MARINE BIOTECHNOLOGY

A SEA GRANT INITIATIVE

Competing in the 21st Century

Contents

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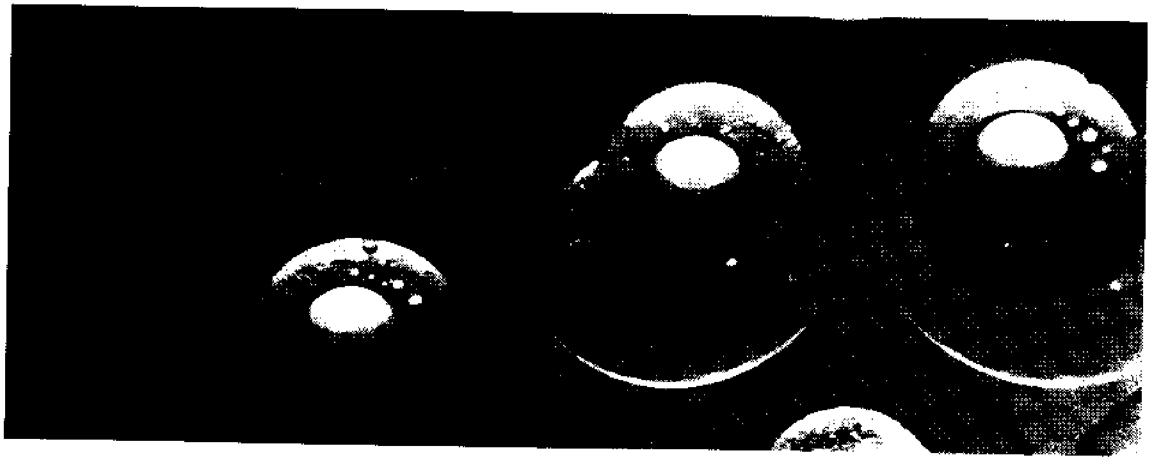
Competing in the 21st Century

"A revolution in the ocean sciences has begun, with the application of the modern tools of biotechnology, molecular and cellular biology to marine organisms and ecosystems. The change is expected to be fundamental in nature, exponential in pace and unprecedented in its scientific and economic impact. . .within a decade, quantum leaps will have been made, not just in the amount of knowledge, but in the types of insights into the fundamental and longstanding problems of ocean sciences. . . ."

— *National Science Foundation 1990*

Marine Biotechnology
Research, Competitiveness and Technology Transfer



The United States has an important lead in the rapidly burgeoning field of marine biotechnology. But the country stands to lose its lead to other nations, which are aggressively pursuing new technologies.

For millennia the oceans have been a source of food, minerals and other natural products. But as population and human needs continue to increase, so too do the pressures on those resources. If we as a nation are to meet these growing needs, if we are to take advantage of the bounty the oceans offer, if we are to protect the viability of our coastal environments, we must commit ourselves to a national program that will build on our current scientific achievements and develop national expertise for the future.

No element of science and technology can match the potential of molecular biology and biotechnology to transform the lives of people around the world. While powerful molecular technologies already are being applied to the study of marine organisms, we have hardly begun to take advantage of their potential. To meet this challenge, research communities, government agencies and the private sector must interact more effectively. Current efforts simply are not adequate: the United States has no national program in *marine* biotechnology. It is only through a comprehensive plan that we will be able to meet the technological challenge at home and competition from abroad.

Economics of Biotechnology — Challenge and Competition

The public sector in the United States has provided less than $50 million annually for research and development in marine biotechnology; in Japan, that figure is between $900 million and $1 billion. Over the next ten years, for example, the Japanese Ministry of International Trade and Industry will bring an additional $200 million to the field and will support the creation of two centers of marine biotechnology, reflecting its assessment of marine biotechnology as the "the greatest remaining technological and industrial frontier." The United States, while assisting other governments in building capabilities in marine biotechnology, has only scattered efforts supporting research and development.

A national vision for development of marine biotechnology will lead to applications useful to many industries and, ultimately, the American consumer and world markets.[1] It will reverse the current trend where products from marine microbiology are already being imported into this country from abroad, further eroding the country's trade deficit.

Achievements and Potential

Since 1983, limited public funding of marine biotechnology has still resulted in more than 170 United States patents. Sea Grant programs across the nation have been instrumental in helping to support developments such as new classes of anti-inflammatory agents that have become the basis of major investment by the pharmaceutical industry, vaccines that combat major microbial diseases of salmon and a new assay that a commercial firm has used to produce a kit for rapid detection of contaminated seafood.

To exploit the unlimited possibilities that aquatic organisms have for protecting public health, for restoring degraded ecosystems, for improving seafood production and safety and for developing an array of new products, Sea Grant proposes an initiative with clear national goals that will help guide marine biotechnology in the United States.

[1] Biotechnology for the 21st Century. 1992. Federal Coordinating Council for Science, Engineering and Technology, Washington, D.C.

MARINE BIOTECHNOLOGY IN THE UNITED STATES

The United States is the current world leader in research expertise in marine biotechnology. However, our leadership faces stiff competition from other countries that are moving ahead with strong national investment and planning. Focused research in marine biotechnology in concert with commercial development offers the promise of economic and social opportunities: it will lead to new industries and new jobs; it will help upgrade and advance higher education to meet United States needs in an increasingly technical and competitive world. It will assist in reversing our trade deficit, which in seafood alone is $2.4 billion dollars a year, second only to petroleum; it will lead to new international markets and overall economic development.

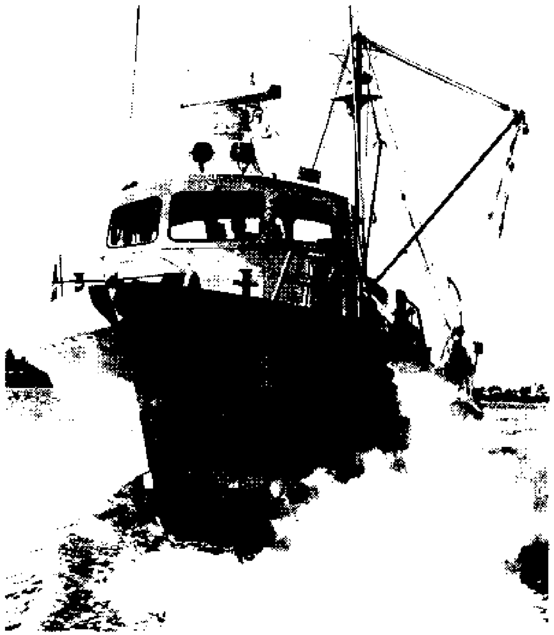
A national commitment to research and development in marine biotechnology will also help us respond to the critical needs of society; it will:

- Open new avenues for monitoring health and treating disease
- Provide innovative techniques to restore and protect aquatic ecosystems
- Increase the food supply through aquaculture
- Enhance seafood safety and quality
- Develop new types and sources of industrial materials and processes
- Expand knowledge of biological and geochemical processes in the world ocean

This document describes the framework for initiating this important and exciting program in marine biotechnology. Three broad elements — Molecular Frontiers in the Ocean Sciences, Applications of Marine Biotechnology, and Marine Biotechnology and Society — encompass the range of research, application and education needed to exploit this new technology and ensure its wise use. Each element, in turn, includes examples of significant advancements to date and outlines directions for future research and development in this challenging field.

Marine biotechnology has made an auspicious beginning — this Sea Grant initiative gives the United States a means to chart a course through the 90s and into the 21st century.

SEA GRANT: THE NATION'S MARINE RESEARCH NETWORK

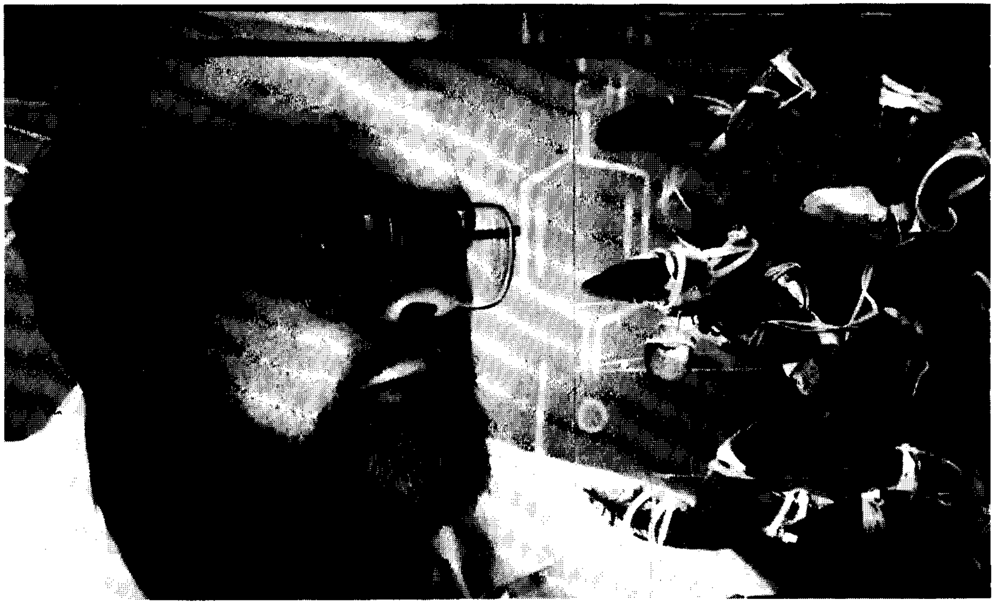


For more than a quarter century, the National Sea Grant College Program has promoted excellence in marine research and outreach. Sea Grant operates through twenty-nine coastal programs and numerous discrete inland projects, involving hundreds of universities, scientists, educators and students. During the past two decades, Sea Grant has provided benefits to resource management agencies and to a range of marine-related industries — to those in commercial and recreational fishing, aquaculture, shipping, mining, boating, seafood processing and biotechnology.

Productive research in support of marine biotechnology has been well underway in Sea Grant programs around the country. Despite modest funding, this effort represents a core of experience for the nationally coordinated initiative.

The key to the Sea Grant concept is partnership. One-third of each Sea Grant program's funds must come from nonfederal sources, from state or private funds. Federal funds, provided through the National Oceanic and Atmospheric Administration (NOAA), often serve as a catalyst, helping to mobilize support for important marine-related research and education activities. Sea Grant, then, provides an excellent mechanism for pursuing coordinated efforts, where corporate and other support can be joined with state and federal monies to fund research, technology transfer and scale-up activities.

As the outreach arm of NOAA, Sea Grant provides a link to academia, business and industry, other federal agencies, including the Environmental Protection Agency, the National Science Foundation, the National Institutes of Health, Departments of Agriculture, Interior and Defense, and the general public.



Sea Grant and Marine Biotechnology

Productive research in support of marine biotechnology has been well underway in Sea Grant programs around the country. Despite modest funding, this effort represents a core of experience for the nationally coordinated initiative in marine biotechnology that Sea Grant now proposes. Sea Grant programs have developed an effective managerial infrastructure with a strong track record and are clearly capable of directing a major national initiative. In addition, Sea Grant is the only agency in the marine sector with experience in outreach programs targeted to the transfer of marine science and technology and to the improvement of education in aquatic sciences.

Sea Grant has demonstrated experience in integrating public policy considerations into multi-disciplinary research and education programs. Part of Sea Grant's primary mission includes working together with federal and state agencies and other organizations, public and private, to consider critical marine-related issues. New developments in molecular biology — and their commercial application — will have significant ethical, ecological and economic implications. Sea Grant programs have a strong record of approaching such issues, helping to bring together expertise from across disciplines to foster balanced analyses and workable solutions.

The Need to Act Now

Marine biotechnology has the potential to develop rapidly — and to provide major economic returns to the nation. To take advantage of this opportunity, however, we must have an aggressive program of marine biotechnology research and development that draws on cutting-edge science underway in laboratories around the country. We must encourage and support multi-disciplinary research and ensure rapid technology transfer. We must actively plan for partnerships with industry and commercial enterprise, and facilitate efficient technology transfer. Most importantly, we must train the next generation of scientists and technologists or risk losing the slim competitive edge we now have.

To assure the relevance of program elements, a focused initiative, open to researchers throughout the nation, will be guided by an advisory panel of academic and industrial scientists, economists and managers and will build upon the ideas and recommendations in the Biotechnology Research Initiative put forward by the Federal Coordinating Council on Science, Engineering and Technology, under the guidance of the President's science advisor.

The United States has an important lead in the rapidly burgeoning field of marine biotechnology. But as has happened with other emerging technologies, the country stands to lose its lead to other nations, most notably Japan, which are aggressively pursuing new technologies. By strengthening its leadership role now, the United States can help assure its competitive position as the stage is set for the next century.

A national initiative in marine biotechnology will fund the best science throughout the nation, to exploit this new technology.

Molecular Genetics

Knowledge of the genetics of marine organisms will yield important information about their phylogeny, evolution, disease, symbiosis, ecological adaptation and physiology. This information will improve our ability to understand and manage complex ecosystems. Also, information gained about simpler organisms such as bacteria, algae or invertebrates will give us insights into the functioning of more complex mammalian systems.

The introduction of foreign genetic material into organisms and genetic manipulation to induce sterile, polyploid or hybrid stocks has been accomplished with a few marine species. In some cases, these results have been immediately applied in commercial aquaculture. These methodologies can also be used in the management and enhancement of wild stocks. For example, genetic markers can be used to differentiate various strains, or to separate hatchery from wild populations. Examination of DNA has shown that some fish and shellfish populations consist of various substocks, often with differing abilities to adapt to environmental stresses. This observation suggests that genetic engineering could be used to improve survival and productivity of cultured stocks.

Marine viruses, recently shown to infect many phytoplankton and bacteria, are thought to play a major role in plankton dynamics. They may also provide a means for incorporating foreign genetic material into marine algae, which have proved resistant to genetic engineering, but which have great potential as sources of new chemicals and materials.


THE CHALLENGE

Determine the Molecular Genetics of Aquatic Organisms:

- ***Identify the basis for unique adaptations***
- ***Characterize environmental factors controlling gene expression***
- ***Use aquatic organisms as molecular models***
- ***Develop new techniques in molecular biology***

Exciting new areas of research in this promising field include:

■ Certain Antarctic fish produce novel glycoproteins which inhibit ice crystal growth in their tissues; use of molecular techniques may permit their large-scale production for research and practical application. It may be possible to transfer the genes coding for this natural antifreeze to other species in order to improve their growth and survival in cold environments.

■ The unique physiology of hydrothermal vent organisms — in particular their adaptation to life at high pressures, high hydrogen sulfide content and at temperatures which would denature most proteins — is receiving deserved attention. Molecular genetic methods are being used to clone the genes of barophilic (high-pressure) bacteria with the goal of studying the mechanisms of pressure control on gene expression.

■ DNA-fingerprinting techniques demonstrated that traditional stock assessments of Atlantic salmon were erroneous, resulting in a large share of valuable United States stocks being given over to European fishing interests.

■ Basic studies of fish gene expression have shown that carp contain many genes similar to those of mice. This has led to the use of fertilized transgenic fish eggs as a substitute for transgenic mouse embryos in genetic studies, with considerable savings in cost and handling effort.

Bio-Organic Chemistry and Pharmacology

While the number of truly novel chemicals from terrestrial plants and microbial fermentation has declined, marine natural products chemists have shown that almost every class of marine organism elaborates a wide variety of molecules with unique structural features. Pharmacologists, physiologists and biochemists have demonstrated that many of these novel marine products modify fundamental life processes in ways suggesting biomedical applications. These molecules can serve as leads to guide the pharmaceutical and chemical industries in developing new products.

Because marine species may have evolved the production of chemicals for protection against predation, infection and competition, some of these chemicals are proving useful in agricultural and medical applications. By determining the biochemical pathways by which these compounds are produced — and the environmental or physiological triggers controlling their production — techniques of enhanced commercial production can be developed.

Ground-breaking discoveries in marine chemistry, pharmacology and biotechnology will provide essential help that the American pharmaceutical industry needs in its efforts to cure intractable forms of cancer, inflammation, arthritis and viral infections. New classes of marine invertebrates and microorganisms are needed as a source of medicinal, agricultural and industrial agents; in particular, bacterial symbionts (now known to be the source of many novel marine compounds) hold a tremendous potential for biotechnological exploitation. Further examination of molecular systematics and biogeography of compound-producing organisms can greatly improve the search for new sources.

New fermentation and related production technologies must be developed which


apply to marine bacteria, fungi and related species. Such advances will make possible further research on how drugs work and will provide new leads to bio-organic chemists studying the structure and production pathways of these materials. New approaches in defining molecular structure, including the use of powerful new computer techniques, will lead to synthesis of marine-derived drugs and will offer new potential solutions to production of larger quantities of rare drugs. Genetic engineering — the transfer of genes encoding for synthesis of the compound of interest into more manageable organisms —holds particular promise for mass production.

Recent developments include:

■ Manoalide, an anti-inflammatory and analgesic agent isolated from a Pacific sponge, is now in clinical trials. Its action differs from that of standard drugs and it appears free of the side-effects of steroids.

■ A substance isolated from shark cartilage inhibits blood supply to tumors, thus restricting their growth. Bryozoans and tunicates have also yielded novel compounds with highly specific antitumor activity; some are now undergoing clinical trials.

■ Halenquinone, isolated from a sponge, is a powerful new antibiotic, while didemnin, from a tunicate, exhibits antiviral and anticancer activity.

■ Extracts of a sponge have yielded a potent insecticide; especially interesting is the finding that this compound may actually be produced by a bacterium living in the sponge. Accumulating evidence suggests bacteria as the source of many bioactive marine chemicals such as tetrodotoxin.

THE CHALLENGE

Discover New Bioactive Materials:

- ***Identify new sources of drugs***
- ***Determine biosynthetic pathways***
- ***Define structure of marine-derived drugs***
- ***Characterize drug-receptor interactions***
- ***Define molecular mechanisms of action***

Immunobiology and Pathology

Animals and plants in the marine environment are subject to disease, parasitism and tissue pathologies such as tumors. And as with terrestrial species, these pathologies may be due to microbial agents or to environmental stress. The ecological or economic impacts of these diseases can be costly. For example, a probable virus has been implicated in the recent massive die-off of the black sea urchin, *Diadema*, in the Caribbean; grazing of fouling algae by this urchin is considered vital to coral reef health. Disease and parasites have brought about the virtual elimination of oyster populations in many areas, with corresponding economic and societal dislocations. Tumors in fish from coastal waters have been ascribed to exposure to toxic pollutants, though the exact mechanism of such impacts remains unclear. Determining the causes of these tumors, whether pollutants, infectious agents or other factors, will have a direct effect on public use and management of the resource. There is growing evidence that aquatic viruses, abundant in most systems, play a major role in the control of bacterial and algal populations. Infection by viruses thus may impact production and nutrient cycling, as well as plankton diversity and abundance.

The inability to culture many marine bacteria and viruses, or to elucidate the life cycles of parasites, hampers the diagnosis and treatment of disease. The immunological response of marine species, particularly invertebrates, to disease agents is not well-understood, nor is the impact of pollutants on the immune system. The aquatic environment itself makes the isolation, identification and quantification of potential disease agents difficult. The techniques of marine molecular biology and biotechnology show tremendous promise for addressing these problems. Of particular importance would be the development of gene probes or immunochemical agents for disease diagnosis; establishment of fish and shellfish cell cultures to support basic research on the molecular basis of pathogenesis; production of vaccines by recombinant DNA technology; production of molecular probes or biomarkers for assessing effects of environmental stress on organisms; and determination of the relationship between environmental stress and disease resistance in fish and shellfish.

Recent examples of such advances are:

■ The induction of specific enzymes in deep-sea fish, established using monoclonal antibody techniques, indicates biological changes due to anthropogenic chemicals are occurring even in the deep ocean. Molecular techniques have identified specific mutations, of uncertain cause, in critical genes associated with diseases such as cancer in fish.

■ Gene probes have been developed for several viral diseases of shrimp. These have made possible the establishment of disease-free brood stock for United States shrimp hatcheries and will help prevent the loss of stocks during grow-out.

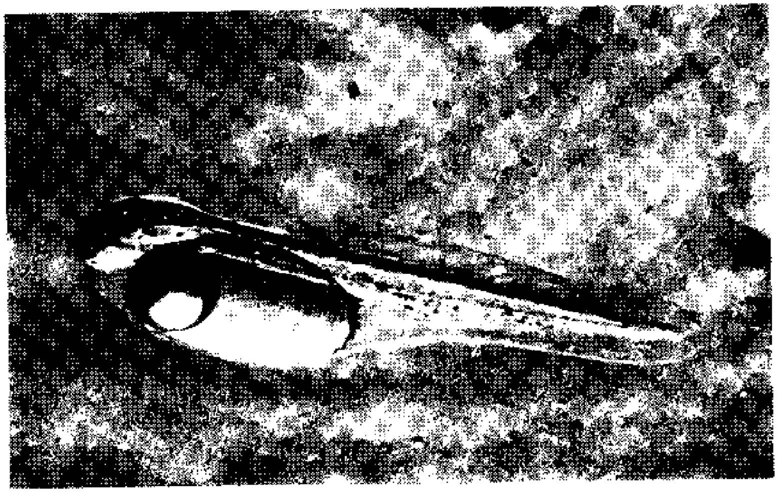
■ Flow cytometry and specific immunological labeling are being employed to identify and enumerate pathogens, including cryptic life stages of parasites, in natural waters. Molecular probes are being developed to elucidate life cycles or identify alternate hosts of parasites which infect fish or shellfish.

THE CHALLENGE

Investigate Disease Processes:

- *Use marine organisms as biomedical models*
- *Develop new diagnostic techniques*
- *Prevent and treat diseases in aquatic organisms*
- *Determine the role of environmental stress on disease*
- *Explore cell-to-cell recognition*

ENDOCRINOLOGY AND DEVELOPMENTAL AND REPRODUCTIVE BIOLOGY



Reproduction, development and growth in marine organisms, as in all animals, are regulated through the orderly release of hormones. These hormones are produced by the neuroendocrine system which integrates information from the genome and the environment. The central role of the neuroendocrine system in the regulation of growth and development can be exploited to generate the technology necessary for the efficient and reliable propagation of important food species. A knowledge of hormones and of endocrine regulation of growth can also be used to stimulate individual growth and improve productivity while reducing feed and other costs to the farmer; hormonal growth promoters of farm animals were among the first products of the biotechnology industry. For these same reasons, the application of cooperative research between endocrinology and molecular biology is critical if aquaculture is to significantly increase the food supply of both the nation and the world. A knowledge of hormone and gene interactions in the control of growth, reproduction and other aspects of physiology provides important new avenues for restoring populations of endangered species and in limiting populations of noxious organisms.

These approaches must be combined with a thorough understanding of the developmental biology of marine organisms. Many organisms pass through periods of development called critical periods in which mortality is high. While the factors involved in these critical periods remain largely undefined, they can have a strong negative impact on the economics of aquaculture and on the fisheries mitigation efforts of hatcheries. Characterizing the sources of such critical periods and overcoming their limits will provide essential tools in producing and growing many important marine organisms.

The intersection of endocrinology, molecular biology and developmental biology can provide still other important assets. Thus, research is essential not only in terms of developing useful products and technologies, but also in ensuring that they are applied responsibly.

Recent research areas include:

■ Cloning of growth hormone and growth promoting factor genes of some commercially important fish has led to production of rapidly-growing stocks. Injections of growth hormone biosynthesized by bacteria containing this gene increase the growth rate of trout, while transgenic carp and catfish containing copies of this gene grow up to 50% faster than controls.

■ Identification of factors controlling spawning and settlement of abalone and oysters has allowed synchronized spawning in captivity, leading to the development of commercial hatcheries for these valuable shellfish.

■ Development of technologies for delivering growth hormones and gonadotropin-releasing hormone (GnRH) to fish in culture, either through micro-encapsulation or through implants, has made it possible to induce spawning in captive stocks.

THE CHALLENGE

Describe the Molecular Basis for Reproduction and Development:

- ***Identify genetic factors controlling reproduction***
- ***Discover biomedical uses for hormones of aquatic organisms***
- ***Identify internal factors directing growth and development***
- ***Uncover the molecular adaptive mechanisms of aquatic organisms***

Environmental and Evolutionary Biology

Rapid advances in marine biotechnology make it possible to answer some of the most intractable basic problems in modern biological oceanography. This was emphasized at a 1990 workshop supported by the National Science Foundation, the Office of Naval Research, and NOAA/Sea Grant, which concluded that "the impact of this new technology is similar to the impact of computer technology; both are completely revolutionizing the capability to address complex scientific issues." Marine biotechnology will provide ocean scientists with *a new means of knowing*, a new set of tools with which to examine basic ocean processes and to link these processes to the global ecosystem. Questions involving the distribution, characterization, recruitment and movements of marine organisms, their evolutions, adaptations, and interactions, and the elucidation of production, consumption and cycling rates are particularly amenable to biotechnological tools.

For example, the vast majority of micoorganisms in the oceans cannot be cultured, yet they play essential roles in the cycling and transformation of materials in the biosphere. Nucleic acid hybridization, monoclonal antibodies and other techniques of molecular genetics now are being used to characterize these cells and examine their activity.

This improved understanding will, in turn, enhance our ability to develop practical uses for the unique adaptations of marine organisms, to manage marine ecosystems and populations, and to restore impacted environments. However, significant barriers exist to the rapid incorporation of new technologies into the ocean sciences. Among these are the relatively small number of ocean scientists experienced in the tools of molecular biology and biotechnology, which emphasizes the need to incorporate training and interdisciplinary communication into any program in marine biotechnology.

Promising areas of research include:

■ Use of gene probes to rapidly identify and enumerate marine organisms, particularly small but ecologically important forms such as phytoplankton and zooplankton. This work is currently extremely labor intensive, and can be a major bottleneck in oceanographic research.

■ Investigation of harmful effects of the ozone "hole" in the Antarctic are being carried out by examination of DNA of marine organisms exposed to increased UV radiation. UV-tolerant forms are also being examined for potential natural sunscreens.

■ Employing molecular techniques to examine symbiotic relationships between species, such as the identification of nitrogen-fixing symbionts, as well as the molecular and genetic basis which controls "self-recognition" of cells. The latter is important to questions such as coral bleaching (the expulsion of algal symbionts by stressed coral animals) and the infective mechanisms of certain protozoan parasites.

THE CHALLENGE

Explore Ecological and Evolutionary Processes:

- ***Determine ecosystem processes relating to environmental change***
- ***Characterize the phylogeny and evolution of marine organisms***
- ***Delineate stocks and natural populations***
- ***Clarify biogeography and biodiversity***
- ***Assess the ecological roles of microorganisms***

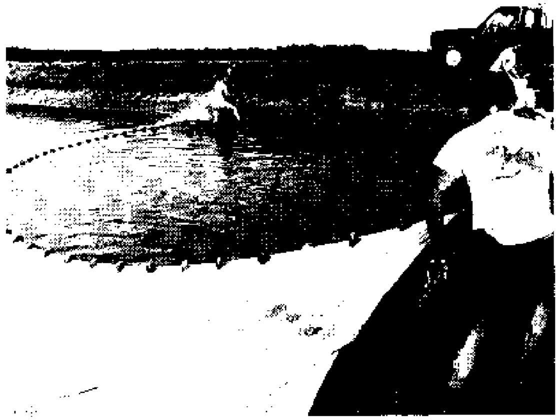
AQUACULTURE

Aquaculture is the growth of aquatic organisms in a controlled environment. Such environments may be bioreactors, open or closed raceways, ponds or natural bodies of water. The aim of such culture is to be able to produce items of economic import such as pharmaceutical agents, feed additives, isotopically enriched chemicals, polymers, lipids with petroleum potential and foodstuffs. The United States has a significant annual trade deficit in seafood, so increasing our nation's capability in aquaculture of food species would provide considerable economic benefit. There is also a growing market for production of ornamental fish, including captive breeding of tropical species now rare in their native countries. Economically viable aquaculture requires that the entire life cycle of a species be completed in captivity, yet many forms of potential importance do not reproduce under these conditions. Currently, fundamental research in physiology and endocrinology is directed primarily to understanding reproduction and growth of cultured species, and developing means for its control.

Other major areas of activity include research on means to increase the productivity or food value of the cultured species; drugs and vaccines to enhance immunity to disease or other stresses; genetic improvement of strains and identification of deleterious or desirable genes within stocks; vaccines, drugs and feeds tailored to specific species and means to increase the palatability, quality and safety of cultured food products.

Sea Grant has supported — and continues to support — groundbreaking research in aquaculture. Among recent accomplishments have been:

- Development of a triploid oyster that makes possible a year-round rather than



THE CHALLENGE

Produce More and Healthier Seafood:

- ***Enhance growth and productivity***
- ***Control reproduction of cultured stocks***
- ***Improve disease resistance and diagnosis***
- ***Produce drugs and feeds tailored to each species***
- ***Improve genetic makeup of strains***

seasonal oyster production — this achievement has contributed greatly to the revival of the West Coast oyster industry.

■ Use of DNA markers to differentiate between wild and hatchery-released stocks of fish, such as salmon, steelhead and striped bass, particularly in areas where wild, genetically-diverse stocks are threatened by habitat loss, overharvesting or competition from non-native species.

■ Development of vaccines against two major diseases of salmon, IPN and IHN virus. This research sets the stage for commercial development of improved vaccines to increase survival in cultured trout and salmon.

Seafood Safety and Human Health

Fish and shellfish provide an important component to the global diet — in many countries, seafood represents the major (or only) source of protein for thousands of people. Population pressure and the need to maximize the global food supply will mean an increased reliance on seafood in the future. There are, however, obstacles to this goal. In many tropical areas, the presence of ciguatoxin in reef fish greatly limits utilization of this resource. Other naturally-occurring toxins, such as paralytic shellfish poisoning, may prevent consumption of shellfish over wide areas. Virulent pathogens can contaminate the seafood supply, putting large segments of the population at risk. Some of these disease organisms, such as *Vibrio cholerae*, are endemic to marine systems; others indicate contamination with human or animal wastes. The presence of chemical toxicants in fish or shellfish is an increasing problem in industrial societies. Transfer of products across national boundaries, and the relative lack of inspection and certification at most stages of harvest and processing makes the issue of seafood safety a growing concern world-wide.

Marine biotechnology can give us the capability to prevent or detect these problems, and to ameliorate their impact. Most importantly, it can help us answer questions about how these problems arise — what are the processes which lead to the contamination of reef fish with ciguatoxin, what ecological conditions favor the microorganisms which produce the toxin, and how can their presence be detected? What processes enable endemic species of bacteria to cause epidemics of acute disease? How are toxic chemicals made biologically available to fish and shellfish, and what metabolic processes lead to their accumulation in tissues of exposed organisms? What are the long-term human health impacts of prolonged exposure to these contaminants?

Because of the direct threat to human health, as well as the need for increasing the world's food supply, the issue of seafood safety is receiving considerable attention from scientists, policy makers and the public. The potential of marine biotechnology to address many of these issues is demonstrated by successes to date:

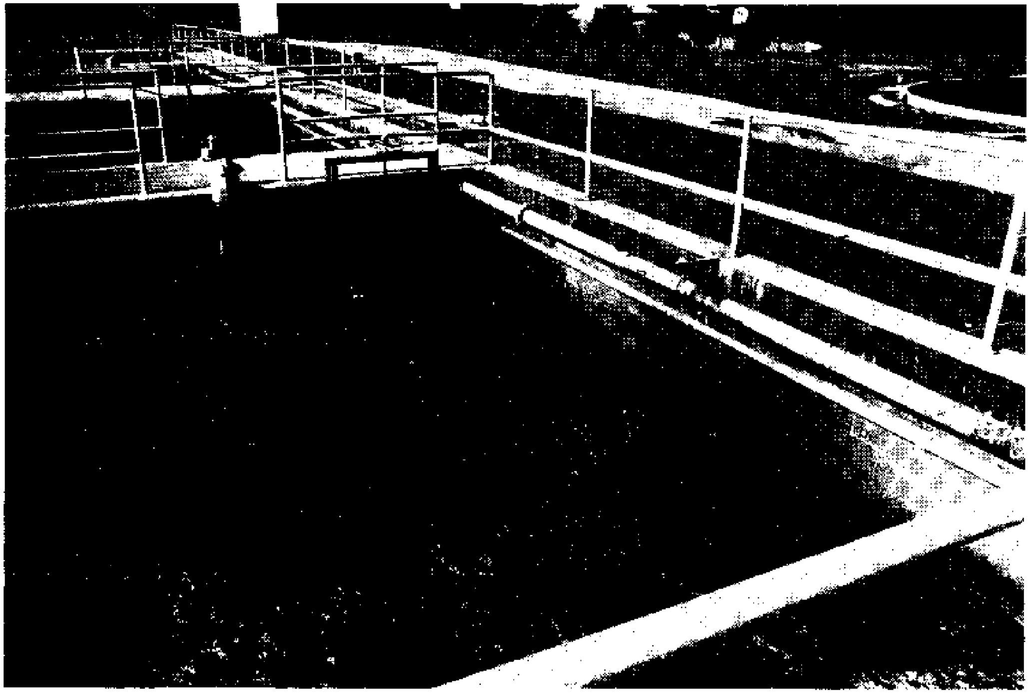
- Development of an inexpensive biochemical "dip-stick" monoclonal antibody assay for ciguatoxin, which can affect finfish from most tropical areas. This test kit is being produced commercially in Hawaii.
- Use of molecular techniques to elucidate the pathways leading to toxin formation by marine organisms, to chemically characterize these toxins and to determine the genetic coding governing their production.
- Development of a rapid enzyme-linked immunosorbant assay (ELISA) for *Vibrio cholerae* in oysters. A commercial firm has adapted this technology to produce a small hand-held kit for detection of contaminated seafood; millions of these kits will be sent to South America to help combat the cholera epidemic.

THE CHALLENGE

Safeguard Human Health:

- ***Detect contaminated seafood using rapid and sensitive methods***
- ***Characterize ecology of disease organisms***
- ***Improve depuration methods***
- ***Enhance shelf life of seafood***

Environmental Remediation



Marine organisms are also sources of novel bio-mediated pathways for processing and degrading a wide variety of natural and manmade substances. Degradation in aquatic environments by naturally occurring organisms or communities, or in bioreactors containing these organisms, offers potential for the disposal or cleanup of hazardous materials. In fact, waste processing in most modern sewage treatment facilities relies to a great extent on the manipulation of bacterial metabolism. Similarly, toxic industrial wastes or other effluents can be treated by properly selected aquatic microbes which have the capability (or have been genetically manipulated to acquire the capability) to break down these materials. Biosensors are being developed which use naturally-occurring marine proteins combined with information technology, for example, fiber optics and microcircuitry, to monitor for very low concentrations of toxicants.

Research is being initiated to identify the biochemical pathways of these processes, and to understand how these activities are distributed, regulated and maintained. There is a need to accelerate the search for naturally-occurring organisms which have the potential to degrade or process materials of interest — bacteria occurring near natural oil seeps — and to identify the genes responsible for these capabilities. Significant commercial potential for industrial use will follow, either by employing the original organisms or by inserting the appropriate genes into more traditional species.

A number of applications are already being developed which employ marine species, and others are being currently studied. These include:

■ Oceanic bacteria have been discovered that directly oxidize and precipitate iron, manganese, cobalt, nickle and other valuable and strategic metals. The genes and enzymes of these bacteria may be the key to separating these metals from low-grade ores, bypassing more expensive and environmentally-damaging industrial processes now employed.

■ Related to the above, bacteria have also been found that reverse these processes — that is, they can reduce or solubilize many metals (some toxic), and thus may be able to play a role in the remediation of pollution.

■ In Israel, a new petroleum-emulsifying agent has been developed from marine bacteria. A multi-million dollar business now produces this material for use in the petroleum industry, with production licensed in over a dozen countries including the United States.

■ Chitosan, a product extracted from shellfish waste, is being used to treat wheat seeds to reduce fungal infestation, with resultant increases in germination and ultimate yield (up to 10%). Many other applications of chitin and chitosan are currently under development, including medical materials; much of the original research in this material was supported by Sea Grant.

■ A test plant employing naturally occurring bacteria which degrade phenols has demonstrated a 99% drop in the concentration of chlorinated phenols from 100 to 1 part per million in a bioreactor system. These procedures were tested successfully by the Environmental Protection Agency at a Superfund site, and two firms have contracted to use these procedures in commercial applications.

THE CHALLENGE

Protect and Restore the Aquatic Environment:

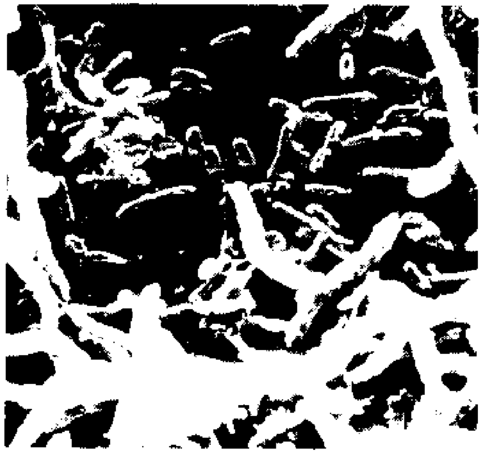
- ***Isolate or bioengineer organisms that degrade pollutants***
- ***Determine metabolic pathways to degrade hazardous substances***
- ***Develop processes to clean up waste streams***
- ***Design biological controls for nuisance species***

Biofilms and Corrosion

Biofouling and corrosion are major costs in commercial and U.S. Naval operations in marine environments. The occurrence of natural biofouling or corrosion-resistant substances has been demonstrated and these compounds merit intensive investigation at a molecular level to develop new methods for controlling these processes. Such approaches require an understanding of the attachment mechanisms of organisms that form biofilms, the growth of these films and how these processes influence fouling and the electrochemistry of corrosion.

There is an urgent need to develop newer, less toxic means of controlling biofouling of surfaces — both because of increasingly restrictive environmental regulations, such as the ban of tributyltin paints, and the current problems with exotic, invasive fouling organisms such as zebra mussels. Similarly, corrosion of marine materials can be intensified by microbial activity, and clarification of the biochemical and electrochemical pathways involved may allow control of these processes. Biofilms, and the invasion of tissues by surface-active bacteria, are also primarily involved in a number of human health problems, including dental caries, prosthesis septicemia and interstitial cystitis. There are also many potential benefits from a better understanding of biofilm formation, such as improved waste water treatment (trickling filter design), aquaculture (setting of oysters and other shellfish), agriculture and industry.

The naturally evolved chemicals which serve to reduce competition between surface-living organisms and bind organisms to surfaces under adverse adhesive conditions are potential areas for investigation, as are the cues which signal setting or metamorphosis in fouling macrofauna. These and other avenues are being pursued, including the following:



THE CHALLENGE

Reduce Fouling and Corrosion of Marine Structures:

- *Develop non-toxic fouling controls*
- *Determine the role of microorganisms in marine corrosion*
- *Describe molecular processes involved in fouling and corrosion*
- *Develop biomedical applications*

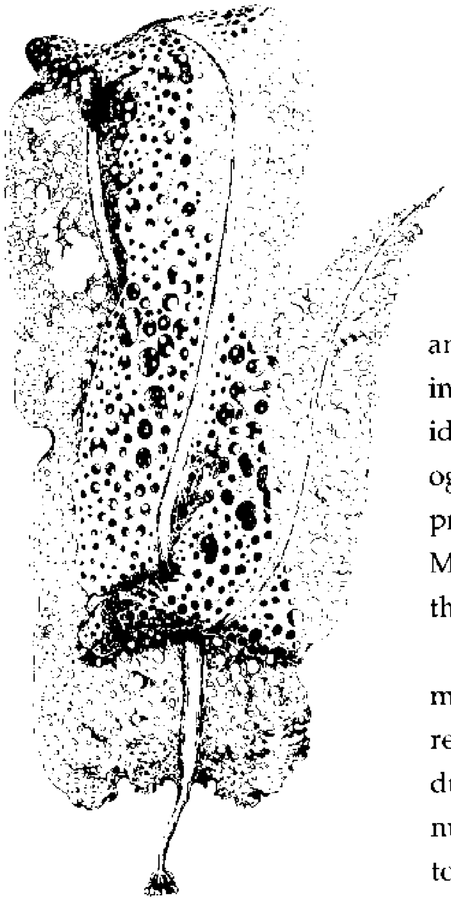
■ The organic matrix in oyster shell has been found to be a potent inhibitor of growth of crystalline calcium carbonate, the principal component of mineral scaling on marine surfaces. Synthetic substances modeled after this matrix also inhibit deposition of mineral scale. Research on the exact mechanisms involved is continuing while earlier results are being developed into commercial applications.

■ Recent developments in the study of bacterial exopolymers have shown that considerable differences exist in the metal-binding activity of these materials. This observation is now being expanded to look at the implications for corrosion of metal surfaces through enhanced microbial activity.

■ The observation of antagonistic interactions between closely related strains of film-forming bacteria, whereby one strain inhibits adhesion of the other clone, has led to the isolation of a material which shows a broad range of activity against film-forming bacteria and phytoplankton. This substance is now being further characterized and tested with Sea Grant support. It may have wide potential applications as a safer fouling-control agent.

■ Bacterial biofilms promote the successful settling and metamorphosis of oyster larvae, and in fact these films produce DOPA-like substances which act as cues and inducers of settlement. Research in this area has led to the patenting of a product, derived from these substances, and its use in a number of oyster hatcheries to produce cultchless spat. DOPA proteins may have application as inhibitors of corrosion, as well.

Biomaterials and Bioprocessing

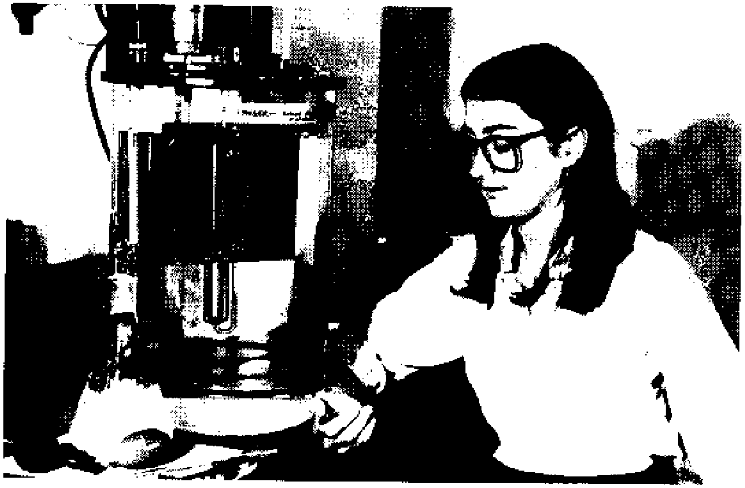


Marine organisms synthesize numerous chemicals with bioactive properties: metabolites, proteins, enzymes, polysaccharides, lipids and other materials which have direct application in health and life science. Determining the physiological and environmental role of many of these materials is likely to lead to new industrial processes and applications, for example, in bioremediation and in control of pests or diseases.

While most research on marine natural products has focused on identification, isolation and characterization and testing of the material for activity or utility, these are only the initial steps: elucidation of metabolic pathways by which materials are produced and identification of the genes responsible also will provide the bases for advanced technology. Because biosynthetic production, rather than extraction from the organisms, holds promise for more economical processes, this approach needs thorough exploration. Marine-derived polymers have potential application in a variety of products, among the most exciting being superconductors.

Photobioreaction may warrant attention both for production of energy and for material production. Light-harvesting chlorophyll and protein complexes can be reconstituted onto artificial membranes and are capable of collecting energy and transducing electrons with efficiencies comparable to photovoltaic cells. Under conditions of nutrient limitation, some microalgae produce hydrocarbons and lipids in quantities up to 65 percent of their dry weight. Application of DNA technology and other modern methods may provide economically viable approaches to exploring this biosynthetic capability.

For some applications, the quantities of materials required or the cost of obtaining them may limit — for a time — the commercialization of natural products. In these cases, the study of models may reveal alternatives that are more cost-effective to produce than the naturally-derived materials, but which retain their desirable characteristics.



Related areas of marine biotechnology are already highly productive, and many new products are in use or in development. Among these are:

■ Technology for the controlled farming of seaweed has allowed the production of biopolymers with many uses in industry. For example, carrageenan is widely used in the food and cosmetic industries and is the basis of hundreds of millions of dollars of commercial trade. Other algal polymers, such as agar and alginic acids, are also essential to a variety of biotechnological research and processing.

■ Marine glues which adhere firmly underwater are being developed from the naturally-produced byssal glues of mussels. Such adhesives can be used in bone and teeth repair and may have other medical applications.

■ Highly-charged oyster-shell proteins have been used as models to launch a new polymer technology. These new biodegradable and non-toxic compounds can be used to replace the hundreds of millions of pounds of non-degradable acrylic-based polymers employed annually as additives in detergents, dispersants and in other industrial applications.

■ Marine biomass production as a source of energy or useful chemicals is now being studied; these investigations focus primarily on macroalgae but microalgae, marine grasses and marsh plants, are also potential sources. It may be possible, for example, to apply molecular genetic techniques and produce rapidly growing strains that are able to grow in normally unproductive saline soils, providing animal fodder, fuel or raw materials in marginal or degraded environments.

THE CHALLENGE

Exploit Marine Natural Products for Human Benefit:

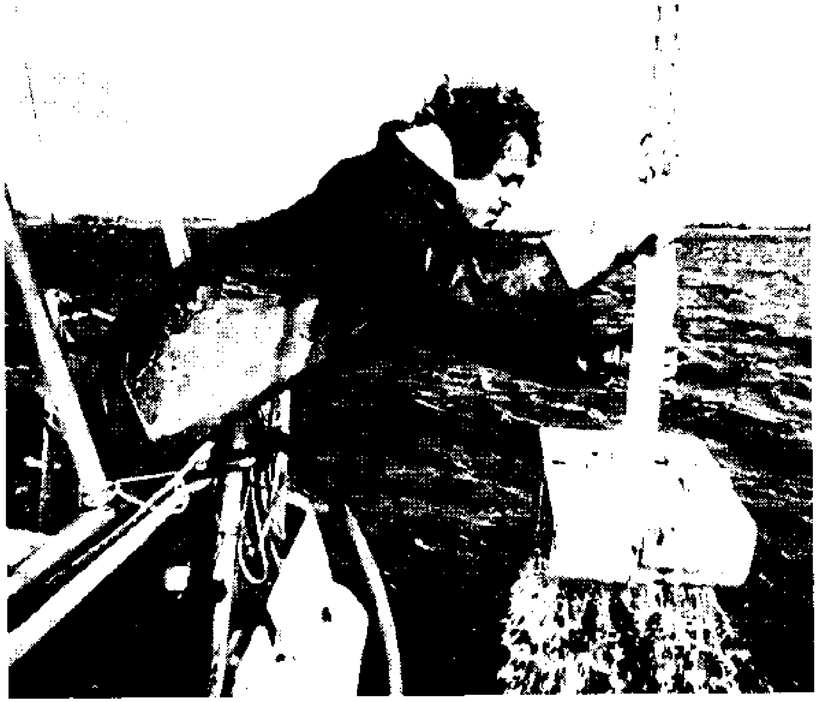
- ***Develop sensors for continuous monitoring***
- ***Discover new prosthetic materials***
- ***Develop new uses for industrial byproducts***
- ***Use marine materials for novel applications***
- ***Engineer marine organisms for innovative industrial uses***

Policy and Public Responsibility

THE CHALLENGE

Promote Responsible Use of Marine Biotechnology:

- *Develop new approaches for establishing protocols*
- *Adopt policies to promote research and investment*
- *Encourage cooperative projects between institutions, agencies and governments*
- *Facilitate the transfer of information and technologies*



Biotechnology generally has engendered complex ethical, legal, political and social issues. As more and more research institutions use the advanced techniques of biotechnology in their work, as genetically engineered organisms are made ready for field testing, and as the products produced by genetically engineered organisms multiply in the market places, the number and complexity of public issues are certain to increase. Neither researchers nor their home institutions can ignore these issues or fail to become involved in the public debate that will influence how rules governing research and its applications are formulated and how funding for scientific activities is apportioned.

It is unclear to what extent marine biotechnology raises issues beyond those raised in other biotechnology fields. While the majority of products generated by marine biotechnology research may differ in kind and should pose few special problems to industries, regulatory agencies or the patent office, major differences are likely to be in regard to future field testing of genetically manipulated organisms. Since in the aquatic environment there are few natural barriers to prevent an organism from mov-

ing from one site to another, containing test organisms within one area becomes very difficult. Moreover, the transfer of genetic materials among organisms is problematical: for example, a recent discovery of vast numbers of viruses in ocean waters leaves open the question of whether introduced genes may be transferred from one species to another via virus vectors.

Marine biotechnology research, and the application of these results for public benefit, must include due consideration of ethical and regulatory issues. There will be the need to balance freedom to conduct research and to develop products with appropriate oversight and guidance. Existing policies, especially the protocols governing work in genetic engineering, will need to be adapted to guide research and development in marine biotechnology.

ECONOMICS OF MARINE BIOTECHNOLOGY

THE CHALLENGE

Evaluate Economic Benefit of Marine Biotechnology:

- ***Perform cost-benefit analyses***
- ***Evaluate economic potential of new products and processes***
- ***Develop new markets for products***
- ***Facilitate investment in marine biotechnology***
- ***Provide guidance for new businesses and industries***

Marine biotechnology has received relatively scant attention in the United States, despite its obvious potential and despite the remarkable productivity that current research has demonstrated. It is unlikely that this level of achievement can be sustained if funding levels are not improved, and consistent support provided to research and development efforts.

Considering its potential, the present support for marine biotechnology is modest. Total research investment at academic and public institutions in the United States, excluding industry research, was approximately $44 million in 1991. Funding grew from $38 million in 1988 and is expected to reach $50 million by 1994. After adjusting for inflation, these figures represent no significant growth in investment for marine biotechnology research over this period.

Total support since 1984 for marine biotechnology research and development, exclusive of private sector investment, is estimated at $181 million. This support has resulted in 170 patents for funds expended to date, a large number of patents per research dollar given the academic orientation of most institutions which emphasize basic research.

With limited funding, marine biotechnology has been very successful in increasing our stock of economically important information. The ultimate value, however, will come from how this knowledge is applied to new product and process development. Although figures on overall value of marine biotechnology products are not available, there are estimates that on average the stock market value of a company increases by $810,000 with the awarding of a patent.

Education and Technology Transfer

Development of new methods, products and knowledge is only the first step; to be fully utilized, this new information must be transferred — to entrepreneurs, educators, institutions and industry. Formal mechanisms to disseminate research results, to instruct potential users, to facilitate the granting of patents and the licensing of products will facilitate this process. Sea Grant has a network of extension and communications personnel that could meet many of these needs. It is a base upon which to build additional efforts in technology transfer. This new direction will be both a challenge and an opportunity. Certainly expertise in many areas, such as intellectual property law, must be augmented. But the infrastructure is in place and needs no duplication.

Four major program elements are proposed:

■ **Scientific Entrepreneurship**: Scientists need to learn the realities of the business world, and those involved in business must understand the principles of the scientific method. We will establish programs which emphasize cross-training, and which allow students from each discipline to become familiar with the approaches and needs of both fields.

■ **Scientific Training:** A lack of trained personnel to drive the emerging marine biotechnology industry is one factor causing United States industrial growth to lag behind our foreign competitors. We will establish training programs to develop the

THE CHALLENGE

Develop Human Resources and New Partnerships:

- ***Train scientists and technicians***
- ***Educate the public***
- ***Promote interdisciplinary activity***
- ***Provide links between research and industry***
- ***Develop protocols for technology transfer***

skills necessary for students at the college level, for faculty at colleges, universities and vocational institutions and for scientists in both industry and academia.

■ **Public Awareness:** While marine biotechnology has great economic potential for this nation, it is essential that the public be informed about this science and feel comfortable with its use. The public awareness program will employ on-site training, seminars and production of teaching materials aimed at students from elementary grades to college, as well as the general public.

■ **Minority Outreach Program:** The scientific and economic challenges of the next century will require action and input from our entire population. Demographic realities, as well as the moral imperative to share benefits of technological and economic progress with all segments of this society, mandate the increased involvement of women and minorities in science and industry. This program element will include a mentorship program to encourage students on an individual basis, and provide them with research experience in a supportive environment.

Sea Grant Institutions

Alaska Sea Grant College Program
University of Alaska

California Sea Grant College Program
University of California, San Diego

Southern California Sea Grant Program
University of Southern California

Connecticut Sea Grant College Program
University of Connecticut

Delaware Sea Grant College Program
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Virginia Sea Grant College Program
University of Virginia

Washington Sea Grant College Program
University of Washington

Wisconsin Sea Grant Institute
University of Wisconsin

Woods Hole Oceanographic Institution Sea Grant Program
Woods Hole Oceanographic Institution

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Hawaii Sea Grant College Program
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Honolulu, Hawaii 96822
(808) 956-7031

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