
Molluscan Shellfish Research and Management

Charting a Course for the Future

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Cover: based on "Birth of Venus," Sandro Botticelli (1445-1510)

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PREFACE

This document is the result of a workshop supported by the Cooperative Research and Information Institute (CRII) and attended by 50 of the top molluscan shellfish scientists, industry representatives and policy makers in the United States. The purpose was to develop a National Shellfish Plan, charting a course for the future in shellfish research and management. The report outlines what the collective wisdom of the group understood to be known about this field of science, what needs to be known and how to gain the additional knowledge. This report is a major step forward for the shellfish community and provides a basis for research and funding priorities in the new millennium.

This report would not have been possible without the early efforts of Dr. Nancy Foster. It was Dr. Foster who had the vision of the Institute, a network of scientists, educators and businesses working in concert to put good science in action. The overarching goal of CRII was to focus scientific research efforts on the information needs of industry, policy makers and the public. Specific aims were to: 1) strengthen collaborative links between research, industry and other users to influence and guide policy design and implementation; 2) develop teams of research and communication specialists to disseminate the information broadly; and 3) stimulate education and training through government and university outreach programs.

In the three-year life of CRII, three tasks were completed: a world summit of the top medical researchers on the benefits of omega-3 fatty acids, a workshop covering the latest research and developing technologies for detection and management of pathogens, and this workshop to develop a National Shellfish Plan. Dr. Foster's untimely death left CRII without its top supporter and too limited in funding to begin new efforts.

Drs. Michael Sissenwine, Garry Mayer, and Roger Hutchinson, of NOAA-National Marine Fisheries Service, displayed extraordinary leadership and support while shepherding the final publication of this document. Without them, this important task would not have been possible and their efforts are greatly appreciated. Richard DeVoe and his staff at South Carolina Sea Grant organized the workshop in their usual efficient and pleasant manner. Steven M. Allen provided assistance in innumerable ways during the meeting and Maille Lyons has since provided much needed editorial assistance. Their support and constant attention to detail are greatly appreciated. In our effort to provide an integrated document representative of the cooperative spirit of the workshop, authorship of chapters is not attributed to specific individuals. We therefore issue a special thanks to all of the participants for their unstinting efforts and patience in finalizing this report.

INTRODUCTION

Bivalve shellfish (clams, oysters, mussels and scallops) provide the basis for a wide spectrum of jobs, products and services for the U.S. population and economy. The most obvious product is food. In colonial times oysters and clams were a poor man's food because their natural protective shell allowed them to be harvested, moved, and stored without refrigeration. This convenient packaging, coupled with the highly digestible and nutritious quality of the flesh, made bivalves the food of choice. Traditionally bivalves were consumed raw. The desire for variety in the diet led to fritters, chowders, stews, and steamed, fried and roasted preparations. As people became more affluent, variations such as bisques, gratins, scalloped and souffles developed. These were followed closely by elegant presentations such as casino, Rockefeller and Benedict. Throughout this culinary evolution, the raw product has remained a highly valued item, often highlighted as an appetizer in the form of a cocktail or on the half shell. Food is, however, certainly not the only virtue of bivalve molluscs.



Restaurant presentation of oysters.

BIVALVES AS SENTINELS

Because clams and oysters are consumed raw and because they feed by concentrating small particles from the seawater in which they live, bivalves were the impetus for the first environmental monitoring programs in our coastal zone. These programs were designed to protect public health and, to this day, these programs are one of the few coastal monitoring efforts that produce immediate action. If certain bacterial parameters are exceeded, an area is closed to harvest. Other areas may be closed for bivalve harvest (such as near marinas) simply because the potential for pollution exists, and still other sites may be closed because they are not monitored according to the standards. One outgrowth of the monitoring program to reduce human health risk associated with consuming raw bivalves has been a program designed to determine if areas are safe for swimming. The standard for bivalves harvested for human consumption is over 14 times (93%) more stringent than that for swimming: Anywhere bivalves can be grown for harvest is biologically safe for humans to swim.



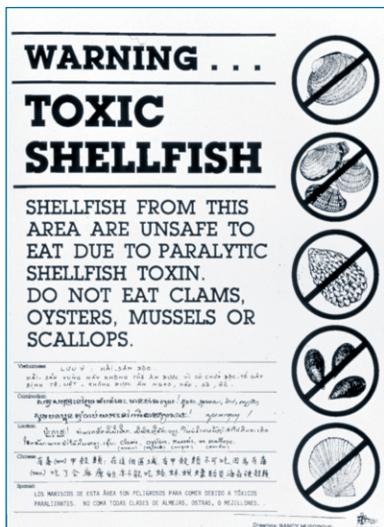
Hesse's Oyster & Lunchroom. Stapleton Staten Island, New York. Photography by George Bear, November 27, 1898. Photo Courtesy of Staten Island Historical Society.



A Don Bousquet cartoon (Bousquet, 1994; Quahogs Are A Girl's Best Friend).



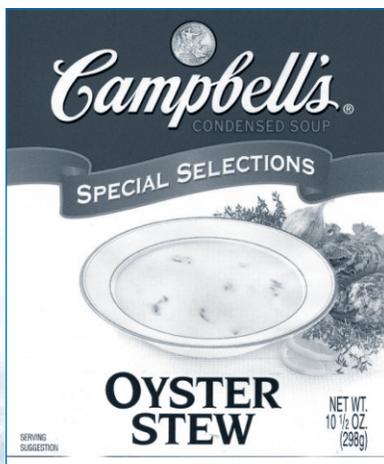
A sample closure sign warning of contaminated shellfish.



An example of a harmful algal bloom closure sign.



Cover photo from a scientific journal depicting a size range of hard clams in a rake. Photo courtesy of Ray Grizzle.



Campbell's Soup® label.

Because they remain in place and filter large quantities of water, bivalves also form the basis for a large-scale, long-term monitoring program designed to determine whether the water quality of bays, sounds, and other nearshore waters is declining or improving. This program (Mussel Watch) collects mussels or oysters annually from 285 stations located in estuaries in every coastal state of the US. These bivalves are analyzed for such pollutants as heavy metals and pesticides. The trends in these analyses are quantified to provide a time series that reflects the base environmental conditions each year.



A hard clam filtering for food.

BIVALVES PROVIDE JOBS

The pursuit of bivalves for food either through fisheries or aquaculture provides a myriad of jobs in every coastal state. While more than 20 species of bivalves are harvested from U.S. waters, only about half are commercially significant. In the late 1990's, between 160 and 170 million pounds of bivalve meat was harvested in US waters. Virtually all of this was



Digging soft-shell clams.

utilized as food for humans, and was worth approximately \$310-\$320 million in dockside value. This amounts to \$1.90 per pound of bivalve meat. The largest quantity of meat (averaging 98 million pounds per year) comes from ocean quahogs and surf clams. These inhabitants of the east coast continental shelf are processed to yield a variety of canned and frozen products.

No readily available records are kept that allow an estimate of the numbers of boats or individuals that are devoted to shellfisheries. We do know, however, that in the Northeast from 1994 to 1996 an average of 300 boats devoted at least part time to scallop dredging. These boats employed an average crew of 6.3 and spent approximately 174 days at sea. Because of the nature of the gear, boats dredging for surf clams and ocean quahogs cannot easily pursue other fisheries. Between 1994 and 1996 an average of 102 boats with crews of approximately 12 individuals harvested surf clams and ocean quahogs. These numbers reflect the employment in the shellfish industries, but do not begin to indicate the large numbers of small boats with crews of 1 to 3 that harvest bivalves in virtually every shallow bay in every coastal state. This harvesting activity creates jobs in boat building and repair industries, food processing, food distribution, advertising, and many others are employed to be sure shellfish are safe to eat and stocks are maintained and production is increased. The discarded shells are stockpiled and used for parking lots, driveways and roads.

BIVALVES IN AQUACULTURE

Because harvest areas have been closed due to pollution, harvestable stocks of many shallow water bivalves have been reduced. To increase the supply of these high value products there has been a related growth in bivalve aquaculture. Traditional culture (moving naturally produced oysters from high survival low/growth areas to areas where growth or meat quality is improved) has existed at least since the early 1800's. This traditional oyster culture, and modifications of these techniques for mussels has continued production of these highly desirable products, but in the last 25 years the hatchery production of bivalves has become economically possible. Scientific breakthroughs in culturing algae and rearing the larval and juvenile stages have led to the establishment of clam and oyster farms along both coasts. The development of effective methods of protecting newly planted seed has reduced losses from predation. Advances in hatchery rearing have permitted the use of breeding technology to produce stocks that are disease resistant, grow faster or have some other desirable quality. These stocks are becoming more common and are destined to increase production per unit area and supplement wild harvest.

In 1997 bivalve aquaculture in the U.S. produced nearly 27 million pounds of clams, mussels and oysters worth \$89 million, or \$3.30 for each pound of meat. These were produced on 738 farms.



One of the many boats that harvest clams.



A pile of oyster shells for rehabilitating oyster beds.

BIVALVES AS RECREATION

Bivalves also form the basis for a large recreational industry. Who hasn't scoured a beach to pick up shells? Sanibel Island, Florida is famous for the variety of shells to be found and has developed an entire recreation industry based on this natural abundance. In addition to those who simply pick up a shell as a fond memory of a special place or time, there are those who collect shells as objects of beauty. These individuals support the shell shops that can be found in most coastal communities. There are also shell collectors who may spend hundreds to thousands of dollars to acquire a particularly desirable specimen. At the other extreme are the shells that are glued together to form figurines and curios that are sold by the thousands throughout the country.



Oyster Aquaculture.

In Rhode Island, one professional cartoonist has even published a collection of his work based on the culture surrounding the harvest of quahogs (hard clams) in Narragansett Bay.

In addition to simple shell collecting, live bivalves are harvested recreationally on all coasts. In the State of Washington, large numbers of individuals flock to ocean beaches to harvest razor clams. Towns on Cape Cod retain traditional local recreational harvest by supplementing the natural stocks with seed from hatcheries. Throughout the country clams are dug for sport and in the Southeast intertidal oystering is a popular local activity.



Mussels being cultured on ropes. Photo courtesy of Richard Langan.



An assortment of shells found on Sanibel Island, FL beaches. Photo courtesy Sanibel Shellfish Industries.



Shell art.



A Don Bousquet cartoon (1989 Don Bousquet's New England; Yankee Publishing Inc., Dublin, NH).

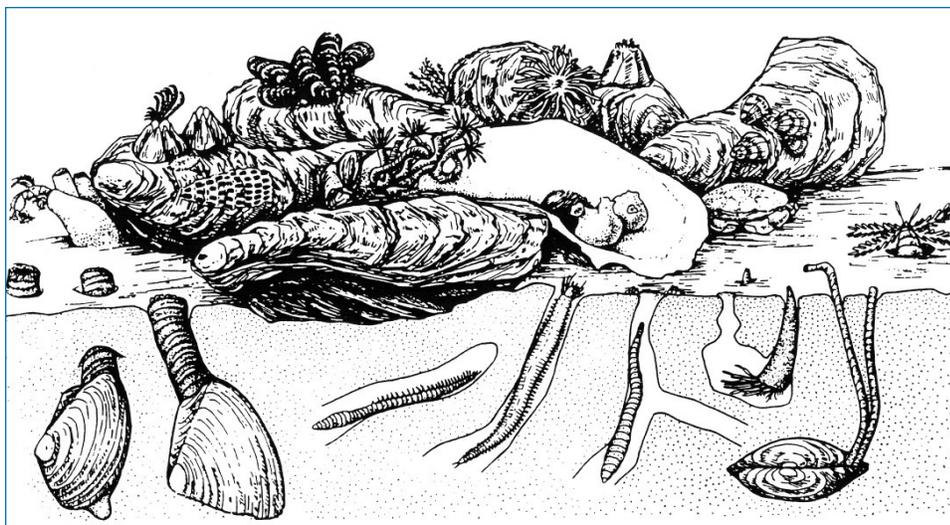
When bay scallops are abundant in Florida, New England and North Carolina, individuals who consider this harvest to be part of their heritage remove large numbers of scallops. Demand for these shallow water resources has allowed some individuals to seed the areas and create jobs by offering recreational digging on their leased area. This mirrors the development of “u-pick your own” agriculture.

BIVALVES AS ECOLOGICAL INTEGRATORS

A critical link between having bivalves available for food, objects of beauty or recreation is their ecological role as cleansers of our coastal waters. Bivalves provide uniquely important ecological services. These organisms are among the largest long-lived, bottom dwelling organisms in the coastal zone. They are also responsible for developing and maintaining hard substrate in what would be an extensive sand or mud habitat. In 1800, Mobius described the oyster reef and the animals that depend on the reef structure as a community within the marine habitat. Oysters form an elevated, porous, hard structure in an otherwise flat habitat. This structure and its interstices are occupied by large numbers of other species. In areas without other structure these habitats become havens for juvenile fish and crustaceans as well as an attachment point for many other species.

In sand and mud habitats, clams often dominate the total biomass, and are long-lived relative to other members of the community. Thus they are often one of the forms used by scientists to describe the community type. In addition to providing physical structure and stabilizing the substrate, bivalves continuously pump large quantities of water. While this pumping rate varies with species, temperature and the amount of suspended material, this pumping, coupled with bivalve feeding by filtering, removes large quantities of suspended material as well as food and potential pollutants. In areas such as Chesapeake Bay, reestablishment of oyster reefs should lead to clearer water, and Virginia has instituted a program to reestablish reefs that occupy the entire water column. Early studies in San Francisco Bay reported that bivalves were important factors in controlling plankton blooms. In subsequent years, researchers in the Great Lakes found that accidentally introduced zebra mussels were the major cause of increased water clarity and exerted substantial control of phytoplankton concentrations. In some areas, increasing water clarity resulting from large beds of cultured bivalves has led to the reestablishment of sea grasses. This synergistic relationship between high density of bivalves and sea grass is an important theme in our efforts to maintain and improve the water quality of our nearshore waters.

It has been calculated that, in pre-colonial Chesapeake Bay, the oysters could filter the entire water volume of the bay in 3 days. It is currently estimated to take close to a year – a direct result of the reduced oyster population. In contrast, the surf clam and ocean quahog populations of the Federal waters of the Mid Atlantic have been estimated to contain 18 billion surf clams and 59 billion ocean quahogs. These figures do not include the large surf clam populations that are in State waters (the inner 3 miles of the continental shelf). The populations in Federal waters can filter approximately 300 billion gallons per day, and the total volume contained out to the 300 foot contour is 1.7 trillion gallons. Based on

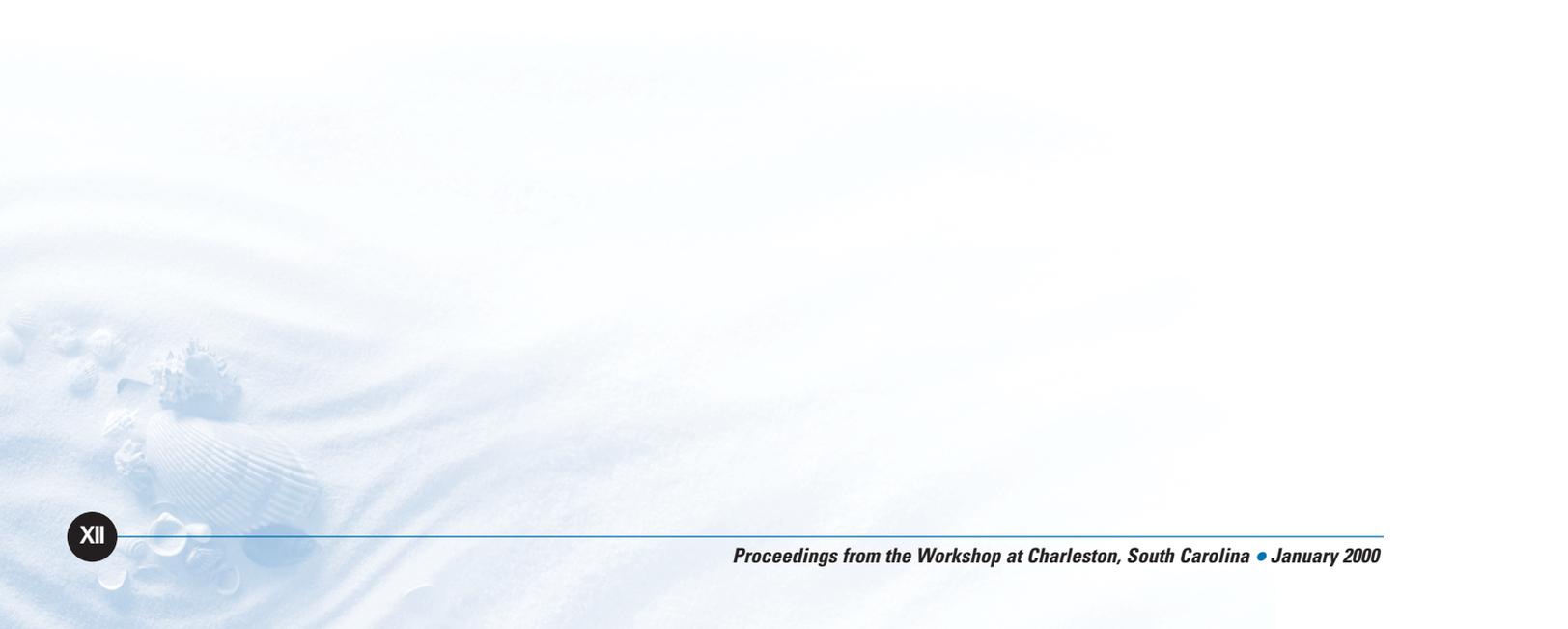


Line drawing of a typical natural oyster community showing ~20 associated species.

these values, the Federally controlled clam populations, filtering half the time, could filter the entire water volume of the shelf from Cape Cod to Chesapeake Bay out to the 300 foot contour once every 3 years. Including the clams in the inner 3 miles would substantially reduce this time factor. Obviously this does not happen because the clams are on the bottom of a deep water column and the water is in constant motion, but it does indicate the filtering capacity of these animals and their potential to influence the bottom water of their habitat. Individuals in the Pacific Northwest have calculated that 500,000 oysters can process enough suspended material to be equivalent to that sent to sewage plants by 123 people in one year.

BIVALVES ARE IMPORTANT!

The above examples illustrate the often overlooked importance of clams, oysters, mussels and scallops in maintaining both the economic base and the ambiance of our coastal communities. Details on specific aspects of bivalve management, biology, aquaculture and their relations to economic, public and ecosystem health are presented in the following chapters. The importance of maintaining healthy bivalve populations and the type of information needed to sustain these structural and functional resources are emphasized in these reviews. After a brief summary of the information, the sections have outlined the areas where more data, experiments or information are needed.



Chapter 1

Trade, Policy and Management Issues

In this chapter we propose a National Shellfish Research Plan and suggest how it might be developed and managed (implemented, updated, promoted).

Status: Currently in the United States there are a number of independent programs and activities related to shellfish research as well as management of wild shellfisheries, shellfish aquaculture, shellfish restoration and human health issues related to consuming shellfish. While these shellfish programs often address similar issues, they have limited, little, or no coordination between them. There is no single entity that links these efforts together, facilitating essential national and international coordination.

Solution: A National Shellfish Management Program (NSMP) administered by a new Office of Shellfish (OS). The future of the Nation's molluscan shellfish resources depends on the development of a National Shellfish Management Program.

Molluscan shellfish are a significant and vital national resource for a variety of reasons.

Shellfish are an important, wholesome, nutritious food in ever increasing demand. A NSMP will ensure that production from existing wild shellfisheries will be sustainable and that depleted stocks are restored. A NSMP will ensure that production of cultured shellfish will continue to increase with practices that are environmentally responsible. Demand for US produced shellfish continues to grow worldwide. A coordinated NSMP represents a unique opportunity to make a dent in the significant seafood trade deficit existing today in the United States.

Aside from their economic value, shellfish serve as a universal indicator of the environmental health of our nations estuaries. They work much as the canaries once did in coal mines, providing an early indication of problems in the ecosystem (mine shaft). Shellfish provide essential habitat for a variety of marine vertebrate and invertebrate species. Functionally, shellfish beds provide structure, habitat, refuge and forage.

Shellfish serve a critical role as grazers in coastal estuaries. Populations of microscopic, free floating plants (plankton) can explode, causing complications in estuarine ecosystems when nutrients from agricultural or development sources reach marine waters. Shellfish feed by filtering these microscopic plants from the water column. Healthy populations of shellfish actively graze down these massive algal blooms with phenomenal efficiency keeping the water clear, and allow sunlight, crucial to seagrass photosynthesis, to penetrate the depths.

A NSMP would provide coordinated planning and a link for all of the existing independent state, federal and private programs related to the management of wild shellfisheries, shellfish aquaculture, shellfish restoration and human health issues and shellfish research. A NSMP would ensure that the Nation's shellfish resources are optimally utilized for both their food and ecological functions. A NSMP would ensure that policy and management decisions were made based on sound science, balancing the economic viability of the shellfish wild harvest and aquaculture industries with the need to protect the estuarine ecosystems.

The Office of Shellfish administering the NSMP would provide national coordination for all national shellfish programs. A variety of options currently exist for where the Office of Shellfish could potentially be housed. These options include, but are not limited to, the Interstate Shellfish Sanitation Conference, the Sea Grant Marine Advisory Program or the Molluscan Shellfish Institute (MSI), which is part of the National Fisheries Institute (NFI).

It is envisioned that the OS would be limited in size to just a couple of individuals. It could conceivably be a virtual office. The programs to be coordinated are largely in place, but occur currently in a fragmented manner.

Four Major Components of the National Shellfish Management Plan

The four major components of the proposed National Shellfish Management Plan are graphically illustrated here and in greater detail in an organizational chart.



1. The National Shellfish Sanitation Program (NSSP)

The NSSP is an established and highly effective program that assures that any shellfish harvested from the coastal waters of the United States or imported from foreign countries are safe for human consumption.

The NSSP is administered by the Food and Drug Administration and implemented through the Interstate Shellfish Sanitation Conference (ISSC). This arrangement would not change under the National Shellfish Management Program other than it would be more closely coordinated with all other shellfish programs in the country. A Memorandum of Agreement (MOU) exists between USFDA and ISSC defining the current relationship. A Model Ordinance has been developed by the ISSC for shellfish producing and receiving states to adopt. The Model Ordinance defines the necessary guidelines to assure shellfish growing waters are clean and that shellfish products are not adulterated during harvest, processing, transportation or sale.

The ISSC is convened annually to update the Model Ordinance and address public health issues related to human consumption of shellfish. Participants include shellfish industry representatives from around the country, representatives from each State Shellfish Control Authority that have been designated to implement the NSSP in their respective states, the Food and Drug Administration, USEPA and NOAA.

Foreign countries wishing to export shellfish into the United States have to enter into Memoranda of Understanding with the USFDA that ensure the shellfish sanitation programs in those countries meet or exceed the standards set forth in the Model Ordinance. Delegates from these MOU countries typically attend the annual Interstate Shellfish Sanitation Conference.

The Office of Shellfish in administering the National Shellfish Management Program would attempt to strengthen the existing National Shellfish Sanitation Program by:

- Promoting a risk based shellfish consumption program that includes policies for effective risk management and communication both with the shellfish industry and the general consuming public. The Office of Shellfish would work through ISSC to ensure public communication balances the need to protect public health with consideration for economic impact to the industry resulting from inappropriate public communication;
- Securing a commitment from the USDA to support and participate in the NSSP/ISSC process, contributing to research and enforcing Best Management Practices (BMPs) on agricultural operations adjacent to shellfish growing areas;
- Restoring America's confidence that shellfish are safe to consume raw. Included in this effort would be encouraging Health and Human Services to promote consistent policy between USFDA and the Center for Disease Control (CDC) that implementation of the NSSP is predicated on the ability to ensure shellfish are safe to consume raw. Also included in this effort would be a request that the USFDA prepare an annual report to Congress regarding the effectiveness of the NSSP;
- Strengthening regulatory control to ensure imported processed shellfish from MOU countries meet NSSP requirements and that shellfish from non-MOU countries that do not meet the standards required of our domestic shellfish program do not enter the country;
- Encouraging NOAA to reaffirm their commitment to the NSSP by supporting research related to issues of human health concern and promoting coastal zone management that recognizes the values of shellfish in estuarine systems;
- Encouraging the USEPA to reaffirm their commitment to the NSSP by supporting research related to shellfish, water quality and human health; assisting with the development and standardization of improved water quality indicators and source tracking tools to identify pollutants; and by providing additional funding and support to states developing Total Maximum Daily Loads (TMDL) for shellfish growing areas listed on Clean Water Act 303d lists.

2. A National Shellfish Research Plan

This would be the plan whose evolution began at the Cooperative Research and Information Institute (CRII) workshop in Charleston, South Carolina on January 8-12, 2000.

The new Office of Shellfish would facilitate the development, maintenance and implementation of the National Shellfish Research Plan as a component of the National Shellfish Management Program.

Existing research planning efforts: The West Coast shellfish industry under the guidance of the Pacific Shellfish Institute has just recently (December, 1999) completed an eighteen-month effort resulting in a document titled North American West Coast Shellfish Industry 2010 Goals, Research and Initiative Priorities. This document was developed with input from several workshops involving shellfish growers, academia, extension agents and resource managers. It establishes goals for the year 2010 and identifies the research priorities and initiatives necessary to achieve those goals.

The Gulf Oyster Industry Council has established the Gulf Oyster Industry Program with dedicated funding within the National Sea Grant program of approximately \$930,000 annually. This funding is directed predominantly at shellfish research related to human health issues, which the Gulf shellfish industry has identified as a priority.

Sea Grant also continues to make \$1,475,000 available annually for the National Oyster Disease Research Program. Projects funded under this program address diseases that are affecting oyster populations around the country.

Development of the National Shellfish Research Plan: Extensive effort has gone into the development of the West's 2010 Goals and the Gulf Oyster Industry Program. To maintain the critical support and involvement of these industries in the National Shellfish Research Plan, the integrity of these efforts should be acknowledged and preserved. The National Shellfish Research Plan should attempt to merge the ten chapters represented in the West's 2010 Goals document and the categories discussed at the January 8-12 CRII workshop. Color-coding or some other designator could potentially be used to reflect whether the research need is national or regional. Many of the research needs already identified by the West or the Gulf are national in nature and could be designated as such in the Plan.

Research needs in the national plan should be prioritized in some fashion. The West Coast industry chose a simple High (H), Medium (M) or Low (L). This is a model that could perhaps be followed in the National Shellfish Research Plan. Clearly the National Shellfish Research Plan will include needs beyond those likely to be funded. The prioritization will assist researchers and granting entities in targeting the most important research needs. The National Shellfish Management Plan should include a process agreed upon by the participants for research prioritization in the National Shellfish Plan.

In the future, the Office of Shellfish would facilitate periodic workshops on the Pacific, Gulf and East Coasts to establish (in the case of the East Coast) or update (in the case of the Gulf and West Coast) research priorities for inclusion in the national plan. These workshops should include industry, academia, extension agents and resource managers. The Sea Grant Marine Advisory Program is a likely sponsor of these workshops. The workshops could be used as an opportunity to disseminate recent research results, evaluate progress on previously identified priorities and establish new priorities.

The Office of Shellfish should electronically update the Plan every two years and produce an updated printed version every sixth year.

3. A National Shellfish Aquaculture Plan

The National Shellfish Aquaculture Plan would be developed and implemented by the Office of Shellfish as a component of the National Shellfish Management Program.

A National Shellfish Aquaculture Plan is needed to orchestrate the continued growth and economic viability of the shellfish aquaculture industry. Expanding human populations in coastal regions are impacting water quality and increasing competition for the use of the marine waters and

viewsheds. West Coast listings of salmon under the Endangered Species Act, identification of Essential Fish Habitat under the Sustainable Fisheries Act and generally a more environmentally conscious society have raised the level of public and regulatory scrutiny on the shellfish culture industry.

A National Shellfish Aquaculture Plan will:

- Ensure the development of Environmental Codes of Practices (Best Management Practices). The Plan will ensure that the industry will be accountable for implementing the Codes and furthermore, that the Codes will incorporate adaptive management when research identifies improved, economical, environmentally sensitive culture practices. To the greatest degree possible, the Codes of Practices should be based on environmental outcomes and avoid prescriptions which may stifle industry innovation;
- Ensure that ecological changes (both positive and negative) associated with shellfish culture and harvest are documented, understood and incorporated into the Environmental Codes of Practices;
- Encourage demonstration projects which provide education, training, technology transfer and public outreach regarding economical and environmentally sensitive culture practices;
- Ensure coordinated, comprehensive Aquaculture Planning as provided for under Sections 309 and 306 (a) of the Coastal Zone Management Act. These efforts would include guidance to states on aquaculture permit streamlining and zoning for aquaculture development;
- Promote programs which foster interstate and international coordination and uniformity with regard to health and certification requirements. These requirements apply to the movement of larvae, seed, broodstock and mature crops across political boundaries for culture or market. These programs should be designed to control the spread of disease, pests or aquatic nuisance species utilizing a Hazard Analysis Critical Control Point (HACCP) - based shellfish health management program.

Coordination provided through the Office of Shellfish would ensure that the shellfish culture industry's research priorities are identified and prioritized accordingly in the National Shellfish Research Plan.

Department of Commerce Secretary, William Daley, signed a new Aquaculture Policy in August of 1999. The goal of the policy is to build a robust, competitive, environmentally sound aquaculture industry by 2025. DOC is striving with this policy to increase aquaculture production from \$900 million to \$5 billion annually. Other goals in the policy include developing aquaculture technologies and methods to improve production and safeguard the environment; increasing jobs in aquaculture from 180,000 to 600,000; developing codes of conduct for responsible aquaculture. A National Shellfish Aquaculture Plan will ensure shellfish contribute significantly towards achieving DOC's ambitious goals.

As an outcome of a workshop hosted by the DOC in August of 1999 to assess the obstacles to implementing their new aquaculture policy, regional caucuses have been organized. The caucuses are developing strategies for implementing DOC's policy. The shellfish industry is represented on these regional caucuses. The caucuses could well serve as the forum for developing a National Shellfish Aquaculture Plan.

USDA has five regional aquaculture centers to coordinate research for the nations aquaculture industries. To a large degree, much of the focus of the research done by these centers has been on fresh water species. The Western and Northeast Regional Aquaculture Centers (WRAC and NRAC) have historically supported molluscan shellfish aquaculture research. The WRAC is currently supporting

shellfish ecology and genetics research at roughly \$200,000 annually. The Office of Shellfish should coordinate the involvement of these USDA regional aquaculture centers in the process of developing the National Shellfish Aquaculture Plan as well as the National Shellfish Research Plan.

The Joint Subcommittee on Aquaculture will be releasing its latest draft of the National Aquaculture Development Plan. This plan does not include any species-specific guidance such as a National Shellfish Aquaculture Plan would provide.

4. A National Shellfish Restoration Plan

The National Shellfish Restoration Plan would be developed and implemented by the Office of Shellfish as a component of the National Shellfish Management Program.

Currently there are a variety of projects around the United States that are directed at restoring depleted wild shellfisheries, restoring shellfish reefs for their ecological function or restoring degraded water quality in shellfish growing areas. These projects are occurring independently with limited coordination or communication between them.

On the East Coast there have been monumental efforts to restore Eastern oysters to the Chesapeake Bay for both their economic benefit to the waterman as well as for their ecological function to the estuary. Efforts are underway to restore Eastern oysters to New York Harbor for their ecological and filtration benefits. Connecticut has successfully restored commercial populations of Eastern oysters to Long Island Sound and rejuvenated what was a struggling shellfish industry. In the west there are efforts underway to restore the native Olympia oysters to estuaries in Washington and Oregon. Examples like this abound; however, they are independent efforts that often lack the benefit of each other's experiences and any centralized national planning effort.

Independently, shellfish culturists and wild harvesters struggle to address pollution sources that have degraded water quality in their growing waters. Success stories abound with projects involving small community sewer systems with upland disposal, innovative alternative septic systems appropriate for shoreline use, grants and low interest loan programs to assist home owners with expensive septic system repairs, operation and maintenance programs adopted by local governments to stay vigilant on septic systems surrounding shellfish growing areas. There does not currently exist a central clearinghouse for such shellfish related water quality mitigation projects. Nor does there exist any coordinated, prioritized national plan for addressing water quality problems in shellfish growing areas.

A National Shellfish Restoration Plan will:

- Develop and implement a coordinated approach to restoring and managing the Nation's shellfish resources and associated growing waters;
- Prioritize restoration based on economic and ecological benefit and by evaluating likelihood of success;
- Provide a national clearinghouse for examples of successful shellfish restoration and water quality mitigation projects;
- Facilitate future International Shellfish Restoration Conferences;
- Improve assessments of wild shellfish resources through improved science and models;
- Promote upland municipal waste water disposal alternatives (e.g., water re-use for golf course irrigation, lawn watering, street sweeping, tertiary treatment for ground water recharge) in areas adjacent to shellfish growing waters.

Conclusion: It is in the Nation's best interest to support an Office of Shellfish implementing a National Shellfish Management Plan that coordinates the National Shellfish Sanitation Program addressing whether shellfish are safe to consume, with a National Shellfish Research Plan, prioritizing the nations shellfish research needs, with a National Shellfish Aquaculture Plan facilitating a robust and environmentally sound aquaculture industry and with a National Shellfish Restoration Plan which serves as a clearinghouse and provides a prioritized roadmap to restoring the Nation's shellfish resources and growing water quality.

Chapter 2 Aquaculture and Enhancement

INTRODUCTION

The demand for shellfish has increased in the United States while the wild shellfish stocks have declined. Overfishing and environmental degradation resulting from human and natural impacts have contributed to the decline in suitable habitat. Aquaculture, both for commercial purposes and for public stock enhancement, provides a means for increasing shellfish resources and at the same time protecting an important part of our fishing heritage.

The industry recognizes a need for public education programs that stress the environmentally sound and sustainable nature of shellfish culture. These programs should include the essential role that shellfish culture can play in improving water quality, creating fisheries habitat, reducing harvesting pressure on wild stocks, providing jobs and reducing the national balance of payments deficit. Cultured shellfish are a high quality source of protein and other essential nutrients. As primary consumers, they provide an efficient, sustainable and healthful food source for a growing global population.

Need for public education programs that stress environmentally sound and sustainable aquaculture.

STATUS

Shellfish aquaculture is in its infancy despite the fact that oysters have been cultured in the United States for over a century, and more recently clam culture has become a multi-million dollar industry. Rapid advances in shellfish hatchery, nursery and growout technology promise to increase our ability to culture not only these traditional species, but also alternative species. The same techniques may also provide the mechanisms for restoring wild shellfish stocks. In order to accomplish these goals, we are faced with challenges involving questions of genetics, diseases, population dynamics, physiology and molecular biology. In addition there are important issues involving public awareness, water quality such as harmful algal blooms and human pathogens and broadscale questions involving habitat restoration for cultured shellfish.

AQUACULTURE TRAINING

OBJECTIVE — *Provide entry level and continuing aquaculture training.*

Rationale

Training of culturists for the developing industry.

Training is a critical requirement for both new and existing aquaculturists. Successful retraining programs for displaced commercial fishers should serve as models for further efforts to train new culturists for the developing industry. New employees and small business entrepreneurs often have backgrounds unrelated to the shellfish business with little understanding of shellfish culture, safe food handling or business / managerial practices.

Future Directions:

- Develop and provide information on the availability, type and cost of aquaculture training.
- Establish short-term training programs including on-the-job training (algae culture methods, Best Management Practices, outreach) with links to annual grower meetings or workshops (for regulatory training).
- Develop distance learning curricula (with video tools and web-pages) in association with accredited colleges and universities.

OBJECTIVE — *Continue support for formal education and training in colleges, universities and other research facilities.*

Rationale

Shellfish aquaculture advancement requires substantial commitment to research.

Continued advances in the shellfish industry require a substantial commitment by research institutions. Students and faculty are required to support these programs. The industry, private sector research, and consulting communities will need students with training in shellfish biology, culture techniques, aquaculture business and management and related disciplines. In addition, some fraction of the student and faculty effort should be directed toward meeting applied research priorities.

Future Directions:

- Promote recruitment to bring students and faculty into these programs.
- Ensure there is significant shellfish industry participation in strategic planning efforts at universities and colleges.
- Promote the continued development of aquaculture research and training programs at the high school, technical school, and college levels.
- Assess possible direct support of students and faculty in on-campus training and teaching programs.

OBJECTIVE — Offer “hands-on” educational programs.

Rationale

Formal academic training and laboratory exercises cannot replace experience gained in working in the field or commercial sector. Short-term to long-term (more than 1 month) study with industry and field research/agency partners should always be part of the educational experience.

Internships are vital.

Future Directions:

- Work with the industry to develop intern opportunities.
- Provide funding to support short-term internships.
- Provide universities and colleges with information on internships for students.

OBJECTIVE — Promote shellfish education in public schools, FFA/4-H program, tribal schools, marine science centers, and public / private aquaria.

Rationale

Schools and marine science centers provide an opportunity to educate a wide range of students in classroom, laboratory and field settings. Students gain entrepreneurial skills and an understanding of the biological and water quality requirements of shellfish culture.

Future Directions:

- Integrate shellfish and aquaculture concepts into selected school curricula.
- Incorporate FFA/4-H aquaculture instructional packages and curricula for high school students.

OBJECTIVE — Make aquaculture information readily available to the shellfish industry.

Rationale

Many growers have requested creation of a shellfish library dedicated to the commercial industry. Information resources should be made available on web-pages or other accessible locations.

Future Directions:

- Assist in improvement and cataloging of a reference collection, a video library, and materials for training.
- Improve and expand aquaculture web-pages.
- Create an aquaculture information database cataloging existing information relevant to the shellfish industry and link it to associated web-pages.

GENETICS

OBJECTIVE — *Establish effective shellfish breeding programs (selective and/or hybridization) and have it adopted and supported by the industry.*

Rationale

This research is crucial for maintenance and expansion of the shellfish industry. Several shellfish breeding programs are currently underway and these programs are showing exciting results. These programs will need continued financial support to ensure long-term genetic improvement of broodstock lines.

Recent bivalve genetics research on the West Coast and in the Gulf of Saint Lawrence in eastern Canada indicate increased heterozygosity results in decreased metabolic needs and increased survival and growth. “Natural” triploid oysters produced from tetraploids have increased heterozygosity. They have demonstrated increased survival and growth as they have moved into commercial application in 1998 and 1999. These recent findings have immediate and exciting implications for the future of bivalve genetics research.

Need for long-term genetic improvement of broodstocks.

Improvement of shellfish currently under cultivation by the shellfish industry is desired. Long growout periods, losses due to disease and other mortality factors, and seasonal problems with marketability of product contribute to high costs of production, product loss and reduced market share on an annual basis. Improvement of stocks may be achieved *via* genetic methods (including selection, hybridization, ploidy manipulation and transgenic approaches (specifically for disease-resistance)).

Future Directions:

- Develop a selection and breeding program for enhanced growth rate and survivorship of shellfish, partnering with industry growers for rearing experimental stocks under different grow out conditions.
- Develop record-keeping practices to allow determination of the performance of individual stocks as they pass through the hatchery, nursery and growout to market.
- Develop protocols to facilitate the transfer and utilization of broodstock lines by commercial hatcheries.
- Develop protocols to minimize disease risks in transferring broodstock and seed among growers.
- Continue biotechnology research on improving broodstock (genomic research, marker assisted selection, heterozygosity/homozygosity, transgenics *etc.*).
- Develop methods to produce triploid and tetraploid shellfish for sale during the summer months to improve growth rate, reduce market loss due to poor condition and reduced shelf-life of oysters during this season.
- Develop genetics tags for tracking and survival studies.
- Establish and maintain facilities designed to support the above research needs/initiatives.

HUMAN HEALTH AND SHELLFISH

OBJECTIVE — *Predict changes in toxic phytoplankton and bacterial levels in the water and sediments.*

Rationale

Toxic phytoplankton and excessive levels of bacteria can close shellfish growing waters very quickly. Occasionally levels of toxins or bacteria increase so rapidly that contaminated product escapes detection, getting into the market place and causing illnesses. Once detected these products need to be recalled. The illnesses and recall process not only erode consumer confidence in the products, but also represent a considerable expense for the shellfish grower. Inexpensive tools which would allow the early and rapid detection or prediction of developing blooms or increasing bacteria levels would prevent unnecessary illnesses and recall situations. It would also allow growers the opportunity to manage around the closures by shifting to new harvest areas or by supplying alternative product.

Toxic phytoplankton and pathogenic bacteria can close shellfish growing waters.

Future Directions:

- Communicate economic impacts of marine biotoxins to funding sources to encourage additional research funding.
- Develop rapid identification tools for toxins such as PSP, including field kits.
- Identify bacterial diseases such as *Vibrio* sp. triggers, abundance and implications for management of harvesting.
- Collect information about virulent species, abundance and prevalence and environmental interactions. Build comprehensive database for phytoplankton occurrences.
- As energies are focused on new shellfish species, assure the harmful algae bloom relationships are understood.

OBJECTIVE — *Improve public understanding of shellfish pathogens and good shellfish handling practices.*

Rationale

Many shellfish borne illnesses are the result of individuals mishandling shellfish after they have purchased it in a retail outlet or harvested it recreationally. A public education campaign could reduce the number of illnesses associated with this mishandled product.

Public education could reduce shellfish induced illnesses.

Future Directions:

- Improve public understanding of shellfish pathogens and good shellfish handling practices.

OBJECTIVE — *Improve food handlers understanding of shellfish pathogens and good shellfish handling practices.*

Rationale

The National Shellfish Sanitation Program, which governs the activities of commercial shellfish operators, ensures that commercial growers possess a reasonable understanding of good shellfish handling practices. Retail marketers and restaurant workers often do not have this same level of understanding. Shellfish borne illnesses are often associated with mishandling of the product once it is in the distribution system.

Future Directions:

- Improve food handlers understanding of shellfish pathogens and good shellfish handling practices; work through local health departments and the Conference for Food Protection to disseminate this information.

OBJECTIVE — *Improve tools to identify specific pollution sources.*

Rationale

When growing areas are impacted by fecal coliform bacteria, it has historically been very difficult to determine the source and to quantify it. The result is a broad, expensive, slow, non-targeted assault on all non-point source pollution (birds, waterfowl, cows, cattle, failing septic systems, storm water runoff *etc.*). Recently new DNA tools are being used to trace fecal coliform bacteria back to the source. While this has been helpful, there is not yet a technique available which can be used to quantify the contribution, nor does it assess the human health risk associated with the source.

Future Directions:

- Support on-going and new research to identify specific pollution sources (for restoration/remediation).
- Support research that determines the human health risk associated with various sources of pollution.

OBJECTIVE — *Improve indicator organisms to identify potential for human health impacts.*

Rationale

Fecal coliform bacteria, the current indicator organism used to classify shellfish growing waters, are present in the feces of all warm-blooded animals. It does not, however, correlate well with the presence of human pathogens and human health risk. An indicator that is more directly representative of human health risk has been desired by the industry for many years.

Future Directions:

- Monitor progress of ISSC indicator study review; identify and prioritize additional research needs as appropriate.

Need for new indicators of pathogens and rapid diagnostics.

OBJECTIVE — *Improve epidemiology/reporting systems to identify accurate level of problems so the effectiveness of management controls can be identified.*

Rationale

Typically, only a small percentage of shellfish borne illnesses are reported. Many shellfish illnesses result in gastroenteritis and individuals often are not ill enough to seek medical attention. When they do report an illness to a local health authority, they are often reluctant to submit a stool sample, which is essential for verification. Different states have different reportable diseases resulting in inconsistent reporting. Reporting problems exacerbate the epidemiological investigation of the illness outbreaks and complicate assessment of the effectiveness of management controls.

Illness reporting and epidemiological investigations need improvement.

Future Directions:

- Encourage the institution of a nationwide policy, based upon that of Washington State, which requires mandatory reporting of shellfish-borne illnesses to local health departments and the State/Provincial shellfish authority, by healthcare workers.

OBJECTIVE — *Improve understanding of dynamics of HABs and shellfish.*

Rationale

A clearer understanding of HAB dynamics in shellfish is essential to adequately protect public health and minimize economic impact to the shellfish industry.

Understanding of HAB dynamics in shellfish is essential to protect the public.

Future Directions:

- Continue and improve Harmful Algal Bloom (HAB) monitoring programs.
- Encourage further research on HABs in shellfish, looking at variables such as depth, harvesting effects, seasonality, water column, intertidal vs. subtidal, cultured vs. wild, age variability.
- Encourage further evaluation of the risk assessment associated with HAB's and other toxins levels currently established in the National Shellfish Sanitation Program and current methods of testing.

OBJECTIVE – *Support research directed at developing effective methodologies to eliminate marine biotoxins, bacteria and viruses from live shellfish.*

Rationale

Naturally occurring marine biotoxins (Paralytic shellfish poisoning (PSP), diuretic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP)), *Vibrio* spp. and other marine bacteria, and viruses are a public health risk associated with shellfish. Monitoring of shellfish meats and growing areas is expensive and growers are economically impacted by closures, product recalls and lost consumer confidence. While depuration is a proven technology for eliminating harmful bacteria from shellfish, it is not effective at eliminating viruses or marine biotoxins. The development of post harvest treatments for live shellfish that are effective at eliminating marine biotoxins and viruses as well as bacteria would improve public health protection and reduce the associated economic impacts to the industry.

Future Directions:

- Conduct research to identify possible inactivation or removal (depuration) of marine biotoxins from live shellfish.
- Conduct research to develop an effective process for the removal of viruses from live shellfish.
- Conduct research on technology to eliminate or control the growth of bacteria in live shellfish.

MARKETING SHELLFISH PRODUCTS

OBJECTIVE — *Develop a marketing strategy to increase demand and price for species currently under cultivation and identify market opportunities for new species.*

Rationale

Marketing of shellfish products has relied largely on the efforts of individual companies. Further development of shellfish products can be enhanced by unified marketing plans.

Unified marketing plans will further the development of the industry.

Future Directions:

- Establish marketing strategies committees.
- Investigate examples of organized marketing efforts (i.e., programs developed by the Alaska Seafood Marketing Institute, The Catfish Institute, the various agricultural commodity commissions and the Sustainable Agriculture Program).
- Identify funding options to support the development and implementation of marketing programs.
- Conduct market surveys to identify the market potential for new and existing product forms, new packaging and new species.
- Organize and/or participate in promotional activities such as the San Francisco and Boston Seafood Shows.
- Produce generic promotional information regarding environmentally sound culture techniques, shellfish quality, handling, nutritional and product safety for distribution (brochures, flyers, table tents, recipe cards) in retail outlets, trade shows, county fairs, farmers markets, press releases and on web-pages.

PRODUCTION TECHNOLOGY

OBJECTIVE — Support development and expansion of hatchery, nursery and growout techniques for rearing shellfish species with current or emerging potential and examine the restoration and mitigation opportunities for these techniques; investigate alternative seed sources including wild spat collection.

Rationale

Chronic seed shortages are currently limiting growth of the industry. The continued development of hatchery techniques for rearing species of shellfish for commercial production and the enhancement of wild fisheries is required for growth of the industry. Basic research on maturation and conditioning techniques, spawning and larval rearing, nursery techniques and planting/distribution methods are critical to establish prior to large-scale seed production by public and private hatcheries. In addition, the genetic integrity of the native stocks in the areas being enhanced should be investigated and preserved. Wild spat collection has potential to be a successful alternative means of seed procurement for public and private growout of some species.

Chronic seed shortages limit the industry.

Recent research indicates that shellfish (especially bivalve molluscs) contribute greatly to the overall health of near-shore environments by providing habitat to other organisms and by serving as a means of cycling nutrients through the system. More work is needed to determine the effectiveness and feasibility of using hatchery produced shellfish for use in mitigation and restoration projects.

Future Directions:

- Develop hatchery, nursery and growout techniques for private enterprises and public enhancement programs.
- Investigate use of hatchery cultured shellfish as a means of contributing to the effectiveness and efficiency of mitigation and restoration efforts in near shore habitats.
- Continue investigations of algal nutrition with the goal of developing strains which improve larval survival and growth.
- Further develop the methodology to alleviate bacterial and viral induced losses in hatcheries including improved sanitation and further development of effective therapeutics through USDA/INAD programs.
- Encourage the development of new hatcheries and improvement of hatchery techniques in existing facilities to meet the chronic seed shortages.
- Continue to optimize existing nursery systems and engineer new gear technologies.
- Investigate environmentally friendly methods to control biofouling in nursery and growout systems.
- Improve field growout techniques to reduce environmental impacts.
- Reduce the effects of predation and pests in private growout systems and public enhancement efforts.
- Develop carrying capacity models to assist growers in optimizing production while minimizing environmental effects.
- Investigate cost efficiency of public enhancement programs.
- Investigate optimum densities for seed plantings and spawning stocks to increase the probability of reproductive success in enhancement projects.

- Investigate successful Japanese and New Zealand spat forecasting and collection methods for domestic application.
- Address the species specific concerns of hatchery and translocated stocks on the genetic integrity of native populations.
- Address regulatory constraints to the construction of new hatcheries and deployment of nursery systems.
- Support and encourage programs to monitor current non-indigenous species invasions and to prevent the introduction of unwanted species.

DEVELOPMENT OF NEW SPECIES FOR DIVERSIFICATION IN SHELLFISH AQUACULTURE

OBJECTIVE – *Develop an appropriate list of candidate species by region with current or emerging economic and market potential for commercial production and establish hatchery, nursery and growout techniques for rearing these species.*

Rationale

The development of hatchery techniques is desired for species not currently under cultivation and which have a high, or potentially high, value in the market place. Basic research on maturation techniques, spawning and larval rearing, nursery techniques and adult grow out methods to market size are critical to establish prior to large-scale adoption by the shellfish industry. Demonstration projects should precede full-scale commercialization of techniques.

Future Directions:

- Develop aquaculture techniques suitable for large-scale commercial production of regional candidate species.
- Address regulatory concerns and/or constraints that may limit the development of these species (i.e., water column usage for scallop growout, use of non-native species).

OBJECTIVE – *Evaluate the use of polyculture techniques for the potential of biofouling and predator control for shellfish currently under cultivation by the shellfish industry.*

Rationale

Improvement of shellfish technologies currently in use by the industry is desired. Long growout periods and losses due to fouling and predation contribute to high costs of production, product loss and reduced market share on an annual basis. The use of natural control techniques, or bioremediation, negates the need for labor intensive methods and does not contribute to ecosystem degradation.

Future Directions:

- Develop, implement and demonstrate effective techniques.

Develop alternative species for culture.

SITING AND LEASING

OBJECTIVE - Promote the development of user-friendly procedures for the continued expansion of the shellfish culture industry.

Rationale

Lease siting considerations are crucial for the maintenance and expansion of the shellfish aquaculture industry. Further, with the development of new species for culture comes the need for water usage other than traditional bottom, submerged lands.

Rational leasing, siting considerations and regulations are necessary for industry growth.

Future Directions:

- Identify criteria for lease siting that would be acceptable to multi-users, regulators, managers, and lead to regulatory consistency.
- Investigate zoning concepts to allow for water column usage for specific technologies.
- Develop effective mechanism for conflict resolution between competing user groups.
- Establish and maintain a current list of the required procedures and subsequent permits and licenses necessary to farm shellfish in the various coastal states.
- Work to simplify the leasing and permitting process.

SHELLFISH HEALTH

OBJECTIVE – Foster the growth of the sustainable aquaculture industry through the development of disease resistant stocks and management approaches.

Rationale

Shellfish diseases are a top concern for the shellfish aquaculture industry. Diseases (such as MSX and Dermo for the eastern oyster, QPX for the hard clam, withering disease for abalone and summer mortality for the Pacific oyster) cost the industry millions of dollars in lost production annually. The best hope for solutions to these diseases rely on genetic selection programs for disease resistance which require a long-term commitment of funding and resources to maintain selected stocks over several generations.

Diseases impact not only on the stocks themselves, but also affect industry indirectly through the regulations which have been established in an effort to control the spread of disease; (regulations restricting the interstate/intrastate/ and international transport of seed and broodstock). A better understanding of the dynamics of disease transmission is necessary for the formulation of rational, science-based regulations. Rapid diagnostic assays are needed to detect diseases to facilitate management, and remediation of disease.

Regulations should be based on an assessment of the relative magnitude of risks from all sources including shellfish aquaculture, ballast water, wet storage and natural processes.

Shellfish diseases cost the industry millions of dollars.

Future Directions:

- Continue and expand the monitoring of shellfish diseases in growing areas.
- Research the mechanisms of disease transmission so that high-risk activities can be properly managed and regulated.
- Evaluate the effectiveness of potential therapeutants, treatments and management approaches on minimizing the economic impacts of disease outbreaks.
- Establish management plans for infectious shellfish disease outbreaks in shellfish growing areas.
- Improve interstate coordination in regard to health and certification requirements for shellfish movements as related to larval and seed sales and brood stock movements.
- Develop a regional capability to gather and disseminate information on shellfish diseases and management strategies for the control of diseases from world-wide sources (i.e., the Organization of International Epizootics).
- Determine the specific nature of hatchery and nursery bacterial pathogens of all important cultured shellfish species and provide means to diagnose the type, source and hatchery location of these pathogens. Include evaluation of hatchery handling methods and protocols and nutrition on shellfish larval and seed health and infectious diseases.
- Determine management and control methods for hatchery and nursery bacterial pathogens including: development of probiotics; assessment of water quality factors that influence pathogens; nutrition; and hatchery methods that may influence (or be used to manage and prevent) infectious diseases in shellfish hatcheries and nurseries.
- Work with industry associations to develop Shellfish High Health standards to support brood stock and seed stock transfers (intra / interstate and international).
- Determine the role of algal toxins in both shellfish production areas and hatcheries as they are related to mortality and poor performance.

POST-HARVEST / PROCESSING

OBJECTIVE – *Establish optimal processing and post-harvest handling techniques/protocols for live and processed shellfish.*

Rationale

Most shellfish aquaculture and fisheries products have historically been sold whole in the shell. Despite the high value of the product and the quality conscious market receiving it, additional work is needed to optimize processing and handling from harvesting through shipping to maximize product quality.

Future Directions:

- Improve shelf life (survival in refrigerated storage) of live products (shell stock).
- Develop value added and new product forms.

- Investigate the effects of irradiation and pasteurization on product quality, production, economics, and consumer acceptance.
- Further evaluate applicability of depuration, relaying and wet storage alternatives to shellfish post-harvest handling.

QUALITY ASSURANCE

OBJECTIVE – *Develop and implement a total quality assurance program for the shellfish industry.*

Rationale

The recently implemented Hazard Analysis Critical Control Point (HACCP) program focuses on seafood safety issues, but does not address concerns regarding product quality. In order to maintain consumer confidence, it is necessary to implement an industry-developed quality assurance program through all phases of shellfish aquaculture and wild harvesting.

Optimize processing and handling to maximize product quality.

Future Directions:

- Develop and implement programs for wild harvesters, or “diggers”, growers and processors.

ENVIRONMENTAL AND ECOLOGICAL INTERACTIONS

OBJECTIVE — *Develop, adopt and promote best management practices (environmental code of practices) for the shellfish aquaculture industry and wild fisheries.*

Rationale

The shellfish industry is under increasing regulatory and public scrutiny as a result of the Endangered Species Act, the Sustainable Fisheries Act and associated identification and protection of essential fish habitat. The animals that are cultured by the shellfish industry are an integral part of the marine ecosystem and commercial shellfish growers are clearly dependent upon a healthy ecosystem. Adopting BMPs or ECOPs will be crucial to the survival and continued prosperity of the shellfish industry.

An industry-developed quality assurance program will restore consumer confidence.

Future Directions:

- Develop, adopt, publish and promote a bottom-up development of BMPs or ECOPs.
- Implement an outreach program to educate growers and resource managers on BMPs or ECOPs.
- Periodically update the BMPs or ECOPs as science and technology find new economical and environmentally sensitive practices.

OBJECTIVE – Determine and document the ecological impacts (positive and negative) associated with shellfish growing and harvesting.

Rationale

Cultured shellfish are an integral part of estuarine ecology. Much of the existing research and public perception has focused upon the negative impacts associated with commercial shellfish culture. Little is said about the positive effects that shellfish culture activities have on estuarine ecology. It is crucial that industry, resource managers and the general public have a clear understanding of the role these cultured shellfish play in the ecosystem.

Future Directions:

- Establish a research database related to shellfish ecology.
- Identify gaps in current understanding of shellfish ecology and pursue research to fill those gaps, the final goal being a clear understanding of the ecological impacts of shellfish aquaculture and wild harvesting. Issues needing to be addressed include:
 - ◆ Cultural practices;
 - ◆ Gear impacts;
 - ◆ Predator control;
 - ◆ Substrate modification;
 - ◆ Carrying capacities;
 - ◆ Water quality;
 - ◆ Species diversity;
 - ◆ Habitat;
 - ◆ Genetics;
 - ◆ Submerged aquatic vegetation.

OBJECTIVE — Respond to water quality problems in grower watersheds in a coordinated, knowledgeable, constructive manner.

Rationale

With coastal populations on the rise, shellfish growing areas are threatened with degrading water quality from failing on-site sewage systems, stormwater runoff, domestic animal waste and increased recreational use without adequate facilities. Existing commercial dairy and livestock are frequent sources of pollution to shellfish growing waters as well. Collectively, growers have considerable knowledge on these various problems and solutions. Responding in a coordinated, cooperative fashion could prevent future growing area classification downgrades. It will also reduce the time necessary to resolve problems, improve and maintain quality of approved growing areas as well as upgrade polluted growing areas.

Future Directions:

- Develop appropriate methodology to detect sources of bacteriological pollution.
- Become involved in local and regional watershed plan development and implementation.
- Implement programs that augment the sanitary shoreline surveys.

Best Management Practices (BMPs) - Environmental code of Practices (ECOPs) will foster environmental stewardship.

Cultured shellfish are an integral part of estuarine ecology and can enhance water quality.

PROMOTION OF SHELLFISH CULTURE

OBJECTIVE — Educate the general public, consumers, waterfront owners, resource managers, regulators, boaters and others regarding the benefits of shellfish, shellfish culture and the industry's need for a clean healthy environment.

Rationale

Farmers and shellfish processors share a common need to educate a wide array of people on a variety of topics. Some of these needs include educating the general public, consumers and resource managers on topics ranging from proper handling and preparation of their products and nutritional information to environmentally sound culture practices and the industry's need for a clean healthy environment in which to culture their shellfish.

Shellfish are key to a clean, healthy environment.

The industry recognizes a need for public education programs that stress the environmentally sound and sustainable nature of shellfish culture. These programs should include the essential role that shellfish culture can play in improving water quality, creating fisheries habitat, reducing harvesting pressure on wild stocks, providing jobs and reducing the national balance of payments deficit. Cultured shellfish are a high quality source of protein and as primary consumers, provide an efficient and sustainable healthful food source for a growing global population.

Future Directions:

- Develop a list of experts and a speaker pool for public presentations, general meetings, etc. —include agency, industry, tribal and academic representatives.
- Develop educational information for inclusion in printed materials, informational kiosks, interpretive center displays and on web-pages.
- Develop videos showing strengths of industry, tribal and research interactions, important elements of shellfish production/harvesting and environmental interactions.
- Stress the environmentally sound nature of shellfish culture to foster a positive regulatory and social environment.

Chapter 3

Water Quality and Public Health Concerns: Molluscan Shellfish Consumption

INTRODUCTION

Water quality is a general term that encompasses a range of topics and issues that are broad and complex. The role of water quality in the initiation of harmful algal bloom/biotoxin events, in detrimental effects on shellfish habitats and in effects of toxic chemicals on shellfish physiology are addressed in other sections of this report. The following sections address the relationship between water quality and consumption of shellfish as food.

The priority focus of this topic area is the risk of human infectious disease associated with the consumption of raw molluscan shellfish. Etiologic agents of concern include those associated with sewage and fecal matter such as *Salmonella* sp., viruses such as Norwalk and hepatitis A, and naturally-occurring estuarine pathogens *Vibrio vulnificus* and *Vibrio parahaemolyticus*. Research needs identified herein are directed at decreasing the occurrence of disease transmitted through consumption of raw molluscan shellfish by providing cost-effective and improved tools to assess potential health risk in growing areas. This will lead to improved water quality and a safer resource. A second focus considered is accumulation of pollutants (e.g., PAHs) that present real or perceived risks to human health.

A combination of federal and state agencies regulate water quality criteria, sampling, health and risk considerations. Both programmatic and scientific research needs are identified to unify and continue effective public health practices. Ultimately, consideration must be given to management practices and economic support that minimize the introduction of pathogens, reduce the levels of indicators and prevent release of undesirable chemicals into shellfish growing waters. Approaches include adequate support for new alternatives to existing on-site wastewater treatment and watershed pollution abatement strategies.

Bivalve shellfish have for centuries been associated with a recognized set of risks for the consumer. This particular, essentially unique risk derives from the fact that bivalves derive their nutrition by extracting small particles from large volumes of the water in which they live. This is referred to as suspension or filter-feeding. During feeding, bivalves can accumulate and bioconcentrate infectious or toxic microorganisms and compounds. The risk associated with infectious or certain toxic agents can be exacerbated by the widespread custom of consuming bivalves uncooked (raw).

REGULATORY PROGRAM

Most of the public health risks associated with bivalves are directly related to the quality of the water in which they live prior to harvest and distribution. Contemporary recognition of risk of infectious disease carried by shellfish from polluted waters was documented in the scientific literature in the late 1800s (typhoid fever caused by a species of *Salmonella*; Bulstrode, 1896; Herdman and Boyce, 1899; De Giaxa, 1889). Religious and cultural traditions surrounding shellfish consumption bear witness to recognition of the risk at much earlier times. We now know the presence of infectious disease agents is attributable not only to fecal contamination of human origin,

but also to microorganisms naturally-occurring in estuarine and marine waters. Other organic and inorganic toxic materials that might be concentrated and sequestered by bivalves can be of natural or human origin (Canzonier, 1988).

Historically, pathogenic microorganisms in human sewage have been the dominant contaminant of concern to shellfish growing waters. More recently, naturally-occurring pathogens in marine waters, as well as chemical contaminants from point sources and non-point run-off from varied land use practices have become concerns to the continued safe harvest of shellfish resources. Although a variety of animals can harbor human pathogens, the degree of health risk associated with warm-blooded, feral animals found in watersheds remains to be determined. Molluscan shellfish are filter feeders that can bioaccumulate harmful microorganisms and chemical constituents from the water column. Eating shellfish raw or not fully cooked can present special hazards either from particular contaminants or for certain high-risk consumers. Some chemical contaminants can be a concern even in cooked shellfish.

For the past 100 years, most illnesses from molluscan shellfish consumption have been linked to human fecal contamination of harvest areas. A major U.S. typhoid epidemic in 1924 in New York, Washington, D.C., and Chicago caused by contaminated oysters initiated development of water quality guidelines and other regulatory measures adopted through collaboration of the U.S. Public Health Service (now the Food and Drug Administration) and agencies in the coastal states. These controls have led to implementation of a state-federal cooperative program that has evolved into the current National Shellfish Sanitation Program (NSSP).

Though the public health aspects of shellfish production, processing, distribution and consumption have been generally defined in North America since the latter part of the 19th century, a specific program to address the etiology and control of shellfish-related disease was not developed until 1925 (Frost, 1925; ISSC, 1997), following epidemics of typhoid fever. A cooperative program was initiated involving state regulatory agencies and the shellfish industry, with encouragement and guidance from the U.S. Public Health Service. Protocols were developed to assure that only shellfish from growing areas having acceptable water quality would enter interstate commerce. This National Shellfish Sanitation Program (NSSP) places major emphases on identifying and eliminating sources of fecal contamination in watersheds, evaluating shellfish growing water quality and pursuing the application of detailed technical and administrative criteria for classifying the waters in which bivalves are cultivated and harvested (ISSC, 1997).

The NSSP objective of reducing public health risks associated with shellfish consumption has been eminently successful over the intervening years, and instances of epidemic disease of enteric bacterial etiology have diminished markedly. As the program evolved, the systems and methodology for evaluating sanitary water quality were improved and expanded, resulting in the currently accepted classification criteria based on a comprehensive

sanitary survey of the growing waters and identification of actual or potential sources of pollution within the watershed. Over time, as other etiological agents of public health concern have been identified - including enteroviruses, naturally-occurring phytoplankton toxins, heavy metals, synthesized organic compounds and potentially pathogenic bacteria that normally occur in estuarine waters - the program has responded to address and reduce the associated risks.

Responsible state and federal agencies have been able to garner financial support for monitoring and classification operations of coastal waters. Likewise, due to recognition of the economic, social and environmental benefits of shellfish production activities (both culture and wild stock utilization), a significant number of major initiatives have been implemented to reduce inputs of pollutants into estuarine and coastal waters. In many cases the need to protect or improve the quality of shellfish growing waters has been the pivotal argument in gaining both public and agency support for undertaking major pollution abatement programs. Another benefit of shellfish sanitation initiatives is the long-term commitment to monitoring programs, often impossible to justify on other grounds. These programs generate a continuum of both extensive and intensive environmental data sets for resolving a variety of environmental problems. The National Academy of Science (NRC, 1990) report on marine environmental monitoring found that this is one of the few monitoring programs in which the results of the program can be directly linked to immediate governmental action.

Although the basic public health principles of the NSSP have remained unchanged, the NSSP is updated and improved through periodic deliberations and agreements within the Interstate Shellfish Sanitation Conference (ISSC). The ISSC was formed in 1982 to promote the safety of molluscan shellfish through the adoption of uniform standards, laws, regulations and procedures for use by State shellfish control agencies. It has since added the goal of restoration of shellfish growing waters to its mission. The Conference is a partnership of environmental, natural resource and public health state agencies, Food and Drug Administration (USFDA), National Oceanic and Atmospheric Administration (NOAA), Environmental Protection Agency (USEPA), and representatives from the shellfish industry, academia, and foreign governments. Twenty-eight states are members of the ISSC, including all 23 coastal states. The primary mission of the ISSC has been the sanitary quality of shellfish. State programs must meet guidelines covering classification of growing areas for harvesting as well as shellfish processing, handling, and distribution concerns that would affect the safety of shellfish. The sanitary safety of shellfish is ensured by identifying and preventing human and domestic animal fecal contamination of shellfish waters and restricting harvest and shellfish utilization in growing areas that exceed established microbiological criteria. Coastal states conduct surveys of shorelines for point and non-point sources of fecal input as well as water testing for evidence of fecal contamination. There are also tolerances and action levels for certain chemical concentrations in shellfish.

INDICATORS FOR WATER QUALITY CRITERIA

Pathogenic microorganisms of fecal origin or naturally-occurring in marine systems, as well as chemical contaminants from point sources and non-point run-off from varied land use practices pose threats to the continued safe harvest of shellfish resources. The safety of raw shellfish for human consumption is dependent on the quality of harvest area water impacted by watershed inputs both upstream and directly into shellfish growing areas. For various technical and economic reasons, current microbiological water quality standards are not based on direct measurement of the wide variety of enteric pathogens that can be present in water samples. Instead, indicator microorganisms, generally associated with gastrointestinal tracts of warm-blooded organisms and shed in feces in large quantities, are measured as surrogates to reveal fecal contamination and the potential presence of pathogens.

Water quality criteria for shellfish harvesting are based on standards for fecal indicator bacteria established in the 1920s. These standards have been modified and become more specific over the years with regard to analytical methodology and sampling requirements. USEPA is the federal agency directed by the Clean Water Act to maintain water quality that provides for public health protection and propagation of shellfish. Approved microbiological indicators currently used by participants within the NSSP are the total coliform or fecal coliform groups. Either coliform group may be used by states for classifying waters for shellfishing. It is often pointed out that coliform-based standards have served well in protecting public health since the inception of shellfish sanitation programs in North America and Europe.

Indicator water quality standards for direct marketing as raw shellfish (Approved Classification category) are:

Total coliform median or geometric mean most probable number (MPN) of 15 water samples shall not exceed 70 MPN per 100 ml; and not more than 10 percent of the samples exceed an MPN of (a) 230 MPN per 100 ml for a 5 tube dilution test or (b) 330 MPN per 100 ml for a 3 tube dilution test.

Fecal coliform median or geometric mean most probable number (MPN) of 15 water samples shall not exceed 14 MPN per 100 ml; and not more than 10 percent of the samples exceed an MPN of (a) 43 MPN per 100 ml for a 5 tube dilution test or (b) 49 MPN per 100 ml for a 3 tube dilution test.

[See NSSP Guide for the Control of Molluscan Shellfish, 1997, pp. 24-27 and 209-213 for specific sampling plans under various environmental conditions and for total and fecal coliform criteria for other more restricted shellfish water classification categories.]

The success of the NSSP cannot be attributed solely to the intrinsic validity of the “standard” *per se*. Microbiological monitoring is one component of the overall assessment protocol as applied to classification of the growing waters. It is also probable that the values selected for the water quality standard are extremely conservative, thus giving a very wide margin of safety for even extreme situations of potential pathogen contamination. Correlations of fecal indicator levels with disease risk due to viral pathogens or naturally-occurring bacterial pathogens are weak to nonexistent. Indeed, lack of a consistent relationship between viral loadings in shellfish and bacterial indicators has been demonstrated by laboratory studies (Carter and Cantelmo, 1989; Canzonier, 1971; Sobsey et al., 1987; 1991) and epidemiological evidence (FDA 1983; Feingold, 1973; Truman et al., 1987).

Though the classification of growing waters has been effective in dramatically reducing exposure of the consumer to shellfish-borne infectious agents of human origin, a system of directly determining the health risk for a particular lot of shellfish is still lacking. Initially, coliforms were used as the indicator group; this was later refined to use the so-called fecal coliforms or even *Escherichia coli* et affin. (Regan et al., 1993). Other bacterial indicator groups, including the enterococci, clostridia, and *Bacteroides fragilis* have been suggested for use as replacements or in conjunction with coliforms in evaluating contaminant loadings in bivalves. In addition to bacterial indicators, alternate indicators of human sewage or feces for growing waters and shellfish have included bacteriophages of selected enteric bacterial hosts (coliphage, male-specific coliphage, phages to *Bacteroides fragilis*) and chemical compounds of gastrointestinal or waste treatment system origin.

It has been argued that direct detection of pathogens should replace the use of indicators. Such arguments have become more persuasive with improved technology, and researchers now talk of polymerase chain reaction (PCR) “cocktails” designed to simultaneously detect multiple pathogens. For the most part current analytical methods to directly detect pathogens remain labor intensive, expensive and time-consuming compared with detection of indicators. Moreover, use of surrogate indicators to detect fecal pollution remains valid for reasons that include the unpredictable nature of pathogen occurrence and their distribution patterns in natural environments, differences in pathogen virulence and host susceptibility and because failure to detect a given pathogen(s) cannot be interpreted as the absence of a health risk. Pathogen detection is also complicated by the fact that even sensitive molecular methods do not provide information on genome integrity, and may detect genetic fragments or non-infective entities. Finally, direct detection of pathogens has no intrinsic predictability when defined in the context of an USEPA health effects criterion, whereby a health effect is related to indicator density. Therefore, it is expected that current and improved indicators will continue to be used as new indicators and/or pathogen detection techniques are developed and validated on the basis of indexing actual health risk.

SOURCE OF FECAL CONTAMINATION: HUMAN VERSUS ANIMAL

Fecal coliforms represent an operationally defined group of heterogeneous bacterial species not all of which are restricted to the gastrointestinal tracts of humans and other warm-blooded animals. *E. coli* is a fecal coliform whose presence is taken to reflect frank fecal contamination and the possible presence of enteric pathogens. Despite greater specificity, numerous reports in the literature suggest *E. coli* suffers from many of the same limitations as fecal coliforms (Kator and Rhodes, 1994). When measured using approved methods, *E. coli* is a nonspecific fecal indicator with respect to its source. The extent to which human disease risk from raw shellfish consumption differs for fecal contamination derived from human versus domesticated or wild warm-blooded animal sources has not been established. Some bacterial and parasitic diseases can be transmitted from animals to humans, while enteric viral agents appear more species specific; however, a case has not been made to quantitatively elevate the public health importance of human-derived fecal input over that from animals. Therefore, the current classification system is conservative with respect to preventing disease.

Benefits to determining the source(s) of indicator organisms to shellfish growing areas lie in focusing on management controls to reduce or eliminate specific types of fecal inputs. If a source is predominantly human, control points are sewage treatment plants, septic systems, or overboard discharge from water craft. Fecal contamination from domesticated or wild animals may require management strategies to minimize animal contact with growing waters, use of runoff abatement and other management practices. The ability to identify and locate contamination sources has been elevated in importance as a result of USEPA's recent commitment to implement Total Maximum Daily Loads (TMDLs) that intrinsically mandates contaminant source identification and remediation.

Fecal coliforms are defined as all the facultative anaerobic, gram negative, nonspore-forming, rod shaped bacteria that ferment lactose in EC medium with gas production within 24 h at $44.5 \pm 0.2^\circ\text{C}$ in a most-probable-number technique (APHA, 1970).

Molecular and biochemical techniques have recently been applied to determine the sources of fecal indicator bacteria from a variety of sample types by comparison with databases in "libraries" constructed using strains from known fecal sources. Like other bacteria, *E. coli* can develop resistance to multiple antibiotics. Hypothetically, *E. coli* from humans should possess broader and more specific antibiotic resistance profiles than that of wild or domesticated animals owing to drug therapies for human illnesses. Studies have been completed with hundreds of *E. coli* isolates collected directly from animal and human feces as well as from sites of sewage discharge and remote waters where wild animals are the only known source of fecal input (Parveen et al., 1997). Comparisons of multiple antibiotic resistance (MAR) patterns indicate statistically significant distinctions between point and nonpoint sources. Confidence is enhanced by increased sampling and analysis, thereby extending the database of profiles for comparisons with individual sample results. Similar studies have been performed using the fecal streptococci (Wiggins et al., 1999; Hagedorn et al., 1999).

From a molecular perspective, various methods and approaches have been recently applied to identify sources of bacterial strains. These methods also require creation of source-specific "libraries". Methods such as pulsed field gel electrophoresis (PFGE) rely on extraction and restriction (cutting into fragments using selected enzymes) of genomic DNA from *E. coli* isolated from various animal or human sources. Separating fragments by molecular weight produces patterns of DNA fragments (called a DNA "fingerprint") that can be distinct for each species. "Fingerprints" can also be generated using the polymerase chain reaction (PCR) to amplify selected genomic DNA targets. Another procedure used to separate *E. coli* from different sources is based on the gene that codes for small subunit ribosomal RNA (SSU rRNA). Interpretive difficulties can arise with these techniques because individual animals in a population, as well as geographically distinct populations of the same animal species, e.g., raccoon or deer, can exhibit multiple patterns. Therefore, it is important to sample and analyze sufficient numbers and types of authentic fecal samples to build a comprehensive "fingerprint" library to identify the within-and between-species variation and effects of geographic isolation.

Molecular techniques are undergoing rapid evolution and their application in natural waters requires continued data collection for library and method development. Molecular fingerprinting methods are expensive and methods based on bacterial growth assays are labor intensive and time consuming. Database size is also a consideration if the methods are to be applied on a national basis. Cost and labor considerations will be an important factor if these methods are to become routine.

Other methods to discriminate human from animal contamination are based on the selective detection of human specific indicators. These include male-specific coliphage, human secretory immunoglobulin (sIgA), caffeine and sterols such as coprostanol.

Needs/Recommendations

- Review and disseminate the complete technical results of the National Indicator Study. A number of research projects focused on use of candidate indicators to discriminate human from animal contamination and to index health risk.
- Develop, validate and improve methods for identification of sources of fecal contamination in shellfish waters affected by non-point source contamination.

ENTEROCOCCUS AS AN INDICATOR/MEMBRANE FILTER-BASED ENUMERATION METHODS FOR SHELLFISH WATERS

The USEPA (1986a) recommended bacteriological ambient water quality criteria for fresh and marine recreational waters and provided a scientific rationale for developing water quality standards to maintain the safety of the nation's waters. Data supporting the new water quality criteria were obtained from a series of studies conducted by USEPA examining the relationship between swimming-associated gastrointestinal illness and the microbiological quality of the waters used by recreational bathers. Results of these studies demonstrated that the current approved indicator, fecal coliforms, was poorly correlated to incidences of swimming-associated gastroenteritis. Two other indicators, *E. coli* and enterococci, exhibited strong correlations with disease incidence, the former in fresh waters only and the latter in both fresh and marine waters. Consequently, the USEPA (1986b) recommended the use of *E. coli* and enterococci rather than fecal coliforms for recreational waters. The "steady-state" geometric mean values of these indicators were established on the basis of accepting a given level of risk of acute gastrointestinal illness.

USEPA recreational health effects criteria are: for fresh waters: geometric means of 33 enterococci per 100 milliliters (ml) and 126 *E. coli* per 100 ml; for marine waters, a geometric mean of 35 enterococci per 100 ml.

The USEPA would like state shellfish programs to adopt both the enterococci as the basis for shellfish growing area classification and membrane filter-based enumeration methods. The NSSP currently uses standards based on total or fecal coliforms to classify shellfish growing waters, and densities of these groups are generally measured using the most-probable number technique (APHA, 1970). USEPA would like to see the NSSP program adopt membrane filter-based methods which provide significantly better precision than MPN methods, and thereby could require collection of fewer samples. Although definitive studies relating levels of enterococci to disease risk associated with consumption of shellfish have not been conducted, research shows the enterococci are a better indicator of gastrointestinal illness in marine recreational waters suggesting it may lead to improved risk assessment in the shellfish program as well. Although USEPA has recommended the shellfish program utilize its enterococci method, transition to this indicator is not a simple straightforward adoption, and epidemiological studies to correlate the incidence of shellfish-transmitted disease with enterococci levels are unlikely to be funded. For the NSSP to adopt the enterococci indicator and filtration method, the density of enterococci which corresponds to the current growing area standards based on total or fecal coliforms must be determined, the membrane filter methodology should probably be evaluated on a regional basis, and the NSSP must adopt and implement the method through the ISSC's formal methods review process.

Needs/Recommendations

- The ISSC should determine if there is sufficient legitimate justification to consider adoption of the enterococci as an indicator for use in shellfish growing waters. If there is consensus to support such action, the ISSC should move to identify and evaluate appropriate analytical procedures and to determine what steps are necessary for development of an enterococcus-based shellfish growing area standard.
- Continue to develop and evaluate new indicators and supporting methods that are based on prediction of human health risk. Rapid and/or real-time detection methods would be particularly beneficial.

IMPAIRED WATERS AND TMDLS

Differences now exist between water quality and shellfish administrative groups in the states concerning sampling frequency, laboratory methods, classification criteria and application of the water quality standards. States establish TMDLs for water quality-limited waters such as shellfish growing waters that do not meet their designated use. A TMDL must identify and quantify pollutant sources and allocate allowable loads to point and non-point sources so that the water quality standards are attained for that water body. TMDLs now being developed for shellfish growing waters by water quality agencies or departments could be derived using methods that differ from those used by State Shellfish Control Authorities (SSCAs). As noted the latter are obligated to follow most probable number (MPN) methods for total and fecal coliform bacteria adopted by the National Shellfish Sanitation Program (NSSP). USEPA and their state counterparts generally follow *Standard Methods for the Examination of Water and Wastewater* (APHA, 1998) for monitoring recreational water quality and can use membrane filter techniques.

Although approved membrane filter-based methods provide superior precision, their application to shellfish waters requires more experience, and comparability between the membrane filter and the MPN methods and its interpretation in relation to the approved shellfish growing area standard has not been determined. Following a literature report jointly prepared by USFDA, USEPA and NMFS, *Evaluation of Comparability of the Most Probable Number and Membrane Filter Method for Enumerating Coliforms and Fecal Coliforms in Shellfish Growing Waters*, this is an issue now being evaluated by the ISSC. Similarly, USEPA's adoption of *Escherichia coli* or the enterococci as indicators of choice cannot be used for classification of growing waters by the NSSP until the methods are formally adopted and comparable standards determined.

Current sampling methods to establish microbiological water quality are based on "grab" sampling. That is, single or multiple samples collected at a single moment in time. These samples are then analyzed and interpreted as representative of water quality over a more extended time interval. To a very great degree this sampling method is dictated by the exigencies of field sampling in general and financial and analytical constraints. Estuarine and marine environments supporting shellfish are dynamic systems where tide, wind, hydrodynamics and climate can interact to produce changes in water quality parameters over short periods of time and space. The NSSP attempts to address natural variation through selected sampling strategies using a low sampling frequency. Shellfish, on the other hand are intrinsically integrative water samplers because they bioconcentrate pathogens, chemicals and indicators over time. New sampling methods utilizing integrated sampling combined with new indicators, and making use of improved detection methods and automated sampling have the potential to improve water quality characterization and resource protection.

SSCAs classify shellfish waters using a soundly conducted sanitary survey, a part of which is the Bacteriological Survey. Samples collected under the guidelines of the NSSP are examined in the laboratory using Standard Methods for the Examination of Sea Water and Shellfish (1970) also published by the APHA. This method uses the Most Probable Number (MPN) method of enumeration.

Needs/Recommendations

- The ISSC and relevant federal agencies should support and identify appropriate funding for research to determine the most appropriate indicators, laboratory methods and standards for shellfish growing water classifications as well as sampling methods/techniques that use integrated approaches rather than those based on grab sampling.
- Establishing TMDLs for waters designated for shellfish use should utilize those SSCA sanitary surveys and the appropriate approved APHA and NSSP methods for the examination of seawater and shellfish. It may be appropriate to establish workshops whereby both SSCA and state agencies responsible for collection of water quality data are brought together to insure that appropriate methods are used in classifying shellfish growing areas.

PATHOGENS FROM FECAL SOURCES

Shellfish have been convincingly indicted in the transmission of infectious doses of enteric pathogens to humans. The primary evidence for association of human disease outbreaks with consumption of molluscan shellfish has been, and will continue to be, based on retrospective epidemiology. The classification of growing waters has been effective in dramatically reducing exposure of the consumer to shellfish-borne infectious agents of human fecal origin. A method for directly determining the infection potential of a particular lot of shellfish is still lacking.

The limitations associated with use of a nonpathogen indicator to establish infection risk for specific pathogens have been recognized (Regan et al., 1993; Richards, 1999). Though bacterial pathogens of fecal origin are still of concern, diseases of viral etiology have assumed a primary position in the context of public health (GAO, 1984; Richards, 1985; 1987; 1991; Rippey, 1994; NAS, 1991). Correlation of “bacterial indicator” levels with infectious risk due to viral pathogens is poor. These and other concerns related to indicator shortcomings have focused attention on the need for new candidate indicators as well as methods for the direct detection of pathogens. Molecular methods based on PCR are particularly attractive in this respect, providing high sensitivity and specificity, attributes essential for detection of individual pathogens in environmental samples.

NEEDS/RECOMMENDATIONS

- Review/evaluate developing techniques and rapid methods to identify pathogens directly and consider use in risk analysis/public health.
- In all instances new methods must be validated through studies that couple risk-assessment with detection. This is especially true with regard to molecular methods which do not currently assess pathogen infectivity.

NATURALLY-OCCURRING PATHOGENS

Concurrent with an apparent decrease in large outbreaks attributed to “classical” infectious agents of fecal origin, increased attention is being directed toward pathogens which are not “traditional” enteropathogens. Several species of bacteria belonging to the genus *Vibrio* naturally occur in coastal waters where shellfish grow. The presence and densities of *V. vulnificus* are not related to fecal pollution of shellfish growing waters, thus current harvesting regulations do not prevent these illnesses. Consumption of vibrios or contact through open wounds by susceptible individuals can cause illnesses ranging from gastroenteritis to acute septicemia. Those vibrio species of most concern to shellfish consumers are *V. vulnificus* and *V. parahaemolyticus*.

Vibrio vulnificus

Vibrio vulnificus is a common component of bacterial communities in near shore and estuarine environments. Although it has been isolated from all U.S. coastal waters, *V. vulnificus* densities are highest in warmer waters with low to moderate salinities such as found in the Gulf of Mexico. Environmental conditions favoring growth include water temperatures of 20°-30°C, salinities of 5-20 psu (practical salinity units) and neutral to alkaline pH (Tamplin, 1994).

V. vulnificus illnesses are relatively rare and are caused almost entirely by wound infections or consumption of raw bivalve shellfish. Although the illness incidence is very low, its severity and associated high mortality are of great concern to public health authorities and the shellfish industry. Raw oysters from the Gulf of Mexico have been the primary vehicle of infection in the U.S. (CDC, 1997). These illnesses appear as single cases, not as outbreaks, and occur almost exclusively in individuals associated with certain medically compromised groups. Infections are manifested in three ways: gastroenteritis, wound infections and primary septicemia.

Healthy individuals are at very low risk of becoming infected by *V. vulnificus* through raw shellfish consumption. Certain health factors increase the risk of infection. Nearly 75% of cases resulting in primary septicemia occur in persons with liver disease or heavy alcohol intake. Almost all the remaining cases occur in individuals with an iron storage disease (hemochromatosis), diseases that affect the immune system such as diabetes, cancer, or in persons who take immunosuppressive drugs (Whitman, 1994; Tacket et al., 1984; Oliver, 1981; Blake et al., 1980). Most illnesses are reported from April through September when the level of *V. vulnificus* is highest in water and shellfish. In 1988, the CDC began a voluntary illness reporting system in states where the majority of cases were reported, including all the Gulf States and California. From 1989 through 1998, CDC summaries indicate 204 cases and 105 deaths, for an average of 20 cases and 10 deaths per year.

Risk Reduction Strategies

Federal and state health authorities along with the shellfish industry through the ISSC are cooperatively developing and implementing several strategies to reduce the risk of this illness from shellfish consumption. The focus is on education and risk communication to shellfish consumers and those in the medically-compromised groups, improved post-harvest shellfish handling practices, shellfish processing to mitigate levels present in shellfish prior to consumption, and research on *V. vulnificus* and factors affecting its virulence. It is hoped that these multiple approaches will combine to satisfy consumer risk concerns and the shellfish industry's interest that regulations not be overly restrictive.

The USDA has distributed health advisories through the medical community, its foodborne illness website (<http://vm.cfsan.fda.gov/list.html>), a seafood hotline and media releases published in newspapers for the general public. The ISSC, USDA, National Oceanic and Atmospheric Administration (NOAA), universities, state public health agencies and others have cooperated in developing educational brochures and messages, and several states have begun educating shellfish consumers and high-risk populations through targeted programs and messages at restaurants and other points of sale (Welch, 1994). The ISSC, through a grant from NOAA Fisheries in 1996, is developing and producing educational kits for states and coordinating the best means for distributing health advisories through targeted health associations to improve the effectiveness of education.

In 1995 and 1996, the Gulf States and shellfish industry instituted voluntary and then mandatory practices to reduce the maximum duration allowed between time of harvest and refrigeration. These practices were designed to limit the replication of vibrios in shellfish prior to temperature control. Analytical studies testing the effectiveness of these procedures over previous handling methods in reducing *V. vulnificus* densities are now being performed.

Various post-harvest treatments to reduce levels of *V. vulnificus* in shellfish at the time of consumption have been explored. Treatments such as freezing, mild heat followed by rapid chilling, irradiation and the use of ultra high pressure are known to reduce the levels of vibrios in oysters to nondetectable levels. Commercial processes for post harvest treatment of oysters using mild heat, chilling in low temperature baths, freezing and hydrostatic pressure have been developed and studies are being conducted to consider the economic impact on the industry. While not yet approved for use on shellfish, irradiation can also reduce *V. vulnificus* levels in oysters. Currently there is a petition before USFDA to allow irradiation of oysters. These and other post harvest treatments will continue to be considered to decrease the risk of illness from *V. vulnificus*.

The ISSC has funded two projects to explore various aspects of *V. vulnificus*. One project has focused on the question of using gene(s) controlling capsule expression as a marker to detect virulent strains, since virulent strains produce capsules. This research has shown capsule expression to be complex and probably controlled by more than one gene and identification of virulence markers will require considerably more effort. Another study has demonstrated that low temperature chilling, even without initial mild heat treatment, is an effective means to reduce viable counts of *V. vulnificus* in freshly harvested oysters.

Vibrio parahaemolyticus

V. parahaemolyticus is seasonally present in many fishery products, and some strains causes acute gastroenteritis in consumers. Until recently, molluscan shellfish in the U. S. were only sporadically implicated as a vehicle for transmission of this pathogen. However, in 1997, outbreaks attributed to *V. parahaemolyticus*-contaminated bivalves were responsible for more than 250 illnesses in the Pacific Northwest, from California to British Columbia. In 1998, outbreaks caused by *V. parahaemolyticus* in bivalve shellfish occurred in three areas of the U.S., the Pacific Northwest, Galveston Bay (TX) and in Long Island Sound (NY). Though no outbreaks were reported in 1999, the consumption of raw *V. parahaemolyticus*-contaminated shellfish, particularly oysters, is a seafood safety concern. Unlike *V. vulnificus*, *V. parahaemolyticus* infects healthy humans. Not all strains of *V. parahaemolyticus* are enteric pathogens and nearly all isolates obtained from shellfish, sediments and waters are nonpathogenic. This means that simple detection and determining the densities of this species in seafood or water is inadequate for assessing the risk of illness. A thermostable direct hemolysin (produced by a gene called *tdh*) is produced by most pathogenic strains of *V. parahaemolyticus*. Assays to detect this gene product provide a reasonably reliable means of differentiating pathogenic from nonpathogenic isolates.

In June 1998, a *V. parahaemolyticus* serotype called 03:K6, and transmitted by oysters, was identified for the first time in the U.S. in Texas and New York. Moreover, this serotype was confirmed from a patient in the New York outbreak. Serotype 03:K6 has previously been associated with outbreaks in Asian countries whereas most other serotypes are primarily associated with sporadic cases. Based on all information available, CDC has described the 03:K6 serotype as “an outbreak strain” of *V. parahaemolyticus*. Other pathogenic serotypes, such as 04:K8 seen predominantly in Japan, may also merit special concern. Because dissemination of pandemic cholera (*Vibrio cholerae*) strains has been linked to discharge of ballast and other nonpotable waters from foreign cargo ships (McCarthy and Khambaty, 1994), a similar mechanism may underlie the introduction of pandemic “outbreak” strains of *V. parahaemolyticus* to United States shellfish waters.

The dose-response relationship for *V. parahaemolyticus* is unknown. From volunteer studies conducted more than 25 years ago, along with investigations of U.S. outbreaks caused predominantly by cross contamination of cooked crabs, FDA had previously indicated the *V. parahaemolyticus* level in seafood should not exceed 10,000 cells per gram. This guideline now appears inadequate to protect consumers.

Levels of *V. parahaemolyticus* found in oysters from implicated harvest sites during the 1997 and 1998 U.S. outbreaks suggest that the minimum number of infective cells required to cause illness is probably between 100 and 1,000.

Many parameters influencing the natural occurrence of this species have been reported. Factors specifically related to the occurrence of epidemic strains in shellfish remain uncertain, as do specific conditions that serve to limit the organism. The temperature range for *V. parahaemolyticus* is approximately 7 to 43°C. The organism can tolerate a pH of about 5 for extended periods, with a range of 6.0 to 9.8 and an optimum pH of 8.5. Preliminary studies have shown that in unrefrigerated oysters, 100-1000 viable cells/g will increase ten-fold within 5 h, one hundred-fold within 10 h and one thousand-fold by 24 hr. *V. parahaemolyticus* will grow over a salinity range of about 5 to 33 psu with an optimum of about 20 psu. Other potential factors influencing its occurrence and proliferation include the zooplankton which may promote its growth and transport, the role of fish and vessel ballast water as vectors for its dispersion, recent warm weather and the effects of critical environmental temperatures on uptake and maintenance of pathogenic (tdh) *V. parahaemolyticus* strains in shellfish.

Risk Reduction Strategies

Strategies focus on the need to understand the ecology of virulent strains in the environment, particularly factors that control the appearance of such strains, and monitoring to prevent the presence of pathogenic (tdh) strains in oysters from areas historically implicated and during seasons when virulent strains might be likely to appear in oysters. Additionally, targeted educational messages and improved shellfish handling and processing techniques are being developed and applied for *V. vulnificus*.

Needs/Recommendations

- Evaluate public health significance of vibrios in shellfish (infectivity, virulence, overall human health risk factors).
- Develop improved methods to isolate and quantify pathogenic vibrios in water and shellfish.
- Improve understanding of water quality parameters that affect the ecology and pathogenicity of naturally occurring *V. vulnificus* and *V. parahaemolyticus* and identify those factors that can be used to predict their occurrence.
- Determine the significance of ballast water from foreign shipping on the introduction of pathogenic strains of *V. parahaemolyticus* and other vibrios.

CHEMICAL CONTAMINANTS

The food safety of bivalve shellfish can be compromised through contamination by a variety of toxic organic contaminants. This is most common for wild populations, but is also can occur with cultured shellfish, such as following oil spills or other accidental releases of harmful substances. There are several reasons why contamination with toxic chemicals is especially important for bivalve molluscs, compared to crustacean or fish species. For example, many organic toxic contaminants cannot be readily eliminated from tissues until they are metabolized into more water-soluble forms. It is well known that molluscs have a very low capability to metabolize, or biotransform, a range of contaminants, including polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons (CHs, including PCBs and dioxins). Moreover, molluscan species tend to have high levels of lipids (fats) in their tissues, and compounds such as PAHs and PCBs readily

accumulate in lipid stores. And finally, because many molluscan shellfish are filter feeders, and toxic contaminants tend to be associated with particulates rather than being truly dissolved in water, these species can be exposed to large quantities of chemical contaminants as a result of their ingestion of large volumes of particles. Thus, even though the body burdens of chemical contaminants in molluscs are affected by season, spawning status, species, etc., these species are often used as sentinel organisms for assessing chemical contamination of nearshore marine and estuarine ecosystems.

Needs/Recommendations

To better determine the potential effects of chemical contaminants on the safety of shellfish for human consumption, research in the following key areas is needed:

- Improved understanding of elimination kinetics of chemical contaminants from bivalve molluscs, especially via production of gametes, and under “natural” (i.e., non-laboratory) conditions.
- Assessment of toxicokinetics of contaminant compounds in long-lived (i. e., decades or more) shellfish species such as geoducks, which are increasingly being utilized as a food resource.
- Moreover, because there are often large differences between expected and observed contaminant body burdens in field monitoring of molluscan species, focused research needs to improve predictive capabilities in this important area including:
- Better measurement of bioaccumulation factors and biota to sediment accumulation factors (BAFs and BSAFs, respectively) and improved understanding of how sediment physico-chemical factors affect contaminant bioavailability and shellfish uptake/elimination kinetics;
- Investigations of the differential fates and roles of dissolved contaminants versus contaminants adsorbed to particulate materials in shellfish; and
- Improved understanding of temporal variation on contaminant body burdens in molluscan shellfish.

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Chapter 4

Harmful Algal Blooms

I. INTRODUCTION

A. WHAT IS A “HARMFUL ALGAL BLOOM (HAB)?”

“A harmful algal species is in bloom when its occurrence has harmful consequences regardless of biomass.” Smayda, 1997.

Phytoplankton—microalgae—are the base of the marine food web. They are responsible for over half of the primary production on earth and are, thus, essential to all life on this planet. Of more than 5,000 described species of marine microalgae, less than ninety are known to be inherently harmful to other organisms or to have caused documented harmful environmental effects. Harmful microalgae can produce a suite of toxic or noxious bioactive compounds (metabolic by-products) that can negatively influence other organisms. Some microalgae may not produce toxins, but they can also be harmful to other organisms and to the environment. Some harmful effects of microalgae may be attributed to mechanisms used for defense against predators or for competition. For example, some microalgae have spines or setae that can damage the gills of finfish and shellfish. Many microalgal toxins, however, have no apparent ecological function, including some compounds that are toxic to mammals that never consume microalgae.

B. DEFINITIONS

Microalgae are microscopic plants that are usually found in different types of aquatic systems, e.g., lakes, seas, estuaries. Most species are pigmented and when present in high concentrations can be visible as discolored water e.g., red tide (Figure 1). Microalgae are classified into groups that share common biological features (e.g., morphological structures, pigments) and include dinoflagellates, diatoms, raphidophytes, prymnesiophytes, pelagophytes, cyanobacteria and silicoflagellates. Some “microalgae” that exhibit “animal” characteristics (e.g., lack of photosynthetic pigments, targeted predatory behavior) are also included in these groups, because of other common features that they share with microalgae.

Harmful algal blooms (HABs) are caused by species of microalgae that can be harmful to aquatic and terrestrial organisms and man, and can cause impacts to the environment. HABs can include both toxic and non-toxic species. Although more traditionally defined as being associated with high concentrations of microalgae, HABs can also affect organisms at low concentrations.

Toxic HABs include those species of microalgae that produce toxins that can accumulate in shellfish and fish. When consumed by man these toxins can cause severe illness or even death. Some of the same toxins can be produced by microalgae from different groups. Examples of microalgal species, the group they belong to, the toxins they produce and the poisoning they are associated with, are:

- *Pseudo-nitzschia* spp., diatoms, domoic acid, amnesic shellfish poisoning (ASP);
- *Karenia brevis*, dinoflagellates, brevetoxin, neurotoxic shellfish poisoning (NSP);
- *Alexandrium* spp., dinoflagellates, saxitoxins, paralytic shellfish poisoning (PSP);
- *Dinophysis* spp., dinoflagellates, dinophysistoxins, diarrhetic shellfish poisoning (DSP);
- *Gambierdiscus toxicus*, dinoflagellates, ciguatoxins, tropical fish poisoning or ciguatera.

As well as affecting humans, toxic microalgae can also cause animal mortality and contribute to disease in aquatic animals.



Bloom of the dinoflagellate *Cochlodinium* in the Chesapeake Bay. Darkest region is the most dense part of the bloom, lighter areas have been diluted by tidal action. Photo S. Shumway.

Non-toxic harmful microalgae can affect animals by producing other bioactive compounds that may affect their health. High concentrations of non-toxic algae can cover large areas of water and use up oxygen. Some of these blooms can contribute to mass mortalities of shellfish and fish.

Many molluscan shellfish—including living marine resources such as oysters, clams, scallops and mussels—feed directly upon microalgae suspended in the water. When shellfish feed on toxic algae they can accumulate the toxins in the tissues, and if not controlled, these toxins can pose a risk to human consumers. In some cases, harmful effects by specific microalgae upon shellfish have been documented, including death, ill-health, or poor condition of harvestable populations from wild or farmed stocks. The most widely-known harmful algal-shellfish interaction, however, involves the poisoning of human seafood consumers. These poisonings have resulted in the death or sickness of many people. Public health agencies, usually at the state level, have instituted

monitoring, analysis, and regulatory mechanisms to protect the American consumer from shellfish poisoning. These programs are effective, where instituted, but they are not optimized for cost-effectiveness or coordination between agencies. In addition, resource shellfish populations with high potential value remain unharvestable because monitoring programs have not been instituted (e.g., the Alaskan coast).

The monetary value of the molluscan shellfish landed in the U.S. is in excess of \$305 million (NMFS data). The impact of harmful and toxic algae upon this commercial resource is difficult to ascertain, but probably is in the range of \$42 million (WHOI estimate) in cost of public health protection, lost revenue from mortality and unharvestable populations, and consumer avoidance of all seafood products in response to localized closures. There also is an intangible economic loss to shellfish production that results from poor growth, disease, or lowered reproduction as a result of shellfish exposure to harmful microalgae. Aquaculture operations are particularly vulnerable to harmful algal events because the farmers have a considerable investment in livestock that is difficult or impossible to move when a harmful algal event threatens. Improved information about, and management of, harmful and toxic algal-shellfish interactions will likely have a direct, measurable and significant economic payoff.

II. STATUS:

Many molluscan shellfish, including most commercially-important species, depend upon phytoplankton for a major portion of their food. Recognized interactions between harmful and toxic algae and shellfish include instances wherein 1) shellfish are rendered unfit for human consumption and, 2) algae that have lethal and sub-lethal effects upon the mollusks themselves. Conversely, the filtering activity of feeding molluscs may influence the population dynamics of harmful microalgae. Grazing inhibition by harmful microalgae may be involved in harmful algal bloom initiation and persistence. The following list categorizes the potential interactions between harmful microalgae and molluscan shellfish and provides some documented examples:

- HABs that make shellfish toxic for human consumers—the critical linkage between toxin-producing, marine microalgae and human health has resulted in both scientific and regulatory activities; these will be described in a later section. Examples include PSP, DSP, ASP and NSP (see Definitions, above).
- HABs with intrinsic lethal or sub-lethal effects upon the shellfish themselves—some shellfish are susceptible to harmful effects of the same algae that produce mammalian toxins, but additional microalgae with no known mammalian effects cause health problems in shellfish. Examples include the following algal genera: *Gymnodinium*, *Gyrodinium*, *Alexandrium*, *Prorocentrum*, *Aureococcus*, *Heterosigma* and *Prymnesium*. (See also Box for a list of documented, lethal and sub-lethal effects of harmful microalgae upon molluscan shellfish).
- HABs of exceptional biomass accumulation by otherwise benign algae—unusually high population densities of almost any microalga can stress bivalves by interfering with feeding behavior or causing hypoxia when the algal biomass decomposes in the benthic boundary layer. Examples include past blooms of *Ceratium*, *Noctiluca* and *Gonyaulax*.
- Grazing suppression of HABs, and grazing inhibition by HABs—although these interactions have been demonstrated in laboratory and field experimental studies, the impacts of these interactions upon natural events are poorly documented.
- Pelagic-benthic coupling—the filtration and subsequent translocation of microalgal biomass by suspension-feeding bivalves serves to move energy and materials from pelagic to benthic environments, as well as depositing living cells or resting stages of harmful microalgae into the benthic layer; environmental implications of these interactions are largely unexplored.
- In environments where filter-feeding shellfish represent a large portion of the grazing community, chiefly in shallow, coastal systems, removal of shellfish populations by human harvest, disease, or other natural processes may lead to blooms of microalgae that would otherwise be controlled by the shellfish grazing.
- Trophic cascades—few human activities have done more to alter marine food-web structure than commercial fishing, and changes in higher trophic dynamics may cause oscillations in shellfish recruitment that subsequently alter grazing pressure, as described in the previous bullet.

The potential role of molluscan shellfish as vectors of algal toxins to human seafood consumers, and concomitant public health concerns, have driven many of the economic and social consequences of harmful and toxic algae. Technical methods for sampling and analyzing shellfishery products or the waters they inhabit for toxins have been devised and instituted in public laws and regulations. Such programs are functional and remarkably successful in protecting public health from known, recurring toxic algal outbreaks, but they are not optimized, technically or economically, and are not designed to respond to new toxic algal events. Shellfish harvest closures because of toxic algal events represent a hardship for harvesters of both wild and cultured shellfish with subsequent

Known chronic and sublethal effects of HABS on shellfish (Burkholder, 1998)

- *Narcosis (prolonged lethargy, reduced fright responses, reduced shell valve closure, reduced burrowing).*
- *Depressed or altered feeding.*
- *Decreased activity of lateral cilia.*
- *Increased mucus production.*
- *Hemocyte abnormalities.*
- *Poor shell growth.*
- *Depressed general growth and shell disease.*
- *Disseminated neoplasia and germinomas.*
- *Mantle and gill lesions.*
- *Decreased absorptive cell height, increased lumen diameter.*
- *Blood, gastrointestinal, and reproductive diseases.*
- *Increased protozoan and bacterial infections.*
- *Improper setting.*
- *Recruitment failure.*
- *Impaired recruitment timing.*
- *Reduced reproduction and early development.*

economic ripple effects. Public perception that seafood is generally unsafe when local closures are publicized has a “halo effect” upon the entire seafood industry. This “halo effect” could be offset with better public education. Economic impacts of harmful algae that are not public health risks are much less recognized, if not uncommon (e.g., the commercial extinction of the bay scallop from Long Island, NY waters because of brown tide, and the recruitment failure of scallops in North Carolina following an unusual *Gymnodinium* event).

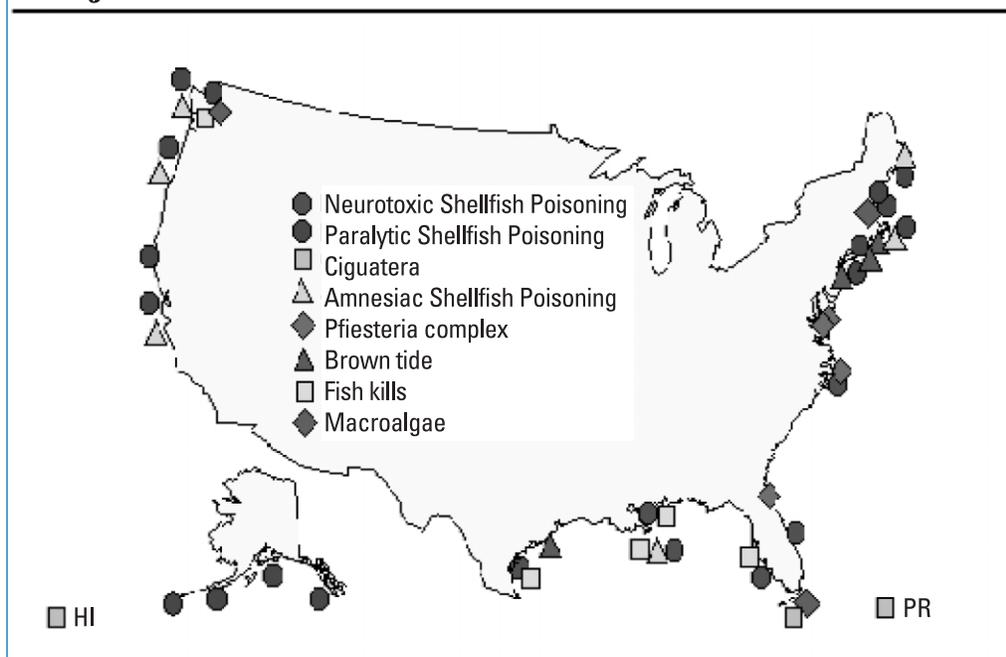
Research, management and regulatory activities related to harmful algal-shellfish interactions often are regional or limited to the state level, which is appropriate because implications often occur in waters under state jurisdiction, and the distribution of harmful algae often is regional. The provincial nature of programs, however, has resulted in efforts that tend to be factious and poorly-coordinated on a national level. Understanding and management of harmful algal-shellfish interactions would benefit from improved information availability, dissemination and coordination.

A national program (ECOHAB) has been established to coordinate research on the ecology and oceanography of harmful algal blooms (Anderson, 1995). This program has been successful in bringing together Federal Government funding agencies to issue joint requests for proposals that address common information needs and share investments. Justification for the ECOHAB investment is predicated, largely, upon the public-health exigencies of shellfish harvest from bloom waters; however, the focus of this program has been upon elucidating environmental parameters associated with bloom initiation and perpetuation, rather than on the effects themselves. Accordingly, a coordinated, national program on harmful algal-shellfish interactions would complement ECOHAB by providing practical information needed by shellfish resource managers, harvesters, farmers and U.S. seafood consumers.

III. REGIONAL CONSIDERATIONS

One clear example of a product from existing harmful algal programs having direct application in the shellfish field is the mapping of coastal waters that have experienced harmful or toxic algal events. An on-line map, updated regularly as new information becomes available, is maintained on a National Harmful Algal Bloom internet web page (<http://www.habs@whoi.org>). The version of this map available at the time of this writing is reproduced in (Figure 2). The assumption must be made that any area that has experienced a harmful or toxic algal event has the potential for recurrence. It is clear, however, that no single harmful alga is distributed throughout the coastal waters of the U.S.; therefore, approaches to many research and management issues will be regional in nature. In cases where a harmful algal susceptible region extends across jurisdictional boundaries—both between states as well as between neighboring nations—coordination will be appropriate. There are, however, some issues that are national and international in nature, including both basic biological questions, e.g., the role of molluscan feeding behavior in harmful algal-shellfish interactions, and establishment of consistent standards for public health protection. Research needs in these areas will be specified in the recommendations section of this chapter.

Major HAB-related Events in the Coastal U.S.



Map depicting location of different types of harmful algal bloom occurring in U.S. coastal waters.

IV. OUTREACH

A. GENERAL PUBLIC

A majority of U.S. seafood consumers have heard the term, “red tide”, and are aware that shellfish can “be toxic.” Beyond this minimal understanding, there is much misinformation about harmful and toxic algae and shellfish. Efforts to inform the public that effective programs to protect public health have been instituted successfully must be continued and emphasized. The benefits of improved methods, including sampling regimes, assay technologies and dissemination of findings must, however, be stressed so that complacency does not impede support for innovations. Most importantly, the often local and species-specific nature of harvest closures must be communicated so that safe seafood products are not avoided by consumers frightened by toxic shellfish reports.

B. SHELLFISH HARVESTERS AND FARMERS

Individuals who derive their livelihoods from the harvest of molluscan shellfish have, perhaps, the most to gain from an improved understanding of harmful algal-shellfish interactions. These individuals need clear and current information to help them manage their activities to minimize the impact of harmful algae upon their businesses. New technologies for detecting and diagnosing effects and toxins must be made available to the harvesters; the ISSC fills this function to some extent at present, but modern media, such as internet web pages and email list-servers, would enhance the dissemination of this information more thoroughly and in a more timely fashion.

C. PUBLIC HEALTH AGENCIES

Public health agencies, ranging from federal (USFDA) to state and even municipal levels, also are direct users of research findings and technological developments. They must be made aware of advances in knowledge and techniques that are scientifically-valid and tested in practical trials. The goal of this outreach effort will be to replace current methods with procedures that offer improved technical and/or cost effectiveness and to formalize these improvements in regulations and laws. The ISSC provides a reasonable forum for this effort.

D. HAB RESEARCH COMMUNITY

Much of the present HAB research community is focused upon oceanographic, water-column ecological questions or the fine points of toxin chemistry. Shellfish research will benefit from some developments in this field, but improved communication between HAB researchers and shellfish researchers should serve to re-focus biological oceanographers and analytical chemists on shellfish issues, especially through collaborative studies, possibly funded jointly by ECOHAB and a national shellfish program.

E. GENERAL RESEARCH COMMUNITY

A partial list of academic disciplines that could provide valuable skills and tools to research on harmful and toxic algal-shellfish interactions include: medical science and practice, epidemiology, economics, physiology, population and community ecology, veterinary medicine, toxicology and many others as difficult technical questions are addressed.

V. SUMMARY

There is no question that Harmful Algal Blooms continue to pose a serious threat to the shellfish industry and to public health. While impacts of HABs upon human activities continue to increase in frequency, our ability to mitigate impacts is hampered by the lack of a coordinated, unified, and integrated approach. This approach must include not only all facets of shellfish and HAB research and management, but also a publicly-recognized need to improve consumer safety standards for shellfish.

A. PRIORITY ISSUES THAT NEED TO BE ADDRESSED INCLUDE:

- Continuation, improvement and coordination of programs that minimize health risks to seafood consumers;
- Improved public education and awareness;
- The development of a nationwide response capability to HAB events that can impact shellfish resources;
- Provision of services, i.e., information and financial assistance, to affected harvesters;
- Improved technological methods for rapid toxin identification and quantification;
- Implementation of technical training courses for harvesters and resource-agency personnel to broaden technical expertise and communicate improvements in methods;

- Provision of consistent financial support specifically to assist priority research efforts;
- Determination of the full range of effects that harmful and toxic algae may have upon shellfish, and the role of shellfish in HAB dynamics, so that;
- The full economic impacts of HAB-shellfish interactions can be estimated.

Beyond economic consequences, harmful and toxic algal blooms represent a very visible indicator of the health of the environment in the eyes of the public. Although algae that are harmful or toxic are part of the natural ecosystem, their effects upon shellfish represent a very real health threat to seafood consumers, and their environmental effects impact on human uses that range from commercial to aesthetic. An increased understanding of harmful and toxic algal-shellfish interactions will serve to set realistic expectations for improving our positive relationship with the sea.

B. PUBLIC HEALTH

- Develop rapid, reliable, inexpensive “dip-stick” tests for the various HAB toxins (as discussed by Boesch et al., 1997, and reiterated here) and identify other specific technological developments that need to be implemented for appropriate toxins.

Existing methods for the detection of toxins in shellfish principally rely on the standard mouse bioassay. While the mouse bioassay has numerous advantages, it is controversial and can be expensive. There is a need for assessment methods that are fast and can be used reliably in the field (see linkages to Molecular section).

- Develop financial strategies to support technique development.

Complacency and perceived legislative difficulties concerning current toxin-detection methods should not impede support for technical innovations.

- Establish action levels for other known HAB toxins, including domoic acid, okadaic acid and brevetoxins and determine the human health consequences of long-term, low-level exposure to consumption of toxin-contaminated shellfish.

For obvious reasons, algal species known to be associated with short term human health risks have received the most attention. Many of the potential longer-term effects on human health associated with these biotoxins are not well known, nor are the fates of these toxins in ecosystems clear.

- Investigate possible means of depuration of toxin-contaminated shellfish.

Research information is needed to establish procedures, where appropriate, whereby toxin-contaminated shellfish can be rendered safe for human consumption through such procedures as relay, land-based depuration facilities, etc.; these procedures then will need to be included in the regulatory framework.

- Include innovative use of volunteer and other public and shellfish industry programs in development of future monitoring programs.

Government agencies may be able to supplement dedicated staff and resources with inclusion of public or industry groups with an interest in safe shellfish and healthy coastal environments. Models developed in some regions (e.g., California) should be considered for expansion into other regions as appropriate.

C. NETWORKING AND ORGANIZATIONAL ISSUES

- Develop a national response plan that identifies existing personnel and financial resources, expertise, financial coverage, and provides advice on management strategies (GIS-based information management).

At the national level, there is no coordinated response plan for the sudden appearance of toxic shellfish resulting from an unexpected bloom. As an example, when a recent DSP event occurred in the Pacific northwest there were delays in responding because a response infrastructure was not established. The ISSC has begun to address this need, but they or other organizations need the charge and resources to complete establishment of a national plan.

- Develop an emergency research fund to facilitate rapid response, e.g., sampling, sample analysis, information dissemination, to unexpected harmful or toxic algal events.

Deployment of an emergency HAB-shellfish response should not be impeded by questionable reimbursement of those who act.

- Develop strategies that provide financial aid to aquaculturists/farmers who experience HAB-caused crop losses.

When unexpected HAB events occur the economic impact to the individual aquaculturist/farmer can be financially devastating. Programs being developed to protect farmers from crop losses should include HABs within the definition of “natural disasters.”

- Provide training and develop teaching tools that will assist in the identification, recognition and enumeration of HAB species and toxicity analyses.

Reliable monitoring of HABs depends upon qualified personnel who can routinely survey local waters for potential blooms. In many cases existing personnel resources are limited and could be supplemented by additionally trained staff. Development of training programs, at the appropriate regional or national level, is needed.

D. SHELLFISH HEALTH

- Investigate effects of harmful or toxic algae upon filtering and feeding behaviors of molluscan shellfish.

The feeding mechanism of molluscs is the interface between suspended microalgae and the shellfish; this interface will control most other interactions. Laboratory and field studies elucidating feeding responses of molluscs to harmful or toxic algae will benefit many other research objectives.

- Investigate the effect of harmful microalgae on basic physiological and behavioral functions and their resulting impacts on all shellfish life-history stages.

Sub-lethal effects of harmful or toxic algal exposure of shellfish species may vary with life-history stage such that the timing of a harmful algal event may be critical in individual- and population-level effects. Laboratory and field research are needed to understand these interactions.

- Conduct epizootiological surveys to investigate potential correlations between HAB events and disease outbreaks in wild and cultured shellfish populations (GIS-based information management).

Shellfish stressed by exposure to harmful algae exhibit pathologies, and these pathologies may increase susceptibility to secondary infections, possibly associated with compromised immune function. These interactions remain largely unexplored and will require both laboratory and field research.

- Conduct post-bloom monitoring for all life stages of HAB-exposed shellfish for chronic effects.

Because many effects associated with harmful algal blooms tend to be ephemeral, acute and lead to fast-acting shellfish poisoning events or mass mortalities of aquatic organisms, many of the potential longer-term chronic effects associated with these biotoxins are unknown.

- Conduct long-term health evaluations on populations exposed to repetitive blooms.

Only recently has attention been drawn to the fact that microalgal toxins and their chronic effects need to be considered at all biological levels. Preliminary observations must be expanded for effective, long-term management of bloom-prone areas.

E. ECOSYSTEM STRUCTURE

- Determine the impact of toxic shellfish in food webs involving critical fisheries resources and endangered species.

As well as being a public and shellfish health threat, toxic shellfish are also a risk to other animal consumers up the food chain. Toxic shellfish may act as vectors for the transfer of toxins which can threaten other natural resources such as endangered marine mammal and turtle species, and perpetuate the transfer of toxins through the food web. The fate and effect of numerous HAB toxins in the food web is not well known nor indeed is the ultimate loss of shellfish resources within the food web.

- Investigate the potential for altered trophic structure resulting from overfishing of higher trophic levels to lower shellfish populations (especially non-commercial shellfish) thereby relaxing grazing pressure that normally controls algal blooms.

Global changes in marine trophic structure caused by overfishing have occurred at the same time as a generally-recognized increase in HABs; a possible biological basis for this correlation, and the possible role of shellfish, needs to be explored.

F. MITIGATION

- Develop forecasting methods to warn shellfish harvesters and resource managers of impending HAB events.

Foreknowledge about the advent of a HAB is extremely important in terms of public and animal health protection and for the prevention of unnecessary economic losses. Although there are some existing technologies for predicting the occurrence of HABs the responses available for protecting shellfish resources are somewhat limited. There are certain types of early warning systems that may help to minimize certain losses.

- Implement phytoplankton monitoring programs in areas not currently surveyed or identify the existing knowledge base on phytoplankton and toxins by region.

The potential for new, unanticipated toxic algal events poses a threat to public health and such events may result in catastrophic economic and ecological losses to the shellfish industry and to natural resources. Surveys of coastal areas for seed populations of potential HAB species and map-based distribution databases should be a part of future siting of shellfish harvesting and aquaculture operations.

- Develop formal risk analyses for harmful or toxic algae in sites being considered for shellfish aquaculture activities.

Geographic-information databases being developed for aquaculture site selection should include risk estimates for recurring and future harmful or toxic algal events, based upon research information identified in the previous paragraph.

G. EDUCATION/ OUTREACH

- Provide ear-marked support for education and outreach activities identified elsewhere in this document.

Only an educated and well-informed public will react appropriately to harmful algal-shellfish interactions in issues ranging from seafood consumption to political support for research and management.

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Chapter 5

Population Dynamics

INTRODUCTION

What is population biology? It is the study of biological features that exist only at the population level. Typically, population studies measure where individuals of a species can be found in the environment (population distribution), how many individuals are present (population abundance or per unit area population density), the pattern of how individuals are distributed with regard to each other (population dispersion), how fast individuals grow, birth and death schedules and the factors that alter these features. Shellfish population biology can be subdivided into three general areas of investigation – stock assessment, population dynamics and population genetics. Each of these three areas has different goals although overlap of the information produced by each does occur. **Stock assessments** estimate the number of individuals in a population that can be harvested in a subsequent harvesting season. **Population dynamics** is a general title for studies that describe the temporal and spatial changes in the number (or biomass) of individuals in a population and define the processes that produce these changes. **Population genetics** are studies that describe the genetic variation within populations and try to reveal the processes that produce differences in population-specific genetic characteristics. (Discussion and details of the last area are presented in the genetics section.)

How is information produced by population studies used? Stock assessments serve as the primary means for fisheries managers to determine whether harvests will occur, how long harvest will be allowed and what size will the harvest be (McHugh, 2001). To set the size of an upcoming harvest, fishery managers combine stock assessment data with some theoretical model or management goal (Ulanowicz et al., 1980; Malinowski and Whitlatch, 1988). Generally the models require age- and size-specific mortality and fecundity data (see the model section of this report). Managers have the competing goals of allowing harvest to occur (to preserve the fishermen) while retaining some portion of the shellfish (to preserve the resource) for future harvests. Setting harvest targets that have a high probability of achieving these competing goals are improved by having a good understanding of the relative importance of many biological processes (e.g., recruitment, predation, disturbance, critical population sizes) uncovered by studies of population dynamics and genetics (Mann et al., 1994; Whitlatch and Osman, 1994).

Stock assessments provide no direct information for aquaculturists, but information on population dynamics has some value. It can help aquaculturists set the boundaries for specific aspects of their operations (for example, how many shellfish can we put in an area before they exceed the ability of the habitat to provide food? What predators are problems for shellfish of a given size?). Population genetics can be very important to aquaculturists. Attempts to improve the level of domestication of their shellfish by breeding or to anticipate potential problems associated with inbreeding would benefit from understanding how genes change in natural populations.

Information derived from population dynamics and genetic studies have their greatest value for a broad audience, who rarely sees the details of those studies. All people who live or recreate in coastal waters rely on natural processes to help keep those areas clean, safe and natural. Over the past decades, it has become increasingly clear that populations of shellfish play vital roles in maintaining coastal waters in conditions that are desirable to the general public (Newell, 1988). In order for us to rehabilitate local habitats, protect and enlarge existing natural populations, or make informed

decisions about integrating human needs in coastal areas with the environment, we must understand how the native shellfish populations work. Actually, we must understand how populations work in great detail. The level of knowledge necessary to manipulate and maintain natural populations, effectively and economically, exceeds that which would be sufficient for academic interest. Aspects of cultural heritage would be enhanced as well by having abundant shellfish. Cookouts, beachcombing and coastal aesthetics (watermen in their boats plying their trade, mussels and oysters on intertidal rocks) depend on readily available and consistent numbers of shellfish in the natural habitat.

What needs to be done to improve our knowledge of shellfish population biology? The short answer is, “a lot.” Population studies have faced demanding challenges not encountered in many areas of biological study. Shellfish populations occur over broad spatial areas, 10’s to 100’s of kilometers (Appeldoorn, 1995). Important changes sometimes happen only over long intervals of time such as years to decades (Peterson and Summerson, 1992; Lindegarth et al., 1995). The early stages in the life cycle of shellfish are microscopic and cannot be studied directly in the natural habitat. The majority of shellfish species lie hidden in mud or sand throughout their adult lives, making it very difficult to know just how many individuals are present and where they are located. These challenges have handicapped many previous studies and constrained the value of their results. Consideration of previous studies does provide insight into which topics need to be examined most carefully in the future and where research effort will likely be rewarded with useful results.

STATUS

EARLY LIFE HISTORY

Problem: Commercial bivalve species have low natural (not fishery-related) mortality as adults. It appears that the effects of factors controlling population abundance are concentrated during early life history. Yet, we know very little of the specific details of population dynamics associated with early life history stages (Malinowski and Whitlatch, 1988).

Rationale: Bivalves have unique early life-histories among animals. They have a tremendous potential for reproduction that is much greater than other animal groups, but this potential is very rarely realized. Individuals often produce millions of eggs during a single spawning season (Loosanoff and Davis, 1963; Bricelj and Malouf, 1980). Fertilization of the eggs occurs externally in the water and the developing larvae spend days to weeks drifting with the currents. The developing larvae are very small, only 100-250 microns in size. They have the beginnings of a shell after only a day, but do not look like adult bivalves. They possess a specialized swimming structure called a velum that helps them orient themselves in the water. Larvae (and adults) of commercially important species feed on phytoplankton, microscopic plants drifting in the water. In turn, the larvae are food for a variety of small animals, the zooplankton, that are also present in the water. Among the zooplankton predators of bivalve larvae are small shrimp-like animals called copepods, jellyfish, comb jellies and fish larvae (Kennedy, 1996; Krauter, 2001).

During the time bivalve larvae are drifting in the water their numbers are reduced tremendously. Predators may eat up to 5% of the larvae every day (Carriker, 1961; Kennedy, 1996). Currents can carry the larvae to locations that are not suitable for survival or may carry the larvae completely out of the bay or area where they originated. Larvae will die from starvation if enough food is not present or if it is not nutritious for them. Diseases may cause additional mortality. At the end of the larval period, only a small fraction of 1% of the number of initial larvae remain to settle on the bottom.

When the few surviving, fully developed bivalve larvae find their way to the bottom after drifting in the water for several weeks, they are faced with a whole new set of problems. At this time they are still very small, only about 200 microns (Chanley and Andrews, 1971), no larger than the size of the period at the end of this sentence. As the larvae are carried along the bottom, it is their goal to find a suitable place to live. In places where the current is slow, they can crawl along the bottom with a specialized foot. If they manage to find a suitable area, they burrow in or attach to large objects on the bottom using byssal threads (a process called setting). Once they make the decision to settle, they lose their velum and look more like a miniature version of an adult.

Even though they have a shell, the young, post-settlement bivalve is small and is viewed as food for a whole new set of predators (Seed, 1993; Kraeuter, 2001). Shrimps, crabs, marine worms, and fish are among the predators that feed on them. Juvenile bivalves eventually grow large enough so that their hard shell or depth of their burrow protects them from most predators, but that often requires a year or more. During this first year of life on the bottom, juvenile bivalves must maintain close contact with the water and sediment surface so they can feed and grow. They can also be “washed” out of suitable habitat by currents and waves (Emerson and Grant, 1991). Very few juveniles survive.

We know this life-history in only very general terms (Fegley, 2001). Much of our knowledge is derived from laboratory rearing studies where conditions are not like natural systems. Field observations and field experiments are limited and often rely on guesswork to fill in large gaps. The small size of bivalves during their early life history is one limitation to understanding their population biology. Bivalve larvae are very small and difficult to identify to species (Lutz et al., 1982). Juvenile bivalves are easily overlooked when sorting through the sand, shell, and other material that makes up the bottom. The tremendous decline in numbers, from the initial millions of eggs produced by an individual to just a few surviving juveniles, is another critical limitation to our understanding. The chances of an individual fertilized egg surviving to adulthood is miniscule, but a population can maintain itself if only one larva for each living adult eventually survives to reach adulthood. The odds of an individual bivalve surviving are among the lowest in the animal kingdom. We have limited experience with studying such populations.

Future directions

- For larvae:
 - ◆ Role of primary production, especially timing and size of blooms on survival;
 - ◆ Role of hydrography, temperature, salinity, turbidity in transport, dispersal, settlement, and development;
 - ◆ Effects of adult fecundity and dispersion in association with near-bottom flows on fertilization efficiency;
 - ◆ *In situ* growth rates;
 - ◆ Estimates of predation rates associated with specific predators;
 - ◆ Survivorship vs. time during larval period; presence or absence of critical periods; changes associated with duration and season (early vs. late spawners);
 - ◆ Vertical distribution of late-stage larvae in the water column;
 - ◆ Patterns of settlement and immediate post-settlement transport;
 - ◆ Origin of larvae; coherence of set (are the individuals setting in an area related in any way?) and fraction of larvae retained near parent population;
 - ◆ Stock size vs. settlement relationships;
 - ◆ Comparative studies of different species present in the water at the same time.

- For post-settlement and juveniles:
 - ◆ Role of primary production, especially timing and size of blooms on survival;
 - ◆ Role of temperature, salinity, turbidity on survival and growth;
 - ◆ *In situ* growth rates;
 - ◆ *In situ* estimates of predation rates associated with specific predators. Also changes in rates in the presence of alternative prey;
 - ◆ Survivorship vs. time; presence or absence of critical periods, especially first winter;
 - ◆ Patterns of post-settlement transport;
 - ◆ Stock size vs. recruitment relationships.
- Determine the relative importance of larval versus post-settlement processes in limiting population size. If the larval period is the most important then essential management strategies must include the means to increase the number of larvae, e.g., spawner sanctuaries, improved water quality, etc. If post-settlement survival is critical, then management efforts should focus on habitat restoration, habitat creation, predator protection, etc.
- Research is needed to develop rapid, reliable identification and enumeration procedures for shellfish early life-history stages so that population parameters can be estimated.
- Develop the means to age larvae and early post settlement stages.

SAMPLING

Problem: Data collected by sampling is often inadequate for population assessments and rarely allows us to address questions related to fundamental processes and population dynamics.

Rationale: Bivalve populations are large and usually consist of many millions of individuals, even for a population in a small embayment. It is impractical to measure the characteristics of each individual in a population, such as its size, age, growth rate or fecundity, and so these characteristics are estimated by sampling a small fraction of the population. Even though only a relatively small part of the population is measured, sampling can be a highly accurate and precise approach to studying the population. Such an approach is used every day by organizations conducting public opinion polls and has been the preferred approach by the U.S. Census Bureau to estimate the size of the U.S. population and its composition. The need for accuracy and precision is evident when you consider that mistakes in the U.S. Census cost regional locales hundreds of millions of dollars in federal funding.

Sampling methods vary between studies. Many individuals or organizations that collect samples of a bivalve population use an individualized protocol (Fegley, 2001). The differences between studies can include: 1) what device is used to collect samples, 2) how many samples are collected at each location, 3) how samples are processed, 4) what measurements are taken on collected individuals, 5) how many locations are sampled, and 6) when and how often samples are collected. Traditionally sampling protocols are developed based on the experiences of the individuals involved or may be adapted from an existing program elsewhere. Since the habitats to be sampled, the funding, the number of personnel involved, and other factors vary from place to place, sampling methods are rarely the same.

Many bivalve species require different sampling methods. Oysters and mussels are visible on the bottom in dense, but highly patchy assemblages. Other species, such as hard clams, surf clams, and ocean quahogs bury themselves just below the sediment-water interface, while some species, like the

soft shell clam, are buried very deep in the bottom and maintain contact with the overlying water using long extendable siphons. Specific methods that work for some species are totally inappropriate for others. In addition, because of the tremendous differences in size, different sampling methods may be needed for juveniles and adults. However, within one of these “ecological” types of bivalves there should be greater consistency in sampling methodology. Differences in sampling methodology are currently so great that regional comparisons are not possible.

Sampling can be inefficient. With limited knowledge about a given bivalve population, the only practical approach is to sample all life stages and to distribute sampling locations throughout the embayment or body of water. This concentrates as much effort on noncritical life stages and places where there are few bivalves present as it does on critical stages and places where there are many bivalves. As knowledge about a population increases, more efficient sampling is possible since it can focus on the particular life stages, locations, and processes that need the most effort (Burd et al., 1990; Constable, 1999). Sampling methods evolve as more information is gathered and sampling can become more efficient; however, incomplete knowledge about the specific biology of the population places limits on this progression. Mistakes arising from incomplete knowledge can result in sampling problems such as under- or overestimating population size, or the inability to detect when the population has changed in size.

Sampling is generally expensive. For all their good intentions, individuals and organizations have limited resources that they can expend on sampling. Field assessments can cost thousands of dollars a day to mount, taking into account vessel costs, fuel and labor. The field component may also be only one part of a sampling effort. Some of the samples collected need to be brought back to a laboratory for additional processing. This high cost limits the extent and size of data sets that exist on bivalve species.

Future directions

- Develop **standardized** species-specific methods for sampling each life history stage (larvae, juveniles and adults) so that data collected across regions are comparable.
- Establish the average efficiency as well as the variability in efficiency of frequently-used sampling gear.
- Encourage the collection of long-term, regional census data that can be used to accurately assess the status of populations, identify trends in population parameters and provide basic information to calibrate and verify predictive models (Kassner et al., 1990). This will require fundamental changes in the policy of agencies responsible for sampling bivalve populations. Traditional monitoring programs should be redesigned to specifically address the information needs of the managing authority.
- Existing stock assessment data sets should be analyzed to: 1) define the specific inadequacies of past sampling protocols (so that future stock assessments can avoid the mistakes of the past), 2) provide estimates of expected sampling variability at increasing spatial and temporal scales (to allow future stock assessments to be designed efficiently and with sufficient replication), and 3) develop hypotheses about patterns in the data that could be tested in future studies.
- Continue to develop and utilize acoustic techniques for identifying and delineating shellfish habitats; acoustic methods such as side-scan sonar and RoxAnn can form the basis for efficient and economical sampling of bivalve populations.

- Develop new techniques for field sampling and sample processing. Sampling possibilities include “bounce” cameras to photograph small areas of the bottom and sub-bottom acoustic imaging to detect clam species that burrow into the bottom. A laboratory method that could rapidly identify and count shellfish larvae in water samples would revolutionize the study of early life-history population dynamics. A molecular identification approach, involving monoclonal antibodies targeting larval stages of a shellfish species, is possible and could lead to computer assisted, semi-automated analysis of larval samples.
- Expand the use of existing statistical and modeling approaches that maximize the information obtained by sampling (Cerrato, 1990). Encourage the development of new statistical and modeling approaches for situations where the data are not amenable to existing methods. For example, several approaches to size-frequency analysis are available for estimating population age structure, and some also provide estimates of recruitment, growth, and mortality (MacDonald, 1987). These procedures are sophisticated and require expert application, but they can reduce the overall sampling effort and cost required to get the equivalent information by traditional means.
- Establish a web page or equivalent system for the rapid communication and exchange of sampling methods and analysis techniques that work.

POPULATION PROCESSES

Problem: The information we have about how shellfish populations actually work (e.g., what controls the size of a population, why do populations occur here and not there?) is insufficient to allow us to manage these populations or predict how they will behave in the future.

Rationale: Many natural and anthropogenic processes affect population size. Predators, disease, harsh environmental conditions, starvation, storms, physical disruption of the habitat, toxins and many other factors all have been demonstrated capable of decreasing the size of shellfish populations (Duggan, 1973; Shumway, 1996; White and Wilson, 1996; Ford, 2001; Kraeuter, 2001). Processes that increase population size have also been identified (e.g., spawning and settlement of larvae from the water column). What we do not know is the relative importance of these different factors (which has the biggest effect on population size?) to a particular population from time to time or from place to place. Are they all important all the time? Are only a few important during certain times? Without information of this kind, anyone interested in assuring the health of a shellfish population is reduced to guessing and relying on luck to achieve their goal.

We have identified three general areas that merit attention. Studies that compare the population dynamics of different species of shellfish are needed. Currently, we tend to treat almost all shellfish populations as if they are the same. Anyone observing different shellfish populations knows this is fiction. Some shellfish species occur in dense concentrations, some do not. Some shellfish species occur over large geographical areas, some do not. Some shellfish species are present in the local habitat every year, some seem to come and go. As most of these species have similar life histories (see above) the differences we see in the adult populations must be a consequence of species-specific differences in population processes. Studies, conducted in the natural habitat, that examine different shellfish species at the same time and in the same way are very few in number.

We also need to know whether population processes remain the same for a single shellfish species in different locations at the same time or at different times in the same location. Many shellfish species extend over broad ranges of the continental coast. Within that range the environmental conditions and the types of predators, competitors, and diseases change (Appeldoorn, 1995). Do the processes that determine population size remain constant against this varying background? Similarly, everyone

is aware that years differ (unusually warm winters, summers with lots of rain). Do these annual differences have an effect on which population processes are important to a shellfish population? We have numerous pieces of information that suggest populations respond differently to changes in location and time (Abbe, 1986; Reusch and Chapman, 1997). Yet, this information has not been collected in comparable or sufficiently rigorous conditions to permit anyone to draw general conclusions, much less predictive paradigms.

Finally, it is important to emphasize that some of the species-, site-, and time-specific information be gathered using experimental methods. Descriptive studies are required, but only experimental studies can uncover which processes are more or less important. Only experimental studies can show whether our existing paradigms are correct. Hypotheses can be generated from either descriptive or experimental studies but the development of theories requires a greater certainty of knowledge than can be provided by descriptive studies alone (Hairston, 1989; Underwood, 1997). The ability to use population information for generating mathematical models, restoring habitats, and relating population processes to other types of biological information (physiology, community and ecosystem processes) rests on developing testable general, realistic theories of bivalve population dynamics.

Future directions

- Gather data that would allow the use of commonly-used analyses of populations such as life history tables or K-factor analyses. If these cannot be done then comparable analyses need to be developed for shellfish.
- Estimate the amount of gene flow / dispersal between populations.
- Elucidate changes in genetic composition of a population in response to harvesting, natural mortality sources, and catastrophic events (such as large storms).
- Examine the effects of spatial and temporal scale on the outcome of experimental studies (Thrush, 1991; Hewitt et al., 1998; Legendre et al., 1997; Constable, 1999). Are the results of experiments conducted at 1 m scales representative of the results conducted at 10x or greater spatial scales? What are seasonal and interannual effects on results?
- Compare the same population processes of different species within the same habitat at the same time.
- Compare the same population processes of the same species between different locations (or times) using the same methodology. This should be done at different locations within the same population, between different populations within the same part of the species range, and between different populations within different parts of the species range.
- Determine whether populations are near carrying capacity (relative importance of density-dependent and density-independent processes) and how carrying capacity changes through time.
- Follow population processes for periods of time appropriate to the process (not the length of the funding cycles). In some cases, a minimum of a decade or more of continuous study may be required to provide meaningful data.
- Examine the role of non-lethal toxins or diseases on population dynamics (e.g., on adult fecundity, spawning behavior or growth rates).
- Document population dynamics of introduced species; determine why some introduced species cause major changes to invaded communities and others do not.
- Develop methods that increase our ability to follow changes in the soft-tissue (internal) portions of bivalves such as for following soft-tissue growth over daily intervals or estimating soft-tissue condition without destroying the whole animal (non-destructive sampling; Lewis and Cerrato, 1997).

- Increase the utility of shell growth patterns for population studies by: 1) developing rapid preparation methods that are automated and can be done at lower cost and effort than are necessary with current methods, 2) developing image analysis procedures that are more automated, 3) developing methods for analyzing shell growth patterns in larvae and early juveniles, and 4) exploring the use of shell patterns for providing information on physiological processes (Cerrato, 2000).
- Conducting elemental analyses of shells using ICP-MS. These results should be evaluated for their utility in determining the origin of larvae (in combination with environmental data on spatial distributions of contaminants). Incidentally, elemental analyses of shells may also be useful in pollution studies or revealing physiological mechanisms regulating shell deposition (Rosenberg and Hughes, 1991; Thorn et al., 1995).

REGIONAL CONSIDERATIONS

Regional differences exist in population functionality. Unfortunately, it is difficult to identify which patterns or problems are common or unique between systems. Managers and researchers in different regions have not attempted to coordinate their activities. Their methods are different enough that reaching conclusions by comparing existing studies cannot be done with confidence. As indicated above, there is a critical need for standardization of methods and goals across regions.

No region is demonstrably better or worse in terms of understanding the function of their respective shellfish populations (although the offshore clam fisheries are the best monitored). No region is unarguably better or worse in terms of their shellfish populations. They are threatened everywhere. Greater numbers of shellfish are needed everywhere.

OUTREACH

Although there many stories about shellfish life history that would interest the public, little of the technical aspects of shellfish population biology will excite general audiences. Outreach efforts should concentrate on the role that larger shellfish populations would play in providing more habitat for other, desirable organisms (e.g., oyster reefs), in improving water quality, in serving as “canaries” in monitoring water quality, and in increasing the opportunity for recreational harvest.

SUMMARY			
	Knowledge level	Assessment ability	Potential importance
Techniques and methods	low – moderate	moderate	moderate
Species specific knowledge	low	moderate – high	major
Monitoring	high	high	major
Management capability	low – moderate	low	major
Policy development	low	low	moderate
Public outreach	low	N/A	minor

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CHAPTER 6

Broadscale Ecological Implications for Efficient, Optimal and Sustainable Shellfisheries

INTRODUCTION

As a society we want to develop sustainable optimal harvest when managing naturally-native fisheries or in designing and implementing aquaculture-based fisheries. In so doing we also want to insure the environmental integrity. To accomplish these goals we need to recognize that the benefits derived from understanding basic broad-scale ecological principles will be essential in determining what we do or do not know or can predict about how marine ecosystems function and thus how our manipulation of “natural” ecosystems will affect environmental integrity. More specifically, with current knowledge we can highlight characteristics, potential effects and special problems of marine ecosystems, whether they are in natural environments of the coastal zone or in closed systems. We reemphasize that engineer-designed aquaculture systems also exhibit ecological structure and function, and become part of, and affect the structure and function of the greater coastal ecosystems in which they are placed.

Potential problems of disease and disease management are associated with any intense culture of organisms (see disease section), and there can be beneficial and/or adverse effects of introducing exotic species associated with aquaculture activities (see shellfish habitat rehabilitation section). Information on the physiological processes of reproduction and feeding of commercially-valuable shellfish is vital to sound management practices. Such information is either currently available or discrepancies have been identified elsewhere in this document (see physiology section). Stock assessment models are routinely used to manage shellfisheries, but we need to develop better estimates and indicators (see population dynamics section).

We recognize that all these processes function, interact and are expressed within what are termed ecosystems. This part of the report seeks to identify those key broad-scale ecological principles underlying ecosystem integrity that are particularly germane to a discussion of effective and prudent fisheries/aquaculture practices. In the following paragraphs we address the potential effects of shellfisheries *per se* on: (1) habitat modification; (2) biotic community composition and organization; (3) food web structure; and (4) material transport and cycling. We then suggest the need for: (1) exploring interactions of reef, submerged aquatic vegetation, marsh, and mud flat subsystems in sustaining ecosystem integrity; (2) addressing differences in scales of size— both in total system size and in relation to ecological subsystems— on how we need to manage shellfisheries; and (3) discussing potential role of stratigraphy, geomorphology, and hydrography (including mesoscale turbulence) on the functioning of shellfish populations and their associated biotic communities.

HABITAT AND BIOTIC COMMUNITY MODIFICATION BY SHELLFISH

The importance of bivalves in creating structure in shallow coastal waters and the development of an associated biotic community has long been recognized. The distinct assemblage of species associated with oyster reefs led Mobius (1880) to propose the concept of the “biocoenosis,” the foundation of community ecology. Bivalves also develop much of the horizontal and vertical

textural structure of sand and mud bottoms (soft sediments) through burrowing and the organic matter they contribute through feces and pseudofeces. Introduced aquaculture based on raft, tray and pole structures further add structure to coastal ecosystems.

The structural complexity of oyster and mussel beds as well as the less obvious biostructures of soft sediments create a heterogeneous environment in which numerous species can find food, shelter from exposure and currents, and protection from predators. For these reasons it seems clear that bivalves contribute in important ways to the biocomplexity and biodiversity of coastal ecosystems. Similarly, their burrowing and reworking of soft sediments must influence the biogeochemical cycling of elements that move through the sediments (e.g., Aller, 1978).

FOOD WEB STRUCTURE AND CARRYING CAPACITY

Reliable estimates of carrying capacity do not exist and are key to effective shellfish and broader ecosystem management.

On theoretical grounds, it is also possible that energy transfer from phytoplankton through bivalves to fish or crabs may be more efficient than the grazing food chains of the water column (Dame, 1996). The Eularian life style of the bivalve takes advantage of the physical energy of the currents to acquire food. Moreover, bivalves are commonly much larger than the other grazers (i.e., the zooplankton) and their size relationship yields a lower respiratory energy cost per unit weight. Bivalves also maintain a standing biomass capable of responding immediately and efficiently to intermittent blooms of phytoplankton whereas zooplankton standing biomass often lags behind an effective carrying capacity to exploit intermittent phytoplankton blooms (Tenore et al., 1984; Heip et al., 1995).

The development of intensive shellfish culture in the field would benefit greatly from an improved ability to estimate the carrying capacity of coastal areas. In the past, it has been common to increase shellfish production until the growth rate of the bivalves declined or water quality deteriorated to an unacceptable level. The problem is challenging because of the non-linear relationships between the shellfish, their food supply and the water transport mechanism.

As a first approximation of carrying capacity, we define bivalve carrying capacity as *the total bivalve biomass supported by a given ecosystem as a function of the water residence time, primary production time and bivalve clearance time.*

Water residence time. (RT) is the theoretical time it takes for the volume or mass of water within a basin to be replaced with water from outside the system. The water flow from outside the system, also sometimes known as advection, can enter as ocean currents, tidal exchange, rainfall, surface water runoff from the surrounding landscape, ground water input or river flow. In general, the smaller the basin the shorter the residence time of its water mass. Estuarine and coastal systems are strongly influenced by river flows that are often seasonal. In large estuarine systems, the residence time of water may vary depending on the location within the system. Thus, although water residence time is an ecosystem scale parameter, it can vary greatly depending on season and location. In most of the systems described here, salinities are seldom very low and tidal exchange dominates freshwater flow.

Primary production time. In this discussion, primary production time is defined as the ratio of yearly averages of phytoplankton biomass (B) to phytoplankton primary production (P) within the particular ecosystem. Primary production time (PPT) or B/P describes the time it takes for primary production within the system to replace the standing crop biomass of phytoplankton within the system. This measure is analogous to the organismic-population measure known as phytoplankton doubling time, but PPT is an ecosystem scale parameter. Primary production time is influenced by import and export of phytoplankton and seasonal variations in light and nutrients.

Bivalve clearance time. From Smaal and Prins (1993), the time that is theoretically needed for the total bivalve filter feeder biomass within an ecosystem to filter particles from a volume of water equivalent to the total system volume is termed as bivalve clearance time (CT). This turnover time is a function of the biomass of the bivalves and the seasonal influences of particulate concentrations, seston quality and temperature on the filtration rate of the bivalves.

A recent comparison of ecosystems that have or have had major populations of bivalve filter feeders (Dame and Prins, 1998), clearly indicates each system is unique in many ways. The focus on turnover times as defining parameters of bivalve filter feeder carrying capacity strongly couples the major abiotic and biotic components in a straightforward way. Based on ecosystem scale parameters, a number of bivalve dominated or cultivated systems were compared. From a practical perspective, the identification of the 3-D space for the system turnovers of residence time, primary production time and clearance time provide an indication of the fundamental parameters of highly productive and sustainable bivalve filter feeding dominated systems. Those interested in initiating bivalve cultivation in a given system can probably determine rough approximations of these turnovers in order to compare their perspective system to those that have been extensively studied. Such information can then be used to determine if a pilot cultivation study is worth while.

MATERIAL TRANSPORT AND CYCLING

Bivalve shellfish increase the removal of suspended particles from the water column in two ways: (1) by removing particles from suspension through the pumping of water across their gills and filtering the particles (>2-3 μm) and (2) by creating structure that increases bottom roughness and drag. This roughness reduces water velocities and increases sedimentation rates. Further, natural bivalve beds are often found in zones of turbulent water flow that they enhance with their 3-D structure. This interaction of bivalves with the water flow results in the increased probability of the shellfish interacting with food particles and eventually higher growth rates. In turn, the turbulent flow increases the flushing of dissolved and particulate waste products of shellfish metabolism from the animals. Finally, the filtration capacity of bivalves, particularly dense populations (reefs and beds) and cultured populations, gives these animals the potential to control the density of suspended particulate materials, plankton and other particles, in the water column. The removal of suspended particulate material and the excretion or production of waste defines the major couplings between the benthos and water column. These exchanges also describe the major pathways for the recycling of the potentially limiting nutrients of nitrogen, phosphorus and silicon. In essence, the shellfish may increase the recycling rate for their ecosystems. The negative aspect of the filtration capacity of shellfish is that they may also concentrate toxic chemicals and disease agents. The toxics are adhered to particles in the water column and some disease causing organisms are often the result of human or agricultural waste.

ECOSYSTEM LANDSCAPES: HABITAT INTERACTIONS

In estuaries there are a number of major habitats that interact with each other. Bivalves often have positive effects on these other ecosystem components. In salt marshes, the presence of marsh mussels has been shown to increase the productivity of the grasses by increasing the movement of nutrients from the water column to the sediments surrounding the grasses. Submerged aquatic vascular plants are often associated with environments that also support bivalves (clams and scallops; Peterson and Heck, 1999). The submerged aquatic vegetation (SAV) provides a mechanism of passive sedimentation that leads to sediment stability and some protection from predators for the bivalves. The bivalves increase the removal (active process) of suspended sediments (particulate nutrients) from the water column to the bottom. The bivalves with the associated vascular plant habitats provide increased habitat heterogeneity and diversity. Mudflats are found interspersed among all the

preceding habitats. These systems are devoid of vascular plants, but are subject to sedimentation as a result of very low water velocities (passive process). This sedimentation is increased via the active filtration of the overlaying water by infaunal bivalves and the decomposition of organic material on the flats. Nekton take advantage of these attributes to enter these habitats to feed, develop and to move between components. Thus each of these components through passive and active coupling processes, often mediated by shellfish, potentially magnify material fluxes between the water column and the bottom.

SPATIAL SCALING

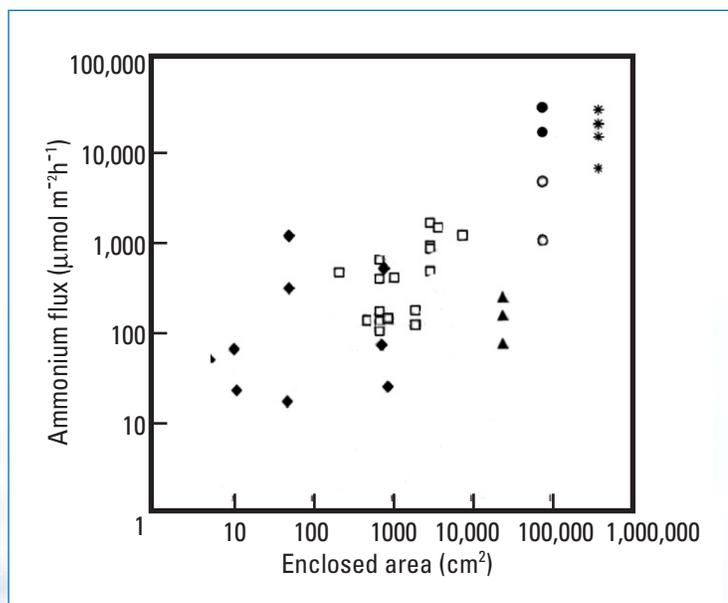
Traditional scaling up approximations of critical parameters can be misleading; we need multi-scale estimates.

Shellfish commonly influence spatial scales and temporal scales across 5 to 6 orders of magnitude. Physiological processes typically influence spatial distances of cm – m and temporal scales of minutes, while epidemics span scales of 10 – 1000 km and years of duration (Dame, 1996). To better understand the involvement of bivalves in their environment, recent evidence suggests that simultaneous measurements at multiple scales is imperative (Asmus et al., 1998).

These investigators observed the flux of ammonium from mussel bed sediments in cores, bell jars, tunnels and flumes on spatial scales between 10 and 10⁶ cm² (see Figure 1 taken from Asmus et al., 1998). The ammonium fluxes ranged from 10 μmol m⁻² h⁻¹ for the smallest core to 10⁶ μmol m⁻² h⁻¹. These data reflect that the largest rates of ammonia fluxes come from the measurement over the largest areas that integrate the most factors at a given time. The lesson from this study is that scaling up from small area measures to examine ecosystem level processes is dangerous. Depending on the process of interest, direct field estimates at the appropriate scale(s) is preferable.

GEOMORPHOLOGY AND HYDROGRAPHY

From a physical perspective, shellfish structure can influence water flow patterns and the geomorphology of streams. In shallow intertidal systems, reefs are typically found as a fringe reefs or a string (long) reefs. Fringe reefs are generally on the outer (concave) edges of meanders where water velocities are higher, even turbulent (Keck et al., 1973). In some systems the reefs actually build out from the stream edge and effectively damn the stream. At this extreme, the geomorphology and water flow of the system becomes severely altered. Intertidal string reefs are often located where turbidity is high. In subtidal systems, reefs are found as fringes, strings and patches with predation and stratification becoming major influences (Kennedy and Sanford, 1999). All bivalve reefs generate turbulent flow that aids in bringing food to the bivalves and flushing away waste. Further, this positive feedback of building hard structure may eventually change the shape of streams and channels as well as the flow dynamics within them.



Flux of ammonium from mussel bed sediments (from Asmus et al. 1998) showing the scale effects of the size of the experimental containers.

An understanding of ecosystem level processes is an essential part of an integrated approach to the management of coastal ecosystems. The almost unknown effects of scale affect our interpretation of the potential impacts of fishing, aquaculture and other human enterprises on individual ecosystem components,

and the interaction between these components. Thus linkages between the water column and the benthos; estuaries and water exchange between either the freshwater or oceanic ends of the spectrum, or between bivalves and phytoplankton all require an integrated approach that can only be derived from a fundamental understanding of the effects of scale on each experiment. Without such an understanding of ecosystem level processes we have the potential for providing the wrong advice about management of our coastal waters.

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Chapter 7

Modeling

INTRODUCTION

Mathematical models are an important tool for scientific studies because they provide a structure for integrating and synthesizing conceptual ideas and observations to make predictions about the future state of a system or to test hypotheses about the effect of certain actions. Within the past decade the state of the art of mathematical modeling of marine systems, especially environmental and biological interactions, has advanced to the point where it is now feasible to expect model simulations to reflect reality and to have predictive and forecast capability. Thus, mathematical models are a relatively inexpensive alternative for testing the effect of a wide range of potential perturbations for a given system, far beyond what can be done with experimental manipulations. The simulations obtained from the mathematical models can then be verified with experiments that are designed from the outset to focus on specific aspects of a given system.

The primary areas in which mathematical models have been used to understand and investigate shellfish processes are in studies of post-settlement population dynamics, larval ecology and disease. The mathematical models used in these studies range from optimal density models of varying complexity (Incze et al., 1981; Heral et al., 1988), to correlative models of population growth (Ulanowicz et al., 1980; Eldridge and Jackson, 1993; Hawkins et al., 1998), to population dynamics models (Allen, 1979; Ulanowicz and Tuttle, 1992). During the last decade, progress has been made in developing mathematical models that explicitly couple environmental variability (Bacher, 1989; 1991; Hofmann et al., 1994; Raillard et al., 1993; Gerritsen et al., 1994; Kobayashi et al., 1997), and the effect of diseases, such as Dermo (Powell et al., 1996; Hofmann et al., 1995) and MSX (Ford et al., 1999; Paraso et al., 1999; Powell et al., 1999) to shellfish population dynamics. The latter group of models, although formulated for specific species, such as the eastern oyster (*Crassostrea virginica*), are general in approach and have been applied successfully in a range of environments. These models, therefore, allow testing and simulating the response of a specific shellfish species to environmental variations in a wide range of environments.

Larval models have typically been developed independently and then coupled to post-settlement models when modeling the entire life cycle was desired. Larval recruitment is an important factor controlling any shellfish population and the factors that underlie this are many, encompassing environmental, ecological and biological processes. Mathematical models that allow testing of the effect of these many processes are now available (Deksheniaks et al., 1993; 1996; 1997) and results of these modeling studies have highlighted the importance of temperature and ontogenetic changes in larval behavior in maintaining larvae in a given region. A more recent modeling study (Deksheniaks et al., 2000) has shown the importance of adult population dynamics and environmental factors in determining larval recruitment in a large bay system. Most recently, models have attempted to describe larval growth at the biochemical level and this direction has provided sufficient advantages to suggest that future models will routinely be formulated in this way.

The studies cited above have clearly demonstrated the importance and utility of modeling in understanding the factors regulating shellfish populations. Continued development of models is needed and we believe that the next generation of shellfish models can make important contributions in policy and management decisions and in investigation of population dynamics and environmental interactions in the areas of:

- Effects of marine diseases;
- Management strategies for commercial shellfish populations;
- Optimization of aquaculture;
- Ecosystem management;
- Environmental monitoring; and
- Climate change.

CHALLENGES IN MODEL DEVELOPMENT

The development of the next generation of mathematical models that are capable of addressing the issues encompassed in the above topics face a number of challenges inherent to the modeling process. It is not simply the development of new models formulated to address these topics that is required. It is also the development of new ways to model that will be required to further the value of modeling in shellfish biology and ecology. These challenges to modeling that must be overcome in the next few years are as follows.

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DermoWatch

DermoWatch is a **SeaGrant** project which estimates a time to a critical level of the oyster parasite *Perkinsus marinus*, commonly called "Dermo".

Galveston Bay										
Spl	Date	Location	Sal ¹	Temp ²	j-WI ³	j-PI ⁴	jCrit ⁵	c-WI ⁶	c-PI ⁷	cCrit ⁸
423	05/08/2003	Redfish Reef	16.0	26.7	0.58	26.67	6	0.51	33.33	6
431	06/30/2003	Lease 415	21.0	30.0	0.67	46.67	4	0.98	80.00	3
430	06/30/2003	Lease 403	19.0	30.0	1.60	86.67	0	1.18	80.00	2
429	06/30/2003	Lease 301	18.0	30.0	0.69	33.33	4	1.31	53.33	1
425	05/29/2003	Hannah's Reef	16.3	26.3	0.00	0.00	1000	0.04	6.67	17
426	05/29/2003	Frenchy's Reef	15.4	26.2	0.00	0.00	1000	0.49	26.67	7
432	06/05/2003	Fisher's Reef	13.5	23.9	0.00	0.00	1000	0.02	6.67	34
427	06/28/2003	Confederate Reef	26.0	33.0	2.31	100.00	0	1.82	93.33	0
428	06/25/2003	April Fool Reef	20.5	30.5	0.56	53.33	4	1.07	93.33	2

Figure 1. Home page from the DermoWatch web site (www.blueblee.com/dermo) showing the input variables that are needed to calculate time to critical infection levels of *Perkinsus marinus* (= *Dermocystidium marinum* for juvenile (j) and commercial-size oysters (c) oysters). Calculations of weighted incidence (WI) and percent infection (PI) for juvenile (j) and commercial-size (c) oysters are also displayed.

- We need to streamline the development of user-friendly model codes to permit widespread use by fishermen, aquaculturists, ecosystem and fishery managers and the public at large, but that is not enough. We also need to increase the accessibility of these models to the diversity of users (e.g., DermoWatch).

MODELING DISEASE RISK

Web sites provide a means of delivering useful information to the general public. A model embedded in the web site <http://www.blueblee.com/dermo> uses the initial infection level of the parasite *Perkinsus marinus*, that causes Dermo disease, oyster size, water temperature and water salinity to predict a time to a critical level of disease (Figure 1). Critical level is the level of infection where significant oyster mortality occurs. Estimating time to critical level permits informed decisions about when to harvest oysters before they die or when to move them to a lower salinity site where the risk of loss to disease is reduced.

- We need to develop fully coupled physical-biological and climate-biological models for a range of uses as diverse as ecosystem management and application of optimal density farming practices in aquaculture. These models require state-of-the-art formulations to allow for the spatial data.

COUPLED PHYSICAL-BIOLOGICAL MODELS

Coupled physical-biological models represent the combination of a circulation model with models of the lower trophic levels, which usually include nutrients, phytoplankton and zooplankton. In these models, the circulation field provides the overall spatial structure of the biological distributions, while the biological model provides the local changes in the magnitude of the biological variables. The addition of a planktonic larval component in which the larva has behavior (e.g., vertical migration) introduces the need to accurately resolve the vertical current structure and high frequency flows, such as tidal circulation. While it seems that coupling a circulation and biological model is straightforward, this is not the case. There are serious issues associated with ensuring that the two models resolve the same space and time scales and that each contains the needed dynamics. Matching scales in the two models may require alternate solution grids, inclusion of additional dynamics or even reformulation of existing dynamics. Numerous examples exist in the oceanographic literature where *ad hoc* approaches (e.g., Rothlisberg et al., 1983; Walsh et al., 1988) were used after the fact to attempt to match the dynamics of circulation and biological models. In all cases, the result was less than what was needed. The development of physical-biological models needs to be done with the idea that the models will be coupled from the outset. Since these models are new to shellfish research, the opportunity exists to develop these properly from the outset.

- We need data collection programs that are long-term and provide rate as well as standing stock estimates with concurrent environmental information with spatial and temporal resolution resolution that adequate for model calibration, verification, and simulation.

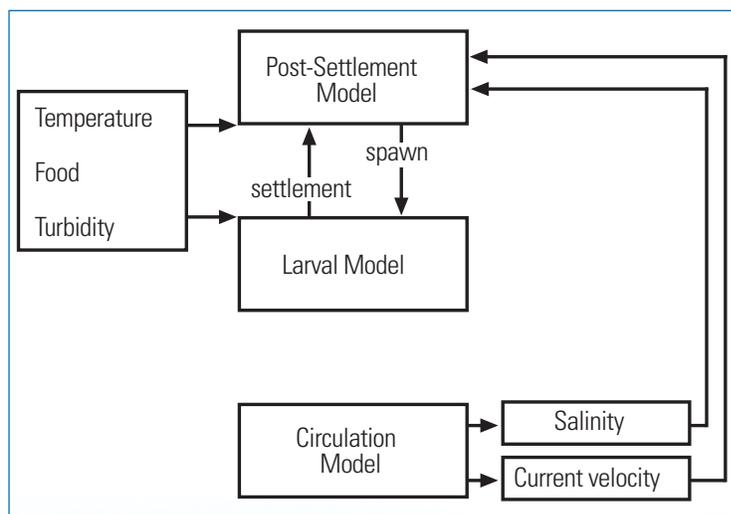


Figure 2. Schematic showing the model components needed for developing a fully coupled physical-biological model for oyster populations. Figure adapted from Deksheniaks et al. (2000).

KNOWLEDGE AS A LIMITING FACTOR

All models are eventually limited by the amount of information available on the system being modeled. Shellfish modeling has identified critical limitations in our knowledge of shellfish and their diseases that impose barriers to the use of models as forecasting tools, which are as follows.

Post-settlement Physiology: Although most commercial species have been studied for many years, research has tended to focus on certain areas of physiology and not on others. More information is needed on the:

- ◆ influence of environment, especially total suspended solids and variations in food quality on feeding rate;
- ◆ operation of physiological processes at the environmental extremes of high and low temperature and low salinity;
- ◆ time course of mortality at the environmental extremes of low temperature and low salinity;
- ◆ controls on the division of assimilated energy into somatic growth and reproduction; effects of extreme flow velocities on feeding; and
- ◆ genetic variability in physiological processes affecting efficiency of production.

Larval Physiology: Generally speaking, limitations in modeling larvae are more profound than limitations in modeling post-settlement life stages. The accuracy of coupled larval/post-settlement models are always limited by our knowledge of larval processes, especially the:

- ◆ metabolic processes affecting the use and fate of ingested lipid, carbohydrate and protein;
- ◆ genetic variability establishing the efficiency of feeding and respiration;
- ◆ adult processes affecting egg quality, particularly neutral lipid content;
- ◆ environmental effectors establishing position in the water column;
- ◆ influences of food quality and quantity and environment on feeding efficiency; and
- ◆ physiological and environmental controls on larval metamorphosis, settling and survival following settlement.

Environment: Models require data on the environment for simulation and for verification. For shellfish, these data are typically food supply, total suspended solids, temperature, salinity, and flow velocity. Simulations show that time series are required of at least several years duration because time-dependent processes typically impact simulations on time scales of 1 to 3 years. Model simulations emphasize the desirability of frequent measurements with increasing frequency generating increased accuracy of model results. Emphasis on environmental data collection should focus on:

- ◆ simultaneous measurements of all environmental factors at locations pertinent to the modeled population; and
- ◆ identification of variables best describing food supply.

Disease: Disease models are usually two-component models. One component simulates disease intensification and is usually important for diseases in which the disease organism can reproduce in the host. The second component is the transmission component by which uninfected hosts become infected. Research has focused on the intensification component of disease and this is generally well-modeled. The process of transmission is poorly known and requires epidemiological studies directed at the:

- ◆ local, within-population, processes affecting transmission and susceptibility;
- ◆ large-scale, between-population, processes affecting disease introduction into virgin host populations.
- ◆ importance of flushing time in modulating transmission rates; and
- ◆ epidemiological studies for model formulation and verification (reference Box 7 methods box).

Contaminant monitoring: Bivalves are used today as sentinel organisms to monitor long-term changes in contaminant loadings in estuaries. The processes of contaminant uptake and loss, which control body burden, is affected by climate cycles through such physiological processes as feeding and reproduction (e.g., Wilson et al., 1992). Development of models describing this process requires:

- ◆ information on the influence of environment and
 - ◆ physiology on contaminant uptake and loss.
- We need focused research efforts in basic shellfish ecology and physiology to repair the deficiencies in knowledge of shellfish that limit the accuracy and applicability of shellfish models. The most recent 5-year phase of shellfish modeling has taxed the state of knowledge in a number of fields including physiology, ecology, genetics and environmental monitoring.

FOOD

What exactly constitutes food for bivalves is one of the most enduring questions in shellfish biology. It is of great practical importance since the energy content of the food is a major determinant of the success or failure of shellfish populations and aquaculture businesses. A major component of the food is certainly phytoplankton, as measured by chlorophyll *a*. However, modeling studies have consistently shown that the use of chlorophyll as an indicator of food is inadequate to support observed shellfish growth and feeding studies have consistently observed differences in growth and, often, survival using foods of varying food quality. Particulate lipid, carbohydrate and protein measurements have consistently resulted in improved simulation accuracy and models based in the biochemical conversions of these key food components have been shown to offer promise in producing simulations of improved accuracy and of wider applicability.

- We need a strong theoretical base in shellfish biology and ecology that can provide a basis for formulating the next generation of shellfish models. Theoretical studies have provided the underpinnings in such fields as human and mammalian disease epidemiology and finfish fisheries management, but the field of shellfish modeling has developed without this same grounding.

NEED FOR THEORETICAL MODELING

The development of modeling as a component of shellfish research has improved from the use of, sometimes sophisticated, correlation-based models to simulation models that permit forecasting. In basic ecology, in the fields of human and mammalian disease epidemiology, and in the control of agricultural pests, theoretical modeling has played an important role. Theoretical models permit the development of relatively simple basic principles that describe important relationships. One of the most famous is the development of a basic theoretical model for human and mammalian diseases that describes the initiation and cessation of epidemics using the base reproductive number, the number of new infections expected from a single infected individual. When this number drops below 1, the disease becomes locally extinct and that relationship can be used to maintain animal densities at levels that do not promote the disease process. A theoretical component to shellfish modeling has not developed. Theoretical work is urgently needed in several areas:

- ◆ disease modeling of the conditions leading to local extinction of parasites based on the application of the base reproductive number concept;
 - ◆ fisheries management models permitting rapid evaluation of the biomass at maximum sustainable yield (B_{msy}), population carrying capacity and yearly catch allocations;
 - ◆ development of the carrying capacity concept for diseased populations;
 - ◆ maintenance of disease resistance and other preferred genotypes within wild populations or after introduction of new genotypes into wild populations;
 - ◆ the competitive interaction of disease organisms as it determines relative virulence.
- We need to move into a phase of sustained model development of community-based general models that can be adapted to specific species, regions and specific space and time scales. This will dramatically increase the flexibility and applicability of models while minimizing the cost of development by eliminating much of the repeated development of core model codes.

COMMUNITY-BASED MODELING EFFORTS

In the late 1960s the physical oceanography community made a decision to produce a general circulation model that could be used to simulate the flow in the world ocean. The outcome of this commitment was the Geophysical Fluid Dynamics Laboratory ocean general circulation model (Bryan, 1969; Bryan and Cox, 1972), which was the first of several ocean general circulation models (OGCMs) that are now freely distributed within the oceanographic community. The current generation of OGCMs are available via web sites, come with extensive documentation, and graphics output and diagnostic packages. The community approach has made models accessible to researchers who have not traditionally used models, and has allowed the different OGCMs to be tested in many more environments under a wider variety of conditions than would be possible by a small group of investigators. Free access to the OGCMs has also resulted in careful scrutiny of the model dynamics by a much larger community which in turn has improved model capability and has resulted in the development of OGCMs that are moving towards forecast capability. More recently, the biological oceanographic community has started a similar trend towards community model development that was stimulated by the availability of the Fasham et al. (1990) biogeochemical model. The community-based modeling approach has been of enormous benefit to the oceanographic community. It has allowed empirical and theoretical studies to advance together

thereby improving overall integration and understanding. A similar effort is needed within the shellfish community. Such an effort will require a long-term commitment from federal and state funding agencies.

- The movement of models to the broader community requires extensive documentation of the model codes (e.g., user manuals), long-term maintenance of web sites where the documentation and model codes are available, and long-term maintenance and archiving of model versions. This has been successfully accomplished in other disciplines (e.g., physical oceanography).

CHALLENGES FOR MODEL APPLICATION

Shellfish disease

Diseases have been the focus of significant research since at least the late 1940s. Modeling efforts recently completed and now underway focus on the effect of diseases on the dynamics of oyster populations and in particular on understanding the factors that trigger and then terminate epizootics of these diseases (Figure 3). Models now exist that allow investigation of Dermo (Hofmann et al., 1995; Powell et al., 1996) and MSX (Ford et al., 1999; Paraso et al., 1999; Powell et al., 1999) disease effects on eastern oyster populations.

These modeling efforts have made good use of the large body of physiological measurements that have been made for the host and for the disease organisms. Model development efforts have suggested some areas in which additional physiological studies would be beneficial, but overall the development of models to study host-disease interactions has not been limited by lack of information in this area. This is not the case for disease transmission where significant empirical studies must coincide with further model development.

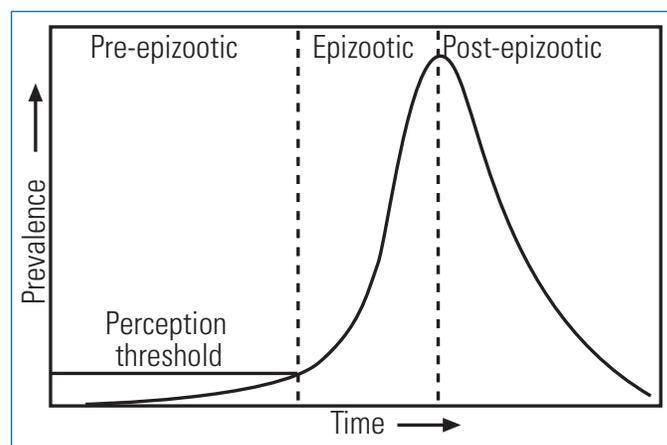


Figure 3. Schematic showing the phases of a disease epizootic, including the pre-epizootic phase when the existence of the pathogen is unknown. The factors that trigger an epizootic occur during the pre-epizootic phase which emphasizes the need for continuous monitoring of shellfish populations.

Methodological recommendations for epidemiological studies

For epidemiological data to be valuable and of use for calibration and verification of models of shellfish disease requires:

- ◆ tracking of individuals;
- ◆ sampling of size frequencies within populations;
- ◆ collection of coincident environmental information, including temperature, salinity, food supply and suspended sediment;
- ◆ sufficient space and time resolution, e.g., monthly versus seasonal samples;
- ◆ use of a uniform, semi-quantitative or quantitative scaling for disease prevalence and intensity; and
- ◆ use of a uniform methodology, such as tissue sections versus blood samples.

The majority of existing epidemiological studies for shellfish populations have not met these criteria and are of limited use.

With the increase in fishing pressure, the expansion of shellfish aquaculture, and the advent of climate warming, diseases have increased in their impact on shellfish populations. Recent predictions all point to a continuing increase in disease pressure in the coming years (Harvell et al., 1999). Adapting to this new age of disease prevalence will require increased application of disease models in problem solving. Improved disease models are required for:

- epidemic forecasting;
- evaluation of shifts in virulence and susceptibility;
- optimization of farming practices, including transplanting, crop rotation, density management, and harvest management;
- investigation of the dynamics of disease transmission and disease range extension;
- optimization of the introduction of improved genetic stocks;
- evaluation of strategies to modify genotype frequency in wild populations and low-intensity aquaculture.

MANAGEMENT STRATEGIES FOR COMMERCIAL SHELLFISH POPULATIONS

All commercially-exploited shellfish populations are managed through state and/or federal regulatory agencies. These management plans vary in their sophistication and the state of the commercial fishery varies in the demands it makes on the management plans. Over the past decade, through overt shifts in policy, and through necessity imposed by demands of the fishing industry, management programs have relied more and more heavily on fisheries models. These models are of two types, those that seek to estimate maximum sustainable yields and those that seek to forecast population dynamics. The two approaches are inherently different enough that one normally does not suffice for the other. No effort has yet been made to couple these two approaches into one holistic management model. The Magnuson-Stevens Fisheries Conservation and Management Act requires that fisheries be managed on the basis of the relationship between the stock size and the population carrying capacity.

Fisheries Management

Federal fisheries are managed under the Magnuson-Stevens Fisheries Conservation and Management Act. This statute requires that commercial species be managed for maximum sustainable yield. Ecological theory relates maximum sustainable yield and the carrying capacity of the environment by the relationship that the biomass at maximum sustainable yield is one-half of the carrying capacity, K . Carrying capacity is normally defined as the abundance of a commercial species that was present prior to the initiation of fishing activities; that is, the abundance that can be supported by the environment under pristine conditions. We rarely have information about population abundance prior to fishing and we believe that the carrying capacity varies with climate change.

It is the task of a fisheries model to (1) estimate K ; (2) derive from that B_{msy} ; and (3) carry out a forward prediction, usually of one year, that estimates the allowable yield to the fishery for that period of time. Fisheries models have been developed for finfish. No fisheries model has been developed specifically for a shellfish species. In addition, no fisheries model today includes disease and its impact on K .

This approach is likely to continue and to slowly expand in its purview to state-managed shellfisheries. The current models used to manage shellfisheries often work poorly, due principally to the history of fisheries model development that has stressed finfish, rather than invertebrate

animals, which influences the types of data that are collected. Models used to manage shellfisheries must better fit the life history of the animal. In addition, disease-based maximum-yield models for shellfish populations are urgently needed as more and more fished populations succumb in some measure to the influence of disease.

Population dynamics models are also needed because issues related to density-dependent growth cannot be answered by the classical fisheries model of today. This is especially important in moving towards area management to optimize yields on a geographically intensive basis. Initial forays into this area, the ocean scallop fishery on the east coast being a prime example, have proven successful, and many states, throughout most of their history, have used area management approaches for oysters.

Eventually, combined fishery and population dynamics models will be needed to help understand how to effectively conduct area management, e.g., crop rotation, for shellfish populations. Models provide an efficient and cost-effective means for evaluating different strategies. These combined models will also be able to take a longer view and include the potential effects of climate change. Much recent work has shown that reproductive potential, disease prevalence and basic physiological processes of shellfish populations are moderated by long-term climate effects, including climate warming.

Challenges for the development of the next generation of fisheries management models include the following:

- development of shellfish models for fisheries management plans;
- development of carrying capacity-based disease models;
- development of improved stock monitoring programs. Stock assessment all too often limits modeling by limiting the validity of the data set describing the population; limitations include the trade off between random and fixed sampling approaches and the need to develop unbiased means of establishing strata in stratified-random sampling approaches;
- forecasting effects of management policy changes including variations in allocation, fishing practices such as changes in dredge efficiency, area management options, establishment of brood stock sanctuaries and transplantation techniques;
- maximization of yield per individual. Most fisheries fish for biomass but impact the population by taking individuals; thus maximizing biomass per individual can increase profits and also reduce population impact; and
- evaluation of climate change as it influences broodstock-recruitment relationships and surplus production.

Optimization of aquaculture

Aquaculture continues to expand in the United States and a premium continues to be placed on the application of intensive farming practices to improve yields per unit area and to reduce costs by improving efficiency. Evaluating farming options places unique and specific burdens on models, but their use is crucial in permitting the examination of the widest variety of options without costly empirical experiments.

Models must be developed to test scenarios that can be implemented to optimize:

- hatchery yield, including evaluation of different food qualities, feeding rates and growing densities, and nursery utilization of water flow and food;

- grow out strategies, emphasizing the evaluation of optimal planting densities and plot sizes and/or shapes;
- transplant techniques, emphasizing timing and location and predator control; and
- genetics, emphasizing the evaluation of the economic value of improved genotypes.

In addition to intensive aquaculture, a number of shellfish species are farmed with low-intensity approaches that depend upon natural set. Optimizing the use of natural set is key to this approach. In addition, the advent of improved genotypes through genetics will put additional emphasis on evaluating the benefit of changing from natural to hatchery-based seed. Models that include an economic overlay will be necessary to address these issues.

Ecosystem Management

The populace is placing more and more value on ecosystem management. Key decisions will be made that will affect shellfish populations. Shellfish, unlike most other marine taxa, are also viewed as tools for improving ecosystem quality. This places shellfish at the hub of the decision-making process and imposes critical requirements on the use of models to evaluate potentially costly options designed to improve ecosystem function.

For example, an important aspect of shellfish management is the determination of strategies that make optimal use of existing habitat and designing approaches for restoration of habitat that will support shellfish populations. Associated with management of existing and proposed shellfish habitat are issues related to the quality of the water that will affect these areas. For example, changes in nutrient loading and turbidity in a given region will affect food supply and subsequent growth of shellfish populations. The creating and rehabilitation of habitat, the siting and design of reefs, and actions that result in changes in water quality are potentially expensive propositions and therefore need careful advance consideration and planning. Mathematical models provide cost-effective frameworks for testing the effects of different strategies and designs. The type of model needed for these studies, however, differs from what has been previously discussed in that a fully-coupled environmental and biological model is needed in addition to the model of shellfish population (adult and larva) dynamics. The environmental component of the needed model includes a full hydrodynamic component that may often need to be interfaced with a sediment transport model. The biological components must also contain a model of the lower planktonic trophic levels which has sufficient detail to allow evaluation of the effects of changes in nutrient loading on shellfish food supply.

Specific challenges facing models for ecosystem management include:

- evaluation of the effects of proposed measures to improve water quality, particularly nutrient loading and total suspended solids reduction;
- use of shellfish to render desired changes in ecosystem trophodynamics, typically by improving substrate stability and water clarity and thus facilitating the development of submerged aquatic vegetation and demersal fish components;
- habitat restoration/rehabilitation designs and locations maximizing impact per unit cost; evaluation of proposed changes in physiography, including channelization, spoil disposal, and reef creation; and
- use of reef creation to generate desired changes in hydrology through manipulation of physiographic structure.

Environmental Monitoring

Various species of shellfish have been shown to be useful indicators of contaminant loading in the environment. As a result, considerable funds have been allocated to set up and maintain contaminant monitoring programs using shellfish.

Shellfish as indicators of environmental quality

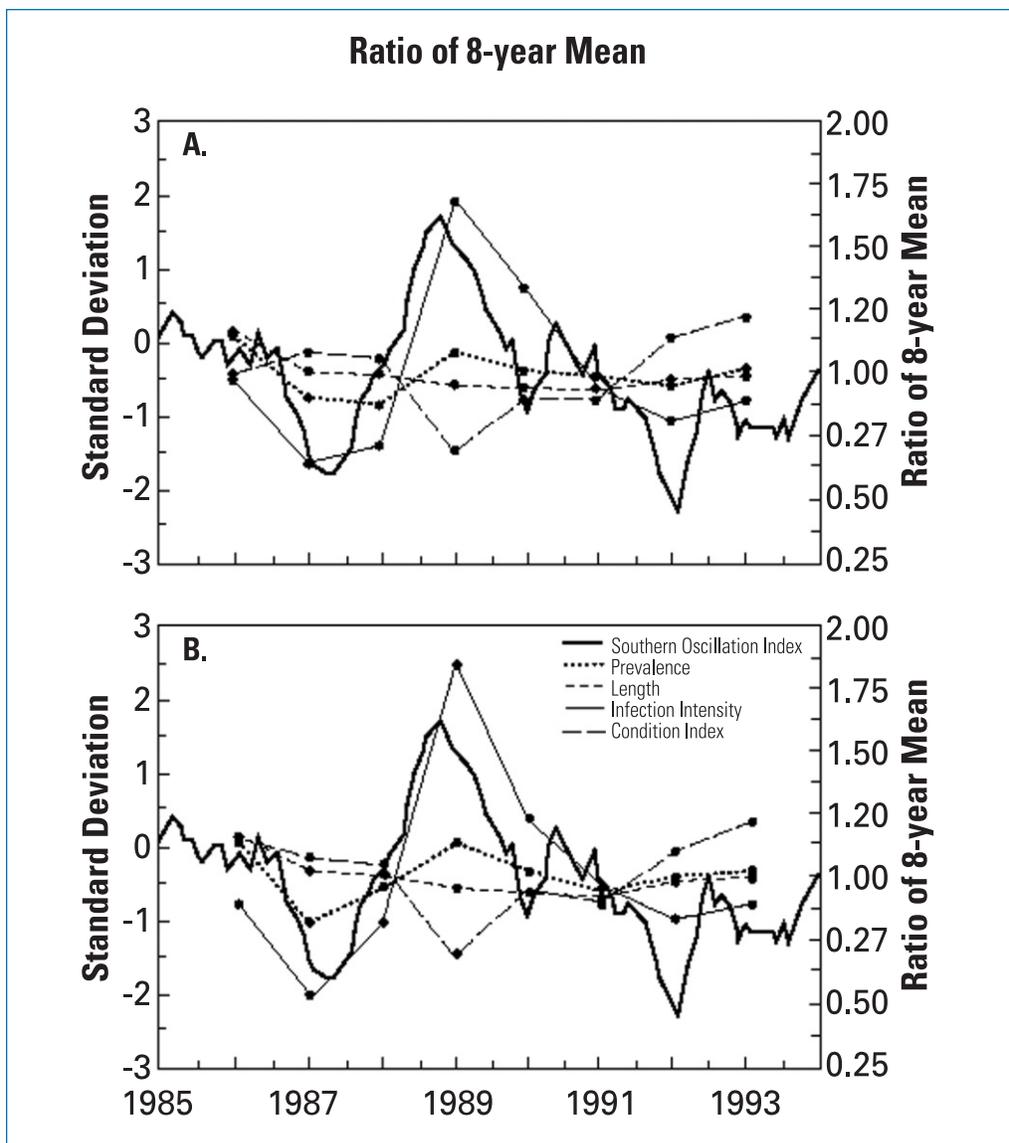
Stationary filter-feeding bivalves accumulate a broad suite of contaminants including heavy metals such as lead, cadmium and zinc, polynuclear aromatic hydrocarbons such as naphthalene and chrysene, pesticides such as DDT, dieldrin, and chlordane and PCBs. As shellfish feed, they take up contaminants, mostly associated with their food, and concentrate them in their bodies. Measurement of the amount of contaminants in their tissues has been used to evaluate the water quality of the estuary in which they live and to monitor long-term changes in that water quality. In the United States, bivalves have been used as monitoring tools by the National Oceanic and Atmospheric Administration Status and Trends Mussel Watch Program since its inception in 1986. This program monitors contaminant water quality in all of the significant bays and estuaries of the United States (Figure 4). In addition, analyses of bivalve tissues are often used to evaluate the extent and severity of contamination after point source introductions that occur, for example, from oil spills or catastrophic containment failures during floods. Analyses of Mussel Watch data have revealed time-dependent patterns linked to climate cycles, probably originating from the influence of climate on body mass, spawning frequency and food source and feeding rate. Simulation models are required to describe the influence of physiology on body burden in order to evaluate the significance of changes in body burden as indicators of changes in water quality.

The processes and mechanisms that underlie the observed variations in contaminant body burdens are not well understood and, so, evaluation of temporal and spatial variations in body burden is weakly grounded in theory and, in practice, interpretable only with great difficulty. To date contaminant loading in shellfish has been investigated primarily through empirical and correlative studies (e.g., Phillips, 1976; Phelps et al., 1985; Wilson et al., 1992). Models with predictive capability or models with the ability to test ranges of possible environmental and biological effects remain to be developed. Such models are needed to separate the potentially-important impact of changing host physiology in determining changes in body burden. The development of models that can be used to describe, evaluate and forecast contaminant body burden in a variety of shellfish species, in a range of environments, is urgently needed. The same model, properly formulated, would expand the abilities of the shellfish sanitation program by permitting a better understanding of the accumulation of potentially significant human disease organisms in commercially-exploited shellfish populations.



Figure 4. Map showing distribution of benthic surveillance and mussel watch sites that are included in the National Oceanic and Atmospheric Administration Mussel Watch program (<http://ccmaserver.nos.noaa.gov/NSandT/NSandTMWsites.html>).

Figure 5. Time series of the standard deviation of the Southern Oscillation Index (SOI), calculated as the five-month running mean of the difference between the standardized sea level pressure anomalies at Tahiti and Darwin, and mean condition index, length, and *Perkinsus marinus*, causative agent of Dermo disease, prevalence and infection intensity, calculated for *Crassostrea virginica* populations from the Gulf of Mexico for the period 1986 to 1993 for A) the entire Gulf of Mexico and B) only the northern Gulf of Mexico. Increases in Dermo disease prevalence and intensity are associated with positive SOI anomalies, which are indicative of the occurrence of an El Nino event. Figure adapted from Kim and Powell (1998).



Climate Change Issues

Each of the previous subject areas describes issues that will be directly impacted by climate change. Climate affects the most basic environmental parameters of temperature, salinity and indirectly food supply and these factors directly determine many facets of shellfish physiology. The current trend towards a warming climate has serious implications for shellfish and disease effects on shellfish populations. The last decade has produced an increasing understanding of the importance of climate cycles in shellfish production and health.

Climate change and climate warming

El Nino cycles modify temperature and precipitation throughout most of the continental United States. Shellfish are particularly susceptible to warmer than average winter temperatures and droughts which increase bay salinity. Models provide a means of determining the likely effects of these environmental changes on shellfish diseases such as MSX and Dermo and, in combination

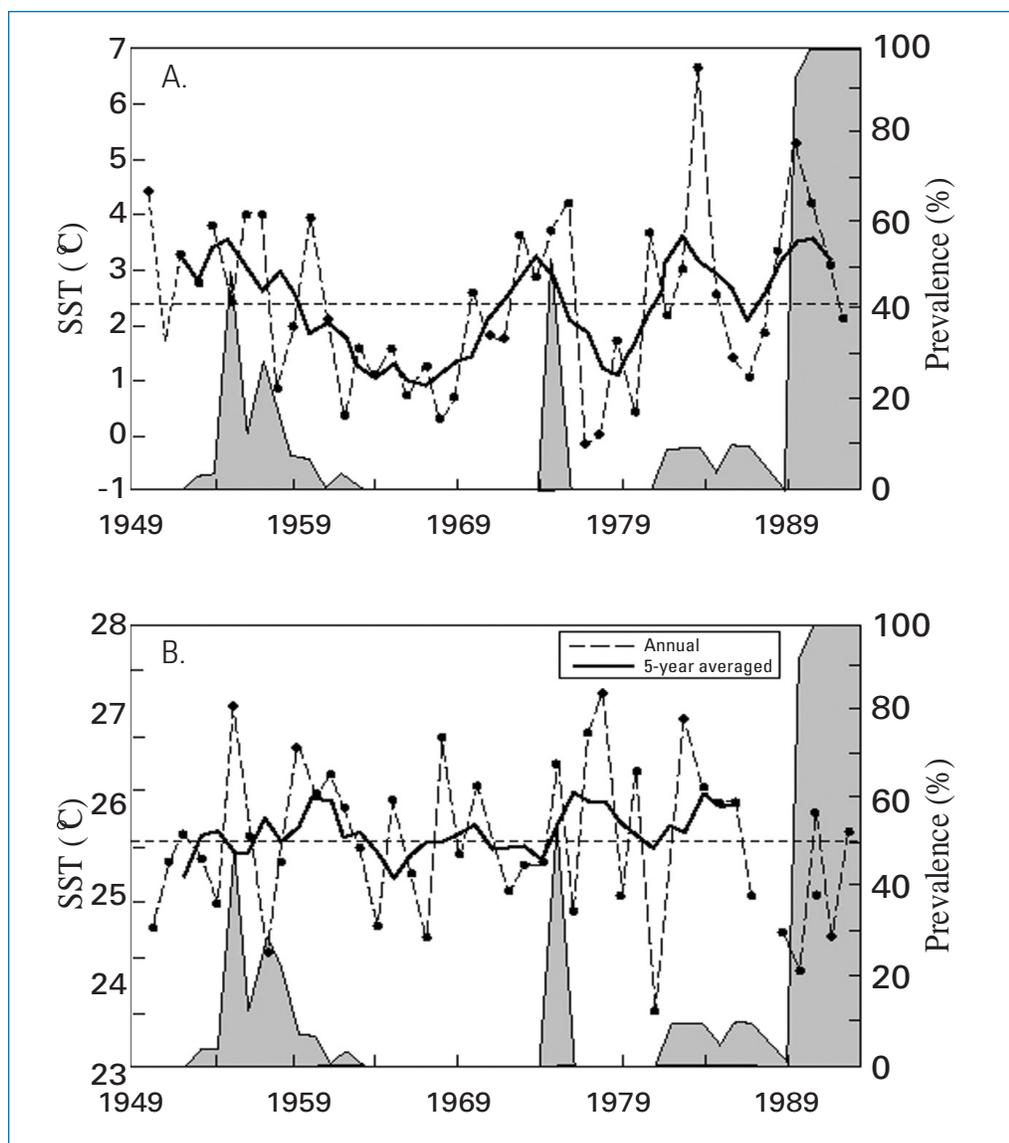


Figure 6. Annual (dashed line) and five-year averaged (solid line) sea surface temperature measured at Bivalve, NJ for A) February and B) August. Superimposed on the temperature time series are the prevalence and intensity of *Perkinsus marinus* in Delaware Bay oyster populations (shaded area), as reported in Ford (1996). The horizontal dotted line represents the mean sea-surface temperature of 1950-1994. The time series show a correlation between increased Dermo disease prevalence and intensity and increased winter temperatures. Figure adapted from Cook et al. (1998).

with climate models, offer the possibility of forecasting disease epidemics (Figure 5). Climate warming is predicted to increase winter temperatures. Milder winters favor diseases and permit diseases to extend their range northward, thereby infecting virgin populations. Recent northward progressions of Dermo and MSX disease in oyster populations are thought to be a product of climate warming in the northeast United States (Figure 6). As climate cycles and climate warming become better understood, model predictions from coupled climate/disease models will be used to modify fisheries management programs and aquaculture farming practices to minimize the effects of changes in climate on production.

We can anticipate that issues associated with climate change will impact nearly all of the areas challenging model development in the coming years.

Particular emphasis will be placed on long-range forecasting capability and this will require the coupling of shellfish models to the sophisticated climate models available today.

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Chapter 8

Protection, Rehabilitation, Restoration and Enhancement of Shellfish Habitat

INTRODUCTION

Shellfish populations and associated communities in coastal habitats provide numerous human services, including food production and important ecological functions. Many of these habitats face ongoing threats from disturbance posed by water quality degradation (contaminants, harmful algal blooms (HABs), suspended sediments and eutrophication), loss or increase of vegetation and alternative habitat uses (e.g., recreation and navigation). Further, the use of these habitats for one or more of these human services may alter its value for other uses. Anthropogenic activities, including the direct and indirect effects of fishing and boating, loss or increase of vegetated habitats, the introduction of non-indigenous species, aquaculture operations, and the maintenance of harbors and waterways, alter physical and biotic components of shellfish habitats.

HUMAN SERVICES PROVIDED BY SHELLFISH HABITAT

1. *Harvest of shellfish*
 - a. *Commercial*
 - b. *Recreation*
 - c. *Aquaculture*
2. *Benthic-pelagic coupling, materials processing*
3. *Habitat*
 - a. *Support economically important fish and crustaceans*
 - b. *Enhance species biodiversity*

In recent years there has been a growing discussion in the U.S. about the need to “restore” shellfish habitat in an effort to recover, maintain, or enhance one or more of the services outlined in the box above. In the case of habitat degradation associated with contaminants, HAB’s, or eutrophication, shellfish habitat restoration has a clear meaning. Reduction or elimination of the stressor can result in more of these services. In other cases, the emphasis of some services may be at the expense of others (e.g., harvest versus ecological functions). Still other cases exist in which attention to the restoration and maintenance of critical habitat features (e.g., complex reef habitat for oysters) is critical to support both fisheries production and ecological services. Often the objective of intervention is to enhance one of these services beyond historical levels—for instance seeding for recreational harvest or the development of intensive aquaculture. In fact, we suggest that *restoration* is an inappropriate term in many instances and should be replaced by a hierarchy of terms dependent on the level of intervention.

We emphasize that **protection is easier than restoration** and that efforts to restore habitats and associated populations are futile if the disturbances responsible for the degradation continue unabated. In some cases active restoration of shellfish habitat may be warranted following rare, stochastic events (e.g., restoration of *Crassostrea virginica* habitat in Louisiana after Hurricane Andrew; Perret et al., 1999). For other recurring or ongoing disturbances, such as the loss of scallop habitat in Great South Bay, NY, to brown tide, there is little basis for attempting to physically restore habitats or shellfish populations in the face of continued disturbance, unless there is a parallel effort to reduce the disturbance. Thus, the first need with respect to shellfish habitat restoration is to recognize the nature of the disturbance, clarify specifically how this disturbance affects shellfish species and ecological functions of interest, and seek means of reducing that disturbance.

Definition	Ease of implementing/cost
Protection - Reduction or management of anthropogenic disturbance	Relatively easy and cheap
Rehabilitation - Repair of anthropogenic disturbance to recover desired ecosystem services	Difficult, often expensive*
Enhancement - Intervention to increase specific human services associated with shellfish habitat, e.g. stock enhancement or private aquaculture	Variable difficulty and expense
Restoration - Return to some past condition	Exceedingly difficult, often undefined and prohibitively expensive*

*Costs may be dependent upon the rate of implementation.

A return to past conditions is rarely possible. As indicated above, reconstructing past conditions is often not reasonable, owing to economic, ecological and societal constraints. A good case in point is the numerous social and technical issues surrounding attempts by State and Federal programs directed towards rebuilding oyster populations in the Chesapeake Bay. Clearly, the goal of oyster reef restoration in the Chesapeake Bay cannot be a return to late nineteenth century harvest levels, which were unsustainable (Kennedy and Breisch, 1981; 1983), but rather to achieve a sustainable balance of fisheries production and ecological services. Likewise, restoration of native oyster fisheries on the U.S. West Coast, where a shellfish aquaculture industry is based largely upon exotic species, is not a reasonable proposition. We must ask the critical questions about what fisheries- and ecosystem-related functions of the habitat have been degraded and what can reasonably be rehabilitated. Clearly, if the disturbance was associated with overexploitation of the shellfish, restoring former fisheries production levels will require enhancement of the resource. Coastal and near-shore ecosystems are increasingly highly managed systems subjected to numerous uses and impacts, as well as long-term climatic changes. With regard to shellfish habitat, we must be quite specific about those habitat functions which society most values—be they supporting recreational or commercial shellfish harvesting, private aquaculture, finfish production and/or other ecosystem services—and target recovery efforts accordingly. Achieving more than one of these outcomes in a particular region will require management of a mosaic of habitats. **Rehabilitation of specified ecosystem services is usually the goal of recovery efforts.**

Rehabilitation of lost human services may be achieved by means other than replicating past conditions. For instance, a non-indigenous species may serve the goal of commercial production following the demise of a native shellfish species; intensive aquaculture may serve a benthic-pelagic coupling function formerly provided by wild stocks. The most fundamental need in the future for the preservation and restoration of shellfish habitat will be justifying its value in relation to non-shellfish dependent uses.

STATUS

A. FISHING IMPACTS

It is clear that the direct and indirect impacts of fishing activity on shellfish habitat can have effects upon the status of shellfish populations and their associated services.

In soft sediment habitats mobile fishing gear—dredges and trawls—have been shown to have significant impacts on shellfish habitats and populations. On the continental shelf in habitats ranging from the North Sea (Jennings and Kaiser, 1998) to Georges Bank (Watling and Norse, 1998), the Gulf of Maine (Pilskaln *et al.*, 1998) and the Gulf of Alaska (Freese *et al.*, 1999) disturbance of the seabed from mobile fishing gear not only reduces the standing stocks of resident benthos (including shellfish), but alters the habitat.

In inshore shellfisheries, traditional “clam digging” in some areas has been replaced by towed and hydraulically operated dredges. Both the scale and the intensity of these harvest methods have the potential to alter shellfish habitat. In Virginia, the intensive use of heavy towed dredges to fish for both blue crabs and hard clams alters the structure of sedimentary habitats (Luckenbach and Wesson, 1996), but the long-term effects on shellfish populations have not been studied. Adverse effects of hydraulic clam harvesting in Washington State (Vining, 1978) has resulted in most clam harvesting there being restricted to hand rakes. Lenihan and Peterson (1998) have shown that oyster harvesting with dredges in North Carolina estuaries has resulted in significant degradation of reef habitats, reducing oyster survival.

For soft-sediment shellfish habitats that have been altered by fishing activity, active rehabilitation or restoration on larger scales may be impractical simply because of the volumes of sediment involved. In such cases protection must be the primary mode of recovering lost services of the habitat. Since the major dredge fisheries in some regions are specifically targeting shellfish stocks (e.g., *Spisula solidissima* and *Placopecten magellanicus* fisheries in the mid-Atlantic Bight), it is important to understand the impacts of the fishing activity on habitat suitability for the shellfish as well as for other members of the benthic community. Towards that end we will need an improved understanding of the frequency and intensity of disturbance in relation to life histories and population dynamics of target shellfish species in order to develop efficacious management strategies (see section on Population Dynamics).

For some areas, stock enhancement may be a useful tool for rehabilitating impacted shellfish habitats and populations. However, this should only proceed after the disturbance causing the original degradation has been controlled (see sections on Aquaculture and Enhancement and Genetics).

In some instances shellfish habitat may be enhanced or created by biogenic structures such as submerged aquatic vegetation and oyster reefs. In the case of the former, above ground vegetation provides important habitat for juvenile *Argopecten irradians* (Garcia-Esquivel and Bricelj, 1993) and roots and rhizomes provide refugia for infaunal species, including *Mercenaria mercenaria*, from predators (e.g., Peterson 1982). Protection and restoration of seagrass beds has become widespread in the U.S. within the past 15 years, largely because of the acknowledged role of these habitats in supporting fisheries species, including shellfish. Much of the research in this area to date has focused on the habitat requirements of seagrasses (e.g., Dennison *et al.*, 1993; Thom, 1995). To most effectively restore shellfish populations to such habitats it will be necessary to advance our understanding of recruitment limitations (both settlement and post-settlement processes; Peterson *et al.*, 1996) and to develop spatially explicit, species-specific population dynamics models with an emphasis on early life history stages (see Population Dynamics section).

Biogenic reef structures created by the eastern oyster *Crassostrea virginica* serve as important habitat for the oysters, other shellfish and non-molluscan fauna (Wells, 1961; Bahr and Lanier, 1981; Lenihan, 1999; Coen *et al.*, 1999; Luckenbach *et al.*, 1999) and the same may be true for *C. gigas* in the Pacific Northwest. Widespread degradation of oyster reef habitats has occurred largely as the result of fishing activity (Kennedy and Breisch, 1981; 1983) exacerbated by disease, increased sedimentation and water quality degradation in some areas. Rehabilitation of these habitats is currently practiced in varying manners (and with varying degrees of success) through the addition of substrate to reconstruct reef bases (reviewed in Luckenbach *et al.*, 1999), but there are several important research avenues that require further attention. We need to develop a better understanding of how the architecture of the substrate provided as reef bases affects the development of oyster populations and other reef assemblages. Specifically, we need to clarify how the availability and scale of interstitial space, elevation above the seabed, relation to tidal height, aerial extent and position within the landscape of reef bases affect their development. Finally, successful rehabilitation of oyster reefs will depend on developing an adequate understanding of recruitment dynamics (see Population Biology section) to determine whether natural recruitment is sufficient or whether active enhancement efforts are required (see Aquaculture and Enhancement section). If active enhancement is required, then population genetics issues become an important consideration (see Genetics section).

B. EXOTIC SPECIES

Non-indigenous species may be agents of disturbance to shellfish habitats through predation, competition or direct habitat modification, but they may also provide a means of rehabilitating or enhancing specific functions. The introduction of predatory species, such as green crabs to both the Atlantic and Pacific Coasts of North America, has had obvious impacts on shellfish populations (e.g., Ropes, 1968; Quayle, 1969; Grosholz and Ruiz, 1995; Walton *et al.*, 1999). An interesting example of non-molluscan exotic species impacting shellfish habitat comes from the Pacific Northwest where several species of cordgrass (*Spartina*) have been accidentally introduced. Dense, monotypic low salt marsh habitat has developed on formerly open mudflats, resulting in a reduction in available habitat for both native shellfish, such as *Protothaca staminea*, and cultured populations of introduced, but now established species, such as *Tapes philippinarum* and *Crassostrea gigas* (Dumbauld *et al.*, 1997). Also, some examples exist of molluscan shellfish introductions leading to declines in indigenous shellfish species (e.g., the mussel *Musculista senhousia*; Crooks, 1998 and the clam *Potamcorbula amurensis*; Nichols *et al.*, 1990).

Exotic species may be beneficial agents of rehabilitation. They may serve as alternative fisheries species and provide valuable ecological functions in circumstances that discourage rehabilitation of native species (e.g., disease epizootics). The expected beneficial impact must, however, be tempered by evaluation of wider ecological implications from accidental importation of associated species or unanticipated effects of the target species.

Once established as an actively reproducing population, exotic species may be difficult, if not impossible, to control. Thus, adequate monitoring and timely response to their discovery is essential. Interstate and international commerce provide unintended avenues for future invasions. Coordinated efforts to track potential avenues of invasion and reduce impacts are warranted. Additionally, continuing documentation of life history data for non-indigenous species in their new environments, in accordance with International Council for Exploration of the Seas (ICES) guidelines, is needed to improve our ability to predict impacts of invasions.

C. AQUACULTURE

Intensive aquaculture is designed to enhance shellfish production per unit area, but in the process it modifies natural systems by co-opting resources from non-target populations. Shellfish aquaculture often involves the placement of large numbers of shellfish, structures and/or substrates into the environment (e.g., racks, pens, longlines, nets, rafts, gravel and shell). Relatively few studies have been done in North America to examine ecological changes associated with these activities (but see references, primarily from Europe, in the Large-Scale Ecology section). There clearly exists a need to understand the functional similarities and differences between “natural” shellfish populations and those of more engineered aquaculture systems. Some studies have examined these issues on limited temporal and spatial scales (e.g., Simenstad and Fresh, 1995; Everett et al., 1995; Thompson, 1995; Kaiser et al., 1998; Feldman et al., 2000; for a review see Dame, 1996). Kaiser et al. (1998) recently reviewed the available data on the environmental effects of bivalve aquaculture and, while they report both some positive and negative impacts, it is clear that there is much that we do not know. For instance, we need to know more about the role of bivalve aquaculture systems in energy and nutrient flows through habitats (see both Large-scale Ecology and Aquaculture sections). Similarly, details about the role of aquaculture in the provision of habitat for other species need to be elucidated.

Virtually unstudied in North America are the landscape-level effects of shellfish aquaculture on nearshore ecosystems. For instance, we do not generally understand how shellfish aquaculture affects food web dynamics or its role as sources and sinks of recruits in relation to adjacent shellfish populations. Further, we need to better understand how the use of predator exclusion devices affects predator suites within the landscape. Both the temporal and spatial patterns and scales of disturbance associated with harvesting cultured shellfish are likely to differ from those in natural systems and we need to develop a better understanding of their consequences for shellfish habitats. In particular, it will be important to improve our knowledge of how shellfish aquaculture affects regional patterns of biodiversity by further elucidating species-specific interactions with non-target organisms.

D. WATERWAYS PROJECTS

The maintenance and operation of waterways and harbors has impacts on coastal marine habitats that are generally addressed through the environmental impact statement process. Important research issues to support this process include improved understanding of shellfish population dynamics and recruitment (see Population Dynamics section) and the refinement of simulation models (see Modeling section). One response to impacts on shellfish from waterways and harbors projects is to attempt to mitigate those impacts through the movement of wild shellfish, the planting of hatchery-produced shellfish or habitat creation (e.g., Dumbauld et al., 2000). If they are to be successful, these efforts will need to be guided by good population dynamics models and enhancement strategies (see sections on Population Dynamics, Modeling, and Aquaculture and Enhancement).

Large-scale waterway diversion projects (e.g., the Cooper-Santee Basin in South Carolina; lower Mississippi in Louisiana) have the potential for widespread impacts on shellfish habitat and associated populations, such as those documented in the Oosterschelde estuary in the Netherlands by Nienhuis and Small (1994). To fully understand and manage these impacts it will be necessary to integrate knowledge of disease dynamics, population biology, systems modeling, and landscape-ecology.

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Chapter 9

Genetics

INTRODUCTION

The biology of molluscan shellfish, like that of all other organisms, is affected by variation in genes and environment. Understanding, conserving and utilizing natural genetic variation in molluscan shellfish furthers the goals of maintaining healthy natural populations, managing capture fisheries, restoring threatened populations and domesticating farmed stocks. Traditional methods in genetics are being applied to molluscan shellfish in many of these areas. Moreover, conceptual and technological advances in the science of genetics, particularly those derived from molecular biology, biotechnology and genome projects for human and other model and agricultural species, are rapidly transforming biology into an informational science. Applications of genetics and genomics will be a key element in solving problems facing shellfish farmers and managers, that substantial part of society living near coastal ecosystems facing increasing urbanization.

We know a lot about the genetics of molluscan shellfish, though no recent comprehensive review of the scientific literature exists (Bonhomme and Blanc, 1987; Beaumont and Fairbrother, 1991; Gaffney et al., 1996; Longwell and Stiles, 1996). While standard methods of genetics clearly apply to molluscan shellfish, research has uncovered a number of unusual features, which make the genetics of molluscan shellfish fundamentally interesting and which impact how genetics is applied to specific problems.

- The population biology of most molluscan shellfish differs radically from that of terrestrial animals, having more features in common with perennial plants and trees than animals like the mouse or the fruit fly. Ecologically and economically important shellfish have, by far, the highest fecundities among animals. The female oyster produces hundreds of millions of eggs in a lifetime, whereas the female mouse produces tens of eggs and the fruit fly, a few thousand eggs in a lifetime. This enormous fecundity makes possible large variance in reproductive success—a single female oyster can, in principle, stock an entire hatchery or bay—with implications for maintenance of genetic diversity (Hedgecock, 1994). Widespread geographic populations can be connected by dispersal of planktonic larvae, so that natural populations comprise billions of individuals.
- Molluscan shellfish, unlike most terrestrial animals, can live with extra sets of chromosomes (polyploidy) or with additions or deletions of individual chromosomes (aneuploidy), both in somatic tissues and the germ line. Triploids, having three rather than the normal two sets of chromosomes, tend to be sterile, which can be a useful property in aquaculture of native and non-native species.
- Many molluscan shellfish show evidence for substantial hybrid vigor in growth or yield. Hybrid vigor in major crops, like corn, and its utilization by crossbreeding, is the basis of a multi-billion dollar agricultural enterprise and arguably represents the most valuable contribution of genetics to society. In contrast, hybrid vigor is not nearly as important in animal breeding programs, which are typically based instead on selection. Crossbreeding, in addition to selection, is likely to be important in improving the yield of farmed molluscan shellfish.

- Pacific oysters and, by inference, many bivalve molluscs, carry unusually large loads of lethal mutations, which cause substantial deviations from the normal rules of inheritance and probably explain hybrid vigor in growth or yield observed upon crossing of inbred lines. The theoretical and practical implications of this genetic load have not yet been explored, but survival of larvae in hatcheries, which is notoriously low, might be improved dramatically by purging lethal genes from brood stock populations. Distortions of inheritance, which have heretofore impeded the construction of genetic linkage maps, can be avoided by typing early larvae.
- Sex determination in many molluscan shellfish is much less fixed than in most terrestrial animals. Individuals can change sex through their lives or can even be both sexes simultaneously (hermaphrodites). This has practical implications in aquaculture breeding programs, if sexes differ in performance or productivity. Females of the Pacific oyster, for example, are about 15% heavier than their male siblings at harvest.
- Genetics has important applications to the conservation of natural populations and management of captive fisheries, as well as to the improvement and domestication of farmed stocks. In the following sections, the goals, approaches, and tools of applied genetics research on natural and captive populations are illustrated by example. Review of current status exposes research needs and future directions. The predominance of work on oyster genetics, particularly in the area of aquaculture, reflects their importance in fisheries and aquaculture. However, the molluscan shellfish culture industry is highly diversified, and will require extension of the promising progress described for oysters to several other species (mussels, clams, scallops and abalone) in the coming decade. This demands an investment in research capability and careful planning of the general research strategies most likely to achieve breeding goals.

STATUS OF APPLIED RESEARCH

NATURAL POPULATIONS – ECOLOGY AND FISHERIES

Goals

Genetic approaches can play an integral role in the fisheries management of molluscs. Resolution of stock structure facilitates the assessment of management units, the elucidation of recruitment processes, which may provide the capacity to predict larval settlement and design spawning sanctuaries, and all forms of shellfish management that utilize hatcheries, which have the capacity to alter the genetic composition of populations in nature. The role of genetics in stock resolution has been well established, but its use in recruitment studies and the recognition of hatchery impacts on genetic composition of populations has only recently begun.

Approaches

Stock Structure

The mainstay of population genetics is the partitioning of genetic variation into within- and among-population sources. Bivalves have been the subjects of considerable genetic research both in applied and basic sciences. Most of these studies rely on analysis of protein or DNA markers and

more recently on DNA sequence analysis. These methods provide a great capacity to resolve subtle genetic structure of populations and, in many cases, to infer the mechanisms responsible for the development of population structure.

Genetic analysis has been particularly valuable in resolving cryptic stock structure. For example, the blue mussel is one of the most intensively studied marine invertebrates yet it was not recognized until the 1980s that what was thought to be one species is actually composed of at least three species and that additional genetic substructure exists within these species. Genetic analysis likewise resolved taxonomic disputes in Pacific oysters and demonstrated that *Crassostrea gigas* and *Crassostrea sikamea* are distinct species. In other cases commercially important species contain genetically distinct subpopulations. For example, the eastern oyster *Crassostrea virginica* is composed of Atlantic and Gulf of Mexico races that are genetically and functionally distinct (Figure 1). There is recent evidence of genetic differentiation among Atlantic populations of *C. virginica*. Hard clams, scallops and many other species exhibit similar intraspecific variation. Cryptic species are commonplace throughout marine invertebrates (Knowlton, 1993) and previous studies have clearly just hinted at the magnitude of this problem. Genetic analysis will continue to be a major tool in the delineation of stock structure.

Biological differences among stocks can be revealed by “common garden” experiments, an alternative to population genetic analysis of markers whose biological functions are usually not known. Common garden experiments require controlled crosses and extensive field trials, entailing substantial effort and cost. Consequently, few such studies of this type have been made for molluscan shellfish, almost all in the context of stock selection for aquaculture. Perhaps the best example is the retention of local reproductive cycles by geographic stocks of American oysters spawned in a common locality. This was first demonstrated by Loosanoff and Nomejko (1951) at the Milford laboratory, and confirmed over many generations by the stability of differences in reproductive cycles among lines selected for MSX resistance by Dr. Hal Haskin (Barber et al., 1991).

Recruitment ecology

Recruitment dynamics are poorly known, but marine molluscs are notorious for erratic recruitment with geographic and temporal heterogeneity. An understanding of the regulation of recruitment variation borders on non-existent. Certainly, part of the variation is due to post-settlement ecological success, but variation in dispersal dynamics also plays a major role. Traditional ecological mark-recapture techniques have been unsuccessful due to the small size of larvae, morphological similarities among larvae of different species, and the logistic difficulties of recapture following dispersal over large geographical areas.

Molecular genetic techniques, however, can be a powerful approach (and sometimes the only approach) for elucidating the recruitment or dispersal dynamics of marine bivalves, because these indirect methods overcome problems associated with physically tracking small, highly dispersive larvae. In circumstances where genetically differentiated populations occur in close spatial proximity the dispersal of larvae from one distinct patch can be followed (Figure 2). Strong genetic differentiation can occur where two related species co-occur and/or hybridize, or when genetically unique strains or species are either intentionally or unintentionally introduced to an area. In the latter cases the genetic distinctiveness is likely to break down and biologists must be prepared to exploit these opportunities when they arise. Also required are genetic markers that can distinguish

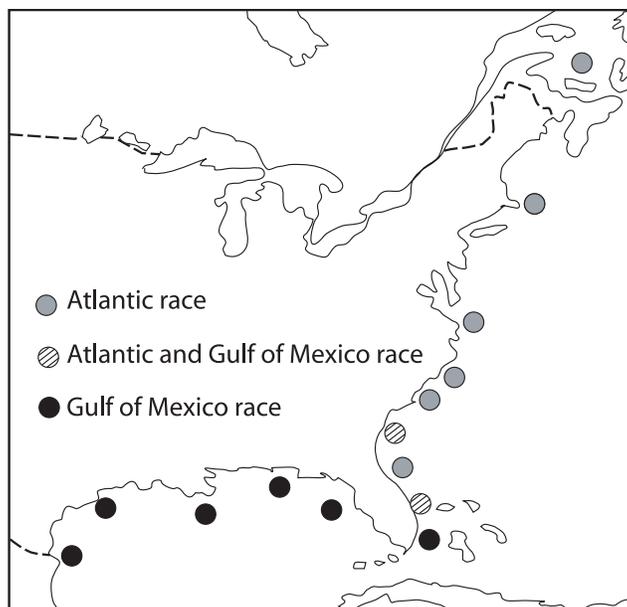


Figure 1. Atlantic and Gulf clades of the American oyster (after Reeb & Avise 1990) defined by differences in the sizes of fragments produced by enzyme digestion of whole mitochondrial DNA. The two races also show differences in susceptibility to Dermo disease.

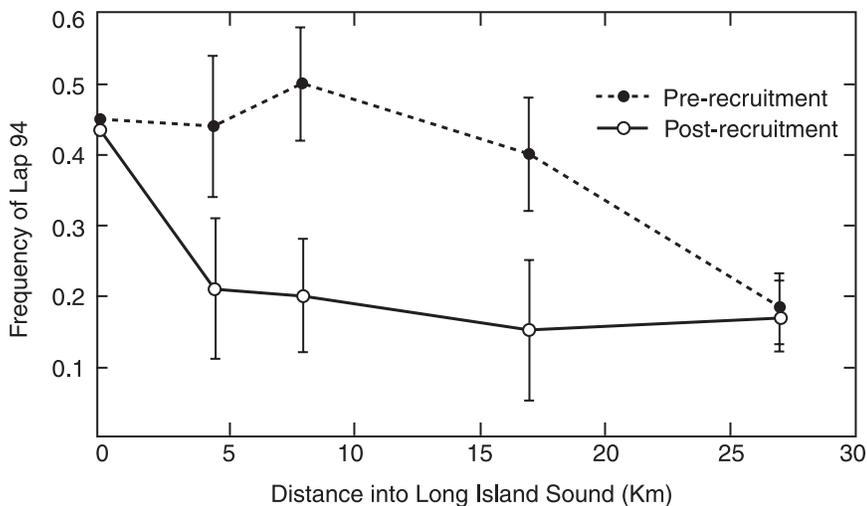


Figure 2. Frequencies of Lap⁹⁴ in adults and larvae of *Mytilus edulis* in Long Island Sound suggest delivery of larvae from oceanic populations, where this allele has a high frequency, followed by strong, clinal selection against this allele.

assayed by PCR from individual larvae have been developed and are being used to analyze the dispersal of larvae across juxtaposed genetic patches.

The opportunity to conduct genetically based recruitment studies is sometimes afforded by the inadvertent introduction of non-native stocks or species. The initial reproductive events following an introduction provide invaluable information about recruitment processes yet funding infrastructure is not well posed to support such opportunistic science. Funding agencies should seek to establish programs, similar to Sea Grant's Rapid Response, which are geared to take advantage of these opportunities.

Several lines of evidence suggest that highly fecund marine organisms, like molluscan shellfish, have substantially smaller genetically effective sizes (N_e) than census sizes (N). Indeed, the ratio, N_e/N , which in terrestrial animals is rarely less than $1/4$, may often be less than $1/100$ or even $1/100,000$, because of large variance in reproductive success. A relatively small minority of spawning adults can, by chance, contribute the majority of individuals in the next generation by happening to match its reproductive activity to oceanographic conditions conducive to spawning, larval development and settlement. Evolutionary genetics studies indicate that long-term average effective size of the world population of some bivalve species may be as low as approximately 1 million individuals. Other studies have shown that genetic variation among larvae or newly settled year-classes implies that the number of breeders producing particular larval cohorts can be as low as 10s of individuals. These results are in strong contrast to expectations based on the census sizes of most marine bivalves, which are many orders of magnitude greater than the estimated effective sizes. Ecological and breeding studies demonstrate a wealth of biological mechanisms that reduce the reproductive success of individuals so that even though thousands of individuals may spawn only a few successfully produce recruits. Nevertheless, very few studies have attempted to assess N_e in natural populations of marine bivalves or the impact of hatchery practices on N_e (see below).

Approaches of this type can be used to answer questions on the regulation of larval recruitment by local hydrodynamics, the scale of larval dispersal and other essential information for developing management plans that protect key areas of larval production and identify areas of high larval recruitment. Recruitment information is pivotal in positioning of spawning sanctuaries or reserves.

Key requirements for the genetic analysis of larval dispersal and recruitment are the availability of suitable genetic markers, collaborations among scientific sub-disciplines and the ability to exploit genetically distinct populations when they arise or to conduct large-scale comparisons of adults to larvae or newly settled juveniles. The development of genetic markers is straightforward and often a valuable by-product of stock assessment studies (see above) and can be rapidly developed as

the progeny of genetically distinct adult populations, which can be assayed on larvae and recently recruited juveniles. Molecular genetic markers that are amplified by PCR is ideally suited for this application, but species or strain markers must be derived on a case-by-case basis.

For example, a study of this type is presently underway to analyze transport of mussel larvae in southwest England. In this case, two species of mussels in the genus *Mytilus* come into contact and hybridize providing genetically distinct patches that are over 100 km in extent and grade into another such patch over distances of only a few km.

Species-specific genetic markers that can be

needed. An information clearinghouse of prospective genetic markers for various species would be valuable to avoid duplication of effort in marker development and can enhance response time. Collaborations, particularly amongst geneticists, marine ecologists, oceanographers, and modelers are important to maximize the information that can be obtained from any given opportunity.

Hatchery Enhancement of Natural Populations

The use of hatcheries to augment natural populations of bivalves is an expanding enterprise to increase recreational and commercial shellfish harvests or to restore fisheries or habitats. Successful enhancement programs have the potential of seriously altering the genetic compositions of wild shellfish populations. Of necessity, such programs use limited numbers of brood stock to produce large numbers of individuals for release into the wild. If a program is successful, then a substantial fraction of the post-enhancement population originates from the hatchery, possibly resulting in the dilution of genetic variation in the natural population. Restrictions in the genetic diversity of a natural population can have a number of severe long-term consequences; the population may become increasingly inbred with a concomitant loss of performance, lose the capacity to respond to environmental challenge, and lose the diversity necessary for successful selective breeding programs. The actual genetic impact of an enhancement depends upon three parameters, (1) the magnitude of augmentation, (2) the effective size of the hatchery contribution to the native stock, and (3) the effective size of the recipient wild population (Figure 3). This information is essential to understanding the genetic risk of enhancement programs and developing appropriate hatchery practices to mitigate these genetic impacts. Unfortunately, we do not know these three parameters for any large-scale enhancement program, but limited information suggests that risks of enhancement on biodiversity need to be assessed. The effective population sizes of most hatchery stocks of molluscan shellfish, for example, are less than 50 (Hedgecock et al., 1992). Contributions of hatchery stocks to natural populations can be extensive; the frequency of the *notata* color morph in the northern quahog clam *Mercenaria* has been dramatically increased in many local populations through hatchery releases of stocks with marked shells. Indeed, a thorough investigation of *notata* frequencies along the Atlantic coast could provide an excellent case study, not only for assessing the impacts of artificial propagation on natural stocks, but also for estimating rates and distances of larvae dispersal.

Molecular genetics can also be used to evaluate the “success” of enhancement efforts. Such assessments are possible because even the best hatchery practices can not produce stocks that mimic wild populations with respect to genetic variation. These subtle differences can be used to estimate the contribution of specific enhancement efforts to subsequent cohorts. An enhancement program using bay scallops, *Argopecten irradians*, is employing such an approach in Florida. The strategies for developing the appropriate genetic markers are well established, but specific markers suitable for evaluating these parameters for any given species and/or enhancement effort may be required.

For example, eastern oysters from the Atlantic and Gulf coasts, while belonging to the same species, show distinct genetic differences at the DNA level. These differences are being exploited in a program designed to evaluate the effectiveness of an oyster enhancement program in the Chesapeake Bay. As part of a larger oyster rehabilitation program, hatchery-produced oyster seed derived from Gulf coast parents were used to seed several sites in a Chesapeake Bay tributary. All of these seed bear a unique genetic marker that is not found in Atlantic coast oysters; when they

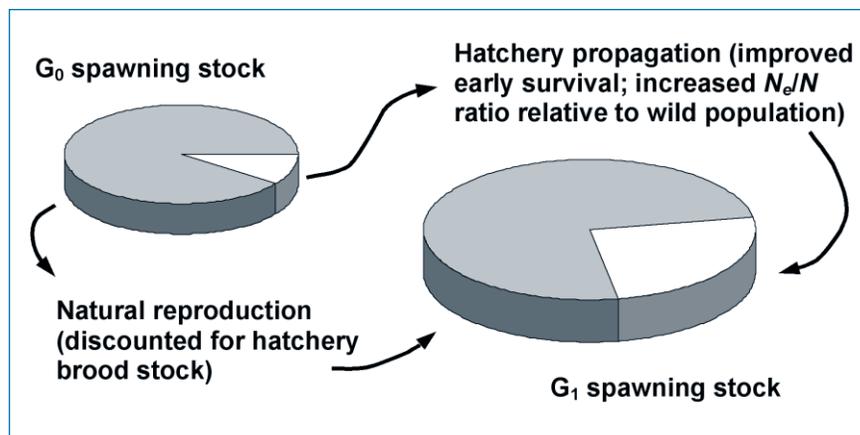


Figure 3. Genetic diversity of a stock can be reduced by artificial propagation of a small fraction of the spawning population at time G₀. If early survival in the hatchery is greater than in nature, as expected, the hatchery stock will contribute disproportionately to the natural population at time G₁. Stock size can increase, as illustrated by the relative sizes of the pies. The genetic diversity of the stock will decrease unless the ratio of effective to actual number of breeders (N_e/N) is greater in the hatchery than in the wild. The consequences of hatchery enhancement need not be negative, but the risk to diversity ought to be evaluated, particularly for highly fecund shellfish.

reproduce, their offspring will also carry this marker. If newly settled oyster spat bearing the unique marker are found in the Chesapeake, this will be direct evidence that the outplanted hatchery oysters were able to successfully survive and reproduce.

In years past, direct evidence on the efficacy of enhancing marine stocks was hard to obtain and interpret. For example, analysis of protein variants in populations of California red abalone suggested that animals from a 1979 outplanting effort by the California Department of Fish and Game were alive in 1992 (Gaffney et al., 1996). This interpretation may not be consistent with the sizes or locations of the individuals sampled, however, and the allozyme data could have resulted from artifacts (R. S. Burton and M. Tegner, pers. comm.). Still, the increased availability of molecular markers is expected to make future studies of this type more robust and conclusive.

Tools

Molecular (genetic) markers

Modern molecular techniques are providing fisheries managers with a powerful array of tools. The most versatile among these are molecular genetic markers, which make use of naturally occurring genetic variation found among individuals and among species. Two features of molecular markers are particularly relevant to their use in fisheries and aquaculture. First, markers are available at any level of resolution desired, ranging from species identification to the analysis of stock structure to the identification of individual families or individuals. Second, in many cases the marker technology is virtually universal, i.e., markers developed for one species may with little additional effort be employed for another species.

For the identification of species, or understanding stock structure within a particular species, a few well-chosen molecular markers are often all that is needed. Several examples will illustrate the power and flexibility of these tools for use in fisheries.

A major use of DNA-based molecular techniques is species identification. By using a tiny snip of tissue, it is possible to determine the species-specific genetic “signature” of an individual, using the technique of polymerase chain reaction (PCR) to amplify target regions of DNA. The potential applications of this technology are numerous, particularly in the fields of forensics and pathology. For example, using a portable DNA analysis system that could be operated in a hotel room, investigators were able to demonstrate that fillets sold at commercial fish markets were often misidentified, and in fact belonged to species not legally harvestable.

Another increasingly important application of molecular markers for species identification is for the detection and tracking of invading or introduced species. In many cases, the first indication of the appearance of a new species comes from a specimen provided by a local observer. Even poorly preserved tissue samples can be used to provide a clear species diagnosis, if a comparative genetic database is available. In some situations, it is even possible to identify the region of origin of the new arrival.

The most widespread current use of molecular markers in fisheries is for identifying the number and distribution of subpopulations. The eastern oyster *Crassostrea virginica* illustrates the ways in which modern molecular methods can enhance our understanding of population structure. The species is distributed from the Maritime Provinces of Canada to the Yucatan peninsula. Because its larval stage may spend several weeks drifting in the plankton, it was long believed that oysters throughout their range would be genetically similar as the result of genetic mixing during the larval phase. Indeed, this view was supported by the first genetic analyses done in the 1970s, which examined genetic variation in proteins. When DNA tools became available, it was discovered that Gulf Coast

and Atlantic Coast oysters were genetically distinct (Figure 3). Recent studies of DNA sequence polymorphisms in the mitochondrial ribosomal gene revealed distinct subpopulations even along the Atlantic Coast. This information has several implications for oyster fisheries and aquaculture. First, transfer of oysters throughout the species range, a common practice in years past, may result in the mixing of genetically distinct oysters, potentially altering the resident gene pool. Although this is not always a problem, it merits serious attention.

For many marine fish and shellfish, the larval stage is ecologically critical: the successful recruitment of the next generation depends on the fate of the larval population. Consequently, fisheries managers are keenly interested in understanding the dynamics of larval pools. In practice, this can be difficult. Species identification at the larval stage is often difficult, and even when it is possible, it is tedious, time-consuming, and requires substantial expertise. By using molecular species markers, it is possible to unambiguously identify even the most nondescript larvae in a consistent, repeatable way. Unlike traditional means of identifying organisms, molecular methods lend themselves to high-throughput automation. For example, automated processing of water samples could allow one to determine the numbers and types (species) of organisms present in numerous water samples collected at various times and places. This approach can be broad-brush, enabling scientists to monitor species diversity in the plankton, or narrowly focused—for example, monitoring daily fluctuations in the larval abundance of a single species. Early detection of aquatic species invasions through periodic “molecular monitoring” of coastal and estuarine waters is another potential application of marker technology that will be increasingly important.

In summary, the rapid development of molecular techniques during the last two decades has provided us with a powerful toolbox to use for various problems facing contemporary fisheries and aquaculture. For the immediate future, one useful development would be the development of a public catalog of such tools, available to interested users. This would reduce the amount of duplicated effort, and through the pooling of information enhance our overall rate of progress.

AQUACULTURE

Goals

The goal for genetics in aquaculture is domestication and improvement of wild species. For agriculture, domestication has been a 10,000-year process, although more sophisticated applications of genetics are only 100 years old. For example, it took corn breeders 90 years to develop modern hybrid corn, which yields approximately five times what outcrossed corn did at the turn of the 20th century. The challenge of geneticists and breeders in aquaculture is to compress this process to a decadal scale. There is some indication that progress will be more rapid in aquaculture. For example, it only took 10 years between the creation of the first polyploid shellfish (1976) and their full commercialization by industry (1986). Fortunately, today there is a huge array of tools available for accelerating the domestication process. On the other hand, the species we are trying to domesticate are totally wild, with very little incipient domestication or vast storehouse of basic genetic knowledge as for agricultural species.

The advantages of domestication are clear. Domestication not only forges strains with improved characters of economic importance but reduces the variance among animals so that crops are uniform. Simply stated, mounting a selection program to exploit heritable variation is most likely to succeed and improve the product. But there are cautionary notes. First, an ill-planned selection program can have a decidedly negative effect, especially where it inadvertently promotes high levels of inbreeding. Second, because breeding is a relatively new endeavor for aquaculture, especially shellfish, and partly because of the unusual features of molluscan shellfish, the “optimum” breeding

approach for shellfish is unknown. In fact, maybe the optimal method varies with species, or even with characters within a species. Is the most effective mode of selection for growth or yield the same, for example, as that for disease resistance? In fact, there are a variety of approaches under evaluation today, and some are described below.

There are some clear recommendations that are universal to any breeding scheme.

- Identify the available genetic stocks and choose the best one for improvement;
- Separate the breeding effort from production;
- Clearly identify the traits or characters to be improved and know their economic value;
- Determine the nature of genetic variation in traits of interest. If heritable, design a selection program. If non-heritable, as in the case of traits showing hybrid vigor, develop inbred lines for crossbreeding;
- Design a long-term, sustainable breeding program and stick to it;
- Multiply the improved stocks for the broadest possible application;
- Develop control populations or methods with which to assess the success of the program (gain).

Approaches

Stock choice – Clam Breeding Project

Begun in 1998, the Clam Breeding Project is testing commercial “strains” of *Mercenaria mercenaria* on the Eastern shore of Virginia, under the auspices of the Virginia Institute of Marine Sciences (VIMS). The initial phase of the project is designed to evaluate the “lines” that have been developed by industry along the entire East coast. Brood stocks have been collected from Massachusetts to Florida and about 10 F₀ spawns of these stocks will be tested head-to-head over the next two years. From there the most interesting, defined by yield, coloration and shelf-life, will be subject to more intensive selection. One of the principle questions to be addressed in this work is to determine the effect of different farms on the same stock of clams, the environment x genotype effect. For example, does clam strain X perform well on all or most farms or does it vary considerably in its ranking across farms, relative to the other strains? The Clam Breeding Project will do its testing on commercial leases in Virginia under realistic grow-out conditions

Ployploidy

Plants and lower animals, such as fish, amphibians, and molluscs, can tolerate the condition known as ployploidy. Ployploids contain more than two sets of chromosomes, the normal condition in sexually reproducing species. Two forms of ployploids have been produced in oysters, triploidy (with 3 sets of chromosomes) and tetraploidy (with 4 sets). Both triploids and tetraploids are produced commercially. In other shellfish—scallops, clams, pearl oysters, geoducks, for example—triploids have been produced for research, but are not yet in commercial production. Triploid mussels are produced on a limited scale in the Pacific Northwest, and triploid abalone have been produced experimentally.

Triploids are useful because they are reproductively almost sterile, or neutered (Allen et al., 1989). Sterility yields several useful features in triploids. First, triploids may attain a greater size due to the redirection of energy from reproduction to somatic growth. In scallops, for example, dramatic improvements in adductor muscle size result. Second, triploids maintain their peak market

condition even in the summer when their diploid counterparts are maturing sexually. Third, triploids will not normally reproduce with each other because the gametes produced by triploids are highly abnormal. This last feature has a number of applications, some realized and some under intense investigation.

Lack of reproduction in triploids might allow aquaculture of non-native species without the attendant risk of naturalization. This avenue of investigation is underway in Chesapeake Bay where native oyster populations have declined drastically in the last 50 years (also see Non-natives below). The enabling technology for “all-triploid” production is tetraploidy (Guo and Allen, 1994). Unlike triploids, tetraploids are fertile, although their gametes have twice as many chromosomes as normal diploid ones. Therefore, tetraploids can be used for parents in a tetraploid x diploid cross to obtain 100% triploid progeny. It is because the progeny are 100% triploid that they can be used for aquaculture in a foreign place, i.e., the Chinese species *C. ariakensis*, for example, might be grown in the Chesapeake Bay.

Aside from the issue of non-natives, triploids might also become quite useful in addressing the commingling of hatchery and wild stocks. That is, where stocking programs have the potential of reducing the genetic diversity of wild populations (see *Enhancement* in the previous section), triploids may provide a tool for obviating this problem.

Finally, the abnormal gametes created in triploids can be exploited to create unique genetic constructs normally not possible in other animals, but of particular use in the dissection of genetic principles for shellfish. For example, triploids have been crossed with diploids to create aneuploids, an organism in which there is an incomplete complement of chromosomes. For example, an oyster containing two full complements of 10 chromosomes (20 chromosome in all) plus an additional chromosome derived from the triploid parent is called trisomic. Some trisomics of oysters have shown properties that ultimately could either be of direct use in breeding programs or pave the way for understanding the genetic organization of the shellfish.

A number of challenges are still in front of us for polyploid technology. Polyploidy is commercialized in only the oyster industry, and at present, only for *C. gigas* on the West coast of the U.S. The potential of polyploidy for clams, scallops, mussels and other molluscs is still unknown. Consistent with the “unusual features” of molluscan shellfish is a phenomenon recently uncovered about polyploids, reversion. Apparently, at least for *Crassostrea* species, the polyploid condition is unstable. In *C. gigas*, *C. ariakensis* and *C. virginica*, long-term studies of triploids have demonstrated the loss of entire chromosome sets from the somatic cells from some triploids. The resulting phenotype of an individual is mosaic, an oyster with a composite of both diploid and triploid cells. Whether reversion compromises the sterility of triploids, which might increase the risk of non-native introductions or large-scale hatchery enhancements, is unknown and under investigation.

Artificial selection

A trait is said to be heritable or to have additive genetic variation when an individual expressing that trait (e.g., rapid growth) passes it to offspring, causing their performance to exceed the previous average of the population. Artificial selection, in which individuals or families are selected for breeding on the basis of their relative performance, steadily enriches a population with genes that improve traits of interest. Selection is the most common type of animal breeding program. Several large-scale selection programs are underway with molluscan shellfish.

Improving yield—The first large-scale selection program for improving yield of farmed Pacific oysters started in 1996, by Dr. Chris Langdon, at the Hatfield Marine Science Center, Oregon State University, Newport, Oregon, funded as a USDA Special Project. This Molluscan Broodstock Program (MBP) was designed by industry representatives and scientists participating in a USDA

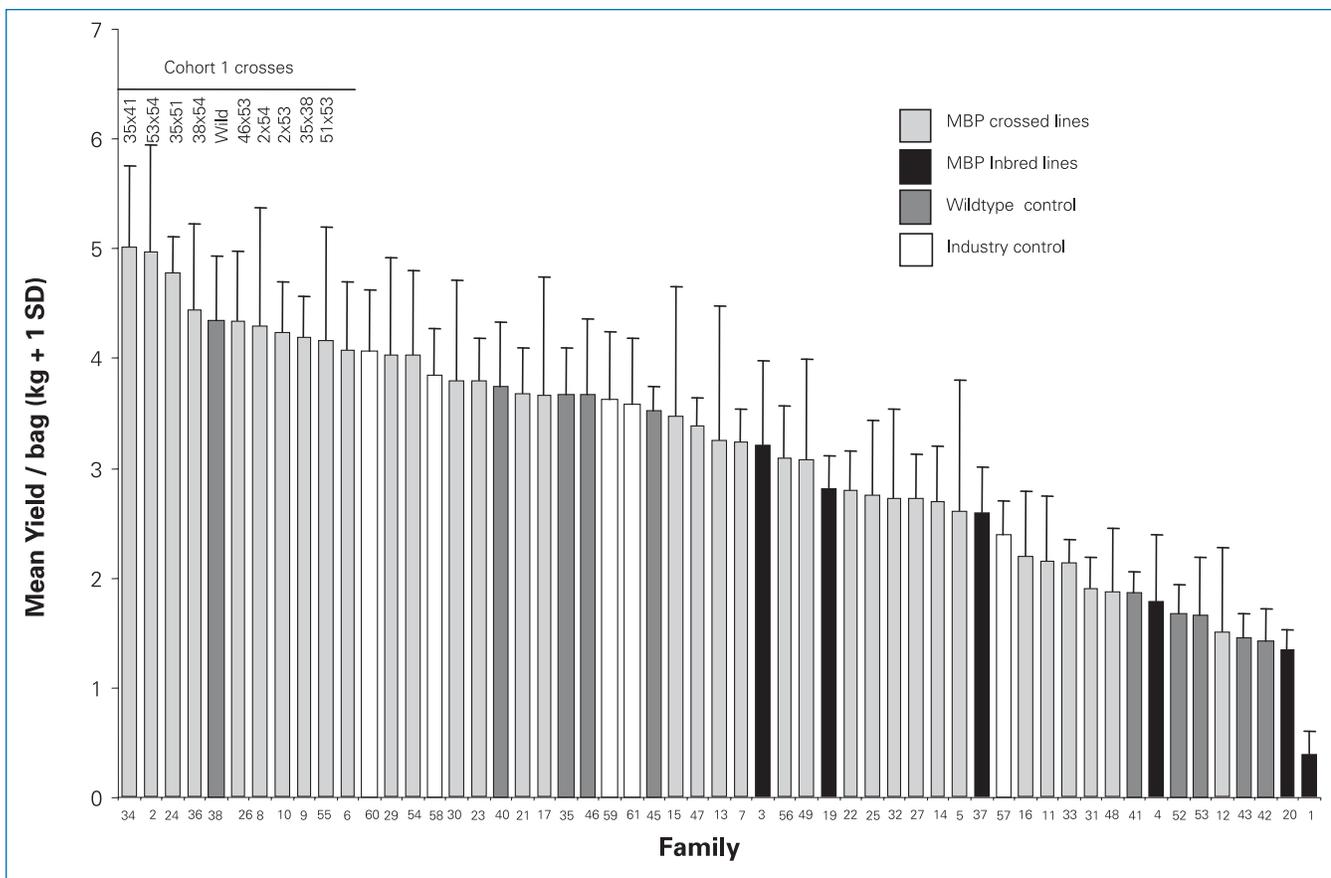


Figure 4. Interim yield for families in the first selected generation of the Molluscan Broodstock Program (family crosses) suggests a response to selection. Selected stock has higher yield than inbred lines made from select stock or wild or industry controls. The significance of the gain in yield will be fully assessed at harvest in 2000.

regional project (WCC-99, Shellfish Breeding and Genetics), and is described in detail by Hedgecock et al., (1996). The overall program is patterned after a highly successful selection program for a strain of Atlantic salmon, although the plant-like features of oyster biology and genetics necessitated several modifications of methods. Like the salmon program, the MBP is expected to evolve eventually from a university-based research project, involving industry partners, to a commercial breeding program.

The MBP focuses on improving the Miyagi strain of the Pacific oyster, which was adopted long ago by the West coast industry after testing of several varieties from Japan. The initial broodstock was formed by four sets of 50 pairwise matings among oysters obtained from natural populations in Washington and British Columbia. Families are reared separately at all life stages, so that the pedigree of each oyster is known and inbreeding can be avoided. Selection is made among families, whose relative merits are judged from mean and variance of yield (meat weight) for 10 cages at each test site. Seed from each set of 50 families is distributed to several test sites at cooperating commercial oyster farms, which are responsible for managing the 500 cages (50 families x 10 cages per family) per cohort. The first randomly bred generation of families was evaluated across several localities and the families that performed the best across all localities (the “generalist” families) were spawned and crossed in all combinations to produce the first selected generation. An interim measurement of yield at six months of age, for selected and control families grown in South Puget Sound, WA, suggests superior performance by certain select families (Figure 4). MBP will produce the second selected generation in 2000.

Additional features of this selection program are worth mentioning. 1) The MBP broodstock are certified as being a “high health” stock, reducing the risk of spreading diseases as seed are disseminated throughout the industry. 2) Industry is involved in two different ways. First, growers from all West

Coast states provide the testing stations for evaluating family performance. Second, commercial hatcheries are given select broodstock for propagation and wide dissemination of select seed to farmers for testing and marketing. Commercial hatcheries presently lack the expertise and infrastructure for breeding programs, so there is now need for transferring breeding technologies and expertise to industry. 3) At this stage, families with high yield in specific localities or bays (specialist families) can also be propagated by the commercial hatcheries for particular farms, instead of, or in addition to the generalist families propagated by the MBP.

The MBP represents a significant advance in the scale and sophistication of breeding programs for molluscan shellfish. Its status as a Special Project means that it is in jeopardy of losing funding at the completion of each 2 or 3 year funding cycle. It is critically important to demonstrate success to the industry so that they will make the same kind of investment in continuing a breeding program as the Norwegian salmon farmers made. That such breeding programs can and should be initiated for other species is also obvious, but facilities and resources for initiating such programs are not available.

Disease resistance—XB, NEH, DEBY—Selection for disease resistance in *C. virginica* is the most critical goal of oyster breeding on the East coast of the U.S. Currently, there are three lines of disease resistant oysters that are being used commercially, and other lines are still under development. The one enjoying the most widespread testing – under the support of the NOAA Sea Grant Oyster Disease Research Program – is the CROSBreed (XB) strain. The XB strain was derived from a number of MSX-disease resistant strains, some bred for almost 30 years by Dr. Hal Haskin of Rutgers University. In 1992, these resistant lines, which were beginning to show inbreeding depression for growth and fertility, were used to produce a new “synthetic population” by mating the strains among themselves. Along with the XB line, another derived from oysters of Long Island descent was produced and named the Northeast High Survival Resistant Line (NEH for short). The principle reason for creating the synthetic lines from the inbred, MSX-disease resistant stocks was to build a more robust line for the onslaught of Dermo-disease that had invaded Delaware Bay (home to these lines) in 1990. Simply put, XB and NEH lines were under development for disease resistance to both MSX- and Dermo-disease. Under heavy pressure from Dermo disease in their first generation, survival of XB lines were 10 times better than controls (from artificially spawned Delaware Bay and Maine derived brood stock).

XB lines are now being tested in a regional program in Delaware Bay and the upper and lower Chesapeake Bay sites spanning the entire salinity range (Figure 5). In 1998, the XB line was adopted by a commercial hatchery in Virginia. They have by now been distributed widely through this and other non-commercial hatcheries. Some have found their way onto new reefs that have been built in Virginia waters, and the role of these stocks in reef restoration is very much an open issue (see Fisheries, Enhancement). At the same time, another disease resistant line was created by the VIMS and is named the DEBY strain. It too has been “released” to industry. Other disease resistant lines are under development and ultimately, a variety of superior strains will be available to the industry.

Although we have made significant progress in establishing programs for selection of disease resistance, our overall methods are still somewhat primitive. Strains are reared for their entire generation time (3 years) before disease resistance (i.e., survival) can be assessed. Needed are molecular or physiological (see next section) markers indicating resistance at earlier points in the

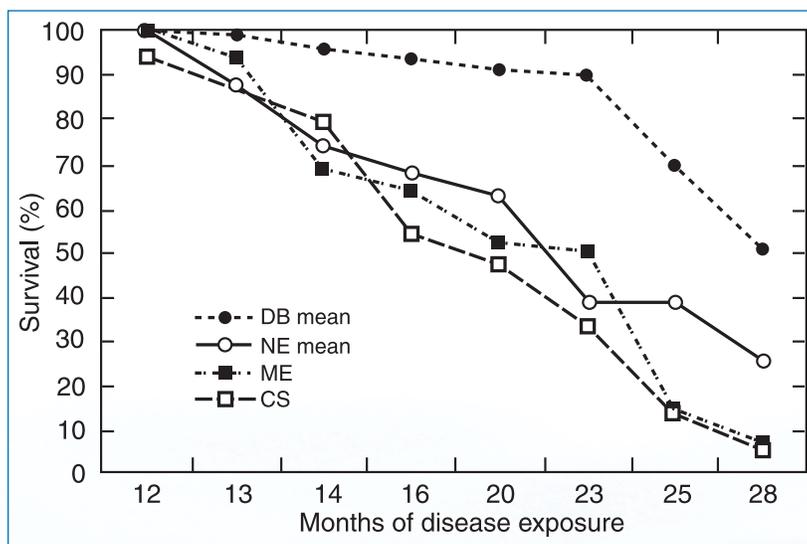


Figure 5. Comparison of survival for two selected lines of Eastern oyster, DB (=XB in text) and NE (=NEH in text), against unexposed F1 controls from Maine (ME) and Delaware Bay (CS).

life cycle. In addition, the disease resistance programs are largely non-quantitative and rely on the overall assumption that there is significant variation for disease resistance in natural populations. This approach may or may not be the “optimal” approach.

Multidisciplinary approaches to understanding disease resistance

As individual disciplines grow and become more sophisticated, the connections among them often become more clearly defined. This is true in the case of shellfish aquaculture, which relies on information from several disciplines that were formerly considered quite distinct.

A case in point is our understanding of the factors affecting disease resistance. In years past, the phenomenon had been approached in distinct and apparently non-overlapping ways by geneticists and physiologists. The traditional genetic approach to understanding a phenomenon such as variation in resistance to MSX or Dermo was to determine whether a species or population possessed enough genetic variation for disease resistance to make a successful selective breeding program likely. The methods used (quantitative genetics) have proven very useful in designing and executing selective breeding programs, without ever providing any information on the actual genetic mechanisms underlying the trait. Thus, several generations of directed selection yielded lines of eastern oysters with improved resistance to MSX, but the mechanism(s) responsible for the improved resistance remained obscure.

The physiological approach to elucidating the mechanisms of disease resistance has sought to describe the cellular/biochemical interactions occurring between host and disease organism, with the hope that an increased understanding might provide some means of intervention. The existence of physiological variation among individuals of either the host or the disease organism was often viewed as an annoying source of experimental noise that complicated our understanding of the disease process.

Ultimately, both forms of knowledge are essential for genetic improvement, and interactions between the disciplines can be mutually beneficial. A good example comes from recent work on the genetics and physiology of Dermo resistance in the eastern oyster.

Previous physiological evidence suggested that the Dermo pathogen produces a protein-degrading enzyme or protease, that the oyster host attempts to counter by the release of protease-inhibiting compounds. It was hypothesized that the amount of protease inhibitor an individual oyster is able to produce directly affects its susceptibility to Dermo, and ultimately its chances of survival in the face of disease pressure. However, direct experimental manipulation of the system is not simple. Instead, investigators asked whether naturally occurring genetic variation could be used to test the hypothesis that variation in protease inhibitor levels affected Dermo susceptibility, which in turn affects survival.

Ten genetically distinct oyster families were reared under similar conditions in the field, exposed to natural levels of the Dermo pathogen. Protease inhibitor levels, disease status and survival were monitored. At the end of the experiment, a significant correlation was found: families with higher protease inhibitor levels had on average significantly lower Dermo infection levels and higher survival rates in the field (Figure 6). This combined genetic/physiological approach confirmed the physiologists' hypothesis that the oyster protease inhibitor is an essential component in the disease process and the existence of significant genetic variation in both this component and resistance to Dermo. The implication of these observations for the geneticist is that sufficient genetic variation for Dermo resistance exists to enable genetic improvement of this trait by selective breeding. Moreover, the multidisciplinary approach has provided breeders with a tool for indirectly assaying an oyster's capacity for resisting Dermo. Such a tool enables breeders to select resistant oysters without doing disease challenge experiments, which are difficult, expensive, and time-consuming.

Crossbreeding

Crossbreeding is an alternative to artificial selection for improving farmed molluscs when the genetic variation in a trait of interest is non-additive or non-heritable. Hybrid vigor for growth, for example, depends on a combination of genes, which cannot be passed directly to offspring because sexual reproduction breaks up such gene combinations. Thus, if superior growth results from hybrid vigor, selecting the fastest growing individuals will not improve growth rate in the next generation. For such traits, it is usually more efficacious to develop inbred lines, which when crossed to each other, yield a superior hybrid. Crossbreeding is very important in plant breeding. Large-scale crossbreeding trials to improve yield have been initiated for the Pacific oyster on the West Coast.

Controlled crosses among inbred lines of the Pacific oyster produce hybrid offspring that grow faster than either of their inbred parents (Hedgecock et al., 1995; Figure 7). The fundamental genetic and physiological causes of this hybrid vigor are emerging from multidisciplinary research funded by the USDA's National Research Initiative Competitive Grants Program. Inbred lines were first initiated in 1989 by self-fertilization of simultaneous hermaphrodites and later, in 1993, by crosses of full brothers and sisters.

The genetic explanation of hybrid vigor for growth in oysters appears to be the inheritance, from one parent or another, of dominant, functional genes that cover up or ameliorate harmful or defective genes carried by the inbred lines. This conclusion, inferred from experimental evidence that oysters carry an unusually large number of lethal genes and likely an even larger number of deleterious genes, accords well with the growing consensus regarding the basis of hybrid vigor in corn, rice, and other crops. We note that this fundamental discovery relied upon a three-generation study, requiring almost ten years from the time the initial inbred lines were derived to the formation of the second hybrid generation. This illustrates why applied research on molluscan genetics requires long-term institutional support of culture facilities and staff.

Considerable effort has gone into understanding the physiological basis of hybrid vigor in growth. Hybrids often show increased metabolic and feeding efficiencies compared to inbred parents (Hedgecock et al., 1996; Bayne et al., 1999). The interaction of genetics and physiology in this research again illustrates, as in the case of determining the role of protease inhibitors in Dermo disease

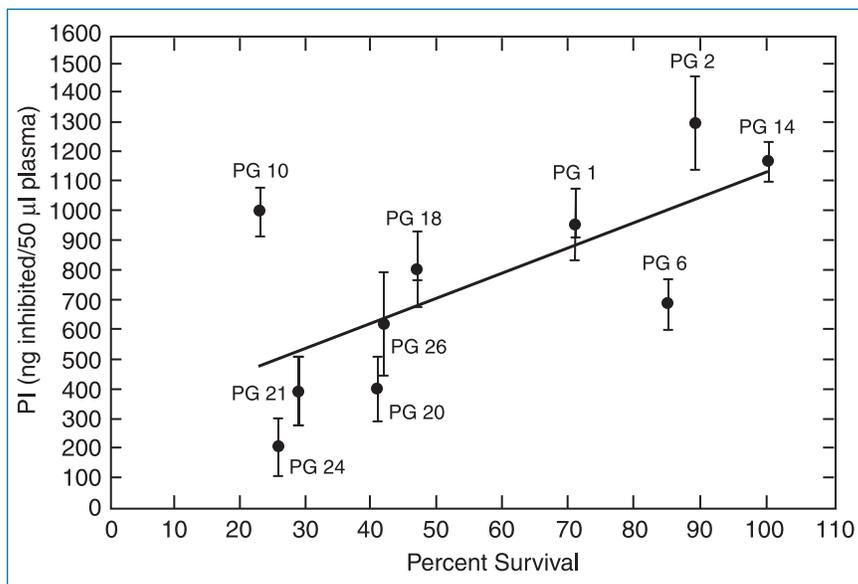


Figure 6. Correlation of survival of oyster families exposed to MSX (text says Dermo) with plasma levels of protease inhibitor (PI) suggests that PI, rather than survival to 2 years, might be used to determine the breeding value of families under selection for disease resistance.

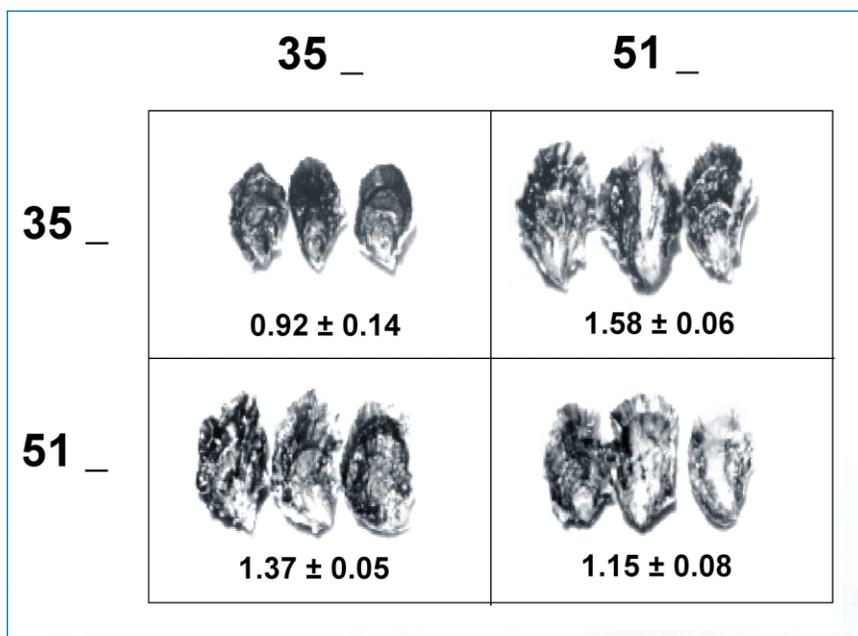
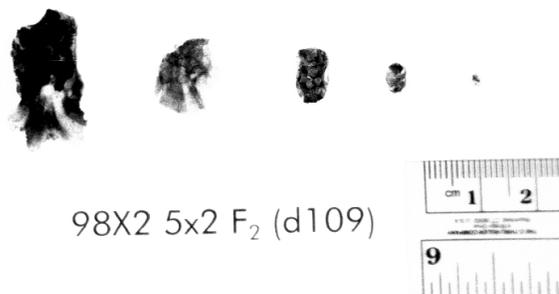


Figure 7. Hybrid progeny from a cross of inbred lines 35 and 51 are heavier than inbred progeny at 4 months age, showing heterosis for growth. Mean live weight (± SE) per oyster based on biomass and counts of replicate cages.

A. Shell height in an F₂ hybrid family



B. Provisional Mapping of Growth QTL

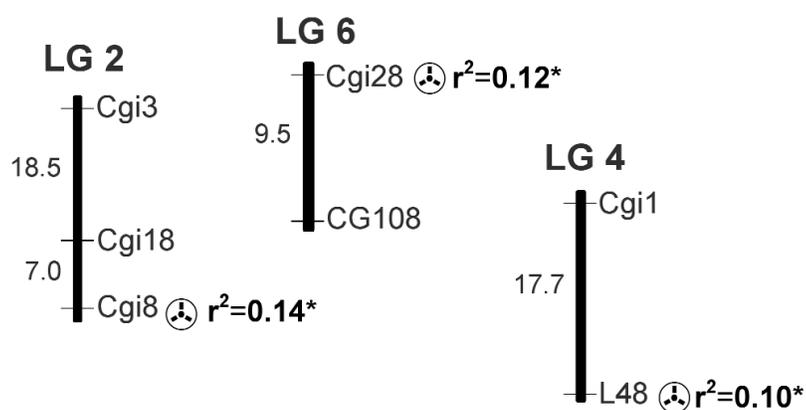


Figure 8. From 10 to 14 percent of shell-height variation, in an F₂ hybrid family of Pacific oysters at 3 months of age, is explained by three quantitative trait loci on linkage groups 2, 4 and 6. Marker loci are named on the right of each linkage group; distances in centimorgans are given on the left.

age, have been identified (Figure 8). These preliminary results suggest that genes affecting commercially important traits can be located and eventually identified and cloned for functional analysis.

The practical applications of hybrid vigor to the improvement of commercial oysters are presently being investigated in a regional research project funded by the USDA's Western Regional Aquaculture Center (WRAC). In the controlled crosses just described, the performance of hybrids is measured relative to their inbred parents. The question remains how hybrids perform relative to standard industry stocks, especially since inbred families yield less meat than do industry and wild controls and the first selected generation of MBP stock (Figure 4). Direct comparisons in a commercial hatchery, however, showed that hybrid larvae grew faster and set earlier than typical, randomly bred larvae. Growth of these same groups in the field will be assessed in January 2000.

Crossbreeding requires the maintenance and testing of numerous inbred lines. In the seed corn industry, for example, thousands of inbred lines are crossed each year, and the millions of hybrid offspring are reared in Mexico to identify elite lines for producing hybrid seed for the Corn Belt. Commercial shellfish producers are never likely to have this scale of testing capacity; indeed, it will be challenging to test dozens of inbred lines in existing facilities. Thus, applying crossbreeding to the improvement of commercial molluscan shellfish populations will depend greatly on our ability to identify promising hybrids at the earliest possible stage, using the tools of biotechnology and genomics, to obviate the labor and expense of large-scale rearing and testing. Differences between hybrids and inbreds in growth rate can be detected by day 6 or 7 of larval life. Differences in

resistance, the power of a multidisciplinary approach in applied research. The geneticist can create, in a repeatable fashion, experimental populations of oysters differing greatly in complex traits like growth. This material enables physiological analysis of the components of growth, which, in turn, enables the geneticist to focus attention on traits that can be measured more precisely than growth. Multidisciplinary interaction accelerates knowledge of fundamental biological mechanisms and the entire enterprise of genetic improvement.

We are presently attempting to map the genes causing hybrid vigor in growth and its underlying physiological components. A moderately dense linkage map (see Tools section below) of the Pacific oyster is under construction, which will enable the mapping of economically important genes, such as those causing hybrid vigor. This advance was made possible by the research showing that typing early larvae could circumvent the distortions of inheritance caused by lethal genes. A preliminary map of six linkage groups has been constructed, and three chromosomal regions, each explaining about 10% of the large variation in shell length at 3 months of

respiration have been detected as early as 12 hours. Patterns of gene expression in larvae, which can be instantaneously visualized on gene-chip arrays, for example, could eventually offer a powerful means for predicting growth potential (see Tools section).

Non-natives

Culture of non-natives is the basis of several highly successful molluscan aquaculture industries, including those in Australia, China, the Pacific Northwest of the U.S., and Europe, to name a few. The Pacific oyster, which was introduced by Portuguese traders to Europe as early as the 17th century, is now cultured on all continents but Antarctica (Mann, 1979). Introductions of marine species, however, are fraught with technical difficulties, genetic and logistical. Much has been written about the ecological peril that non-natives impose. These dangers can generally be described in three categories: *i*) co-introduction of organisms carried on, or within, introduced species; *ii*) genetic “pollution”, or negative genetic consequences of interbreeding between the introduced and other species; *iii*) invasion of the introduced species to the ecological detriment of the carrying system. There are ways around these problems. For example, concerns about the unintended dispersion of pathogens or other “hitchhikers” with the non-native can be avoided by quarantine of imported brood stock and use of F1 progeny or higher. In fact, the International Council for the Exploration of the Seas has quite clearly provided guidelines for this concern (also see section on Diseases). The potential for *ii*) genetic “pollution” and *iii*) invasion may be reduced or eliminated by the use of triploids.

An example of ongoing application of non-native breeding attempts comes from work conducted at VIMS, starting in 1996. The genus *Crassostrea* is rich in species of potential value to oyster breeding. In the mid-Atlantic in particular, the Pacific oyster *C. gigas* and the Suminoe oyster *C. ariakensis* have been tested in Chesapeake Bay (as triploids) to determine their suitability. *C. gigas* seems to be unsuitable for the Bay proper, because it is not tolerant of the low salinity, high temperature, and turbidity often seen there and is susceptible to infestation by the worm *Polydora*. They are more suited to the higher salinity regions of the Eastern Shore where they thrive and grow much faster than *C. virginica*. Moreover, they are resistant to both MSX- and Dermo-diseases so survival is high. *C. ariakensis* is resistant to both diseases and unlike its congener *C. gigas*, is an estuarine oyster found in river drainages in Japan, China and possibly the Indian subcontinent. Field tests of triploid Suminoe in Chesapeake Bay have demonstrated prodigious growth rate and general hardiness at all salinities. The plan for using the Suminoe oyster (or *C. gigas* for that matter) for commercial aquaculture in Chesapeake Bay, and perhaps other similarly disease afflicted areas of the mid-Atlantic, depends on creation of all-triploid (and therefore sterile) seed. Currently, development of tetraploid *C. ariakensis* is the rate-limiting step in this development. Tetraploids of *C. gigas* are already available and commercialization of this species in the mid-Atlantic is not technically constrained.

There are a number of constraints to working with non-natives. The taxonomy and systematics of molluscan shellfish, particularly Asian and tropical taxa, are still relatively murky, so that unequivocal identification of species is not always straightforward. Furthermore, elucidation of population structure is similarly problematic because some of the most interesting species occur in parts of the world that are difficult to sample. Beyond the questions of the genetics of non-natives lie the infrastructure challenges. Non-native work requires significant capital commitment to quarantine facilities, especially when initially imported. After that, less restrictive but nonetheless significant facilities are needed for carrying adult brood stock over many years without allowing them to reproduce in the wild. Moreover, hatchery effluent waters, which are typically discharged directly into receiving waters, need to be treated to avoid impacts on native species.

In response to this need, scientists working with the aid of NOAA Sea Grant support developed molecular markers that allow clear species identification from small tissue samples. Tiny bits of gill tissue removed from hundreds of individually tagged oysters in Washington and Oregon hatcheries have been used to determine the species identity of each oyster, and to ensure that only pure Kumamoto oysters will be employed in future broodstock development. Native stocks of *C. sikamea* and *C. ariakensis* were recently rediscovered by application of these markers to samples collected from Ariake Bay, Kyushu, Japan (Hedgecock et al., 1999). These populations can now be tapped for additional brood stock for commercial hatcheries.

Species-specific genetic markers will be needed for all species used in aquaculture, whether native or non-native, to verify the identity of material used in broodstock development programs. The same molecular methods are also valuable for making use of naturally occurring genetic variation *within* species. The ability to define genetically distinct subpopulations of bivalves is important for aquaculturists, who want to exploit the native genetic variability of the species for the development of cultivated lines. Without an understanding of the geographic distribution of genetic variability in a species, selection of brood stock for founding new hatchery lines is a haphazard process at best.

Cultures of molluscs can be easily mixed, especially in hatcheries, and genetic markers are required to detect and eliminate such mixtures. Reports in the literature of successful interspecific hybridizations were not verified by markers and could not be repeated in experiments. Allozymes confirmed that putative hybrids in culture resulted from hatchery contamination by purebred species. In the crossbreeding research, allozymes were used to verify the integrity of the inbred lines used (Hedgecock et al., 1995; McGoldrick and Hedgecock, 1997). Use of more highly polymorphic microsatellite DNA markers, which can be typed on biopsy samples, enables the pedigree of potential parents in experiments to be verified prior to spawning. Commercial breeders will need access to such technology in order to verify the pedigree of brood stock in their breeding programs.

Genetic sterilization

Genetic sterilization methods have been widely used in insect control programs and in plant agriculture. Male-sterile genes were important in the development of hybrid corn, for example, obviating the need for laborious hand de-tasseling. In the context of aquaculture, genetic sterilization methods could be an alternative to the use of triploidy to sterilize molluscs released to the wild. Australian researchers have found conditional lethal genes that kill larvae not reared in a special hatchery environment, for example.

Genetic maps and genomics

Another major use of genetic markers in aquaculture is for the genetic improvement of cultured species. In its simplest sense, genetic improvement is simply changing the genetic composition of a population so that it more closely conforms to the breeder's wishes—for example, by showing enhanced disease resistance, superior growth rate, or improved meat characteristics. This has traditionally been done through the application of breeding schemes guided by the principles of quantitative genetics, without any knowledge of the particular genes affecting specific traits. Until recently, this was unavoidable, because animal genomes were complex and unapproachable, given the available tools.

With the advent of the DNA revolution, the genome is no longer a black box. Using the same techniques being used to study the genomes of humans, plants and agricultural animals, aquaculture geneticists are beginning to accelerate the process of genetic improvement in their species of interest. A key element in this process is the establishment of a genetic map, using genetic markers as signposts.

Interspecific hybrids

During the first 50 years of this century, hybridization was the primary source of new varieties of self-pollinated crops. By-and-large, hybridization is unexplored in molluscan shellfish. Interspecific hybridizations might be useful in several ways. The transfer of one or a few characters from a non-native or unimportant species might significantly improve the performance of an important native species, for example. Interspecific hybrids might also produce characters that are absent in either of the two parents, i.e., novel expressions of genes unique to the hybrid itself. Finally, interspecific hybrids may be reproductively sterile, which might certainly obtain in allotriploids – triploids of interspecific hybrids.

Hybridization was thoroughly examined between *C. virginica* and the two Asian species, *C. gigas* and *C. ariakensis*. Both Asian species are resistant to the two major diseases affecting the eastern oyster. Hybridization failed between *C. virginica* and both Asian species (Allen et al., 1993), although the Asian species were able to hybridize with each other (Allen and Gaffney, 1993). Other approaches to rapid introgression of genes from the Asian species were explored, and one of the most novel was that of partial gynogenesis where fragments of chromosomes are incorporated into developing embryos of *C. virginica* (Guo et al., 1996). Early work on hybridization of oysters often suffered from the lack of genetic verification of the progeny so that results were equivocal in many cases. Recent work has included genetic verification of parents and progeny.

Transgenesis

Transgenesis, the movement of specific genes from one organism to another, using methods of molecular biology and recombinant DNA, could be important in the improvement of traits with a simple genetic basis, like disease resistance. For more complex traits, such as growth or yield, the traditional methods of improvement discussed above are likely to be more cost effective. Moreover, although the results of interspecific hybridization and transgenesis are similar, the current controversy over transgenic plants and animals in agriculture will probably retard their acceptance and use. Nevertheless, transgenesis is likely to be useful tool in molluscan research in the near term. Transgenic abalone (Powers et al., 1996) and dwarf surf clams (Lu et al., 1996) have been produced experimentally.

Tools

Genetic markers

Molecular markers are currently being used effectively to aid shellfish aquaculture. As in the case of fisheries, there are valuable uses for markers that signal variation among, as well as within species.

In the Pacific Northwest, oyster growers are interested in expanding production of the Kumamoto oyster, a species that was introduced inadvertently in North America with shipments of the more commonly cultured Pacific oyster. Although the Kumamoto oyster is smaller and slower growing than the Pacific oyster, its deeply cupped shell and distinctive flavor have made it a valuable part of the half-shell trade, and growers are seeking to increase hatchery production. Here is where a problem arises: it is not always possible to separate the two species when they are juveniles, and the two species are capable of interbreeding in the hatchery. As a result, many of the Kumamoto oyster populations in commercial production also are contaminated with Pacific oysters, or Kumamoto x Pacific interspecies hybrids. Before expanding production and initiating any long-term broodstock development programs, growers are anxious to make sure that their hatchery lines are pure Kumamoto.

The genetic material of an organism is organized into long strings of DNA called chromosomes; an oyster, for example, has two copies of each of ten chromosomes. The goal of the gene-mapper is to locate genetic markers or signposts at regular intervals throughout all of the organism's chromosomes; the overall description of the location of these signposts is termed a *genetic map*.

The primary uses of genetic maps for aquaculture species are, at this stage of development, twofold. First, genetic maps are used to locate the existence of unseen genetic factors affecting key features of interest (i.e., commercially or economically important traits). If the researcher is able to discover the chromosomal locations of major genes affecting a key trait, the nearby genetic markers can be used to identify individuals carrying the genes responsible for the favorable traits, for use in selective breeding programs. The advantage this offers is most pronounced in organisms with moderate to long generation times. For example, it takes at least a year to determine how susceptible an individual oyster is to a disease such as Dermo, and during years when the disease is not prevalent, it may be difficult to evaluate at all. In contrast, it is possible even at the earliest larval stages to determine whether an individual oyster is carrying a particular molecular marker(s). The key challenge to the molecular breeder is to construct a genetic map populated with numerous genetic markers, some of which will prove to be closely linked to genes affecting a trait of economic interest. The use of markers as proxies for the actual genes of interest in a selective-breeding program is termed *marker-assisted selection*.

In the same way that genetic markers are used to tag genes of interest within a population, in order to increase their frequency in the population by selective breeding, they can be used to identify portions of the genomes of other populations or species that contain genes of particular value. These tagged parts of the donor genome can be inserted into the species of interest, improving its performance, without bringing in other unwanted genetic material. This procedure, termed *marker-assisted introgression*, is particularly valuable when a donor species possesses a feature that is not pronounced in the target species. For example, the eastern oyster is particularly susceptible to the diseases Dermo and MSX, whereas its Asian cousins appear to be highly resistant. If marker genes indicating the location of disease-resistance genes in the Asian species could be located, they could be used in gene transfer experiments to determine which host animals possessed only the desired fragment of the donor species genome.

One particularly promising development in the rapidly moving field of genomics is the ability to simultaneously monitor the expression of thousands of genes in any life stage of an organism. For aquaculture organisms, which are generally poorly known in a genetic sense, this offers the prospect of rapid identification of genes of interest. For example, it is now feasible to monitor the expression levels of thousands of genes in a bivalve before and after disease challenge; those genes whose expression changes as a result of disease exposure are prime candidates for further analysis and eventual incorporation into selective breeding schemes. This approach is especially exciting for researchers in molluscan genetics, as it provides a means to characterize basic genetic processes associated with critical events such as metamorphosis, larval settlement, and the disease response, which are presently only poorly understood.

Because the science of genomics requires a substantial technical infrastructure, as well as substantial contributions from fields not normally associated with fisheries or aquaculture (e.g., bioinformatics), a key need is the development of collaborative linkages with more established genomics projects. Several informal linkages have recently been initiated, such as high-throughput sequencing of oyster DNA by both Japanese and American genomics labs, or the analysis of gene expression in oyster larvae by a private genomics company. However, formal shellfish genomics/gene mapping projects similar to those in progress for major agricultural species have yet to begin.

REGIONAL CONSIDERATIONS

REGIONAL DIFFERENCES IN SPECIES AND TRAITS OF INTEREST

There are dramatic differences between the West Coast and the Gulf and East Coasts in shellfish communities and industries. West coast aquaculture, for example, is dominated by non-indigenous Pacific oysters, most of which are propagated by large commercial hatcheries. The East and Gulf Coasts are dominated by native bivalve species, including the eastern oyster, which historically supported substantial harvests, but has been decimated by diseases. Thus, in the west, the emphasis has been on the improvement of yield by selection and crossbreeding of hatchery propagated stocks. In the east, the emphasis has been on developing hatchery stocks with disease resistance or using naturally resistant non-native stocks rendered sterile by triploidy.

Collaboration

Despite these differences in species and traits of interest, molluscan shellfish geneticists from all regions of the U.S. and even internationally work collaboratively. There have been regional research projects on both the West and East coasts since the inception of the USDA Regional Aquaculture Centers in the late 1980s. In the mid-1990s, the first USDA Coordinating Committee on a marine topic, (WCC-99) Shellfish Breeding and Genetics, was established through the Oregon State University Coastal Experiment Station. This committee, which draws participants from all across the U.S., meets annually, usually in conjunction with a national or regional shellfish meeting. The primary purpose is the exchange of technical information and the reporting of progress in individual laboratories as well as in major breeding programs, like CROSBreed, MBP, and WRAC. The committee played a key role in the design of the MBP (Hedgecock et al., 1997) and still actively advises that program. Finally, oysters are included in a regional research project, NE-186, Genetic Maps of Aquaculture Species, which has international participation and holds a workshop at the Plant and Animal Genome meetings each year in January. Collaboration in the area of genetic maps and genomics is vital because no one laboratory has the capacity to do all the work, there is need to exchange reference families, reagents, and markers, and parallel work should accelerate the mapping of the eastern and Pacific oysters.

The need for infrastructure in all regions

U.S. agriculture was modernized in the late 1800s and early 1900s by the establishment of the Agricultural Experiment Station, which enabled scientists at Land Grant Universities to carry out long-term, large-scale experiments to improve crops and animals. George Shull published the first experimental demonstration of hybrid vigor in corn in 1908, but it took three more decades of research in the Agricultural Experiment Stations of the Corn Belt before hybrid seed was widely available from commercial seed producers. **The need for long-term infrastructure and support for research, particularly in genetics, is as crucial for aquaculture today as it was for agriculture at the beginning to last century.** Research in aquacultural genetics or genomics requires culture facilities and staff, in order to carry out the long-term, large-scale experiments that will be necessary to improve stocks and establish a commercial seed industry. Unfortunately, there is little institutional commitment to providing this infrastructure. Most university research facilities are bootlegged off of extramural grants, which do not provide long-term stability. Many programs in the past could not be sustained when funding expired; consequently, breeding goals were not attained and research investments and stocks were lost. At present there are dedicated facilities for aquaculture genetics research in some states on the East Coast (Maine, New Jersey and Virginia) but none on the Gulf or West Coasts.

OUTREACH

TECHNOLOGY TRANSFER

Breeding programs require close cooperation with industry for transfer of technology. Initial contact and technology transfer is accomplished by convincing farmers to host test sites for strains under development. Breeding programs benefit in a number of ways. First, strains are tested under realistic conditions that mimic those of commercial farming practices. Second, the farmer and breeder have similar investment in the project. Third, the farmer is acquainted with the process and intricacies of selective breeding and especially, how it differs from production.

Technology transfer of the product of breeding programs can be various. For example, one product is the brood stock themselves. Transfer of brood stock needs to be accompanied by recommendations for their use and propagation in the hatchery. Another form of technology transfer might be to turn the breeding program itself over to the industry. In a sense, divestment of the breeding program is the ultimate indicator of success. It would require that there was a measurable “added value” to the select stock that would justify the transfer of the program to industry or an industry member. In agriculture, breeding in most of the important commercial crops is accomplished by private enterprise. At the moment, the cost of shellfish breeding programs is higher than their value as a private enterprise. For example, the cost of the CROSBreed program for disease resistance on the East Coast is about \$120,000 a year. In order to recoup this operating cost at a royalty rate of 10% on seed, sales would have to be on the order of \$1,200,000. Of course, disease resistant stocks may yield up to 6-10 times better survival than non-resistant seed, so perhaps the royalties are accordingly worth more than 10%.

INTELLECTUAL PROPERTY ISSUES

Intellectual property (IP) is any technology whose origins are attributable to a source of invention. Typically, both the inventor and the developer exploit IP for a commercial end. Today, major research universities foster the development and sale of IP and often, the proceeds of its commercialization return to the home research program. In other cases, the IP is licensed by corporations and developed into commercial product, as in the agricultural biotechnology, pharmaceutical and food science industries. The amount of development an invention goes through before it is licensed varies greatly. Because of the nature and small size of the aquaculture industry, IP would surely have to be well developed before licensing begins.

There are two examples of licensing of IP in shellfish genetics. A corporation in New Jersey licensed the IP rights to tetraploids, developed and patented at Rutgers University, for further development and marketing. The IP resides entirely in the private sector now, and patents protect tetraploid technology. Protection of the technology depends almost entirely on active patents.

IP of CROSBreed oyster brood stocks are under a different formula. IP rights are also held by Rutgers University (where they were derived) but have been partially transferred to collaborating institutions via a Memorandum of Understanding: the Mid-Atlantic Aquaculture Genetics and Breeding Technology Consortium. Licensing agreements, emanating from the IP offices of Rutgers, will be negotiated with private hatcheries on behalf of the Consortium and royalties will accrue to the Consortium via Rutgers for continued support of the CROSBreed selection efforts. All-triploids might be useful for the protection of intellectual property of XB oysters. Triploids would prevent unauthorized use of the proprietary strains. Tetraploid *C. virginica* were produced for the first time in 1999. Efforts are underway in two labs to produce tetraploids of disease resistant strains.

Whether these examples suffice as a model for the dissemination of the brood stocks from other programs such as MBP, or not, is still an open question. However, successful technology transfer may rely on exploiting the value added by a genetic improvement, and licensing of IP may be a condition of release.

SUMMARY

All of the biological issues raised in this workshop report concerning molluscan shellfish can be affected by research in genetics. Applications of genetics to ecology, fisheries, and aquaculture have been reviewed. A primary role of genetics is not surprising. Genes contain the information necessary for life. At the same time, there is an explosion of new genetic techniques and approaches, driven largely by the needs of human biology and medicine, which has culminated this year in the Book of Life, the complete sequence of the human genome. Biology has become an informational science. Molluscan shellfish genetics and biology will undoubtedly benefit from this revolution, which will provide tools to accelerate domestication and improvement of cultured stocks. We can capitalize on advancements in genetics, however, only if we have the necessary infrastructure for carrying out long-term, large-scale culture experiments, if we learn to work collaboratively, and if we bring multiple disciplines to bear simultaneously on biological problems.

FUTURE DIRECTIONS FOR MOLLUSCAN GENETICS

- *Infrastructure (Need for Aquacultural Experiment Stations)*
- *Transfer of breeding technology to industry*
- *Selection programs for more species besides oysters*
 - *Domestication strategies*
 - *Germplasm resources*
 - ◆ *Genomics*
 - ◆ *Multidisciplinary and collaborative research*
 - ◆ *Fisheries enhancement and conservation of biodiversity*

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Chapter 10

Disease

INTRODUCTION

SHELLFISH HEALTH MANAGEMENT: INDUSTRY NEEDS

Why is disease important? Landings and culture of commercial shellfish are important economic components of our coastal economies. For example, in 1996 clam landings alone were valued at \$127,799,000 and \$129,686,000 in 1997 (Johnson, 1998). Shellfish aquaculture production in the U.S. approached \$94,000,000 in both 1996 and 1997 (Johnson, 1998). The U.S. shellfish industry is based upon a combination of natural fisheries, managed fisheries, extensive aquaculture, and intensive aquaculture. Shellfish are also an integral and often dominant component of coastal ecosystems (see Broad-scale Ecology). As such, shellfish play important roles in maintaining the health and productivity of coastal ecosystems. Thus, the continued maintenance and growth of many coastal economies will depend upon a proactive system to address shellfish health problems. Many shellfish health problems exist and many more will undoubtedly arise as demands for shellfish increase, human populations increase and pressure on coastal and marine resources intensifies. The following review outlines the status and needs to meet current and future demands for maintaining viable, healthy shellfish resources.

In order to maintain healthy and renewable shellfish resources a thorough understanding of the biology (e.g., ecological niche, life history, physiology, nutrition, genetics), environmental requirements, stress tolerances and disease susceptibility/resistance of the host is needed. A disease occurs when the combination of parasite, host(s) and environmental conditions favor the pathogen (Figure 1). It is crucial to understand and maintain a current bank of data on the population dynamics of species of commercial importance, those of potential commercial importance and other species that may not be of commercial interest, but are ecologically important under both 'normal' and stressful conditions. In addition, optimal culture and husbandry practices are needed for shellfish aquaculture. A significant part of husbandry involves a thorough understanding of pathogenic mechanisms, modes of transmission, treatment and management of disease-causing organisms. This knowledge is also crucial to the successful conservation, maintenance and culture of shellfish species and populations. This information will enable the industry, resource managers and researchers to identify when a health problem arises, and may also help modelers predict disease outbreaks. Once a disease and pathogen are identified, rapid, specific

Importance of Healthy Shellfish

Healthy shellfish support coastal economies.

Shellfish maintain and protect healthy and productive coastal ecosystems.

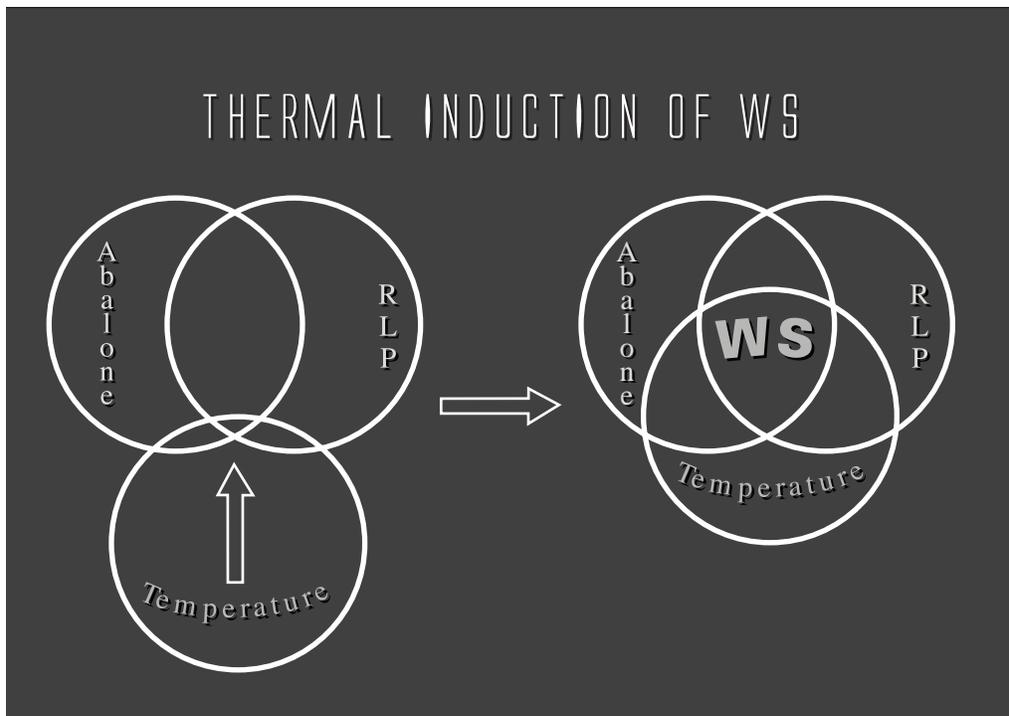
Healthy shellfish provide abundant ocean harvests of shellfish.

Healthy shellfish are needed for viable aquaculture.

Shellfish Health is determined by a combination of interacting factors, including:

- *Environmental Quality*
- *Life History*
- *Nutrition*
- *Population Dynamics/Ecology*
- *Disease*

Figure 1. The expression of disease typically involves the interaction of a host, a pathogen and the environment. In this example, withering syndrome (WS), a chronic wasting disease in abalone along portions of the west coast of North America, is triggered by increases in temperature. Below a certain threshold temperature, the pathogen, a Rickettsiales-like prokaryote (RLP), may infect abalone without causing any clinical signs of disease (Moore et al. 2000).



Presence of a shellfish disease does not indicate presence of a human health risk but does indicate a threat to the future of the shellfish resources.

and sensitive diagnostic tools are needed for disease management. These assays need standardization and must be validated and interpreted properly in relation to health management. Resource managers need this information to make educated decisions and allow states and federal governments to standardize regulations of health testing and animal transfers.

STATUS AND LINKS WITH OTHER PROGRAMS

Biology of Shellfish

Determination of optimal environmental parameters such as salinity, temperature, appropriate water column location, etc., and parameter ranges and interrelationships for shellfish species is a crucial need.

Optimal Environmental Conditions. Knowledge of environmental parameters is essential in the establishment of correct culture conditions for shellfish aquaculture. Determination of environmental parameters for bivalve and gastropod molluscs involves the definition of appropriate salinity temperature, water column location, food sources, oxygen concentration and other variables for various life stages. Such definitions involve not only discovery of the optimal value for these parameters, but the ranges and interactions between these parameters. Definition of the environmental parameters for various species of commercially important bivalves is necessary to understand and promote the development and growth of the larvae and juveniles, the reproductive potential of adults and the maintenance of animal condition. This information helps us understand population fluxes in fished species, establish protected areas (e.g., ecological reserves; Tegner, 1993) as a method of fisheries management, understand how environmental change will affect fisheries and aquaculture production, and identify sites most likely to be successful in restoration efforts. Data have been collected on several species and on many levels (resulting from observations of natural habitat laboratory and commercially cultured populations). This information is patchy, not comprehensively reported, and ranges of these parameters, and their interactions, are not well established.

Life History

Although life histories of marine shellfish are complex, their characterization is crucial for development of appropriate resource and fishery management policies and decisions, and successful aquaculture. Knowledge of an organism's life history provides the basis with which to understand how diseases recur and methods for reduced losses or prevention. Studies of normality are needed to define abnormalities and their causes. Studies investigating the biology of various marine shellfish have been conducted, particularly those involving the eastern oyster. Current information concerning the life history of oyster, clam and scallop biology has been published (Kennedy et al., 1996; Kraeuter and Castagna, 2001; Shumway and Sandifer, 1991). Essential knowledge of shellfish life history includes, but is not limited to, understanding larval behavior (Rogers-Bennett and Pierce, 1998; Hovel and Morgan, 1997), determining thresholds of population density and determining the proximity (maximum distance) needed between both sexes of reproductive animals for successful fertilization of broadcast gametes (e.g., oysters, Mann pers. comm. and abalone, Tegner, 1993); descriptions of the development of the pre and post metamorphic stages including integrated sequential descriptions of both the important cellular and cytological changes, fisheries recruitment (larval, juvenile and adult) and better descriptions and understandings of reproduction in bivalves and those factors affecting the reproductive potential. Life histories of commercially important bivalves, especially the eastern oyster, are presently being studied, but large voids in our knowledge still exist (Figure 2).

Physiology. Considerable data exist describing various physiological processes of several bivalve species. Work defining the general physiology of bivalves' (especially oysters), filtration rates, respiration and reproduction have resulted from studies of apparently healthy animals (Newell, 1979). Further investigation of these processes, as they relate to development and growth in healthy animals, is needed. Importantly, the study of physiological mechanisms in healthy animals can be used to evaluate important pathogenic effects of disease agents on infected individuals and the general population. Of particular importance is the need to understand the physiological mechanisms of host responses to shellfish pathogens.

Needed Life History Information:

- *Determination of carrying capacity (proximity and density) for effective maintenance or expansion of feral populations.*
- *Determination of larval recruitment factors for important bivalve fisheries.*
- *Detailed descriptions of pre and post metamorphic development for commercial important bivalves.*
- *Better understanding of the reproductive cycles and the associated metabolic and biological controls.*

Oyster Life History

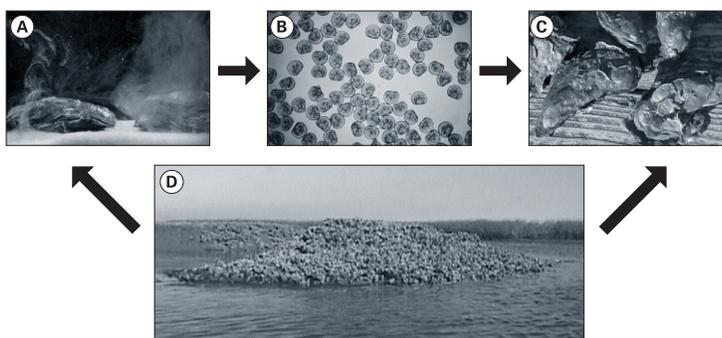


Figure 2. Simplified oyster life history. Adults spawn millions of gametes (A) that produce free-living larvae (B). The microscopic larvae drift in the plankton for about 2 weeks feeding on even smaller plankton until ready to settle and permanently attach themselves to a hard substrate (C) where they grow and eventually form beds and reefs (D). Many questions remain about spawning and settlement cues, food resources and survival for larvae and adults, impacts of environmental conditions and pollutants, etc.

Use physiological processes to understand host response and the pathogenesis of disease in shellfish.

Identify affordable methods to rapidly quantify "stress" in shellfish.

Investigate effects of pollutants including naturally produced toxins on shellfish.

Characterize immune response of healthy and diseased shellfish.

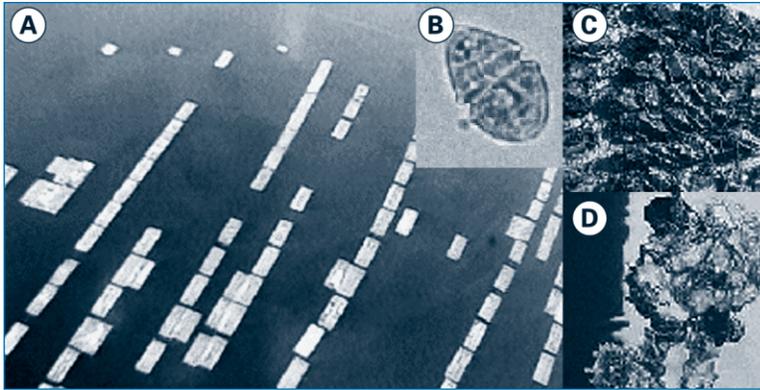


Figure 3. Red tide of *Heterocapsa circularisquama* over Japanese aquaculture farm (a). The red tide organism (b). Resulting dead pearl oysters (c) and pacific oysters (d). Photos courtesy of Yukihiro Matsuyama.

possible stress monitoring methods would help us understand the pathogenesis of disease and how to detect and manage disease.

Inadequate or detrimental environmental conditions, resulting from loading by various pollutants (e.g., toxins, metals and polyaromatic hydrocarbons or PAHs) and the effects of poor nutrition on various life stages can result in important sublethal effects, lesions, or at the very least, physiological stress (Fisher et al., 1999). Evaluation of the sublethal effects of various metals and some organic pollutants on selected bivalves has been done. Understanding the differences in effects of these contaminants between species as well as determination of the sublethal detrimental effect of other pollutants, such as toxins resulting from dinoflagellate blooms, needs to be investigated (Shumway et al., 1990; Figure 3). Sublethal effects have important negative implications for persistence and growth of natural and cultured shellfish populations.

The study of bivalve defense mechanisms has been sporadically investigated over several decades, primarily in eastern oysters and hard clams (Ford and Ashton-Alcox, 1999; Fisher, 1988). Bivalve defense mechanisms include both cellular actions of hemocytes resulting in phagocytosis and encapsulation, as well as production of various humoral factors such as lysins and agglutinins. Similarities and differences in immune response between species is important in understanding how disease agents successfully cause morbidity and mortality in a healthy bivalve and how we might modify or prevent diseases in affected bivalve species (Bramble and Anderson, 1998). Presently, our understanding of the cellular and humoral defense mechanisms in molluscs is rudimentary. Development of new molecular tools can augment and expand our study of these defense systems in molluscs and lead to a better understanding of the development of disease resistance in bivalves.

We need to characterize:

- Optimal diets and rations- especially for early life stages and grow out as they relate to shellfish health.
- Availability of natural food.
- Methods to assess current/recent nutritional levels.

Nutritional Requirements. Nutrition is of paramount importance to the sustained health of any organism; shellfish are no exception. The acquisition of data on nutritional requirements of marine shellfish has evolved from characterization of particle size selection studies and growth on selection of algal species to current efforts to define the bivalve's biochemical needs. These requirements vary between species. Current research on oysters and scallops is focused on lipid and fatty acid needs in the larval phase prior to metamorphosis (e.g., Soudant et al.,

1999). Manipulation of algal cultures (composition of algal and probiotic bacterial species, growth stage of algae, nutrient availability and culture temperature) to optimize nutritional value to shellfish larvae is currently being investigated (Lewin et al., 1996). The focus in abalone culture lies in the formulation of artificial feeds for juvenile and adult life stages. Once the nutritional requirements are better understood, optimization of feeding via combining feeding rations and regimes with qualitative aspects of good diet profiles are needed for each life stage. Agricultural feed standards

have been defined for many terrestrial food animals and are based on biochemical components. These standards may be used in the formulation of both artificial and mixed live diets for shellfish culture. The relationship between nutrition and the ability of shellfish to combat disease and other stressors has received little attention. Research along these lines would provide an opportunity for improving shellfish health as a preventative measure.

Selection of sites for aquaculture facilities, fisheries and restoration efforts should consider food fluxes and carrying capacity not only as it relates to growth and production, but also as it relates to maintenance of healthy shellfish. Improved indicators of food availability (such as particulate organic nitrogen levels) are needed. Placement of culture facilities in locations with optimal food levels will enhance animal health and reduce disease problems.

We are just beginning to understand the effects of food limitation and aerial exposure on various aspects of shellfish health (Carefoot et al., 1993; Winstead et al., 1998). These methods need to be refined and expanded for application to a variety of shellfish species.

Natural Load of Parasitic and Commensal Symbionts.

All organisms harbor parasitic and commensal symbionts; these symbionts may be viruses, bacteria, protozoa or other types of microorganisms. Not all symbionts cause disease. Under certain conditions, such as a change in environment that favors enhanced growth of the symbiont (obligate or facultative parasite) and/or reduced fitness of the host, these relationships may result in disease. Thus, it is crucial to identify, characterize and monitor the levels of symbionts in shellfish populations that are commercially important, particularly during the initial domestication of a previously unexploited species. Identification of the cause of an epizootic or mass mortality is enhanced by a thorough knowledge of the parasites or commensal endosymbionts of new or established aquaculture species. This information also allows culturists to identify potential disease-causing organisms. It is also imperative to characterize symbionts in species that are not exploited as these species often play ecologically important roles (e.g., pathogen reservoirs) or may become commercially exploited in the future. Natural loads of symbionts of shellfish species with a history of commercial exploitation by the aquaculture industry and, to a lesser extent, the fishing industry have been well characterized in specific regions of the world (e.g., feral and cultured oysters and wild and cultured abalone in the U.S. (Elston, 1999; Bower et al., 1994)). Detection and characterization of emerging diseases in feral shellfish populations or fisheries are often hindered by a lack of baseline data describing natural loads of parasitic and commensal symbionts. For example, a lack of this information made it difficult to identify the etiological agent of withering syndrome of abalone in California, *Candidatus Xenohaliotis californiensis*, a bacterium of which little was known (Friedman et al., 1997; 2000).

The health of new species developed for culture must be carefully monitored for early detection of infectious agents and to develop appropriate management plans for protection of both wild and cultured populations.

Population Dynamics and Ecology

An understanding of population dynamics is essential for assessing shellfish health in natural or cultivated populations. Changes in population structure (size-frequency distribution and/or density) may indicate health related threats driven by environmental change, pollution or disease. The health status of a population provides an estimate for the potential recovery and the rate of recovery when affected by a disturbance, including disease. More importantly, knowledge about population dynamics, provides an important tool that can be used to manage shellfish populations to minimize disease transmission and other health problems. A fundamental understanding of these population processes is lacking for virtually all shellfish species, particularly for the early life stages. As a result little is known about how shellfish population dynamics relate to shellfish health. This void must be filled to facilitate the success of shellfish aquaculture, shellfishery management and the restoration of shellfish populations. Furthermore, as dominant benthic organisms in many coastal ecosystems bivalve

Disease: health condition that is a departure from normal.

Infectious: agent or disease capable of transmission between hosts (e.g., virus, bacteria, protozoa, fungi).

Non-infectious: not transmissible (e.g., most tumors, harmful algal blooms, toxins).

Symbiont: close relationship between two organisms, the host and symbiont (which is usually a micro-organism).

Parasite: metabolically dependent upon its host.

Pathogen: micro-organism capable of causing disease.

molluscs play a vital role in maintaining healthy ecosystems. It follows that the health of the shellfish themselves is crucial to this role. Therefore it behooves us to develop a thorough understanding of disease processes of shellfish in a broader population dynamic/ecological context if we harbor hope of maintaining healthy coastal ecosystems.

Ecological interactions between shellfish and other organisms can have dramatic impacts on shellfish health. Abalone cultured on the west coast, for example, are susceptible to infestations of sabellid polychaetes. These worms settle on the shells, inhibit the formation of respiratory pores and cause the shells to dome. The latter condition limits abalone mobility and increases morbidity and mortality (Culver et al., 1997). Similarly, oysters, mussels and

scallops along the East and Gulf coasts are susceptible to infestations of the mud worm *Polydora*. Infested bivalves are weakened physiologically and structurally; heavy infestations can lead to death. A better knowledge of the ecological parameters that control these types of interactions will lead to better management capabilities and more successful fisheries and aquaculture operations.

DISEASE

Diseases are a natural part of the marine environment and it is very difficult, if not impossible, to eradicate them from natural populations. Therefore, it is necessary to manage around diseases for both fisheries and aquaculture. In order to advise resource managers on disease mitigation strategies or to develop control measures for aquaculture, it is important to understand, as completely as possible, the taxonomy and basic biology of disease agents. Determination of closest relatives may provide clues to life cycles or pathogenic mechanisms and is necessary for development of specific DNA-based diagnostics. Geographic strains likely exist in viruses, bacteria and protists. These strains may have different virulence, and it is important to understand the extent of strain variation and virulence difference of pathogens for proper management of host transfers (see Bushek and Allen, 1996; Reece et al., 1997). Important biological parameters include life cycle and transmission dynamics; physiology, growth and survival at various temperature and salinity combinations; and mechanisms of pathogenesis.

The current status of knowledge for these parameters varies depending on the pathogen. For some diseases, usually those that have seriously impacted fisheries or aquaculture, there is considerable information. For example, *Perkinsus marinus*, the causative agent for Dermo disease in oysters along the Gulf and Atlantic coasts has been relatively well studied (Burreson and Ragone-Calvo, 1996). Nonetheless, critical information is still lacking on transmission rates at combinations of temperature/salinity with varying durations. For emerging diseases, like QPX disease in hard clams, withering syndrome in abalone and many diseases occurring in shellfish hatcheries or those that have not caused serious mortality, far less is known (Bower et al., 1994; Moore et al., 2000).

Better diagnostic tools are needed for diseases, pathogens and parasites.

Diagnosis

Rapid, specific and sensitive diagnostic tools are critical for early detection of pathogens. Early detection will result in more effective animal health management and the diagnostic tools can also be used for monitoring programs. DNA-based detection methods have been viewed as a panacea because of their extreme sensitivity and specificity. Although these methods hold great

promise, further research and evaluation is necessary before unambiguous interpretation of results will be possible.

Current diagnostic tests include paraffin histology, which is useful for detection of a variety of pathogens (Figure 4). This test provides information on location in the host, pathogen intensity, host response, tissue pathology and presence of co-occurring pathogens; no other test provides so much information on host/parasite interactions. Although costly and time-consuming, histology is still the “gold standard” for many pathogens. Fluid thioglycollate culture is routinely used for detection of the genus *Perkinsus* which includes important pathogens of bivalve and gastropod molluscs (Bushek et al., 1994). This test is time-consuming, only semi-quantitative and not species specific. Various rapid bacterial identification kits are available, but they often don’t work with bacteria isolated from marine environmental samples. Development of diagnostic tests for shellfish viruses has been hindered by the lack of cell lines for marine molluscs. Molluscan cell lines are needed to isolate and characterize intracellular pathogens of molluscs and, perhaps, humans (e.g., Hepatitis A virus), conduct toxicological and physiological studies and to produce diagnostic reagents (Burns, UCSD pers. comm.). The lack of cell lines for marine molluscs is a substantial detriment for molluscan health management that needs to be addressed (Elston, 1999).

Modern diagnostic tools include DNA-based methods or antibody-based methods. For all diagnostic methods, but especially the new DNA-based tests, we need to determine what detection of a parasite or pathogen means for the health of the host population. DNA-based diagnostics may detect non-viable pathogens, or even DNA fragments, thus a positive test does not necessarily indicate that a viable pathogen is present. Because the sensitivity of these modern diagnostic tools is so high it is important to determine what a positive diagnosis means with respect to disease status and the potential for transmission if the shellfish are moved (Figure 5, see shellfish transfers on the following page). Before DNA-based diagnostics can be recommended as the sole diagnostic method, much more research is necessary that compares early detection of a pathogen using multiple diagnostic tests (e.g., PCR and histology) coupled with population monitoring (e.g., health status and environmental conditions) to determine when a particular parasite becomes pathogenic.

Standardization is needed for tissue subsampling, fixative, primer and probe sequences and threshold determination. For DNA-based and antibody-based techniques, standardized positive controls are critical. Laboratories responsible for diagnosis of shellfish pathogens should be accredited and should use standardized techniques. Diagnosticians need to be adequately trained and certified.

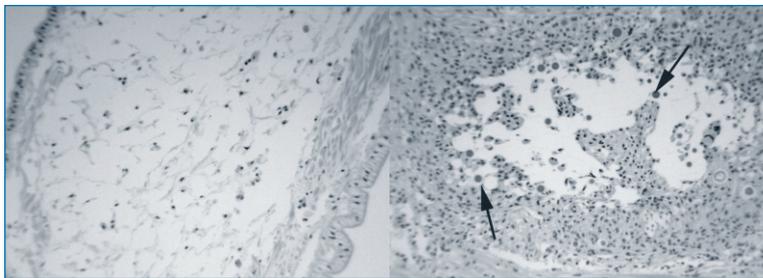


Figure 4. Histology as a diagnostic tool. The image on the left shows a healthy hard clam (*Mercenaria mercenaria*) tissue section. The image on the right shows the immune response of a hard clam infected with the QPX disease causing parasite (arrows). Courtesy of Roxanna Smolowitz.

Molluscan cell culture is needed to allow isolation of shellfish viruses in order to understand and control viral infections, study host and parasite cell physiology and for the development of diagnostic tests.

Modern diagnostic tools are needed to supplement existing methods in order to improve sensitivity, specificity and efficiency but must be calibrated epidemiologically for use in management.

Standardization is needed for all diagnostic tests.

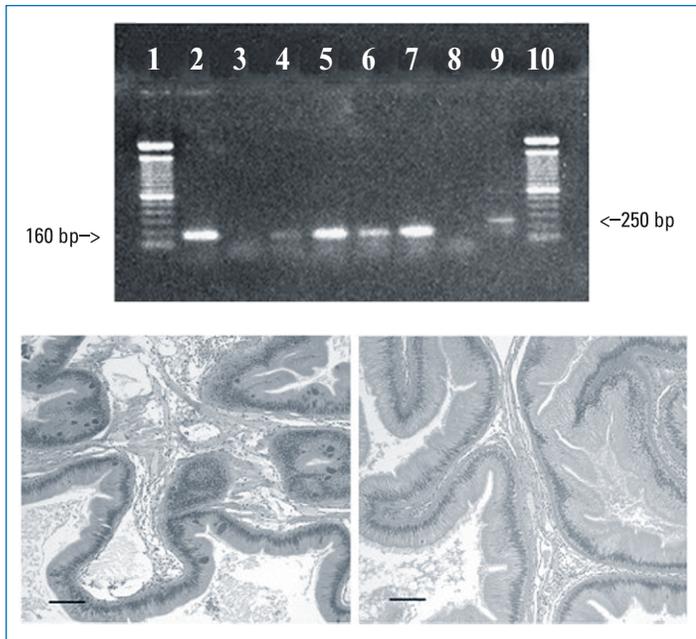


Figure 5. Comparison of DNA-based molecular diagnostics and histology. The top image is a photograph of a PCR (polymerase chain reaction) gel from Friedman et al (2000). It shows the detection of DNA from the rickettsial bacterium (called RLP) that causes withering syndrome in abalone. Each numbered column is referred to as a lane into which DNA samples were added and induced to migrate from one end of the gel to the other. The bright bars represent how far the DNA moved during the assay. Lanes 1 and 10 contain 'DNA ladders', that is, pieces of DNA of known sizes measured by the number of base pairs that are linked together (bp). Lane 2 is a positive control: DNA isolated from a culture of the RLP. Comparison with the DNA ladder indicates the RLP DNA is 160 bp long. Lane 3 is DNA isolated from an uninfected abalone and serves as a negative control to ensure that the assay is working properly. Note that no band is present. Lanes 4–9 are samples of DNA from various tissues of infected abalone or a bacterium isolated from an infected bacterium (lane 8). Lanes 5, 6 and 7 are positive while the others appear to be negative indicating that the RLP organism is concentrated in certain types of tissue. The bottom images show histological sections of RLP infected abalone tissue (arrows in right image) and uninfected tissue (left image). Histological samples typically only detect more advanced infections whereas the molecular assays are much more sensitive and can sometimes detect DNA from a single infective cell. Images courtesy of Carolyn Friedman.

Disease Outbreak Response Plans need to be developed prior to the disease outbreaks.

There is a need to develop **Decision Making Flow Charts** to translate the results of diagnostic tests into management actions.

SHELLFISH DISEASE MANAGEMENT FOR RESOURCE CONSERVATION AND AQUACULTURE

Resource managers need to make decisions related to management and control of infectious shellfish diseases. Such decisions may be related to animal transfers or disease outbreaks. These decisions may have significant consequences in regard to environmental quality, value of natural resources and economic viability of businesses based on edible shellfish production. There is an urgent need to support the decision making process with information, guidelines and protocols that will provide consistent and effective management.

Application of disease diagnosis information. Diagnostic information is used to make management decisions, but a more systematic approach is needed. Difficulties can result from the highly technical nature of disease diagnostic information compounded by a lack of baseline information regarding existing geographic ranges of diseases and their significance. While more technical information is always needed to support this decision making process, a more fundamental need in resource management is to have a basic decision making approach and to have one that is consistent across political boundaries. This information needs to be coupled with fisheries management and aquaculture so that diagnosis is coupled with specific actions.

Response to disease outbreaks. Infectious disease outbreaks are not predictable phenomena and often may not be discovered until sometime after they occur. Therefore, a decisive and rapid response is critical in order to minimize the effect of an outbreak. Unless a plan has been developed and is available prior to the disease outbreak, the response is not likely to be effective. Therefore it is important that such plans be developed for each governmental jurisdiction responsible for shellfish management. In addition, response plans can be developed by private producers of shellfish as part of a quality control/quality assurance program. Such plans should be readily available to Extension Agents (e.g., Sea Grant Marine Advisors) to improve their ability to assist the industry. An example of a disease response plan is contained within the Shellfish High Health program of the Pacific Coast Shellfish Growers Association. Such approaches need to be expanded and incorporated into resource management agencies.

High Health Programs. In order to reduce the risk of dissemination of infectious diseases through aquaculture activities and to make shellfish aquaculture as efficient as possible, health standards, programs and operating protocols are needed. The National Poultry Improvement Plan is an example of a voluntary, producer-based program to promote animal health. Similarly, Shellfish High Health Programs are needed for the intensive production of shellfish. Producer associations need assistance to develop framework programs that

will then be adopted and endorsed by appropriate governmental agencies such as the Animal and Plant and Health Inspection Service of the U.S. Department of Agriculture. Such a partnership will provide an essential element that will allow healthy, live shellfish produced for export in the United States to have access to foreign markets. In addition, High Health Programs will set health standards for husbandry operations and will help ensure that only healthy shellfish are distributed from hatcheries and nurseries and may reduce the costs of shellfish production.

Shellfish Transfers. Shellfish must be moved within and across state boundaries in order to maintain the viability of the shellfish industry. Shellfish may be moved for aquacultural production, stocking fisheries, habitat restoration, marketing, or even research. For example, U.S. producers of shellfish export in excess of 100 million shellfish larvae and seed annually. At the same time, researchers may be moving isolates of cultured parasites for critical research. It is imperative that native and farmed populations of shellfish are protected from the introduction of exotic pathogens and non-indigenous species via these movements.

Movement of shellfish (destined for culture or planting in the environment) across state boundaries usually requires the approval of the responsible resource management agency in order to reduce the risk of transfer of infectious shellfish diseases or undesirable species. As a result, inspections and permits are required. Both on an interstate and international basis, these inspections are a significant cost of production. Due to the lack of science based standards for such inspections, the requirements and thresholds that must be met are inconsistent. In addition, the inspection procedures are heavily weighted toward the inspection of aquaculture products while neglecting other pathways of shellfish disease transfer. There is an urgent need for science-based standards in regard to the inspection of shellfish for diseases. Such standards are needed for regional and distant transfers and intentional exotic species introductions. The importance of local ecology, particularly in geographically restricted systems, should be considered when transfers are evaluated. Harmonization of state transfer regulations and standards for acceptance or rejected are needed.

In addition, disposal of pathogen contaminated materials (including effluents from processing facilities and research laboratories) represent potential dispersal mechanisms for a disease agent that threatens the continued health and stability of shellfish resources (e.g., *P. marinus* and *Candidatus Xenohalotus californiensis*, Friedman et al., 2000). Disposal methods are needed that will eliminate or minimize the potential inadvertent dispersal of shellfish parasites and pathogens via these pathways. Presently, no standard protocols exist for the safe disposal of pathogen contaminated tissues or effluents. Given the extensive commercial transport of mollusks potentially harboring various races of parasites, the establishment of standards for treating and disposing of contaminated effluents and tissues is long overdue. In addition, a means of compensating a farmer for destroyed stocks needs to be evaluated.

Shellfish High Health Programs
are needed to establish health standards for producers of live animal seed and brood stocks.

Scientifically based standards for animal transfers are needed.

An expert panel should be convened to develop and recommend standards.

Evaluation of animal transfer risks should account for all pathways such as:

- *Seafood markets, restaurants.*
- *Shipping ballast water.*
- *Public (individual transfers, e.g., of edible retail shellfish products).*
- *Shellfish culture transfers.*



ANIMAL HEALTH MANAGEMENT FOR INTENSIVE CULTURE SYSTEMS

An increasing portion of our shellfish is produced by intensive aquaculture, including the production of seed (Figure 6). This trend is expected to continue and intensify. As shellfish aquaculture develops, there is an increasing need to develop and apply health-based culture methods, health management approaches for husbandry systems, and to train shellfish hatchery and nursery workers to use methods that optimize the health of their shellfish products. These approaches will reduce production costs and result in healthy shellfish products. Specifically, more information, technology development and standards are needed to:

Figure 6. Intensive bivalve culture in Maine. Clockwise from upper left: larval production tanks in a hatchery, nursery upwellers for producing seed, long-lines with trays for grow-out, sorting and cleaning trays during grow-out phase. Photos courtesy of David Bushek.

Develop culture methods to optimize shellfish health including:

- Hatchery and nursery site selection,
- Training for hatchery workers in health and disease management,
- Improved water treatment, conditioning and disinfection methods,
- Sanitation procedures with proven efficacy for specific disease agents,
- Use of probiotic approaches to prevent infectious diseases,
- Shellfish health and condition indices and standards,
- Criteria for grading and culling animals.

Improve brood stock management including:

- Establishment of shellfish strains with genetic resistance to disease,
- Collection and use of specific pathogen free brood stock,
- Standards and procedures for the maintenance of brood stock to insure health status.

Prevent and control infectious diseases in the hatchery and nursery through:

- Improved field based diagnostic procedures,
- Improved hygienic and health management procedures (e.g., feed, water, management),
- Established procedures for culling of diseased organisms and their disposal.

Modeling

Modeling plays an important role in many aspects of shellfish research (Soniati et al., 1998). Application of models can improve our understanding of shellfish health. The most valuable and useful models will be process driven and verified with independent data. As models are developed their initial utility will be to highlight gaps in our knowledge of disease processes. Later they will highlight potential intervention mechanisms to prevent disease or improve shellfish health. Models need to be developed that investigate shellfish health and disease processes at various scales ranging from molecular and cellular

Development of culture practices that promote animal health will increase the efficiency of culture and reduce the risk of disseminating infectious diseases.

through populations, communities and ecosystems. Of vital importance is a need to examine transmission processes and the factors that influence those processes. Efforts to encourage the independent development of models from different groups will encourage multiple perspectives.

Environmental Quality

A healthy environment equals a healthy resource. Water quality is of paramount importance to shellfish resources. With increasing coastal development, efforts are needed to understand the relationships between changing land-use patterns, water quality and impacts on shellfish populations, especially transmission and progression of shellfish diseases. Geographic Information System technologies should be exploited to identify these relationships (Figure 7; White et al., 1998). Some relationships have been suggested between pollution and disease from laboratory studies, but field data remain equivocal. More effort should be placed on experiments to elucidate these relationships so that better management can be developed.

Monitoring

Monitoring is a crucial tool in establishing the presence of infectious agents, and the resulting diseases, within a population or between different populations in various geographic areas (Richards et al., 1993). Continuous, standardized monitoring of shellfish populations, disease agents and environmental parameters provides information concerning both the past and present status of infection and disease in a population. These data may also illustrate relationships with environmental factors and the potential to predict future morbidity and mortality events due to that disease (link to modeling). Such epidemiological information is critical to the development of management methods for both fisheries and aquaculture. These data may also elucidate the pathogenic relationship between a host and a potential disease causing agent, and how that relationship changes within an area over time. More importantly, immediate access to monitoring data is vital to disease response efforts. Monitoring is often haphazard and poorly timed or reactive rather than proactive. Standardized methods of proactive monitoring need to be established to (1) better understand the relationships between infection and disease and (2) provide appropriate data for production of disease prediction models and (3) facilitate disease prevention and response plans. Factors to consider in the formulation of appropriate monitoring methods include:

- Detection method used (See Diagnostic Methods);
- Spatial and temporal scales, frequency and intensity;
- Collection of relevant environmental data;
- Identification of appropriate monitoring locations;
- Correlation of monitoring data with land-use patterns using GIS technology; and
- Ability to “scale up” monitoring when unexpected increases in morbidity and mortality occur.

Modeling:

- *Important for predictions.*
- *Process models should be accomplished first and verified with actual data.*
- *Research needed on component processes, especially transmission rates, for all diseases.*
- *Different groups of modelers should be encouraged to get multiple perspectives.*

It is important to:

- *Identify the spatial relationship between hydrography, land use patterns, fisheries, and aquaculture.*
- *Maintain and improve water quality.*
- *Understand relationships between pollution and disease.*

Monitoring is crucial for effective management of shellfish resources and aquaculture species.

Important Monitoring Needs:

- *Convene a group of professions to develop standardized methods of monitoring.*
- *Increase basic agent and disease monitoring in geographical locations of the infectious agent.*
- *Correlate that data on a regional and national scale using GIS technology.*
- *Provide a mechanism to allow “rapid monitoring responses” to unexpected increases of disease.*

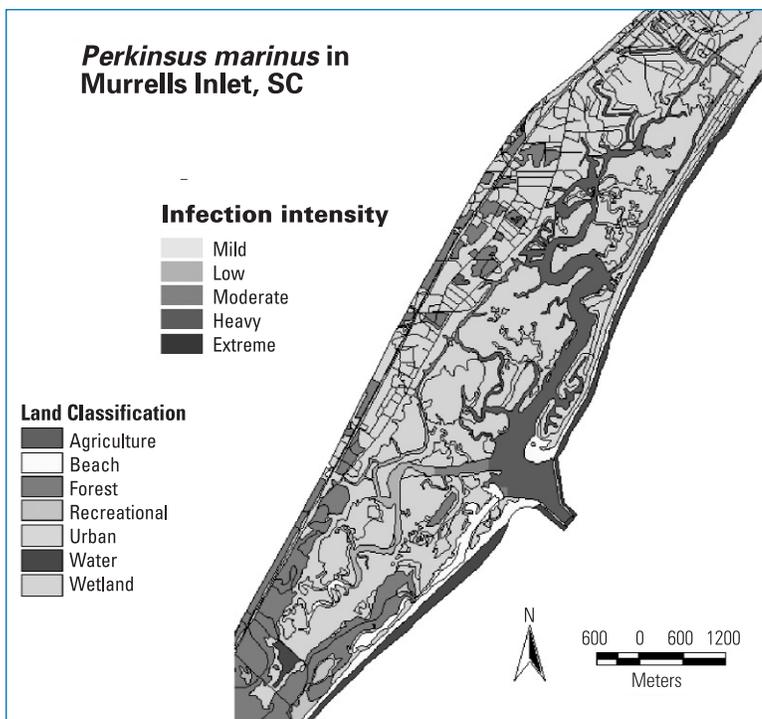


Figure 7. Geographic Information Systems provide powerful tools to examine and model spatial patterns of disease. This example depicts data collected by White et al. (1997) as part of NOAA's Urbanization of Southeastern Estuarine Systems (USES) project. It shows the associations between Perkinsus marinus infection intensities, landscape, and land-use patterns in Murrells Inlet Estuary, South Carolina, USA.

Successful Resource Management Relies Upon:

Improved links between researchers (universities), industries and environmental conservation groups (e.g., NPS) and resource agencies.

Access to diverse individuals with expertise in statistics, sciences in general, fisheries (fishery independent and dependent data collection/analysis experience), and aquaculture.

Education of managers and user groups (researchers, general public, and industry members).

Resource Management

Resource managers are faced with the difficult task of preserving our natural resources for ecological, recreational, and commercial uses. An interdependence exists between these three primary uses of near shore marine environments. Preservation of the ecological balance of our oceans will, in turn, enhance our ability to maintain sustainable fisheries and aquaculture. Despite this, the different user groups are often in conflict. Some of these conflicts are justified as certain activities are inherently destructive or prohibitive to the success of other user groups. In many other situations, these conflicts may arise from a paucity of information, lack of familiarity with some of these issues, or jurisdiction. Sound resource management relies on the ability of managers to make informed and educated decisions. Improved links between resource managers, scientists, industry members, environmental conservation groups, legislators and the general public are needed to facilitate the development and success of new policies and regulations. These links will also enable the resource agencies to acquire adequate funding and attract a diverse pool of specialists (e.g., statistics, ecology, zoology, pathology, fisheries and aquaculture) which are crucial to achieve these diverse goals.

Regional Considerations

West Coast Region. Shellfish production occurs in all of the West Coast states (Alaska, Washington, Oregon, California and Hawaii). Abalone have become an important cultured species in California, and Washington state is the nation's leading producer of cultured oysters. In addition, there are many other species of commercially and recreationally harvested and cultured shellfish, a number of which are affected by infectious diseases. Examples of these shellfish species and the diseases that affect them are shown in the following table.

Table 1. Harvested and cultured shellfish from the west coast and known west coast disease conditions.

Shellfish Common Name	Scientific Name	Disease conditions
Pacific oyster	<i>Crassostrea gigas</i>	Nocardiosis Haplosporidiosis (two types) Oyster velar virus disease Hinge ligament disease Vibriosis Invasive ciliates <i>Polydora</i> infestations
Abalone (7 species)	<i>Haliotis</i> spp.	Withering Syndrome (rickettsiosis) Coccidiosis Sabellid polychaete infestations
Manila clams	<i>Tapes (Ruditapes) philippinarum</i>	Nursery wasting syndrome Vibriosis Hinge ligament disease
Pacific razor clam	<i>Siliqua patula</i>	Nuclear inclusion X (NIX) Branchial rickettsiosis
Eastern oyster	<i>Crassostrea virginica</i>	None observed
Kumomoto oyster	<i>Crassostrea sikamea</i>	Vibriosis
Olympia oyster	<i>Ostrea conchaphila</i>	Hexamitiasis
European flat oyster	<i>Ostrea edulis</i>	Bonamiasis
Littleneck clam	<i>Prothaca staminea</i>	Coccidiosis Vibriosis
Geoduck clam	<i>Panope abrupta</i>	Vibriosis
Bay mussel	<i>Mytilus trossolus</i>	Hemic neoplasia
Horse clam	<i>Tresus capax</i>	Haplosporidiosis

Gulf Region. Extensive fisheries for *C. virginica* still exist in the Gulf of Mexico and the Gulf oyster fishery often produces more than half of the nations oysters. Gulf oyster populations suffer losses, however, from a number of parasites, pests and predators, but the predominant threat comes from *P. marinus* which causes Dermo disease. Largely as a result of this disease, Gulf oyster harvests fluctuate widely from year to year. Smaller fisheries and aquaculture operations also exist for a few other species, mainly hard clams (*Mercenaria mercenaria*) and scallops (*Argopecten gibbus* and *Argopecten irradians*) along the west coast of Florida, and these species have also been threatened by various disease epizootics (E. Powell pers. comm.).

Southeast and Middle Atlantic Region. Important mollusc fisheries in the middle and southeast Atlantic include eastern oyster, *Crassostrea virginica*; hard clam, *Mercenaria mercenaria*; soft shell clam, *Mya arenaria*; channeled whelk, *Busycotypus canaliculatus*; knobbed whelk, *Busycyon carica*; surf clam, *Spisula solidissima*; ocean quahog, *Arctica islandica*; scallop, *Placopecten magellanicus*; bay scallop, *Argopecten irradians*; and squid, *Loligo* sp. Major diseases in the middle Atlantic include

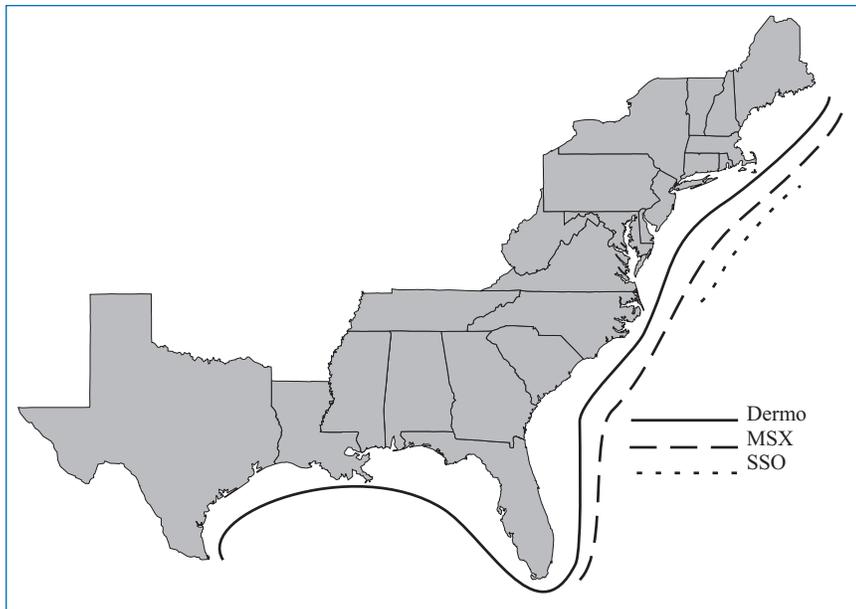


Figure 8. Geographic distribution of bivalve molluscs and key diseases along the east coast of the U.S.

Northeastern Region. The primary cultured species of bivalves in the northeast consist of the eastern oyster and hard clams. Other species include the surf clam, sea and bay scallops, razor clams (*Ensis directus*), blue mussels (*Mytilus edulis*) and soft shell clams (*Mya arenaria*). Prevalence, intensity and resulting oyster mortality from *Perkinsus marinus*-induced disease have increased significantly over the last three years (1996 to 1999) (Smolowitz, pers. comm.). Such changes appear to be directly related to increased length of warm weather periods and lack of sufficiently long and cold winters in the area. Recently (1999) MSX was identified at high prevalences and resulting mortalities in several areas of the Northeast. Whether this reflects a temporary change due to decreased rainfall (1999 was a drought year in most locations) or is also a reflection of decreased “winter weather” (or both) is not known. Bivalve shellfish hatcheries have suffered from repeated losses due to Juvenile Oyster Disease and vibriosis, both of which vary greatly on an annual basis. The etiological agent of Juvenile Oyster Disease has not been identified, but this disease causes mortality primarily in New England. Leukemia and gonadal neoplasia in *Mya arenaria* continues to be found at static levels in already defined locations. Disease associated with QPX infections in hard clams has been identified in 4 areas of the northeast and continues to cause morbidity and mortality in the most northern of those locations. Other populations of QPX infected hard clams have not yet been identified. Additional monitoring is needed to determine the distribution of QPX infections.

Outreach

Education of those who use the shellfish resources and application of shellfish health information and technology must receive increased emphasis in order to optimize the health, productivity and sustainability of shellfish resources. Shellfish culturists must receive training in health management techniques to produce high health shellfish. Resource managers must receive procedures, information and training regarding the use of shellfish disease data to make resource management decisions. Outreach efforts are needed to reduce the risk of disseminating shellfish diseases by the geographic transfer of live shellfish by industry, scientists or transfer of live retail-purchased shellfish products by the public. Diagnostic tools must be used to improve shellfish culture systems and the health status of husbanded shellfish. This can occur through the identification and subsequent elimination of sources of infectious diseases within culture facilities. This, in turn, will greatly increase the efficiency of shellfish production. Further, the dissemination of healthy shellfish from hatcheries and nurseries, resulting from the application of health and disease research and technology, will ensure that enhanced shellfish populations are initiated only

using “high health” shellfish. To accomplish this outreach mission increased support should be directed toward the development of support systems for shellfish aquaculture and fisheries, perhaps modelled after the agriculture experiment station system. These efforts should include research programs, education, technical assistance, reporting of events (e.g., disease outbreaks) and rapid dissemination of this information.

SUMMARY

Achievement and maintenance of optimal shellfish health relies on a thorough knowledge of the biology, life history, ecology, culture, nutrition, physiology, and presence of natural loads of parasitic and commensal symbionts. This will enable the diagnosis of a disease and subsequent sanitation, management and treatment of cultured shellfish and the management of diseases in natural populations or commercial fisheries. Modern molecular diagnostic methods and development of marine shellfish cell lines promise enhanced diagnostic abilities. The limitations of these newer and traditional diagnostic methods must be determined and must be placed in perspective when evaluated. The presence of a pathogen must be differentiated from the presence of disease and better standards must be developed for shellfish health certification and shellfish disease risk management. A national panel of experts must be convened to develop these standards and recommendations. Procedures for systematic decision making to manage shellfish diseases are needed as well as shellfish producers High Health Programs and successful application of health management procedures for cultured shellfish. Comprehensive shellfish health and disease monitoring is needed to support shellfish resource management and disease control. Education of all user groups is needed for successful health of shellfish in natural populations, fisheries and aquaculture.

FUTURE DIRECTIONS FOR SHELLFISH HEALTH MANAGEMENT

- *Develop thorough biological knowledge base (Physiology, nutrition, defense mechanisms, population dynamics, genetics).*
- *Diagnostic tool development.*
- *Develop broader understanding of disease transmission dynamics and pathogenesis.*
- *Establish proactive disease monitoring and rapid dissemination of information.*
- *Determine and implement optimal aquaculture conditions for shellfish health.*
- *Establish Shellfish High Health Programs, intensive health management practices, and disease response plans.*
- *Establish health certification standards.*
- *Provide framework for systematic approaches to decision making for management of shellfish resources and shellfish health issues.*
- *Implement information transfer regarding shellfish health for resource managers and shellfish hatchery workers.*
- *Contribute health data to a central data base.*

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Chapter 11

Physiology

INTRODUCTION

Understanding the physiological processes that govern feeding, digestion, growth and reproduction is of fundamental importance. These processes are used to assess a variety of questions related to populations of molluscan shellfish. In near-shore waters, molluscan shellfish strongly influence and interact with biotic and abiotic components of the ecosystem. Bivalves for example, join the water and bottom environments through their feeding. They filter organic matter (and other materials) from the water and deposit undigested material on the sediment surface. This affects nutrient regeneration (Newell and Ott, 1999).

Molluscan shellfish physiology is linked closely with all other elements of molluscan shellfish with the possible exception of “Economics, Trade and Policy.” Physiology can address the factors underlying development, growth, reproduction and survival of molluscan life stages. Many shellfish studies need physiological data to achieve their objectives (e.g., models require information on the partitioning of assimilated energy between tissue and eggs and sperm production). In turn, physiological research often requires input from other research elements (e.g., appropriate indicators of parasite and disease burden) to design and conduct studies. Therefore, basic physiological information often underlies an understanding of important issues and problems related to fisheries management, aquaculture, habitat rehabilitation and enhancement.

There is a large body of information on molluscan physiology, including mechanisms of feeding, metabolism, growth and reproduction of both gastropods and bivalve molluscs (Wilbur, 1983; Bayne and Warwick, 1998). Much of our understanding of molluscan physiology, however, is based on laboratory studies in which relatively few species were examined in almost complete isolation of natural environmental conditions. A more complete understanding of the complex physiological processes of commercially important molluscs will require studies that are performed under more natural conditions using a broader range of environmental variables. Physiologists also need to broaden their studies to include a diversity of molluscan species and to consider the full range of molluscan life stages including larvae, post-set, juvenile and adult stages. Moreover, because we still lack a complete understanding of the physiological responses at the cellular, tissue, individual organism and population/community level, future studies must also span a range of organizational scales from the cellular to the ecosystem level. Finally, because of the multidisciplinary nature of physiological studies, it is important that uniform research protocols be established (e.g., Bayne et al., 1985). These protocols might include establishment of common terminology (e.g., clearance vs. filtration rates), agreement of defined sampling frequency and scale, standardization of instrument calibration in different environments and clearer definitions of environmental parameters being tested.

In the following sections, we have identified a number of critical issues that relate to the physiology of natural and artificially enhanced populations of molluscs. These issues are presented as questions that are discussed in general terms, followed by several approaches that could be used to address the particular issue raised. We recommend that future funding initiatives focus on research that addresses questions relevant to the goals established below.

GOALS:

1. To understand the role of molluscan shellfish in natural ecosystems and to provide information for models that predict carrying capacity and nutrient processing.
2. To assure population stability of molluscan shellfish, thereby maintaining maximum ecological benefits and sustainable harvests.
3. To understand the response of molluscs to environmental stressors on five levels of organization: biochemical, cellular, tissue, organismal and population.
4. To optimize yields and product quality for economic gain, without negatively impacting the environment.

II. PRIORITIES FOR FUTURE DIRECTIONS

GOAL 1: *To understand the role of molluscan shellfish in natural ecosystems and to provide information for models that predict carrying capacity and nutrient processing.*

Status and Importance

Bivalves. Most species of bivalve molluscs use their gills to capture food particles suspended in the water column. Because such food is often at low concentrations, bivalves are highly efficient at pumping large volumes of water and trapping and sorting food particles prior to ingestion. The animals' particulate wastes (biodeposits) fall to the sediment surface where they provide organic food for other benthic animals and also serve to change the site of nutrient cycling from the water column to the sediments (Dame, 1996; Newell and Ott, 1999). The combined feeding activity of dense assemblages of bivalves can deplete the suspended particle concentrations from the surrounding waters. This can have diverse ecological effects, some of which are considered beneficial because their mode of action supports management objectives (Figure 1).

For example, in many coastal locations man's nutrient enrichment stimulates excess phytoplankton production which generates bottom water anoxia that kills benthic life. Greater consumption of phytoplankton in near-shore waters by bivalves reduces the amount of suspended organic carbon available to stimulate low oxygen levels (Newell and Ott, 1999). Additionally, bivalve feeding reduces turbidity, thereby increasing light availability to the bottom and enhancing the growth of seagrasses (Newell and Ott, 1999) (see Figure 1). One potentially undesirable effect of enhanced bivalve populations is that they may out-compete other species for a limited food supply and thus alter the food web at that location.

Only by gaining quantitative information on the feeding processes of bivalves under field conditions can we quantitatively assess both the possible beneficial and detrimental effects bivalves have on complex coastal ecosystems. This information can then be integrated into predictive ecosystem models, such as those generated by Kemp and Bartleson (1990) and Ulanowicz and Tuttle (1992). The ultimate goal of such ecosystem models is to predict the number of bivalves that can be grown

in any particular location which is useful when granting aquaculture permits (e.g., Grant and Bacher, 1998; Newell et al., 1998; Scholten and Smaal, 1998).

Non-filter-feeding molluscs. Most gastropod molluscs feed by grazing on either the organic coatings of particles or surfaces (e.g., diatoms, bacteria, detritus, encrusting organisms), or on macroalgae. Their impact and its importance on the succession of plant colonization of substrates has been described by ecologists. High grazing pressure by intertidal gastropods, for example, can reduce the rate of seaweed colonization on rock surfaces and inhibit the development of associated communities (Carefoot, 1977; Johnson and Mann, 1986). A few species of gastropods are carnivorous and prey on sessile invertebrates, including commercially important bivalve species such as oysters. Such predation can cause high rates of mortalities and heavy losses of individuals in an artificially enhanced population. More research needs to be undertaken to determine the chemical cues and behavior that allow predatory gastropods to find and feed on their prey. Such an understanding would result in better strategies to prevent the adverse effects of predation on commercial bivalve production, including better predator exclusion devices and traps or bivalve decoys.

Approaches to meet this goal

- *Develop methods to measure physiologically relevant processes in situ (e.g., feeding rates) to gain new information and corroborate laboratory based data.*

Although much is understood about bivalve feeding, these data have largely been generated under experimental laboratory conditions (e.g., Winter, 1978; Bayne and Newell, 1983; Ward, 1996). Many environmental factors, such as food availability and quality, are highly variable, and so the response of bivalves to complex variations of many environmental parameters needs to be determined in multi-factorial experiments (e.g., Widdows, 1978). For example, it is apparent that in order for ecosystem models to be useful, feeding rates must be measured under the range and complexity of environmental conditions found in the field (e.g., MacDonald and Ward, 1994; Cranford and Hill, 1999; Hawkins et al., 1999). We need to develop *in situ* methods to continuously collect physiologically relevant data over different temporal scales (e.g., tidal cycle, seasonal cycle). Such *in situ* studies could be conducted in collaboration with physical oceanographers who routinely deploy instrumentation packages that monitor water column parameters such as current speed and direction, tidal stages, density, turbidity and chlorophyll concentration. By integrating continuous physiological measurements with environmental data we will gain a real knowledge of how bivalves compensate for environmental change.

- *Determine the effects of changes in environmentally relevant food regimes on digestive physiology.*

The particle composition of seawater is a temporally and spatially variable mixture of living and dead plant material, bacteria, and single-celled animals, as well as suspended mineral particles, such as clays. Because bivalves can ingest this entire spectrum of food particles, quantifying their diet under natural conditions is complex. We have little quantitative information on factors affecting the efficiency with which bivalves digest their food and the various processes that can affect the energy costs associated with food processing. This lack

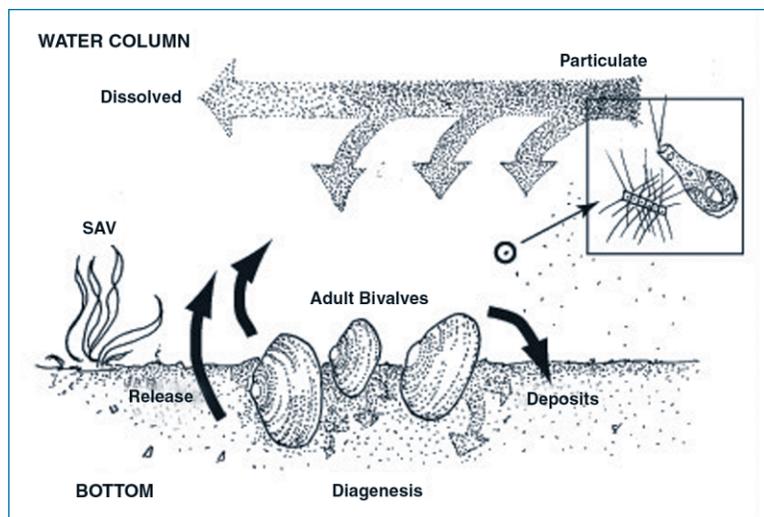


Figure 1. Some interactions between bivalves and the ecosystem. Through filter-feeding processes, dense beds of bivalves remove phytoplankton and other particles from the water column using some of the material as food to grow. Unwanted or undigested material is deposited on the bottom. Such removal can reduce suspended particle loads and enhance the clarity of the water allowing for better growth of submerged aquatic vegetation such as sea grasses (SAV). Dissolved materials (nutrients) are released back into the water column by the bivalves and by microbes inhabiting the bottom (adapted from Dame, 1996).

of understanding is due, in part, to the fact that bivalves have a complex digestive system in which some food particles are digested in the stomach and gut, and other particles are digested intracellularly within the digestive diverticula (reviewed by Morton, 1983; Langdon and Newell, 1996). Therefore, in order to understand the factors that govern digestive adaptations, we need to refine techniques for quantifying the exact food (seston) composition and the bivalves ability to capture and digest major dietary sources. These processes need to be examined over different temporal scales because physiological processes can be greatly influenced by both short (tidal) and long term (seasonal) changes in temperature and salinity (Newell, 1979; Widdows et al., 1979; Berg and Newell, 1986; Langdon and Newell, 1990).

In connection with digestive adaptations, we also need to determine factors that influence rates and quality of biodeposition, and influence nitrogen and phosphorous excretion. Although there are data on rates of nitrogen and phosphorous excretion by bivalves (reviewed by Bayne et al. 1976), such data need to be obtained under varying environmental conditions. Similarly, rates of nitrogen and phosphorous biodeposition under varying seasonal and food conditions need to be collected. These data are important in understanding how large populations of bivalves affect primary production in nearshore waters.

- *Investigate the influence of genetic diversity on key physiological processes.*

We understand very little about the possible genetic basis of the highly variable physiological responses found within natural bivalve populations. In some species, this variation could be altered even before it is understood as a consequence of interbreeding between natural and aquaculture stocks. Management restoration activities are transplanting bivalve stocks between locations possibly reducing the physiological fitness of these stocks if they are not genetically adapted to their new location. Genetic variation can underlie complex behaviors such as larval recruitment (Manuel et al., 1996 a,b; 1997; Manuel and O'Dor, 1997), so it is imperative that collaborative research between physiologists and geneticists take place at all scales from the sub-cellular to whole animal to population level.

- *Investigate the competitive physiological processes (e.g., feeding and recruitment) of co-occurring species.*

The physiological studies described above must be performed both on the target species associated with aquaculture and commercial shellfisheries, as well as species native to the culture area. In this way data will be available to model the ecological role of cultured species as it relates to non-commercial indigenous species. For example, it is important to understand how an increase in shellfish, e.g., through oyster aquaculture, may result in competition for resources with native shellfish, such as infaunal clams, thereby potentially reducing populations of the latter. If the infaunal clams, for example, serve as an important food source for native predators, then a decline in predators may also occur.

Potential links to other groups

The initiatives outlined above directly link to issues addressed by other working groups including Ecosystem Modeling, Broad-scale Ecology, Genetics, and Protection and Restoration of Shellfish Habitat. In particular, better interactions need to be established between physiologists and modelers. Model development should include evaluation of the questions that physiologists need to address, so that specific model parameters can be determined.

GOAL 2: To assure population stability of molluscan shellfish, thereby maintaining maximum ecological benefits and permitting sustainable harvests.

Status and Importance

Over the past fifty years, the basic life-cycles of many commercially important molluscs have been elucidated (Figure 2), however, many questions concerning the factors that mediate molluscan population dynamics remain. Many molluscs exhibit the capacity to shift or reallocate resources between growth and reproduction to meet reproduction requirements (Peterson and Fegley, 1986).

For example, the gonadal-somatic index of the hard clam, *Mercenaria mercenaria*, in subtidal locations is greater than the indices in intertidal locations. Although this is evidence that ecological conditions influence the allocation of resources between growth and reproduction, it is not clear to which environmental factor(s) the hard clam is responding, and what factors affect the allocation process. Similarly, in eastern oysters, *Crassostrea virginica*, it is not clear what biochemical or physiological mechanisms regulate reproduction. In general, there is little information on what regulates reallocation of energy and how this reallocation affects the physiological state of the individual.

Considerable variation occurs in the number of eggs spawned by a female bivalve or at least in our estimates of fecundity (as reviewed by Thompson et al., 1996). Fecundity may be affected directly by stressors such as parasites and disease, or physiological condition. For example, gonadal neoplasia in the soft shell clam, *Mya arenaria*, disrupted egg development and caused a 66% reduction in production of gametes (Barber, 1996). Some individual and population variation in gonad development and fecundity of bivalves may also be explained by genetic differences (Barber et al., 1991). An individual's condition can also influence the quality of gametes it will produce, which has been demonstrated for oysters (*Crassostrea gigas* and *C. virginica*) and hard clams (Lannan et al., 1980; Gallagher and Mann, 1986). Moreover, larvae from larger eggs of hard clams survive better than those from smaller eggs (Kraeuter et al., 1982) and larval hard clam survival is correlated with lipid content of eggs (Gallagher and Mann, 1986).

Bivalve recruitment requires successful completion of a planktonic stage after fertilization (Figure 2). Despite extensive research, there are numerous gaps in our knowledge of larval ecology and the settling process both on hard substrates (Kennedy, 1996), and soft sediments (Snelgrove and Butman, 1994; Olafsson et al., 1994). For example, under laboratory conditions hard clam larvae actively selected an appropriate substratum in still water but failed to select this substratum under defined flow conditions (Butman, 1987; Butman et al., 1988). Laboratory studies need to be more realistic in order to elucidate the mechanisms affecting larval survival, dispersal and metamorphic/settlement success.

The relationship between broodstock abundance and dispersion and larval recruitment has been of interest to biologists and resource managers for years. Although our knowledge of recruitment processes have improved over recent years, there is still much to be learned. Recently, for example, Marelli et al. (1999) failed to detect a significant relationship between adult bay scallop (*Argopecten irradians*) density and subsequent recruitment. Population abundance is as important as the spatial proximity of broadcast spawning invertebrates in achieving successful egg fertilization (Levitan et al., 1991). Success or failure of recruitment relies on three factors: reproduction, water column processes (larval survival) and benthic processes (e.g., predation on post-larvae). When examined

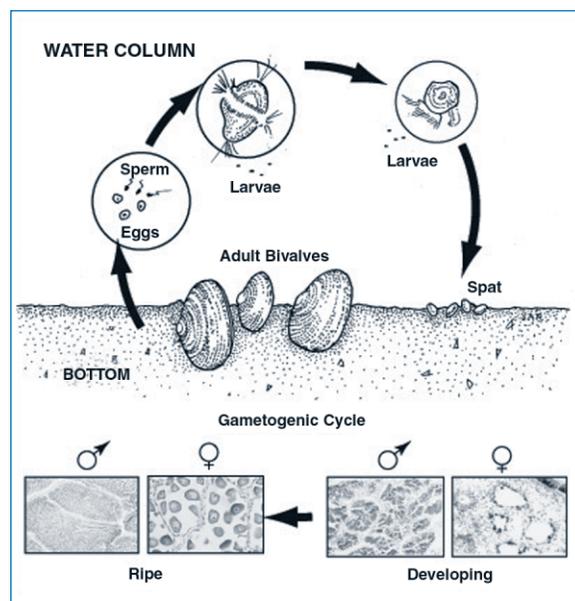


Figure 2. Generalized life-cycle of a bivalve. Adult bivalves enter a gametogenic cycle whereby their gonads develop and become ripe (see inset). Sperm and egg are shed into the water column where fertilization occurs. Larvae pass through several developmental stages and eventually 'set' on the bottom as spat where they grow into adults.

over several years, however, none of these factors alone is the dominant determinant of successful recruitment; therefore, an integrated approach with links to other research programs is needed in the future.

Maximization of ecological benefits and sustainable harvests of bivalves requires successful recruitment. As mentioned above, several factors are important in defining recruitment, including fertilization of a large number of high quality gametes, completion of the larval phase, successful settlement and growth and survival of post-larvae. Research has focused on the more descriptive aspects of recruitment, such as the gametogenic cycles, fecundity, estimates of induced spawns, larval development and morphology, and field observations of larval abundance and dispersion patterns. Although this information is important, much is to be gained from understanding factors that influence each component of the recruitment process and the relationships among recruitment components. For example, the effectiveness of spawning sanctuaries has never been demonstrated because the importance of population density and dispersion in successful recruitment is poorly understood. Improved understanding of the population-dispersion relationship and its supporting processes will go a long way in helping natural resource personnel manage bivalve populations for maximum ecological benefit and sustainable harvest.

Approaches to meet this goal

- *Identify factors affecting energy partitioning between somatic and gonadal growth and influencing reproductive potential.*

Insights into the reproductive capabilities of individual or populations of bivalves can be gained if the interrelationship between energy allocation and reproductive condition are better understood. In many species of bivalves, such as mussels (Gabbott, 1976), scallops (Sastry, 1979) and oysters (Thompson et al., 1996), glycogen reserves are stored during periods of high food availability in late summer and fall. At these times the major energy requirements for tissue and reproductive growth have already been satisfied. The reserves are then subsequently mobilized and used to initiate gametogenesis (gamete development) in late winter and early spring, and to maintain metabolism during periods of low food supply and reduced feeding in the winter. In this manner, the majority of bivalves partially uncouple the processes of energy intake and gamete production, allowing gametogenesis to begin during the winter when food intake is at a minimum (Sastry, 1979). Also, the stored nutrient reserves may be an adaptive mechanism that allows bivalves such as mussels to reduce their dependence on the spring phytoplankton bloom for acquiring all of the nutrients required for reproduction (Bayne, 1976). Spring spawning is synchronized in these bivalves to take advantage of seasonal warming and increased larval food in temperate waters. In species such as hard clams, a more continual supply of food needs to be present for gametogenesis (Loosanoff, 1937; Heffernan et al., 1989). Species that have a close coupling between current food availability and gametogenesis may have a greater capacity to respond to favorable environmental conditions, by increasing fecundity. Obviously, the reproductive response of a given bivalve species to any set of environmental factors may vary among species. Crucial to the development of fisheries models is a knowledge of those environmental factors which affect the processes of energy allocation and reproductive condition and thereby influence production of numerous high quality gametes.

- *Investigate factors affecting quantity and quality of gametes, and the influence of egg quality on larval development.*

Most bivalves are broadcast spawners that produce larvae that feed on plankton (Figure 2). In order for larvae to survive, the phytoplankton food must be adequate both in terms of amount and digestibility throughout the various larval life stages. The cyclic annual pattern of reproduction found in many species of temperate shallow-water marine bivalves is

primarily determined by temperature and secondarily by other seasonal factors (Bayne, 1976), including variations in food supply associated with patterns of primary production (see reviews by Gabbott, 1976; 1983). The reproductive cycle includes: 1) accumulation of energy and nutrients to be used during gametogenesis, 2) proliferation of the gonad and differentiation of eggs and sperm, 3) ripening of the gametes, 4) spawning and 5) a reproductively quiescent period.

Under conditions of reduced phytoplankton abundance or the blooming of non-nutritious species (Langdon and Newell, 1996; Grizzle et al., 2001), adults use food for metabolism rather than for gametogenesis. The number of eggs spawned and quantity of nutrients in the egg yolk of bivalves is dependent on the nutritional status of the female parent (Sastri, 1979; Gabbott, 1983). If the adults are subject to nutritive stress after the initiation of gamete development, the eggs spawned may be smaller in size than normal because of reduced yolk content, and such eggs have a lower rate of fertilization and development than those spawned by unstressed females. The influence of adult nutrition on subsequent larval viability has been observed under laboratory conditions in a number of commercially important bivalves, including the hard clam, *M. mercenaria* (Gallager and Mann, 1986), the European flat oyster, *Ostrea edulis* (Helm et al., 1973), the blue mussel, *Mytilus edulis* (Bayne et al., 1978), and the Pacific oyster, *C. gigas*, (Robinson, 1991). Future research must be directed towards identifying the role of nutrition in governing larval survival and ultimately recruitment in natural populations of bivalves.

- *Determine the role of biological, physical, chemical and anthropogenic factors in affecting larval survival and metamorphic/settlement success.*

The number of bivalve larvae that successfully settle is a function of the number of competent larvae produced and those larvae that survive the planktonic phase and reach a suitable settlement site. As discussed above, the supply of larvae is closely linked to the production of numerous high quality gametes and successful fertilization. Survival and dispersal of competent larvae in the plankton is influenced further by biological (e.g., available food, predators and competitors in the plankton), physical (e.g., temperature, hydrodynamic forces), chemical (e.g., salinity and turbidity) and anthropogenic factors (e.g., contaminants; Butman, 1987; Olfasson et al., 1994; Snelgrove and Butman, 1994; Kennedy, 1996; Eversole et al., 2000). Although research indicates that these factors may affect larval supply, the relative contribution of these processes to the variation observed in successful settlement and bivalve distributions is poorly understood (Eversole, 2001). Sorely needed are laboratory and field measurements and experiments that use new technology to test hypotheses concerning how environmental and physiological factors influence the survival, transport, metamorphic and settlement processes.

- *Establish an integrated approach to assess the relationship between stock abundance and recruitment.*

Although the concept of spawner transplants and sanctuaries to enhance recruitment is not new (Kassner and Malouf, 1982), this concept embodies a series of untested hypotheses concerning how enhancement practices and resident populations contribute to recruitment. Because an individual's health impacts both its reproductive output and success of larvae it produces, a population's contribution to recruitment needs to be quantified within an array of environmental and physiological variables. For example, coordination of spawning and the synchronous release of gametes increases the chance for successful fertilization in broadcast spawners. The optimum gamete ratio for successful fertilization is 1,800 sperm to 1 egg in laboratory cultured hard clams (Bricelj and Malouf, 1980). Larger hard clams allocate an increasingly larger proportion of assimilated energy to gamete production (Peterson, 1983). Equally important may be the density and dispersion of spawning

individuals in the population. Unfortunately, we have no data to indicate what the optimum gamete ratio might be in nature and how the size distribution of the resident bivalve population influences reproductive success.

Potential links to other groups

The initiatives outlined above directly link to issues addressed by other working groups including Ecosystem Modeling, BROADSCALE Ecology, Genetics, Disease, Aquaculture, and Protection and Restoration of Shellfish Habitat sections. In addition, model development requires key information to parameterize reproductive and recruitment success for more predictive population models.

GOAL 3: *To understand the response of molluscs to environmental stressors on five levels of organization, namely biochemical, cellular, tissue, organism, and population.*

Status and Importance

A fundamental question with any stressor(s) is the site of response within the individual cell. Is the site of action a particular enzyme system, a cell membrane function, a particular metabolic pathway(s) or some other site? At the cellular level of function we remain hampered by the inability to maintain lines of cells in culture for specific assays of dose response relationships. Results of some studies suggest intriguing possibilities as to cellular mechanisms for functional detoxification. For example, sequestering of metals to render them functionally unavailable for physiological processes was described for European flat oyster, *Ostrea edulis*, over twenty years ago (George et al., 1978). But the actual cellular mechanisms responsible for this sequestration remain to be fully explained and many questions remain. How do whole body burden data relate to physiologically available concentrations? How does this translate to physiological action levels in the realm of public health?

At the tissue level, aggregations of certain cell types not only reflect commonality of host function, but also offer specific targets for stressor accumulation and action. A notable example is the accumulation of lipophilic organic compounds in lipid rich tissues, with subsequent impact on reproductive activities that utilize stored lipid in egg production (reviewed by Capuzzo, 1996). Public health considerations also can become paramount where specific tissues are consumed (e.g., gonad products in ethnic recipes).

Physiology at the level of the individual is driven by considerations of size specific rates and functions. Uptake and depuration/detoxification processes are also presumed to be size related. In turn, the previously mentioned tissue specific actions of anthropogenic lipophilic materials may have direct affect on size specific fecundity and senility relationships at the individual level. Given that some stressors show cumulative impacts over a period of years, then demographics will skew towards younger individuals with consequent impacts on population and community level functions. Premature death of older age classes will alter recruitment success and push the population closer to instability. Death of older age classes may also alter fertilization efficiency in mass spawning events because of the altered product of size specific fecundity and absolute population densities.

Approaches to meet this goal

- *Define uptake and depuration kinetics of pollutants, toxins and harmful microbes.*

Depuration of shellfish to reduce or eliminate harmful pathogens associated with sewage pollution has been practiced successfully in many countries (Fleet, 1978; Richards, 1988). Elimination processes and rates for harmful bacteria from the guts of molluscan bivalves are understood, but optimal depuration conditions for elimination of pathogenic viruses and harmful toxins still need to be developed. Understanding the physiological basis for these processes will be important for developing depuration protocols.

The effects of some pollutants on the feeding physiology, growth and survival of shellfish have been previously studied (Cappuzo, 1996; Roesijadi, 1996); however, the underlying physiological and biochemical processes that determine depuration rates of these harmful substances have often not been described. More research needs to be undertaken to describe rates and processes of depuration of harmful viruses and toxins so that better predictive tools can be developed for aquaculturists and resource managers. The sequestration process is one form of detoxification, but is not depuration in the classical sense wherein a stressor is gradually purged reducing total body burdens. We need to better understand the physiological processes that govern sequestration of toxins (a form of detoxification) versus elimination of toxins (depuration). In particular, we need more information on how processes of sequestration and depuration are modulated at the cellular level.

- *Examine how whole body burden data relate to physiologically available concentrations and determine how this translates to physiological action levels in the realm of public health.*

The Food and Drug Administration in concert with other state and federal agencies is charged with insuring the safety of foodstuffs available to the public marketplace. A simple approach to food safety is to assess the possibility of ingestion of a harmful substance based on consumption rates by the public and the concentration of the target substance in the food. With these data in hand, food products can be identified that have elevated levels of potential or known harmful substances and their consumption controlled either through their removal from the marketplace or by guidelines suggesting limited consumption. Elevation of body burdens of suspected harmful substances above a predetermined level is reason for removal of a food from the marketplace. A guiding principle in determining harmful levels might be the overt disturbance of physiological function(s) in the targeted food organism. The problem with this approach in marine molluscs is that although a host organism may have an unacceptably high whole body burden of toxins, these substances can be sequestered and rendered physiologically unavailable. Physiological function *per se* of a harvested organism is therefore not a practical barometer of suitability for human consumption.

- *Understand the response of molluscs to diseases and parasites.*

As with anthropogenic stressors, the facility to maintain cell lines in culture would vastly accelerate the explanation of the mechanism(s) by which disease and parasites debilitate cells, how cells respond, how we should consider the modeling of such interactions and what both the ecological and economic impacts could be. By integrating data obtained at the cellular and tissue level with size considerations, information can be obtained on the relative susceptibility of different age classes in a population. This leads to consideration of the non-linear processes of estimating impacts on fecundity, recruitment success and the stability of the population. The possibility of missing year classes due to recruitment failures and juvenilization of the population makes the population, and the food web in which it is a key benthic-pelagic coupler, more unstable.

In addition, we need better information on how fishing practices impact the physiological performance of a given bivalve population. Continued parasite and disease pressure on a population will select for less susceptible individuals. This resistant sub-population will be more fit for their environment and exhibit higher growth rates than the more susceptible individuals. From an ecological perspective, it is critical to maintain the resistant individuals in the population to pass on their traits to future generations. Because of their improved survival and growth rates, however, the resistant individuals will be most targeted by commercial fishing activities. Therefore, commercial fishing can lead to the removal of the most fit individuals and the exact opposite of the desired effect. In short, fishing of the resistant individuals can select against traits of increased survival and growth in the population.

Potential links to other groups

The initiatives outlined above directly link to issues addressed by other working groups including the Water Quality, Broad-scale Ecology, Disease and Genetics.

GOAL 4: *To optimize yields and product quality for economic gain without negatively impacting the environment.*

Status and Importance

Researchers have typically explained growth of molluscan shellfish in terms of energy budgets (Bayne and Newell, 1983). Intake of energy is balanced against losses due to respiration and excretion, with the surplus being available for growth (scope for growth) and any deficit being supplied from body reserves. This approach has been very successful in explaining growth of several molluscan species under both laboratory and field conditions; however, discrepancies between predicted and actual physiological processes have been observed, indicating that growth-determining processes in natural conditions are not fully understood.

In particular, very little is known of the nutritional requirements of molluscan shellfish, except for abalone where defined artificial diets have allowed researchers to determine their requirements for many nutrients (Fleming et al., 1996). The main difficulty in studying the nutritional requirements of suspension-feeding shellfish is that satisfactory microparticulate diets have not been developed due to technological difficulties. This lack of knowledge prolongs aquaculture's dependence on expensive live algal feeds and impedes understanding of the effects of the biochemical composition of seston on shellfish growth in coastal waters.

The tolerance and acclimation capacity of many species of molluscan shellfish in response to a wide variety of environmental stressors and diseases have been described (Newell, 1979; Cappuzo, 1996; Ford and Tripp, 1996; Roesijadi, 1996; Shumway, 1996). In contrast, underlying molecular mechanisms that determine responses are less understood. This lack of understanding makes it difficult for researchers and aquaculturists to improve tolerance or resistance of stock to environmental stress and disease through genetic or other forms of manipulation.

Approaches to meet this goal

- *Determine physiological and biochemical processes that influence growth rate and food conversion efficiencies in both juveniles and adults to determine the best approach to manipulate these processes for optimal yields.*

One way to improve yields of farmed shellfish is to obtain a better understanding of key physiological and biochemical processes that determine growth and survival under culture conditions. Little is known, however, about the physiology of juvenile bivalves (pre-reproductive) and how it compares to reproductive adults. With this information it could be possible to develop methods to manipulate physiological processes at different life stages in order to improve growth and survival in the field. For example, little is known of the role of hormones in shellfish growth or reproduction. Studies also need to be undertaken to identify key genes (or associated genetic markers) that have a major impact on growth and survival. Breeding programs need to be developed to manipulate the frequency and expression of these genes.

Triploidy has been successfully applied to reduce energy allocated to reproduction in cultured bivalves in order to improve meat quality. It will be necessary to obtain a better understanding of underlying mechanisms that cause partial reversion of triploids to the diploid state before triploidy can be used as a reliable tool to “sterilize” potential introductions of non-indigenous species.

- *Determine physiological and biochemical processes that are important in choosing candidate species for aquaculture systems or habitat rehabilitation.*

The physiological requirements of candidate molluscan shellfish species or strains for aquaculture or habitat rehabilitation should be determined in order to evaluate their suitability for these applications. For example, selecting for strains with higher food conversion efficiency and improved growth might be valuable for aquaculture applications, whereas disease resistance and high fecundity might be more valuable for habitat rehabilitation purposes. One caveat, however, is that over time mortality and cross fertilization with non-selected “wild” types may alter the original strain that is established in the field. An assessment of potential competition between candidate species and indigenous or established aquaculture species should be carried out as part of this evaluation.

- *Define nutritional requirements of life stages.*

Elucidating the nutritional requirements of bivalves at various life stages will require the development of artificial diets with defined nutrient complements. Presently such research is hampered by available microcapsule types which rapidly lose low-molecular weight nutrients, such as water-soluble vitamins and amino acids, when suspended in seawater. Such loss makes it difficult to quantify the actual nutrient content ingested by a given individual. We need to develop better artificial diets that are resistant to nutrient loss and are readily ingested and digested by different life stages of bivalves. Advances will depend on the development of new methods for nutrient delivery, such as novel microencapsulation types. Such novel diets will greatly enhance the production of intensively cultured suspension-feeding bivalves.

Potential links to other groups

The initiatives outlined above directly link to issues of focus of other working groups including Genetics and Habitat Rehabilitation workgroups.

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LIST OF ACRONYMS

Referent	Acronym
American Public Health Association	APHA
Amnesic Shellfish Poisoning	ASP
Best Management Practice	BMP
Centers for Disease Control	CDC
Coastal Zone Management Act	CZMA
<i>Perkinsus marinus</i>	Dermo
Deoxyribonucleic Acid	DNA
Diuretic Shellfish Poisoning	DSP
Ecological Code of Practice	ECOP
Environmental Management System	EMS
Food and Drug Administration	FDA
Geographic Information System	GIS
Gulf Oyster Industry Council	GOIC
Harmful Algal Bloom	HAB
Hazard Analysis Critical Control Point	HACCP
Inductively coupled plasma -Mass spectrometry	ICP-MS
Intellectual Property	IP
International Council for Exploration of the Seas	ICES
Interstate Shellfish Sanitation Conference	ISSC
Investigational New Animal Drug	INAD
Multiple Antibiotic Resistance	MAR
Molluscan Broodstock Program	MPB
Most Probable Number	MPN
<i>Haplosporidium nelsoni</i>	MSX

Haplosporidium costaleSSO

National Estuary ProgramNEP

National Marine Fisheries ServiceNMFS

National Oceanic and Atmospheric AdministrationNOAA

National Shellfisheries AssociationNSA

Neurotoxic Shellfish PoisoningNSP

National Shellfish Sanitation ProgramNSSP

Polycyclic Aromatic HydrocarbonPAH

Polychlorinated biphenylsPCB

Polymerase Chain ReactionPCR

Pacific Coast Shellfish Growers AssociationPCSGA

Pacific Shellfish InstitutePSI

Paralytic Shellfish PoisoningPSP

Pulsed Field Gel ElectrophoresisPFGE

Quahog Parasite UnknownQPX

Ribonucleic AcidRNA

Secretory immunoglobulinsIgA

State Shellfish Control AgencySSCA

Small Subunit Ribosomal RNASSUrRNA

Sewage Treatment PlantSTP

Total Maximum Daily LoadTMDL

United States Department of AgricultureUSDA

The United States Environmental Protection AgencyUSEPA

The United States Food and Drug AdministrationUSFDA

Western Regional Aquaculture CenterWRAC

Woods Hole Oceanographic InstituteWHOI

World Aquaculture SocietyWAS

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