; b) a fish culture unit, with the gilthead seabream, *Sparus aurata*; c) a seaweed biofilter/culture unit, with the seaweed *Ulva lactuca*.

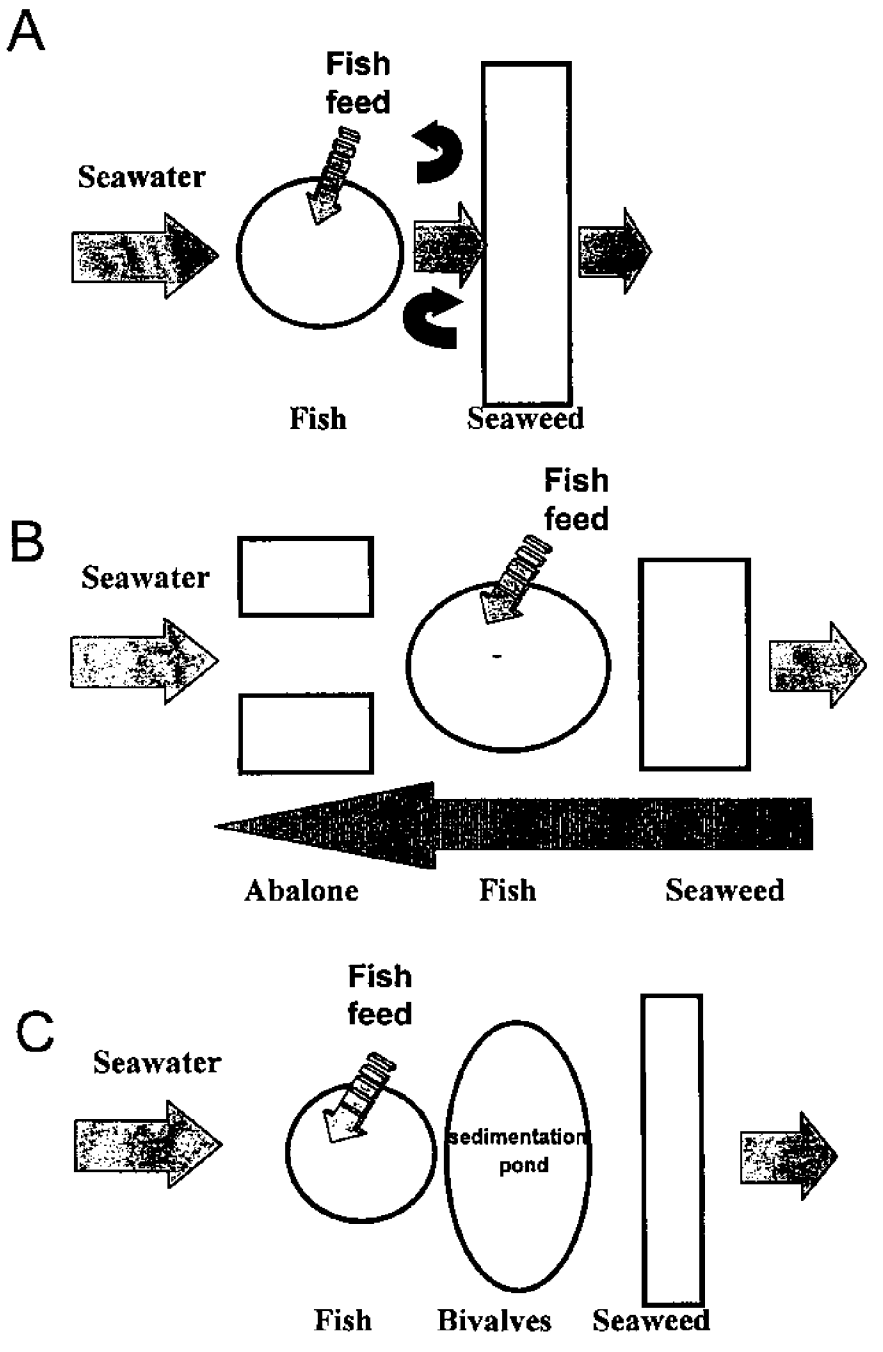


Figure 1. Three models of land-based integrated systems.

The three components were integrated in the following manner: unfiltered seawater was pumped into two abalone units, drained first through a fish unit, and then through two *Ulva lactuca* filtration and production units. Fish feed was the only source of nutrients for the fish, and the fish excretions were the only source of nutrients for the seaweed. The seaweed was transferred to the abalone tanks, where it was the only food for the abalone.

In one year, the sea bream grew from 20g to commercial size of 470g, with average daily growth rate of 0.6% with average food conversion ratio of 2. Juvenile abalone grew from 11 to 36 mm. Growth rate averaged 0.3 % per day and was negatively correlated with abalone size and water temperature. Fish assimilated about 26% of the nitrogen input. The harvested seaweed not consumed by the abalone, assimilated about 25% of the nitrogen, while the abalone assimilated about 8% of the nitrogen.

In the third approach, fish-excreted nutrients are assimilated by microalgae, which is used to feed clams. This system consists of three units (Figure 1C): a) a fish culture unit, with the gilthead seabream, *Sparus aurata*; b) a sedimentation pond, which serves as both a microalgal biofilter and a bivalve culture unit with the Japanese oyster, *Crassostrea gigas*, and the Manila clam, *Tapes semidecussatus;* and c) a seaweed biofilter unit, with the seaweed, *Ulva lactuca*. Microalgae, especially benthic diatoms, do much of the nutrient biofiltration. The main cultured herbivores are filter-feeding bivalves, which grow in the sedimentation pond. The seaweed biofilter only polishes the remaining dissolved nutrients from the final effluents. In this system, fresh seawater enters the fish ponds. Effluents from the fish ponds drain through an earthen sedimentation pond. The high nutrient content in the fish pond effluents, coupled with solar radiation, result in extremely high microalgal production in the sedimentation pond. Clams that grow on the bottom of the sedimentation pond consume the microalgae. From the sedimentation pond, the water drains into bivalve particulate-polishing reactors and finally to a seaweed dissolved-polishing unit, and then it is discharged into the sea. In this approach, the harvested yields (fish, bivalve and seaweed) contain 63% of the N introduced into the system, feces contain 33% and suspended and dissolved outflow, approximately 4% of the N budget.

Different modes of integrated mariculture vary in their complexity. Complex modes require highly skilled operators, and hi-tech culture systems, but provide increased product diversity. Depending on practical factors such as market prices, quality and cost of manpower, seed and fingerlings supply, environmental conditions, market size and property cost, the operational protocol can be shifted between the three product types (fish, algae and invertebrates) to maximize profits. The NCM wishes to cooperate with the industry, to scale up this technology and begin using it on a commercial scale in countries with coastal deserts.

Recent Advances in Transferring Foreign DNA into Finfish, Crustaceans and Shellfish

A. Sarmasik, S. Siri, C. Zoon, I. Jang, J.K. Lu, and T.T. Chen
Biotechnology Center
University of Connecticut
Storrs, Connecticut 06269

Foreign genes can be introduced into many finfish and shellfish species by microinjection or electroporation. However, these methods are ineffective in some invertebrates and live-bearing fish species. Recently, a new gene transfer vector, a defective pantropic retroviral vector, has been developed. Therefore, it might be possible to use this vector as an alternative method for transferring foreign genes into invertebrates or live-bearing fish. To test this hypothesis, the retroviral vectors containing reporter genes were introduced to the vicinity of the testis or ovary of *Poeciliopsis* and crayfish. The progeny of the treated fish were raised and the presence of the introduced gene was determined by PCR. The results demonstrated the presence of the introduced genes in progeny, thus indicating the successful transfer of the gene into gametes. Crossing the F1 fish with nontransgenic fish resulted in 50% transgenic fish which suggested the association of the retroviral vector with the fish genome. In addition, southern blot analysis indicated the integration of the gene into the genome. The pantropic retroviral delivery method was also tested in crayfish since the removal of the attached embryos from the female crayfish abdomen causes death of the embryos. Results from PCR analysis of crayfish progeny demonstrated the successful gene transfer in crayfish. Further crossing of the transgenic crayfish with nontransgenic animals showed that 50% of the animals carry the gene, thus indicating that the P1 animals' ovaries were transformed. In addition to this transgenic technique, electroporation was explored in bay scallops since viable newly-fertilized eggs can be obtained from these animals. New embryos were collected from bay scallops and the rainbow trout growth hormone gene was transferred successfully into these embryos by electroporation. PCR data indicated that the gene construct was transferred successfully in this shellfish species. We conclude that gene transfer can be conducted in a wide range of taxonomically unrelated species using a defective pantropic retroviral vector and electroporation.

Chromosome Engineering and the Biotechnology Potential of Shellfish from Long Island Sound

Sheila Stiles, J. Choromanski, and C. Cooper
U.S. Department of Commerce
NOAA/NMFS/NEFC
Milford Laboratory
212 Rogers Avenue
Milford, Connecticut 06460

Marine aquaculture lags behind agriculture with respect to progress in genetic improvement through breeding or biotechnology such as chromosome engineering. However, some successes have been reported, notably that of triploid induction to increase growth rate by diverting energy from reproduction with sterility in Japanese oysters (*Crassostrea gigas*) for commercial production on the West Coast of the United States (Allen *et al.*, 1989). Triploidy in oysters has been considered an example of successful marine biotechnology.

Previous chromosome engineering efforts at the Milford Laboratory for the induction of cloning or polyploidy in 15 experiments with American oysters (*Crassostrea virginica*) from Long Island Sound revealed that the ploidy level of early embryos developed from eggs treated with cytochalasin B, high pressure and/or exposed to irradiated sperm in general ranged from haploidy through pentaploidy (Stiles and Choromanski, 1986; Stiles *et al.*, 1983). Additional information was collected from trial induction of triploidy in interspecific crosses of the American and Japanese oyster (*C. gigas*) and on other interspecific crosses (Stiles and Choromanski, 1990). Outcomes depended on the female, experimental conditions, synchronous development and whether or not the sperm were genetically inactivated with irradiation (Longwell and Stiles, 1996). Triploidy occurred as high as 66%, but generally ranged from 3% to 38%. Some embryos were chromosomal mosaics or aneuploids. Triploid induction in American oysters by others elsewhere demonstrated increased growth rates but at different percents, depending on whether triploidy was induced by blocking meiosis I or meosis II (Stanley *et al.*, 1984). Results should be considered with regard to suggestions that sterile polyploid or hybrid oysters be used to rehabilitate fisheries devastated by disease.

Similar approaches to those conducted on oysters are being explored for the genetic improvement of bay scallops from Long Island Sound, with selective breeding (in progress) and chromosomal manipulation (planned). A major goal for genetic improvement of bay scallops (*Argopecten irradians*) is to increase muscle yield through increased growth rate to market size. Increased size prior to the onset of winter could also improve overwintering survival, a constraint to commercial production of bay scallops in the area. Preliminary experiments conducted by Tabarini (1984) in Maine demonstrated an increase in muscle yield of triploid bay scallops. Further development and refinement of this technology should allow improvement in muscle yield of bay scallops from Long Island Sound.

Achieving Sustainable High Yields in a Closed Shrimp Mariculture System

Craig Strong
Bluepoints Company, Inc.
P.O. Box 8
West Sayville, New York 11796

The Bluepoints Company reacted to the collapse of its cultured shrimp production from Taura Syndrome in the mid-1990's by testing a new technology to recirculate and treat water with ozone and other supplementary filtration methods. By employing this new system, developed at Bluepoints' two shrimp farms in the Guayas River Delta, our shrimp farms have doubled their per hectare shrimp production from their pre-Taura Syndrome production levels. In the spring of 1998, yields at the two Bluepoints farms were at the 5,000 pounds per hectare annual rate while neighboring farms continue to suffer low yields. The patented invention, the Bluepoints Mariculture System has broad implications for the $15 billion world shrimp farming industry, as well as for pond-based aquaculture situations.

The Bluepoints system combines ozone treatment and special filtration methods to destroy pollutants from outside and inside the closed network of ponds and canals that comprise the modern shrimp farm. Pesticides, fungicides, pathogens and other water-borne pollutants are largely destroyed by the closed-circuit, open-air system. Water drawn from the Guayas River, and recycled from the farm ponds, is ozonated and filtered to destroy bacteria and other pollutants. The result is a water condition that is ideal for raising shrimp.

The production advantages of a closed ozone system are that the system (1) maintains ideal temperatures and salinity levels; (2) eliminates bluegreen algae blooms which cause musty odors; (3) reduces the cost of fuel and wear-and-tear because of the lower rate of water exchange; (4) introduces clean water for shrimp culture; (5) facilitates more flexible shrimp harvest times independent of tides; (6) reduces the stress on the shrimp during harvest leading to higher quality shrimp; and (7) minimizes the loss of beneficial algae, nutrients and feed due to the lower water exchange rate.

Environmentally, the Bluepoints shrimp farming system has distinct advantages over competing open-loop shrimp production methods. For example, shrimp farms that have been decimated by bacterial or viral infection are often abandoned, and the farms relocated to pristine land. By restoring these contaminated farms to commercial viability, our system reduces the demand for further development of pristine coastal land and the resultant destruction of mangrove trees. Further, the use of antibiotics and other drugs to treat illnesses in farm-raised seafood is restricted by the U.S. Food and Drug Administration. The Bluepoints system eliminates the need for expensive and potentially harmful drugs in aquaculture. Our system creates virtually no effluent. The closed nature of our system and the removal of pathogens and pollutants from the water through ozonation eliminates contamination of water sources. All effluent is rendered cleaner than the source water.

As environmental concerns grow, the shrimp industry will continue to face regulatory constraints. Because the effluent from our system is cleaner than the water into which it flows, and, because our system permits sustainable, intensive yields, it will reduce the risk of ever-increasing environmental regulation. The Bluepoints Closed Mariculture System has the potential to revolutionize the shrimp aquaculture industry throughout the world by enabling shrimp farmers to increase yields and profits while maintaining an environmentally-friendly system.

New England Bay Scallops as a One-Season Aquaculture Crop

Gary H. Wikfors, James C. Widman, Sheila Stiles, Richard A. Robohm, and Anthony Calabrese
NOAA Fisheries, NEFSC
Milford Laboratory
Milford, Connecticut 06460

The native New England bay scallop, *Argopecten irradians*, is a high-value seafood species with an existing market, but an unstable yield from the wild-caught fishery. Wide inter-annual fluctuations in bay scallop populations can be attributed largely to the short life-history of the bay scallop — most live less than two years and spawn only once (Orensanz *et al.*, 1991) — and its sensitivity to environmental stresses, particularly winter mortality (Mercaldo & Rhodes, 1982; Bricelj *et al.*, 1987). The bay scallop is, however, an extremely fast-growing bivalve, reaching a market size of 40+ mm in less than one year (Oesterling & Rose, 1996). For comparison, oysters require 3-5 years to reach market (Burrell, 1985), and quahogs grow even slower, requiring 4 years or more to reach littleneck size in New England waters (Menzel, 1989). When available, New England bay scallop adductor muscles (the only portion of this animal currently marketed widely) sell for $9-12 per pound or more. The high market value, coupled with limited wild-fishery yield and rapid growth, make the bay scallop an attractive candidate for aquaculture (Gates *et al.*, 1974; Webber & Riordan, 1976).

An aquaculture strategy for the bay scallop must address the basic biological constraints that limit the wild fishery; in fishery biology terms, these constraints are recruitment and survival to market size. Recruitment is the addition of new individuals to the population. In aquaculture, "recruits" are referred to as "seed". There are two ways to obtain bivalve seed: one is collection of wild "spat", or metamorphosing larvae, in natural waters, usually employing an artificial substrate deployed in the water. The other source of seed is a land-based hatchery. Spat collection is dependent upon a sufficient population of "spawning stock", i.e., reproductive adults, and conditions suitable for development and retention of planktonic larvae in the estuary. Efforts to collect bay-scallop spat in southern New England estuaries have met with limited and inconsistent success (Tammi *et al.*, 1997). We, therefore, have based our strategy for bay scallop aquaculture development in New England upon hatchery production of seed scallops.

Practical methods for artificial spawning of bivalve mollusks and rearing of larvae and young spat were developed at the Milford Laboratory over four decades ago (Loosanoff and Davis, 1963). Preparation of adult bivalves for induced reproduction (referred to as "conditioning" or "ripening") can be accomplished at almost any time of year by raising or lowering water temperature incrementally over several weeks to 17°C and providing sufficient phytoplankton food to fuel the energy-intensive process of gamete formation. "Ripeness" of bay scallops is easier to determine than for other bivalves because the scallop gonad can be inspected visually without killing the individual. When spawning of animals maintained at 17°C is desired, water temperature is raised 7-8°C to 25°C in a few hours, and release of gametes is induced. Eggs and sperm released into the water are collected and mixed according to desired genetic composition of the offspring. Embryos develop without feeding for 24-48 hr; energy and biochemical components for these pre-feeding stages are provided by compounds stored

in the eggs, therefore nutritional status of broodstock females is important in determining early development.

When larvae reach the veliger stage, about 70 µm in size and characterized by presence of a locomotory and feeding organ called the velum, they are fed a diet of cultured microalgae. Effective microalgal diets for larval bay scallops must be: 1) non-toxic, 2) sufficiently small to be ingested, 3) digestible, and 4) nutritionally complete. Whereas toxicity and size of potential microalgal diets are evaluated easily, digestibility and nutritional composition are more difficult to ascertain. Work at Milford pioneered a technique of evaluating algal-cell digestibility in larval bivalves by observing the extent and rate of decomposition of individual microalgal cells within the digestive system of a living larva using the fluorescence microscope (Babinchak and Ukeles, 1979). We now use this "gut-fluorescence" method routinely to screen potential algal diets. More recent research in our laboratory has focused on biochemical aspects of bivalve nutrition. For bay scallop larvae, we have shown that algal diets high in specific, "essential" fatty-acids and sterols support more rapid growth and result in a higher percentage of larvae successfully undergoing metamorphosis to the post-set stage (Alix *et al.*, 1996).

After 8-10 days of larval development and growth to about 200 µm, scallops undergo a process of metamorphosis, also called "setting," whereby adult morphological characteristics are established. As this process includes loss of the velum and development of a new, gill-feeding apparatus, feeding may be interrupted or become less efficient. Thus, nutritional condition of larvae at the pre-setting stage may be important in determining setting success.

Metamorphosing scallops attach to surfaces by means of byssal threads similar to those seen on adult mussels. It is thought that byssal attachment to eelgrass and other living and non-living structures in nature serves to keep scallops off bottom to avoid crustacean and other predators and to ensure a current-mediated supply of phytoplankton food (Brand, 1991). In the hatchery, scallops are set on mesh "onion bags" filled with nylon monofilament line, on plastic strips, on fiberglass, or directly on the container sides. Setting success in the hatchery generally ranges from less than 1% to about 10%; this variation offers a potential area for improvement, but from a practical standpoint, does not constrain hatchery production seriously because of the large number of larvae (millions) that can be obtained from spawning of several individuals. Post-set scallops then enter a stage of culture referred to as the "nursery". Traditionally, young, post-set scallops, as well as other bivalves, are reared in tanks through which coarse-filtered seawater is pumped (Rhodes & Widman, 1980) or in protected enclosures deployed in the natural environment (Widman & Rhodes, 1991). In either case, young animals are exposed to ambient seawater temperatures and depend upon natural phytoplankton, not cultured microalgae, for food. It is in the nursery stage of culture that the Milford Laboratory's current strategy departs from established procedure; the main reason for this departure involves timing.

Returning, briefly, to the natural history of bay scallops, timing of the life cycle in southern New England involves: spawning in mid-summer (July-August); growth to about 25 mm by November, when temperatures below 10°C preclude growth; winter mortality of young-of-the-year scallops; resumption of growth in survivors when temperatures rise above 10°C in April; and finally spawning of survivors in mid-summer. Winter mortality is the constraining factor that limits survival to market size in New England coastal waters. Previous experience with hatchery production of bay scallop seed for field grow-out has followed the natural life-history "time-line" fairly closely, in that scallops were spawned only slightly earlier than natural reproduction — in late-spring or summer — so that a size of 50 mm or less was obtained before winter (Rhodes & Widman, 1980). The choice then becomes whether to over-winter scallops at this size and incur losses to winter kill (not a viable option), or to market scallops at the smaller size of 50 mm. An economic analysis of the latter strategy showed

it to be marginal in terms of potential profit, chiefly because adductor muscle yield from scallops of this size is modest — only half the yield from a 70 mm scallop generally caught in the second year. Marketing of whole, "half-shell" scallops is being investigated as a possible means of making short-season aquaculture of small bay scallops an economic option. In addition, we are pursuing experiments to investigate the reasons for winter mortality and whether a viable means of circumventing this phenomenon can be developed. Nevertheless, our leading strategy for development of a bay scallop aquaculture industry in New England is based upon the production of 65-70 mm scallops in one season without over-wintering. To accomplish this, we must have in hand 25 mm seed scallops in early spring when water temperature rises above 15°C. The nursery stage of the production cycle becomes shifted to a time-of-year when in situ water temperatures will not support growth of young scallops. A new approach to bivalve nursery culture is needed.

To produce 25 mm scallop seed for April planting, broodstock are conditioned during December and January, spawned early in February, and must spend the 8-12-week nursery stage in heated water. A flow-through system, similar to the traditional, land-based nursery, with sufficient flow rate to provide natural phytoplankton food is not economically viable because of energy costs to heat large volumes of water. In addition, discharge of heated water would not be environmentally compatible. We, therefore, made the decision to build a bivalve nursery system around the recirculating technology that has been developed for intensive, land-based finfish and crustacean culture. In these recirculating systems, water is retained for periods of days to months, and wastes created by animals being reared are treated and/or removed using a variety of technologies. Chemical wastes (chiefly ammonia) are oxidized to less-toxic forms by "biological filters" that consist of attached bacterial cultures over which water is circulated. For finfish and crustacean systems, solids separators of various designs are employed to remove uneaten feed and feces. Small particles can be removed on surface tension of bubbles in so-called "protein skimmers". Our evaluation of these technologies for nursery-rearing of bivalves is the first serious effort in this area since studies conducted at the University of Delaware two decades ago (Pruder *et al.*, 1976). The Delaware experience was not positive in most respects, but recirculating water systems and process-control technologies have matured since then.

The Milford Laboratory's facility for researching recirculating seawater systems for bivalve nursery culture is housed in a secondary enclosure within an existing tank farm building to maintain a constant temperature of 25°C. The enclosure contains four identical sets of three 2 m x 0.75 m x 0.15 m tanks and one 1.75 m x 1.3 m x 0.8 m tank for holding scallops. Each tank array currently is plumbed to a different biological filter under evaluation (Figure 1). The four filters being tested are: 1) an aerated biological contact filter, 2) a chenille fiber unit, 3) a rotating biological contact filter, and 4) a sand filter. A PC-based monitoring and control system will log data on temperature, dissolved oxygen, pH, in vivo fluorescence, conductivity, and ORP. Feedback control loops respond to changes in pH, conductivity, and algal feed density to maintain system parameters within selected ranges. Experimental data on effectiveness of the four biofilters under evaluation are being collected, and different stocking densities, feeding rates, and flow rates are being evaluated at present.

The second new facility constructed to support the scallop research effort is the Greenhouse for Research on Algal Mass Production Systems (GRAMPS). GRAMPS is a 100 m^2 hard-plastic-on-steel-frame structure housing eight 425-liter, 3 m-tall tubular tanks and two 20,000-liter, 3 m x 2 m x 1.5 m oval tanks. The system was scaled to produce 5,000 liters of dense microalgal feed per day — enough to feed one-million 10 mm scallops. Algal feeds produced in GRAMPS are pumped through underground pipe chases into the scallop nursery facility. In addition to the practical requirement for algal feed to test the one-season scallop strategy, research objectives of the GRAMPS facility are to evaluate contemporary process-control technologies for improving the effectiveness and economics of microalgal feed

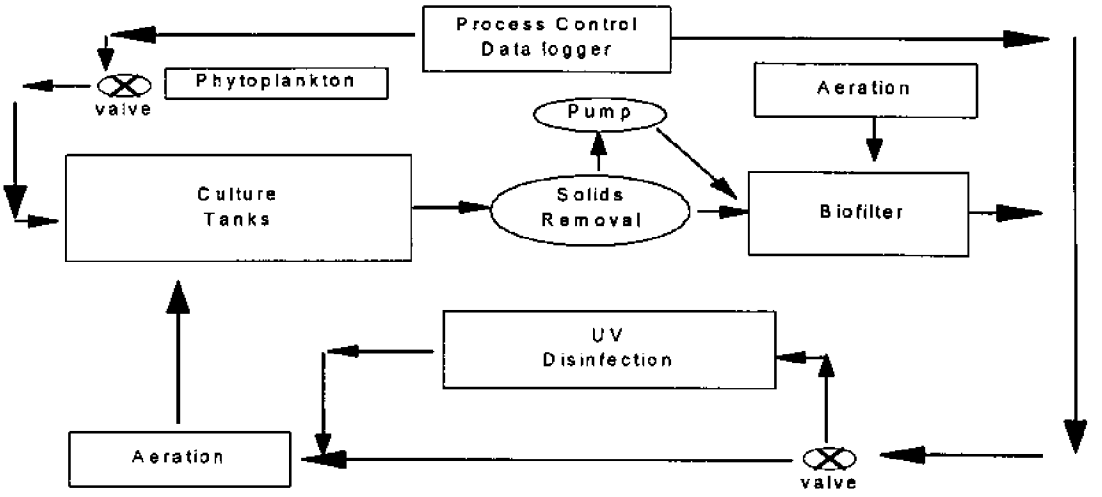


Figure 1. Schematic diagram of recirculating system for bay scallop nursery culture.

production and to transfer appropriate technologies to the private sector. Automated processes currently under development are: 1) use of venturi eductors to mix concentrated nutrients into seawater during tank filling, 2) level switches to automatically refill cultures during semi-continuous operation, 3) pH control loops to inject carbon dioxide into cultures as needed to keep pace with photosynthetic uptake, 4) control of artificial lights to supplement sunlight when daily photosynthetically-available radiation totals fall short of production needs, and 5) cooling with thermostat-activated freshwater misting. Nutrition studies done in preparation for nursery feeding of seed scallops have identified practical feeding standards in terms of: What — high-lipid *Tetraselmis* strains; How much — 2-5% of scallop live weight in dry weight of microalgae per day; and How Often — 16 times per day at the optimal ration. This technology is being transferred to the aquaculture industry.

Genetic studies of native bay scallop populations are being pursued to evaluate the genetic diversity of potential founding stocks and to explore the possibility of selective breeding to improve morphological and/or growth characteristics. As a functional hermaphrodite, the bay scallop can be bred through self-fertilization in-breeding, in addition to more conventional mass selection techniques. Results to date show that inbreeding depression often leads to decreased survival in early stages, but mass selection shows early signs of success in identifying individuals with faster growth as a heritable trait (Stiles *et al.*, in press). Opportunities for further improvements in domesticated lines are suggested by a relatively high genetic diversity in Stonington wild populations upon which our selective breeding studies are based.

Epidemiological studies in other species show that diseases are more easily transmitted when animals are in close proximity; however, factors such as genetic traits (Hildemann, 1974; Zinkernagel, 1979) and inadequate nutrition (Chandra, 1980) can contribute to disease susceptibility. Cultured bivalves are no exception; hatchery animals

sometimes experience severe mortalities from infections. While no definitive studies have been done to link genetic traits with immunity in bay scallops, the work of Ford and Haskin (1987) on selective resistance to MSX disease in oyster lines suggests a similar possibility for scallops. Nutritional effects on disease resistance are unexplored in scallops.

We are monitoring hatchery, nursery, and grow-out areas for microbial disease conditions. This includes histopathological examination of moribund animals, isolation of potential bacterial pathogens, and testing of bacterial isolates for pathogenicity in adult or larval scallops. Pathogenic isolates are identified by their phenotypic biochemical reactions and then cryo-preserved in liquid nitrogen. These organisms are expected to be useful in searching for immunological markers for disease resistance in scallop blood cells. This will include assays for: 1) cytotoxic cells (Volgmann *et al.*, 1989), 2) lysosomal enzymes in phagocytic cells (Twining, 1984; Campanelli *et al.*, 1990), and 3) cell-surface receptors (Erbe *et al.*, 1992). Establishment of disease-resistance markers that can be identified by in vitro methods may allow us to recognize disease resistance in scallop genetic lines without resorting to complicated, scallop-challenge studies with living microbes. The markers also may aid in evaluation of the effects of algal diets in boosting disease resistance.

Following nursery culture, grow-out of 25 mm seed scallops to 65-70 mm between April and November is expected to be accomplished using existing technology (Rhodes *et al.*, 1984). Bay scallops will be stocked into pearl nets and/or lantern nets that are suspended from long-lines held in place with anchors and buoys. Evaluation of grow-out will be done in collaboration with the Bridgeport Regional Vocational Aquaculture High School and several commercial and municipal scallop growers, thereby assuring that grow-out results will be applicable to commercial production.

All steps in the overall strategy for a one-season scallop crop are being evaluated economically through a cooperative agreement with the University of Rhode Island. Established modeling techniques are being used to account for costs associated with individual steps in the process and to project how specific improvements are likely to affect the overall economics of this strategy.

In summary, we have developed a strategy for farming of bay scallops in New England waters in which scallops are spawned during the winter, reared to a size of 25 mm in a recirculating-seawater nursery system, and grown-out in suspension culture to market in one year (Figure 2). Critical evaluation of this process will require development of recirculating-seawater technology for bivalves—a new application—and improvements in the culture of phytoplankton feeds using

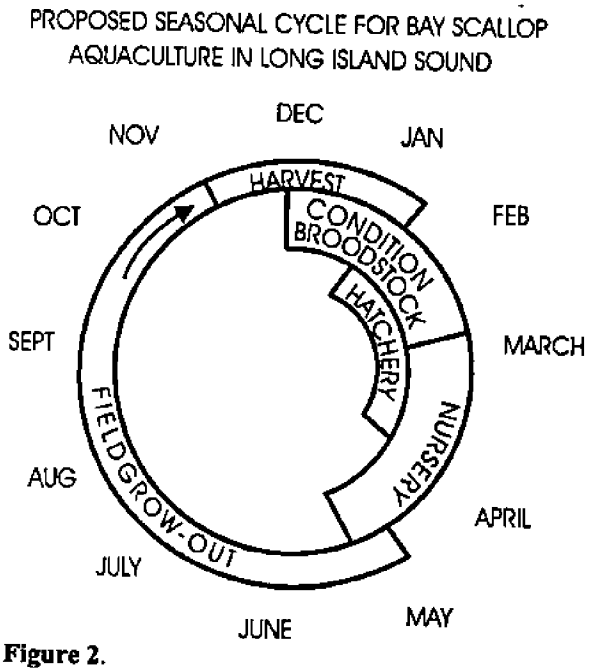


Figure 2.

natural light and process-control technology. Domestication of the bay scallop also will include genetic evaluation of New England populations and selective breeding of scallop varieties suitable for this farming application, as well as an understanding of health management and disease potential in the new, recirculation nursery environment. Successful solution of technical problems and a favorable economic analysis

should encourage development of New England bay scallop farming.

Acknowledgements

Funding for the Milford Laboratory bay scallop aquaculture development project has been provided by the Northeast Fisheries Science Center of NOAA Fisheries, the NOAA Fisheries Saltonstall-Kennedy program, and the University of Connecticut Marine Sciences and Technology Center.

References

Alix, J.H., M.S. Dixon, B.C. Smith, and G.H. Wikfors. 1996. Scallop larval feeding experiments: Some surprises and unanswered questions. *J. Shellfish Res.*, 15: 451 (abstract).

Babinchak, J. and R. Ukeles. 1979. Epifluorescence microscopy, a technique for the study of feeding in *Crassostrea virginica* veliger larvae. *Mar. Biol.*, 51: 69-76.

Brand, A.R. 1991. Scallop ecology: Distribution and behavior. In: Shumway, S.E. (ed.), *Scallops: Biology, Ecology, and Aquaculture*, Elsevier, New York. Pp. 517-584.

Bricelj, V.M., J. Epp, and R.E. Malouf. 1987. Comparative physiology of young and old cohorts of bay scallop *Argopecten irradians irradians* (Lamarck); mortality, growth, and oxygen consumption. *J. Exp. Mar. Biol. Ecol.*, 112: 73-91.

Burrell, V.B., Jr. 1985. Oyster culture. In Huner, J.V. and E.E. Brown (eds.), *Crustacean and Mollusk Aquaculture in the United States,* AVI Publishing Company, Inc., Wesport, CT. Pp. 235-274.

Campanelli, D., P.A. Detmers, C.E. Nathan, and J.E. Gabay. 1990. Azurocidin and a homologous serine protease from neutrophils. *Journal of Clinical Investigation,* 85: 904-915.

Chandra, R. K. 1980. *Immunology of Nutritional Disorders.* Year Book, Medical Publishers, Inc., Chicago.

Erbe, D.V., B.A. Wolitsky, L.G. Presta, C.R. Norton, R.J. Ramos, D.K. Burns, J.M. Rumberger, B.N. Narasinga Rao, C. Foxall, B.K. Brandley, and L.A. Lasky. 1992. Identification of an E- selectin region critical for carbohydrate recognition and cell adhesion. *J. Cell Biol.*, 119:215-227.

Ford, S. E. And H.H.Haskin. 1987. Infection and mortality patterns in strains of oysters *Crassostrea virginica* selected for resistance to the parasite *Haplosporidium nelsoni* (MSX). *J. Parasitol.*, 73: 368-376.

Gates, J.M., G.C. Matthiessen, and C.A. Griscom. 1974. Aquaculture in New England. Univ. Rhode Island Tech. Rpt. Ser. No. 18, Kingston, RI. 77 pp.

Hildemann, W.H. 1973. Genetics of immune responsiveness.*Ann. Rev. Genetics*, 7: 19-36.

Loosanoff, V.L. and H.C. Davis. 1963. Rearing of bivalve mollusks. In: Russell, F.S. (Ed.), *Advances in Marine Biology I*, Academic Press, London. Pp. 1-136.

Menzel, W. 1989. The biology, fishery and culture of quahog clams, *Mercenaria.* In: Manzi, J.J. and M. Castagna (eds.), *Clam Mariculture in North America,* Elsevier, New York. Pp. 201-242.

Mercaldo, R.S. and E.W. Rhodes. 1982. Influence of reduced salinity on the Atlantic bay scallop, *Argopecten irradians* (Lamarck) at various temperatures. *J. Shellfish Res.*, 2: 177-181.

Oesterling, M.J. & L.A. Rose. 1996. Bay scallop culture in a Virginia saltwater pond. *J. Shellfish Res.*, 15: 458-459 (abstract).

Orensanz, J.M., A.M. Parma, and O.Iribarne. 1991. Population dynamics and management of natural stocks. In:

Shumway, S.E. (ed.), *Scallops: Biology, Ecology, and Aquaculture,* Elsevier, New York. Pp. 625-713.

Pruder, G.D., E.T. Bolton, and P.H. Sammelwitz. 1976. Controlled-environment bivalve molluscan mariculture. Delaware Sea Grant Publ. DEL-SG-23-76, College of Marine Studies, University of Delaware, Newark, DE. 14 pp.

Rhodes, E.W. and J.C. Widman. 1980. Some aspects of the controlled production of the

bay scallop (*Argopecten irradians*). *Proc. World Maricult. Soc.*, 11: 235-246.

Rhodes, E.W., R. Goldberg, and J.C. Widman. 1984. Density-dependent growth of the bay scallop, *Argopecten irradians irradians*, in suspension culture. International Council for the Exploration of the Seas, C.M./1984/K:/18.

Stiles, S., J. Chrormanski, and C. Cooper. (In press). Selection studies on growth and survival of bays scallops, *Argopecten irradians*, from Long Island Sound. *J. Shellfish Res.*

Tammi, K.A., E. Buhle, and W.H. Turner. 1997. Making the perfect spat bag for collection of the bay scallop, *Argopecten irradians*. *J. Shellfish Res.*, 16: 295 (abstract).

Twining, S.S. 1984. Fluorescein isothiocyanate-labelled casein assay for proteolytic enzymes. *Analytical Biochem.*, 143: 30-34.

Volgmann, T., A. Klein-Struckmeier, and H. Mohr. 1989. Fluorescence-based assay for

Webber, H.H. and P.F. Riordan. 1976. Criteria for candidate species for aquaculture. *Aquaculture*, 7: 107-123.

Widman, J.C. and E.W. Rhodes. 1991. Nursery culture of the bay scallop, *Argopecten irradians* irradians, in suspended mesh nets. *Aquaculture*, 99: 257-267.

Zinkernagel, R.M. 1979. Associations between major histocompatibility antigens and susceptibility to disease. *Ann. Rev. Microbiol.*, 33: 201-213.

Domestication of Indigenous *Porphyra* Species for Commercial Cultivation

Charles Yarish*, R. Wilkes, T. Chopin, X.G. Fei, A.C. Mathieson, A.S. Klein, C.D. Neefus, G.G. Mitman and I. Levine
*Department of Ecology & Evolutionary Biology
University of Connecticut
Stamford, Connecticut 06901-2315

In view of the broad-based support of several federal agencies for enhanced mariculture of coastal resources, including the National Sea Grant College Program, Northeast Regional Aquaculture Center, Departments of Commerce and Agriculture and the National Science Foundation, we have embarked upon a study of domesticating indigenous species of *Porphyra* for commercial cultivation. A variety of field and culture studies are being made in order to clarify the taxonomic status and ecological requirements for developing the mariculture of several *Porphyra* species from coastal New England and the Canadian Maritimes. At least six different species of *Porphyra* are being examined using a variety of traditional morphometric parameters, cytological and molecular techniques. Detailed seasonal and spatial collections, from diverse coastal and estuarine habitats, are being used to delineate the seasonality and habitat preferences of *Porphyra* in northeast America. Over 130 unialgal cultures of *Porphyra amplissima (Kjellman)* Setchell & Hus in Hus, *P. miniata* (C. Agardh) C. Agardh, *P. umbilicalis* (Linnaeus) J. Agardh, *P. linearis* Greville, *P. purpurea* (Roth) C. Agardh, and *P. leucosticta* Thuret in Le Jolis have been established and are being maintained for comparative molecular genetic and ecophysiological investigations. Several strains of each of the species of *Porphyra* have successfully completed their life cycles in culture and F2 individuals have been obtained for *P. amplissima, P. leucosticta, P. purpurea* and *P. umbilicalis*. Conchocelis cultures have also been successfully established in bivalve shells, presently an important stage of the domestication process. Whether or not nori aquaculture will ultimately succeed in New England and the Canadian Maritimes will depend in large part upon several key factors, including: (1) successful transfer and modification of Chinese and Japanese cultivation technologies to local coastal environments; (2) development of genetically improved strains (cultivars) of marketable nori that will extend the growing and harvest season; (3) establishing a constant supply of a "seedstock" of juvenile organisms that will be readily available; and (4) the expansion of the area presently used for cultivation (i.e. beyond northern Maine).