

THE GLOBAL CHALLENGE OF MARINE BIOTECHNOLOGY



A Status Report on the United States,
Japan, Australia and Norway

Raymond A. Zilinskas, Rita R. Colwell,
Douglas W. Lipton and Russell T. Hill



A Maryland Sea Grant Publication
College Park, Maryland

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With Assistance from
Li Sheng Kong



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EXECUTIVE SUMMARY

This report addresses the emerging science and developing technologies encompassed by marine biotechnology. Marine biotechnology is defined as “the application of scientific and engineering principles to the processing of materials by marine biological agents to provide goods and services.” Specifically, the report contains a broad overview of marine biotechnology, sets forth current industrial realities, and assesses the future potential of this new field of biotechnology. Information to accomplish the foregoing was derived from the scientific literature and in-depth interviews with researchers, industrialists, and policy makers. We undertook primary data collection relevant to the United States (U.S.) by conducting a survey designed specifically for this study of research institutions and industry; information derived from the survey was entered into a database dedicated to marine biotechnology, MARBIO. Accordingly, research and industrial activities in the U.S. related to marine biotechnology are given the most thorough scrutiny, but developments in Japan, Norway, and Australia are also analyzed.

The report has eight extensive chapters. The first contains a wide-ranging review of major scientific achievements in marine biotechnology. The subject areas encompassed within marine biotechnology are grouped within six areas: aquaculture; marine animal health; marine natural products; biofilm and bioadhesion in the marine environment; bioremediation; and marine ecology and biological oceanography.

- **Aquaculture.** Research in marine biotechnology will benefit aquaculture in many, many ways, two of which are as follows.

By genetic manipulation, scientists can enhance finfish, shellfish, algal, or microalgal growth rate, disease resistance, and ability to survive adverse environmental conditions. Second, hormonal treatment can be used to control growth and development of commercially important fish and shellfish species to obtain consistent, controlled reproduction that also is economical. By strengthening the scientific basis of intensive aquaculture, increased yields can be expected.

- **Marine Animal Health.** Through biotechnology, new vaccines to counter bacterial and viral diseases that can have devastating impacts on marine organisms are being developed. These vaccines will protect fish, shrimp and other organisms from diseases that are responsible for decimating stocks and causing significant economic damage to the aquaculture industry throughout the world. During the past few years, for example, infectious diseases have destroyed 60-80% of shrimp aquaculture in China and Taiwan. Marine biotechnology research on control and prevention will reduce the incidence and scope of many of the microbial agents of disease.
- **Marine Natural Products.** Many marine organisms produce metabolites that help them survive and thrive. In addition, many of the intermediates of the metabolic pathways have properties beneficial to mankind. Recent surveys of algae, corals, sponges and tunicates have led to the discovery of such metabolites that show antibiotic, anti-tumor, anti-viral, or anti-inflammatory activity. As screening procedures improve, additional marine organisms producing, among others, anti-parasitic, pesticidal, immune-enhancing, growth-promoting, and wound healing-promoting chemicals will be added to the list. This aspect of marine biotechnology has significant potential for growth, since it is estimated that less than 1% of all marine species have been screened for potentially useful bioactive substances.

- **Biofilm/bioadhesion.** When an object is immersed in seawater, the exposed surfaces are colonized by a variety of marine organisms. The colonizing organisms become enmeshed in a film and produce acid, which corrodes piers, derricks, and other structures. The surface film also increases hull drag in ships, decreasing speed, and raising operating costs, as well as fouling equipment such as canal locks, cooling systems for power plants and engines of ships, and sensors used in remote monitoring instrumentation. Research being done in marine biotechnology is addressing the molecular basis of the settling and adhesion processes. Findings from this research will be used to develop methods for preventing settling by marine organisms on ships and marine structures and to manufacture adhesives that bond wet surfaces, notably in surgical and dental procedures.
- **Bioremediation.** Bioremediation is the use of microorganisms to break down pollutants and wastes in soil or water to harmless or less toxic end-products. Bioremediating microorganisms generally can be controlled successfully after the substance on which they feed has been depleted. Microorganisms employed in bioremediation usually are isolated from sites in nature, but can have their natural capability for breaking down pollutants enhanced through research. Because bioremediation is environmentally benign, in certain situations, it holds significant advantages over the usually employed chemical and heat treatment methods. As bioremediation is perfected, it will find increased use to clean polluted harbors, waterways, and marine structures, as well as to decontaminate sensitive geographical areas, such as estuaries and mangroves.
- **Marine Ecology and Biological Oceanography.** The techniques of molecular biology and biotechnology are increasingly employed in fundamental studies on marine ecology and biolog-

ical oceanography. Specifically, these techniques are being applied to identify fish, shellfish, algal, and microbial species and to detect genetic variability within species; to investigate species composition, variability, and metabolic activity of natural marine communities, and to monitor water quality by direct, more precise detection of bacteria in coastal and other waters that potentially are pathogenic for humans. Further, biosensors, i.e., biological molecules that selectively bind to specific agents or substances, offer sensitive methods for detecting low levels of nutrients, toxic compounds, DNA, and related chemicals. Thus, data gathering in the marine environment will be significantly improved, generating information useful for aquaculture and fisheries, public health, and environmental regulation agencies.

The second and fourth chapters of this report present the results of a survey of U.S. marine biotechnology research institutions and analyze economic aspects of marine biotechnology research and development. Analysis of the MARBIO data revealed that, in the U.S. in 1991, about \$40 million was spent by federal agencies, state governments, and industry to support marine biotechnology research at universities, public research centers, and industrial laboratories. Of this total, by far the greatest share (86%) originated from the federal government. Thus, of the total amount spent by the federal government to support marine biotechnology research, the National Institutes of Health (notably the National Cancer Institute) contributed 28%, the National Science Foundation 13.5%, the Office of Naval Research (ONR) 9.4%, and the National Oceanographic and Atmospheric Administration (Sea Grant) 8.8%. In addition, state funding provided ca. 7%, while private industry funded a little less than 7%. Although funding for marine biotechnology research grew rapidly with the emergence of this technology in the early 1980s, funding was essentially level during 1988-1991, and survey respondents expected no real growth in the next several years.

The analysis of MARBIO data also shows that microbiology and molecular biology were the areas in which most marine biotechnology research was being done, followed by natural products

chemistry. The type of research being performed most often was classified as basic research (34%). Other areas of applied research drawing attention were pharmaceuticals/fine chemicals (14.6%), environment/bioremediation (12.6%) and aquaculture (11.7%). Clearly the basic biological sciences are driving progress in marine biotechnology, indicating that this field still is in its formative stage.

Although not a major factor in the total U.S. science program, marine biotechnology research to date has been productive, in terms of generating new applications. From the MARBIO data, we estimate that, for every \$1.1 million spent in research at university and public research centers, one patent has been approved. We expect that this patent activity will increase as researchers change from their current focus on basic research to research with more possibilities for commercial application.

The MARBIO survey also encompassed industry. Approximately 85 companies in the U.S. were identified as being dedicated to marine biotechnology or sponsoring marine biotechnology R&D, either in-house or extramurally. The analysis of MARBIO data showed that most of the effort by industry was devoted to natural products development. It is a notable achievement that three pharmacological agents derived from marine biotechnology are in clinical trial, since they demonstrated, in one case, activity against tumors, in another, an ability to inhibit viruses and, in the third, efficacy in the treatment of psoriasis. Approximately five additional compounds have shown similar promise and are in pre-clinical trial.

In contrast to natural products development, the aquaculture industry in the U.S. is essentially nascent and there is a need for significant technological advancement for it to develop to its fullest capacity. The general notion regarding aquaculture has been that it is demand driven, when in fact, it is technological advancement, particularly genetic selection that has allowed this industry to spin off a few products. Marine biotechnology will contribute significant innovation, as closed system production becomes economically feasible in the U.S.

The relatively low level of interest shown to date by the wider industrial community for marine biotechnology may stem from the fact that most companies are not aware of this field or, if they are

aware, have misconceptions about the difficulty of working in the marine environment, and/or are also uninformed about the scientific/technical advances that have been achieved in marine biology to date.

Our analysis of MARBIO data clearly shows that industry interest and, more importantly, investment in marine biotechnology in the United States is relatively meager, compared to U.S. investment in biotechnology over-all. As noted above, total funding for marine biotechnology research was ca. \$40 million in 1991. For purposes of comparison, this sum is between 7% and 11% of what the Japanese spent on research in this field (see below). Shortage of capital in the U.S. for marine biotechnology has limited it from achieving the explosive growth that has occurred in other areas of biotechnology.

The politics and funding policies of U.S. federal agencies and state governments, related to marine biotechnology, are discussed and analyzed in Chapter 3. Our review of policies adopted by the Reagan, Bush, and Clinton administrations indicate that all have supported important initiatives in biotechnology, but marine biotechnology specifically has not been favored. Neither of the first two administrations had been particularly favorably disposed towards the ocean sciences in general, so that funding decreased, in real terms. However, the Clinton administration appears to be attempting to reverse this situation. For example, strong support has been given the National Sea Grant Program of NOAA. We expect the Clinton administration will continue to favor biotechnology and especially initiatives supporting marine biotechnology, including the 1980 National Aquaculture Act and the 1994 Marine Biotechnology Investment Act.

The U.S. Congress has been a strong supporter of the ocean sciences and biotechnology since the early 1980s and we expect this to continue for the foreseeable future. The concrete steps that Congress is likely to take, which will favor marine biotechnology, are to fund the National Aquaculture Act passed in 1980, but which, until now, has not been funded, Congress has passed the 1994 Marine Biotechnology Investment Act, which provides \$20 million per year in new funds to support marine biotechnology research.

In Chapter 3 we also discuss funding of marine biotechnology by federal agencies. As part of an inter-agency process initiated by the Federal Coordinating Council on Science, Engineering, and Technology (FCCSET), each agency estimated the amount of funding it dispersed in support of various areas of biotechnology including marine biotechnology. Unlike MARBIO data, which is derived from those who are recipients of federal funding, the FCCSET estimates are made by the providers of federal funding. From MARBIO data we estimate that federal agencies provided ca. \$34 million in 1991 in support of marine biotechnology; FCCSET estimates that these same agencies provided approximately \$40 million. The difference is not easy to reconcile; perhaps the discrepancy is due to problems with defining which activities should be considered marine biotechnology. Since there is no single agreed upon definition of the term among federal agencies, some may be overly generous in what they place under the rubric of marine biotechnology.

Between 1988 and 1991, three states, Maryland, North Carolina and California, established dedicated marine biotechnology centers. While the centers in California and North Carolina were built with funds provided by the respective states, major funding for the Maryland center was provided by the Office of Naval Research under the University Research Initiative. This program currently is funding the establishment of another research center in California that will largely focus on marine natural products research and development. The direct contributions that state governments make to support marine biotechnology in general is not so significant, a mere \$2.8 million in 1991.

The issue of the safety of marine biotechnology research and testing is presented and analyzed in Chapter 5. We conclude that the biosafety issue to date has not been a barrier to the advancement of marine biotechnology. Approaches that have been developed to ensure safety in biotechnology research generally apply directly to marine biotechnology. However, most of the activities within marine biotechnology so far have related to research, which typically is not controversial. When large-scale field trials of marine transgenic organisms are imminent, the level of public concern may rise. It will be

important to allay such concerns, which easily could hinder progress in marine biotechnology, by developing risk assessment schemes applicable to the marine environment and taking steps to educate the public about the precautions being taken to ensure safe testing.

Marine biotechnology is an emerging scientific/technical field throughout the world. Significant marine biotechnology research and development is taking place in Australia, France, Germany, Italy, Norway, Sweden, the United Kingdom and other countries. However, limits on resources and time allowed us to survey and analyze the status of marine biotechnology in just three foreign countries: Australia, Norway, and Japan.

Marine biotechnology in Australia and Norway are covered respectively in Chapters 6 and 7. We deduce that marine biotechnology will fuel advances in these countries that are likely to have significant national economic effect and will incrementally increase scientific knowledge in general. Thus, Australian investigators are probing the spectacular undersea territory in their coastal zone and should discover a large number and range of marine species producing chemically unique and biologically active substances. It can be predicted that some will be useful as medicinal agents, generating profits for their developers. However, the commercial climate in Australia, which tends to be risk averse, does not appear to be conducive to the type of imaginative, long-term programs required to bring the results from marine biotechnology research to the market. Therefore, most such products are likely to be exploited with the aid of affluent foreign companies.

Based on their achievements to date, Norwegian scientists will join the front ranks of research and development in targeted areas, e.g., transforming wastes from aquaculture and fisheries into useful products, such as animal feed, industrial enzymes, and specialty chemicals. The aquaculture industry in Norway, already the world's largest in terms of bulk production, will be positioned to utilize marine biotechnology-derived diagnostics, therapeutics and vaccines to improve its fisheries output, thereby becoming even more efficient and competitive in seafood markets worldwide. However, in view of predictable market conditions from increasing supply, as well as in-

creasing competition from the growing aquaculture industry in developing countries, improvements to Norway's aquaculture will have to turn to being more cost effective and more technologically efficient for it to remain at the front of the pack of seafood producers.

The growth of Norwegian aquaculture has stimulated the formation of other types of companies, those that offer services and products useful to aquaculture. These Norwegian companies will be in a powerful position to compete in the sectors of the international market constituted by marine animal feed, diagnostics, and therapeutics. Although these sectors are relatively small, potentially they are lucrative and serve as entry-points for smaller biotechnology-based companies, which are likely to be the basis for major economic development in the 21st century.

As made clear in Chapter 8, both the Japanese government and the industrial sector of Japan early realized the importance of the marine environment for economic progress and took steps to promote marine biotechnology, going much beyond simply promoting aquaculture and fisheries. The data indicate that Japan spent ca. \$357 million to \$519 million in 1992 on marine biotechnology research and development, a sum that certainly has increased every year since then. About 80% of this funding is supplied by industry, in contrast to the current U.S. investment pattern where government funding predominates. However, the Japanese government provides significant indirect support of industry in the form of special tax advantages, loan programs, well-funded schemes for industry-university cooperation in R&D, and regional promotional activities. The major areas of emphasis selected by the Japanese for research and development are aquaculture, marine natural products, and biosensors, although investments in environmental applications are rapidly increasing.

As a consequence of the scientific research and development effort that the Japanese have made to date with their focus on discovering new marine natural products, significant discoveries have already been made. We predict that 10-15 years from now, results from these discoveries will become manifest—i.e., a cascade of new drugs derived from marine organisms and developed by Japanese scientists

will appear in Western pharmacies and will be used to treat a wide range of infectious and non-infectious diseases, including cardio-vascular diseases, cancers, immunological disorders, bacterial, fungal, and viral infectious diseases. In addition, it is highly probable that Japan will be the world's major source of biosensors for medicine and environmental monitoring. A smaller commercial market in comparison to pharmaceuticals but, nevertheless, of importance to the U.S. in terms of balance of trade, Japanese aquaculture will benefit significantly from marine biotechnology applications, especially those related to preventing and treating diseases of finfish and shellfish and from marketing of genetically improved finfish and shellfish species. In addition to fisheries biotechnology, Japanese scientists are focussing on advances in biological oceanography, especially for determining the role of plankton and picoplankton in the world oceans and their effect on global climate. This work is voluminous and will offer benefits by the 21st century, particularly to international efforts aimed at improving the environment.

Chapter 9 concludes the substantive part of the report. Researchers and funding agencies in the U.S. were the first to recognize the importance of marine biotechnology; this recognition resulted in an initial spurt of growth in R.&D. However, funding levels have stayed about the same over the last three years, so no real growth is taking place in this field. As the funding level is not likely to grow in the foreseeable future, the explosive growth that has occurred in general biotechnology cannot take place in marine biotechnology. Some additional growth could take place if industry became more interested in marine biotechnology, but this is an unlikely prospect since so few applications from this field are in the pipeline. Unless there is a major effort to focus U.S. activity in this area, advances in the U.S. will be limited to the occasional chance discovery of a process, activity or application. National effort designed to produce marine biotechnology products and processes would be much more successful, and produce a far greater return to society, than one that relies mostly on chance. Fortunately, the elements of such an effort are discernable with the passage by the U.S. Congress in 1994 of the Marine Biotechnology Investment Act, which makes available signif-

cant new funding (\$20 million per year for the first two years) to scientists in both private and public research institutes and laboratories.

This report also includes a glossary of technical terms commonly used in marine biotechnology and four appendices. The first appendix contains definitions of the term "marine biotechnology" by scientists throughout the world; the second is a copy of the questionnaire sent out to research units; the third is a copy of the questionnaire sent out to industry; and the fourth appendix lists foreign institutions known to us wherein marine biotechnology or marine biotechnology-related research is performed.

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GLOSSARY

Aerobic—requiring oxygen.

Amino acid—any of a group of 20 chemicals that are linked together in various combinations to form peptides or proteins.

Anabolism—see metabolism.

Anaerobic—without oxygen.

Antibody—a specific protein molecule produced by an organism's immunological defense system when it is challenged by a foreign substance (the antigen). The antibody neutralized the antigen by binding to it.

Antigen—a substance that when introduced into an organism elicits from it an immunological defensive response. Many living microorganism or chemical agents can under appropriate circumstances become antigens.

Applied research—experimental or theoretical work directed towards the application of scientific knowledge for the development, production or utilization of some useful product or capability.

Bacteriophage (phage)—a virus that attacks or colonizes a bacterium.

Basic research—experimental or theoretical work that is undertaken to acquire knowledge of fundamental principles of phenomena and observable facts and that may not be directed towards a specific application.

Biodegradation—the natural process whereby organisms break down organic molecules.

Biodiversity—the totality of the world's life forms, ecosystems, and ecological processes, which can be characterized at the genetic, taxon, and ecosystem levels.

Bioremediation—a technology that uses biological activity to treat contaminated soil or water in order to reduce or contain the contaminant(s).

Biosafety—in activities involving life forms or their parts, the observance of precautions and preventive procedures that reduce the risk of adverse effects.

Biotechnology—a collection of processes and techniques that involve the use of living organisms, or substances from those organisms, to make or modify products from raw materials for agricultural, industrial, or medical purposes.

Bivalve—one of a class of sessile or burrowing mollusks, including clams, mussels and oysters.

Capability—the ability to produce or apply a particular set of scientific techniques or technologies.

Carroageenan—generic term for certain hydrocolloids (gums) extracted from red macroalgae. For industrial purposes, three types having differing characteristics have been identified—iota, kappa, and lambda.

Catabolism—see metabolism.

Catalyst—a substance that affects the rate of a chemical reaction but remains itself unaltered in form or amount.

Cell culture—the propagation in culture of cells removed from a plant or animal.

Cell fusion—combining nuclei and cytoplasm from two or more different cells to form a single hybrid cell.

Clone—a group of genetically identical cells or organisms asexually descended from a common ancestor. In case of a cloned organism, all cells making up that organism have the same genetic material and are exact copies of the original.

Cloning—the use of genetic engineering to produce multiple copies of a single gene or a segment of DNA.

Crustacean—one of the class Crustacea, which breathe by gills and whose bodies are covered by shell or crust, including barnacles, crabs, lobster and shrimp.

Culture—the growth of cells or microorganisms in a controlled artificial environment.

Cytokines—peptide hormones produced by the immune system and other diffuse tissues. At this time cytokines encompass about 25 distinct peptides, including colony stimulating factors, interferons, interleukens and tumor necrosis factors.

Dispersant—a substance that reduces surface tension of a floating pollutant, causing it to sink.

Database—a collection of data, defined for one or more applications, which is physically located and maintained within one or more electronic computers.

Development—the process of applying scientific and technical knowledge to the practical realization or enhancement of a specific product or capability.

DNA—deoxyribonucleic acid; the carrier of genetic information found in all living organisms (except for a small group of RNA viruses). Every inherited characteristic is coded somewhere in an organism's complement of DNA.

Emulsant—a surface-active substance that allows a normally immiscible liquid (for example oil) to disperse or become mixed into a second liquid (for example water).

Enzyme—a special protein produced by cells that catalyze the chemical processes of life.

***Escherichia coli* (E. coli)**—a species of bacteria that commonly inhabits the human lower intestine and the intestinal tract of most other vertebrates as well. Some strains are pathogenic, causing urinary tract infections and diarrheal diseases. Weakened strains are often used in laboratory experiments.

Expression—the translation of a gene's DNA sequence by RNA into protein.

Fermentation—the anaerobic bioprocess in which yeasts, bacteria, or molds are used to convert a raw material into products such as alcohols, acids, or cheeses.

Filter feeder—an organism that obtains its food by straining water passing through some part of its body and recovering suspended organisms. Filter feeders include baleen whales, corals, mussels and sponges.

Finfish—vertebrate fish, as opposed to invertebrate shellfish.

Fraction—a chemical agent or compound that may be separated out by chemical or physical methods from a solvent containing a mix of substances.

Gene—the fundamental unit of heredity. Chemically a gene consists of ordered nucleotides that code for a specific product or control a specific function.

Gene splicing—the use of site specific enzymes which cleave and reform chemical bonds in DNA to create modified DNA sequences.

Genetic engineering—a collection of techniques used to alter the hereditary apparatus of a living cell enabling it to produce more or different chemicals or to be deficient in a normally produced chemical. These techniques include chemical synthesis of genes, the creation of recombinant DNA or recombinant RNA, cell fusion, plasmid transfer, transformation, transfection, and transduction.

Halophilic—requiring high concentrations of salt for existence.

Hazard—the likelihood that an agent or substance will cause immediate or short-term adverse effects or injury under ordinary circumstances of use.

Host—a cell whose metabolism is used for growth and reproduction of a virus, plasmid, or other form of foreign DNA.

Host-vector system—compatible host/vector combinations that may be used for the stable introduction of foreign DNA into host cells.

Hybridoma—a special cell produced by joining a tumor cell (myeloma) and an antibody producing cell (lymphocyte). Cultured hybridoma produce large quantities a particular type of monoclonal antibodies.

Hydrocarbon—one of a large and diverse group of compounds, consisting of only carbon and hydrogen, which constitutes petroleum.

Infection—the invasion and settling of a pathogen within a host.

Intellectual property—the area of the law encompassing patents, trademarks, trade secrets, copy rights, and plant variety protection.

Interferon—a type of cytokine discovered in the 1950s having potential as anti-cancer and anti-viral agents. Three types of interferons are known, alpha (IFN- α), beta (IFN- β) and gamma (IFN- γ).

In vitro—literally “in glass”; pertaining to biological processes or reactions taking place in an artificial environment, usually the laboratory.

In vivo—Literally “in the living”; pertaining to biological processes or reactions taking place in a living system such as a cell or tissue.

Metabolism—the sum of the chemical and physiological processes in a living organism in which foodstuff are synthesized into complex biochemicals (anabolism); complex biochemicals transformed into simple chemicals (catabolism), and energy is made available for the organism to function and procreate.

Metabolite—a substance vital to the metabolism of a certain organism, or a product of metabolism.

Microinjection—the injection of DNA into a cell or cell nucleus using a fine needle under a microscope.

Microorganism—a microscopic living entity that can be a virus, bacterium, or fungus.

Mollusc—invertebrate member of the phylum Mollusca, including clams, mussels, octopuses, snails and squids.

Monoclonal antibody—an antibody produced by a hybridoma that recognizes only a specific antigen.

Nucleotide—the fundamental molecule that makes up DNA and RNA. Each nucleotide constituting DNA consists of one of four amino acids (adenine, guanine, cytosine or thymine) linked to the phosphate-sugar group deoxyribose; each nucleotide constituting RNA consists of one of four amino acids (adenine, guanine, cytosine or uracil) linked to the phosphate-sugar group ribose.

Pathogen—an organism that causes disease.

Peptide—a linear polymer of amino acids. A polymer consisting of many amino acids is called a polypeptide.

Plankton—microscopic organisms inhabiting sea water in high numbers. Plankton may be phytoplankton (microscopic plants) or zooplankton (microscopic animals).

Plasmid—small, circular, self-replicating forms of DNA often used in recombinant DNA experiments as acceptors of foreign DNA.

Plasmid transfer—the use of genetic or physical manipulation to introduce a foreign plasmid into a host cell.

Polymer—a linear or branched molecule of repeating subunits.

Production—the conversion of raw materials into products or components thereof through a series of manufacturing processes.

Real time—a characteristic of a system which makes information available about a process so quickly it allows the operator to act to change the outcome of the process while it is still underway.

Recombinant DNA—rDNA; the hybrid DNA resulting from the joining pieces of DNA from different sources.

Risk—the probability of injury, disease or death for persons or groups of persons undertaking certain activities or exposed to hazardous substances. Risk is sometimes expressed in numeric terms (in fractions) or qualitative terms (low, moderate or high).

Risk management—the process of determining whether or how much to reduce risk through regulatory action. Decisions usually depend on data from risk assessment and take into account economic, ethical, legal, political and social factors.

RNA—ribonucleic acid; found in three forms—messenger, transfer, and ribosomal RNA. RNA assists in translating the genetic code of a DNA sequence into its complementary protein.

Shellfish—an indistinct term for marine and freshwater invertebrates, but commonly refers to any crustacean or mollusc.

Synthesis—the production of a compound by a living organism.

Technology—the scientific and technical information, coupled with know-how, that are used to design, produce and manufacture products or generate data.

Toxicity—the quality of being poisonous or the degree to which a substance is poisonous.

Trait—a characteristic that is coded for in the organism's DNA.

Transduction—the transfer of one or more genes from one bacterium to another by a bacteriophage (a virus that infects bacteria).

Transfection—the process in which a bacterium is modified in a way which allows the cell to take up purified, intact viral or plasmid DNA.

Transformation—the introduction of new genetic information into a cell using naked DNA (i.e., without using a vector).

Vector—a transmission agent, usually a plasmid or virus, used to introduce foreign DNA into a host cell.

Virus—an infectious agent, containing either DNA or RNA as its genetic material, which requires a host cell for its replication.

Wild-type—the form of a given organism that occurs in nature; i.e., one that has not undergone mutagenesis.

INTRODUCTION

Marine biotechnology has emerged as a major component of the biotechnology revolution. It is rooted in the traditions of marine biology, with its richness of literature and poetry of scientific discovery. Marine biology has contributed greatly to the over-all discipline of biology, weaving in the tapestry of the biological sciences. Information from more than 100 years of observation, experimentation, and careful record-keeping is available for the prepared minds of molecular biologists to refine, probe, and to apply to societal needs in the most environmentally protective way ever possible. The fishes of the sea, strange and bizarre organisms living in the abyssal depths of the oceans, legions of creatures from the reefs, produce molecules with potential healing and nurturing properties that may be harvested by cloning the genes responsible for the pathways by which these substances are produced. The pharmacopoeia of the sea now can become a reality, a truly remarkable source of new drugs, new cures, and new chemicals, new foodstuffs, and a rich source of protein and nutrients. The tools of molecular biology should make this a reality by the turn of the century.

The poetic sweep of the possibilities of marine biotechnology runs counter to the harsh fact that it is, however, an underdeveloped science and technology, with its own history tracing back only a decade. It is far from the highly sophisticated stage that medical biotechnology has moved to, where the potential of gene therapy is very nearly reality. Marine biotechnology, in contrast, is only in its infancy, still at the discovery stage. There is little or nothing known about the molecular genetics of most of the invertebrates of the sea, nor about algae and marine bacteria. The available information is

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much less than for the commercially valuable crustaceans, such as the shrimp. It is ironic that countries like Thailand, Ecuador, and Indonesia have created major industries out of shrimp farming (Rosenthal, 1993), yet, little is known about genetic mechanisms controlling fertilization, development, growth, and health of shrimp species.

Although much research and development related to the marine environment is being done throughout the world, it is only recently that a subset of these activities has been termed marine biotechnology. In fact, marine biotechnology was first defined only a decade ago (Colwell, 1993, 1984a,b). Because of its newness, few outside the immediately affected scientific community even now are aware of the richness of the field, its promises, or its problems. Our study has been hampered by the lack of a universally accepted definition of the term "marine biotechnology," as well as by the field's great diversity. In a strict sense, marine biotechnology may be defined as "a set of scientific techniques that use living marine organisms, or parts of marine organisms, such as cells, to make or modify products, to improve plants or animals, or to develop organisms for specific applications" (OTA, 1984). However, this definition is believed by many researchers to be too narrow, i.e., some hold that a host of different R&D activities rightfully are areas of marine biotechnology, including aspects of biological oceanography, e.g., use of restriction fragment pattern analysis to monitor biological resources of the sea.

Taking into account the lack of consensus on how marine biotechnology is bounded, we do not draw rigid disciplinary lines in this report. Instead we view marine biotechnology as a field that encompasses broad scientific and technological activities relating directly to marine organisms or their parts and employing classical biotechnology and/or molecular biology techniques. Thus, a broad definition, and the one we use in this report, is a derivation of the Organization of Economic Co-operation and Development's (OECD) definition (Bull et al., 1982), namely, marine biotechnology is "the application of scientific and engineering principles to the processing of materials by marine biological agents to provide goods and ser-

vices." When molecular biology techniques, such as recombinant DNA, hybridoma/monoclonal production, protein engineering, polymerase chain reaction, and DNA hybridization, are used for research and development in certain applied fields, such as aquaculture, fisheries, and natural marine products, we include them under the rubric of marine biotechnology (see Figure 1). This point is important because, as we discuss below, some researchers in, for example, the natural products chemistry area claim that they are doing marine biotechnology, while others involved with similar work assert that they are not. To illustrate the diversity of opinions as to what does and does not constitute marine biotechnology research and development, we include in Appendix 1 definitions of marine biotechnology formulated by scientists located throughout the world.

Even with an agreed upon definition, it is problematic to generalize about marine biotechnology research and development (R&D). This is because marine biotechnology does not define an industry but is, instead, a broad category of technological advancement involving a variety of industries, products, and production processes. In some cases, tracing the marine biotechnology origins of

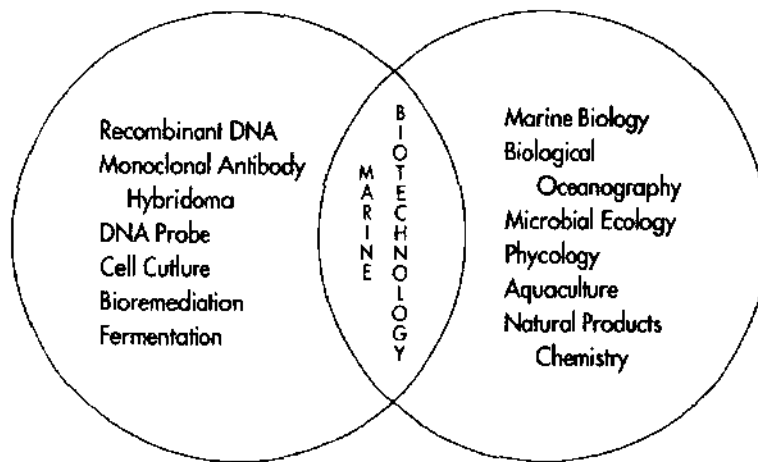


Figure 1. Elements of marine biotechnology.

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a product or process is straightforward. For example, an aquaculture-produced fish, using the methods of molecular biology and biotechnology, is closely linked to its marine biotechnology origins. However, the results of marine biotechnology R&D may also be detected in an application far removed from the marine environment, such as development of a new pharmaceutical product from sea urchins for use against human disease. Although these two very different products, chosen as an example for purposes of discussion, are linked by their common origin in marine biotechnology research and development, the potential markets for such products or processes are quite disparate and require separate analysis.

Marine biotechnology is an emerging, science-based, technological area. "Emerging" means that the technology is at a stage in its development cycle where attempts are being made to identify practical applications the technology can engender and to put laboratory processes and techniques into practice. Looking at the term from another perspective, an emerging technology is one that the public and its representatives begin to recognize as having the potential to generate new scientific knowledge and produce useful new products and processes. Marine biotechnology, as is characteristic of an emerging field, has, to date, produced only a few applications that are in the marketplace. However, its major economic effects are, therefore, yet to come.

In recognition of its emerging character, the first requirement to conducting an assessment of marine biotechnology was to analyze primary data about the research being conducted within the field, the objectives of research, the amount of funding that was financing this research, and the major sources of research funds. We quickly found out that this type of primary data did not exist, so we had to collect it ourselves. Accordingly, we undertook primary data collection relevant to the United States (U.S.) by conducting a special survey of research institutions and industry; information derived from the survey was entered into a database dedicated to marine biotechnology, MARBIO. Eventually, as is explained in Chapters 2 and 4, sufficient data were collected to make possible statistically meaningful analysis of marine biotechnology research in the U.S.

In view of its future potential, we have made an attempt to determine the applications and products of marine biotechnology research and development that are likely to be generated in the short term (one to three years), medium term (three to six years), and the long term. The best source of information required to perform this type of prospective analysis is industry. Therefore, we interviewed scientists and managers of the small number of companies that could be identified as being dedicated to marine biotechnology, and the larger number of companies that had made investments in this field. Information derived from these interviews was also entered into MARBIO. The analysis of MARBIO data allowed us to discern corporate strategies for marine biotechnology, explore strengths and weaknesses in corporate R&D programs, and identify links between industry and universities.

A related objective of the project was to evaluate the economic potential of marine biotechnology products. We developed a conceptual framework of product life cycles and demonstrated the usefulness of this framework in assessing the importance of marine biotechnology for a given application, e.g., aquaculture.

Another major objective of the study was to evaluate the competitiveness of U.S. researchers and industry, compared to other countries expending resources to develop marine biotechnology as an industry. As we discovered, this is a difficult endeavor since most foreign researchers have only a hazy notion of the term "marine biotechnology" and few governments even use the term in their biotechnology strategic planning. Of the countries we investigated, Australia, Japan and Norway provided substantial, useful information about their research efforts in marine biotechnology. In each of these cases, we were able to estimate, with a high degree of confidence, those resources that the government and industry was allocating to this field. However, detailed information about research units, such as we have been able to compile concerning U.S. researchers, was not available from either official or private sources. Due to limited resources, we were unable to survey Japanese or other foreign-based scientists, to develop the kind of database we have compiled on U.S. marine biotechnology. Nevertheless, sufficient information was col-

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lected from the literature and interviews to permit meaningful comparisons of accomplishments among the three countries and to draw some conclusions.

Information about marine biotechnology research in countries other than Australia, Japan, and Norway is very hard to obtain. One major difficulty lies in the difference in definitions, as discussed above. Thus, government and private reports on biotechnology in most countries do not list marine biotechnology as a distinct category. If a country supports R&D activities that fall within the purview of what we term marine biotechnology, these activities are usually listed under such headings as agricultural, chemical, environmental, or pharmaceutical biotechnology. The task, then, of compiling data on marine biotechnology in most countries is arduous and sometimes impossible, especially primary data required for accurate assessments of marine biotechnology in specific countries. Therefore, scientific accomplishments in marine biotechnology described or referred to in this report, especially in Chapter 1, originate mostly from U.S. and Japanese research institutions. We regret that a lack of resources precluded us from recording the many significant accomplishments in this field by researchers from other nations.

It has to be pointed out, however, that in general we found the level of interest in marine biotechnology to be extraordinarily high in many countries of the world, including industrialized countries (e.g., Canada, France, Germany, Italy, Sweden, and United Kingdom), the newly industrialized countries (e.g., Korea, Taiwan, and Thailand) and developing countries (in particular, China, Ecuador, India, Indonesia, and Philippines). It is unfortunate that we were unable to consider marine biotechnology developments in these countries and include them in this report.

The information presented in this report is organized as follows. In Chapter 1, the scientific basis of marine biotechnology is surveyed. Considering the diversity of marine biotechnology, a review of the field is more manageable for analysis by dividing it into six areas. Examples of research being done in each of these areas are provided, as well as specifics about the researchers and laboratories involved. Chapter 2 contains a general report of the survey results derived from questionnaires distributed to researchers staffing universi-

ty and public institutions that had been identified as performing marine biotechnology R&D in the U.S.. In Chapter 3, we discuss the support that past presidential administrations and U.S. Congresses have given to the ocean sciences, in general, and to biotechnology and marine biotechnology, specifically. We also consider support of the Clinton administration and the U.S. Congress as constituted in 1993-1994. Major federal funding agencies and their support of marine biotechnology research are described, as well as state-funded support. Chapter 4 is a discussion of the economic issues related to marine biotechnology R&D, including product life cycle and its application to aquaculture. Chapter 5 discusses safety aspects of marine biotechnology and analyzes possible regulatory constraints and consequences for the field. Next, in Chapter 6, an overview of marine biotechnology in Australia is provided, including estimates of singular strengths possessed by that nation. In Chapter 7, an overview of marine biotechnology in Norway is presented. Chapter 8 is focused on Japan and its endeavors in marine biotechnology. In the final chapter, the degree of U.S. competitiveness versus other countries in marine biotechnology is assessed and some predictions about the future direction of marine biotechnology in the U.S. and Japan are offered.

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Chapter 1

SCIENTIFIC BASIS OF MARINE BIOTECHNOLOGY

INTRODUCTION

Marine biotechnology, similar to general biotechnology, has many aspects and directions. For manageability, we subdivide the field into six areas: marine aquaculture and biotechnology, marine animal health, marine natural products, biofilm and bioadhesion in the marine environment, bioremediation, and marine ecology and biological oceanography. Examples of notable research in each of these areas are provided below.

MARINE AQUACULTURE AND BIOTECHNOLOGY

During the past decade, the farming of marine finfish, shellfish, crustaceans, and seaweed has grown significantly in economic value world-wide. However, marine aquaculture in the U.S. has remained relatively limited in scope. For example, total world fish production yields from aquaculture reached circa 14 million metric tons (mmt) in 1991, yet only 0.3 mmt was produced in the U.S, nearly three-quarters of which comprised freshwater organisms. Marine aquaculture in the U.S. is dominated by oyster culture, followed, in order of volume and market value, by clams, mussels, salmon, and shrimp. Another perspective is that the U.S. trade deficit in edible fish products was \$2.2 billion in 1992 (\$2.8 billion, if non-food fishery products, such as algal-derived polysaccharides and chemicals, are included; National Marine Fisheries Service, 1993), representing a significant

negative influence on the trade balance for the U.S. There is no question that expansion of marine aquaculture in the U.S. could contribute greatly to reducing the U.S. trade deficit, as well as meet the growing demand for fresh seafood. Furthermore, as is shown below, aquaculture of seaweed and phytoplankton can yield high-value products, especially algal-derived polysaccharides and chemicals that comprise a significant fraction of the fisheries contribution to the trade deficit.

The marine aquaculture industry faces many problems that must be solved before it can achieve significant growth as an industry in the U.S. Non-biotechnology problem areas include the following. Further research is needed to advance the husbandry of important fish and shellfish species, and conventional breeding techniques could improve many of these species. Federal and state regulatory and funding frameworks must be changed if growth of the industry is to be encouraged. Resolution of a number of policy issues at the federal level would also assist the industry enormously (National Research Council, 1992). There are, however, contributions from marine biotechnology that could make the aquaculture industry far more productive than it is at the present time. Key are genetic manipulation of marine organisms of commercial importance and improved knowledge of molecular and biochemical processes of marine animal and plant species. Both would allow for better utilization of the biological resources of the sea. A good example is the progress being made in regulation of the hormonal control of reproduction of finfish and shellfish in fish farming. Another example of a significant potential contribution that marine biotechnology can make to aquaculture is the diagnosis and treatment of diseases of marine organisms grown in aquaculture facilities, an area of research that awaits the full attention that it deserves.

Most of the contributions of biotechnology to aquaculture benefit both freshwater and marine aquaculture. In fact, the distinction between the two is rather artificial, since some species have both freshwater and marine stages in their life cycles. In general, the following discussion applies to marine organisms, but some fundamental advances of relevance for marine aquaculture have been made in freshwater organisms.

Genetic Manipulation of Marine Organisms

While we recognize that selective breeding of fish historically has played an important role in the aquaculture industry (Hershberger, 1990) during the past decade, direct genetic manipulation of fish, using recombinant DNA techniques has revolutionized the aquaculture industry and made it possible to develop an entirely new approach to fish farming. Fish are highly amenable to genetic manipulation, because fish eggs are characteristically large and, therefore, can be micro-injected with DNA constructs, after which external fertilization and subsequent development will take place. Major research efforts in genetic manipulation have been directed at enhancement of growth and production of fish with superior resistance to cold temperatures. Also, developing disease-resistant fish is an increasingly important objective, since intensive mariculture is becoming more common worldwide.

Growth enhancement in animals induced by the introduction of foreign growth hormone (GH) genes was first demonstrated a decade ago, using mice. Mice eggs micro-injected with rat GH gene constructs yielded larger animals (Palmiter et al., 1982). This principle of growth enhancement was subsequently employed in fish species of importance to aquaculture. The first successful growth hormone experiments using fish was the transfer into goldfish of the human GH gene fused to a mouse metallothionein promoter (Zhu et al., 1985), yielding transgenic offspring significantly larger than the untreated, control fish (Zhu, 1992). Since then, several vertebrate genes have been fused to a variety of promoters and introduced into fish species. Examples include the expression of a mouse metallothionein-human growth hormone fusion gene in Atlantic salmon (Rokkones et al., 1989) and bovine growth hormone (expressed from the Rous sarcoma virus long terminal repeat and carp β -actin gene promoters) in walleye salmon (Moav et al., 1992).

Of more practical significance has been the achievement of growth enhancement using fish GH. T. Chen, at the Center of Marine Biotechnology, University of Maryland Biotechnology Institute (UMBI/COMB) and D. Powers, then at the Johns Hopkins University and a joint faculty member of the UMBI/COMB, demonstrat-

ed that growth hormone in rainbow trout is encoded by two separate genes (Agellon et al., 1988a). A large amount of biologically active rainbow trout GH was prepared by expressing one of the rainbow trout GH genes in the bacterium *Escherichia coli*. This hormone was administered to rainbow trout by injection or dipping, yielding enhanced growth of the trout (Agellon et al., 1988b). Obviously, exogenous GH application is not very practical for large-scale aquaculture, since it is labor-intensive and requires individual treatment of each fish. A better approach is the generation of transgenic fish. This was first achieved by the transfer of a rainbow trout GH gene to common carp and channel catfish (Zhang et al., 1990). However, a non-fish gene element was included in this construct since the GH gene was fused to the long terminal repeat of Rous sarcoma virus.

“All-fish” gene constructs have also been used for growth enhancement of fish. P. Hackett and colleagues at the University of Minnesota developed expression vectors containing the proximal promoter and enhancer regulatory elements of the carp- β actin gene and the polyadenylation signal from the salmon growth hormone gene (Liu et al., 1990). Growth enhancement was subsequently obtained in Atlantic salmon using an all-fish gene construct. The construct was an antifreeze protein gene promoter linked to a chinook salmon GH gene. These transgenic Atlantic salmon demonstrated enhanced growth (Du et al., 1992).

Inheritance and expression of GH genes in transgenic fish are complex genetic and metabolic processes. Important considerations in successful manipulation of these processes include tissue specificity and developmental stage specificity of the transcriptional control elements (Moav et al., 1992), as well as stable inheritance and expression of the GH genes in the offspring. The fast-growing, transgenic fish, however, cannot yet be used in commercial aquaculture until further progress is made in research on these animals. For example, the physiological, nutritional, and environmental factors that maximize performance of individual transgenic fish must be determined. Also, safety and environmental impact issues must be resolved before the large scale, commercial production of transgenic fish will be permitted by regulatory agencies (Chen and Powers, 1990).

Many marine fish inhabiting cold waters produce proteins which act as an "antifreeze," i.e., protect fish by inhibiting the formation of ice crystals in their serum. These proteins are termed antifreeze glycoproteins and antifreeze polyproteins/polypeptides. Atlantic salmon lack genes coding for these proteins and, therefore, cannot survive in icy waters (Hew et al., 1991). The mechanism whereby antifreeze proteins bind ice crystals and inhibit ice formation has been described (Raymond et al., 1989). Genes coding for antifreeze proteins in Arctic flounders have been transferred, expressed, and inherited in Atlantic salmon (Shears et al., 1991). Expression of an adequate concentration of antifreeze proteins in salmon blood would extend the geographical range within which this fish can be cultured. Interestingly, antifreeze proteins from fish may also prove valuable for hypothermic preservation of mammalian organs, especially for transplant operations (Lee et al., 1992b).

Not only finfish, but also shellfish are amenable to genetic manipulation, especially for enhancing both the rate of growth and size of the adult. The exogenous application of bovine GH enhanced the growth rates of California red abalone (Morse, 1984). Similar results were reported when biosynthetic rainbow trout GH was applied to juvenile oysters (Paynter and Chen, 1991).

In contrast to finfish and shellfish, arthropods, such as lobsters, shed their exoskeletons during growth, in a molting process which is under hormonal control. Thus, endocrine regulation of molting may improve growth efficiency in the lobster. The endocrine regulation of molting in Crustacea has been reviewed by Chang (1989). However, knowledge of the molecular genetics of marine crustaceans (shrimp, lobsters, prawns, etc.) must be elucidated before commercial production of these species will be totally efficient and reliable, i.e., growth, development, and disease resistance have yet to be controlled, and, unfortunately, none has yet been fully achieved.

Hormonal Control of Reproduction

Successful exploitation of a fish or shellfish species in aquaculture requires the ability to obtain consistent, controlled reproduction

as economically as possible. Many fish species demonstrate poor ovulation, and spawning in captivity. Manipulation of water temperature and photoperiod has been used with some success in attempts to improve spawning. Fortunately, important advances have been made recently in the use of hormone treatment to control reproduction of fish species that are important in aquaculture.

Spawning in fish is initiated by a surge of gonadotropin (GtH) secretion from the pituitary. There is growing evidence that this surge is frequently absent in fish raised in captivity (Zohar, 1988a). An important factor controlling induction of a GtH ovulatory surge is gonadotropin releasing hormone (GnRH). Administration of GnRH, or its analogues, offers, therefore, an efficient method for control of ovulation and spawning. Analogues of GnRH have been synthesized, which have been found to possess increased resistance to degradation (Zohar et al., 1990b), as well as possessing higher affinity to pituitary receptors (Pagelson and Zohar, 1992). These analogues have subsequently proven to be superactive in the induction of spawning. Also, in some fish species, the efficiency of induction of spawning by GnRH can be enhanced by the use of dopamine antagonists, which counteract the inhibitory effect of dopamine on GtH release. These interactions have been explained in more detail by Zohar (1989).

As a further development, based on these findings, controlled release delivery systems for the hormones show potential for inducing and synchronizing spawning in several fish species important in aquaculture, including Atlantic salmon (Crim and Glebe, 1984), trout (Breton et al., 1990), and seabream (Zohar, 1988b). Novel approaches have been developed by utilizing advanced polymer technology to produce implants that slowly dissolve and release hormones at a steady rate. Manipulation of spawning in farmed fish has been achieved by sustained administration of GnRH analogs via polymer-based delivery systems (Zohar et al., 1990). Low intensity ultrasound has been shown to enhance dramatically the uptake of test peptides into the circulation of fish blood and offers an alternative approach to the use of polymer-based delivery systems. The use

of low intensity ultrasound has tremendous potential for improved hormone and drug delivery in aquaculture (Zohar et al., 1990a) because it is non labor-intensive and minimizes handling of the treated fish.

There is no doubt that molecular genetic information, when obtained, will elucidate the mechanism of failure of some fish species to spawn in captivity. Deficient expression of the GnRH gene or lack of GnRH secretion appear to be the best explanations for failure of spawning, at the present time. Understanding the regulation of GnRH at the molecular level may suggest new approaches to obtain reliable spawning. Clearly, by understanding fish endocrinology at the molecular level and applying this new information to the control of fish reproduction, the aquaculture farmer will employ knowledge derived from molecular genetics increasingly in the industry.

Great benefits in aquaculture can be achieved by the production of monosex, or sterile, populations of fish. For reasons not yet understood, in some fish species one sex grows faster or is more disease resistant. Furthermore, sterile populations usually achieve better growth rates, as no energy is diverted to gamete production. Monosex populations can be obtained by steroid treatment or manipulation of the set of chromosomes, both methods having proved useful in the production of sterile fish. This subject has been discussed in detail by Zohar (1989) and Yaron and Zohar (1993).

Triploid Pacific oysters (*Crassostrea gigas*) produce greatly reduced numbers of gametes, compared to diploids. Triploid oysters contain an extra copy of chromosomes. The successful production of triploid oysters has resulted in a superior product during summer months. At this time of the year, diploid oysters become sexually mature, form soft reproductive tissue throughout the body and deplete energy rich glycogen stores, which results in a less tasty product (Allen, 1988). Conditions for production of triploid oysters by treatment with the chemical cytochalasin B have been optimized (Downing and Allen, 1987). At the present time, triploid oyster production has grown rapidly and represents over 50% of the total oyster production of hatcheries in the Pacific Northwest (Allen, 1988).

The success of the triploid Pacific oyster in aquaculture is a dramatic illustration of the commercial potential of manipulation of reproduction in invertebrates.

Endocrine regulation of reproduction may have applications in aquaculture of certain invertebrates, although these applications, in contrast to the situation with finfish, are not yet at a practical stage of use. Hormone treatment surely will be useful in shrimp culture, since these invertebrates do not become reproductively mature when grown in aquaculture ponds. In fact, reproduction of shrimp must be stimulated by manual ablation of the eyestalk, which has negative effects on the animal, such as increased susceptibility to disease. Purportedly, the purpose of eyestalk ablation is that it removes a gonad-inhibiting neurohormone (GIH) produced by the neurosecretory complex located in the eyestalk. By elucidating the structure and function of GIH, it should be possible to devise strategies to counter the inhibitory effects of GIH by peptide biotechnology, ensuring efficient shrimp reproduction thus without eyestalk ablation (Keeley, 1991).

Algal Aquaculture

Aquaculture of marine macroalgae, i.e., seaweeds, has been practiced for several centuries in Asian countries, particularly Japan, and products from these algae have been widely used as medicines and food. Microalgae culture is practiced in Australia, Israel, and the U.S. Macro- and microalgae yield a wide range of products, including food additives and supplements, culture media, pesticides, plant growth regulators, and antibacterial, anti-cancer and antiviral agents (Harvey, 1988). Some of the bioactive compounds isolated from marine algae are discussed on page 25.

Microalgae have proven useful for large-scale production of the long chain fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kyle et al., 1991). Diets rich in these omega-3 oils have been suggested to reduce the risk of coronary vascular disease. The green microalga, *Dunaliella salina*, is grown in large-scale, intensive culture in California and Australia to produce

beta-carotene (Kranzfelder, 1991), a vitamin A precursor associated with the prevention of cancer. *Dunaliella* cells can accumulate β -carotene to up to ten percent of their dry weight and are therefore an excellent source of this substance (Avron and Ben-Amotz, 1992). It has even been suggested that "oceanic farming" of marine algae can reduce global carbon dioxide levels (North, 1991) by increasing rates of carbon dioxide fixation into organic material.

Application of biotechnology to cultivation of marine algae presents an opportunity for riparian countries, especially developing countries, with extensive coastal regions. This potential is most likely to be realized by the formation of partnerships with industrialized countries. However, to achieve success, understanding of the molecular genetics and application of the techniques of modern molecular biology will be required (Singleton and Kramer, 1988). Although molecular techniques have not yet been widely applied to achieve strain enhancement or production of transgenic plants and algae of commercial importance, this approach is being taken in several laboratories in the U.S., Asia, and Europe. Most of this work employs protoplast fusion. However, protoplast fusion has been somewhat limited in application to seaweeds because of difficulties in obtaining plant regeneration from protoplasts of complex algae. Successful genetic manipulation, using protoplast fusion, has been achieved in a few cases. For example, protoplast fusion has proven to be a useful tool in production of red algae hybrids, specifically the commercially valuable, agar-producing seaweed *Gracilaria* (Cheney, 1990). Successful protoplast fusions and regeneration have also been reported for *Porphyra perforata* (Polne-Fuller and Gibor, 1984) and *Porphyra nereocystis* (Waaland et al., 1990); these are important achievements since the edible product, nori, is derived from *Porphyra* species. Direct DNA manipulation, using vectors for gene transfer or techniques such as electroporation or biolistics, have not yet been employed successfully in the genetic studies of the macroalgae. Development of these methods for macroalgae depends on advances in the molecular genetics of algae and algal aquaculture.

A curious twist in the short history of marine biotechnology is that several products of marine macroalgae are essential in many of

the techniques used in biotechnology. Notably, agar and its purified derivative, agarose, are mixtures of polysaccharides extracted from red seaweeds, particularly *Gracilaria*, *Gelidium*, and *Pterocladia* species. (Renn, 1990). Agar forms the basis of solid media used extensively in microbiology, whereas agarose, a thermoreversible, ion-independent gelling agent, is used to prepare gels for the electrophoretic separation of proteins and nucleic acids. Agarose is also widely used in immunological assays and cell culture. Algin (obtained from several species of brown algae), carrageenan (extracted from red algae), and agarose are all used for encapsulation and immobilization of cells of yeast or bacteria (Renn, 1990). An example of a commercial process based on encapsulation of cells in a cage of kappa-carrageenan is conversion of glucose to ethanol and production of L-aspartic and L-malic acids (Renn, 1990). Algal polysaccharide beads also can be used for bioconversions, either directly or after further modification (Guiseley, 1989).

MARINE ANIMAL HEALTH

Natural fish and shellfish populations, as well as marine mammals, are susceptible to viral, bacterial, fungal, and protozoan infections. Animals raised in intensive aquaculture are especially vulnerable to disease. Since massive use of antibacterials and/or antibiotics in aquaculture can be counterproductive, molecular techniques employed in marine biotechnology will have increasingly important application in the detection of infectious disease, elucidation of pathogenesis, development of preventative measures (such as vaccines), and treatment of disease, including epidemics and epizootics.

Studies of bacterial, viral, and fungal diseases of marine fish and shellfish have been carried out since the earliest days of microbiology. *Vibrio* diseases of fish have been a consistent problem in aquaculture. In the 1950s, it was found that several species of *Vibrio*, including *V. cholerae* (Colwell et al., 1981), that cause serious gastrointestinal disease and wound infections in humans (Fujino et al., 1951; Joseph et al., 1982) are autochthonous to estuarine and brackish water environments. Thus, there is both a human and veterinary interest in fish disease prevention and health.

Fish Diseases

Bacterial Diseases

Vibrios, most notoriously *Vibrio anguillarum*, are common bacterial fish pathogens and vibriosis occurs in both cultured and natural fish populations, including Pacific and Atlantic salmon, red sea bream and sea bass (Ezura et al., 1980). Other bacterial pathogens of fish include *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in salmon, *Aeromonas salmonicida*, which causes furunculosis in salmonids, and *Yersinia ruckerii*, the causative agent of enteric redmouth disease in rainbow trout and other salmonids. *Piscinickettsia salmonis*, a rickettsial pathogen of salmonids has recently been described (Fryer et al., 1991).

Fortunately, several very useful vaccines have been developed which confer immunity in fish to bacterial diseases. *Vibrio* vaccines are currently the most successful, although the constant presence of vibrios in seawater make vibriosis always a potential threat. Multivalent *Vibrio* vaccines effective against several *Vibrio* species will probably prove most effective in diseases caused by vibrios (Smith, 1988). Many of the vaccines in commercial use are killed vaccines, based on bacterial cultures inactivated with formalin. Molecular methods will be useful in future vaccine development, notably in identification of specific immunizing antigens which can then be produced on a large scale. Vaccines prepared using this approach should be less variable in reported efficacy than those based on whole killed cells.

A major contribution of marine biotechnology to disease control in aquaculture has been the use of molecular techniques for rapid, sensitive diagnosis of fish pathogens. Specific DNA probes have been developed for detection of *Aeromonas salmonicida* (Barry, 1990) and *Vibrio anguillarum* (J. L. Powell, University of Otago, New Zealand, personal communication). Monoclonal antibody detection systems have been used to detect several important bacterial pathogens, including *Yersinia ruckerii* (Austin et al., 1986) and *Renibacterium salmoninarum*, a bacterium difficult to detect by conventional culture techniques because of its slow growth rate (Arakawa et al., 1987).

Molecular approaches have been used to investigate mechanisms of pathogenesis in several bacterial diseases of fish. For example, the role of three proteins in iron uptake and virulence in *V. anguillarum* was conclusively demonstrated by the construction of mutant strains in which the genes coding for iron uptake proteins were disrupted. These attenuated strains, which persisted in fish, were nonpathogenic and may ultimately prove useful as live vaccines (Singer et al., 1991).

Viral Diseases

Important diseases of fish include those caused by infectious pancreatic necrosis virus (IPNV) and infectious hematopoietic necrosis virus (IHNV) that infect salmonids. Recombinant DNA technology has been used to construct viral subunit vaccines for IPNV (Manning and Leong, 1990) and IHNV (Gilmore et al., 1988). These vaccines have induced protective, long-lasting immunity in laboratory trials (Leong et al., 1991). Because there are no effective antiviral treatments available, unlike bacterial diseases where antibiotic treatment is frequently used with good effect in aquaculture, vaccines protective against viral diseases are badly needed. Killed viral vaccines have been of limited use in aquaculture because of the cost of vaccine production and lack of efficacy of the vaccines (Leong and Munn, 1991). Genetic manipulation is likely to be useful, therefore, not only in vaccine production but also to obtain disease-resistant and, especially, virus-resistant, fish.

A gene has been identified in rainbow trout which shows homology to the Mx1 gene, which confers resistance to influenza virus in mice (Staehli et al., 1989). This gene is inducible by double stranded RNA (IPNV and IHNV are double stranded RNA viruses) and, although its function in fish has not been determined, it is interesting to speculate that it might be used to create disease resistant fish (Leong et al., 1991).

Detection of viral disease, in fish as well as humans, relies mainly on molecular techniques. IPNV can be detected in immunoassays using a monoclonal antibody (Caswell-Reno et al., 1989). DNA

probes (Deering et al., 1991) and a PCR-based method (Arakawa et al., 1990) have also been developed for detection of IHNV.

Parasitic Diseases

Protozoa, mainly ciliates and flagellates, are common parasites of fish and are found on the skin and gill epithelium (Hoffman, 1968). They are serious pathogens in aquaculture. A useful model system for the study of dinoflagellates, which are common ectoparasites of marine fish, is the dinoflagellate, *Amyloodinium ocellatum*, which has been successfully propagated on a fish gill cell line (Noga, 1987).

A recently discovered "phantom" dinoflagellate has been identified as the causative agent of major fish kills in estuaries of the southeastern U.S. and may also be active in other geographic regions. This dinoflagellate requires live finfish or their fresh excreta for excystment. After excystment, it releases a potent neurotoxin that causes fish death. Within several hours of death, the dinoflagellate encysts and settles back into the sea-bottom sediment, to await favorable conditions, when the cycle begins anew (Burkholder et al., 1992). Molecular techniques will be useful in elucidation of the mechanisms of pathogenesis and development of host resistance to these and other fish parasites. Because so little is understood of the mechanisms of fish immunity to parasites, further research is necessary to assess the potential for vaccine development (Houghton et al., 1988).

Diseases of Marine Invertebrates

Bacterial, fungal, viral, and protozoan diseases also adversely affect both natural and cultured stocks of marine shellfish. Commercially important pathogens include the infectious hypodermal and hematopoietic necrosis (IHFN) virus which causes stunting and mortality in several species of shrimp (National Research Council, 1992) and the microsporidian protozoan, *Ameson michaelis*, which infects the blue crab, *Callinectes sapidus*, in a variety of habitats from Chesapeake Bay, throughout the Southeastern Atlantic, to Louisiana (Overstreet, 1978). Bacterial infections of invertebrates, as in finfish, are frequently caused by *Vibrio* species, *V. parahaemolyticus*, *V. algi-*

nolyticus and *V. anguillarum* are associated with crustacean septicemia and mortality. *V. parahaemolyticus* is present in wild stocks of blue crabs from the Chesapeake Bay and constitutes the most common *Vibrio* species isolated from diseased crabs (Colwell et al., 1972). Vibriosis also occurs in oysters and other commercially important mollusks, such as abalone. In the eastern American oyster, *Cassostrea virginica*, the European oyster, *Ostrea edulis*, and other bivalves such as the clam, *Mercentaria*, vibrios infect the conchiolin-containing ligament and the periostracum, preventing normal ligament function and shell deposition (Elston et al., 1982). Vibrios present in invertebrates can also present serious health risks for humans (see page 54).

The potentially serious impact of shellfish diseases is well illustrated by the example of oyster production from the Chesapeake Bay. Oyster production plummeted from a high of 2.5 million bushels harvested annually a decade ago to less than 1% of this level in the 1992/1993 season. Mass mortality due to protozoan infections was a major reason for this decline in populations of the eastern oyster *C. virginica*. The major disease was "Dermo," caused by the parasite, *Perkinsus marinus*, although MSX ("multinucleated sphere unknown") disease has also contributed to the decline in oyster populations. The disease caused by *Perkinsus* has now extended to areas of the James River that were sources of oyster seed stock. No natural resistance to this parasite has developed in natural oyster populations. *In vitro* continuous cultures of *P. marinus* in the absence of oyster cells have recently been established at the Center of Marine Biotechnology, University of Maryland Biotechnology Institute (Gauthier and Vasta, 1993), providing an abundant and reliable source of the parasites, which will be used to develop nucleic acid and monoclonal antibody probes. With the probes, molecular techniques can be employed for early detection of parasites in seed stocks of oysters. In addition, these continuous cultures will be useful in studying growth and regulation of all stages of the life cycle of the parasite and will assist in evaluation of anti-parasitic compounds (G. Vasta, personal communication).

The molecular mechanisms for defense against disease in invertebrates are not well understood. Lectins appear to be an important

part of the immunological system in invertebrates. Lectins from the blue crab, *C. sapidus*, have been shown to interact with certain serotypes of the pathogen, *V. parahaemolyticus* (Cassels et al., 1986). Serum lectins may act as opsonins in the crab internal defense mechanism (Lackie and Vasta, 1988; Yang and Yoshino, 1990). Lectins are present on the surface membrane of invertebrate hemocytes in the blue crab (Cassels et al., 1986) and the oyster, *C. virginica*, (Vasta et al., 1982; Vasta et al., 1984; Vasta, 1986). These lectins may function by binding to non-self substrates (such as bacteria) bearing specific glycosyl moieties. Molecular analysis of the interaction between invertebrate lectins and pathogenic bacteria will be useful in the elucidation of mechanisms of non-self recognition, a critical bit of information for the understanding of the pathobiology of invertebrates (Vasta, 1992).

As the demand for aquacultured seafood products increases, and the harvest of shellfish from the wild decreases, microbial diseases of shellfish will receive increasing attention and, perhaps, this area of research will then receive the research funding needed to solve the disease problems associated with aquaculture.

MARINE NATURAL PRODUCTS

Ironically, enzymes resulting from marine biotechnology have made possible some important new techniques in biotechnology, e.g. high-temperature-resistant polymerases, which are employed in the polymerase chain reaction. The polymerase chain reaction makes possible the selective amplification of DNA sequences of interest and this important new technique has many applications in molecular biology.

Bioactive Compounds From Marine Invertebrates

The marine environment is characterized by physical and chemical properties that are markedly different from those of the terrestrial environment. Furthermore, these properties comprise the complex ecosystems which include many sessile organisms. The production of bioactive chemicals is a common means of defence, espe-

cially in sessile organisms and in vulnerable soft-bodied organisms. Groups of organisms that have been found to produce bioactive natural products include marine bacteria, dinoflagellates, algae, coelenterates (namely the corals), echinoderms (such as sea cucumbers and starfish), bryozoans, sponges, soft-bodied mollusks (such as sea hares and nudibranchs), and tunicates. The chemical basis of some of the marine ecological interactions amongst invertebrates has been discussed by Scheuer (1990), who has also edited two comprehensive review volumes on organic chemicals of biological marine origin (Scheuer, 1987, 1988). Bioactive substances from marine organisms have been studied for several decades and thousands of these chemicals have been described. Recent discoveries of marine natural products with interesting biological and pharmaceutical properties have been the subject of a series of comprehensive reviews by Faulkner, dating to 1984 (Faulkner, 1984a, 1984b, 1986, 1987, 1988, 1990, 1992), the most recent of which (Faulkner, 1992) contains 438 references. The majority of the publications describe marine natural products of biological origin published in the previous year. It is, of course, beyond the scope of this review to cover this huge range of natural products that have been described in marine organisms. However, it is useful to list some of the major laboratories in the US that are involved in this important aspect of marine biotechnology and some examples of significant products that have emerged from the research. Approaches likely to yield significant results in the future are outlined.

Work in Faulkner's laboratory at Scripps Institution of Oceanography, La Jolla, CA has resulted in the discovery of numerous bioactive compounds from a wide range of organisms, including sponges (James et al., 1991; Kushlan and Faulkner, 1991; Stierle and Faulkner, 1991) and algae (Trimurtulu et al., 1992). Fenical and coworkers have also isolated a number of bioactive compounds, including anti-inflammatory and anti-viral agents, mainly from corals (Groweiss et al., 1988; Roussis et al., 1990) and antifungal disulfides from ascidians (Lindquist and Fenical, 1990).

A promising group of antitumor compounds, ecteinascidins, has been isolated from the Caribbean tunicate, *Ecteinascidia turbinata*, by

K. Rinehart and colleagues, University of Illinois-Urbana. Rinehart is one of the true pioneers in marine natural products R&D (Rinehart, 1988). One of these ecteinascidins, designated Et 729, is undergoing evaluation by the National Cancer Institute on the basis of having shown potent activity *in vivo* against a variety of tumors in mice (Sakai et al., 1992); another form, designated Et 743, has demonstrated "very potent" activity in melanoma and breast cell lines. The latter compound has been licensed to PharmaMar, S.A., Spain (Anonymous, 1994).

Sponges have proven to be an important source of bioactive compounds. Dysirin-type sesquiterpenes with antihelminthic activity (Horton et al., 1990) and several cytotoxic heterocycles (Quinoa et al., 1986) are among the compounds isolated by researchers at the University of California, Santa Cruz. Researchers in Scheuer's laboratory at the University of Hawaii in Manoa have isolated a number of cytotoxic compounds from marine sponges (Akee et al., 1990; Carroll and Scheuer, 1990). The Harbor Branch Oceanographic Institution, Ft. Pierce, FL, team of researchers has isolated many bioactive compounds from sponges, including an antitumor compound (Sakai et al., 1986) and many cytotoxic and antifungal substances (Wright et al., 1987a, 1987b; Gunasekera et al., 1990a, 1990b; Wright & McCarthy, 1994).

The wide range of bioactive compounds produced by marine microorganisms emphasizes the great potential of compounds for biomedical applications, which has encouraged further large-scale systematic screening of marine organisms. For example, the National Cancer Institute (NCI), U.S. Public Health Service, has established a screening system consisting of 60 *in vitro* cell lines representing seven cancer sites: blood cells; brain; colon; kidney; lung; ovary; and skin (Ansley, 1990). Extracts from many marine organisms are tested for their cytotoxic activity and additional tests are performed to detect anti-HIV activity, using a human lymphoblastic cell line infected with the AIDS virus. Some pharmaceutical companies also screen marine isolates for anti-inflammatory, insecticidal, and herbicidal activities, in addition to cytotoxic and anti-viral screening (Cardellina, 1986).

Bioactive Compounds From Marine Algae

Marine algae have been a major subject of investigation by Gerwick's group at Oregon State University, Corvallis, OR. A mammalian insulin release modulator (Moghaddam et al., 1990) and a potent mammalian immunohormone (Bernart and Gerwick, 1988) are among the biomedically important compounds isolated from these algae. Red marine algae have been shown to be a rich source of eicosanoid-type natural products. They produce several expensive and rare biochemicals that have previously been isolated only from mammalian sources (Gerwick et al., 1990).

Researchers at the Harbor Branch Oceanographic Institution, Ft. Pierce, FL have isolated a compound from the marine alga, *Halimeda tuna*, that shows antiviral activity (Kochm et al., 1991). However, the potential of marine microalgae has not yet even begun to be exploited and, therefore, a major search program is clearly warranted.

Bioactive Compounds From Marine Bacteria

Marine bacteria have enormous potential for the production of bioactive compounds and pharmaceuticals, but have been relatively little investigated. William Fenical and his group at Scripps Institution of Oceanography are perhaps the most active and successful U.S. researchers studying bioactive metabolites in marine bacteria. For example, they have isolated from deep-sea bacterium a series of novel cytotoxic and antiviral macrolides, the macrolactins and have produced them by bacterial fermentation (Gustafson, et al, 1989). Also, Fenical working with others isolated antibiotics with selective activity against Gram positive bacteria from a *Streptomyces* species obtained from the surface of a jellyfish (Trischman, et al, 1994).

It is possible that many of the compounds isolated from marine organisms, such as sponges, are produced by bacteria associated with those sponges. For example, several diketopiperazines previously ascribed to the sponge, *Tedania ignis*, have been shown to be produced by a marine *Micrococcus* sp. associated with this sponge (Stierle et al., 1988). It has also been observed that secondary metabolites from certain mollusks, sponges, and tunicates closely resemble natural

roducts from cyanobacteria, formerly taxonomically described as blue-green algae." In mollusks, these metabolites are generally derived from ingestion of cyanobacteria, whereas in sponges and tunicates these products are apparently produced by symbiotic cyanobacteria (Moore, 1991). One example of the circumstantial evidence that indicates a cyanobacterial origin of certain natural products from marine animals is the similarity in structure of scytophilin C (Shibashi et al., 1986), isolated from cyanobacteria, to swinholide A (Kitagawa et al., 1990), an antifungal compound from the sponge, *Theonella swinhoei* (Moore, 1991).

Isolation of marine bacteria, particularly from sponges, that are subsequently screened for production of bioactive substances is in progress at the Center of Marine Biotechnology, University of Maryland Biotechnology Institute, in Baltimore, MD. There are several practical advantages in the use of bacteria as sources of natural products. Many bacteria can be readily grown in fermenters under controlled conditions, providing consistent yield of compounds of interest. Marine invertebrates, on the other hand, need to be collected from natural ecosystems where they may be inaccessible or present only in low numbers. Furthermore, large scale collection of invertebrates for natural product production may threaten endangered populations. Alternatively, specialized conditions can be established to grow invertebrates in captivity, but it has taken literally years to get some invertebrates into productive culture. Of course, the possibility exists for cloning genes from invertebrates into bacteria for production of the described natural product. However, the molecular genetics of invertebrates is poorly understood, as cited previously (*vide supra*).

Production of compounds by bacteria can be enhanced, often by several orders of magnitude, by optimization of fermentation conditions and by selection of high-producing mutants. In addition, genetic manipulation of bacteria is relatively easy, and genes of interest can be cloned into expression vectors and transferred to bacterial species that are well-adapted for production of compounds by fermentation processes. For these reasons, it is advantageous to investigate whether natural products ascribed to marine invertebrates may,

in fact, be produced by bacteria associated with the invertebrates and to investigate specific species of marine bacteria, in particular those bacteria closely associated with invertebrates, e.g., symbionts, as potential sources of natural products. In some cases, bacteria may be present in extracellular associations and readily cultured, for example as found with isolates from the Caribbean sclerosponge, *Ceratoporella nicholsoni* (Santavy et al., 1990), some of which produced compounds with antibacterial and antineoplastic activity (Colwell et al., 1989).

There is a range of interdependence between bacterium and host and there is evidence that some bacterial-invertebrate symbioses may date from the Precambrian era (Wilkinson, 1984). In cases where true symbiotic relationships exist between host and bacterium, it may be extremely difficult or impossible to isolate and maintain the bacterium in pure culture. Molecular approaches are very useful in such cases. For example, luminescent symbionts of some marine fish have not yet been isolated into pure culture but have, instead, been characterized by 16S ribosomal RNA (rRNA) (Haygood and Distel, 1993).

Total DNA can be extracted from the invertebrate (and its resident microbial population) and a composite gene library of the total DNA can be produced in an appropriate host. The genes coding for useful products can be isolated from the composite library by using appropriate screening procedures. This "genetic fishing" procedure obviates the need for prior determination of the cellular source of the products of interest and has the potential of facilitating rapid, large-scale production of marine natural products.

Actinomycetes in the marine environment are a group of gram-positive bacteria that have been little investigated but which are known to be metabolically versatile and to produce many bioactive compounds, including antibiotics. Terrestrial actinomycetes produce over two-thirds of naturally-occurring antibiotics, including many of medical importance (Okami and Hotta, 1988). Although not commonly regarded as an important microbial group in marine ecosystems, in which gram-negative bacteria are believed to dominate the microbial populations, several recent reports indicate that marine environments are an important new source of actinomycetes. One of

the primary innovators in the search for new metabolites in marine actinomycetes is Y. Okami's group at the Institute of Microbial Chemistry in Tokyo. For example, they isolated new aminoglycoside antibiotics, istamycins A and B, from culture broths of marine actinomycetes Okami, et al. (1979). A coryneform or actinomycete-like group was isolated from the Caribbean sclerosponge *Ceratoporella nicholsomi* (Santavy et al., 1990). Scheuer (1990) raises the interesting possibility that a series of isoquinolenequinones, among them the antibiotic nimosamycin, isolated during an investigation of a nudibranch predator and its sponge prey and found independently in a sponge, *Reniera* sp., might be produced by a *Streptomyces* sp. Jensen et al. (1991) reported that actinomycetes are widespread in tropical marine sediments.

Workers at COMB, UMBI, have developed an efficient method for isolation of actinomycetes from marine samples and demonstrated that a wide range of unusual actinomycetes, different from those typically found in terrestrial samples, are present in sediments from the Chesapeake Bay (Takizawa et al., 1993). Intensive isolation and screening of actinomycetes from marine environments is warranted, in view of the enormous range of important compounds that have been isolated from terrestrial actinomycetes during the previous several decades.

Marine Toxins

Marine toxins may be defined as marine natural products that have specific pharmacological activities resulting in adverse effects in animals, generally at very low concentrations. Many marine toxins are produced by dinoflagellates and may be retained or concentrated through several trophic levels before exerting adverse effects on predators higher in the food chain (including man). Examples of toxins from dinoflagellates capable of causing fatal poisoning in man are ciguatoxin and saxitoxin. Ciguatoxin is a sodium channel agonist and is generally considered to be produced by dinoflagellates associated with coral reefs (Swift and Swift, 1993). There are, however, indications that ciguatoxin may be produced by bacteria, including the

cyanobacterium *Oscillatoria erythraea* (Hahn and Capra, 1992).

An important marine toxin found in many marine animals is the potent sodium channel blocker, tetrodotoxin, also known as puffer fish toxin. However, this toxin also has been found in a wide range of marine bacteria (Yasumoto et al., 1986; Simidu et al., 1987). The presence of this neurotoxin in many distantly related animal genera therefore may indicate production of the toxin by bacteria associated with these animals. Kogure and colleagues have discovered tetrodotoxin to be produced by sediment bacteria and burrowing animals (Kogure et al., 1988). A variety of bacteria, including *Vibrio* species, have been shown to produce tetrodotoxin (Simidu et al., 1987). The production of tetrodotoxin by *Vibrio cholerae* was reported by Tamplin et al. (1987). Molecular approaches will be useful in understanding toxin production and in devising methods for detection of toxins, such as the method recently described for the detection of tetrodotoxin (Raybould et al., 1992).

Soft corals of the genus *Palythoa* contain the marine toxin, palytoxin, that may have application in treatment of cancer. A palytoxin prodrug was shown to be activated by a monoclonal antibody-penicillin G amidase conjugate to give a thousand-fold increase in toxicity to carcinoma and lymphoma cell lines (Bignami et al., 1992a). A sensitive monoclonal antibody-based immunoassay has been developed for the measurement of palytoxin in biological samples (Bignami et al., 1992b).

Toxins are of interest in the context of marine natural products because they may have useful medical applications, if appropriate dosages and delivery systems can be devised. They also have application as research tools, particularly in studies on neuromuscular systems (Colwell, 1983). It has been speculated that toxins from a single genus of predatory cone snails may prove to have pharmaceutical potential comparable to that of plant alkaloids or the fermentation products of microorganisms. *Conus* species (approximately 500 in number) produce a vast range of pharmacologically active, small peptides, the targets of which include calcium channels, sodium channels, N-methyl-D-aspartate receptors, acetylcholine receptors, and vasopressin receptors (Olivera et al., 1990).

The study of marine toxins promises to be a particularly productive area of marine biotechnology, as evidenced by the more than a thousand compounds described in the literature during the past decade. More recently, intensive screening of marine natural products for bioactivity has been initiated by commercial laboratories. With more methodical and expanding screening programs now underway, the range and scope of bioactivity of marine natural products will yield a plethora of new compounds of biomedical interest, as well as provide a better understanding of organism-organism and organism-environment interactions in the sea.

Cell Culture and Marine Natural Products

Production of natural products, from marine invertebrates in particular, is frequently limited by ecological factors. Natural products are often present in trace concentrations and the harvesting of a large number of marine invertebrates may be necessary to produce sufficient quantities of natural products for therapeutic use or even for characterization of their molecular structure to enable organic synthesis. An attractive possibility to circumvent this barrier is the production of natural products by cell cultures derived from invertebrates which synthesize the important product, i.e., the product of interest.

Work to develop cell culture of marine invertebrates lags seriously behind that of other animal species, even finfish, for which many cell lines have been established. There are no established marine invertebrate cell lines available to date, although primary cultures of several mollusks, crustaceans, and echinoderms have been reported (Pomponi, 1991). For example, the culture of unspecified oyster cells (Perkins and Menzel, 1964) and cardiac tissue cells (Li et al., 1966) were described as early as the 1960s and the *in vitro* culture of presumptive nervous tissue from the oyster has recently been reported (Kleinschuster and Swink, 1992). There remains a large gap in developing cell lines of invertebrates, especially crustaceans.

Thus, it is not surprising that there are no reports of the production of natural products from cell cultures of marine inverte-

brates, given the very limited success of cell culture in these animals. Efforts to develop marine invertebrate cell culture techniques for large-scale production of bioactive compounds should be directed to specific groups of organisms, such as the sponges, bryozoans, and ascidians, which most frequently yield compounds of substantial pharmaceutical interest (Pomponi, 1991). Further, cell culture of terrestrial plants is well established, but marine plants are essentially uncharted territory, with respect to tissue culture.

Enzymes From Marine Organisms

Enzymes from marine bacteria are important in biotechnology because they are likely to be salt-resistant, a characteristic which is often advantageous in industrial processes. Proteases, i.e., enzymes which digest proteins, are of particular importance (Kalisz, 1988) and have application in detergents and as components of membrane cleaning formulations (Marshall et al., 1991). *Vibrio* species have been found to produce a variety of proteases. The marine bacterium, *Vibrio alginolyticus*, produces six proteases, including an unusual detergent-resistant, alkaline serine exoprotease (Deane et al., 1987). This bacterium also produces collagenase (Reid et al., 1980), an enzyme with a variety of industrial and commercial applications.

An unusual group of marine microorganisms from which enzymes have been isolated are the hyperthermophilic archaea isolated from hot water seeps and hydrothermal vents. The Archaea form one of the three domains of organisms defined by Woese et al. (Woese et al., 1990) (the other two domains are Bacteria and Eucarya) and are typically found in extreme environments (see page 50). Hyperthermophilic archaea grow at temperatures over 100°C and, therefore, require enzyme systems that are stable at high temperatures.

Thermostable enzymes offer significant advantages in industrial processes. Furthermore, thermostable DNA-modifying enzymes, such as polymerases, ligases, and restriction endonucleases, have important applications in molecular biology. The use of thermostable DNA polymerases in the polymerase chain reaction (PCR) (Saiki et al., 1988), a powerful technique in which a specific DNA sequence

of interest from complex mixtures of nucleic acids is selectively amplified, has been instrumental in major breakthroughs in diagnostic kit development and in detecting genes associated with inborn errors of metabolism. A thermostable DNA polymerase from the hyperthermophilic archaea, *Pyrococcus furiosus*, possesses both polymerase and error-correcting capabilities, giving the advantage of high fidelity PCR products (Lundberg et al., 1991).

A recent development is the emergence of the ligase chain reaction (LCR), a new technique for detection of mutations in DNA (Murray, 1989). The ligase chain reaction employs a thermostable DNA ligase to detect, amplify, and distinguish specific DNA sequences. Thus, thermostable DNA ligases will have significant, new applications in the future.

Metabolic enzymes isolated from thermophilic bacteria and archaea typically are markedly thermostable, as mentioned above. The optimal temperature for enzyme activity of glutamate dehydrogenase, a key enzyme in nitrogen metabolism, originally isolated from *P. furiosus*, is 95°C, with a half-life of more than 3.5 hours at 100°C (Klump et al., 1992). The glutamate dehydrogenase and glutamine synthetase (also important in nitrogen metabolism) enzymes from *P. furiosus* represent the most thermostable versions of these enzymes described to date (Robb et al., 1992). Similarly, the majority of enzymes involved in the primary metabolic pathways of *P. furiosus* and the most thermophilic bacterium, *Thermotoga maritima*, are dramatically more thermostable than their counterparts from mesophilic organisms (Adams et al., 1992). Expanded study of the characteristics of enzymes from thermophilic marine microorganisms will contribute to the understanding of mechanisms of enzyme thermostability and should enable directed modification of industrially important enzymes to enhance thermostability. Alternatively, from these microorganisms the isolation of enzymes suitable for particular industrial applications will be a productive path of research, as well.

Abundant Marine Natural Products

There are several marine products that are available in very large quantities that may increase in importance as valuable resources

through the applications of marine biotechnology. Three of these, chitin, polysaccharides, and kelp offer good examples and are considered in detail here.

Millions of pounds of chitin are generated annually as waste products by the seafood industry. This abundant biopolymer and its byproducts, chitosan and N-acetylglucosamine, can be useful in several industrial and medical applications. Chitin has made it to market in a variety of forms, i.e., as poultices to heal wounds, extenders, emulsifiers for photography. Applications of chitin products include paper additives (Muzzarelli, 1986), pharmaceuticals (Nagai et al., 1984), and absorbable sutures (Nakajima et al., 1984). Demonstrating its wide scope of applications, when added to soil chitin has been found to inhibit the growth of fungi pathogenic to plants (Mitchell and Alexander, 1962). Further, the marine polymer chitosan, which can be derived from crustacean shells, is effective in protecting certain crops against pathogenic fungi and other disease-causing pathogens by activating disease-resistance genes (Hadwiger et al., 1984). Thus, chitin and chitosan may have applications beneficial in agriculture.

Current mechanical and chemical methods for isolation of chitin from shellfish wastes are relatively harsh and inefficient. Enzymatic methods would be preferable for the purification of chitin and production of chitin byproducts. Many bacteria, including marine bacterial species of the genera *Vibrio* (Wortman et al., 1986) and *Streptomyces* (Pisano et al., 1992), produce chitinases which may be useful in this regard. Chemotaxis of the marine bacterium, *Vibrio furnissii* (Bassler et al., 1991a), to chitin oligosaccharides and degradation of chitin oligosaccharides by this bacterium have been demonstrated (Bassler et al., 1991b), indicating that this bacterium also has potential for chitinase production.

If and when the genes for chitin synthesis are cloned, production of this compound will increase immeasurably since a stable source will then be available. Marine biotechnology has an essential role to play in the discovery and large-scale production of many other marine natural products like chitin. Cloning of chitinase enzymes from marine bacteria (Wortman et al., 1986) is one approach that

can be followed to develop enzymatic systems for production of useful chitin derivatives.

Many marine bacteria produce abundant polysaccharides, which may have commercial application as viscosity-increasing agents, gelling agents, or adhesives (Colwell et al., 1985). Another marine source of polysaccharides is seaweed. Polysaccharides from seaweeds are important economically and are widely used in industry. For example, carrageenin from the red seaweeds is an extender in foods and related products, ranging from toothpaste to evaporated milk (Witt, 1985). Marine algae and seaweeds have many commercial applications and are discussed on page 15.

One aspect appropriately discussed here is the use of kelp as a source of methane. The Giant Pacific kelp, *Macrocystis pyrifera*, is particularly suitable as a substrate in view of its high growth rate, high ratio of readily degradable organic compounds in its composition, and the soluble nature of its major organic constituents. A marine methanogenic consortium of bacteria capable of methane production from kelp was characterized by Sowers and Ferry (1984). Methanogenesis from biomass offers several advantages. Notably, up to 90% of biomass energy may be recovered as methane. The low solubility of methane facilitates collection and the methane is a readily usable and transportable fuel (Sowers and Ferry, 1984).

BIOFILMS AND BIOADHESION IN THE MARINE ENVIRONMENT

Biofilms rapidly form on clean surfaces in seawater. The components of these films are, initially, organic molecules, followed by bacterial attachment and, subsequently, other microorganisms and larger animals, such as oyster larvae and barnacles. The study of biofilms has been directed at devising strategies to control biofouling of surfaces, understanding mechanisms of bioadhesion, and investigating ecological relationships within biofilms and between biofilms and organisms in the surrounding water.

The formation of biofilms on hulls of ships leads to biofouling, which greatly reduces fuel efficiency. It has been estimated that a 200

μm -thick layer on a ship's hull can decrease speed by 20% (Curtin, 1985), with a concomitant reduction in fuel efficiency. Application of anti-fouling paint to the hulls of ships decreases biofilm formation, but these paints frequently contain toxic compounds, such as heavy metals, including tributyltin, which are toxic and pollute the marine environment. The anti-fouling agents also inhibit larval development (Calabrese et al., 1973; Boyden et al., 1975; Calabrese et al., 1977). Copper and zinc were shown to accumulate in microbial biofilms and inhibit oyster set even at sublethal water column concentrations of these metals (Chang et al., 1993). Research on factors controlling formation of the initial film by marine bacteria on surfaces should lead to the development of non-toxic methods for prevention of biofouling. Possibilities include use of alternative materials to inhibit biofilm formation and "biological control" of biofouling, using thin bacterial biofilms which resist further colonization. New types of "non-stick" paints depend for their effectiveness on the inability of organisms to adhere strongly to them, rather than preventing all fouling (Cooksey and Wigglesworth-Cooksey, 1992). The physiological activities of biofilm bacteria frequently differ from those of freely suspended cells. Improved understanding of the underlying mechanisms of biofilm metabolism will be important in dealing effectively with biofilms (Fletcher, 1992).

Biofilms have been implicated in corrosion processes and control of the biological processes involved in formation of biofilms should, therefore, be important in preventing metal corrosion in seawater. Microorganisms are hypothesized to be involved in hydrogen embrittlement, which results in loss of ductility and tensile strength of susceptible metals. The generation of sufficient hydrogen to affect susceptible metals has been demonstrated with bacterial films prepared using pure cultures (Walsh et al., 1989a). However, the relative importance of these laboratory findings with complex biofilms in natural marine environments is not yet clear. Bacterial biofilms have been implicated as factors in corrosion of copper and nickel in seawater (Little et al., 1990). Corrosion of bare steel in seawater was increased two-fold by a mixed culture of marine bacteria but, interestingly, was decreased seven-fold by a different mixed culture (Walsh

and Jones, 1990). The interactions between biofilms, metals, and protective coatings applied to metals are complex and "decoating" processes, whereby consortia of marine bacteria initiate corrosion, will require a great deal more research. The molecular biology of these interactions is under study and this information should prove helpful ultimately in developing methods to prevent corrosion.

The other, more positive side of biofilms is that they play an important ecological role in settlement and metamorphosis of marine invertebrates. This interaction has been studied in detail using larvae of the commercially important eastern oyster, *Crassostrea virginica*, and the Pacific oyster, *Crassostrea gigas*, at COMB, UMBI. Biofilms of the marine bacterium, *Shewanella colwelliana*, were shown to be beneficial to "set" of oyster larvae (Weiner et al., 1989). "Set" is a general term that refers to both settlement and metamorphosis of larvae, although these processes may be triggered by different cues. Settlement behavior appears to be initiated by L-DOPA and may also be triggered by ammonia produced by bacteria in the films (Bonar et al., 1990). *S. colwelliana* synthesizes two tyrosinase enzymes, MelA and MelB. MelB is a conventional tyrosinase, catalyzing the hydroxylation of tyrosine to L-DOPA, which appears to be important in the induction of settlement behavior by *S. colwelliana* biofilms. The *melA* gene has been sequenced (Weiner et al., 1991) and codes for a unique enzyme that mediates melanogenesis and may provide a positive cue, influencing larvae to cement permanently onto a surface. The *melA* gene is essential for melanin biosynthesis in this bacterium (Fuqua and Weiner, 1993). In addition, an acidic exopolysaccharide is produced by *S. colwelliana* and this also appears to be an important cue for oyster set (Weiner et al., 1991). Detailed elucidation of factors important in oyster set is being used to develop methods to enhance the setting process in natural waters and aquaculture facilities in commercial culture.

Bioadhesion of bacteria, other microorganisms, and invertebrates is a sequence of events characteristic of biofilm formation that has been studied by many investigators. One of several important applications is the production of water-resistant adhesives. Results of work at the University of Maryland on the marine bacterium, *Al-*

*teromonas colwelli*ensis, indicates that an exopolymer, designated "polysaccharide adhesive viscous exopolymer" (PAVE), produced by this bacterium has great potential as an adhesive (Abu et al., 1991).

The adhesive strategies of marine mussels have been explored by H. Waite and coworkers at the University of Delaware, who have studied formation of byssal threads, by which the mussels attach to solid surfaces (Waite, 1983). The threads comprise a core of collagen and elastin, surrounded by a tough, durable varnish derived from a polyphenolic protein and catecholoxidase. The polyphenolic protein has a highly repetitive structure, rich in L-DOPA (Waite, 1991). Analogues of this protein have been produced by recombinant DNA technology (Filpula et al., 1990) and may be useful as protein-based medical adhesives (Strausberg and Link, 1990). Cell-Tak™, a crude preparation of the polyphenolic protein extracted directly from mussels, is used to enhance attachment of cells and tissues in culture (Notter, 1988).

Although some progress has been made in the study of certain aspects of biofilms and bioadhesion, many basic questions remain concerning natural biofilms. Complex factors involved in the interactions between bacteria and surfaces have been reviewed by Fletcher (1987, 1990). Interesting scientific questions pertaining to biofilms that remain to be answered include: what organisms are present in natural biofilms, what are their metabolic and genetic interactions, and what is the role of extracellular polymers in biofilm structure (Walsh et al., 1989b).

Molecular techniques are becoming increasingly important for the study of natural biofilms. For example, advances in the use of species-specific DNA probes for identification of single microbial cells in nature (Giovannoni, 1988) (discussed on page 48) have proven extremely useful for investigation of the community structure of biofilms. With fluorescent rRNA-targeted hybridization probes, it is now possible to quantify the cellular content of ribosomes of single cells of a specific population of sulfate-reducing bacteria in multispecies, anaerobic biofilms. This scientific advance makes it possible to infer the generation time of cells *in situ* and, therefore, to compare cell growth in young and established biofilms

(Poulsen et al., 1993). Application of novel microscopic techniques to study bacterial adhesion to surfaces (Fletcher, 1988) and of confocal scanning laser microscopy and environmental scanning electron microscopy to examine intact biofilms are providing new insights.

BIOREMEDIATION

Bioremediation of pollutants in the marine environment is an field of marine biotechnology that is still in its infancy, but one that has great potential for dealing with pollutants that may be extremely difficult or impossible to remove using other approaches. The aspect of bioremediation in the marine environment that has received most attention is degradation of hydrocarbons, in particular petroleum products, which enter the marine environment from oil drilling, loading of tankers, catastrophic oil spills, and by natural seepage from oil-bearing sediments. Environmental parameters that affect microbial degradation of hydrocarbons in the environment and metabolic and genetic factors that are important in this process have been extensively reviewed (Walker and Colwell, 1977; Atlas, 1981; Atlas, 1984; Leahy and Colwell, 1990). Biodegradation of petroleum in the marine environment is a complex process. Physical and chemical factors that affect this process include chemical composition, physical state, and concentration of the oil or hydrocarbon, as well as temperature, salinity, oxygen and nutrient availability, and water activity. Important biological factors influencing rate of biodegradation include the suite of bacteria, fungi and other microorganisms that are present and adaptation of these microorganisms by prior exposure to hydrocarbons (Leahy and Colwell, 1990).

In view of the complexity of microbial degradation of hydrocarbons, it perhaps is not surprising that the effectiveness of attempts to enhance natural degradation processes is not completely clear. Bioremediation attempts to treat oil spills have to date used nutrient addition to enhance the growth of the indigenous bacteria, the addition of laboratory-grown inocula, or a combination of the addition of an inoculum with specific fertilizer (Prince, 1993). Only the former has been shown to work unequivocally (Bragg et al., 1994), although promising results have also been reported for the combina-

tion of selected bacterial inoculum plus a fertilizer designed to specifically stimulate the growth of the inoculum (Rosenberg et al., 1992). Many other approaches have been suggested, including the use of bacteria with dispersants, and the use of microbially produced surfactants.

Nitrogen and phosphorus are frequently limiting nutrients in the marine environment and it has been demonstrated experimentally that the supply of these nutrients can limit microbial degradation of hydrocarbons in seawater (Atlas and Bartha, 1972) and in estuarine water and sediment (Walker and Colwell, 1974). Encouraging results were obtained by using oleophilic and slow-release fertilizers (Atlas and Bartha, 1973; Olivieri et al., 1976) in near-shore environments. A potential effect of fertilizer addition is a direct toxic effect on susceptible marine life; ecological and toxicological effects of fertilizers must, therefore, be monitored (Clark et al., 1991).

Seeding oil spills with active hydrocarbon-degrading microorganisms is hypothesized to reduce the initial lag period before the indigenous community responds to the oil spill (Atlas, 1991) or to nutrient addition. In a trial following an oil spill that came ashore in marshes in Galveston, Texas, portions of contaminated marshlands were treated with the bacterial bioremediation agent Alpha BioSea. However, the effectiveness of this treatment was not clear (Mearns, 1991). Conversely, the use of bioremediation to degrade oil on beaches in Alaska after the *Exxon Valdez* oil spill, using a combination of slow-release and oleophilic fertilizer, was generally successful at stimulating the natural rate of degradation several fold, providing the fertilizer was effectively applied (Bragg et al., 1994). In some cases fertilizer nutrients were not delivered to oiled sub-surface sediments despite application to the beach, and it is clear that monitoring nutrient delivery should be an important part of any future applications.

Many bacteria that degrade hydrocarbons produce extracellular surfactants to render the hydrocarbons more bioavailable, and these biosurfactants have often been suggested as tools for spill response. For example, Harvey et al. (1990) reported laboratory experiments where biosurfactants enhance oil removal from beach material. Such

products will have to compete in the market place with chemical products that generally are much cheaper, and it is not clear that bio-surfactants will be commercially viable for remediation. Biological surfactants have been useful in the emulsification and removal of residual oil in oil tankers (Rosenberg et al., 1975), but, again, cost is an important issue, and such products are not in use today. Nevertheless work continues in this area. Thus, the alkane hydrocarbon-degrading bacterium, *Acinetobacter calcoaceticus* RAG-1, has been shown to emulsify hydrocarbons efficiently by production of an emulsifying factor, termed Emulsan (Rosenberg et al., 1979). Emulsan and *Acinetobacter calcoaceticus* RAG-1 have been extensively studied and both topics were reviewed by Rosenberg (1986) and Rosenberg et al. (1989). In a recent study of the role of lipase in emulsification of hydrocarbons by *Acinetobacter calcoaceticus* RAG-1, the physiology and growth of the wild type strain on hydrocarbons was compared to that of lipase-deficient transposon mutants. Lipase-deficient mutants produced less emulsifying activity under most conditions. Lipase and thioesterase enzymes were found to catalyze the esterification and transesterification of fatty acids to the polysaccharide backbone of emulsan (Leahy, 1993). Understanding the physiology and molecular genetics of surfactant production by marine strains, such as *Acinetobacter calcoaceticus* RAG-1, will facilitate production of these surfactants for use in bioremediation in both marine and terrestrial environments.

Clearly, bioremediation was successful following the *Exxon Valdez* spill, where it was used to stimulate the natural degradation of oil that landed on gravel shores (Price, 1993; Bragg et al., 1994). Bioremediation has not yet been shown to be effective on oil spills in the open ocean, and this warrants further investigation. An interesting suggestion made recently is the use of hollow glass beads coated with the catalyst titanium dioxide, which in the presence of light initiates oxidation of large organic molecules such as hydrocarbons. The hypothesis is that natural microbial degradation should proceed more rapidly with more soluble, partially oxidized molecules (Rosenberg et al., 1992). Another approach that has considerable potential is the use of bacterial strains manipulated by recombinant

DNA methods to improve capabilities for hydrocarbon degradation and suitability as seed organisms. The disadvantage of the molecular genetic approach is that use of genetically engineered microorganisms in the environment remains a contentious issue (Leahy and Colwell, 1990).

Degradation of hydrocarbons in marine and estuarine sediments is likely to be dependant on degradative pathways different from those occurring in the water column because of the anaerobic conditions below a thin surface layer of sediment. Preexposure to polycyclic aromatic hydrocarbons was shown to enhance subsequent rates of hydrocarbon degradation in organic-rich, aerobic marine sediments (Bauer and Capone, 1988). Also, degradation of oxidized aromatic hydrocarbons has been shown to occur under anaerobic conditions.

It is important to note that microbial consortia are effective in metabolizing hydrocarbons other than oil. Such anaerobic degradative processes were reviewed by Leahy and Colwell (1990), who concluded that bioremediation of pollutants in the marine environment, other than hydrocarbons, has been too little studied. In general, pollutants frequently are dispersed over very wide areas, becoming greatly diluted in the process. Furthermore, pollutants such as heavy metals and pesticide residues may retain toxic effects for susceptible marine organisms, even at very low concentrations. Unfortunately, there is only very meager information on the fate of pesticide residues in the marine environment, although biodegradation of pesticides in terrestrial and freshwater ecosystems is reasonably well understood (MacRae, 1989). Bioremediation may be the only practicable method for removal of these pollutants since other options, e.g., incineration, landfill, burial, etc. do not apply. In any case, application of molecular genetic techniques, such as the use of gene probes to detect and monitor organisms with specific biodegradative capabilities, will provide useful tools for *in situ* treatments. Interestingly, it may be possible to monitor expression of specific biodegradative genes by detection of messenger RNA transcripts of those genes and these approaches are discussed on page 48. Another approach that has been proposed is the use of regulatory gene promoters that are

highly expressed in low nutrient conditions, such as those typically found in seawater, to give selective expression of desired genes in metabolically sluggish populations of bacteria (Matin, 1991).

In cases where high concentrations of pollutants are confined in a relatively small volume of seawater or sediment, it probably is advantageous to employ a closed system in which degradative processes are easier to study and may be better controlled. This approach is particularly useful in treatment of dredge spoils from harbors, where the sediments are likely to be among the most highly contaminated of all marine and estuarine ecosystems. A disadvantage of closed systems is the requirement for specialized bioreactors or other sophisticated equipment. A novel soil treatment method was developed by Kaake et al. (1992) that avoided the use of expensive equipment, but retained some of the advantages of a closed system. In this instance, bioremediation of herbicide-contaminated soils was achieved by nutrient pretreatment, which stimulated oxygen consumption, leading to anaerobic conditions, thereby establishing an anaerobic microbial consortium capable of complete degradation of the herbicide. Effectiveness of *in situ* (or open system) bioremediation of organic pollutants has been difficult to demonstrate in many cases. Convincing indirect evidence for microbial degradation of polyaromatic hydrocarbons in a contaminated aquifer was obtained by monitoring microbial adaptation to the pollutant and demonstrating pollutant-stimulated *in situ* bacterial growth (Madsen et al., 1991).

Research on bioremediation and biodegradation processes in soil and groundwater can yield information useful for bioremediation of contaminated marine sites. For example, in a study of the biodegradation of creosote and pentachlorophenol in contaminated groundwater, it was found that indigenous microorganisms could degrade the majority of the organic contaminants. However, toxicity and teratogenicity of the biotreated groundwater decreased only slightly, indicating that toxicity and teratogenicity were associated with compounds difficult to degrade by the indigenous microorganisms (Mueller et al., 1991). One approach, of course, is to develop genetically engineered microorganisms with specific capabilities against the most toxic, recalcitrant components.

Heavy metal pollution of seawater and sediments may be particularly damaging to marine ecosystems. Heavy metals persist for long periods. For example, high mercury concentrations were found in marine sediments and in crabs, shrimps, and oysters ca. 21 years after a mercury release from a chlor-alkali plant into Lavaca Bay, Texas (Palmer et al., 1992). High contamination of the coastal marine environment by many metals (and pesticide residues and hydrocarbons as well) has been found to be associated with dense urban areas on the East and West coasts of the U.S. (Valette-Silver and O'Conner, 1992). High heavy metal concentrations were measured in stranded Atlantic Bottlenose dolphins (Haubold and Tarpley, 1992), demonstrating entry into, and concentration of these toxic pollutants in, the food chain. Very likely, bioremediation of heavy metals in marine ecosystems will be a future beneficial application of marine biotechnology.

Bacteria capable of concentrating silver (Goddard and Bull, 1989) and copper (Dunn and Bull, 1983) have been reported and an actinomycete was recently isolated that accumulated uranium and lead (Golab et al., 1992). Metal resistant actinomycetes have been isolated from heavily polluted sediments in the Inner Harbor of Baltimore (Amoroso et al., 1993). Bacteria that tolerate and accumulate metals or that convert metals to less toxic forms may be useful in bioremediation of heavy metal pollution in the marine environment. However, application of bioremediation for effective treatment of pollutants in the marine environment requires further research on the metabolic capabilities of marine and estuarine microorganisms. Fortunately, the substantial progress made in bioremediation of soils and groundwater is likely to spur progress in marine systems. Development of molecular approaches to monitor microorganisms in the environment will be important. Issues associated with release of genetically engineered microorganisms into the environment will also influence progress in marine bioremediation.

High accumulations of polyhalogenated and polynuclear aromatic hydrocarbons and their degradation products have occurred in some aquatic ecosystems (Safe, 1984). However, the long-term effects on humans and marine animals from chronic exposure to low

levels of these pollutants are not fully understood. A known response of many marine organisms to exposure to toxic compounds is induction of cytochrome P-450. Cytochrome P-450-dependant monooxygenases can detoxify many xenobiotics. Polychlorinated biphenyls and polynuclear aromatic hydrocarbons are among xenobiotics that induce cytochrome P-450 activities in fish (Stegeman, 1981). Some investigators hypothesize that measurement of cytochrome P-450 induction in fish is a reliable and reasonably accurate method for assessment of toxic effects of xenobiotics. Induction of cytochrome P-450 in the estuarine killifish, *Fundulus heteroclitus*, has been reported to be under transcriptional control. Increases in specific messenger RNA (mRNA) levels from the gene encoding the P-450 protein may, therefore, be the earliest indicator of environmental exposure to toxic chemicals (Klopper-Sams and Stegeman, 1988). An alternative approach is the use of cDNA clones of rainbow trout estrogen-responsive genes and growth hormone gene as molecular probes (Chen, 1988). In appropriate rainbow trout cell culture systems, these probes could be used to detect the presence of xenobiotics and, thereby, serve to elucidate molecular mechanisms of toxicity.

Microorganisms can be useful indicators of elevated levels of xenobiotics, notably hydrocarbons (Colwell and Saylor, 1978; Sansaverino, 1993). In freshwater ponds polluted with different chemical species of mercury compounds, microbial communities were found to be resistant to Hg^{++} , which was present at near toxic levels, but minimal acclimation was observed for CH_3HgCl , which did not approach toxic levels (Liebert et al., 1991).

Gene probe analysis of soil microbial populations indicated that amendment with the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) resulted in selection and maintenance of a 2,4-D-degrading population (Holben et al., 1992). Application of the techniques of gene probing and detection of gene expression in the environment (discussed on page 48) should make possible development of detection methods for low levels of xenobiotics in the marine environment. Furthermore, the addition of genetically engineered marine microorganisms to degrade pollutants *in situ* should prove to be a

very useful approach to environmental remediation and will be an area of increasing research activity.

MARINE ECOLOGY AND BIOLOGICAL OCEANOGRAPHY

Application of Molecular Techniques to the Study of Marine Mammals, Fish and Invertebrates

The study of marine mammals, fish, and invertebrates has traditionally been regarded as belonging in the realm of biological oceanography. Application of modern techniques of molecular biology to the study of these organisms is included here in recognition of its being an important part of marine biotechnology. Molecular methods that have application in the identification of species and the detection of genetic variation within species include protein-based methods, e.g., immunological methods, the study of isozymes, and nucleic acid-based methods, such as DNA hybridization and restriction analysis, and rRNA (rRNA) analysis.

The application of molecular and biotechnological techniques to the study of large marine ecosystems was recently reviewed by Powers (1993) and the use of molecular techniques in the study of fish and invertebrate populations was discussed in detail (*vide supra*). Therefore, only a brief outline is provided in this section, along with several of the examples also included in the review by Powers (1993).

Separation of proteins by electrophoresis, followed by specific histological or immunological staining of particular proteins, has been widely used to detect variation in homologous proteins. These patterns of variation can be used to distinguish between morphologically similar species and to measure hybridization between species. For example, isozyme analysis was used to distinguish two sympatric species of Hawaiian bonefish that were morphologically identical (Shaklee and Tamaru, 1981).

Management of commercially important species relies on identification of stocks of organisms and the study of isozymes has been widely used in this regard. Isozyme analysis has revealed some cases

of tissue-specific expression of particular isozymes, which may be related to metabolic requirements of those tissues (Powers, 1993). There have been some attempts to correlate isozyme changes to changes in physical environmental parameters, such as temperature and salinity. For example, the gene frequencies of the heart-type lactate dehydrogenase locus from the fish *Fundulus heteroclitus* was found to vary in different populations of the fish located along the east coast of North America (Place and Powers, 1978). These allelic isozymes were found to be structurally and functionally different [reviewed by Powers (1990) and Powers et al. (1991)].

A sensitive method for measuring genetic variability is the use of restriction endonuclease digestion of mitochondrial and chloroplast DNA. Mitochondrial DNA (mtDNA) is an extensively studied cytoplasmic DNA element that is relatively easy to purify and characterize. Since mtDNA is maternally inherited, changes in mtDNA can be used to trace matriarchal lineages and distinguish between populations. Restriction analysis of several populations of *F. heteroclitus* revealed intergradation in these populations (Gonzalez-Villasenor and Powers, 1990). Size variation in mtDNA restriction patterns was used to discern discrete stocks of striped bass along the east coast of the U. S. within the Chesapeake Bay, and along the Gulf coast (Chapman, 1987; Chapman and Brown, 1990). Similarly, the geographic distribution of mtDNA haplotypes in humpback whales revealed differences between populations in the Atlantic and Pacific Oceans, as well as differences within each population (Baker et al., 1990).

Isozyme and mtDNA analysis have usually given confirmatory results. For example, it was confirmed by electrophoretic analysis of 31 proteins and analysis of mtDNA that morphologically similar hake found along the coast of southern Africa comprised two sympatric species, *Merluccius capensis* and *Merluccius paradoxus* (Grant et al., 1987; Becker et al., 1988). Furthermore, in other cases, mtDNA studies have answered questions about relatedness that could not be resolved by isozyme studies. For example, the Atlantic eel of the genus *Anguilla*, which migrates over vast regions of Europe and America, was considered to be a separate species from the European

eel, on the basis of some isozyme studies, while other investigators concluded that small gene frequency differences between populations indicated only partial reproductive isolation (Powers, 1993). This was resolved by mtDNA studies that indicated no genetic divergence among eels along the coast of North America but found that European eels were significantly different from those along the North American coast (Awise et al., 1986).

Chloroplast DNA (cpDNA) has been used in some studies of marine algae and phytoplankton. cpDNA is significantly larger than mtDNA, 120-200 kb, compared to 14.5 to 19.5 kb in higher eukaryotes (Watson et al., 1987). Relationships among red algal species and populations were studied using analysis of restriction patterns by Goff and Coleman (1988) and an evaluation of kelp cpDNA was performed by Fain et al. (1988). Strain improvement, employing biotechnology, for algae in aquaculture (see page 15) relies on fundamental research of the biology and genetics of these marine plants. As new molecular genetic information is accumulated, the mtDNA studies are likely to become increasingly important for the aquaculture industry.

Sequence analysis of ribosomal RNA (rRNA), widely used in taxonomic and evolutionary studies of microorganisms (see below), has also proved useful for studies of eukaryotes. For example, nucleotide sequences from two teleostean fish species, *F. heteroclitus* and *Sebastes altivelis*, from the spiny dogfish, *Squalus acanthias*, and the prickly shark, *Echinorhinus cookei*, were used to elucidate their molecular phylogeny (Bernardi et al., 1992). Comparison with sequences of the coelacanth, *Latimeria chalumnae*, the frog *Xenopus laevis*, and humans was done by maximum parsimony analysis. A single phylogenetic tree was obtained that was in agreement with the expected phylogeny. Another example is the use of restriction fragment length polymorphism (Rowan and Powers, 1991) and rRNA sequences (Rowan and Powers, 1992) to investigate unicellular algae that occur as endosymbionts in many different invertebrate species. Closely related algal zooxanthellae were found to be symbionts in distantly related hosts, indicating a flexible evolutionary relation between algal symbionts and their animal hosts.

Application of Molecular Techniques to Marine Microbial Ecology

Conventional microbiological methods that rely on culturing of microorganisms have very limited application in the study of marine microbial ecology, since it is estimated that the great majority of bacteria in picoplankton communities cannot be cultured, an observation made by marine microbiologists more than thirty years ago. Generally, less than one percent of bacterial cells observed by direct microscopy can be recovered on laboratory media (Jannasch and Jones, 1959; Kogure et al., 1979; Ferguson et al., 1984). It can be shown by microautoradiography that a considerably greater proportion of the bacterial community retains metabolic activity, even though they cannot be cultured (Meyer-Reil, 1978). Bacteria that cannot be cultured may belong to known bacterial groups that can be grown on laboratory media but may have entered a viable but unculturable state (Roszak and Colwell, 1987). Alternatively, some of these bacteria may belong to groups not previously isolated (Giommoni et al., 1990).

Application of molecular techniques is essential in understanding species composition, variability and metabolic activity of natural marine bacterial communities. A relatively rapid and simple method that does not necessarily identify individual species, but that gives general information about the variability of species composition, is community DNA hybridization (Lee and Fuhrman, 1990). This technique gives an indication of the proportion of identical or very closely related bacterial strains present in a given assemblage of bacteria and in other assemblages sampled at different times or locations. This technique can be useful in the selection of the most frequently occurring type of sample composition for more detailed and time-consuming individual species composition analysis (Lee and Fuhrman, 1990), such as by using 16S rRNA sequencing. Another hybridization technique which may have application in marine microbial ecology is reverse sample genome probing, in which total DNA extracted from samples is labeled with a radioisotope and hybridized to relevant bacterial species "standards" arrayed on hy-

bridization membranes (Voordouw et al., 1991). This procedure can be useful in the identification of bacteria.

A powerful molecular technique in the analysis of microbial communities is 16S rRNA sequence analysis (Pace, 1986), in which sequences derived from bacteria in natural samples are compared to known sequences by phylogenetic analysis. The 16S rRNA sequences can be selectively retrieved by cloning and sequencing cDNA synthesized from the 16S rRNA molecule; cDNA synthesis is primed with a synthetic oligonucleotide complementary to a universally conserved region in the 16S rRNA molecule (Weller and Ward, 1989). Another approach is the cloning and sequencing (or direct sequencing) of 16S rRNA genes amplified by PCR (Ward et al., 1992). Analysis of 16S rRNA sequences, for example, revealed that a novel microbial group, the SAR 11 cluster, is a significant component of the oligotrophic bacterial community in the Sargasso Sea (Giovannoni et al., 1990). This discovery and the subsequent identification of two novel eubacterial lineages in samples of bacteria from the Sargasso Sea provide support for the conclusion that a majority of planktonic bacteria are probably new, previously unrecognized species (Britschgi and Giovannoni, 1991). Sequences that were not closely related to any known rRNA sequences from cultivated organisms were obtained from north central Pacific Ocean surface water samples. One of these rRNA sequences was nearly identical to those from some Sargasso Sea bacteria, suggesting a global distribution of these newly discovered bacteria (Schmidt et al., 1991). The presence of broadly diverse microbial assemblages containing many phylogenetically undescribed groups was indicated by the results of Fuhrman et al. (1993) who used 16S rRNA sequencing to compare samples taken in the western California current of the Pacific Ocean and in the Atlantic Ocean near Bermuda. In the 61 clones sequenced, no more than two occurrences of the same sequence were found in a given sample, although identical sequences were found between samples four times, two of which were between oceans.

A profound discovery, resulting from phylogenetic characterization of microorganisms based on 16S rRNA sequencing, was the existence of two primary groupings of microorganisms, the archae-

bacteria and the eubacteria (Woese, 1987). These groupings have since been termed the domains Archaea and Bacteria (Woese et al., 1990). Archae are generally considered to be microorganisms present in extreme environments, i.e., in the oceans primarily found in or near the hydrothermal vent environments. However, 16S rRNA analysis of samples of planktonic bacteria has indicated that archaea may also be part of the planktonic assemblage of microorganisms in coastal and oceanic waters. Fuhrman et al. (1992) found sequences from a previously undescribed archaeal group in oligotrophic oceanic water, and archaea from this group and from a second group, related to the methanogens, were found to be present in coastal surface water samples collected near Woods Hole, MA and Santa Barbara, CA (DeLong, 1992).

In some cases, use of PCR to recover rRNA genes from natural communities can result in differential amplification of different rRNA genes. In particular, rRNAs of extremely thermophilic archaea from hydrothermal vent communities appear to be difficult to amplify (Reysenbach et al., 1992). Therefore, the interpretation of these data needs to be done carefully, i.e., with respect to extrapolation to other sites and other regions. However, the examples described above show that analysis of 16S rRNA sequences has clearly had a major impact on understanding the diversity of microorganisms in marine environments and will continue to provide exciting new findings well into the next decade.

Sequencing of rRNA molecules isolated from strains of marine bacteria in culture collections has been important in determining taxonomic relationships. For example, in addition to the 16S rRNA, the 5S rRNA sequences of *Shewanella*, *Vibrio*, and *Aleromonas* strains has permitted better definition of the species comprising these genera (Ortiz-Conde et al., 1989; Muir et al., 1990), which are important in the marine environment. Recently a large, morphologically peculiar microorganism, *Eputopiscium fishelsoni*, found in the intestinal tract of a surgeonfish, *Acanthumus nigrofuscus*, was confirmed to be a bacterium by rRNA sequence analysis (Angert et al., 1993). This microorganism was previously considered to be a protist because of its large size and is the largest known bacterium to date, with indi-

vidual cells reported to be larger than 600 μm by 80 μm , ca. one hundred fold larger than other bacteria.

Fluorescent-labelled oligonucleotide probes based on rRNA sequences can be used for phylogenetic identification of single cells (Distel et al., 1988) by fluorescent microscopy or microautoradiography of natural samples, an approach used for detection of marine nanoplankton protists by Lim et al., (1993) and for localization of a bacterial symbiont in tissue sections of the shipworm, *Lyrodus pedicellatus*, by Distel et al. (1991).

Phylogenetic characterization of bacterial symbionts of marine invertebrates and fish, many of which cannot be propagated in pure culture, has been possible by using 16S rRNA sequence analysis. Sulfur-oxidizing bacterial endosymbionts in three invertebrates from deep-sea hydrothermal vents and three invertebrates from shallow coastal marine environments were found to be species-specific. Furthermore, the symbionts were concluded to be unique to their respective hosts (Distel et al., 1988). The gill symbionts of a marine bivalve, *Thyasira flexuosa* was identified by 16S rRNA sequencing and shown to be closely related to known symbionts of lucinid clams (Distel and Wood, 1992). Previously, it was reported that the *T. flexuosa* symbiont was identified as *Thiobacillus thyasiris* TG-2 (Wood and Kelly, 1989). However, the bacterium, isolated in pure culture from gill tissue homogenates, was likely to have been on the gill surface and not an authentic intracellular symbiont of *T. flexuosa* (Distel and Wood, 1992). Bioluminescent symbionts of flashlight fish (family Anomalopidae) and deep-sea anglerfish (suborder Ceratioidei), extracellular parasites that cannot be cultured by conventional techniques, were recently shown by phylogenetic analysis of 16S rRNA sequences not to be previously described luminous bacterial species but, instead, new groups related to *Vibrio* species (Haygood and Distel, 1993).

In addition to elucidating phylogenetic relationships and the identification of microorganisms, molecular techniques are useful for the study of metabolic processes of microorganisms in the environment. Variations in the rRNA content of the marine bacterial species *Vibrio alginolyticus* and *Vibrio furnissii*, during starvation-sur-

vival and recovery, was assessed by measurement of the amount of hybridization to 16S rRNA probes. Mechanisms controlling starvation-survival in these marine bacteria were found to be linked to the physiological state at the onset of starvation (Kramer and Singleton, 1992).

A probe for a portion of the 23S rRNA gene of *Pseudomonas* was used to investigate the ratio between growth rate and nucleic acid content in this marine denitrifying bacterium (Kerkhof and Ward, 1993). Identification of particular genes in extracted nucleic acids from environmental samples can provide information about the presence of bacteria with the potential to carry out specific functions. PCR amplification of naphthalene-catabolic genes from sediment samples indicated the presence of bacteria carrying naphthalene deoxygenase genes (Herrick et al., 1993).

An exciting development is the detection of gene expression in natural populations of microorganisms by mRNA analysis. Pichard and Paul (1991) used this approach to detect expression of the gene (*rbcL*) encoding the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (RUBISCO) in natural phytoplankton populations. RUBISCO is a key enzyme in photosynthesis and *rbcL* expression was greatest during the day and least at night, as would have been expected. Expression of the catechol-2,3-dioxygenase gene (*xylE*) on plasmid pLV1013 in a marine vibrio was detectable in the environment and gene expression was expressed, relative to gene dose, in this study (Pichard and Paul, 1993). Measurement of regulation of gene expression at the transcriptional level in marine microorganisms (which complements measurement of enzyme levels) will be increasingly important in the understanding of activities of indigenous populations and microorganisms released into the marine environment for bioremediation purposes.

Gene exchange between microorganisms in the marine environment is currently of interest because of the importance of assessing the probability of gene exchange with genetically engineered microorganisms that may be released into the marine or estuarine environment (see Chapter 5). Gene transfer has been demonstrated in microcosms containing marine water and sediment (Pichard and

Paul, 1991) and a marine vibrio was shown to develop competence, i.e., ability to take up DNA, under a wide range of conditions, similar to those found in tropical and subtropical estuaries (Frischer et al., 1993). It has been shown that bacterial cells maintain plasmids even after entry into the viable but nonculturable state (Byrd and Colwell, 1990; Byrd et al., 1992). Retention of plasmids by bacteria, after release into the marine environment, is significant relative to introduction of genetically engineered microorganisms into the aquatic environment. Molecular techniques, in addition to conventional culturing methods, will be needed to examine gene exchange in natural communities of marine microorganisms.

The recent discovery of large numbers of viruses in marine natural waters is proving to be important for understanding microbial ecology and gene transfer in the environment. Abundances of 10^3 to 10^8 viruses ml^{-1} have been reported in marine waters (Bergh et al., 1989; Proctor and Fuhrman, 1990), estuarine waters (Wommack et al., 1992) and in a coral reef environment (Frischer et al., 1993). There are indications that bacteriophages may be important in marine bacterial mortality (Proctor and Fuhrman, 1990; Heldal and Bratbak, 1991) and that viruses may infect phytoplankton, reducing primary productivity (Suttle et al., 1991). As in other aspects of marine biotechnology, molecular techniques, such as detection of viruses and prophage by gene probing (Ogunseitan et al., 1992) are already proving useful in assessing the ecological role of viruses. It is clear that, although marine viruses have been studied for several decades (Spencer, 1955; Spencer, 1960; Chen et al., 1966; Baross et al., 1978; Torella and Morita, 1979), the recent reports of virus abundance have stimulated new research. The widespread distribution of viruses in the marine and estuarine environment indicates that viruses are likely to play an important role in marine microbial ecology.

An important technique for molecular studies in marine microbial ecology is the extraction of nucleic acids from water and sediment samples. These procedures are also important for detection of pathogens in the environment by gene probing (see page 54). Extraction of DNA from natural planktonic microorganisms can be achieved by filtration, followed by standard lysis procedures

(Fuhrman, 1988; Somerville et al., 1989). Extraction of intact mRNA requires the use of RNase inhibitors and satisfactory results have been obtained using this approach (Pichard and Paul, 1991, 1993). Recovery of nucleic acids from sediment, compared to seawater, is much more difficult and frequently a high content of humic substances can inhibit PCR reactions. Several methods recently have been described which involve extracting DNA in a sufficiently pure form that it can be used as a substrate for PCR reactions, without inhibition of the reaction (Pillai et al., 1991; Tsai and Olson, 1992a; Tsai and Olson, 1992b; Young et al., 1993), making possible the sensitive detection of pathogens and other bacteria of interest.

Advances in marine microbial ecology are likely to be greatly accelerated by application of molecular techniques, in combination with conventional approaches, where appropriate. This is well exemplified by results of recent studies of the impact on benthic microbial ecology of disposal of sewage and sewage sludge into the deep sea. Results of enumeration of *Clostridium perfringens* spores by conventional plating were used to show that sewage dumped at the Deep Water Municipal Sewage Disposal Site (ca. 100 miles off the coast of New Jersey in water depths of 2,600 m) had contaminated the benthic environment (Hill et al., 1993). Application of conventional techniques, such as enumeration of bacteria by microscopy and plate counts, combined with molecular methods for community DNA hybridization (Lee and Fuhrman, 1990), yielded data showing that contamination of the benthic environment had induced changes in the indigenous benthic microbial assemblages (Hill et al., 1993b; Takizawa et al., 1993). These studies advance our understanding of pollution in the marine environment.

Marine Ecology and Human Health

The quality of estuarine and marine water used for recreation and seafood harvesting has traditionally been assessed by enumeration of coliforms and fecal coliforms, rather than by direct detection of pathogenic bacteria and viruses. Direct detection of pathogens has not been adopted as a standard method in the U.S. (American Public Health Association, 1989) because the practical conclusion, before

the advent of molecular genetics methods, was that standard bacteriological culturing methods for direct detection of pathogens were expensive, time-consuming, and not efficient. In addition, direct detection of pathogens by standard microbiological methods is of questionable value. Exposure of bacterial pathogens to the aquatic environment reduces efficiency of recovery, when standard culture methods are employed (Bissonnette et al., 1975). Several important bacterial pathogens, including *Vibrio cholerae* (Colwell et al., 1985), *Salmonella* species (Roszak et al., 1984), and *Campylobacter jejuni* (Rollins and Colwell, 1986) have been shown to enter a viable but nonculturable state (Roszak and Colwell, 1987) in the environment, in which they are no longer culturable by conventional techniques but may retain pathogenicity (Colwell et al., 1985; Colwell et al., 1990). Molecular approaches have made it possible now to monitor water quality by direct and reliable detection of pathogenic bacteria, even cells in the viable but nonculturable state.

Molecular methods for detection of bacterial pathogens in the aquatic environment that have been published to date include DNA probes (Knight et al., 1990) and PCR (Way et al., 1993) for detection of *Salmonella* species and PCR and fluorescent antibody methods for detection of *Shigella dysenteriae* Type I (Islam et al., 1993) and *Vibrio cholerae* (Brayton and Colwell, 1987; Huq et al., 1990). *Escherichia coli* and enteric pathogens (*Salmonella* and *Shigella* species) have been detected in environmental samples with sufficient specificity and sensitivity for monitoring water quality by PCR amplification of the *lamB* gene (Bej et al., 1990), and PCR amplification of the *uid* gene that codes for β -glucuronidase was used to detect *Escherichia coli* and *Shigella* species (Bej et al., 1991). Detection of virulence factors in *E. coli* isolates from water samples was achieved using seven different DNA probes (Martins et al., 1992). PCR amplification of a region of the enterotoxin gene in enterotoxigenic *E. coli* and *V. cholerae* was found to be suitable for detection of these organisms (Knight et al., 1991). Other examples include a biotype-specific probe that reliably differentiates between the El Tor and Classical biotypes of the *Vibrio cholerae* O1 serogroup (Alm and Manning, 1990) and a monoclonal antibody-based test that allows rapid and

sensitive detection of *V. cholerae* 01 in contaminated water (Colwell et al., 1992). Enteroviruses, which pose a public health risk in the environment and can be transmitted via contaminated water, were detected in groundwater by PCR. This assay is applicable for detection of enteroviruses in other environmental samples (Abbaszadegan et al., 1993).

Contamination of shellfish, because shellfish are filter feeders and, therefore, concentrate bacterial and viral pathogens, can present a severe public health risk. To assess the risk, molecular techniques have been developed for detection of several important shellfish-related pathogens, one of which is *Vibrio vulnificus*, a human pathogen, associated with oysters, that can cause septicemia after ingestion of raw oysters. *V. vulnificus* septicemia has a mortality rate of greater than 50% (Morris and Black, 1985). A DNA probe with excellent specificity and sensitivity for *Vibrio vulnificus* was developed from a fragment of the cytolysin gene of this organism (Morris et al., 1987). The hemolysin-cytolysin structural gene *vvhA* was subsequently sequenced (Yamamoto et al., 1990) and an alkaline phosphatase-labeled oligonucleotide probe based on this gene was used for rapid isolation and enumeration of *V. vulnificus* without the need for enrichment or selective media (Wright et al., 1993). A fluorescent-labeled oligonucleotide probe has proven useful for enumeration of *V. vulnificus* (Heidelberg, 1993). Detection of another important shellfish pathogen, *Vibrio parahaemolyticus*, in artificially contaminated oysters, was accomplished using an oligonucleotide probe specific to the thermostable direct hemolysin (*tdh*) gene of the organism (Lee et al., 1992a).

Molecular techniques are now available for detection of many important pathogens in water and shellfish samples. The use of direct detection methods can no longer be ignored. They are important for assuring public health and food safety, and will supplant culture methods in the very near future.

Use of Biosensors and Remote Sensing

There is growing interest in the use of biosensors and remote (usually satellite-based) sensing of the marine environment. Biosen-

sors may offer sensitive methods for detection of low levels of nutrients or toxic compounds. Furthermore, remote sensing allows detection of large-scale changes in such parameters as water temperature, wind-induced mixing, and phytoplankton biomass. This capability will be greatly expanded by the NASA Sea-viewing Wide Field-of-view Sensor (SeaWiFS) and the Earth Observing Satellite (EOS) (Hooker and Esaias, 1993; Hooker et al., 1993) and remote sensing should be considered by biologists as a useful tool for environmental research and monitoring.

Development of biosensors is dependant on the coupling of biological material, such as enzymes, antibodies, or whole cells, to a transducing element which converts the biological signal of interest to some form of electronic readout. An example is the use of mid-ultraviolet range spectrophotometry (MUVS) to detect the redox state of bacterial cells immobilized in a gel membrane. Toxins flowing over the membrane cause changes in redox state, detectable by MUVS (Bains, 1992).

Biosensors will be particularly useful in detection of toxic compounds and may allow development of true toxicity sensors that would interact with any compound that is toxic; this could be followed by conventional chemical approaches that identify the specific toxic compound (Bains, 1992). One assay system of this type, which may be useful for biosensor development, measures luminescence of *Photobacterium phosphoreum*. Light generation by luciferase in this bacterium is dependant on a high level of ATP, which is not maintained in the presence of toxins that affect the intracellular ATP pool (Bulich et al., 1990).

Biosensors are also under development for detection of specific substances, i.e., development of optical and electrochemical enzyme-based methods for detection of very low concentrations of DNA (Downs et al., 1988). A diversity of biological elements has been incorporated into biosensors (Hendry et al., 1990). It is clear that sophisticated approaches to environmental sensing will have a significant impact on the ability to gather information about marine ecosystems. As satellite technology becomes more sophisticated and the molecular biology of biosensing systems is better understood,

this rather futuristic area of marine biotechnology will revolutionize data gathering in the marine environment.

SUMMARY

In 1983, marine biotechnology was first recognized as an area of biotechnology of great potential (Colwell, 1983). In the decade that has since passed, rather astonishing progress has been made. More than 1,000 publications describing new compounds, natural products, discoveries of the molecular genetics of fish and shellfish growth, metabolism, and reproduction, and expansion of marine biotechnology research on bioremediation, biofouling, and related aspects have appeared in literature. Where only a few pioneers toiled in their laboratories, there are now major centers of marine biotechnology research and development in Bergen and Tromsø, Norway, Kamaichi and Shimizu, Japan, and in Baltimore, MD, Monterrey, CA, and elsewhere in the U.S. and across the globe.

Promising new antibiotics, anti-cancer therapeutic agents, improved aquaculture stocks, marine polysaccharides as food additives, and potential new energy sources from the sea, driven by molecular genetic control of marine biological systems, are in the discovery, post-discovery, scale-up, and/or production stages. There is no doubt but that there has already been, and will continue to be, major successes, both intellectual and commercial, in marine biotechnology.

Areas of marine biotechnology in the more-or-less expansive stage are bioremediation and marine biodiversity inventories and assessments. Yet to be fully exploited is the nucleic acid fingerprinting for global marine biological stock assessments and monitoring, although this application is moving rapidly. More futuristic are the applications of bio-signaling and biosensor technology, especially in global change/global monitoring, where the role of microorganisms in weather regulation and weather processes is only vaguely suspected and poorly understood, the latter especially the case for biogeochemical cycling. The notion of a "microbial loop" and its role in global ocean processes has only relatively recently been enunciated (Azam et al., 1983; Ducklow et al., 1986). This aspect of marine biotechnology is very much in the future, perhaps 10-15 years down

the road, although some investigators are currently pursuing biosensors/bioimaging and its applications (Bains, 1992). Similarly, the use of microalgae for addressing global warming is being considered by Miyachi and colleagues (Kodama et al., 1993).

During the 1970s and early 1980s, ecosystem modeling was very much in vogue, later falling into lesser prominence, mainly because the software (and in some cases, the hardware) was not sufficiently sophisticated, nor were the data complete enough to pass the "reality checks." With the extraordinary power of computing and the volume of data now able to be collected, as well as historical data available in data banks, ecosystem modeling will provide a powerful means of interpreting microbial ecosystems of the world oceans.

Marine biotechnology is a long-term investment technology. There are some immediate commercial successes, notably in the food industry, but as the Japanese government has decided, the view should be with an 8-10 year investment strategy. The U.S. Congress has now passed a marine biotechnology initiative that will provide up to \$20 million for marine biotechnology research and development. Industry, over the past decade, has begun to move into marine biotechnology. The development of this aspect of marine biotechnology, industrial marine biotechnology, is detailed in the next chapter.

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Chapter 2

MARINE BIOTECHNOLOGY SURVEY RESULTS (MARBIO)

INTRODUCTION

Assessing marine biotechnology research and development without a reliable base of information is difficult, at the minimum. Obtaining the information needed to establish such a base is difficult since marine biotechnology is rarely demarcated as a program category by academic institutions or private companies where research and development in marine biotechnology is taking place.

In order to learn more about the kinds of research being conducted and the level of activity classifiable as marine biotechnology, a survey of marine biotechnology research and development in the United States was conducted during 1991-1992. In this chapter we discuss the survey methodology, as well as some general results. Where these results are referred to in this document, they are referenced as "MARBIO" referring to the database created to store information derived from the survey.

Two slightly different survey instruments were prepared, one for academic and public research institutions, and another for private industry. Copies of the questionnaires are included in Appendices 2 and 3.

SURVEY METHODOLOGY—ACADEMIC & PUBLIC RESEARCH INSTITUTIONS

The initial survey of academic and public research institutions was conducted during the summer of 1991. The population from

which the survey sample was drawn was identified through numerous existing sources that could be used to associate an individual or research institution with marine biotechnology research. For example, literature searches were conducted in Marine Biotechnology Abstracts and in DIALOG, and the affiliation of authors was noted. From these sources, we estimated that there are roughly 218 distinct academic and public research facilities conducting marine biotechnology research in the United States.

The survey was pre-tested, using a sample of 37 individuals who agreed to assist in refining the survey. Thus, experience in asking the questions and interpreting responses was gained. In this initial step, individuals were sent a letter explaining the purpose of the survey and asking their cooperation. This was followed up by a phone call, during which the surveyor scheduled an appointment to conduct the survey by telephone.

Ultimately, 112 persons representing a variety of research establishments, were contacted—an estimated 51% of the entire marine biotechnology academic research community. Only 3 individuals contacted chose not to participate in the survey. After being given our definitions of marine biotechnology and marine biotechnology related research, 21% of the respondents concluded their work did not meet our definition and the survey was terminated, lowering the number of establishments where marine biotechnology research is being conducted. However, the actual number of establishments where marine biotechnology research is being conducted is probably close to our original estimate since there are facilities we are unaware of that may be conducting marine biotechnology or related research.

SURVEY METHODOLOGY—MARINE BIOTECHNOLOGY COMPANIES

Identification of private companies performing marine biotechnology research was achieved through a combination of database searches and by questioning individual scientists. A list of 59 companies met the criteria for inclusion, but the actual number of U.S. companies involved in some type of marine biotechnology activity may be as high as 110. For purposes of sample expansion, the mid-

point of the minimum estimate of 59 companies and the maximum estimate of 110, and assumed the number of companies to be 85.

Representatives of all 59 companies on our list were contacted, and only one chose not to participate.

GENERAL SURVEY RESULTS

Affiliations

Seventy-seven percent of academic and public research institutions involved in some type of marine biotechnology research were directly affiliated with public or private universities. The remaining units were either national, state, or independent research institutes doing some type of marine biotechnology research. For units associated with private industry, 72% are U.S. corporations and 21% multinational corporations.

Research Area

Survey respondents were presented with a list of 25 major research areas and asked to indicate which area represented their major activities. For academic units, none of the selected areas associated with marine biotechnology yielded more than 10% response (Figure 2). Microbiology and molecular biology were listed by 9.4% of the respondents. Other research areas listed that were mentioned by a significant number of respondents were natural products chemistry (8.8%) and aquaculture (7.7%).

The focus for research in private industry was on aquaculture (19%) and natural products chemistry (15%) (Figure 3), with the next most frequent being bioremediation and microbiology, each 8.2%.

That academic research was less focussed on specific areas was not surprising. Industry, expectedly, showed sharply defined objectives, with 50% of the firms concentrated in just four areas of research. Areas more closely aligned to application such as aquaculture and natural products chemistry require more attention from industry scientists than the more basic science areas of microbiology and molecular biology.

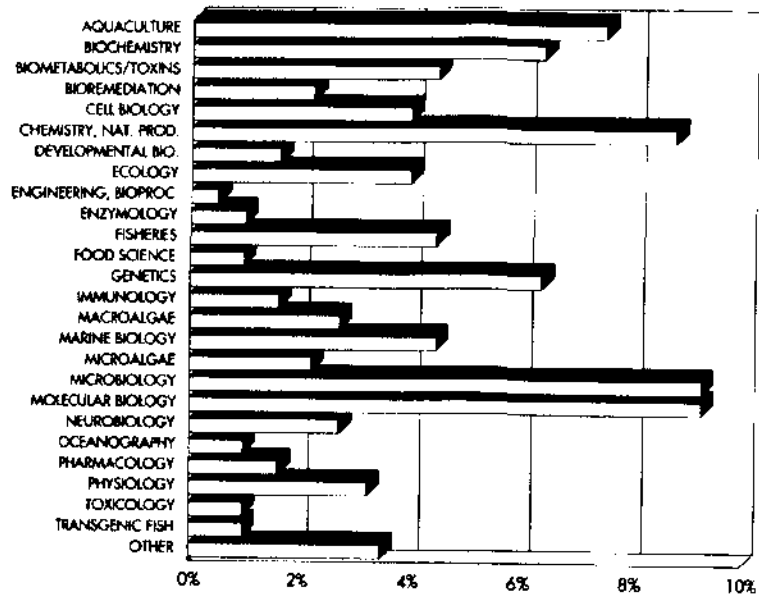


Figure 2. Academic marine biotechnology research areas.
Source: MARBIO.

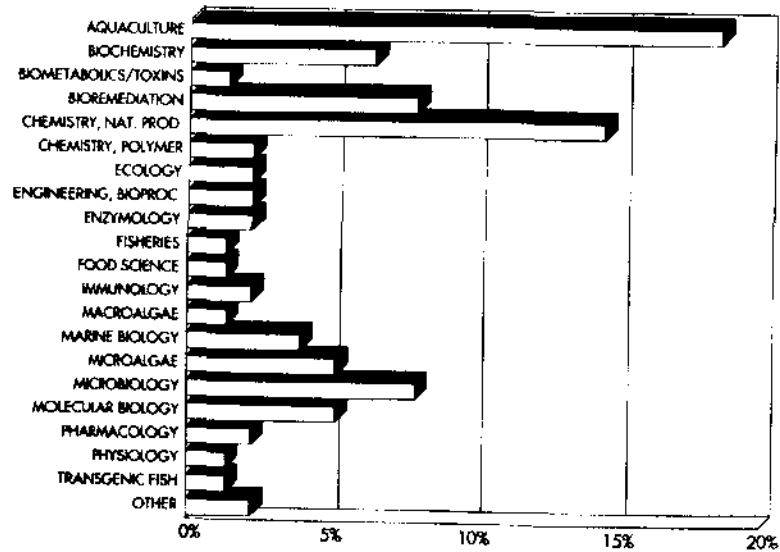


Figure 3. Corporate marine biotechnology research areas.
Source: MARBIO.

Applications

The distinction between academic and corporate research areas was further emphasized by results of responses to the query concerning the eleven areas of applications for research. The most frequent response concerning research applications from academic scientists was basic research (34%) (Figure 4). Aquaculture (16.6%), pharmaceuticals/fine chemicals (13.3%) and environment/bioremediation (11.9%) also were significant in the responses. None of the other areas were cited by more than 5% of the scientists.

In comparison, only 5.7% of the corporate scientists listed basic research as the major focus of their work (Figure 5). The major items of interest were: aquaculture (21.4%); pharmaceuticals/fine chemicals (18.6%); fermentation processes (14.3%); and environment/bioremediation (10%).

Research Methods

Research methods used by research workers in marine biotechnology are driven, in part, by research area and application. In both academic and corporate research, cell culture methodology is dominant (Figures 6 and 7). A slightly higher percentage (19.7%) of the corporate scientists listed cell culture, compared with academic researchers (14.4%). For both groups, research methods in categories not listed on the questionnaire was the second most prevalent response.

Two research methods stand out as being used at significantly different frequency in academia and industry. Classic genetic plant breeding was mentioned as a major research direction by 9.6% of the corporate researchers, but only 1.6% of the academic researchers. Academic researchers, are more involved with DNA probe construction (9.6%), compared with corporate researchers (1.4%).

Organisms Used

Both academic and corporate scientists rely on bacteria as the major organism with which to perform their research in marine

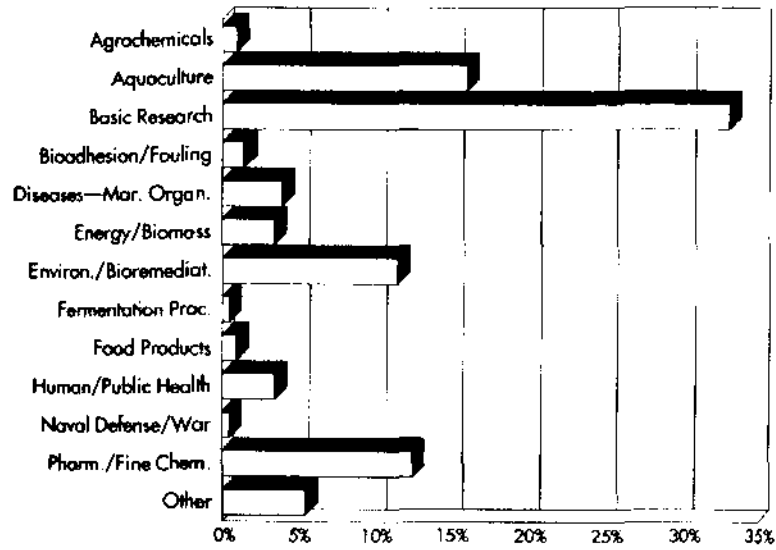


Figure 4. Marine biotechnology applications of academic-related R&D.
Source: MARBIO.

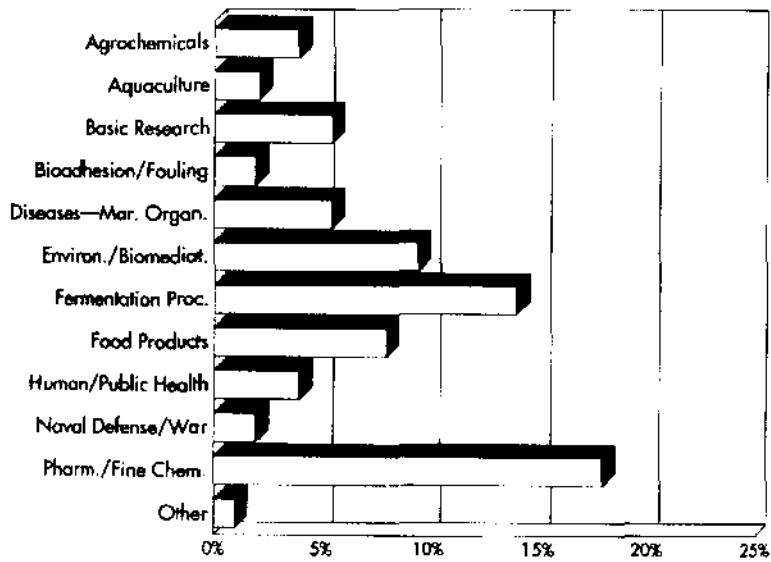


Figure 5. Corporate applications of marine biotechnology.
Source: MARBIO.

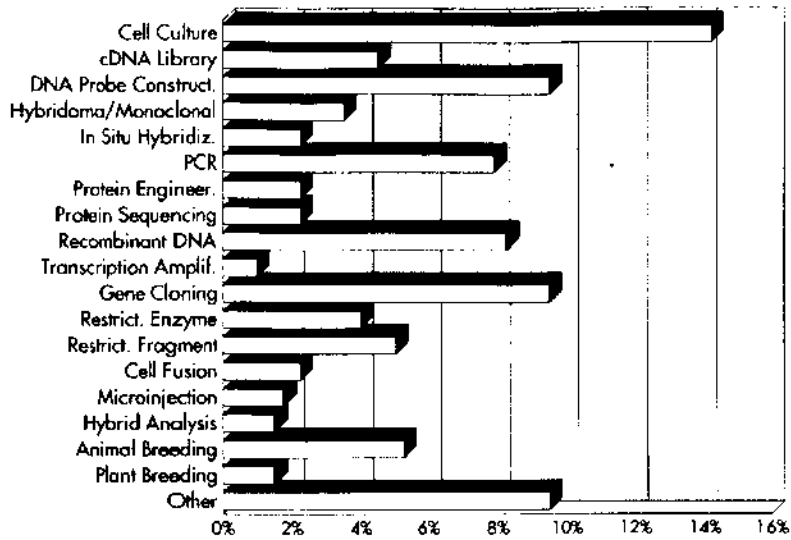


Figure 6. Marine biotechnology research methods at academic-related institutions.
Source: MARBIO.

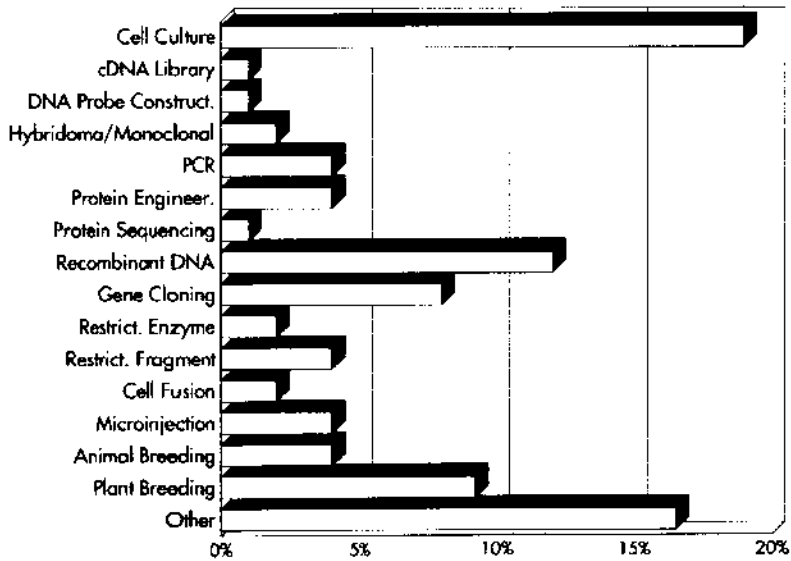


Figure 7. Corporate marine biotechnology research methods.
Source: MARBIO.

biotechnology (Figures 8 and 9). Bacteria were mentioned by 16.2% of the academic scientists and 18.2% of the corporate scientists. Shellfish species (both crustaceans and mollusks) were important for both groups at 13.0%; however, finfish (14.6%) were used more often by academic scientists than their corporate counterparts (10.4%). Fungi and microalgae (11.7%) were used often by corporate researchers. While microalgae were important tools for academic researchers (9.2%), fungi was mentioned as important only by 1.1%.

Industry-Academia Collaborations

Fifty-two percent of the academic scientists interviewed indicated that they had some form of collaboration with private industry. When researchers in private industry were asked about their collaborations with academics, 77% indicated that they did, indeed, have a collaboration underway with an academic unit. These findings are not incompatible since there are many more scientists than companies, and not all academic scientists care to link with industry scientists, although this attitude is changing dramatically.

Foreign Collaborations

More than 60% of the academic scientists indicated that they are carrying out some type of collaboration with foreign scientists in the area of marine biotechnology. In the case of private industry scientists, such collaborations were being undertaken by slightly less than 50% of the firms interviewed.

Commercial Use/Marketable Products

Both groups of scientists were asked if their research had resulted in products that were currently in use or being marketed. A smaller percentage of academic research (23.2%) has resulted in a commercial/marketable product, whereas 48.7% of the private firms indicated that their research had resulted in commercial applications.

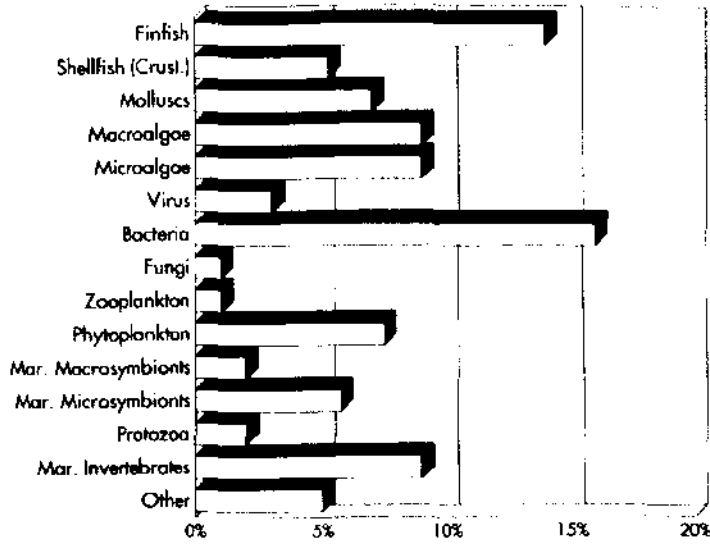


Figure 8. Marine biotechnology organisms used in academic-related R&D.
Source: MARBIO.

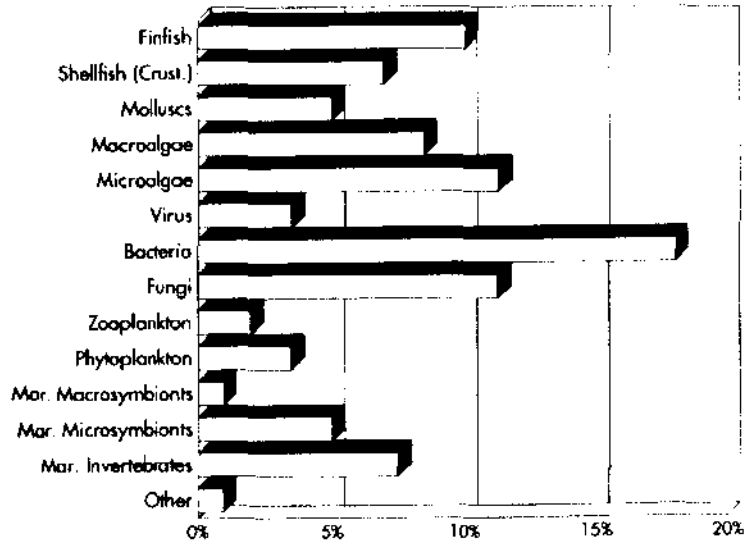


Figure 9. Marine biotechnology organisms used in corporate R&D.
Source: MARBIO.

Patents/Patents Pending

The academic scientists interviewed reported that they had received 72 patents from their marine biotechnology research, and had another 52 patents pending. Corporate scientists had 14 patents awarded and 54 patents pending. Patents are discussed in more detail in Chapter 4.

Laboratory Staffing

Significant variability in the size of laboratory staff and among types of personnel employed were noted. On average, the academic marine biotechnology laboratory comprises 2.6 scientists at the Principal Investigator level, 1.9 post-doctoral appointees, 4.4 graduate students, 2.1 technicians, 0.9 other faculty and 2.0 undergraduates.

The corporate research laboratory typically comprised 4.5 senior scientists, 3.0 junior scientists, 0.3 post-doctorates, 4.5 technicians, and 1.1 individuals listed in the "other scientist" category.

Laboratory Budget

The average research budget for the academic laboratory was \$204,000, in 1991. In private industry, the average budget was \$362,000, for the same period of time. For both groups, the median budget was less than \$200,000. Only a small number of laboratories had significantly larger budgets. The latter tended to skew the average budget to higher than the typical laboratory budget.

Funding Sources

The National Institutes of Health (NIH) of the Public Health Service represent the most important funding source for marine biotechnology research at academic institutions, accounting for circa 25% of the total funding (Figure 10). Other major funding sources are the National Science Foundation (14.4%), followed by the National Oceanic and Atmospheric Administration (NOAA) with

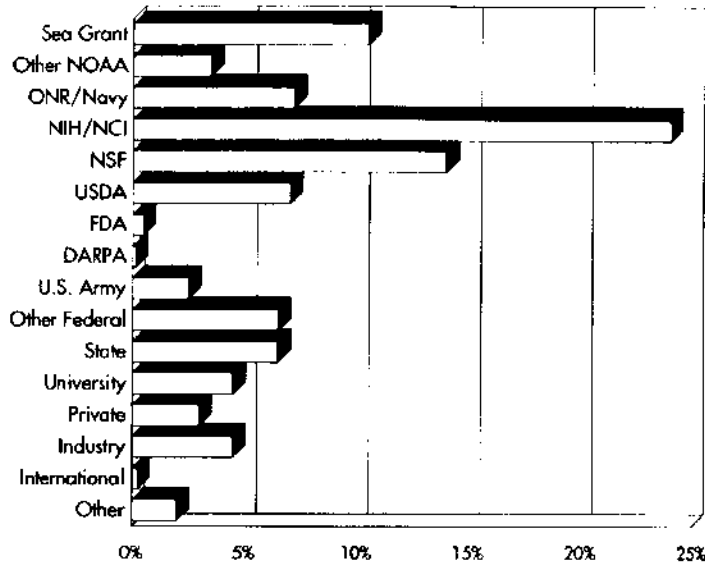


Figure 10. Funding sources for marine biotechnology research at academic-related institutions.

Source: MARBIO.

14.1% of the support. Most of the NOAA funding derives from the National Sea Grant College Program (10.7%). Private industry accounted for less than 5% of the research being done at academic institutions in 1991.

Funding for marine biotechnology research in the corporate sector is nearly entirely generated internally (73.3%) (Figure 11). Joint ventures or contracts with other firms provided an additional 2.8% funding. The federal government, predominantly the NIH, Department of Energy and U.S. Department of Agriculture, accounted for 14.5% of the funding. State governments contributed 2.4% to private research laboratory funding.

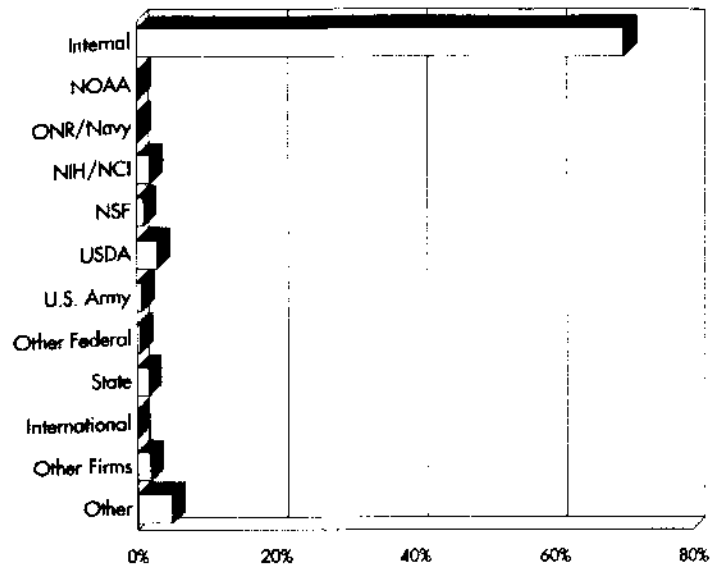


Figure 11. Corporate funding sources for marine biotechnology research.
Source: MARBIO.



Chapter 3

POLITICS AND FUNDING POLICIES IN THE UNITED STATES RELATED TO MARINE BIOTECHNOLOGY

This chapter comprises three sections: analysis of the perception of the executive and legislative branches of the U.S. government, with respect to marine sciences and biotechnology; identification of public and private sources of funds used to support biotechnology and marine biotechnology R&D, with an estimate of the amount of funding each source provides for marine biotechnology R&D; and discussion of future developments in marine biotechnology.

VIEWS WITHIN THE U.S. GOVERNMENT OF THE MARINE SCIENCES

The two branches of the U.S. government which have the most significant influence on policies affecting marine biotechnology, especially funding, are the Executive branch and the Legislative branch, i.e., Congress.

The Executive

The sciences in general were supported relatively well by presidential administrations between 1972 and 1980. However, after the Reagan administration took over the reins of government in 1981, federal spending in support of nondefense R&D decreased sharply and, for ideological reasons, a general effort was made to reorient the role of government vis-à-vis industry. One of the Reagan administration's tenets was that the nation's natural resources could be handled most effectively and efficiently by the private sector. Another

was that the government could best help the private sector by reducing its regulatory load. The Reagan administration, more so than its predecessors, used the federal budget for directing change—by sharply reducing or eliminating funds for specific programs the administration promoted major policy shifts (King and Jennings, 1988).

The marine sciences were not deliberately discriminated against by the Reagan administration, but because most ocean-related programs were funded from the approximately 25% of the federal budget not committed by law, they were easy to cut or eliminate. The U.S. Department of Commerce's (DOC) National Oceanographic and Atmospheric Administration (NOAA) was especially hard hit by cuts. Between 1982-1984 NOAA lost 136 staff, mostly program officers. In addition, three NOAA research vessels were removed from service and some of its fisheries laboratories were closed. The Office of Oceans and Coastal Resource Management was, for all practical purposes, abolished, while the National Undersea Research Program (NURP) was barely able to keep functioning (see below) (King and Jennings, 1988).

In line with the Reagan administration's attempts to eliminate state-federal programs, NOAA's Sea Grant program was targeted for eradication—none of the eight annual federal budgets formulated by the Reagan administration requested funds for either Sea Grant or NURP. The Reagan administration's reasoning appears to have been that the Sea Grant program had proven its success and should revert to the states. However, the Reagan administration made no attempt to assess the ability of the states to take over the Sea Grant program, to evaluate national interest in it, or determine what to do in the transition period before transfer was completed. Only Congressional action limited the damage to NOAA, in general, and retained the Sea Grant program (see the next section).

It was during the time of the Reagan administration that the biotechnology revolution commenced. Several federal agencies soon recognized the importance of the emerging field and responded in various ways. For example, the National Institutes of Health (NIH) early supported efforts to regulate possible risky activities, support which led to the development of the so-called NIH guidelines and

the establishment of the Recombinant Advisory Committee (RAC) in 1976 (see Chapter 5). These developments, in turn, prompted the President's Office of Science and Technology Policy (OSTP) to begin work in the early 1980s that led to the development of the Coordinated Framework for the Regulation of Biotechnology, first published for public comment in 1984 and adopted as policy in 1986. The Agricultural Biotechnology Research Advisory Committee was established that year to develop guidelines for agricultural research employing modern biotechnology techniques and the field testing of genetically engineered plants. The OSTP's Life Sciences Program also cooperated throughout the 1980s with the Congressional Office of Technology Assessment (OTA) as the latter agency performed a series of studies on biotechnology and the major public issues the field was generating that required scrutiny by Congress.

It was also during the Reagan administration that the Biotechnology Science Coordinating Committee (BSCC) was set up under the Federal Coordinating Council on Science, Engineering and Technology (FCCSET), an inter-agency council on which all major government departments and agencies were, and are, represented. FCCSET was authorized by Congress in the 1970s, but had been dormant for many years. The BSCC provided significant interagency coordination in relation to policies relevant to biotechnology promotion and regulation until it was replaced by the Biotechnology Research Subcommittee (BRS) during the Bush administration (see below). Perhaps BSCC's most important accomplishment was to initiate interagency funding for the National Academy of Sciences and National Research Council study, published in 1989, which established fundamental principles regarding biotechnology risk and regulation (see Chapter 5) (NRC, 1989). Finally, the foundation for the National Research Initiative, which was to be authorized and funded during the Bush administration (see below), was laid during the Reagan administration.

The Bush administration indicated early on that it intended to support strengthening science and technology in the U.S. and took steps to transform its words into action. For instance, with respect to the oceans, in the fall of 1989 the Bush administration turned to an

ocean scientist for NOAA leadership, appointing Dr. John A. Knauss, formerly a physical oceanographer at the University of Rhode Island Graduate School of Oceanography, as NOAA Administrator. Further, NOAA's 1991 budget was given a 35% increase over that submitted the year before and Sea Grant was included as a budget item for the first time since 1980. In 1992, the Bush administration again included Sea Grant as a budget item, although at a reduced level.

The Bush administration was very supportive of biotechnology. In 1990, the OSTP Director (and the President's Science Advisor) Dr. Allan Bromley organized an interagency task force on biotechnology, the Biotechnology Research Subcommittee (BRS), under the purview of FCCSET's Committee on Life Sciences and Health. The BRS, which replaced the BSCC, was chaired by Dr. David Galas from the Department of Energy (DOE). In 1991, the Subcommittee organized a Working Group on Marine Biotechnology, chaired by Dr. David Attaway from NOAA. The Subcommittee was given a broad interagency coordination and cooperation role regarding federal support of biotechnology research; its first activity was to examine all government agency activities in biotechnology in order to identify overlaps between programs, gaps that indicated weaknesses, and opportunities for future development. In February 1992, the Subcommittee issued the report *Biotechnology for the 21st Century*, which delineated a far-reaching Biotechnology Research Initiative (BRI) (Committee on Life Sciences and Health, 1992).

The report has been extensively described and discussed in the popular press and professional journals. For purposes of discussion here, consideration of the report is limited to some general comments about budget allocations, objectives, and treatment of marine biotechnology.

The BRI was the largest of five special federal initiatives, often called "crosscuts," for the fiscal year 1993 (FY 1993) budget (the others related to high-performance computing and communications, advanced materials, global change, and math and science education). The goal was to "Sustain and extend U.S. leadership in biotechnology research for the 21st Century to enhance the quality of life for Americans and the growth of the U.S. economy." It comprised four

“strategic objectives”: extending biotechnology’s scientific and technical foundations; developing sufficient human resources to ensure that biotechnology can be extended as planned; accelerating transfer of research results to the applied sectors; and realizing biotechnology benefits for “the health and well-being of the population and the protection and restoration of the environment.” If the strategic objectives were achieved, the hoped-for result would be that U.S. biotechnology industry would benefit. Sales of biotechnology products could be expected to increase from \$4 billion per year to \$50 billion per year by 2000.

The BRI proposed federal funding for biotechnology research, which had steadily increased during the late 1980s and early 1990s to \$3.76 billion in FY 1992, be boosted by an additional 7% for FY 1993, to \$4.03 billion. The twelve federal agencies identified by the Subcommittee as supporters of biotechnology research were integral components of the BRI. But, by far, most of the FY 1993 funding were destined for the Department of Health and Human Services (DHHS), \$3.125 billion, followed by DOE, \$242.7 million, the National Science Foundation (NSF), \$206 million, and USDA, \$167.7 million. The DOC (including NOAA) provided a minuscule \$13 million for biotechnology research, while the Department of Defense (DOD), including the Office of Naval Research (ONR), spent \$86.6 million for this purpose.

Under the BRI, three research areas merited special attention: marine biotechnology; structural biology; and genome research. However, FY 1993 support for these areas was not specified but was included under the headings of BRI’s “six primary research areas” (agriculture, energy, environment, health, manufacturing/bioprocessing and general foundations) and “two supporting components” (social impact research and infrastructure). Nevertheless, 5 pages of the 125 page report was devoted to marine biotechnology. Additional references to activities that could be considered marine biotechnology were scattered throughout the document. Estimates for FY 1992 funding of marine biotechnology by federal agencies were listed. The total federal investment in marine biotechnology research for FY 1992 was estimated at \$43.9 million. The section of the report on marine biotechnology concluded as follows:

"No area of marine biotechnology has yet been completely explored or exploited, and the breadth of research opportunities represented by these areas cannot be addressed adequately with current levels of investment. The Biotechnology Research Initiative is laying the foundation for the development of an integrated, expanded research effort." (Committee on Life Sciences and Health, 1992, p. 60)

In the Clinton administration, Vice President A. Gore was assigned major responsibility for developing the federal government's science and technology policies and to serve as liaison to Congress with respect to these matters. On February 22, 1993, President Clinton gave a speech on technology policy and the next day, the document *A Vision of Change for America* was released by the White House (Anderson, 1993). Later the same year the second FCCSET report was issued (Anonymous, 1994a), but FCCSET itself was to be eliminated as a Presidential initiative in 1995. The newly created National Science and Technology Council (NSTC) will take on many of the functions of the FCCSET. It will include a Biotechnology Research Subcommittee, to be chaired by Dr. Lura Powell from the National Institute for Standards and Technology (NIST).

The Clinton administration considers science and technology key to the future growth of the U.S. In general, it appears that the administration is focused on supporting "small" science, technology transfer, and cooperative projects between industry and government. About 10% of the \$100 billion proposed by Clinton for increased spending over the next four years was slated to support science and technology, including over \$2.3 billion for increasing the NSF budget over current projected budgets and \$1.2 billion for FCCSET "crosscuts" (including biotechnology). "Big" science projects, the space station, and DOE defense laboratories were projected to be losers in the budget. The biotechnology "crosscut" will be continued for FY 1994, but will be eliminated as a Presidential initiative for FY 1995. This comes as the Clinton administration's proposed increases for science and technology are being hammered by Congress.

While the Clinton administration's view of the ocean sciences is

not yet discernible, biotechnology will continue to be a high priority area whether or not the inter-agency "crosscut" stands. The Vice President, in the past, has shown interest in biotechnology, outlining his general position on biotechnology issues in late 1991 (Gore, 1991). In the first instance, the Vice President will concentrate on regulatory issues, perhaps to institute a unified federal regulatory approach towards field testing of genetically engineered organisms and biotechnology-derived foods (Fox, 1992). Based on what he has written, Mr. Gore can be considered a very cautious proponent of biotechnology, borne out by some of his writing and latest pronouncements (Gore, 1994).

How biotechnology will fare during FY 1995-FY 1997 is not yet clear, but as this is written the President's budget projects increased support for this field. In addition, the Presidential Office of Science and Technology Policy (OSTP) has requested that the NSTC's Biotechnology Research Subcommittee prepare a report on issues and opportunities in biotechnology. The report is expected to be released in 1995 and will highlight agriculture, environment, manufacturing/bioprocessing, and marine biotechnology (Grimes, 1994). Somewhat concurrently, several executive agencies, including DOE, NOAA, and NSF, are sponsoring a study undertaken by a committee of the NRC's Commission on Life Sciences entitled "Opportunities for Advancement of Marine Biotechnology in the United States." The committee, headed by Dr. Michael Greenberg, Whitney Laboratory, University of Florida, is tasked to:

examine the importance of marine biotechnology and related research and development policy issues to provide a comprehensive perspective on the current and future direction of marine biotechnology in the United States. The committee will identify and assess opportunities for development of marine biotechnology; research, support, and infrastructure needed to meet these opportunities; and obstacles to the advancement of marine biotechnology in the United States. (NRC, 1994)

The committee is expected to issue its report in late 1995.

Congress

Thirty of the 50 states constituting the U.S. are coastal states (including those bordering the Great Lakes). In 1992, these states were represented by 60 of 100 senators and 355 of 435 House representatives (167 representatives came from districts that touch on an ocean or a Great Lake). Therefore, a fairly strong ocean constituency exists in Congress. Over time, a strong Congressional bipartisan consensus on the U.S. ocean policy had evolved, which was based on several assumptions, including: (1) the U.S. must maintain a strong science and technology capability in the oceans; (2) academic institutions are collectively a critical source of expertise and ideas and, as alternative funding sources are lacking, the federal government must bear the major burden for maintaining oceanographic capabilities at these institutions; (3) the role of the federal government in marine resource management is to balance competing claims between preservation, conservation, and development; and (4) the oceans are vital to national security, hence there are powerful incentives to safeguard their international character.

Due to the efforts of a small group senators and congressmen who sought to maintain a strong U.S. capability in the ocean sciences, most of the attempts by the Reagan administration to curtail NOAA programs were deflected and funding for NURP and the Sea Grant program were inserted in each year's budget. The overall effect was that NOAA funding remained level, in absolute terms, but actually suffered progressive diminution, because of inflation. Between 1980 and 1990 the oceans programs are estimated to have lost more than \$500 million due to inflation (Kitsos, 1988). Some programs received no funding; for example, the 1980 National Aquaculture Act, potentially a boon to U.S. aquaculture, was not funded (although the USDA has increased its funding in this area). In effect, Congress shifted the emphasis of ocean science programs from ocean/coastal/fisheries to atmosphere and satellites. To illustrate, in 1980 the first category received 46% of NOAA's budget; in 1988 just 36% (Kitsos, 1988).

In the Reagan administration's last year and during the Bush administration, interest in the oceans was rekindled in Congress. NOAA was allocated \$613 million over ten years to replace its aging research vessel fleet, and there was a new willingness to strengthen NURP and Sea Grant. A hearing was held in 1989 on the future of aquaculture and marine biotechnology in the U.S. (United States Congress, 1989), but it did not lead to Congressional action until 1993, in the early months of the Clinton administration, when legislation on marine biotechnology authorizing \$20 million in FY 1995 and \$25 million in FY 1996 was introduced in the House and Senate.

Congress has shown a high level of interest in biotechnology since the late 1970s, when the safety of recombinant DNA became a public issue (Zilinskas and Zimmerman, 1986). Beginning in 1979, Congressional committees requested OTA to undertake a series of studies on biotechnology. The first, completed in 1981, explained biotechnology to non-technical persons and delineated its expected impact on American society (OTA, 1981). During subsequent years, OTA studies dealt with biotechnology patents, field testing of genetically engineered organisms, public perceptions of biotechnology, and other subjects (but not marine biotechnology). An OTA study released October 1991 examined the impact of biotechnology on major industrial sectors and efforts taken by governments of 16 nations to influence the development of biotechnology in their countries (OTA, 1991a). It is reasonable to conclude that, as a result of the OTA studies, as well as position papers by the Congressional Research Service, Congresspersons and their staff are well acquainted with biotechnology. Further, this acquaintanceship is in general positive, since Congress voted to support federal programs funding biotechnology research.

Conversely, it is curious to note that the OTA studies, each of which contains a set of options for congressional action, have not been followed up by legislation. That is not to say that bills have not been introduced; several have, dealing with a variety of issues, such as bioinformatics, patents, testing, etc. However, with minor exceptions,

Congress appears to be content with following the Executive lead, regarding government initiatives in biotechnology, as well as science and technology, in general. For example, the funds Congress appropriated for R&D in FY 1992 were within .01% of that requested by the Bush administration (Norman, 1992).

Congress made some attempts to cut the federal budget in the waning days of the Bush administration, when the 1990 budget agreement with Congress was to be implemented, dictating spending caps on three separate categories for FY 1993 (Long, 1992). This was taken up by Congress, in the Clinton administration's first year. Following the Clinton administration's lead, Congress has viewed "big ticket" items, such as the Superconducting Super Collider and the Strategic Defense Initiative with a jaundiced eye, because of the need to cut the budget, but funding for most agencies supporting R&D, including NSF and National Institutes of Health (NIH), will increase. NIH is scheduled to receive only a very modest overall increase, with the biotechnology interagency crosscut uncertain.

In early 1993, Representative G.E. Studds introduced a bill, called "The Marine Biotechnology Investment Act of 1993" (H.R. 1916), which would provide matching project grants through the National Sea Grant College Program (Anonymous, 1994b). At the time, Studds noted that the federal government spends over \$4 billion per year to support biotechnology, but only about 1% of that funding supports marine biotechnology. In late 1993, the Senate Commerce Committee approved the Act, which stipulated that the President's science advisor develop a ten-year national strategy for marine biotechnology and authorizes \$32 million in funding to NOAA (including \$20 million to Sea Grant) to support marine biotechnology research and other activities (Anonymous, 1993). The Act was passed in 1994.

PUBLIC AND PRIVATE FUNDING OF MARINE BIOTECHNOLOGY R&D

Two methods were used to determine the amount of money governments and private agencies provide to support marine biotechnology R&D. The first was to consult official sources, such as

annual reports issued by agencies, OTA studies, and the 1992 and 1993 FCCSET Reports, and extract information relating to marine biotechnology. While this is relatively easy to do, this method has a serious shortcoming—the analyst is entirely dependent on the official source's determination of what constitutes marine biotechnology and how the reporting agency calculates the amount of funds for research in this field. The following example illustrates the problem in achieving accurate tabulations. Certain types of research funded by the USDA that we would think of as aquaculture research was designated marine biotechnology by that agency, while some research supported by the NIH's National Cancer Institute (NCI), which we would consider marine biotechnology, was termed "natural products chemistry" by that institute.

The second method was to define marine biotechnology R&D and marine biotechnology-related R&D (see Introduction), then ask each researcher how much outside support he or she received for research in each category and from whence the funding came. This was done to the extent possible and the information collected was entered in the MARBIO database. Analysis of the MARBIO data and projection of the findings to cover the entire U.S. research establishment permitted determination of the funding amounts and patterns for the period FY 1990-1991. This method also has shortcomings. Specifically, the analyst is dependent on the veracity of the researchers interviewed and their ability to discern research activities according to the definitions formulated. In addition, since less than 50% of the researchers conducting marine biotechnology R&D were actually interviewed, sampling error can bias the analysis when results are projected to represent the entire marine biotechnology research community.

Funding of marine biotechnology is discussed in the following three sections. The first addresses the major source of public funding, namely, the U.S. government acting through federal agencies. The second focusses on state governments, which provide funds for research, but at much lower amounts than the federal government. Private sources, including industry and nonprofit funding agencies, are discussed in the third section.

Federal Funding

Each subsection that follows, except the last, covers one agency. In the first part of each subsection the amount the agency in question spends to support biotechnology, in general, is quantified; second, we express funding that the agency under consideration officially claims that it assigned specifically for marine biotechnology; and, third, amounts of funding determined by the analysis of the MARBIO data. For illustrative purposes, discussion of selected agencies includes examples of the types of marine biotechnology R&D they have funded.

Department of Commerce (DOC)

In FY 1992, the DOC spent an estimated \$13 million on biotechnology research; this sum remained roughly the same for FY 1993. In FY 1992, NOAA was estimated to support marine biotechnology and aquaculture R&D at about \$5.8 million (Grimes, 1991; Committee on Life Sciences and Health, 1992). MARBIO data indicated a similar amount of NOAA funding, circa \$6.1 million.

The NOAA Sea Grant College program was key, in that the National Sea Grant College program aims to direct resources of the nation's universities for wise use of its marine resources (Ragotzkie, 1988). At present, more than 150 institutions in 35 states and Puerto Rico participate in the Sea Grant College program.

Sea Grant has identified marine biotechnology as a program area and employs a specialist at the national headquarters who is assigned to monitor grants in this field (OTA, 1988). Out of its 1989 annual research budget of approximately \$36 million, an official source stated that Sea Grant allocated circa \$2.3 million to marine biotechnology, exclusive of aquaculture (Sea Grant funding for marine biotechnology is not presented in the BRI) (Attaway, 1990). All Sea Grant funds have a multiplier effect, since they must be matched by nonfederal support. Thus, \$1.7 million from various sources should be added to the Sea Grant funding, for a total of \$4.1 million for marine biotechnology R&D derived from this agency. Another

approximately \$5.3 million total was directed to aquaculture research.

Analysis of the MARBIO data indicated that Sea Grant contributed \$4.58 million, or approximately 8.8% of the funds allocated by federal funding agencies, for marine biotechnology R&D. This sum, considerably less than provided by NIH and NSF (see Figure 10 in Chapter 2), placed Sea Grant as the third largest contributor to marine biotechnology R&D.

Of the \$2.3 million Sea Grant reported it provided for marine biotechnology R&D, \$837,000 was spent on biochemistry and pharmacology, \$710,000 on genetic engineering projects (DNA technology applied to microorganisms, algae, finfish, and mollusks), \$361,000 for biochemical engineering (reactors and instrumentation), and \$397,000 for microbiology, phycology and physiology. Although Sea Grant funding for research grants is relatively small, ranging from about \$4,000 to \$93,000 and averaging approximately \$40,000 annually per individual investigator, the results have a significant local impact. For example, a Sea Grant research project begun in Maine on the American oyster *Crassostrea virginica* led to results that were applied in another Sea Grant research project in Washington State on the Pacific oyster *Crassostrea gigas* (Allen Jr., 1988). The first project resulted in the development of triploid oysters (oysters having three times the haploid number of chromosomes), which are sterile. The techniques developed in the Maine project were then applied by oyster hatcheries in the Pacific Northwest to produce triploids. The advantage of triploid oysters have over their wild relatives are that they grow larger and available for harvesting at times of the year when wild oysters cannot be taken. For these reasons, triploid oysters have become a major contributor to the economy of the area, representing approximately 50% of the total hatchery production of oysters in the Pacific Northwest.

In addition to Sea Grant, the National Undersea Research Program (NURP) undertakes projects that include marine biotechnology-related components. NURP, an agency established in 1980, has proved popular with Congress, which has increased its funding from

an initial \$3.3 million to \$16.3 million in 1991, even though the program was opposed by the Reagan and Bush administrations. NURP supports undersea research projects, including studies on coral reefs, submarine volcanoes, and thermal vents on the ocean floor. None of the research institutions surveyed for MARBIO reported receiving support from NURP. It is, therefore, probable that research supported by NURP would not fall into a strict definition of marine biotechnology. However, NURP funds projects that contribute to marine biotechnology. For example, organisms collected from undersea sites are being screened for natural products in marine biotechnology laboratories.

In addition to Sea Grant, NOAA provided circa \$1.7 million for marine biotechnology R&D in FY 1992. The MARBIO estimate of the non-Sea Grant NOAA funding was \$1.52 million, most to fisheries research, i.e., in 1992 NOAA made available \$1 million for research aimed at strengthening and developing the U.S. fisheries industry. Research topics in marine biotechnology included marine biotoxin fishery safety, microbiological safety of fishery products, developing new, low-cost methods to monitor environmental contaminants in fishery products, and developing environmentally sound methods to utilize byproducts and fish wastes. More specifically, an additional \$1.8 million from NOAA was designated for research to improve Gulf of Mexico and South Atlantic fisheries. However, additional fisheries-related research included fish stock assessment and tracking and enumerating pelagic fish, reef fish, coastal herring and groundfish.

Department of Defense (DOD)

DOD provided an estimated \$81 million in FY 1992 for biotechnology R&D (Committee on Life Sciences and Health, 1992). The total amount of DOD funds directed to marine biotechnology was not determined. However, ONR, the U.S. Navy's research arm and the major funder of marine biotechnology within DOD, officially was estimated to have spent \$7 million on marine biotechnology (Grimes, 1991). Data in MARBIO indicated that

ONR provided \$3.09 million, or approximately 7.2% of the total federal marine biotechnology research funding, during 1990-1991.

The ONR supported "molecular genetics," using its terminology, at a level of \$2 million, including basic research, not tabulated as marine biotechnology by the MARBIO interviewees. Also, the ONR includes in its estimate of marine biotechnology funding construction of facilities, fellowships, and other training activities. MARBIO data, however, reflect funds spent directly to support marine biotechnology R&D.

ONR interest in marine biotechnology is pursued mainly through the Biological Sciences Division, which had two programs in FY 1991-1992, Molecular Biology and Systems Biology. The objective of the first was "to extend our basic understanding of the principles of biological processes at the molecular level, with an emphasis on marine organisms," while the second was "to support basic research on responses of complex organisms, particularly man, to the environment" (Office of Naval Research, 1989). Thus, ONR funds extramural basic and applied research at universities and naval laboratories, focussed on biochemistry, marine microbiology, biopolymers, biosurfaces, extreme environmental habitats, and molecular recognition. ONR can support high risk research projects which otherwise would probably go unfunded. However, once a risky project shows promise, ONR encourages the investigator to seek funding from conventional sources (Alberti, 1990). Three categories of ONR funding are available for marine biotechnology research.

6-1 Funding for Basic Research. According to ONR, approximately \$2 million per year, including \$150,000 for training, was provided for basic research in FY 1991-1992, e.g., investigation of biochemical and physiological functions of marine organisms. In particular, ONR funded research that sought to clarify mussel adhesion, biofouling phenomena, formation of biofilms (Byers, 19), and novel processes carried out by marine organisms, including formation of biopolymers. Major recipients of 6-1 funding in 1989 were: the University of Maryland Biotechnology Institute Center of Marine Biotechnology (COMB), Baltimore, MD; University of

California Scripps Institute; University of Delaware; University of Southern California; University of Texas; and University of Washington. In addition, ONR funded training courses relevant to marine biotechnology at Duke University, the Woods Hole Oceanographic Institution, Woods Hole, MA, University of California at Los Angeles, and other higher education institutions.

6-2 Funding for Exploratory Development. Approximately \$700,000 was provided by ONR in 1989 for R&D likely to lead to commercialization of 6-1 project findings. As the title of the program suggests, this activity set the scientific/technical basis for production. Recipients of 6-2 almost invariably worked with industry, and industrial concerns were also eligible for funding. Several projects that started out under 6-1 advanced to 6-2. Some progressed further and are now being commercialized. Commercial products that have been realized (although still in the testing stage) are several biopolymers from marine organisms and a mussel adhesive. The last, developed by Dr. Herb Waite at the University of Delaware, was tested for use as an artificial skin and glue in bone surgeries. Another project close to commercialization involves a natural antifouling compound for treating hulls of ocean-going vessels or adding to paints used on boat hulls. Unlike anti-fouling paints previously available, which contain heavy metals, this compound is both nonpolluting and nontoxic for workers. Other 6-2 projects of commercial interest include compounds from marine organisms that offer possibilities as blood substitutes, immunological adjuvants, agents to speed wound healing and anti-inflammatory compounds.

University Research Initiatives (URIs). The URIs is a tri-service program, that was funded at a level of approximately \$230 million in 1992, that aimed "to strengthen the capabilities of the universities to perform research and to educate scientific and engineering personnel in key disciplines important to the technologies that underlie a strong national defense" (Office of Naval Research, 1987). Of the three services, only the U.S. Navy supports URIs related to

marine biotechnology. Through this program, the ONR assisted in the establishment of scientific centers of excellence in areas that were poorly funded but that ONR believes to be important. Marine biotechnology was included in this category. Accordingly, in 1986 ONR supported establishment of two centers: COMB (circa \$1 million per year for five years—see below) and a joint University of Tennessee/Scripps Institute of Oceanography Center. The latter, supported at about \$750,000 per year, included investigative programs on biofouling, protein dynamics, and the effect of pressure on marine organisms and gene expression. A third URI supports a joint effort between the California Institute of Technology and the University of California at San Francisco, focussed on investigating the effect of pressure on marine organisms and gene expression, funded at circa \$750,000 per year.

The U.S. Navy funds two other programs that touch on marine biotechnology, but whose impact cannot yet be assessed. The ONR Young Investigator Program, which supports 14 academic researchers for three years with each investigator receiving \$75,000 per year in research support (marine biotechnology is an eligible research area); and the Office of Naval Technology postdoctoral appointments at naval laboratories, of which approximately 40 were awarded per year, with length of service one or two years and individual stipends ranging from \$36,000 to \$52,000 per year. Again, marine biotechnology is considered an eligible field of study.

Department of Health and Human Services

The major funders of biotechnology R&D within the DHHS are the NIH and, to a lesser extent, the Food and Drug Administration (FDA).

NIH. In 1992, the NIH total budget was nearly \$9 billion and of this sum, the agency spent approximately \$2.8 billion for biotechnology R&D (Committee on Life Sciences and Health, 1992). The amount the NIH spent on marine biotechnology R&D in 1992 was officially estimated to have been \$11.9 million (Grimes,

1991), correlating well with MARBIO data, which estimated NIH (including NCI) funding to be \$10.54 million for marine biotechnology R&D. This is 24.5% of the total funds supporting researching in marine biotechnology. Thus, NIH has been a major source of support for marine biotechnology research in the U.S. In addition, NIH supports marine biotechnology research in other countries. NIH undertakes or funds marine biotechnology-related research within several of its subsidiary centers and agencies. In biochemistry and chemistry, there are about 12 programs related to investigation of bioactive compounds. In the infectious disease area, a few programs are funded which relate to antibiotic discovery and development, as well as cardio-vascular and cardio-active compounds. However, the major funder of marine biotechnology R&D within the NIH is the NCI.

FCCSET estimates indicated that the NCI spent about \$8 million on marine biotechnology of its total \$1.7 billion FY 1992 budget. MARBIO data are at variance, however, with that estimate, indicating NCI funding of \$3-4 million for marine biotechnology, including marine collections. This discrepancy can be understood after examining projects funded by NCI. As discussed below, NCI supports significant marine biotechnology-related R&D carried out by foreign researchers, the data for which are not captured by MARBIO.

While the sum spent on U.S. marine biotechnology by NCI is relatively small, it is, nevertheless, very important, because it is so strongly targeted. All NCI funding in the marine area is allocated to natural products R&D. NCI has sponsored development of natural products agents for more than 30 years. Among the drugs that have resulted from research supported by this program is taxol, one of today's most promising anti-cancer drug. In addition, NCI scientists have performed clinical research on a host of anti-tumor drugs from natural sources, including adriamycin, bleomycin, etoposide, mitomycin C, vinblastine and vincristine. In 1986, NCI established the Natural Products Repository, wherein are stored extracts from plants collected in 20 tropical countries, as well as marine micro- and macro-organisms, collected mainly in the Indo-Pacific region. NCI

is extremely interested in bioactive compounds that have potential as anti-cancer, anti-bacterial and anti-viral drugs. The primary mission of the NCI's Developmental Therapeutics Program (DTP) is the discovery and preclinical development of promising anti-cancer drugs. To further its mission, the DTP funded four major marine biotechnology-related projects:

The Australian Institute of Marine Sciences (foreign bidders have equal standing to those in the U.S.) received a contract providing total NCI funding of \$2.5-3 million for three years. Over 3,000 organisms were collected between 1986 and the end of 1991, mostly from the Great Barrier reef and New Zealand coastal regions, and were screened and tested by NCI.

A second NCI project at the University of Hawaii focused on natural products from cyanobacteria, beginning in September, 1986, and ending August, 1991. It was funded to a total of \$1.2 million for five years. The contractor propagated 1,000 species of cyanobacteria over the five years of the project and collected 100 mg aqueous extracts and 100 mg organic extracts of each species. These extracts were submitted to NCI for screening. Of the approximately 300 extracts submitted, one glycosulfolipid compound shows promising anti-HIV activity (Collins, 1990).

A third project was directed at protozoans, including microalgae, started in April, 1989, when the Martek Corporation, Columbia, MD, was awarded a contract to cultivate 200 protozoan species per year for three years with total NCI funding of \$800,000. As in the foregoing cases, the contractor cultivated sufficient quantities of organisms to produce two 100 mg extracts for screening. Martek procured requisite strains from the American Type Culture Collection and scientists located in various laboratories throughout the U.S.

A fourth NCI-funded marine biotechnology project investigated marine anaerobes. The contractor was the Michigan Biotechnology Institute; an independent, tax-exempt, applied R&D corporation, involved in mostly industrial and environmental biotechnology. The work on marine anaerobes, which began April 1991, was funded at \$800,000 for three years. The contractor propagates anaerobic

bacterial species, collecting the cell mass from each, and prepares extracts from the cell biomass produced.

The NCI Division of Cancer Treatment administers the National Cooperative Drug Discovery Group Program (NCDDG), the rationale for which is that efficacious research, development, and evaluation of a new treatment requires an interdisciplinary team. It is, therefore, useful for the NCI to assist in the mobilization of creative scientists, regardless of affiliation, into a unit where interdisciplinary cooperation is promoted. Often this means that scientists from both university and industry partake in joint projects. The first two NCDDG groups were formed in 1984; their research focussed on investigations related to the general mechanism of action-based anticancer treatment. By April, 1990, 12 groups had been set up, including two that are marine biotechnology-related (Suffness, 1991).

The first group aimed at discovering new anticancer drugs from cultured and collected marine organisms, including sponges, mollusks, macroalgae, marine bacteria, corals and microalgae. Approximately 3000 extracts were collected and examined each year. The Principal Investigator is J.C. Clardy, Cornell University, and the team includes researchers from Scripps Institute of Oceanography, University of Rhode Island, University of Utah and the Bristol Myers Company. The project will be carried out during 1989-1994.

The second NCDDG group established an objective of discovering antitumor natural products from marine invertebrates, microorganisms, microalgae, and symbionts. Among the specific aims was to identify marine organisms that produce chemicals structurally different than those obtained from terrestrial sources. The Principal Investigator of the group is Dr. P.O. Crews, University of California at Santa Cruz and includes researchers from Oregon State University, University of Oklahoma, and Syntex Corporation. This project runs from 1990 through 1995.

With respect to commercial possibilities of drugs discovered in the course of NIH-supported research, the NIH-supported grantee or contractor has primary patent rights to them. Similarly, suppliers of drugs submitted to the NCI for screening retain patent rights. However, NCI may patent discoveries resulting from internal NCI

research. Further, if the primary person or organization chooses not to exercise these patent rights, the NCI can apply for a patent on the discovery.

In case of patents resulting from internal NCI research, the patent is issued in the name of the NCI researcher(s) who discovered the compound and the NCI. Thereafter, the compound is made available to industry for development. The industry retains profits from commercial successes, if any, except for a share that accrues to the NCI researcher/inventor, who may realize a maximum of \$100,000 per year from any one invention.

FDA. The FDA is officially estimated to be spending \$3.4 million on marine biotechnology, \$3.1 million on molecular genetics, and \$0.3 million on aquaculture (Grimes, 1991). From the MARBIO data, we concluded that the FDA provided research scientists interviewed in our own survey with a total of \leq \$0.13 million. The discrepancy in this case is readily explained; the FDA spends almost all of its funds on intramural research, mainly food safety, including research on marine toxins. Specifically, the FDA Center for Food Safety and Applied Nutrition performs research to develop efficient methods to detect, identify, and quantify pathogens associated with seafood, including *Aeromonas hydrophila*, *Listeria monocytogenes*, vibrios, and gastrointestinal disease-causing viruses. In addition, the FDA supports studies on the incidence of antibiotic resistant pathogens in catfish aquaculture facilities, including the ability of plasmids to transfer resistance among various bacterial species (Salsbury, 1991).

National Science Foundation

NSF's mission is to foster research and education in all fields of science and engineering and promote the interchange of scientific information among scientists and engineers in both the U.S. and internationally. NSF's mandate is to ensure a continuing flow of fundamental knowledge without the constraint of a mission of goal-oriented agenda. Thus, the NSF, with a total budget of \$2.7 billion in 1992, was at that time and remains today the largest funder of basic

research in the U.S. In 1992, NSF provided circa \$178 million for ocean science research activities, funding approximately 70% of all basic ocean science research in the U.S. (Clark, 1993), and spent an estimated \$174 million for general biotechnology R&D (Committee on Life Sciences and Health, 1992). NSF is fourth in the FCC-SET list of agencies supporting biotechnology R&D, far behind NIH but just slightly after DOE and USDA in total expenditures.

Marine biotechnology has been supported mostly through the NSF's Biological Oceanography Program and Polar Biology Program and, to a lesser extent, Medicine Program. The Biological Oceanography Program has supported two workshops that focussed on marine biotechnology: "An Initiative for the Accelerated Transfer of Biotechnology to the Ocean Sciences," held September 16-18, 1988, in Tucson, Arizona (Anonymous, 1990), and "Molecular Marine Biology and Marine Biotechnology Applied to Oceanographic Problems," held March 2-3, 1990, in Monterey, California (which was co-sponsored by the Ocean Studies Board of the National Research Council) (report is as yet unpublished). Both workshops strongly recommended an increased effort in U.S. marine biotechnology.

More directly, the Biological Oceanography Program began in 1989 to invest \$500,000 per year in support of a competitive fellowship program, encompassing both post-doctoral and senior researchers, to facilitate the transfer of biotechnology methods to the areas of ocean sciences and marine ecology. This program also supports the acquisition of equipment for support of marine biotechnology at marine laboratories and field stations. More recently, marine biotechnology was identified as one of five focus areas for the NSF 1993 biotechnology initiative (Committee on Life Sciences and Health, 1992); i.e., it was identified as a growth area in which the NSF has substantial strengths but which is currently under-funded relative to its potential promise (Duguay, 1994).

According to official sources, NSF spent an estimated \$9 million to support marine biotechnology research in 1992 (Grimes, 1991), but an analysis of MARBIO data indicates a lesser figure, i.e., we found that the NSF spent \$6.19 million for this purpose. The latter figure would mean that the NSF paid for 14.4% of all federally sup-

ported research in marine biotechnology. The discrepancy between the two estimates most likely derives from different definitions of marine biotechnology.

U.S. Department of Agriculture (USDA)

The USDA began a competitive grants funding program already in 1981, which grew from approximately \$17 million to \$40 million in 1986. However, total USDA funding for biotechnology in 1992 was \$179.4 million (Committee on Life Sciences and Health, 1992). According to official estimates, the USDA spent approximately \$2 million for molecular genetics and \$4.1 million for biotechnology as related to aquaculture and mariculture (excluding the operation of the National Aquaculture Library in Beltsville, MD) (Grimes, 1991). However, MARBIO data indicates that USDA-funded marine biotechnology R&D was \$3.09 million, or 7.2% of the U.S. marine biotechnology R&D funding in 1992. The most likely explanation for the variance is that the definition of marine biotechnology used to compile MARBIO data differs from that of the USDA; also possible is that the USDA included support for some aquaculture research under the marine biotechnology category.

Department of Energy (DOE)

Total DOE funding for biotechnology in 1992 was \$182 million, including circa \$59 million for the Human Genome Project (Committee on Life Sciences and Health, 1992). DOE funded marine biotechnology at an estimated level of \$560,000 in 1992. In addition, through its Division of Biological Energy Research, it spent an estimated \$2 million on marine algae research, to investigate the promise these plants hold for energy production. Recently, DOE inaugurated a molecular biology initiative within its Ocean Margins Program, and funding for this initiative was set at nearly \$3 million for FY 1994 (Grimes, 1994).

It also bears mentioning that, in addition to the foregoing, the DOE sponsors an extremely interesting and innovative program, called "Deep Probe," as part of its Subsurface Science Program. This Program commenced in 1986 and has funded the collection and

preservation of organisms from subsurface sites, e.g., more than 7,000 microbial organisms are being maintained under this program by Dr. D. Balkwill at Florida State University and by Dr. D. Boone at the Oregon Graduate Institute (Anonymous, 1991b; Grimes, 1994). Research scientists have access to these cultures for investigations of their potential pharmaceutical properties, their ability to degrade and decontaminate pollutants, or other purposes.

Other Federal Agencies

In addition to the federal agencies listed above, other agencies fund marine biotechnology-related R&D, albeit at lower levels. Other federal agencies are officially estimated to have invested a total of \$1.38 million in 1992 on marine biotechnology R&D (Grimes, 1991), e.g., the U.S. Environmental Protection Agency (EPA) spent \$550,000 and the U.S. Fish and Wildlife Service \$270,000. These official estimates compare well with our findings from MARBIO data, which indicated that approximately \$1 million was spent by other federal agencies on marine biotechnology research, comprising 2.3% of the total funding (MARBIO).

Small Business Innovative Research Program

A crosscut program; the Small Business Innovative Research (SBIR) program, whose scope encompasses all major agencies, derives from the Small Business Development Act of 1986 (PL 99-443) which enjoins federal agencies that spend more than \$100 million per year on extramural research to set aside 1.25% of those funds for the SBIR program. The SBIR program was reauthorized in 1993 for five years.

Only companies having fewer than 500 employees and owned at least 51% by U.S. citizens are eligible to apply for SBIR funding. The program constitutes three phases, as follows. Phase I covers projects that are selected for scientific merit and feasibility. Awards are for up to \$50,000 and usually cover no more than six months. Phase II projects are for development of Phase I results. Awards are for up to \$500,000 for no more than two years. Phase III projects are those

that are being commercialized and require private, non-SBIR funding sources. The largest contributor to the SBIR program is the NIH, which spent \$73 million for this purpose in 1990 (OTA, 1991b). The SBIR program, in fact, has provided major funding for risky and/or innovative research performed by small firms in the U.S. and serves to encourage the development of an expanded business base on new technology. By 1991, the SBIR program funded about 15,000 projects at a total cost of about \$1.8 billion.

According to the OTA, biotechnology companies have done well through SBIR, although the exact criteria for success are not specified (OTA, 1991b). The FCCSET Report does not list SBIR funding for biotechnology, nor does any other official source to date. Several biotechnology companies included in the MARBIO survey have had projects funded by the SBIR program, but company officials have been reticent about releasing details of research funding. The MARBIO data indicate funding to the extent of \$2.86 million from "Other Federal Sources," a sum which probably is, to a great extent, SBIR funding.

State Funding

States spent a total of approximately \$1.2 billion in 1988 to support R&D, less than 1% of the total U.S. outlay for R&D (Fleisher, 1990). Most of this funding went for health-related R&D (\$285 million); research aimed at developing natural resources was ranked second. Most state funds come from general funds. Interestingly, thirty-three states actively promote biotechnology, spending about \$150 million per year for this purpose (OTA, 1988). About \$2.8 million, or 6.5% of the total state funding for R&D, supported marine biotechnology in 1991. Three states allocated funds to establish marine biotechnology centers, as components of larger institutions and for other endeavors promoting marine biotechnology.

California

The state of California's major initiative for promoting biotechnology is the System-Wide Biotechnology Research and Education

Program, administered through UCLA. Funded at approximately \$2 million per year, it provides seed money for research projects and training programs. Six projects were funded in 1992, bringing the total number of grants funded by the Program to 56. Funds provided by the Program must be matched, in part, by the recipient. If salary equivalent of instructors is included, the ratio is about 3 parts from the state to 1 part from the university. Program grants have provided seed funds for establishment of several new research centers within the University of California system, including the Marine Biotechnology Center at the University of California at Santa Barbara, which received about \$600,000 over four years to support, in part, training of graduate students and post doctoral positions.

The University of California at Santa Barbara Marine Biotechnology Center is a component of the Marine Science Institute. Other components are the Coastal Resource Center and Marine and Coastal Policy Center. The Marine Biotechnology Center on the edge of the Pacific is housed in a building completed in 1989 at a cost of \$7 million, plus \$1 million for equipment, paid mainly for by the State of California. As this is written, a second large research program dedicated to marine biotechnology has been established in California, namely the Center for Marine Biotechnology and Biomedicine, University of California at San Diego. This center will host a seminar called "Marine Biotechnology: Emerging Economic Opportunities for California" during October 1994, which is organized by the California Consortium for Marine Biotechnology.

Maryland

The Center of Marine Biotechnology (COMB), one of five centers constituting the University of Maryland Biotechnology Institute (UMBI), was established on July 1, 1985. Until completion of the Columbus Center (see below) it has been located on the Harbor Campus of the Community College of Baltimore in the Inner Harbor of Baltimore.

Facilities housing COMB were provided using \$1 million from the Maryland Industrial and Commercial Redevelopment Fund and \$112,000 from the City of Baltimore. Present facilities comprise ap-

proximately 32,000 square feet of building space, housing 14 laboratories.

The permanent home of COMB will be the Columbus Center of Marine Research and Exploration (Columbus Center). The Columbus Center, under construction on Piers 5 and 6 of Baltimore city's inner harbor, is nearly complete at the time of this writing. Other components of the Columbus Center are the Center of Marine Archeology, the Maritime Museum, an exhibition area, and a training center. Total cost of the Columbus Center is \$160 million, of which circa \$55 million is dedicated to facilities for COMB. When fully operational, COMB will occupy 119,000 square feet of the Columbus Center and, by 1997, will be staffed by 213 scientists, graduate students, technicians, and support personnel.

In addition to funding COMB, the state of Maryland established the Maryland Industrial Partnerships (MIPs) program, which incorporates marine biotechnology projects. Under this program, funded at \$1.4 million in 1990, the State seeks to form partnerships between industries and the University in four fields: engineering; computing; life sciences; and physical sciences. The State funds joint cooperative R&D projects between a firm and a laboratory to a maximum of \$50,000 per year for one to three years if: (1) the firm in question supports 50% of the project; (2) the firm and laboratory have Maryland as their home base; and (3) the project is judged to have high potential for creating jobs within the state. Grants are highly competitive, thus specific proposals are critically reviewed.

North Carolina

The North Carolina Biotechnology Center was established in November 1981, making it the oldest center of its kind. In 1985, the Center formed a Marine Biotechnology Advisory Committee to develop a state program in marine biotechnology (Toyota and Nakashima, 1987). The Advisory Committee ended its work by recommending that steps be taken by the state to develop a strong marine biotechnology program, including making certain that steps are taken to "expedite application of research results for economic benefit to North Carolina."

The report of the Advisory Committee languished for five years (Anonymous, 1991a). It was not until January, 1990, that a small step was taken to realize one of its objectives. At that time, the Center for Marine Biotechnology in Botany, which is part of the University of North Carolina Center for Marine Science Research at Wilmington NC, came into existence. The Center's work was supported in 1992 by the NSF (\$110,000 per year); Sea Grant (\$60,000 for two projects); and the North Carolina Biotechnology Center (\$100,000 per year) (Bird, 1990).

Private Sources of Funds for Marine Biotechnology Research

Based on MARBIO data, industry provided \$2.11 million and private sources \$1.49 million for marine biotechnology R&D in 1992.

DISCUSSION AND CONCLUSION

Marine biotechnology is a relatively new component of biotechnology and, as such, has not yet been accorded the status of a distinct budget item by most agencies. R&D activities categorized in the MARBIO study as marine biotechnology are usually included under more well-defined biotechnology areas, e.g., agriculture (aquaculture), environment (bioremediation), and health (marine natural products). More rarely, marine biotechnology projects and programs may be found under the heading of marine biology or biological oceanography. The approach taken in this report to address these difficulties in defining marine biotechnology was to collect and analyze information pertaining to biotechnology-related and marine sciences activities funded by agencies, to determine which of these were marine biotechnology or marine biotechnology-related, according to the definitions given in the Introduction, and to quantify funding levels for these items.

As an aside, as mentioned above, recently a bill was introduced into Congress authorizing funding for marine biotechnology. We would expect that in order to clarify what kinds of projects can be

funded under this bill, it will be necessary for the government to decide on a definition of marine biotechnology. Thus, the uncertain situation we faced when performing this study may change soon.

Federal funding for all biotechnology research rose from \$3 billion in 1988 to \$3.76 billion in 1992, and reached \$4.07 billion in 1993 (CYTA, 1988; Committee on Life Sciences and Health, 1992). Estimates of how much of this total was spent on marine biotechnology R&D differ. One estimate is that between \$5 million and \$20 million has been allocated to marine biotechnology by government funding agencies (Pennisi, 1990), but the basis of this estimate is not clear and the MARBIO data do not confirm such a high estimate, at least up to FY 1991-1992.

An official estimate, prepared by the FCCSET Committee on Life Sciences and Health, indicated that the federal government provided \$44 million in FY 1992 to support marine biotechnology R&D (Committee on Life Sciences and Health, 1992). The methodology whereby this estimate was derived is straightforward, namely that each agency supporting biotechnology R&D was requested by the Committee to examine the projects it funds, identify those it considers marine biotechnology, and calculate the funding devoted to these projects. However, since there was no agreed upon definition for marine biotechnology, agencies differed as to how they designated projects. For this reason, we believe the Committee's final figure can be considered only a very rough estimate.

The estimate based on MARBIO data for the amount of federal funding supporting marine biotechnology R&D during 1990-1991 is \$33.4 million. In addition, marine biotechnology R&D was supported to the extent of \$2.88 million by state governments, \$2.16 million by universities, \$1.49 million by private sources, such as foundations, \$2.11 million by industry and \$0.98 million from other sources. As noted above, our estimate, derived from MARBIO data, is based on actual funding received from public and private sources by research laboratories throughout the U.S., including Puerto Rico and Guam.

The discrepancy of approximately \$10 million between official estimates and the MARBIO-based estimate of federal funding for

marine biotechnology R&D is significant and, therefore, should be accounted for. Unfortunately, closing the gap will require additional data gathering from recipients of funding and from donors themselves, followed by thorough analysis. In particular, funding patterns of three agencies where the greatest discrepancies were found (FDA, ONR, and USDA) need to be clarified. Discrepancies between the MARBIO and official agency estimates will probably be explained by how marine biotechnology is defined and what funding for R&D is included. In any case, the proportion of total funding, made available by the U.S. government to support R&D, that is spent on marine biotechnology, is shown in Figure 12. If the total is the official \$44 billion, the proportion is 0.057%, if it is \$33.4 billion as derived from MARBIO data, the proportion is 0.044%. As is shown in Chapter 8, the amount spent to support marine biotechnology in the U.S. is much less than that spent by Japan.

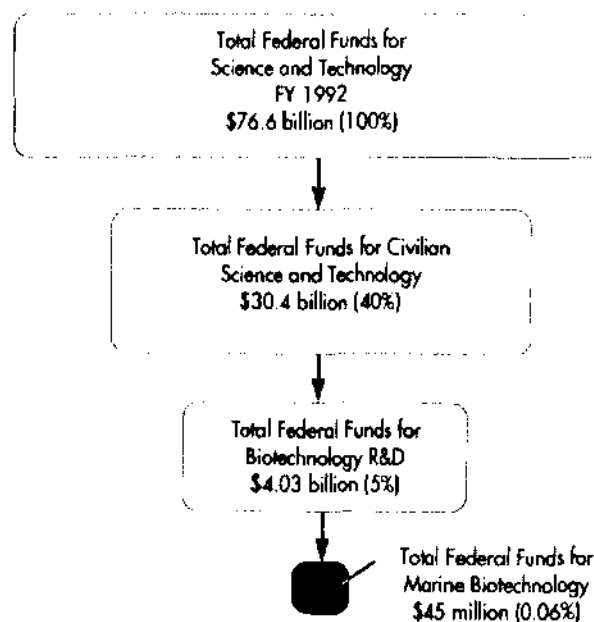


Figure 12. Trickle-down effect in U.S. biotechnology, 1992.

As to the future of marine biotechnology in the U.S., the Clinton administration has signalled a stronger stand on the ocean sciences, in general, than previous administrations. Also, the Clinton administration is likely to view biotechnology favorably, based on the possibilities the field has for technology transfer and commercialization. Whether the subset of biotechnology termed marine biotechnology will receive special attention from the administration is not yet known. Because economically significant applications from this field will be realized only in the longer term, it may be that the administration will support research endeavors in biotechnology that are likely to lead to quicker payoffs. If this was to occur, the U.S. would forego laying the robust scientific/technical base required for establishing a strong competitive position in a subfield of biotechnology that promises abundant returns beyond the year 2000.

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Chapter 4

ECONOMIC ASPECTS OF MARINE BIOTECHNOLOGY R&D

INTRODUCTION

Economic theory can be quite useful in evaluating marine biotechnology research and development. The role of the public sector in providing direct and indirect support for R&D can be clarified when it is examined through a cost/benefit analysis framework. An estimation of the potential for marine biotechnology to improve the overall welfare of the citizenry is an essential component of this type of analysis. Generally, when there is a high potential payoff from R&D, there is greater interest in public sector funding.

However, assessing the potential of an "emerging" technology is difficult, at best. Mistakes are commonly made when a sound framework for assessing the technology is not readily available. One mistake is to project linearly from the existing situation. For example, if a technology shows rapid expansion in a short time, the tendency would be to project future expansion at the same rate. The difference between the "technological optimist" and the "technological conservative" (OECD, 1989) is how the recent history of the technology is viewed, and the time frame they are projecting from. Another frequent error is to assume that all other technologies hold constant while the technology of interest is being analyzed. For example, aquaculture is usually embraced as an area where application of marine biotechnology should overcome the shortcomings of current methods of harvesting wild fish. What is often ignored in such an argument, is that depletion of wild fish stocks can (and in some

cases already has been) reversed by making technologically simple, but often politically difficult, changes in management practices. Two examples are the rebuilding of the Atlantic surf clam stocks, and the Chesapeake Bay striped bass fishery. In the clam fishery, a moratorium on new entrants was placed on the fishery along with an annual harvest quota and minimum size limit. More recently, individual transferable quotas have been assigned to fishing vessels. The surf clam stock once depleted is now harvested at near maximum sustainable yields. The Chesapeake Bay striped bass stock was so severely depleted that by the mid-1980s, Maryland's Department of Natural Resources completely closed down the recreational and commercial fisheries, and made it illegal for anyone to possess striped bass. The ban on fishing remained in effect for about five years, and now the striped bass stock supports a tightly managed recreational and commercial fishery.

The problem is that application of economic theory as a predictor and measurement tool works best in situations where there is no great structural and technological change occurring. This is not the case with marine biotechnology. What economics does offer is a logical construct within which one can understand the growth and development of marine biotechnology. By understanding this construct, or "framework," it becomes possible to affect change in the course of marine biotechnology development through carefully crafted policies. In this chapter, we focus on two frameworks that involve understanding marine biotechnology. One is the framework of the role of R&D in industrial development. The second framework is a broader view of product or industry development in general, the product life cycle. We apply the information gathered in the course of this study to these frameworks to further our understanding of the economics of marine biotechnology.

MEASURING R&D AND ITS RETURNS

Theoretical Considerations

One of several possible ways to view marine biotechnology in a collective and cohesive sense, is to accept the premise that the

knowledge derived from research is an intermediate product or input into the production of other goods. In order to conceptualize this approach, we modify the knowledge production function diagram (Figure 13) from Griliches (1990). Marine biotechnology research, measured by the level of research dollars expended, results in changes over time to the stock of economically valuable knowledge (K.). Patents (P) serve as an indicator of the number of inventions resulting from marine biotechnology research, recognizing that some inventions are not patented, and many others are patented but never developed. This increase in the stock of marine biotechnology knowledge contributes to expected or realized benefits (Z) where i corresponds to various industries, products or processes. For each product, there are a variety of observed (X) and unobserved and random (v) factors contributing to the level of benefits obtained. Benefits (Z) can be measured in a number of ways including growth, productivity, profitability or stock market value of a firm or industry. By following this conceptual approach for examining marine biotechnology, that is, by examining how effectively research

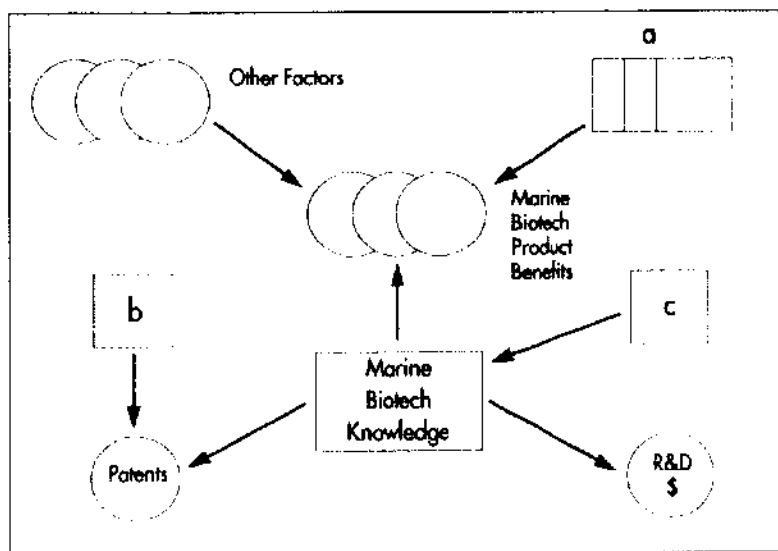


Figure 13. Conceptual model of marine biotechnology R&D. Source: Adapted from Griliches (1990).

dollars have contributed to our stock of knowledge, using patent activity as an indicator, we can assess the state of the field. However, if we are interested in ultimate benefits related to increases in the knowledge base of marine biotechnology, examination on an industry by industry or product by product basis will be required.

Marine Biotechnology R&D

Given the above framework for analyzing research expenditures and the empirical information provided by MARBIO, we analyzed the current results of marine biotechnology research at U.S. institutions. The procedures used were as follows.

Based on the survey data, total research expenditures in marine biotechnology in the U.S. was approximately \$65 million in 1991. Of that amount, \$40 million was being spent at academic institutions and only \$25 million by industry. According to survey respondents, funding would grow from \$50 million in 1988, to reach \$105 million by 1994, if no significant changes in funding policies occur in the interim (Figure 14). Growth in industry R&D is expected to be greater than R&D at academic institutions, so that by 1994, industry will be spending \$65 million, compared to academia's \$40 million. Data are presented in nominal dollars (not adjusted for inflation). In real terms, there has been no significant growth in funding for marine biotechnology research in the academic sector.

To verify the funding estimates, we used information provided by the National Sea Grant Office, the only federal agency listing marine biotechnology as a separate category. Since funding by the National Sea Grant Office in 1989 for marine biotechnology research at academic institutions was \$2.3 million (\$4.1 if matching funds are included), and data from survey respondents showed Sea Grant providing 10.7% of total funds, an estimated budget for marine biotechnology was between \$22 and \$38 million for 1989. The estimate from the MARBIO data for 1989 was approximately \$36 million, within the range estimated from the National Sea Grant Office data.

By extrapolating back from the survey results for budget growth during the last three years, assuming that prior to 1984 there was no

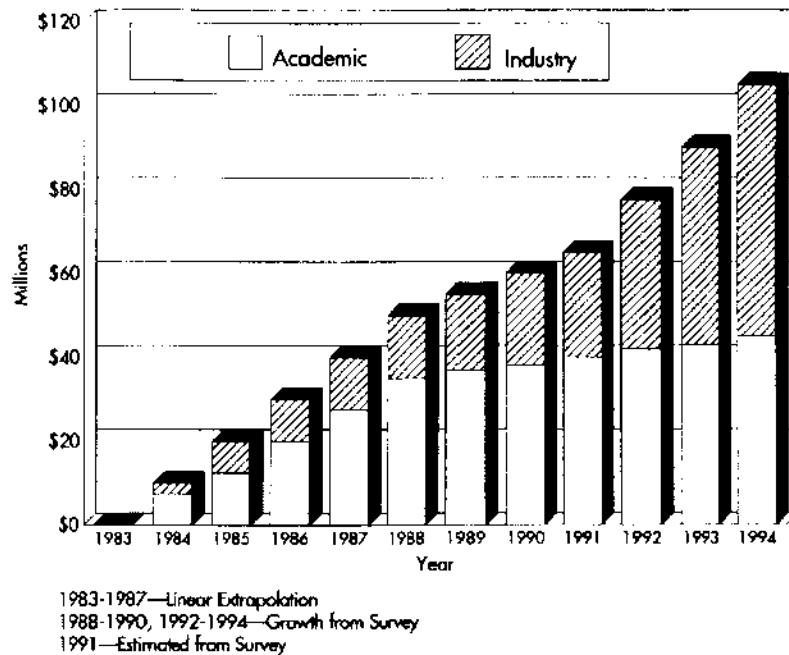


Figure 14. Marine biotechnology research funding in the United States.

funding targeted for marine biotechnology research, since a commonly accepted definition of “marine biotechnology” was not formulated until 1983 (Colwell, 1983), we developed a complete funding history for marine biotechnology research in the U.S. Based on results of this analysis, the U.S. has expended \$218 million through 1991 on marine biotechnology research at academic institutions, and private industry expended \$114 million.

The figure of \$218 million estimated for research expenditures at academic institutions has resulted in 184 patents to date, and an additional 120 patents have been applied for (MARBIO).

According to OTA (1988), the patent acceptance rate in biotechnology is approximately 40.5%. Thus, applying that factor to the 120 marine biotechnology patents being applied for, yields an estimated 49 patents that will be approved from the research expenditures to date, the total then being 233. The ratio of research ex-

penditures to patents is then just below \$1 million per approved patent. Considering the nature of our sample being mainly academically oriented, and therefore with an emphasis on basic research, this is a remarkably large number of patents per research dollar.

In fact, the ratio of patents to research dollars expended by private industry is significantly less than for academic expenditures at this time. By late 1992, only 25 marine biotechnology patents had been granted to industry, with applications for an additional 97 in process. Therefore, we estimate that the \$114 million in R&D expenditures to date will result in a total of 64 patents, or one patent per \$1.78 million in industrial research expenditures.

Following Griliches (1990) framework, one can conclude that marine biotechnology has already been very successful in increasing the stock of economically important knowledge. Value, however, derives from application of the knowledge to new product and process development. Estimates of the value of specific marine biotechnology products are difficult to come by. Griliches (1990) reported that, on average, after a patent was issued the stock market value of the company receiving the patent increased by \$810,000. Marine biotechnology patents are expected to have significant value, especially if the resulting products are high-valued pharmaceuticals or fine chemicals.

THE PRODUCT (INDUSTRY) LIFE CYCLE

Theoretical Considerations

A product (industry) life cycle offers a way to present the broader view in explaining the time path of the sales volume of a product (industry). Simply stated, sales volume equals price multiplied by quantity, so the life cycle explains changes in both the price and quantity of a product. In economic terms, it offers an explanation of the causes of shifts in supply and demand for a product. The product life cycle concept has been discussed in numerous business economic articles (e.g., Wells, Jr., 1972; Rink and Swan, 1979; Onkvisit and Shaw, 1989) and criticized in others (Dhalla and Yuspeh, 1976).

The typical product life cycle model follows an S-shaped curve (Figure 15) which can be divided into four stages (Kotler, 1980). The first stage is the product introduction phase. In this phase, product prices and production costs are high because of the small quantity of output. There may still be technological problems in production, and distributors and customers are reluctant to deal in the product because of supply uncertainty. Lack of familiarity with the products and its attributes and its high price contribute to consumer resistance at this stage.

As the product enters into a growth stage, sales increase as new customers start using the product, and new competitors start producing it. Because the product is expanding into new markets, prices remain high despite the increased production. Production

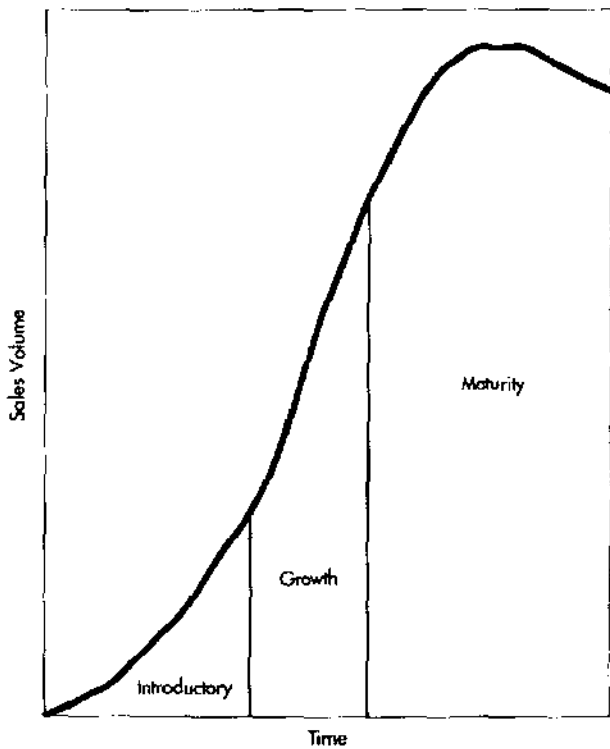


Figure 15. The product life cycle.

costs are lower during this period because technical difficulties in production (e.g., scale-up) have been overcome, and production quantities are high enough to allow firms to enjoy economies of scale. Profit margins are highest during this stage of product development.

The maturity stage of the product life cycle, itself, comprises three stages. Growth maturity represents a period of slow down in the rate of increase in sales as the market becomes saturated with the product. Eventually, the market reaches saturation and enters a phase of stable maturity. At this stage, sales are level on a per capita basis, so that absolute growth is dependent on population growth. Finally, some products reach a stage of decaying maturity where sales actually decline as competition from other products increases. In fact, some products may reach a fourth stage, obsolescence.

A major criticism of the product life cycle concept is that not all products pass through every phase, and the length of time a product stays in a given phase can vary greatly among products. The magnitude of changes in sales growth can also differ greatly among products. Within a product line, change in sales growth between phases can be very large or very small. In other words, the product life cycle concept is so general that it provides little predictive power for projecting the changes in sales for a specific product. Despite these shortcomings, the product life cycle offers a useful paradigm whereby information about shifts in supply and demand can be predicted and/or studied.

Marine Biotechnology and the Product Life Cycle

In the 1989 OECD report, the product life cycle framework was used to discuss the potential for diffusion of biotechnology-related industry. Marine biotechnology has the potential to affect the product life cycle of a given product or industry in a number of ways. A significant contribution of marine biotechnology development is in the downward shift (increased efficiency) in an industry supply curve through development of a new or improved process for producing an existing product. Even though the product may already be in the mature or declining phase of its life cycle, the new

process may allow a lower price of production, and therefore, a lower price to consumers. The lower price may then increase the quantity per demand unit and, thereby, sales per capita increase. Note that total demand does not increase in this case, but the lower price means more of the product will be purchased with the same demand.

Marine biotechnology also has the potential to increase demand for products by producing them via processes appealing to the public in terms of health, safety and ethics. One example is the use of marine cell lines for product assays and testing, eliminating the need to use mammals, alleviating the concerns of consumers who would otherwise avoid the product. Products may be manufactured by methods enhancing the nutritional quality of the final product, or its perceived and/or actual safety to the consumer. For example, one of the virtues claimed for aquacultured fish is that they are grown in a controlled environment, free of harmful chemical residues that may be present in fish harvested from the wild.

There is no question that marine biotechnology will yield a variety of new products and processes in each of the categories cited in Chapter 1. In the following section, application of the product life cycle framework to assess the potential of marine biotechnology potential for a specific industry, namely aquaculture, is discussed.

Economic Aspects of Marine Biotechnology Applications to Aquaculture

At the international level, shrimp and salmon aquaculture represent major success stories of the past decade. Domestically, farm-raised catfish sales comprised approximately 459 million pounds in 1993, with a value of \$325 million pondside (USDA, 1994). Growth of the aquaculture industry has not resulted from consumers increasing their consumption at high prices for aquacultured products. Instead, much of the increase in aquaculture production has been marketed at lower prices. Figures 16 and 17 show Norwegian salmon and U.S. catfish production and farm prices over a several year time span. The real price of both products declined significantly over time. The result, however, was a set of product life cycle curves that closely resemble the classical form (Figures 18 and 19). Fueling

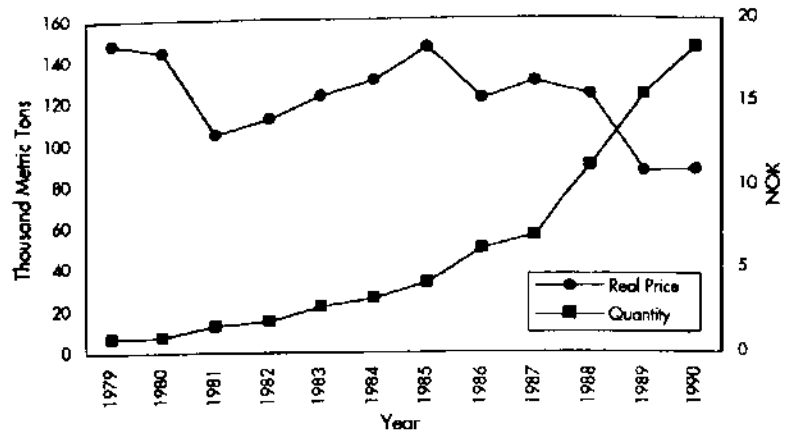


Figure 16. Norwegian salmon quantity and real price, 1979-1990.

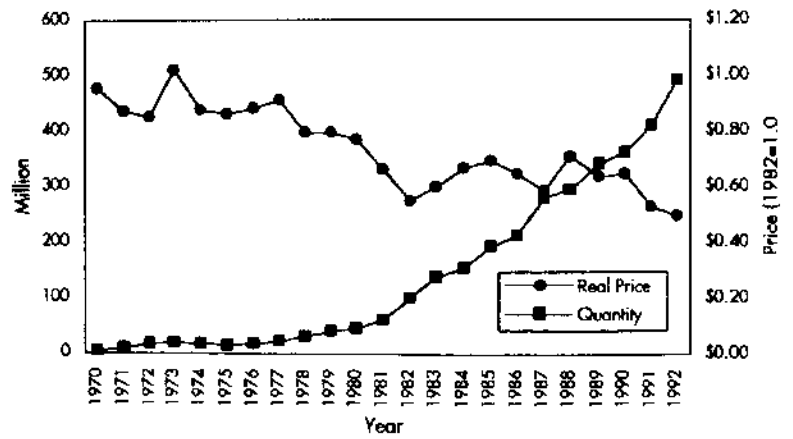


Figure 17. U.S. catfish quantity and real prices, 1970-1992.

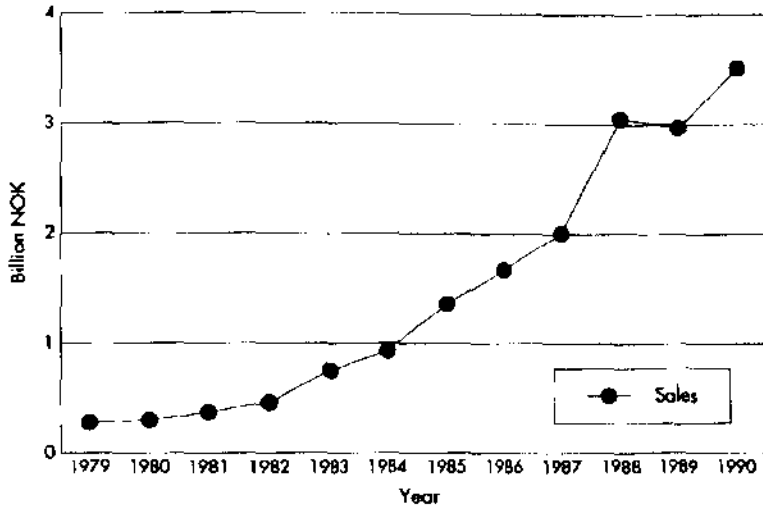


Figure 18. Norwegian salmon sales volume.

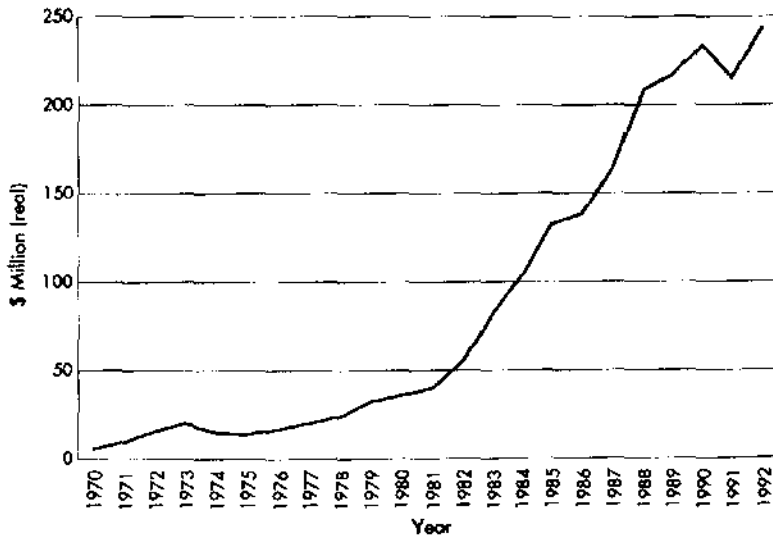


Figure 19. U.S. catfish real sales volume, 1970-1992.

growth in aquaculture is technological change which has been sufficient to overcome market resistance to expansion of these product markets. That is, the product is initially available with many new markets to penetrate, allowing production to expand without putting significant downward pressure on price. As the markets become saturated, with fewer new markets to penetrate (or marginal marketing costs increase), the only way to increase sales in a saturated market is to sell the product at a lower price. Technological change allows producers to lower price while the market continues to expand.

To date, most of the technological change incorporated into aquaculture has been via application of traditional animal husbandry technology to fish production. For example, a genetic selection program, employed for many years in Norway, initially increased the growth rate for Atlantic salmon by 10–12% per generation (Norwegian Fish Farmers' Breeding Center, undated). As application of traditional methods enter a phase of declining marginal returns, marine biotechnology offers an opportunity to return to, or keep the production of some aquacultured products, in the profitable growth phase by lowering production costs through technological improvements. In addition, many high-valued seafood products can not be produced profitably using traditional aquaculture techniques. Marine biotechnology applications offer the promise of economically feasible aquaculture production. Three production areas for which marine biotechnology offers the greatest potential for lowering production costs are growth and feeding, hatchery production, and closed systems or recirculating aquaculture production. Whatever production benefits are derived from marine biotechnology applications to aquaculture will have to be tempered by the as yet unknown level of consumer resistance to foods associated with genetic engineering and other forms of marine biotechnology.

Growth and Feed

Feed is the highest operating cost component of most aquaculture production. Basic research in fish nutrition has provided im-

proved feeds, lowering production costs. For example, a feed that costs \$0.20 per pound that results in 80% survival and a 2.5:1 feed conversion ratio contributes a cost of \$0.83 per pound of fish raised to one and a half pounds. On the other hand a \$0.20 per pound feed yielding 90% survival and 1.5:1 feed conversion ratio contributes only \$0.47 to the cost per pound. In 1992, aquaculture production of salmon in the U.S. was estimated to be about 13,000 tons of fish. From the example above, the savings in that limited market due to improved growth and survival would be at least \$10 million.

Traditional selective breeding programs are aimed at producing fish with a low feed conversion ratio, given the existing feeds and production methods. Faster growth, even with the same feed conversion ratio, lowers costs because of the time cost of money (discount rate), and the ability to increase final output, thus lowering average fixed costs. The ability to produce three crops of shrimp per year from a given pond makes shrimp aquaculture profitable in equatorial regions, but of questionable profitability in the U.S., where only one or two crops can be harvested per year.

Transgenic fish, triploids, and other products of genetic engineering offer the promise of improved feed conversion, better growth rates and enhanced survival of fish in existing aquaculture production as well as new products. Since production costs are most sensitive to a combination of these factors, aquaculture could benefit greatly from these marine biotechnology inputs.

Hatchery Production

Another major operating cost for aquaculture operations is for the "seed," i.e., fry, fingerlings, smolt, spat, etc. A low unit cost and a steady supply of seed for stocking aquaculture operations are important. For example, a major stumbling block for the further expansion of shrimp aquaculture is the availability of post-larval shrimp for pond stocking (National Marine Fisheries Service, 1992). Only recently have hatcheries become the major supplier of post-larvae; most still rely on wild harvest. Marine biotechnology offers the promise of reducing seed cost and increasing availability by control

of fish breeding. Control of disease, also important in grow-out, is another area where marine biotechnology, by increasing survival at all stages of fish production, can have a significant cost lowering effect.

Closed System Technology

Production technology is dependent on the type of aquaculture system employed. Water temperatures maintained for optimal growth and survival have a distinct advantage compared with systems relying on ambient conditions. If large quantities of naturally heated water (e.g., geothermal wells) are not available, then water temperature control makes economic sense only in a water reuse system that keeps the costs of maintaining water temperatures to a minimum. Although several types of closed systems are currently in operation in the U.S. for raising striped bass, tilapia, catfish and other species, to our knowledge, none have demonstrated profitability. Technological constraints of closed systems are associated principally with efficiency of the filtration system and ability to maintain satisfactory oxygen levels. In addition, off-flavor can be a major problem, as well, in closed systems. These technological difficulties are not specific to a particular species, thus aquaculture is improved across the board with each improvement in closed system technology.

Many closed aquaculture systems rely on biological filters for waste treatment. Advances in efficiency of the filters, and resultant lower costs will contribute significantly to economic viability of closed systems. Control of disease will be crucial for economic profitability, especially in systems where fish are maintained in high population density. Genetically engineered vaccines and biotechnology-derived aquaculture drugs will be important components of this fish health system. Doubtless, marine biotechnology will play an important role in the development of closed system aquaculture.

Other Products and Industries

In Chapter 1, an overview of potential products and applications deriving from marine biotechnology research was provided.

The type of framework discussed above for evaluating aquaculture can be applied to each industry or product as they are developed and more information is gained. Diffusion processes in pharmaceutical manufacturing developed through marine biotechnology will be much swifter and more efficient than for most other products. For diseases and illnesses for which there are few treatments, any new drug enters the market with virtually no competition. Obviously, patients are not as concerned about the use of genetic engineering in production of a drug, as long as it is effective and safe. In contrast, when the consumer has choices between genetically-engineered and "natural" products, the fear of genetic engineering enters more strongly into the choice process. The consumer is more willing to accept the life-saving genetically engineered drug without hesitation, but buying the genetically engineered tomato is another matter. In general, the fewer alternatives to any product, and the greater the demand, the more rapidly will the product diffuse through the market, and the greater the absolute level of production. This principle applies dramatically to an area such as bioremediation which holds such great promise. The alternatives for cleaning up contaminated sites are few and very expensive. In comparison to bioremediation, aquaculture holds less dramatic promise because there are so many kinds of seafood, as well as many alternative protein sources.

A study was done by OTA (1991) of the potential of biotechnology in a variety of sectors, including pharmaceutical, agriculture, chemical and environmental. There is no reason to assume that marine biotechnology will play out any differently in its development than biotechnology in general. The finding of the OTA (1988) analysis certainly is relevant here: "Biotechnology is a tool employed by several sectors. Each sector faces its own unique advantages and hurdles in the commercialization process. As biotechnology becomes fully integrated, it is often subsumed into the financial markets, regulatory requirements, patent issues, and personnel needs faced by those industries."

Curtin (1985) suggested another approach to looking at profitable areas for growth in marine biotechnology products. One approach is to produce low-priced substitutes for products that have

broad, high-volume industrial uses, such as agarose, a food additive. Another profitable direction is in manufacture of low-volume, high-value products, including fine chemicals with specialty uses in laboratories or manufacturing of medical products. Highest profits usually are earned with products that are both high-volume and high-value. Many pharmaceuticals have the potential to fit such a market niche, which is why there is significant interest in marine natural products with bioactive characteristics.

Examples of Potential Payoffs from Marine Biotechnology

A question that continually is asked with respect to public and private investment in marine biotechnology is: Will it be worth it? Another is: how much funding is appropriate for this kind of research? These questions are difficult to answer with precision. A realistic approach is to analyze selected industries where marine biotechnology research and development have reasonable potential to make a valuable contribution. It should be pointed out that, in any of these estimates of potential sales or savings, the correct measure of benefits to producers are profits not sales. For consumers, the measure of benefits is the difference between what he or she, the consumer, is willing to pay for a quantity of the product and what is actually paid. For products such as life-saving drugs, willingness-to-pay can be far higher than the amount actually paid.

Examples of potential payoffs from aquaculture have already been discussed. Below, the potential for two other industries, pharmaceuticals and bioremediation, are cited.

Pharmaceuticals

The global pharmaceutical market is estimated to be more than \$150 billion, with a U.S. share of 30% (OTA, 1991). Biotechnology-derived products were valued at \$2 billion in 1990. Marine biotechnology can contribute to this market at least in two important ways: (1) offering less costly alternatives for existing drugs; and (2) yielding development of new drugs for which no substitute currently exists.

Less costly drugs contribute to the cost-containment crisis presently looming before the health care industry. One of the arguments related to containing costs that pharmaceutical firms can recover from the sale of drugs is that this will inhibit investment in research and development of new drugs. But if biotechnology can lower the cost of drug production, or increase the probability that an experimental drug will be effective, this can offset the lower revenues from the sale of the drug.

The market for a new drug can be huge. For example, Amgen's biotechnology produced drug Epogen,TM used to treat dialysis anemia, yielded \$300 million in revenues in 1990 (OTA, 1991). The promise of economic and social benefits from drugs and vaccines derived from marine biotechnology research and development is predicted to exceed, by several orders of magnitude, expenditures proposed in this area.

Bioremediation

According to the U.S. General Accounting Office, American industry and government are currently spending about \$115 billion a year to meet environmental goals resulting from legislation enacted over the last 20 years, and that number is expected to increase to \$160 billion by the end of the decade. In a market this size, any kind of product or process resulting from marine biotechnology research and development has a great potential to garner savings and also be the impetus for the development of a bioremediation industry.

Remedial efforts at superfund and other sites are often prohibitively expensive, and the use of biotechnology may eventually present the only financial viable alternative for cleanup.

Companies faced with liability for lost use value of unique environments due to environmental damage may see biotechnology as a means to limit their liability. The sooner the area can be returned to use, the lower their financial responsibility under Superfund regulations.

There are many other applications in marine biotechnology that can be profitably exploited, such as new enzymes, biopolymers, and both fine and bulk chemicals. Thus, marine biotechnology, in

effect, represents a new, emerging sector of the economy that is, as yet, nascent, but highly promising.

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Chapter 5

MARINE BIOTECHNOLOGY AND BIOSAFETY

INTRODUCTION

As marine biotechnology develops and advances are made, the question inevitably arises whether this field poses additional risks to those of general biotechnology. This question is important not only for public and environmental health, but also because new management procedures and regulations will have to be formulated and instituted to meet any added risks. This could result in delays in research, field testing, and product manufacturing while new measures and regulations are formulated and adopted by legislatures and regulatory agencies. Therefore, it is necessary and timely to here assess whether marine biotechnology activities in fact do pose novel risks and, if so, determine the capability of the existing regulatory framework to meet them.

In view of its short history, and because its activities have so far been limited chiefly to research, marine biotechnology itself offers few examples that may be used to clarify questions about its safety or risk. We instead must scrutinize the history and experience of the two fields from whence marine biotechnology stems—aquatic biology and general biotechnology—and draw lessons from them. Accordingly, in the following sections we: (1) examine aquatic biology, to evaluate the effects of past introductions of exotic marine organisms into new environments and consider national and international policy responses to them; (2) examine general biotechnology, to identify problems that its activities have generated pertaining to biosafety and consider measures that were taken to alleviate them; (3)

use lessons from the preceding two subject areas to analyze marine biotechnology activities in terms of safety; and (4) state some concluding thoughts on the advisability of testing transgenic marine organisms in the open environment.

DISPERSALS OF MARINE SPECIES TO NEW ENVIRONS

Like the atmosphere, oceans are continuous—there are therefore no geographic or geological barriers preventing the spread of organisms from one site to another. Further, except for the abyssal depths, ocean water (and particles suspended in it) is never static; eddies, currents and wind are forever creating movement. The continuity of the oceans and movement of water favor dispersal of marine species, whether by accident or design. Of course, most often a species is well adapted to its native habitat, so its individual members usually will die when carried away from it. Sometimes, however, a species will be transported outside its usual territory, will find an ecological suitable niche in the new environment, and will succeed in establishing itself. In doing so, the introduced species, at times, has caused serious damage to indigenous wildlife and habitat. As scientists and the public have become aware of problems introduced species have caused, more attention has been focussed on developing and applying procedures for alleviating damage from exotic species that have succeeded in establishing themselves, and preventing further introductions. Thus, the subject of damaging dispersals in the marine environment recently has been addressed by one major conference (DeVoe, 1992) and a book (Rosenfield and Mann, 1992), as well as numerous articles in newspapers and magazines.

We believe that past examples of successful dispersals of marine organisms beyond their natural boundaries provide case studies from which data can be drawn that would be useful in assessing risk that may be inherent in certain marine biotechnology activities, including the future field testing of genetically engineered marine organisms. Accordingly, next we consider natural and mediated dispersals and what may be learned from them, and attempts by the U.S. and international community to prevent and control damaging dispersals.

The Dispersals of Marine Species

In the discussion that follows, the terms we use relevant to dispersal are in accord with the definitions of the International Council for the Exploration of the Sea (ICES) (Rosentfield and Mann, 1992). Thus, an introduced species is one that has intentionally or accidentally been transported and released into an environment outside its present range. A transferred species is any species that by intent or accident has been transported and released within its present range. Species may be introduced or transferred through natural actions or in the course of human activities.

Generally speaking, there are two dispersal mechanisms. Of the two, the most common is range expansion, i.e., the normal probing and breaching of territorial boundaries by members of a species. This phenomenon has been insufficiently studied in the marine environment and this lack of information prevents us from fully understanding why and how species at times are able to successfully populate an exotic site. Since we know so little about the forces that stimulate and affect the range expansion of wild species, no predictions can be made about the range expansion possibilities of an organism, whether genetically engineered or not, once it has been introduced or transferred into a new site by human activity. This lack of scientific data about how species disperse naturally creates problems for the investigator who attempts to assess the risks associated with a planned deliberate introduction of a marine species to a new locale.

Mediated dispersals of marine species occur when human activities cause them to be deliberately or accidentally introduced in or transferred to an area where they have never existed before. Since the time when humankind took up sailing, by chance or accident people have affected aqueous habitats throughout the world's oceans, rivers, and lakes, just as people have influenced terrestrial habitats. Ships have carried organisms from one place to another in their ballasts, encrusted on their hulls, and bored in their wooden hulls (Carlton, 1992a; Carlton and Geller, 1993). The ever expanding global network of interoceanic and inter-lake canals have given organisms many new routes for migration. Owners of personal aquariums have released ornamental fish and other animals into local waterways (An-

drews, 1992). Traders have carried crustaceans, fish, and mollusks long distances from fishing grounds to market places. Pathogens that afflict these fishery products have been swept along (Carlton, 1989). Thus, since transoceanic trading commenced humans have moved marine species numbering in the thousands across the globe in innumerable patterns.

Many accidental introductions of exotic species into the U.S. have been recorded but, fortunately, only a few have caused extensive damage (Hedgpeth, 1993). Perhaps the best example of an extremely adverse accidental introduction is the zebra mussel (*Dreissena polymorpha*). It probably originated in the Black Sea and the Caspian Sea, but by the 20th century had spread throughout Europe. Sometime in the 1980s, it was transported to the Great Lakes, probably in the ballast water of a ship that passed through the St. Lawrence seaway. The zebra mussel was first identified in Lake St. Clair in 1988, but its spread since that time has been extraordinary. It is now widely distributed throughout the Great Lakes, as well as in the upper reaches of the Hudson, Illinois, Mississippi and Tennessee rivers. Based on its range in Europe, the zebra mussel potentially can spread over 80% of the continental territory of the U.S. (Aquatic Nuisance Species Task Force, 1992).

The zebra mussel's faculty to inflict severe damage stems from its high reproduction rate and ability to settle on, and colonize, a wide variety of surfaces, including stone, steel, concrete, wood, plastic and glass. Under favorable circumstances, its population density can be astounding—over 100,000 individuals per square meter. As a result of these attributes, its biofouling capabilities are high. Zebra mussels have fouled water intake pipes at electric power plants, public water supply facilities, and conventional and nuclear fuel power plants. The weight of colonizing animals has sunk marker buoys and mussel larva have been drawn into the cooling systems of ships, where they settle and grow, causing overheating that damages engines. Mussel biofouling also has damaged harbor structures, canal locks, and flood control mechanisms. Unless control measures can be developed that slow the spread of the animal into new territories, as well as limit or eliminate present infestations, the cost to pay for

damage caused by the zebra mussel will rise to astronomical heights. The economic damage that the zebra mussel will cause by the year 2000 is estimated at \$5 billion (Aquatic Nuisance Species Task Force, 1992). Having recognized the peril the country faces, in 1991 the U.S. Congress passed a public law that seeks to meet the dangers posed by the zebra mussel (see below).

Besides accidental introductions and transferrals, humans have deliberately transported marine species from their home territories to new sites for some preconceived purpose. Deliberate introductions of exotic marine species, like the deliberate introductions of animals and plants on land, most often have been done to develop aquaculture and fisheries, or for environmental reasons. Introductions have occurred in waves throughout the 20th century as aquaculturists have succeeded in breeding new, more desirable strains of fish, shellfish and crustaceans (Welcomme, 1986; Stickney, 1992). In the 1950s and 1960s, there were large-scale deliberate introductions of fish and shellfish throughout the Third World to establish new aquaculture industries, including the African *Tilapia* to Asia and Latin America, Indian major carps to Southeast Asia and Latin America; and the black tiger shrimp (*Penaeus monodon*) and the white shrimp (*P. orientalis*) to many Asian and some Latin American countries. Deliberate introductions sometimes have been done for purposes other than aquaculture, for instance, to improve human health or alleviate environmental problems. Thus, larva from the fish *Gambusia affinis* and *Lebistes reticulatus* preferentially feed on mosquito larva, so these fish have been introduced into several parts of the world where malaria is endemic. The grass carp (*Ctenopharyngodon idella*), which is a voracious grazer, is used to control the overgrowth of marine plants in canals and other waterways.

In the late 1970s and early 1980s, large-scale introductions included striped bass (*Morone saxatilis*) to the U.S. west coast, the Pacific oyster (*Crassostrea gigas*) to the U.S. and Canadian west coasts and to France, Pacific salmon (*Oncorhynchus* species) to Atlantic waters, the pink salmon (*O. gorbuscha*) to the Arctic Sea coast of the former USSR, a shrimp species from Panama (*P. stylirostris*) to Hawaii; and the Pacific seaweed (*Undaria pinnatifida*) to France (Welcomme,

1986; Sindermann, 1986). More recently, in 1989, the macroalgal species *Euchema spinosum* was transported from the Philippines to Zanzibar, where it is now cultured, and the dried product is exported to Europe where polysaccharide is extracted for use as a food conditioner (Zilinskas and Lundin, 1993).

Many of the deliberate introductions have benefitted local populations and improved the economies of countries. For example, France harvests over 100,000 tons of the Pacific oyster. The introduced fish *Limnothrissa* is a new protein resource in Africa, yielding about 4,000 tons from Lake Kivu and 12,000 tons from Lake Kariaba, and Sri Lanka's entire inland aquaculture production of 32,000 tons consists of introduced fish and crustaceans (Welcomme, 1986; Sindermann, 1986). In the U.S., most of the state of Washington's mariculture production consists of the exotic Pacific oyster *C. gigas* (39,000 tons in 1988) and the Atlantic salmon (Stickney, 1992).

In some cases, however, like introduced species on land, aquatic introduced species have caused damage, ranging in severity from the barely discernible to serious. The mosquito larva-eating fish tends to eat eggs and larva of other fish. The grass carp transmits a cestode causing disease among several species of fish. The Pacific seaweed *Sargassum muticum* was inadvertently introduced with *C. gigas*, eventually growing so dense in the English Channel along the English and French coasts that it interferes with transport and recreational activities. In 1981, scientists discovered that the widely introduced shrimp *P. vannamei* was the carrier of the pathogen infectious hypodermal and hematopoietic necrosis virus, which has decimated shrimp stocks in aquaculture facilities throughout the Pacific rim countries.

Sometimes it appears initially as if an introduction is successful, but in the longer term it proves to be detrimental. This may be illustrated by two examples of deliberate introductions that were initially successful, but which ended up disastrously. First, after several attempts had been made during the 1950s to introduce the Nile perch (*Lates niloticus*) into Lake Victoria, the fish became established in the middle 1960s. For a few years local fishermen benefitted as they

were able to garner an average of 60,000 tons of the fish per annum. But in the 1970s harvests declined and, coincidentally, scientists discovered that as *L. niloticus* colonized Lake Victoria waters, it eliminated native cichlid fish stocks found nowhere else. In addition, the only practical way of preserving perch harvests proved to be smoking, which demanded great quantities of wood, spurring the cutting of bushes and trees, and leading to deforestation. It is now clear that the deliberate introduction of the Nile perch caused extensive damage to aquatic and terrestrial biodiversity, while the initial bountiful harvests could not be sustained (Baskin, 1992).

A second case meriting discussion is the introduction in 1980 of the golden snail (*Pomacea* species) into the Philippines. The edible snail is appreciated by gourmets as *escargot*. The reason for introducing it, therefore, was to provide farmers with an alternative "crop," which could be used locally for food and exported for cash. However, the export market never developed and local consumption remains low. Perversely, the snail settled in rice fields, where it turned into a recalcitrant pest, attacking newly transplanted rice plants and seed, destroying up to 80% of the harvest. By the end of 1991, 426,000 hectares of Philippine rice fields had been infested by the snail, which is resistant to pesticides and other control measures. The International Centre for Living Aquatic Resources Management, which has its headquarters in the Philippines, is trying to develop the integrated use of chemicals, biological control measures and farming methods to control the snail, but with no success so far (Anonymous, 1992a).

A rapidly growing body of literature addresses the causes and effects of damaging dispersals. Some authors discuss and analyze problems pertaining to aquaculture in general (Carlton, 1992b; Courtenay Jr. and Williams, 1992; Davidson et al. 1992). Others write about more specific problem areas, including those pertaining to marine plants (Neushul et al. 1992), mollusks (Farley, 1992), shellfish (Kern and Rosenfield, 1992; Lightner et al. 1992), and finfish (Ganzhorn et al. 1992; Thorgaard and Allen, 1992). Analysis of the effects of past mediated dispersals, whether accidental or incautious deliberate introductions, yields six lessons:

- An introduced animal may disrupt local fauna through competition or predation. In the worst case, the introduction of an exotic species may lead to the extinction of one or more wild species.
- Genetic degradation of the host stock may result from its introduction into a new locale. When an introduced species breeds with wild species, some of its advantageous genetic characteristics may be lost or degraded.
- Genetic degradation of wild species inhabiting the locale where the alien species is introduced may take place. For example, if the introduced species breeds with indigenous wild species, adaptations for survival that the wild species have evolved may become diluted or disappear in hybrid progeny. Even worse, important genes may be lost if the exotic species replaces the wild species.
- When a exotic stock is intentionally transferred to a new locale, exotic disease agents infecting members of the introduced stock may be accidentally introduced at the same time, and these pathogens could attack susceptible indigenous species.
- The exotic species may damage or disrupt some aspect of the habitat into which it is introduced, thereby upsetting natural balances, leading to degradation or destruction of the local environment.
- Once an introduced species colonizes a locale, it may become endemic and impossible to eliminate.

From the foregoing it is clear that the extent of long-term damage of accidental or planned introductions in the marine environment of exotic animal species usually cannot be reliably assessed at the time of introduction, nor is it possible to determine with cer-

tainty whether benefits stemming from deliberate introductions ultimately will outweigh costs.

U.S. and International Measures to Control Dispersal

In view of the problems that dispersals of marine organisms have engendered throughout the world, the U.S. government, other governments, and international agencies have adopted and implemented control measures.

U.S. Measures to Control Introductions

Historically, states have had the major responsibility for protecting their environments from damaging outside influences, including dispersals of aqueous and terrestrial organisms. Some of the states' activities undertaken to manage and regulate marine introductions have been described, including those by California (Collins, 1992), Hawaii (Brock, 1992), Florida (Willis, 1992), Georgia (Smith, 1992), and South Carolina (Tompkins, 1992). However, since U.S. government agencies have the major responsibility for ensuring biosafety (the NIH for laboratory research and the USDA and EPA for confined and open field test experiments), we will not consider further the role of states in this report.

Attempts by the U.S. government to prevent damage to the natural environment by the deliberate introduction of exotic species has a short history. In the 19th century, the Supreme Court enunciated the principle that wildlife is the property of all the people and that government is the public trustee in wildlife conservation. Until 1900, state governments exclusively fulfilled this role. However, that year the Congress adopted the Lacey Act, which seeks to preserve wildlife through the regulation of interstate commerce. As originally intended, it had three objectives. First, it sought to strengthen and supplement the wildlife conservation laws of states. Second, it allows states to prohibit the importation of wildlife killed legally or illegally in other states. Third, it empowers the Secretary of Agriculture to adopt whatever measures are necessary to prevent importation of birds or animals that have the potential of harming U.S. agriculture or horticulture (Bean, 1983).

The original objectives have been considerably expanded during the 94 year old history of the Act. Important additions were the Black Bass Act of 1926, which extended the Act's coverage to include fish, and an 1960 amendment that banned the import of animals that not only could injure U.S. agriculture and horticulture, but also U.S. wildlife and wildlife resources. However, the most important augmentation was the Lacey Act Amendment of 1981.

According to Neushul *et al* (Neushul *et al.* 1992), the 1981 Amendment was adopted by lawmakers who learned about various damaging introductions of exotic species, including the importations of rabbits and prickly pear cactus into Australia, and the walking catfish, Brazilian pepper trees, and water hyacinth into Florida. The Lacey Act Amendment of 1981 simultaneously repealed the Black Bass Act and most of the Lacey Act while consolidating and strengthening their functions. Specifically, the Amendment's provisions pertaining to specimens taken, transported, or sold in violation of state, federal, or foreign laws now apply to all wild animals, including those bred in captivity and to certain plants. Animals that were formerly excluded, such as mollusks and crustaceans, are now covered. Penalties for violations, which can be assessed against importers, exporters or dealers, were substantially increased; the maximum fine is now \$20,000 and the maximum jail sentence is five years (Bean, 1983).

Title 50 of the Lacey Act Amendment prohibits importation of members of the families Chlariidae and Salmonidae. The first includes a particularly damaging species—the walking catfish (*Clarias batrachus*), first introduced into Florida in the late 1960s. The prohibition of salmonids is not absolute; salmonids may be imported if they are certified to be free of viral hemorrhagic septicemia virus and *Myxosoma cerebralis*, the parasite causing whirling disease.

In 1990, Congress passed Public Law 101-646, also known as the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (hereafter the 1990 Act). Although the primary impetus for the 1990 Act was congressional concern about the zebra mussel infestation of the Great Lakes, it provides a framework for addressing

accidental introductions generally. It has five objectives: (1) to prevent the introduction and dispersal of exotic species into U.S. waters through, for example, ballast water management; (2) to coordinate federally supported research and prevention activities on aquatic nuisance species, especially the zebra mussel; (3) to institute control measures to prevent and control non-intentional introductions of exotic species through means other than ballast water; (4) to minimize impacts when exotic species become established; and (5) to establish a national program for assisting states to control zebra mussels (Kern and Rosenfield, 1992).

In response to the 1990 Act, a Federal interagency Aquatic Nuisance Species (ANS) Task Force, whose co-chairmen are Dr. Dennis Lassuy of the Fish and Wildlife Service (FWS) and Dr. Frederick Kern from the National Marine Fisheries Service/NOAA Cooperative Oxford Laboratory, MD, was established. Its major aim is to try to develop a coordinated and cooperative approach among Federal agencies, state agencies and the private sector towards unintentional introductions (Wilkinson, 1992). The ANS Task Force developed an action program to implement the provisions of the Act; a draft program proposal was finished during the summer of 1992, and then was distributed to the public for critique and comments (Aquatic Nuisance Species Task Force, 1992). The ANS Task Force has finished its report, which includes recommendations for congressional actions, and it will be released after review by the U.S. General Administration Office.

In addition to the 1900 Lacey Act, public laws that form a body of federal wildlife law include the Fish and Wildlife Act of 1956, the Endangered Species Conservation Act of 1969, the 1976 Marine Mammal Protection Act, the Fishery and Conservation Act of 1976, and Executive Order 11987 issued in 1977 (which restricts the introduction of exotic species into natural aqueous or terrestrial ecosystems). Most of the U.S. wildlife laws, including the Lacey Act, are administered and enforced by the FWS.

Although it would seem that the federal regulatory structure seeking to prevent and control accidental or deliberate introductions of exotic aquatic species into the U.S. is sufficiently comprehensive

to accomplish its intent, in fact severe problems have impeded its operation. In particular, as explained by Peoples *et al.* (Peoples Jr. *et al.* 1992), the FWS has been thwarted in its attempts to formulate policies for enforcing wildlife regulations by a combination of national politics and inertia by the U.S. executive.

According to Peoples *et al.*, by early 1970s it became generally clear that the reactive approach taken until that time had not prevented damaging introductions of exotic aquatic organisms (Peoples Jr. *et al.* 1992). This approach depends on identifying and listing a small number of species considered as being able to cause enormous damage if they were by chance or on purpose introduced into the U.S.; the importation of the listed organisms was prohibited. Because it did not work well, evidenced by a number of damaging introductions, a proactive approach was proposed, which had as its basis the concept that unless otherwise proven, it should be assumed that the importation of any exotic species will have damaging effects. Thus, only species that were determined by the FWS to pose a small threat to indigenous wildlife, agriculture or forestry can be imported. Clearly, under this approach the importer would have to shoulder the burden of proving the safety of species proposed for import, rather than the FWS having to prove that its introduction would be hazardous.

Due to opposition mainly from the pet industry, the proposal for the proactive approach went through several changes, but was ultimately defeated in 1978. So the reactive approach, with its short list of prohibited species, is currently employed by the FWS. However, the FWS is trying to improve it. Working from the premise that it is neither feasible, nor desirable, to prohibit all importation or introductions of exotic aquatic species, FWS's strategy for controlling introductions include expanding the list of prohibited species, clarifying criteria whereby the potential of species to pose a threat is assessed, and speeding up the procedure whereby species can be added to the prohibited list.

The FWS supports research at the National Fishery Research Center in Gainesville, FL, and two field stations at Stuttgart, AK and Marion, AL. This research encompasses studies on 43 exotic species

that have been introduced into U.S. waters and have become established (Peoples Jr. et al.1992). The Center also serves as an important information exchange center on exotic fish. It is interesting to note that in the early 1980s, the FWS was asked by the American Fisheries Society to identify exotic fish that might have commercial possibilities for the U.S. Over 2,000 species were so identified (Peoples Jr. et al.1992)! This means that the U.S. aquaculture industry has a vast number of possibilities for developing new products to satisfy the growing demand for seafood in the U.S. Of course, as new seafood products are developed, the number of problems associated with introductions will proliferate, potentially creating a nightmare for agencies that regulate these matters.

International Measures

On the international level, several sets of codes and rules have been promulgated dealing with dispersal of living aquatic organisms. Of particular importance was the adoption of the *Revised Code of Practice to Reduce Risks for Adverse Effects Arising from Introductions and Transfer of Marine Species* by the International Council for the Exploration of the Sea (ICES) in 1973 (and revised in 1979). Other codes of practices, position statements and conventions on the subject have been made by the American Fisheries Society (1973), the UN Conference on the Law of the Sea (1982), the Council of Europe (1984), FAO's European Inland Fisheries Advisory Commission (1984) and the International Union for Conservation of Nature and Natural Resources (1987). The overriding objective of these codes and statements is to coordinate international actions to avert future accidental introductions and to prevent adverse effects from deliberate introductions.

Due to the uneven implementation of the ICES code by nations, Dr. Carl Sindermann has suggested strategies for dealing with future proposals for introductions (Sindermann, 1986; Sindermann, 1992). The overriding strategy is for UN agencies and non-governmental organizations to educate the public, policy-makers and national regulatory agency personnel about the potential damage that the importation of a non-indigenous species can do to native stocks

and the local environment. This education, it is hoped, will lead to the understanding among politicians that it is in the best economic interest of a country to have a strong regulatory regime in place to prevent unauthorized introductions and delineate the conditions under which authorized introductions may proceed.

Another strategy is suggested for larger, industrialized countries. It emphasizes regional approaches to controlling the transfer of organisms, where the federal government ensures uniformity and continuity. Whatever approach is adopted, it should be implemented according to the general operating principles set forth in the ICES code. These are based on the assumption that risks from introductions are never zero. National regulatory regimes therefore should be designed to minimize risks from proposed introduction. Risk-reduction includes the thorough study of the organism proposed for introduction in its native habitat; assessing the development of native stocks as an alternative to introducing a new stock; stressing the introduction of non-migratory species over migratory species; establishing a mechanism for the continuous monitoring of the introduced stock; and other measures. It is particularly important that the scientific implications of a proposed introduction be analyzed before the event, including clarifying ecological considerations, such as competition and predation; genetic considerations, including potential for hybridization and change in gene frequency; behavioral consideration, including interactions between the introduced and native species; and pathological considerations, including the possibility that the introduced species will carry new infectious diseases (Sindermann, 1986; Sindermann, 1992).

SAFETY AND GENERAL BIOTECHNOLOGY

Putative risks inherent to biotechnology research, testing and applications have been enunciated but never demonstrated. However, the history of general biotechnology research, the safety concerns it generated, and attempts by regulatory agencies to meet these concerns are useful to review. The potential risks posed by the field testing of inanimate and animate products generated by general biotechnology research and the evolving regulatory regimes that

seeks to lower or contain possible hazards also can be productively assessed.

General Biotechnology Research and Biosafety

Classical biotechnology, which typically utilizes breeding, selection and fermentation techniques, has not generated public fears about hazards. It was not until the early 1970s, after the introduction of recombinant DNA technology, that concerns arose about the safety of some research procedures using genetic engineering (Zilinskas and Zimmerman, 1986). The major worry was that an accidental or chance recombination of genes would alter the bacterial host, endowing it with undesirable characteristics. Citizens, reflecting uncertainties expressed by scientists, voiced their concerns about several aspects of biotechnology. For example, could an entirely new life form with unknown characteristics be created by researchers? Could otherwise innocuous bacteria accidentally be endowed with pathogenic properties during research and escape from research laboratories? Could new recombinant forms of viruses and bacteria cause pandemics of novel diseases among man, animals, or plants? Most bioscientists believed that the possibility of any of these events occurring was diminishingly small, but little data existed to support their opinion. In response to public concern and because of the lack of information on the subject, scientists from throughout the world met during 1975 at Asilomar, California to assess the possible risks of rDNA technology. The conclusions of the Asilomar conference were used by the U.S. National Institutes of Health (NIH) to formulate a set of guidelines for regulating rDNA research. As mentioned earlier, these so-called NIH guidelines for rDNA research were first published in 1976, but have since been revised several times (United States National Institutes of Health, 1986). Although they were binding only on researchers funded by the NIH, the entire U.S. scientific establishment quickly accepted the NIH guidelines.

Initially, the NIH guidelines:

- required total containment for rDNA experiments and set forth the conditions under which this research could take

place. These ranged from the least secure condition, initially designated P1, but now termed Biosafety Level 1 (BL1), to high security containment, or BL4. Some types of experiments were not allowed. BL1 and BL2 work require mostly common sense procedures, such as the cleaning of working surfaces, the wearing of laboratory coats, and the frequent washing of hands. These precautionary measures may be grouped under the rubric of good laboratory practices, which any well-run hospital clinical or research laboratory routinely follow. Research perceived as especially risky could only be carried out in BL4 facilities. These are self-contained units that can only be entered through air-locks; all access is rigidly controlled. All workers in a BL4 laboratory must be specially trained in the handling of extremely hazardous infectious agents, and they wear protective suits resembling space suits when working.

- created a national Recombinant DNA Advisory Committee (RAC) with headquarters at the NIH, which reviews proposals for projects requiring the use of BL3 and BL4 facilities and adjusts the NIH guidelines in view of new scientific knowledge.
- required each institution receiving government funds to set up and maintain an Institutional Biosafety Committee (IBC), which has initial responsibility for reviewing research proposals involving rDNA experiments presented by local researchers and specifying the conditions under which these should take place. Difficult problems that require policy decisions are referred to the RAC.

With the enactment of the NIH guidelines, scientists began to perform a series of risk assessment experiments to deliberately try to create pathogens. These confirmed that the possibility of accidentally creating pathogens in the laboratory was infinitesimal. There are three major reasons for the safety of research employing genetic engineering. First, the successful invasion, colonization and infection by

a parasite that causes disease in the host is a complex process. Not only is the number of genes involved on both sides large, but also the interactions between these many genes are to a considerable extent dependent on their locations in a three-dimensional space. The probability of recreating this complex milieu by accident when manipulating only one or a few genes is minute. Second, genes include regulatory DNA sequences, called operons, that control in a positive or negative way the expression of genes in each particular cell. It is unlikely that complex regulatory operons would be created by accident in the laboratory. Third, the insertion of foreign genes in a microorganism usually weakens that organism in some way, diminishing its ability to compete with wild organisms or to survive the many stresses of natural conditions outside the laboratory.

Paradoxically, risk assessment experiments also led to the development of new laboratory procedures whereby certain laboratory procedures could be made safer when genetic engineering was employed. For example, genes from virulent viruses could be safely cloned in nonpathogenic bacteria for further study rather than directly handling the whole virus as is done in conventional research.

In reference to biotechnology-related research in agriculture, the USDA takes the lead. The USDA's Agricultural Biotechnology Research Advisory Committee (ABRAC) develops biosafety guidelines and reviews individual projects on a case-by-case basis. ABRAC's review process is modelled after that of the NIH and makes use of existing IBCs. The USDA also has established the Cooperative State Research Service (CSRS) to facilitate the safe field testing of transgenic organisms (see below).

In addition to directed risk assessment experiments, actual practice has demonstrated the adequacy of the NIH guidelines. Since the NIH guidelines first came into effect about 18 years ago, thousands of research projects have been done in the agricultural, biological, industrial, medical, microbiological, and other fields without apparent negative side effects. This record indicates that properly conducted research employing genetic engineering techniques is safe.

As data accumulated proving the safety of biotechnology research, the RAC progressively relaxed the NIH guidelines. Since the

late 1970s, public fears about rDNA research in contained situations, such as laboratories, have largely disappeared.

Biosafety in Reference to Field Testing

As biotechnology generated promising results with potential applications useful in agriculture and industry, a biotechnology-based industry grew. By 1993, a large bioindustry had developed in the U.S., consisting of circa 1,400 companies that are either dedicated bioindustrial firms, or more traditional firms that use biotechnology techniques in some of their research, development and manufacturing processes. In either case, general biotechnology can engender two types of products—inanimate products and genetically altered living organisms. Each poses serious questions about their safety: Do inanimate products produced by genetically engineered organisms pose unique risks (i.e., risks over and above those posed by conventionally-produced products) to humans, other animals or plants? Would the deliberate release into the environment of genetically engineered organisms pose unique hazards to existing life forms or the environment?

Considering each question in turn, most inanimate products from biotechnology are known chemicals or compounds produced via fermentation. However, some of these products could previously be procured in only very small quantities. Substances such as interferons and interleukins, which have promising anti-cancer and antiviral properties, could not be investigated to the extent they merited since so little of each was available. However, as production systems utilizing genetically engineered bacteria were developed, the large-scale production of an ever-growing number of formerly unavailable bioactive compounds has become possible. Many of these products are now under research and testing, and a few are commercially available. As a result, new marketing niches have been created, the concept of intellectual property has changed significantly and, at times, new treatment regimes and procedures have raised ethical dilemmas. However, to date no genetically engineered biotechnology product has posed risks above existing products or created unique

hazards that were not present in products manufactured by conventional means.

The main lesson from the experience gained by U.S. regulatory agencies since 1981 when the first genetically engineered product, human insulin, came up for review is that the testing of genetically engineered products need not differ from that of conventionally produced products; the same criteria of safety and efficacy apply equally to both. The strictness of the testing protocol will, of course, depend on the product's intended use. If the product is intended for animal or plant use, or is a non-consumable commodity, its testing need not be so rigorous. Conversely, if the product is a human drug or other excipient, its testing must follow exacting procedures, including clinical phases. The U.S. government has met concerns about the safety of biotechnology products by applying existing protocols and regulations to ensure the adequate testing of the product under scrutiny and to monitor testing procedures to make certain they are rigorously followed. The FDA, for example, does not treat biopharmaceuticals produced by advanced biotechnology differently than conventional drugs (Fox, 1992). Similarly, the agency regulates human foods derived from genetically engineered plants using existing approaches (Department of Health and Human Services, 1992).

The situation is similar elsewhere in the world. Biosafety regulatory programs in other developed countries, the European Communities, and elsewhere have evolved to the point where regulators assess inanimate products from advanced biotechnology on the same basis as products from conventional research and development. As far as we are aware, no country has enacted new regulations aimed specifically at inanimate biotechnology products. This is also the approach of the Organization for Economic Cooperation and Development (OECD) and World Health Organization (WHO) (Directorate for Science, 1986). For example, WHO tests a vaccine the same way, whether it was developed and produced using rDNA technology or by a conventional cell culture system.

The second concern, the so-called deliberate release issue, is currently receiving much attention by the scientific community and the public. The two possible major risks of deliberate release are: can

the introduced organism directly harm the environment or any of its inhabitants, and can any of the genes that the introduced organism carries disperse and become integrated in the genomes of non-target organisms? The U.S. National Research Council (NRC) has scrutinized the issues related to the field testing of genetically engineered microbes or plants in terrestrial situations and concluded that there are three essential criteria for evaluating the risks associated with a proposed release (United States National Research Council, 1989):

- Are we familiar with the properties of the organism and the environment into which it may be introduced?
- Can we confine or control the organism effectively?
- What are the probable effects on the environment should the introduced organism, or a genetic trait it carries, persist longer than intended or spread to non-target organisms?

The OECD, which began to consider the biotechnology safety issue in 1983 (Teso, 1992), published its guidelines of the field testing of genetically manipulated organisms in 1992 (Directorate for Science, 1992). For the purpose of evaluating the safety of field testing marine organisms, which is dealt with in the next section, it is useful to review specific criteria developed by the OECD related to evaluating the field testing of plants and microorganisms, as well as the site where to proposed test is to take place (Directorate for Science, 1992). Its approach is substantially the same as that of the NRC. Although the OECD criteria pertain to the terrestrial environment (as do those formulated by the NRC), they provide a framework for our consideration (below) of the carrying out of field tests in the marine environment.

Plants. When evaluating possible risks associated with the field testing of a plant species, the following characteristics must be considered:

- the reproductive potential and biology of the plant, such as its flowers, pollination requirements and seed characteristics, and the history of the plant's controlled reproduction in an environment similar to the test site;
- the mode of action, persistence, and degradation of any newly acquired toxic property;
- the characteristics of the biological vector used to transfer DNA to the plant;
- the possible interactions with other species and biological systems.

Microorganisms. When evaluating possible risks associated with the field testing of bacterial species (or other microorganism), the following characteristics must be considered:

- the organism's capability for dispersal, survival, and multiplication;
- the organism's interactions with other species and biological systems;
- the organism's potential for gene transfer;
- the mode of action, persistence and degradation of any newly acquired toxic property.

Field Test Site. The following characteristics of the proposed field testing site need to be taken into account for the safety evaluation:

- significant ecological and environmental considerations related to the site that might bear on the safe performance of the field test, such as the water run-off pattern, water table, wind

patterns, and other meteorological and geophysical phenomena peculiar to the test site;

- the size of the site, including a possible safety zone;
- the site's geographic location as related to the nearby or distant presence of biota that could be affected by the organism being tested.

Specific methods for safely managing the field testing of genetically engineered organisms are in a state of evolution. Referring to the experience of the U.S. in this area, proposals for testing genetically engineered organisms in the field are dealt with on a case by case basis by the USDA, specifically by its CSRS. As explained by USDA spokespersons (Medley and Brown, 1992),

the USDA has broad regulatory authority to protect U.S. agriculture against adulteration of food products made from livestock and poultry, and to prevent the introduction and dissemination of plant pests. This authority is applicable to genetically engineered animals, plants, and microorganisms.

The first activity inherent to any proposal considered by the USDA is the development of an environmental assessment. The assessment addresses health and environmental concerns by considering both direct and indirect effects stemming from the proposed release. It must convincingly evidence a conclusion that the proposed release would probably not significantly alter or harm any aspect of the environment or its biota. Permission for testing probably would not be forthcoming if the organism to be tested was likely to present high risk to non-target animals or plants; for example, because it possessed characteristics such as enhanced fitness, increased pathogenicity, or contained novel phenotypes. If the USDA assesses a project as having negligible impact on the environment, this finding is published in the *Federal Register* before a final decision is made in order to give the opportunity to the public and its representatives to scrutinize the assessment report and to comment on it. The agency

must take these comments, as well as statements made by other interested parties, into account before it makes its decision. By the start of 1994, the EPA and USDA has given final approval to over 1,000 applications for field trials of genetically engineered organisms, mostly plants but including two types of transgenic fish (see below). No negative effects have so far been observed, indicating that the scheme seems to be working, at least in the short term (Miller et al.1991).

Field tests carried out in the U.S. are recorded and tracked by the National Biological Impact Assessment Program, which is a computerized network for information exchange on field testing of transgenic organisms (Mackenzie, 1989; Mackenzie, 1992). On the international level, the OECD has developed a database called BioTrack in which information about field tests in member countries involving transgenic organisms is recorded. In addition, BioTrack may be used by subscribers to research sources on information relevant to safety considerations of such field testing.

During the last few years the issue of biosafety has become a subject for deliberation by policy-makers on the international level. The OECD has formulated biotechnology guidelines to guide its member nations, all of which are industrialized countries (Directorate for Science, 1986; Directorate for Science, 1992). An interagency working group, established jointly by the Food and Agricultural Organization (FAO), United Nations Environment Program (UNEP), United Nations Industrial Development Organization (UNIDO) and WHO, developed a set of guidelines that are intended to be used by governments of developing countries as models for local laws (United Nations Industrial Development Organization, 1992).

It bears noting that some persons worry about biotechnology industry manufacturing products that could replace natural products. For example, cell culture systems have been developed that mass produce products, such as agar, saffron and vanilla, important to the economies of developing countries that grow the plants from which the natural products are processed for export. Similarly, European farmers have protested against the use of recombinant bovine somatotropin in animal husbandry, claiming that it would result in an

over-production of milk. While these are important socio-economic problems that need to be addressed by governments when they formulate policies for biotechnology research, development and applications, they have nothing to do with the safety of these activities.

BIOSAFETY AND MARINE BIOTECHNOLOGY

In the preceding sections it is seen that both general biotechnology research and products of this research may give rise to safety concerns. It is reasonable to assume that marine biotechnology will follow the same pattern. Thus, in the parts that follow we: (1) consider the safety of marine biotechnology research in light of the experience of general biotechnology; (2) consider the safety of inanimate and animate products of marine biotechnology; (3) analyze special characteristics of the marine environment that bear on the field testing of transgenic marine organisms; and (4) based on the foregoing, assess whether marine biotechnology poses different safety and regulatory issues than does general biotechnology.

Experience of General Biotechnology Relevant to Safety of Marine Biotechnology Research

It is noted above that national guidelines regulating biotechnology research generally focus on containment and are voluntarily followed by scientists and that the stringency of conditions under which research may proceed depends on the level of perceived risk of the organism being researched. When scientists work with a virulent pathogen, they must do so in a high security laboratory and use elaborate procedures to ensure the safety of themselves, other workers, and the surrounding community. Conversely, research involving a non-pathogen requires no more than good laboratory practices. It is probable that over 95% of all biotechnology research is being done under the conditions defined by good laboratory practices.

While marine and terrestrial organisms may differ markedly in chemical and physiological characteristics, the conditions under which scientists do research are similar in marine biotechnology and general biotechnology. Research in marine molecular biology in-

volves the same training and techniques as does general molecular biology. In view of these similarities, we infer that the health and safety issues posed by marine biotechnology research performed in the closed system of a laboratory are similar to those posed by comparable general biotechnology research. For these reasons, the voluntary guidelines that govern biotechnology research generally are pertinent to and adequate for marine biotechnology research. As far as we are aware, to date no scientist, public advocate or regulator has voiced a differing opinion on this matter.

**Experience of General Biotechnology Relevant to
Inanimate Products of Marine Biotechnology
Research**

Natural inanimate products, whether of terrestrial or marine origin, raise the same safety issues. For example, carbamates, lactones, and terpenes will have in common general characteristics whether they were isolated from a sponge or a terrestrial plant, although their structures may vary widely. Undoubtedly, as more organisms from extreme environments are collected, screened and investigated, exceptional compounds, showing antibiotic, anti-viral, anti-tumor and other properties, will be found. However, if the experience of general biotechnology is a guide, no matter how novel the structure of a marine natural product, it will not create a unique situation, or uncommon hazard, demanding a new risk assessment scheme or regulatory regime. For example, if a unique marine toxin is discovered, its physiological action is not likely to differ markedly from that of a known toxin; neither will its toxicity be significantly greater than existing toxins. Therefore, testing done according to established procedures would elucidate the chemical structure of the new compound, explain its mode of action and, eventually, clarify its effectiveness and safety.

Similar to natural inanimate products, when a cell culture system using genetically modified microorganism is developed by marine biotechnology, it will not create an unusual situation demanding extraordinary control measures or regulations. For example, the development of a recombinant killed vaccine against a viral fish disease

would most probably be done using procedures similar to those used to develop vaccines for other animals; the field testing of the fish vaccine would most likely follow established animal vaccine testing procedures; and the developmental and testing process would be monitored adequately by existing national regulatory authorities. Consequently, present protocols for testing products produced by conventional or advanced biotechniques are appropriate for use in the testing of marine biotechnology products.

Field Testing of Transgenic Marine Organisms

No one so far has proposed to field test genetically modified organisms in the open marine environment. However, our review of industrial activities gives us reason to believe that several U.S. research institutions and firms soon will apply for permits to field test transgenic marine fish, algae and marine bacterial species. It is therefore timely to consider the problems that these proposals are likely to generate.

Some U.S. regulatory agencies have begun to review their responsibilities in such endeavors. For example, the prospective intentional introduction of transgenic organisms is mentioned in the ANS Task Force's report, but the issue is not dealt with in depth because the ANS Task Force felt it lacked the expertise to do so. The report suggested that any transgenic marine organism should be considered as an exotic. For a more detailed consideration of this subject the reader is referred to the position paper by the American Fisheries Society (Kapuscinski and Hallerman, 1990). Since no Federal agency has yet developed criteria to evaluate possible risks associated with the field testing of marine organisms, we must refer to the general criteria published by the NRC (e.g., pages 124-125) and the nearly identical criteria by the OECD for guidance on what would constitute the safe and effective field testing of marine organisms.

Several authors have already considered biosafety aspects of the future field testing of various transgenic marine organisms (see Table 1). We draw much information from this body of work to summarize the most important biosafety concerns related to the field testing of marine organisms in general, and the more specific concerns

in regard to macroorganisms (including transgenic finfish, shellfish, mollusks and other invertebrates, and plants) and to microorganisms (marine bacteria and microalgae).

General Marine Biotechnology Safety Concerns

To consider the possible concerns that marine biotechnology activities may generate, it is necessary to regard each step that a product goes through, from research to marketing, before it reaches the consumer. Thus, in the process whereby an idea or concept is transformed into a commercial product (the concept development process), the process moves through differing facilities, each presenting varying challenges (Zilinskas, 1989).

At the first stage of the process, that of R&D, scientists involved with research, development and testing of transgenic marine organisms would endeavor to ensure the containment of the test subject through physical and biological containment, just as usually is done when scientists handle transgenic terrestrial organisms. NIH guidelines specify conditions under which research may proceed, including containment level. As was noted above, most times by far research will be done under BL1 or BL2 containment level conditions. Therefore, at this stage of the development process there is no difference between safety issues posed by research on a terrestrial or marine organism. Further, the likelihood of the organism under investigation, whether a macro- or micro-organism, escaping and establishing itself in the environment is very low.

After advanced research and development has indicated that the test subject has commercial or other promise, it undergoes pilot plant testing. If the test subject is a transgenic marine macroorganism, growing it in a contained outdoor pond is the approximate equivalent to "pilot plant" testing because here characteristics useful for aquaculture, including growth, reproductive and behavioral traits, are studied. The contained, open-air testing of transgenic carp and catfish (discussed below) are being done for these purposes. At this stage of the concept development process, the conditions under which testing is performed are specified on a case-by-case basis by the USDA and/or EPA. Due to the many precautions that have been

taken, the probability of some test subjects escaping and establishing themselves is very low, as is the probability of adverse effects by the test organism.

If outdoor testing demonstrates that the test subject is more valuable to aquaculture than present strains, it would require more extensive field testing; i.e., a large number of test subjects would have to be grown and propagated under conditions closely resembling those of intensive aquaculture, but with greater security. Thus, transgenic fresh water fish would be field tested in large ponds connected with waterways, or in pens sited in lakes or rivers, while marine fish most likely would be grown in net pens located in protected marine sites, such as inlets and estuaries. At this stage of testing, regardless of precautions being taken the probability of one or a few test subjects escaping is rather high. Escapes may occur as a result of inclement weather damaging net pens, predators such as seals tearing holes in nets, boats accidentally crashing into pens, animal rightists "liberating" imprisoned animals, or for many other reasons. Since the probability of small-scale escape is high, it is important to know the likely ecological and biological consequences of these escapes.

Once field testing has proven the commercial feasibility of the test subject, the organism will be raised in large numbers in aquaculture facilities. Since the probability of large numbers of the transgenic organisms eventually escaping would be high, its safety in the environment must have been previously established during field testing, therefore, no additional biosafety issues are raised once the development process has proceeded this far. Of course, aquaculture operators would still be responsible for meeting local and federal environmental laws and regulations, but that is another issue.

As can be seen, the stage of field testing is exceedingly important in the concept development process in that not only does it demonstrate the practical feasibility of the product, but also its safety (Zilinskas, 1994).

Macroorganisms

The field testing of transgenic animals is given very little attention in the NRC and OECD reports on field testing criteria. This

presumably is because transgenic animals usually are easy to contain, and even if set free or accidentally released, the transgenic animal undergoing testing is not likely to disperse easily or cause damage. However, due to the reproductive characteristics of many marine animals, and considering the aqueous environment in which they exist and breed, the field testing of transgenic marine animals may present a special situation that requires more consideration than do transgenic terrestrial animals.

As noted in Chapter 1, several different species of fish have been transformed. However, only two are undergoing testing outside the laboratory, namely transgenic carp and catfish. For our consideration of the safety of marine biotechnology, it is useful to review the events that preceded the decision by USDA's CSRS to allow the test involving carp to proceed, and to scrutinize the conditions under which the testing of carp and catfish are being performed (Office of Agricultural Biotechnology, 1990).

The fish being tested is a scaleless variant of the common carp (*Cyprinus carpio*), called mirror carp, which has been genetically modified by the insertion of two types of foreign genetic material; a fragment of DNA encoding trout growth hormone and the Rous Sarcoma virus promoter, which ensures that trout growth hormone is expressed in the carp. The transgenic carp was developed by a multi-center scientific team, with representation from the Center of Marine Biotechnology, Maryland, Stanford University, California and Auburn University, Alabama (Chen and Powers, 1990; Chen et al., 1992). At the end of 1989, the team requested the USDA to allow the transgenic carp to be grown in outdoor ponds to learn whether the foreign DNA affected the reproductive capacity of the carp, whether the carp's offspring would inherit the foreign DNA, and whether the offspring would develop and behave normally. Ultimately, results from this research should be useful to improve fish genetic lines for aquaculture.

The test proposal was strenuously opposed by various environmental groups, including the Foundation on Economic Trends and the National Wildlife Federation, on grounds that the tested carp could escape from the testing ponds and reach nearby watersheds,

where it could damage insects, plants and other fish in fresh water habitats. In view of these concerns, which had been inadequately addressed in the initial testing proposal, the proposal was remanded and its drafters were asked by the USDA to provide more information about possible environmental impacts.

About six months later, the principal investigators submitted a redrafted proposal to the USDA. It asked for permission to raise 50,000 fry that had been spawned from nine transgenic carp in ten outdoor pools. After three months, the number of fry will be reduced to 300 per pond; these will be marked for identification and studied for the next 15 months. The fish would then be destroyed, before they reached sexual maturity. The ponds stocking the fish would be well-protected by fences, nets and filters, and there would be no direct connection between the ponds and existing waterways. Further, if a natural event, such as a tornado, threatened the integrity of the testing site, the fish being tested could be killed on very short notice.

After the USDA performed an environmental assessment of the proposed project (Office of Agricultural Biotechnology, 1990), and presented its findings at a series of public hearings, and after having received comments and critique from interested members of the public and public interest groups, the USDA decided on a "Finding of No Significant Impact"; i.e., the agency determined that the "...experiment with transgenic carp presents no significant risks to the environment" (Anonymous, 1990a) (see below). It gave approval for the experiment to proceed, beginning in spring 1991. Actual testing of the transgenic carp began in June 1991. A few months later, a similar test was proposed for a newly developed transgenic catfish, which has an inserted growth hormone gene from rainbow trout (Anonymous, 1991a). This proposal was approved by the USDA in early 1992 (Anonymous, 1992b).

It is useful to review the testing conditions of the transgenic carp and catfish in view of the three criteria formulated by the NRC. First, in scientific terms, carp and catfish have been studied for a long time by many investigators and therefore are well characterized. Since the testing is in effect being carried out in a closed, artifi-

cial system, the environment into which the transgenic fish is being introduced is known. For these reasons, the first criterion is largely satisfied. Second, unless a deliberate, criminal attempt was made to release them, the conditions under which the testing of the transgenic carp and catfish are taking place precludes escape. The second criterion therefore is satisfied; the tested organism is confined and controlled effectively. The third criterion, pertaining to the probable effect on the environment should the organism undergoing testing escape, probably is not applicable since the test conditions preclude persistence or spread. However, because there is a small probability that, for example, a typhoon or other natural phenomenon would break the test site's containment, a critical issue in the USDA's environmental assessment of the proposal to test transgenic mirror carp in contained open-air ponds was the requirement to define the environment affected by the proposed activity (Medley and Brown, 1992). Thus, if a mirror carp should escape open-air testing ponds and survive physical barriers and natural predators, it would be carried into the nearby Sougahatchee Creek and, possibly, to the Yates Reservoir, which is 45 miles downstream (Office of Agricultural Biotechnology, 1990). This environment has been studied extensively, so its water quality, indigenous fish populations, other indigenous organisms, aquatic vegetation, public health and safety, and so forth, are well known. The probable effects that the escaped mirror carp would have on these components could be determined with a high degree of confidence. It can be seen that the testers satisfied the NRC criteria.

The testing of these transgenic carp and catfish is so circumscribed and controlled it more closely resembles testing in a closed system than field testing in the open marine environment. Nevertheless, the conditions under which these first tests are being done probably represent a model for the first testing of any aquatic transgenic animal or plant outside the laboratory or indoor tanks.

Duplicating the contained system used in the mirror carp experiment in the marine environment would be extremely difficult. As noted by Medley and Brown (1992):

In marine systems the affected environment could be broadly defined, which would increase substantially the issues and concerns that would need to be addressed in environmental documents such as an environmental impact statement. Therefore, outdoor testing of genetically engineered marine organisms, other than in contained ponds, would require a complex environmental analysis.

While Medley and Brown did not define the elements of "a complex environmental analysis," by analyzing two sets of considerations, we can deduce the major elements of such an environmental analysis. The first set pertains to the characteristics of the marine environment, which were discussed above in reference to dispersals of marine species. The second is related to the attributes of transgenic macroorganisms. The attributes that scientists attempt to obtain in transgenic macroorganisms have been described by several authors (Chen and Powers, 1990; Colwell, 1987; Powers et al. 1991; Chen et al. 1992; Devlin and Donaldson, 1992; Hallerman and Kapuscinski, 1992; Donaldson et al. 1994), and include the following:

- Improved metabolism—for the purpose of speeding up maturation, attaining larger adult growth, increasing reproduction rates, lowering the amount of fat in body tissues, and/or improving food utilization. Examples of relevant research are the development of transgenic carp and catfish that contain growth hormone genes from trout.
- Improved tolerance to physical factors—to make it possible for the target organism to better tolerate colder or warmer water, water of different salinity contents, higher concentrations of metals or pollutants, and/or lower concentrations of dissolved oxygen. An example of relevant research is the attempt in Canada to develop transgenic salmon containing genes from flounder coding for an antifreeze protein.
- Improved chemical constituents—a marine plant may be engineered to overproduce substances that are valuable as phar-

maceuticals or specialty chemicals. For example, several projects are underway to develop macroalgae that produce commercial quantities of carrageenans.

- Improved control over settling—the spat from most molluscan species require specific chemical signals before they will settle and grow on a surface. If species valuable to aquaculture could be engineered to settle in a controlled manner, the efficiency of aquaculture operations would increase significantly. Although research on larval settling is in progress (see Chapter 1), practical applications are far from realization.
- Improved resistance to diseases—an organism's ability to resist pathogens may be increased by engineering it to produce more or different cytokines, higher concentrations of antibodies against common bacterial, viral and fungal pathogens, and/or immune enhancers. Research on the application of marine biotechnology to animal health is discussed in Chapter 1.
- Improved food quality characteristics—traditionally, conventional breeding has been done to improve the characteristics of animals and plants used for human food, such as texture, color, fillet size, and nutritive content. In this vein, thought is being given to modifying edible Pacific seaweed for improved use as *sushi* and transferring food properties from Pacific seaweed to Atlantic seaweed that presently cannot be used as food. Although research in this area is not yet being undertaken, as far as we know, it could commence at any time since sufficient knowledge and appropriate techniques are available.
- Improved behavioral patterns—since some species being raised in aquaculture have the destructive trait of eating their eggs and fry, attempts can be made to change this behavior trait. Such research goals are theoretical.

In view of these research and development objectives, and assuming that some members of the species undergoing field testing will escape, any combination of the six possible effects that have attended past mediated dispersals of introduced organisms could result (see below). For example, if the escapee is a transgenic fish, its larger size may make it a more powerful predator than the wild form, allowing it to disrupt or destroy wild species; or the escapee may interbreed with wild species and in doing so pass on its genetic traits to subsequent generations of hybrids, perhaps to their detriment in terms of them becoming less adapted for survival; or the escapee may be more vulnerable to attack by pathogens than wild species; or the escapee may be able to feed on local fauna or biota that wild species do not, thereby disrupting the local habitat; or the transfer of the antifreeze gene into striped bass may allow the transgenic fish to migrate northward, to Labrador for example, upsetting feeding systems there.

One additional important factor has to be noted when discussing the possible field testing of transgenic macroorganisms, namely the reproductive cycle of marine shellfish and mollusc species. Parent organisms emit clouds constituted by millions of fertilized eggs, which are carried off into the distance by currents and eddies, eventually to settle on surfaces where the correct chemical cues are present. Unless there was a sure method to sterilize these eggs, the genetic material present in them would be dispersed during a field test.

No one has yet proposed the testing of a transgenic marine macroorganism in unsecured facilities or the open marine environment. Before this can occur, technical barriers must be overcome and environmental risks reduced. The technical barriers relate to the cost-effective transfer of valuable genes and promoters into large number of fish; the ready identification of transformed individuals among the treated group; and the selective breeding of transformed fish to develop superior progeny. In 1990, it was estimated that it will take a minimum of ten years to overcome these technical barriers (Kapuscinski, 1990).

The major means whereby risks related to transgenic fish may be reduced could be to sterilize all fish to be used for outgrowth in culture. Fish may be sterilized by two methods. First, hormones can be administered to fish embryos, which render them sterile. Researchers do not favor this method since it cannot achieve 100% sterilization and hormonal residues may contaminate food fish. Second, fish eggs can be treated so the progeny are triploid; i.e., each fish carries three sets of chromosomes rather than two (see Chapter 1). Triploids are sterile. For added safety, triploid induction can be combined with further treatment that produces an all female progeny. Triploid females are 100% non-fertile.

It has been noted that even if only sterile transgenic fishes are cultured, some risk remains because of the necessity to maintain transgenic broodstock. The answer is to maintain broodstock in secure containment facilities, and to educate everyone who works with them of the ecological problems that have resulted from introductions of exotic fish species in the past (Kapusinski, 1990).

Microorganisms

Marine microorganisms encompasses marine bacteria, viruses, and plankton. Plankton, which may be the most abundant form of life in the marine environment (Williamson and Gribbin, 1991), consists of phytoplankton, or microscopic plants, and zooplankton, which are microscopic animals. Unless specifically identified as such, microalgae, including cyanobacteria, can be included under the general designation "plankton" or "ultraplankton."

In view of the technical and environmental difficulties that have to be overcome before a transgenic macroorganism is field tested, it may be more likely that a microorganism will be the first candidate for field testing. Since much research is being done to genetically engineered bacteria (for bioremediation) and microalgae (for increased production of food additives and enhanced CO₂ uptake to counter the "greenhouse effect") one of these should be considered as the primary candidates for the first marine field testing. Further support for this contention comes from the report that the firm Envirogen

Inc. in New Jersey is preparing a proposal for the field testing of a bacterium that has been genetically engineered to improve its ability to degrade the industrial pollutant trichlorethylene (Anonymous, 1991b).

The initial "field testing" of a genetically engineered bacterial, cyanobacterial or microalgae species would probably be done in a contained, open-air system, similar to that used for testing transgenic carp and catfish. The strain to be tested may be "weakened" so it would not survive in the environment should it escape. Parameters that could be tested in a closed system for a microorganism to be used in bioremediation include survivability in the open, ability of the organism to degrade trichlorethylene under various conditions and in the presence of other contaminants, and the extent of synergism between the tested organisms and other microorganisms. Testing of a marine transgenic microorganism under these conditions would be highly unlikely to endanger man or the environment.

However, as with the actual field testing of aqueous transgenic microorganisms, the proposed field testing of a marine transgenic microorganism would pose difficult, possibly unique, problems. It has already been noted that the continuity of oceans and the incessant movement of water favors the dispersal of organisms in the marine environment. In addition to the dispersal and survival of whole organisms, the marine environment also favors the transfer of genetic material. Salt water may preserve the viability of immersed organisms and free nucleic acids by preventing desiccation and absorbing damaging ultraviolet light. A liter of seawater can contain millions of bacteria, tens of millions of viruses, thousands of phytoplankton, and hundreds of zooplankton (Williamson and Gribbin, 1991; Anonymous, 1990b). Microorganisms suspended in water can easily come into direct contact with other organisms and diverse suspended matter, creating many possibilities for the exchange of genetic material. Marine microorganism, especially bacterial species, can exchange genes via one of three mechanisms.

The first mechanism is conjugation, where two bacterial cells directly interact to exchange genetic material. For conjugation to take place, cells have to be closely related. Thus, conjugations works

efficiently between two *E. coli* cells; fairly efficiently between two species in the family *Enterobacteriaceae*, say *E. coli* and *Salmonella*; but not at all or very inefficiently between distantly related genera or between bacterial and yeast cells. Studies have shown that plasmids containing genetic information can be transferred from *E. coli* to a *Vibrio parahaemolyticus* strain native to the Chesapeake Bay. Therefore, it is "highly probable that genetic transfer occurs between bacteria" in the Chesapeake Bay (Colwell, 1987). The possibility of transferring DNA from *E. coli* to a cyanobacter has been demonstrated in the laboratory, but it is not known if this phenomena occurs in nature (Ciferri et al.1989). Therefore, the possibility that a genetically engineered bacterium released in the marine environment could pass on transferred foreign genes to a wild bacterium through conjugation exists, although little is known about conjugation among marine bacterial species in natural waters. The probability of a transformed bacterium passing the gene to cyanobacteria is lower. It is reasonable to believe that dispersal by conjugation would be more likely in water populated by very large numbers of bacteria due to contamination by sewage and human wastes than in oligotrophic oceanic water containing low numbers of bacteria.

It is important to note that large populations of novel bacterial species have been discovered in oligotrophic waters. These species are at present unculturable in the laboratory and nothing is known about their ecology, physiological capabilities, or ability to exchange genetic material (Giovannoni et al.1990; Britschgi and Giovannoni, 1991; Schmidt et al.1991). This recent discovery forcibly illustrates our rudimentary understanding of marine microbial ecology and highlights the need for research in this area.

The second gene exchange mechanism is transduction, where a vector transfers genetic material from one cell to another. For example, viruses that infect bacteria, called bacteriophages (or phages), may transfer genes between bacterial cells. In general, phages are specific, one type of phage will attack only a single bacterial species. Results from recent research demonstrates that an immense number of viruses and viral particles populate the ocean surface layer (Proctor and Fuhrman, 1990; Suttle et al.1990; Wommack et al.1992).

These data indicate that one milliliter of surface water can contain between 10^7 and 10^9 viruses, which means that the one millimeter thick surface layer of the world's oceans would contain a total of 3.6×10^{10} viruses (Anonymous, 1990b)! The role of viruses in the marine environment is unknown, although it is believed that most of them are phages, attacking species of marine bacteria, microalgae, plankton and other organisms. Whether transduction occurs in nature between marine bacteria and cyanobacteria—or between different cyanobacteria—is not known, but may be possible and is under active study (Ciferri et al. 1989).

The third mechanism is transformation, where a plasmid or naked DNA is taken up by a cell from the immediate environment. Transformation can be relatively easily accomplished in the laboratory under appropriate, controlled conditions. As far as is known, transformation is exceptionally unusual in the atmospheric and terrestrial environments. Little is known about the dispersal of genes via transformation in ocean waters.

Terrestrial Versus Marine Biotechnology

Does marine biotechnology pose different safety and regulatory issues than biotechnology related to the terrestrial environment? From the discussion and analysis in the preceding three sections we can deduce that marine biotechnology research does not; neither do inanimate products from marine biotechnology. Present risk assessment and management schemes, as well as existing regulations, adequately cover these areas of marine biotechnology.

The difference, then, is the field testing of genetically engineered organisms. We have noted that the USDA has given permission for the testing in contained, open-air ponds of two genetically engineered aquatic organisms—a transgenic carp and a transgenic catfish (see above for a more detailed description of one of these experiments). These tests are likely to generate much data on the growth patterns of the transgenic species, their behavior, and so forth, but since they are being carried out in closed systems, they cannot be considered more than prototypes to the future field test-

ing of transgenic marine animals in the open marine environment. Here the researcher and the regulator would face special problems not encountered in contained field trials. Similar to field tests in the atmosphere (Stetzenbach et al.1992), in the open marine environment it may be impossible to ensure the biological isolation of the organisms being tested. Biological isolation cannot be guaranteed because of the continuity and movement of the ocean waters and the existence of potentially many unfamiliar biological modes for gene dispersal. The lesson from dispersals is that after release, the subsequent dispersal via natural mechanisms cannot be predicted, so the consequences of escapes also are unforeseeable.

If transgenic fish are intensively cultured in cages or pens emplaced in ponds, lakes, rivers or brackish-water estuaries, the possibility of some of them escaping appears to be high. The consequences of an escape would range from no discernable or minimal effect to severe damage to existing wildlife and/or biota. Past experiences of terrestrial field testing of genetically engineered organisms indicate that ill effects are unlikely. However, we cannot completely discount the possibility that an escape may trigger a low probability, high consequence sequel, similar to what has been described (Thorne-Miller and Catena, 1991):

the dangers of genetic manipulations should be recognized, and biotechnology may prove to be as much a threat to natural species and genetic diversity as it is a justification for maintaining that diversity. The release of individuals with artificially composed genetic makeups into wild populations of the same species could upset the natural distribution of that species as well as the competitive interactions with other species, destabilizing natural biological communities.

In the case of transgenic microorganisms, not enough is known about mechanisms for gene dispersal in the marine environment to predict whether the foreign genes carried by the transgenic microorganism would disperse, the frequency of possible dispersal, the probability of dispersed genes being acquired by wild organisms, or the ultimate effects of dispersal.

CONCLUDING REMARKS ON THE SAFETY OF MARINE BIOTECHNOLOGY

The status today of the field testing of transgenic marine organisms is similar to that for terrestrial and atmospheric field testing when these tests were being proposed a decade ago. However, scientists now can learn from the experience of past field tests to plan for future field testing in the aqueous environment. In addition, better methods for assessing risks have been developed and sophisticated techniques for detecting and tracking genetic material are in use. Scientists thus are in a better position than in former times to undertake effective environmental assessments prior to testing, to design safe test protocols, and institute efficient mechanisms for monitoring test events and measuring the long-term effects of tests. Nevertheless, we cannot lose sight of the fact that for the present it is substantially more difficult to evaluate and determine the possible effects of the field testing in the marine environment of transgenic marine animals, plants and microorganisms than similar tests in the terrestrial environment.

In summary, the present regulatory situation does not favor the field testing of transgenic marine macro- or microorganisms in the aqueous environment. Due to the many uncertainties that would accompany the field testing of transgenic marine organisms regulatory agencies should not allow the field testing of organisms in the marine environment until research in biological oceanography, microbial ecology and environmental toxicology has clarified the details of the mechanisms of dispersal of organisms and genes in the marine environment and a satisfactory risk assessment methodology for field testing in the oceans has been developed. It can be seen that the greatest need at the present time is for research to be done that would lay a scientifically-sound basis for the safe and effective field testing of genetically engineered organisms in the marine environment.

Table 1. Articles Related to Transgenic Marine Organism and Safety.

Subject	Reference Number(s)
General aspects of marine biotechnology safety	(Agricultural Biotechnology Research Advisory Committee, 1992) (Allen Jr., 1992) (Colwell, 1986) (Colwell, 1987) (Donaldson et al. 1994) (Gealt, 1992) (Gregory, 1992) (Hood, 1992) (Mackenzie, 1989) (Mackenzie, 1992) (Saye and O'Morchoe, 1992)
Transgenic finfish	(Office of Agricultural Biotechnology, 1990) (Parker, 1992)
Transgenic shellfish	(Thorgaard and Allen, 1992)
Transgenic mollusks	(Cembella and Shumway, 1994)
Transgenic invertebrates (other)	(Anonymous, 1993)
Transgenic plants	(Meints and Van Etten, 1992)
Transgenic microalgae	(Ciferri et al. 1989) (Skujins, 1992)
Transgenic marine bacteria	(Colwell, 1992) (Cripe and Pritchard, 1992) (Gealt, 1992) (Saye and O'Morchoe, 1992)

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Chapter 6

MARINE BIOTECHNOLOGY IN AUSTRALIA

INTRODUCTION

In this report, we consider in some detail the status of marine biotechnology in three countries—Australia, Norway, and Japan. These countries were selected because each has significant activities underway in marine biotechnology. Analysis of each country is provided in seven sections: background, government support of science and technology, research and development related to marine biotechnology, industrial activities related to marine biotechnology, academia-industry cooperation, international projects related to marine biotechnology, and conclusion.

BACKGROUND

Australia is exceedingly well endowed with marine biological resources. Its marine territory is impressively large, i.e., almost nine million square kilometers encompassing a larger area than its land area. The world's largest coral reef system, the Great Barrier Reef, lies off the eastern coast of Australia, stretching more than 2,200 kilometers. Australia's marine environs include tropical, temperate, and cold-water seas. It is likely that Australia possesses a greater variety of marine life than any other nation. For example, the Great Barrier Reef is populated by approximately 2,000 fish species (which is twice as many as the second richest habitat located in New Caledonia) and 500 coral species (compared with 300 in New Caledonia) (Groombridge, 1992). Other off-coast regions of Australia, particu-

larly western and southern Australia, may have the most diverse algal flora in the world, with an unusually large proportion of endemic species and genera (M.A. Borowitzka, 1994). The rich biodiversity of animals and plants present in Australian waters forms a treasure trove of raw material, amenable to sustainable economic exploitation via marine biotechnology. However, the rich marine biological resources of Australia are only lightly explored and just beginning to be utilized.

Australia has 33 universities and technological institutes and one maritime college, plus a variety of other colleges and advanced education institutes (Department of Industry, 1994). Between 1983 and 1987, an average of 25 doctorates and 15 master of science degrees were awarded annually in the marine field and this was predicted to increase to 35-40 doctorates and 25-30 masters degrees by 1991. Two-thirds of these marine science degrees were in marine biology. The major degree-granting institutions are the Universities of Sydney, Queensland, New South Wales, Western Australia and James Cook (Review Committee on Marine Industries, 1989). It should be pointed out, however, that almost every Australian university offers courses in marine-related subjects.

The biotechnology industry in Australia went through a difficult early period—several of the biotechnology companies established in the early 1980s went bankrupt (Gumm, 1992). For example, one of these, Biotechnology Australia, at one time was Australia's largest dedicated biotechnology company, having developed seven natural products to the point where they were being marketed (Australian Trade Commission, undated). Initial returns from product sales were disappointing, however, so investors pulled out, apparently unwilling to finance research that had only long-term prospects. Thus, Biotechnology Australia failed (Yuan, 1992).

The last three years have shown the bioindustrial climate in Australia to be significantly improved, most likely a result of strict measures, described below, taken by the Australian government. Several small biotechnology companies show signs of success, including Bresatec Ltd., Bioclone Australia Pty. Ltd., Memtec Ltd., Calgene Pacific Pty. Ltd., Australian Medical Research and Development Corporation Ltd. (AMRAD Corporation), Progen Industries Ltd.,

and Peptide Technology Pty. Ltd. (Roseby, 1994). During this period, the Australian biotechnology industry has moved from being dominated by a few large institutions and several smaller specialized firms (often associated with an educational and/or research institution) to being much more diversified, with a wide ranging institutional base and more large multi-interest firms entering the field at both the state and Commonwealth levels (Roseby, 1994). The future indeed appears bright—a recent report, *Biotechnology in Australia*, predicts that biotechnology will grow into a multi-billion dollar industry by the year 2000 (Australian Trade Commission, undated).

GOVERNMENT SUPPORT OF SCIENCE AND TECHNOLOGY IN AUSTRALIA

Direct Support: Science and Technology

According to the Heads of (Commonwealth) Marine Agencies (HOMA), an informal group comprised of representatives from thirteen Commonwealth agencies involved in marine science and policy, total funding from all sources (including business) of marine science and technology (encompassing not only research, but other science and technology) was approximately \$211 million in 1993 (HOMA, 1993). Non-business marine R&D expenditure (mostly funded by Commonwealth and state governments) in 1990-1991 totalled approximately 3% of R&D expenditure in all fields: \$91 million (measured by socio-economic objective of the R&D) or \$96 million (measured by field of research) (Department of Industry, 1994). Of the latter, marine biology accounted for \$25 million, marine aquaculture \$10.6 million, and biological oceanography \$2 million. It is not possible to estimate expenditure on marine biotechnology from either of these surveys since it is not defined as such.

Major Australian federal ministries and departments supporting marine-related research are Industry, Science and Technology; Employment, Education, and Training; and Primary Industries and Energy. Within the latter's portfolio are 18 Research and Development Corporations and Councils, most of which obtain significant funding from industry levies. In the case of the Fisheries Research and Development Corporation, about 20% of the funds derive from the

fisheries industry (Fisheries Research & Development Corporation 1993).

The Department of Employment, Education, and Training is responsible for funding the Australian Research Council (ARC), as well as providing general financial support for universities. The ARC (together with the previously titled Marine Science and Technology Grants Scheme) contributed circa \$1.8 million to marine-related research in 1987 (Review Committee on Marine Industries, 1989). The main areas funded were marine biology, plant and animal ecology, animal physiology, reproduction and genetics, taxonomy, biochemistry and the metabolism of microorganisms, and parasitology and pathology. Grants are provided mainly to researchers in higher education institutions on the basis of scientific excellence. Until 1990, the ARC (incorporating the former Marine Science and Technology Grants Scheme) funded marine sciences as a priority area. However, experience showed that applications in marine sciences (including marine biology) were highly competitive in attracting funds against other applications in the same discipline. Thus, designation as a priority area was deemed unnecessary (Fayle, 1994).

In addition, the Antarctic Division of the Department of the Environment, Sport and Territories has responsibility for Australia's national research effort in the Antarctic and southern oceans, and conducts and supports major research projects in marine biology, fisheries, and oceanography.

The taxation climate in Australia for industrial research and development (R&D) is attractive and highly competitive, compared with other countries (Birch and Shaw, 1993). Government incentives to Australian firms to undertake R&D include a 150% tax concession, and a comprehensive scheme of grants and loans for industrial R&D. Firms wishing to take advantage of a tax concession must make a substantial investment in R&D, typically 30-60% (Rosebush, 1994). Nevertheless, at this concessional rate, a company's after-tax cost of research is reduced by about 50% (Anonymous, 1994).

The Cooperative Research Centre (CRC) program was launched in 1990, to develop R&D links between academic and government research institutions, CSIRO, and commercial compa-

nies, and to build centers of research concentration in order to achieve worthwhile commercial applications of the research. Three CRCs of relevance to marine science are the CRC for Reef Research, the focus of which is the Great Barrier Reef, CRC for Antarctic Research, and a recently formed Aquaculture CRC, which coordinates work in a large number of institutions and aquaculture companies across Australia. Biotechnology aspects of the R&D of the latter two CRCs are described below. The CRC program, which is now reaching maturity and is an integral element of research funding in Australia, by late 1994 will be supporting 61 centres conducting collaborative scientific and engineering research. The CRC program and grants and loans for industrial R&D are funded through the Industry, Science and Technology portfolio. Two-thirds of the budget of CSIRO, Australia's largest research agency, is also allocated through this portfolio, as is the allocation for the Australian Institute of Marine Science (AIMS).

Indirect Support: Industry

In the late 1980s, Australia enacted a series of measures to attract firms. The financial sector was deregulated, tariffs lowered, foreign exchange controls removed, corporate tax rates reduced to 39%, foreign tax credits arranged, and unrestricted repatriation of profits allowed. The corporate tax rate was again lowered during 1993, to 33%.

Both Commonwealth and state governments provide support measures to companies to assist them to: develop and improve business planning, strategies and operations (management, quality, etc.); seek development capital; acquire or disseminate technology; trial and demonstrate Australian products; exploit international business opportunities; and develop strategic networks and collaborative links. The aim is to assist companies in researching, developing and gaining access to markets for internationally competitive goods, services and systems (Fayle, 1994).

During 1994, an Australian Marine Industries and Science Council was established to formulate a marine industries development strategy. It will advise the Government on mechanisms for po-

sitioning Australia's marine industries, including those in marine biotechnology, to capture a greater share of world markets.

RESEARCH AND DEVELOPMENT RELATED TO MARINE BIOTECHNOLOGY

The number of marine biotechnology-related research projects being undertaken at Australian universities is small, compared to Japan and the U.S. However, the research being done is wide-ranging, illustrated by the following examples. At the Queensland University of Technology, research on the ecology of ciguatoxin is being done. At Deakin University at Geelong, Victoria investigators are studying abalone attachment and growth, and at the University of Melbourne nutritional factors for the growth of abalone, and determination of age in both abalone and lobster are being investigated. Research with applications in aquaculture includes the development of virus-free prawn cell lines at the James Cook University, and study of fish growth hormones at Deakin University. A group at the University of Adelaide has isolated cDNA sequences for the growth hormone from both the barramundi, *Lates calcarifer*, and the black bream, *Acanthopagrus butcheri* (Heyward, 1994). In collaboration with the South Australian company Bresatec, these cDNAs were used to produce recombinant fish growth hormones. The same group has successfully evaluated the application of genetically modification techniques to fish, using the widely accepted zebra-fish model. Other research being performed at universities and institutes in Australia includes extraction of biologically active compounds from marine organisms, analysis of marine toxins and anti-venoms, development of industrial adhesives from marine invertebrates, and development of diagnostic probes for pathogens of marine animals (Review Committee on Marine Industries, 1989).

Scientists at Murdoch University in Perth, Western Australia, are working in three areas of marine biotechnology. First, they are developing large-scale closed tubular photobioreactors to culture microalgae for production of long-chain polyunsaturated fatty acids and carotenoids. Second, they are genetically engineering high carotenoid-producing algae to improve carotenoid yields. Third, the

Murdoch University researchers are collaborating with colleagues from the University of Tasmania and CSIRO to develop a PCR-based test for toxic dinoflagellate species so they can be detected and monitored in ships' ballast water, aquaculture farms, and elsewhere (M.A. Borowitzka, 1994).

Besides universities, public research institutions play important roles in carrying out R&D in marine biotechnology. Institutes with a major activity in marine science are described below, but it must be emphasized there are significant projects being worked on at other locations. At the Queensland Institute of Medical Research in Brisbane, for instance, scientists are testing cytotoxic properties of Bistratene A, a compound isolated from the ascidian species, *Lissoclinum bistratum*, collected at Heron Island on the Great Barrier Reef. Bistratene shows activity against certain leukemic cells, providing new possibilities for studying mechanisms governing cell growth and differentiation (Watters et al., 1994).

The following sections describe major Australian institutes with programs in marine biotechnology.

Australian Institute of Marine Science (AIMS)

AIMS, established in 1975, is a Statutory Authority within the Department of Industry, Science, and Technology. AIMS conducts marine R&D and facilitates the use of research results nationally and internationally. The Institute's headquarters are located on the northeast coast of Australia near Townsville, Queensland. In October 1994, an additional research facility will be opened on the northwest coast of Australia at Dampier, Western Australia. The Institute concentrates on research pertaining to tropical coastal and continental shelf waters of Australia. It has three multidisciplinary programs: Coastal and Shelf Processes; Coral Reef Ecosystems; and Environmental Studies and Biotechnology. In 1993-1994, AIMS had a budget of \$16.9 million of which 15% was obtained from nongovernmental sources. External funding will increase to approximately 21% of the total budget in 1994-1995. Several projects undertaken by AIMS endeavor to provide commercial opportunities, especially those in environmental management, resources and conservation,

marine natural products related to fine chemicals and pharmaceuticals, and mariculture. Marine biotechnology features prominently in the Institute's established collaborations with commercial partners.

An AIMS team headed by Drs. B. Chalker and W. Dunlap is carrying out research on ultraviolet-B absorbing compounds found in corals and other organisms occupying shallow water reefal environments. Several type of UV-B absorbing compounds have been isolated, and their chemical structures identified. Environmental studies on the significance of these compounds have been extended to include the UV-photobiology and photobiological chemistry of temperate and Antarctic marine organisms. A cooperative arrangement was established between AIMS and ICI Australia Operations Pty. Ltd. to complete securing patents on analogs of naturally occurring compounds, which might be used for personal sun protection. This collaboration has resulted in the development of two sets of commercial analogs patented internationally. In January 1994, AIMS acquired exclusive rights for further commercialization of these compounds. The project has subsequently extended its research initiative to examination of other photobiological defence mechanisms, including small-molecule photo-antioxidants in marine organisms (Fayle, 1994).

Building on research initiatives of Drs. J. Baker and P. Murphy, AIMS has significantly advanced commercialization of results of research accomplished in marine natural products chemistry by entering into two agreements with the Melbourne-based pharmaceutical company, AMRAD Corporation. In the first agreement, AIMS has become one of eleven member institutes of AMRAD, joining a network of Australian organizations committed to commercialization of Australia's biomedical research.

As part of the agreement, AMRAD has the first opportunity to consider projects arising from the Institute's research in human therapeutics and diagnostics. In return for this consideration, AIMS gains the opportunity for further funding and benefits from AMRAD's substantial commercial experience. The second agreement funds a major research project (\$4.7 million over five years) that comprises screening the collection of marine plants, animals, and microorgan-

isms for biological activity and extension of chemical and pharmacological R&D of biologically active compounds identified in the new project and in previous AIMS research.

In a project lead by Dr. J. Benzie, AIMS scientists are currently developing methods to close the life cycle of prawns and to produce genetically improved strains through a breeding program. The long-term goals of the breeding program are complemented by reproductive research addressing critical and immediate issues of larval supply.

AIMS has also undertaken research on a range of tropical mollusks, such as the pearl oyster. AIMS has a major contribution to make in reproduction, genetics, and broodstock management areas of the CRC for Aquaculture (see page 209) that will link key AIMS expertise with several state and university research groups and with CSIRO. These programs include research on crustacea, especially prawns, shellfish, especially pearl oysters, and tropical fish.

Commonwealth Scientific and Industrial Research Organization (CSIRO)

CSIRO is Australia's national scientific research organization and emphasizes application-oriented research. CSIRO has about 5500 professional and technical staff, and accounts for 13% of all Australian R&D expenditures (Austrade, 1992). In 1994-1995, it is estimated that CSIRO will have a total budget of \$678 million, two-thirds of which is from government appropriation (\$461 million), and the other third from industry, earned revenue, and grants (Commonwealth of Australia, 1994). Its marine laboratories headquarters are located in Hobart near the Southern Ocean, which is the site of two of CSIRO's divisions, the Division of Oceanography and the Division of Fisheries.

CSIRO Division of Oceanography

Research with biotechnological applications is carried out in the Marine Resources and Pollution Program of the Division of Oceanography, led by Dr John Volkman, through projects on Ma-

rine Products, led by Dr Peter Nichols, and Mariculture Nutrition. Research has focussed on exploring fish waste products and converting these to commercial products through collaborative projects with local industry. For example, waste from the deep-water trawl fishery of orange roughy is processed to produce wax ester-based oil marketed as an environmentally friendly degreaser and solvent. Hand creams are also marketed and other products are in development (Nichols et al., 1993). A process has been devised to produce high purity squalene from livers of deep-sea sharks.

Recent work is concentrated on identification of commercially valuable sources of polyunsaturated fatty acids from microalgae, fish, and bacteria. Bacteriological studies are part of the Microbial Processes Subprogram of the CRC for Antarctic and Southern Ocean Environment (see page 209). Antarctic microorganisms appear to have potential for biotechnological applications, particularly as sources of novel biologically active compounds. A screening program has been initiated with support from AMRAD. Other studies include isolation and culture of Antarctic bacteria capable of synthesizing long-chain polyunsaturated fatty acids.

A collaborative project between the CSIRO Division of Fisheries, Division of Oceanography, and commercial partners involves analyzing the biochemical composition of microalgae that are used as live mariculture feeds (Volkman et al., 1991, 1993; Dunstan et al., 1993). An extensive database has been developed and recent studies have shown how culture conditions can be modified to enhance production of lipid, protein, and sugar constituents. This research is designed specifically to underpin future studies examining production of algal pastes and single-cell oils for mariculture and other applications (Volkman, 1994). A collaboration between the two divisions is focussed on studies of a toxic dinoflagellate species linked to paralytic shellfish poisoning (Hallegraeff et al., 1991).

The Division of Oceanography operates one of Australia's premier research vessels, the *RV Franklin*. Through 1991, the vessel was utilized by the Division's environment and climate programs predominantly and had not included marine biotechnology (CSIRO Division of Oceanography, 1992).

CSIRO Division of Fisheries

The Division of Fisheries has a staff of more than 200 and operates major laboratories in Hobart (Tasmania), Cleveland (Queensland), and Marmion (Western Australia). The Division operates a 66.1-meter vessel, *FRV Southern Surveyor*, one of the largest fisheries research vessels operating in Australian waters. Research of the Division is organized into five programs; three focus on pelagic, tropical, and temperate and deep-water fisheries resources, and the remaining two on marine environmental research and mariculture.

The Division obtains 35% of its funding from other government agencies and industry and an increasing proportion of its resources are devoted to mariculture research. The Division provides focus for major new research initiatives by CSIRO in support of aquaculture and is heavily involved in the recently established CRC for Aquaculture (see page 209). The Division maintains the CSIRO Culture Collection, including the largest algal culture collection in Australia (which in 1986 contained about 150 species of algae), housed at the Algal Culture Laboratory, also located in Hobart (Communications Officer, 1986). The Laboratory is the major supplier of algal starter cultures for research agencies, universities and industry. The Division recently commissioned a major new aquaculture facility at its Queensland laboratory (Fayle, 1994).

Research undertaken by the Mariculture Program in the field of biotechnology includes projects aimed at development of long shelf-life storage products (pastes and powders) produced from selected microalgal species and suitable for use in hatchery and nursery systems to control maturation of tropical prawn species through special diets, and development of transgenic methods for use with prawn embryos to enhance performance in culture and provide biological markers for stock improvement (Martin, 1994).

**National Centre for Teaching and Research in
Aquaculture**

The National Key Centre for Teaching and Research in Aquaculture was established in 1988 at the University of Tasmania. As of

June 1994, the Centre had 209 students enrolled, including 15 overseas students from 11 countries, and 20 students reading for doctoral degrees. New laboratories valued at \$8 million opened in July, 1994. The Centre operates an extensive array of aquaria, including a marine finfish hatchery, salmonid hatchery, and oyster hatchery. Areas of expertise include fish health and farm management, fish reproduction, larval finfish and shellfish culture, and aquaculture nutrition. Development of new species for aquaculture is a priority area of research (Fortreath, 1994).

Bribie Island Aquaculture Centre

Bribie Island Aquaculture Centre, located in Southeast Queensland, is an advanced aquaculture research and training centre. Capital investment in the Centre totalled circa \$10 million by mid-1994. Programs of the Centre focus on nutrition and maturation of prawns (particularly Kuruma and Black Tiger prawns), improvement of feeds for prawns and finfish, mud crab aquaculture, and fisheries effluent management. The Centre includes a laboratory, hatchery, prawn maturation facility, growout ponds, conference center, and facility for accommodating visitors. The Research Centre has direct access to ocean quality water via two large-capacity intake pipelines fitted with filters (Williams, 1994). Aquaculture research and training centers similar to the Bribie Island Aquaculture Centre are located at Launceston, Tasmania, and West Beach, near Adelaide (Roseby, 1994).

State Fisheries Research Laboratories

State Fisheries Research Laboratories undertake research on wild fish stocks, aquaculture, and coastal environments (Review Committee on Marine Industries, 1989). Funding comes from both state government appropriations and external sources, predominantly the Fisheries Research and Development Corporation. In 1993-1994, \$3.4 million (45%) of the funding from the Fisheries Research and Development Corporation was directed toward state and territories fisheries research institutions.

CRC for Aquaculture

Participants in the CRC for Aquaculture include: six universities; federal institutes, represented by three divisions of the CSIRO and the AIMS; twelve branches of state research institutes; and nineteen private sector groups. This CRC, which began operation in 1994, includes representatives from every state and territory, except Western Australia and the Australian Capital Territory and, therefore, covers nearly all regions and most aspects of Australian aquaculture (Fayle, 1994).

Scientific research of the CRC comprises six major program areas (Heyward, 1994):

- Techniques for rapid identification of infectious diseases of key aquaculture species and improved methods of controlling these pathogens; gene probes, culture, and immunological procedures for detection tests against endemic viral and bacterial pathogens.
- Improved feed for larval and nursery rearing and grow-out rearing; species under study include bivalves, prawns, finfish and abalone.
- Improved management of finfish broodstock.
- Shellfish genetics and reproduction.
- Aquaculture pond and effluent management and control of biofouling of aquaculture facilities.
- Improved post-harvest technology.

CRC for Antarctic Research

Partners in the Antarctic CRC include the University of Tasmania, CSIRO Division of Oceanography, Bureau of Meteorology,

and Australian Geological Survey Organization. The biotechnology research is focused on Antarctic microorganisms.

Antarctic microorganisms have been determined to be useful for biotechnology, notably in their adaptation to environmental extremes of low temperature and water availability. Production of polyunsaturated fatty acids (PUFAs) essential for growth and survival of larval fish and crustaceans, are used in mariculture, pharmaceutical and biotechnology industries. Currently, algae and fish oils are the major source of these fatty acids. Production of unsaturated fatty acids in many organisms increases with growth at decreased temperature. CRC researchers have targeted the permanently cold sea ice environment as the source of psychrophilic bacteria in which membrane fluidity is maintained by elevated concentrations of PUFAs. Some bacterial strains can produce up to 18% of their membrane fatty acids as PUFAs. Both eicosapentaenoic acid and docosahexaenoic acid have been detected among the PUFAs synthesized by bacteria.

Centre for Marine Biotechnology

The University of New South Wales formed a Centre for Marine Biotechnology during 1994. This is based primarily on marine biofouling research aimed at development of new environmentally benign, biologically derived antifoulants. Additional programs will include research projects on fish pathogens, probiotics in aquaculture, mariculture of seaweeds, and bioremediation of polluted habitats (Kjelleberg, 1994).

INDUSTRY

In 1992-1993, marine industries earned \$21 billion and \$5.5 billion was earned from exports (McKinnon, 1993). However, at this time industries related to marine biotechnology constitute only a small fraction of this total.

The marine natural products industry in Australia has, until recently, been limited to only a few firms, e.g., Betatene Ltd. and Western Biotechnology Ltd., culturing microalgal species, mainly *Dunaliella salina*, to produce beta carotene. Total sales of beta

carotene, used for animal feed, human dietary supplements, and food coloring, were \$2 million in 1987-1988 (Review Committee on Marine Industries, 1989). Sales increased to \$5 million by 1992-1993 and continue to rise (L. Borowitzka, 1994). Reflecting the increasing size of the beta carotene market, Western Biotechnology expanded production facilities at Hutt Lagoon in Western Australia from 50 to 75 hectares of ponds (M.A. Borowitzka, 1994). Both companies remain profitable as this is written.

The Australian aquaculture industry, although larger than the marine natural products industry, is at an early stage of development, with much of the technologies utilized being imported. In 1987, the aquaculture industry comprised small firms, with an average capitalization of \$150,000. It employed 1,700-2,000 workers (Review Committee on Marine Industries, 1989). However, by March 1994, there were 693 aquaculture businesses with at least one employee, and these businesses were operating in 747 locations throughout Australia (Australian Bureau of Statistics, 1994). The vast majority (92%) were firms with less than 10 employees. The largest publicly listed aquaculture company, Tassal in Tasmania, in 1992-1993 had a turnover of \$32 million and employed circa 140 persons (Bureau of Industry Economics, 1994). Tassal aquacultivated 1,400 tons of fish in 1992-1993, mostly high quality Atlantic salmon.

Since compilation of comprehensive annual aquaculture production statistics was begun in 1988-1989, there has been a 29% increase in the tons produced and a staggering 87% increase in value (this does not take into account effect of inflation). In 1988, aquaculture products earned a \$105 million, of which \$93 million came from pearl oysters (IR&D Board Workshop, 1989). In 1991-1992, almost 16,200 tons of product were harvested, with circa 19.3 million juveniles (mostly finfish fry) being produced in hatcheries for recreational and conservation stocking (some 4 million aquarium fish are included in these figures). Together these are worth \$254 million (farm gate value), an increase of 7% over the 1990-1991 value of production. The most important sectors were pearl oysters (in 1990, \$119 million), salmonids (\$55 million), edible oysters (\$45 million), prawns (\$10 million), and southern bluefin tuna (\$5 million) (O'Sullivan, 1994).

Biotechnology in general is a rapidly growing industry in Australia. The number of biotechnology companies per capita (or per GDP) is similar to that of the U.S., though the largest such firm in Australia has under 250 biotechnology employees, and the average size is well below that of the U.S. (Fayle and Playne, 1993; Department of Industry, 1994). These biotechnology companies are very active in bringing new products onto the market, with products for agricultural and veterinary application and medical diagnostics at the forefront. Commercial interest in marine biotechnology has started to grow, indicated by an investment of \$20 million over the five years, beginning in 1994, made by AMRAD Corporation for screening marine and other organisms for pharmaceutically useful metabolites. In addition to pursuing agreements with Australian research institutes to access collections of indigenous terrestrial and marine plants and microorganisms, AMRAD has made an agreement with the Seattle-based company, Panlabs, to use that company's screening systems to identify new leads from biological sources (M.A. Borowitzka, 1994).

ACADEMIA-INDUSTRY COOPERATION

It has been widely recognized that cooperation between academia and industry in the field of R&D has been rudimentary, at best, in Australia. This deficiency is being addressed at several levels in the universities, at CSIRO, and in state research institutions, with all putting higher priority on industry needs and on securing industry funding for research, while rural R&D corporations (including the Fisheries Research and Development Corporation) and the Industrial Research and Development Board emphasize commercial implementation of the research being supported by these agencies.

"Generic" grants for biotechnology R&D, with the primary aim of fostering collaboration between commercial enterprises and research institutions to develop new technologies of strategic significance, have been available since 1983. A good example of the value of this scheme is the recently awarded \$1.5 million grant to AMRAD and the Centre for Drug Design and Development (University of Queensland) to work on the development of peptidomimetic

drugs based on peptide leads from the venom of cone shells (Andrews, 1994).

The marine natural products area specifically is attracting industry interest and, therefore, presents good opportunities for industry-university collaborations. For example, in late 1993, the international company Astra Pharmaceuticals signed an agreement with the Queensland Pharmaceutical Research Institute of Griffith University to invest \$10 million in a program to screen Australian native plants and marine organisms for potential new drugs. The agreement calls for university researchers to collect for analysis more than 100,000 extracts during the next ten years (M.A. Borowitzka, 1994).

INTERNATIONAL TECHNOLOGY TRANSFER AND INTERNATIONAL RELATIONS

Australia participates in several international projects under the auspices of the International Oceanographic Commission of the United Nations Educational, Scientific, and Cultural Organization (UNESCO), including the World Ocean Circulation Experiment and the Tropical Ocean and Global Atmosphere Program. HOMA coordinates Australia's international programs and exchanges of information (HOMA, 1993). International contacts between individual scientists also are coordinated through the Australian Academy of Sciences.

A successful example of international collaboration is the research project between the James Cook University and the International Centre for Living Aquatic Resources Management (ICLARM) focussed on the giant clam (*T. gigas*). The Australian Centre for International Agricultural Research (ACIAR) has committed \$5 million to this project. ICLARM, which became a Member of the Consultative Group on International Agricultural Research network in 1992, includes six countries in the Asia-Pacific region, with headquarters in the Philippines and a Coastal Aquaculture Centre in the Solomon Islands.

The giant clam grows to a 50 kg weight within nine to ten years, producing a delicious meat highly prized in countries throughout the Pacific and Asia. In addition, giant clam shells are

sought after by tourists. Because they are so popular, fishermen, mainly from Taiwan, have decimated the giant clam population in the Pacific islands, almost to the point of extinction. However, a multitude of giant clams thrive on Great Barrier Reef because they are one of Australia's protected natural resources. Work done at James Cook University Orpheus Island facility on the Great Barrier Reef has resulted in an effective flow-through culture technique, including early-stage nutrient supply and microencapsulated foods, allowing control of the settling of giant clam larvae and rate of metamorphosis of larvae to juveniles. This project's operations have expanded to include other sites in Australia, as well as the Philippines, the Cook Islands, Fiji, and Tonga (Smith, 1994).

CONCLUSION

Australia possesses several of the elements that any nation requires to use marine biotechnology successfully for economic development. The marine biodiversity of Australia may well be the richest in the world, both in variety and quantity. There is a strong base of well-educated, highly trained biologists in Australia and many of them are skilled in key biotechnologies, such as genetic manipulation, cell fusion, cell culture, and fermentation processes. The number of Australian bioprocess engineers is small but adequate (Review Committee on Marine Industries, 1989). Australian research institutes usually are well equipped and housed in adequate facilities.

The country-wide elements that can be considered a national marine program in Australia favor basic research, with marine biology a good example, to applied research or technology, although this attitude is changing rapidly. Thus, the program tends to support coral reef/tropical marine studies, marine ecology, physical oceanography, targeted aspects of fisheries research, aquaculture, and utilization of remote sensing techniques for oceanography and meteorology. Applied research pertaining to biological oceanography, marine chemistry, and marine biotechnology, for example, is not nearly so well supported by the government or industry.

Although Australia's scientific capabilities and natural resources are substantial, the capability of industry in Australia to carry research results to commercial application is extremely limited. Applications of biotechnology to aquatic activity has generated only one successful product in Australia—beta carotene. Commercialization of marine natural products is in its infancy, exemplified by development of several products that have experienced protracted delays.

A 1989 report suggested that four areas hold the best opportunities for the Australian marine biotechnology industry in general: aquaculture; marine natural products; bioremediation; and biofouling (Review Committee on Marine Industries, 1989). However, few Australian marine biotechnology companies presently have the resources over the long term to invest in the necessary R&D required for return on investment. Conversely, many Australian companies that might have the resources to fund long-term projects are subsidiaries of large multinational corporations, with research priorities that may not be most applicable to Australia.

From a review of Australian scientific literature focussed on the marine sciences, biotechnology, and industry and from information provided by Australian scientific colleagues we deduce that several factors impede utilization by industry of research findings, including:

- lack of a national plan for the marine biological sciences, including marine biotechnology, delineating objectives for research institutions and industry;
- impatience and risk-aversion of major Australian investors, who therefore are unwilling to invest in long-term opportunities in marine biotechnology;
- lack of widespread interest by industry in commercializing already existing opportunities;
- inadequate support of university research by the federal government;

- poor career structure for marine scientists;
- low level of activity in applied research and technological aspects of marine biotechnology;
- less than optimal communication between research institutions and industry.

Most of these are being addressed by Australia in an effort to compete with Japan and the U.S. in selected areas of marine biotechnology. It is noteworthy, for instance, that an Australian Marine Industries and Science Council is being established to formulate a marine industries development strategy and that a national aquaculture strategy is in preparation. A marine industries development program is already in place, including the MARINET (Australian Marine Industries Network), which allows industries to network through workshops, seminars, and conferences. The institution of a CRC program has greatly improved communication between research institutions and industry.

From the information we have analyzed, it seems to us that a national strategy to analyze Australia's strengths, including its highly qualified marine and biological scientists, and its unsurpassed marine natural resources, and to link these strengths in a circumscribed, target-oriented program in marine biotechnology would be likely to significantly advance marine biotechnology in that country. Government funding should be increased, or shifted to researchers interested in applied marine sciences. The Australian Marine Industries and Science Council could be a strong force for improving coordination and cooperation between government agencies, researchers, and industries. An easily accessible network of information on current research in marine biotechnology should be established and additional R&D programs involving collaboration between researchers and industrialists should be initiated. Specific incentives and support offered by the government to small companies interested in R&D leading to marine biotechnology products could make a significant difference in Australia's competitiveness at the international level.

Lastly, the competitive position of small Australian companies might improve if they formed strategic alliances or joint ventures with overseas firms that have the necessary capital to carry a project to its conclusion. Such private initiatives would be equally as important as government actions.

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Chapter 7

MARINE BIOTECHNOLOGY IN NORWAY

BACKGROUND

Norway is bordered by the North Sea to the south, the Atlantic Ocean to the west, and the Norwegian Sea to the north. Norway's coast is indented by numerous fjords, creating 21,000 km of coastline. Many fjords offer shelter to harbors and aquaculture facilities, enabling Norwegians easy access to abundant marine resources. Traditionally, Norway's economy has been heavily dependent on the sea; its fishing and whaling fleets are ranked among the largest in the world.

Fisheries constitute Norway's third largest export industry (petroleum is the largest), with sales of fish and fish products in domestic and foreign markets totaling over \$1.54 billion per year (Norwegian Fisheries Research Council, 1990) (the conversion rate used here is \$1=7.4 Norwegian crowns). However, in the early 1980s, Norwegian companies, facing steadily growing costs for landing fish and increasing competition from foreign operators, began to invest heavily in the nascent aquaculture industry. The growth of the industry was spectacular, within ten years over 1,400 hatcheries and fish farms were established. By 1992, these farms were producing 70-80% of the world's aquacultured salmon. In 1990, they generated 160,000 tons of fish per year worth \$670 million, or almost 50% of the value of national fisheries landings (Dodet and Malmcrona, 1991). The main crop by far is salmon; most of it is exported, principally to the countries of the European Communities (EC) (80%).

In addition to salmon, other aquaculture and aquaculture-related exports generate export income for Norway. Seaweed products earn \$52.2 million annually, and the supply of aquaculture-support equipment to domestic and foreign markets nets over \$870 million per year (Norwegian Fisheries Research Council, 1990).

GOVERNMENT SUPPORT OF SCIENCE AND TECHNOLOGY

In 1985, Norway elaborated its first national program in biotechnology, which ended in 1989. The first program was immediately followed by a second, expanded program called "National Plan for Research and Development in Biotechnology," which continued through 1992. The second plan, henceforth referred to as the National Plan, specified governmental support for R&D in cell and gene technology, medicine, agriculture, aquaculture, industry, environmental protection, and biotechnology for developing countries. Funding for biotechnology under the program in 1992 was about \$24.1 million, plus support for equipment at \$1.47 million, for a total of less than \$26.1 million. Undoubtedly some additional funding for what may be considered biotechnology research is provided by ministries and research councils under other research areas, such as agriculture, health, environment, etc., but we were unable to quantify these funds.

As this report was finalized during the summer of 1994, we had received information that a far-reaching reorganization of Norwegian research organizations had taken place. Perhaps most important, the former system that consisted of five research councils (Fisheries, Agriculture, Technology, Science, and Humanities) is now organized as a single General Council, subdivided along new lines with Bio-production and Biotechnology in different sub-councils (Dundas, 1994). However, since we are not in a position to take into account these developments in this assessment, what follows is a description and discussion of Norwegian research as it operated in 1993 and before. Much of this structure still exists of course and, as far as we know, no drastic new directions have been taken.

Before 1993, five research councils funded basic and applied research in Norway. Two of the previous councils are relevant to this report; the Agricultural Science Research Council (NLVF) funded agricultural research, including some aquaculture-related research, and the Norwegian Fisheries Research Council (NFFR) was the major sponsor of living marine resource-related research in Norway (Raa, 1990). In addition, the NFFR developed and put into effect the "R&D in fisheries-Innovation plan," which was a public information network that provided easy access by fishermen, industries, government bodies, and others to research results (Central Board, 1990). NFFR was mainly funded through the Royal Ministry of Fisheries; the total budget for NFFR in 1990, excluding capital investments, was \$24.36 million (Norwegian Fisheries Research Council, 1990). The NFFR's three primary objectives were to: (1) promote and finance fisheries and aquaculture research and industrial applications by planning and budgeting research activities; (2) coordinate national fisheries and aquaculture research efforts; and (3) serve as the main advisory and administrative body to the Norwegian government in fisheries research and development policy. In formulating its national research efforts, NFFR was guided by certain objectives, including the continual improvement of natural resources management for fisheries and aquaculture, the improvement of aquaculture technology, support equipment, and production processes, and the development of state and local, market-oriented industry. To ensure meeting its objectives, NFFR established program boards, responsible for evaluating its research system and for choosing, coordinating, and presenting the results of specific research projects (Norwegian Fisheries Research Council, 1990).

NFFR had nine specific goals for 1990-1994. They are to: (1) develop the knowledge that will enable aquaculturists to farm new fish species that conform to the Norwegian environment and present favorable possibilities for commercial development; (2) improve the competitiveness and profitability of salmon and trout production through research that enhances fish health, prevents and treats diseases, improves feed resources, and upgrades production facilities; (3) strengthen marine and ecological research in Norway to the extent

where the country will become an international resource center in these areas; (4) develop information technology so that it can be used to increase productivity and profitability in Norwegian fisheries and may be marketed in foreign markets; (5) inform the populace about policies and regulations related to living marine resources; (6) expand aquaculture industry by providing it with good market information, promoting horizontal and vertical cooperation in the industrial sector, and developing new marketing strategies for the EC and U.S. markets; (7) improve expertise in fisheries technology in the equipment and service industries supporting fisheries; (8) integrate land and sea production to maximize fisheries output and profit; and (9) deploy and support marine biotechnology research (see below) (Central Board, 1990). Other NFFR activities include providing educational scholarships for maintaining and establishing expertise in fisheries, and international scholarships for promoting international cooperation such as supporting foreign scientists in Norway (Central Board, 1990).

In 1992, NFFR provided approximately \$1.54 million per year to support some 25 research projects in marine biotechnology (Ulsaker, 1992; Norwegian Fisheries Research Council, 1991). Seven areas of marine biotechnology research were emphasized: (1) marine natural products chemistry, which concentrates on marine biomolecules such as lectins, natural antioxidants, marine lipids, marine enzymes, antimicrobial substances, and biopolymers from sea weeds and crustaceans; (2) enzyme biotechnology, which uses enzymes as processing agents in the marine food industry and as catalysts to chemically modify marine oils; (3) marine animal health, with a focus on applications for aquaculture; (4) ecosystem management, where systems of cultivation of phytoplankton and zooplankton are developed to bioassay environmental pollutants and to produce live feeds for juvenile fish; (5) genetics/biotechnology, including the study, improvement, and use of microbial genetics, recombinant DNA technology, hybridoma techniques, and production of transgenic fish; (6) marine microbiology/microbial ecology; and (7) fish feed improvement and development (Raa, 1991).

In late 1993, the University of Bergen and the University of Maryland established a cooperative program in research and education, which includes using video-teleconferencing communication between Bergen and Baltimore MD. More joint trans-Atlantic seminars and courses of instruction are to commence in the fall of 1994.

RESEARCH AND DEVELOPMENT RELATED TO MARINE BIOTECHNOLOGY

Most of the remarkable marine biotechnology research is carried out at ten universities and specialized research institutions. Of these, two are located in Tromsø, three in Bergen, three in Trondheim, one in Stavanger, and one in Fyllingsdalen (Ström and Raa, 1993).

In Tromsø, over 100 scientists and engineers are involved in research related to aquaculture. Approximately 50 scientists work in marine biotechnology (Ström and Raa, 1993). Basic research is carried out in various departments of the University of Tromsø and the Norwegian College of Fishery Science (NFH), also within the university. Scientists at the NFH's Department of Marine Biochemistry perform research in microbiology, microbial ecology, genetics, immunology, and biochemistry; research in other departments encompass fish genetics, fish development, and fish maturation. Applied research is done at the Norwegian Institute of Fisheries and Aquaculture (Fiskeriforskning) that focusses on marine enzymes, enzymes in fish processing, and fish health including vaccine development. An important component of the Tromsø infrastructure is the Aquaculture Station and its laboratory where researchers can perform challenge experiments (Ström, 1994).

In Bergen, various universities carry out research in marine biotechnology primarily focussing on aquaculture. In addition, two institutions are involved in marine biotechnology. The Bergen High Technology Center (HIB), owned by the Bergen Foundation of Science, was created to increase collaboration between basic research and industry. In 1990, HIB opened a biotechnology department

dedicated to aquaculture and marine biotechnology. The second institution, the Institute of Marine Research in Bergen, is affiliated with the Ministry of Fisheries and is funded by NFFR and various external sources (Dodet and Malmcrona, 1991). A total of about 30 scientists in Bergen are focussing on marine biotechnology research (Ström and Raa, 1993).

Trondheim is the national center for technological research. Major institutions include the Norwegian Institute of Technology (NTH) and the University of Trondheim. Approximately 80 scientists and engineers are engaged in work on marine biotechnology topics, including marine biopolymers, aquaculture, molecular genetics fermentation, enzyme technology, and environmental engineering related to marine systems. Notable research is performed at the NTH Institute of Biotechnology as part of the "European Cooperation in the Field of Scientific and Technical Research" (COST) program on macroalgae, and on salmon cell culture systems at the University's Center for Molecular Biology (UNIGEN). Also located in Trondheim is the Norwegian Biopolymer Laboratory, which is in charge of the national program on industrial use of biopolymers. It conducts fundamental and applied research on marine polysaccharides, primarily alginate and chitosan, as well as biopolymer engineering based on genetic, enzymatic, and chemical methods. Another institution, the Foundation of Scientific and Industrial Research at the Norwegian Institute of Technology (SINTEF), is the largest institute for contract research in Northern Europe and has a staff of 2,000. It is engaged in work on enzyme technology related to marine products and bioremediation of oil spills and is highly active in the development and feeding of fish fry for aquaculture (Dodet and Malmcrona, 1991). Between 10 and 20 researchers in Trondheim are working on marine biotechnology research projects (Ström and Raa, 1993).

Following are some examples of projects being carried out within Norway's marine biotechnology program related to aquaculture, the utilization of certain marine natural products that have applications in aquaculture, and marine animal health.

Aquaculture

Aquaculture research in Norway focusses on reduction of production costs and improvement of quality and quantity of farmed fish. Most of this research relies on the use of classical techniques to achieve advances in aquaculture production, so they therefore are not considered in this review. However, some research on transgenic fish is being performed in Norway. For example, microinjection and expression of a growth hormone gene in Atlantic salmon has been accomplished by K. Gautvik at the Institute of Medical Biochemistry, Faculty of Mathematics and Natural Sciences, University of Oslo (Zomzely-Neurath, 1989). Dr. Gautvik has also isolated salmon prolactin and growth hormone to develop methods for quantitative determination. His work on transgenic salmon may become an important model system for studying the effects of multiple copies of growth hormone genes.

Having high-quality broodstock is of utmost importance to fish farmers. A program being undertaken at the Institute of Marine Research in Bergen seeks to secure the availability of the best salmon broodstock for future aquaculture operations. Institute researchers used DNA "fingerprinting" techniques to tag a total of 70,000 individual fish representing three different salmon stocks from as many rivers that were released during the spring of 1991. Probes developed in Ireland will be used to detect "fingerprinted" individuals when they return to their release sites in two to three years, and will allow researchers to assess the return rates to select the best performers for broodstock (Anonymous, 1991a).

Also of potential value to the aquaculture of salmon is the research proceeding at the Agricultural University of Norway in Ås. A research team at the university is using fast-growing zebrafish as a model for studies on the influence of insulin on carbohydrate metabolism and on the genes involved in the sexual maturation of salmon. The zebrafish is also used at the Institute of Medical Biology in Tromsø for embryonic development studies (Evaluation Committee, 1992).

An interesting marine biotechnology-related program bears mentioning. In the past, Norway has been a major exporter of fish-meal, but after 1988 the feed demands of the burgeoning aquaculture industry forced Norway to become a net importer of fish feed. One solution being advocated by scientists is the use of Norway's plentiful mineral gas as an energy source to produce microbial biomass (single cell protein, or SCP) for fish feed. SCP could partially replace the protein, vitamins, and minerals that are now used in fish feed and, additionally, could be fortified by components that stimulate disease resistance in fish. If a well-balanced SCP feed is developed, it would help Norway save spending money on imported fish-meal and SCP could in itself be an export commodity (Raa, 1990, 1991).

Marine Natural Products and Products From Fish Wastes

While small-scale and dispersed aquaculture can be environmentally benign, the very large aquaculture industry in Norway has created problems. Some of these problems are amplified by the operation of concurrent fisheries industries. In particular, the disposal of untreated wastes, especially fish viscera, from fish processing into coastal waters has created a pollution problem in Norway. To alleviate the situation, researchers at the University of Tromsø and Fiskeriforskning initiated a study on potential applications of the complex mixture of enzymes and other biomolecules that constitute fish waste. They developed a product, a de-oiled fish silage, that can be used to feed domestic animals, including farmed fish. As a result of this development, fish wastes are now a valuable resource, collected and processed by firms to produce fish silage, thus generating additional income to the aquaculture and fisheries industries. An important side-effect, of course, is that one source of marine pollution has been nearly eliminated (Raa, 1990).

Fish wastes may also be a source for biochemicals useful in research and industrial practices. For example, fish pepsins have a higher pH optimum than other pepsins and are active at lower tempera-

tures and resistant to autolysis at low pH. These enzymes act differently on various tissues due to differing pH optima, thus enabling the pepsins to separate targeted biological tissues. Enzymes that have been separated from fish wastes may be used by researchers as tools to biochemically dissect and separate biological tissues (Raa, 1990). Similar research is proceeding at the Norwegian Institute of Fisheries and Aquaculture in Tromsø for utilizing shrimp wastes. In this case, water from melted ice used to store whole shrimp after they are caught is processed to recover enzymes valuable to industry, including alkaline phosphatase, hyaluronidase, acetylglucosaminidase and chitinase (Olsen et al., 1990).

One problem peculiar to aquaculture pertains to the use of antibiotics to prevent and treat fish bacterial diseases. Antibiotics for controlling fish disease are generally administered through feed. Surplus feed and fish feces containing antibiotics settle to the bottom of fish tanks and ponds and, eventually, spread throughout the marine environment, where they are encountered by various marine microorganisms and bacteria. At one time this problem was particularly acute for Norway, where a significant amount of antibiotics was used by the aquaculture industry to counter damaging fish diseases. In particular, a severe outbreak of Hitra disease (coldwater vibriosis) stimulated a rise in antibiotic consumption, from 4.5 tons in 1980 to 48.5 tons in 1987, but down to 19.4 tons in 1989 (Anonymous, 1990b). During 1990-1992, Norway experienced an outbreak of furunculosis, which led to an increase in antibiotic usage, to almost 39 tons in 1990, but decreasing thereafter to about 30 tons in 1991 and, again, in 1992. With the introduction of new Hitra and furunculosis vaccines in 1988 and furunculosis vaccine in 1992, antibiotic usage fell drastically, to about 8 tons in 1993 (Anonymous, 1994). The fear in Norway, as elsewhere, was that the aquatic environment would become a large reservoir of organisms that are resistant to antibiotics commonly used in aquaculture; some of which were also significant in human medicine and veterinary practices. Resistance genes from these marine bacteria could be transferred to bacteria that are pathogenic to man. Additionally, antibiotics could have adverse effects on marine ecosystems.

In view of the uncertainties associated with antibiotic use, researchers have been searching for alternative ways to manage fish diseases. Possibly the most important alternative is vaccines, which are discussed in the next section. In addition, some Norwegian researchers have focussed their investigations on lower invertebrates and algae, which have elementary immune systems that depend on a set of non-specific defence mechanisms. One of these mechanisms incorporates a secreted, low molecular weight antimicrobial substance that is naturally degraded in the biochemical cycles of the marine ecosystem. Researchers have discovered that ethyl acetate extracts isolated from the mussel *Mytilus edulis* contain a group of compounds that inhibits the development of the bacterial pathogens *Vibrio salmonicida*, *Vibrio anguillarum*, and *Vibrio ordalii*, but which does not affect the flora present in the fish gut (Raa, 1990).

Scientists at the Norwegian College of Fishery Science are studying the properties of specialized proteins called lectins (Raa, 1990). The immune defensive processes of marine invertebrates include lectins, which specifically bind to bacterial and viral structures of carbohydrates, glycoproteins, or glycolipids. These properties mark lectins as having possible application as therapeutic medicines and as diagnostic tools.

Researchers in Norway also have discovered a lysozyme with strong antibacterial activity that functions effectively in cold environments. This enzyme, which has the ability to kill bacteria by breaking down their cell walls, was isolated from the shell of the clam *Chlamys islandica*. The activity of the lysozyme at 4° C is 80% of its optimum level at 45° C, which is up to several hundred times more active at 4° C than lysozymes from warm-blooded animals. In addition, marine lysozymes in general have better antibacterial activity than other lysozymes due to their peculiar molecular structures (Raa, 1990).

Marine Animal Health

Aquaculture in Norway like elsewhere in the world is susceptible to adverse effects from fish diseases. Maintaining marine animal

health is therefore vital to the economic well-being of Norway. The major bacterial fish diseases afflicting Norwegian aquaculture are Hitra disease, caused by *Vibrio salmonicida*, and furunculosis, caused by *Aeromonas salmonicida*; the viral disease of importance in Norway is Infectious Pancreatic Necrosis (IPN), which is caused by the IPN virus. Much of Norway's research effort is concentrated on increasing knowledge about these fish pathogens and their association with hosts, the prevention of disease spread, and developing vaccines to counter bacterial and viral diseases. The research program of a new fish research center, opened in June 1989, encompasses these four areas. This center, called AkvaVet is located at Vikan in central Norway, was built at the cost of about \$2.61 million, provided by the NIVF, has excellent facilities and equipment, including a large hall containing about 80 test tanks, and ten "research cells," each of which have up to 30 300-liter test tanks. The environment of the tanks is computer controlled, allowing the operator to precisely vary salinity, temperature, pH, and flow of water (Anonymous, 1989).

Some publicly funded research has led to commercial development of several fish vaccines. For example, in 1979 much damage was caused by a previously unknown disease, subsequently named Hitra disease or cold water vibriosis. The causative organism of this disease, which has a mortality rate of greater than 80%, was identified and characterized in 1981 by researchers at the University of Tromsø and the Institute in Bergen as a previously unknown *Vibrio* species, named *Vibrio salmonicida*. Close collaboration between the university researchers and industry, including heavy investment in a modern production plant, as well as cooperation from the Norwegian government, resulted in the development of a vaccine that is very effective in salmon, affording 90% protection against Hitra disease and preventing an estimated \$39-52 million in losses annually (Raa, 1990; Central Board, 1990). Hitra vaccine is being produced by Apothekernes Laboratorium in Norway (and Biomed Inc. in U.S.) (Anonymous, 1994).

Furunculosis, imported in 1985 from Scotland, was present in approximately 400 fish farms in 1991. It has a mortality rate of up to 68%; stricken animals usually die within three weeks. Two paths are

being taken to fight the disease. First, research is underway at NVLF's Institute for Aquaculture Research to develop breeding stock resistant to the disease and some particularly resistant stock have been developed whose mortality rate is about 15% (Anonymous, 1990a). Second, the company Norbio in Bergen, which is owned by the Dutch company Intervet, using conventional techniques has developed a furunculosis vaccine that it claims is 87.5% effective. The high efficiency rate is achieved in part through the use of a new, powerful glucan adjuvant. Norbio's furunculosis vaccine, as well as three other vaccines from foreign sources, are presently being tested by AkvaVet (Anonymous, 1991b).

Norbio claims to have isolated an IPN virus strain common to Atlantic salmon cultured in Norway. Norbio has characterized the virus and constructed monoclonal antibodies against some of its structural proteins. Based on this work, the company is able to offer rapid diagnostic services to fish farmers and veterinarians; in addition, its researchers are well on the way of developing a IPN vaccine (Anonymous, 1991b).

Research is proceeding on developing immunostimulants, which increase non-specific disease resistance in fish or act as adjuvants in vaccines, thus increasing their effectiveness. For example, studies at the University of Tromsø have demonstrated that certain glucans from yeast enhance non-specific immunity in fish, increasing their ability to resist diseases. Administration of glucans by commonly practiced procedures, such as through feed or by injection into the peritoneal cavity of the fish, results in a high degree of protection against bacteria pathogenic to fish (Raa, 1990).

Yet another approach to protect fish health involves substances called probiotics, which are harmless bacteria that block the damaging actions of pathogenic bacteria in the gut. This work built on the fact that the fish gut is inhabited by flora that include vibrio-like microorganisms. The biochemical properties and growth characteristics of these organisms are similar to pathogenic fish vibrios and the two seem to compete for the same ecological niche in the fish gut. By selective breeding and controlled feeding, the propagation of probi-

otics is stimulated to the point where they block receptor sites by greatly outnumbering the pathogenic vibrios (Raa, 1990).

Other Marine Biotechnology Areas

While research related to aquaculture seems to be of most interest to the Norwegian research community, some noteworthy research focusses on environmental problems. At the Department of Microbiology of the University of Bergen, investigations are aimed at discovering and developing novel microorganisms that could be used in environmental pollution control (Zomzely-Neurath, 1989). At the Department of Microbiology of the University of Trondheim, scientists are engaged in studies of microbial degradations of environmental pollutants at low temperatures (Zomzely-Neurath, 1989). Norwegian oil companies are supporting research in marine biotechnology areas vital to their interests, such as the bioremediation of petroleum pollution in the marine environment (Dodet and Malmcrona, 1991).

INDUSTRIES

Fourteen small and medium-sized firms constitute the marine biotechnology industrial sector in Norway. Of these, five are located in Tromsø, three in Oslo, three in Bergen, and one each in Skien, Haugesund, and Drammen.

Norway's largest biotechnology company is Protan in Drammen, which is a subsidiary of the chemical and oil company Norsk Hydro. It claims that it is the world's third largest company in the microalgal sector, producing alginates, laminarin, chitin, and chitosan. Protan also has production facilities in Canada and the U.S. (Dodet and Malmcrona, 1991).

In Tromsø, several small, spin-off biotechnology companies have been formed to capitalize on promising research results. These companies typically employ approximately ten people each and maintain close connections with the university and public research institutions. They typify the new biotechnology industry as it is evolving

in Norway, which utilize raw materials generated from aquaculture and fish processing that used to be considered waste. Among them are Marine Biochemicals A/S, Apothekernes Laboratorium A/S, Rieber & Company, and KS Biotec-Mackzymal (Raa, 1990, 1991; Dodet and Malmcrona, 1991).

Marine Biochemicals A/S, created in 1986 as a subsidiary of Norsk Hydro and now owned by private investors, concentrates on extraction of biochemicals from marine raw materials. It produces enzymes, including those from fish viscera, growth media for microorganisms, and lectins. Apothekernes Laboratorium A/S, created in 1986 by the Department of the Norwegian Pharmaceutical Group Apothekernes Laboratorium, produces classical fish vaccines and starter cultures for preservation purposes. Rieber & Company produces fish silage concentrate, attractants in fish feed, and fish feed from waste. However, of the companies located in Tromsø the most interesting, in terms of variety of products and innovative research approaches, might be KS Biotec-Mackzymal. Among its more traditional products are fish protein concentrates to be used in fish feeds, peptones for use as microbial growth media, and food flavoring. More recent products include DNA, nucleosides, and marine enzymes for uses as fine chemicals, and MacroGuard, a glucan from yeast cell wall, which is added to fish feed because of its immunostimulant properties (Hoffman, 1990). Company scientists have developed enzymes found in marine organisms for use as "biological knives," for example, in the production of caviar to separate fish roe particles from the connective tissue of the ovaries, enzymatic deskinning of fish and squid, and enzymatic cleaning of scallop (Raa, 1990, 1991). An enzyme mixture, sold under the tradename Hyzym, is used in automated processes to descale fish. In this process, gutted or ungutted fish are immersed in a waterbath containing Hyzym, which removes slime and loosens scales. After incubation, the treated fish are treated with water jets that remove scales without damaging the fish. A completely automated process, which sells for approximately \$60,000, can be operated by one person and has a claimed capacity of 1.3 tons of haddock per hour (Svenning et al., 1993). Hyzym sells for \$590 per kilogram, an amount sufficient to treat

16.5 tons of haddock. Hyzym can also be used to descale ocean perch, red snapper, white fish, and silver carp.

Other small Norwegian R&D companies are BioNor in Skien, which specializes in rapid diagnostic tests for fish diseases and in bacteriological control methods for fish farms; Primex A/S, which makes a product used as an attractant in fish feed and as food flavor (Raa, 1991); and Martens and Jahres Fabrikker, subsidiaries of Norsk Hydro, which is engaged in the production of polyunsaturated fatty acids from fish oils for food and medical uses (Dodet and Malmcrona, 1991). Norbio A/S in Bergen develops and produces vaccines and diagnostics. In addition to these products, Norbio markets vaccines against various serotypes of coldwater vibrios and Red Mouth Disease (Anonymous, 1991b). It is testing vibrio vaccines for use in cod and turbot (Anonymous, 1990a). Norbio is now developing a second generation furunculosis vaccine using recombinant techniques. This work involves cloning genes that encode certain surface and extracellular proteins (Anonymous, 1991b).

ACADEMIA-INDUSTRIAL COOPERATION

Innovasjonsenteret A/S has been established to facilitate greater interaction between industry and research throughout Norway (Zomzely-Neurath, 1989). In addition, a unifying research foundation for the Oslo region has been established called FOSFOR. It seeks to facilitate and stimulate cooperation between research institutes and firms in Norway's only science park at Gaustad (near the University of Oslo).

INTERNATIONAL TECHNOLOGY TRANSFER AND INTERNATIONAL RELATIONS

NFFR was Norway's primary agency for promotion of international research cooperation and technology transfer. Presently, cooperation is maintained through informal contacts between scientists and research centers around the world, or between organizations through participation in international projects, multilateral and bilateral agreements, or organizations such as the International Council

for the Exploration of the Sea (Central Board, 1990). As this is written, the Norwegian Research Council has a scholarship system to support visiting scientists.

Norway also participates in the "Nordic Collaborative Program on Biotechnology," a collaboration between Scandinavian countries. This program supports marine biotechnology projects through the Nordic Industry Fund and falls under the jurisdiction of the Nordic Council of Ministers (Dodet and Malmcrona, 1991). Another Nordic collaboration, the "Nordic Council Project," commenced recently and involves Norway, Sweden, and Iceland. This project focuses on the control of sexual maturation in salmon, the area's most important aquaculture crop. Currently, Norway is negotiating to develop additional international projects and collaborations with the EC.

CONCLUSION

The condition of marine biotechnology in Norway may be discussed in terms of research, development, and industry. Observers agree that some research teams are performing world-class research, but the general level is mediocre. One analyst has, for instance, compared the Scandinavian countries and found that biotechnology research in Norway is more conservative and less innovative than that in Denmark and Sweden (Zomzely-Neurath, 1989). A more definitive assessment of Norway's biotechnology program supported this view. An international scientific team that reviewed Norway's National Plan and its accomplishments made note of the high quality of some research teams, most of whom we have mentioned above. But in the final analysis, the team summarized its findings about biotechnology related to aquaculture as follows (Evaluation Committee, 1992):

It must be concluded that fisheries, aquaculture and marine biotechnology research is scattered in Norway. The quality and international competitiveness varies among the groups and from location to location. It may be suspected that the patchiness observed is partly the result of the organization

and different grant application policies practiced by the research councils in Norway.

It is particularly important to abolish the conservatism practiced today and to increase the use of modern biotechnological techniques as tools in the research laboratories.

The total area as such is of obvious importance to the Norwegian industry and society in general. It is thus imperative that the research is competitive on an international basis. The potential for strengthening the research is clearly present. However, the research groups today are too small, making it difficult for them to compete at an international level. Furthermore, small groups are liable to sudden changes due to e.g. individuals dropping out of the group. It is believed that a strengthening of the basic science will lead to the required advancement within applied research and, in due time, industrial development.

We have noted that even though Norwegian marine biotechnology research mostly is directed towards aquaculture, its achievements to date are impressive. However, the sector it serves is in trouble, and this may ultimately affect Norwegian research institutions. To illustrate, in 1989 approximately 700 fish farms operated in Norway. Lately this industry has experienced financial reverses, and an estimated 150-200 farms have closed down operations (Larsen, 1992). Production decreased from a record high of almost 160,000 tons in 1990 (Anonymous, 1991c), to 146,000 tons in 1991 and 138,000 tons in 1992 (Hempel, 1993). Four factors have contributed to the crisis in the industry: First, an over-production of salmon has created an oversupply situation in world markets (Anonymous, 1992a). As supplies continued to increase and prices dropped, profits for farmers have become marginal. Second, a general downturn in the market for salmon has occurred and the decreased demand has exacerbated the oversupply problem. Third, U.S. and EC, Norway's primary foreign markets, have raised their tariffs on imported Norwegian salmon (Anonymous, 1992a). Fourth, the bankruptcy of the Norwegian Fish Farmers Sales Association at the end of 1991 has re-

sulted in a loss of faith by investors in the industry (Anonymous, 1992b). These convergence of these factors resulted in diminishment of the profit margins for fish farmers, and the climate of economic recession and elusive profits led to investors being unwilling to inject additional capital into what has become a risky venture. It is reasonable to believe that salmon production in the future will probably reflect these problems and uncertainties.

Norway has several major barriers to overcome before it once again can become an international competitor in marine biotechnology: (1) The lack of financing has led to Norwegian business making minimal investments in production facilities so in many cases only laboratory scale production of products is possible, limiting marketing possibilities. (2) Since Norway's small biotechnology companies do not possess the resources and credibility needed to serve international customers and markets, commercialization of research results may be delayed five to ten years (Raa, 1991). (3) Scientists at universities and research institutions tend to neglect to adequately document their work, thus decreasing the credibility of products. (4) Technical solutions must be developed in order to overcome problems in the aquaculturing of species such as cod that result in 50-90% losses between metamorphosis and harvest, including adequate supplies of live feed, the recapture of cod fry, and the weaning of fry from live to artificial feed (Holm, 1989). (5) The Norwegians' level of distrust towards biotechnology is high, possibly equal to, or exceeding, the level found in Denmark and Germany. This creates problems pertaining to the public acceptance and commercial authorization of biotechnology products. For example, the company Marine Genetics in Bergen worked on the transfer of the growth hormone gene in salmon. Despite what appeared to be successful research, it failed due to difficulties related to public acceptance of its work (Dodet and Malmcrona, 1991). (6) Unlike the past when Norwegian biotechnology companies were able to open and begin operations on shoestring budgets, new companies must invest \$13-18 million in equipment, facilities, and documentation before start-up. This amount of money is considered very high in Norway, making it difficult for entrepreneurs to raise capital (Raa, 1991).

A major difficulty facing Norwegian aquaculture concerns trade barriers. The U.S. once was Norway's second largest market for aquacultured salmon. However, in 1991 the U.S. raised the import tax on fishery products from Norway, primarily because Norwegian products were being sold cheaper than those produced by U.S. fishery companies, helping drive these companies out of business. Practically no Norwegian salmon is now imported by the U.S. Even if Norway succeeds in lowering the cost of aquaculture production through, for example, the application of efficacious fish vaccines and improved feed conversion, the U.S. can offset the gains by continuing to raise import taxes. A similar scenario may evolve in Europe where the EC has placed tariffs on Norwegian value-added products and imposed minimum-price levels on fresh and frozen salmon. Although minimum-price levels and tariffs were discontinued as of, respectively, January and February of 1992, a precedent has been set for the implementing of future restrictions (Anonymous, 1992a).

In 1993, the situation improved for Norwegian salmon producers due to a reorganization of the industry, a relaxation of strict ownership rules by the government, and the sell off of frozen salmon surplus stocks (Hempel, 1993). Total production of salmon increased to 175,000 tons in 1993 and is predicted to surpass 200,000 tons in 1994 (Ström, 1994). Further increases are expected for 1995 and thereafter (Hempel, 1993).

To sum up our assessment of marine biotechnology in Norway, Norwegian research is internationally competitive in selected areas, e.g., fish vaccines, DNA tagging to monitor wild salmon, and development of fish species new to aquaculture. Norwegian research pertaining to the utilization of by-products from fish processing is the most advanced of its kind in the world. Overall, the level of Norwegian marine biotechnology research is one of, as yet, unfulfilled but tremendous potential.

The Norwegian aquaculture industry, although beset with problems, will continue to be the world's foremost. Eventually, production of salmon in Norway could peak at between 350,000 and 400,000 tons in 2010 (Hempel, 1993). Applications from marine biotechnology research can have important local effects, helping the

industry cement its already powerful competitive position, but little of this research is likely to be applicable to aquaculture in other countries. Only limited applications can be expected in other areas of marine biotechnology. Possibly fish vaccines developed in Norway will find world-wide markets, but this particular market niche is a small one and specialty companies in Canada, Scotland and U.S. will be competitive. Perhaps R&D to discover and develop marine products from microalgae and other marine organisms found in Norwegian waters will be productive, but this will not happen unless funding for this purpose is increased and the effort is better coordinated.

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Chapter 8

MARINE BIOTECHNOLOGY IN JAPAN

INTRODUCTION

In this chapter we assess the status of marine biotechnology in Japan. The information presented herein is derived from the scientific literature, Japanese government reports, and interviews with Japanese and non-Japanese scientists. For convenience, all monetary measures are set forth in dollars, on the basis of 110 yen (¥)=\$1. The names of Japanese organizations are spelled out in English according to a format developed by the Foreign Broadcast Information Service (FBIS) (Foreign Broadcast Information Service, 1993). All references that include the notation "in Japanese" were translated by the FBIS.

As is the case with other large, industrialized countries, Japan's activities in marine biotechnology comprise a rather small part of the country's total science and technology program. To analyze how marine biotechnology fits into the national picture for Japan and to determine its relative importance, one must be cognizant of the significant federal agency and local government support of science and technology, as well as the extent of the support in Japan that is provided to marine biotechnology, industrial initiatives in the field, relevant government-industrial cooperation in R&D, and other activities that directly impact on marine biotechnology. These factors are considered in this chapter. Accordingly, the chapter is divided into six sections.

In the first section, we provide background information on Japan, consisting of data on relevant national demographic and geographic characteristics, including marine resources. The second sec-

tion details the responsibilities and functions of Japanese federal ministries and agencies supporting science and technology, as well as a short discussion of the role of the prefectural governments. The third section provides an overview of the role of the private sector in supporting scientific research and briefly discusses industry–university relations. The fourth section reviews research in Japan in the six marine biotechnology areas covered in Chapter 1. The fifth section presents an analysis of publications of Japanese origin, revealing those areas of research that have been emphasized. The concluding section offers an analysis of existing strengths and weaknesses of Japan in marine biotechnology, as well as ideas on future directions in this field.

BACKGROUND

Geographical and Demographic Facts

Japan is an archipelago, comprised of thousands of islands extending 2,500 kilometers (km) in the Pacific. The four main islands of Japan are Hokkaido, Honshu (the largest of the four, where Tokyo is located), Shikoku, and Kyushu. The total land area is 378,000 square km (slightly less than the size of California), representing 0.25% of the world's land area. However, the extremely irregular coastline of Japan stretches more than 33,000 km. Further, Japan possesses an immense 200 mile Exclusive Economic Zone (EEZ), encompassing circa 3.86 million square km, approximately twelve times larger than its land area. For purposes of comparison, the EEZ of Japan is approximately half the size of that claimed by the U.S.

Japan lies in the temperate zone and its climate is significantly influenced by two ocean currents. The warm Japan current (*Kuroshio*) originates north of the Philippines, flows past Taiwan, then divides into two streams—one stream courses along the eastern side of Shikoku and Honshu and the other flows west of Kyushu. The cold, fertile Kurile current (*Oyashio*) begins in the Bering Sea and runs along the eastern shores of Hokkaido and Honshu. These two currents create a large number of marine micro-environments, resulting in an abundant, highly varied marine biodiversity, much of which is protected in Japan's 15 marine parks.

Although the total land area of Japan is relatively large, most of it is mountainous (74%) or otherwise not suitable for agriculture or human habitation. Of the nation's population of approximately 124 million, 77% live in cities, mostly located in the coastal zone where the population density is extremely high. By world standards, the average Japanese citizen is well off economically, with an average income of \$25,430 in 1990 (World Bank, 1992).

Japan's research infrastructure is composed of three types of institutions. The first consists university laboratories. Of Japan's 98 national, 41 prefectural, and 400 private universities, in 1992, it was estimated that 30 of them are performing high level scientific research (Arima, 1992). Similarly, circa 30 universities have faculties, departments, or laboratories that relate to marine biotechnology and/or support basic research in marine biotechnology (Simidu, 1994). Second, federal ministries support a total of 83 national laboratories (Research Development Corporation of Japan, 1993). Third, applied and developmental research is strongly supported at the local level by prefectural governments, which promote and help fund *kosetsushi* and third-sector research centers (see below). In 1991, there were 170 *kosetsushi* and 121 third-sector centers in prefectures and cities (Anonymous, 1993k; Science and Technology Agency, 1993a).

Japan's Marine Resources

Due to the scarcity of arable land, and because past government policies have disproportionately favored industry over agriculture, Japan's agriculture produces only a small proportion of the food required by its population. For example, in 1990, Japan imported 27 million tons of cereal, making it the world's largest importer of this commodity. To make up for shortfalls in agricultural production, the nation imports some of the animal and plant foods it needs and, for the rest, draws on marine resources.

Japan, more than any other major industrialized country, seeks to utilize the oceans maximally. Japan is the world's largest fishing nation, evidenced by its fisheries production of 19.5 million tons in 1990 (Chopin, 1993), and also the world's largest consumer of fish products. For example, in 1989, the per capita consumption was 161.7 pounds per person, live weight equivalent. In comparison, it

was 45 pounds live weight equivalent for the U.S., more than three fold less. Animal protein consumed daily by the Japanese is predominantly seafood (45%), compared to 4% in the U.S. In 1992, Japan was the world's top importer of sea products, accounting for 28% of the world's total trade valued at \$12.8 billion, mostly from the U.S., Korea, Taiwan, India, Indonesia, and Canada (Josupeit, 1994). At the same time, the nation exported sea products worth about \$1.5 billion. In economic importance, fishery products were the second highest valued of the imported commodities in Japan, after petroleum.

Japan is the world's leader in aquaculture, when the entire range of species (over 30 species are being cultured) and culture environments are considered (Fuji, 1987). Aquaculture, which is one of Japan's oldest industries, is growing rapidly, showing an increase of circa four times in production, from 300,000 tons in 1960 to 1.1 million tons in 1984 (Nakahara, 1992). In 1989, the aquaculture industry produced goods valued at \$4.4 billion, equivalent to 22% of the total Japanese fisheries market. Marine aquaculture production in 1989 consisted of 180,000 tons of scallops, 260,000 tons of oysters, and 400,000 tons of nori (an edible dried seaweed of the genus *Porphyra*), as well as smaller quantities of yellowtail jack, seabream, salmon, brown algae, and salmonids (Kano, 1991). Its freshwater aquaculture produces mainly carp, eel, and rainbow trout (Nakahara, 1992).

Japan is making a significant effort to expand its indigenous aquaculture. In particular, Japanese researchers are attempting to add to an already abundant harvest by developing species for aquaculture that no one has yet been able to culture, such as bluefin tuna, devil stringer (a type of scorpionfish), moara grouper, and spiny lobster (Anonymous, 1992a). For example, in early 1994, Deepwater Fish Farming Technology, a research institution based in the Iwate Prefecture, reported success in culturing sturgeon, whose flesh is similar to high-value fish such as blowfish and flounder and whose eggs are used for caviar (Anonymous, 1994f); similarly, the Maruha Group is successfully culturing artificially hatched bluefin tuna in the Nagasaki Prefecture (Anonymous, 1994g). Fully grown bluefin tuna can reach a weight of 300 kilogram, one fish can sell for up to \$27,000 at

Tokyo's Tsukiji wholesale fish market. Because major fishery resources in Japan's coastal waters are being depleted (Chopin, 1993), we expect that the Japanese aquaculture industry will continue to grow in size and diversity for the foreseeable future. However, environmental constraints (see below) dictates that most of the future growth will be net pens emplaced in open water relatively far from shore or in on-shore, closed systems.

Closely associated with the aquaculture, yet a distinct economic entity, is larviculture; i.e., the production of fish fry by hatcheries that, in turn, sell them to aquaculture farmers. Presently, Japan is the world's largest producer of fish fry, having sold circa 200 million fry in 1993 for approximately \$0.75 each (Sorgeloos et al., 1994). In comparison, the second largest producer, the Mediterranean countries collectively, produced 100 million fry that year. Most of the fry produced by Japan are seabream (*Pagrus major*), Japanese flounder (*Paralichthys olivaceus*), puffer (*Takifugu rubripes*), rockfish (*Sebastes schlegelii*), and mud dab (*Limanda yokohamae*). Japanese excellence in this commercial field largely has come about as a result of research applied to improving nutrition and controlling the microbial flora of fish hatcheries, especially bacterial species pathogenic to fish (Sorgeloos et al., 1994). An example of a new initiative in fry production is the attempt by the Japanese Fishery Agency to artificially spawn eel fry. This project, which commenced in 1987 and is expected to be completed in 1996, is extremely difficult because the eels caught in Japanese rivers usually spawn in the ocean east of the Philippines. Promising results have been achieved by injecting mother eels with special hormones (Anonymous, 1994b).

Initiatives in aquaculture and larviculture are strongly supported by all levels of government, especially at the local level. Prefectural governments designate land to be used for aquaculture and fishermen's cooperative associations allocate sub-areas to individual aquaculturists at no charge. Further, one of the most powerful support organizations for aquaculture and larviculture is the Japanese Prefectural Institutes of Fisheries.

Detracting from what should be a rosy future for aquaculture, the Japanese face severe environmental problems, some of which is a consequence of intensive aquaculture practices. In fact, until only a

few years ago, domestic and industrial wastes from Japanese cities were released untreated into the sea, creating a succession of environmental nightmares, the most extreme example of which was Minamata syndrome, a serious physiological condition associated with lesions in the central nervous system caused by organic mercury concentrating in fish from mercury-polluted waters. In 1993, Japan's Environment Agency (EA) estimated that there were circa 20,000 factories and waste disposal plants, each of which dumped more than 50 tons annually of effluents into Japanese bays and seas (Anonymous, 1993). Pollutants originating from on-shore sources have particularly affected Japan's 88 inland seas and semi-enclosed bays, stimulating the multiplication of phytoplankton that constitute toxic "red tides." The Japanese coastal zone also has been adversely impacted by landfills, which began on a large scale in 1956, with many of these arising from construction of Japan's more than 4,000 harbors. Since the number of protected coastal areas acceptable for aquaculture is limited, and because some areas have become over-saturated with net pens, effluence from aquaculture facilities is adding to the total load of pollutants originating from on-shore sources. The consequence of unbridled pollution is enormous damage as the water in extensive areas along Japan's coastal zone is polluted and the shores spoiled (Nakahara, 1992). Natural coast line and rich fishing grounds have been destroyed. In 1985 it was estimated that the natural coast line was reduced to 46% of the total (Shapiro, 1991); coastal zone debasement continued largely unchecked until the Environment Agency acted to protect inland seas in 1993 (see below).

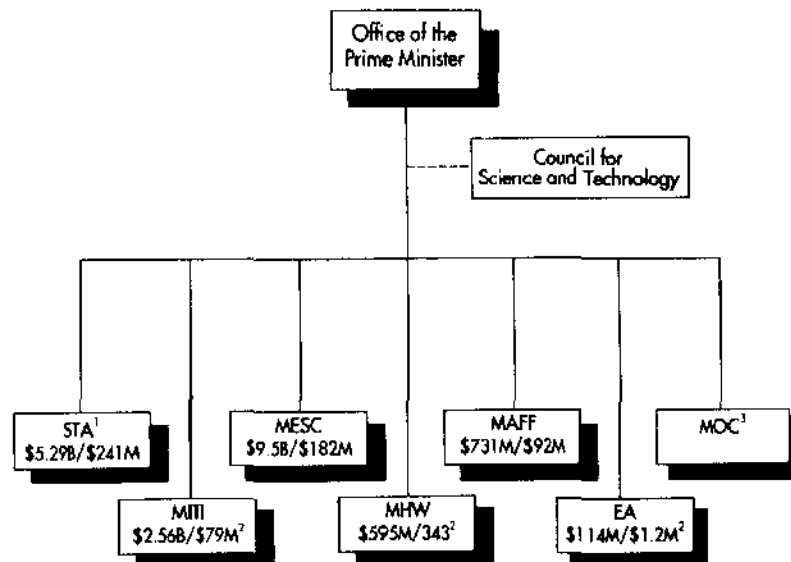
STRUCTURE OF SCIENCE AND TECHNOLOGY IN JAPAN

In this overview, the federal agencies and ministries involved in science and technology, in general, as well as in marine biotechnology, are identified and the major, relevant activities of each agency or ministry are briefly explained. The indirect methods used by the Japanese federal government to support science and technology are described and discussed and the role of prefectural governments in science and technology is considered. Finally, collaborative activities

in marine biotechnology between Japan and other countries are briefly described.

Federal Agencies and Ministries Concerned With Science and Technology

Similar to other highly industrialized countries, the organization of science in Japan is complex (see Figure 20), with governmental agencies having overlapping responsibilities. At the top of the science structure is the Council for Science and Technology, an advisory body connected with the Office of the Prime Minister. Under the Office of the Prime Minister are various ministries and agencies, of which six have substantial responsibilities in science: Science and Technology Agency (STA), Ministry of International Trade and In-



¹First number is total R&D expenditures in science and technology, second number is expenditures in biotechnology.

²Biotechnology expenditure estimate is for 1992, 1993 data was unavailable.

³No direct funding.

Figure 20. Japanese federal structure and R&D expenditures in science and technology, 1993.

dustry (MITI), Ministry of Education, Science and Culture (MESC), Ministry of Health and Welfare (MHW), and Ministry of Agriculture, Forestry and Fisheries (MAFF).

Council of Science and Technology

The Council heads the government science hierarchy. It is composed of ten members, including the Prime Minister, cabinet ministers, the chairman of the Science Council of Japan, and selected corporate executives. Its major functions are to provide advice on scientific matters to the Prime Minister's Office and the ministries and to formulate government policies in science and technology. The general policies are converted to specific programs at the ministerial level. The Council works very closely with STA.

Science and Technology Agency

The STA coordinates basic research supported by the ministries and derived from policies formulated by the Science and Technology Council. It is the leading sponsor of non-university scientific R&D in Japan. STA's budget for supporting R&D was \$5.29 billion in 1993 (Planning and Coordination Bureau, 1993a), including \$241 million for general biotechnology (Anonymous, 1993). A significant proportion of the support it provides to scientific activities are funneled through one of four major programs.

- **Special Coordination Funds for Promoting Science and Technology.** This is a grant-in-aid program which supports basic research and sponsors international scientific meetings. In 1993, the budget was about \$1.1 billion.
- **Exploratory Research For Advanced Technology Program (ERATO).** In 1981, STA set up ERATO, the aim of which is to foster cooperative research projects between industry and universities to develop new industrial technologies. ERATO, which is administered by the Research and Development Corporation of Japan (JRDC), sponsors large projects in the

time frame of no longer than five years and carried out by groups of 15–20 young researchers. Each project's total budget must fall within the range of \$10–15 million. In 1992, ERATO was sponsoring 37 projects and its budget was about \$85 million (Normile, 1994). As far as we have been able to determine, of the 37 active ERATO projects, only one is fully a marine biotechnology project. A group, headed by N. Fusetani from the University of Tokyo, is investigating aspects of biofouling, especially larval settling and metamorphosis (Research Development Corporation of Japan, 1993). Foreign research groups may apply for ERATO support. In early 1994, it was announced that, for the first time, two foreign research groups, both from the U.S., were to be granted ERATO funding (Normile, 1994).

- **Frontier Research Program.** This program, which is administered by the Institute of Physical and Chemical Research (RIKEN), supports research that develops new scientific knowledge that may be used for industrial technologies in the 21st century. Under this program, foreign researchers are invited to set up cooperative projects with Japanese workers. It was budgeted at \$20.76 million in 1992 (Science and Technology Agency, 1993b).
- **Precursory Research For Embryonic Science and Technology (PRESTO).** PRESTO, which commenced in 1991, aims to encourage especially creative individual researchers in Japan, providing them with up to \$500,000 for three years to engage in research of their choice. PRESTO is administered by JRDC (Science and Technology Agency, 1993b).

The JRDC's major objective is to search for promising research being done in universities and public institutes, contracting industry to develop applications from this research. In particular, JRDC funds high risk development projects, i.e., projects that would not otherwise be pursued by industry. Even though JRDC does not have lab-

oratories of its own, it has a significant impact on how Japanese science is utilized. JRDC's budget in 1992 was \$155.5 million (Science and Technology Agency, 1993b).

STA has an important dual role in developing the ocean sciences (Science and Technology Agency, 1993b). It coordinates all federal government programs related to ocean development, including such diverse activities as fishery and aquaculture exploitation, port facilities construction, and coastal maintenance. It also promotes ocean science and technology projects, which usually are executed by the Japan Marine Science and Technology Center (JAMSTEC). Three types of projects are supported. The first is deep sea exploration, including the discovery and investigation of extremophiles. This programmatic area is considered in detail below. The second involves projects focussed on understanding the processes of the oceans, especially those associated with the global environment. This programmatic area has a large international component. The third program seeks to utilize the oceans effectively through new applications of research, for example, development of wave energy technology, creation of calm sea areas for aquaculture, and improvement of the confined marine environment inside bays and inlets. Many of the projects carried out in this third programmatic area have significant local involvement.

In 1992, STA's marine science and technology budget was \$114.5 million. In comparison, the total marine science and technology budget of the Japanese government, including MITI, MAFF, STA, and six other agencies, was \$457.4 million in 1992 (Anonymous, 1992d).

STA directly supports three national research institutes, including RIKEN, one of Japan's leading physics and chemistry research institutions. RIKEN also conducts biotechnology-related research and, through MITI's Frontier Research Program (see below), fosters international cooperation in this field. STA's involvement in marine biotechnology, however, largely is through the operation of JAMSTEC.

JAMSTEC, which was established in 1971 in Yokosuka City, is Japan's leading R&D organization in marine science and technology.

In 1992, its budget was \$110 million (Science and Technology Agency, 1993b).

Perhaps JAMSTEC's most important asset is its collection of deep-sea exploration submersibles. Impetus for constructing a deep sea exploration fleet may have come after Japanese engineers visited Woods Hole in 1973 to inspect the U.S. Navy's submersible, *Alvin*. Two years later, Emperor Hirohito, who was a marine biologist, also visited Woods Hole. In 1987, *Alvin* and its support ship stopped by in Tokyo, where they were visited by Crown Prince Akihito, who now is Japan's emperor. As an aside, Akihito is a marine biologist specializing in the study of a species of carnivorous fish called gobies (Broad, 1994).

JAMSTEC's first submarine, the *Shinkai 2000* (*Shinkai*="deep sea"), with its 1,553 ton support ship, *Matsushima*, was commissioned in 1982. It can operate to a depth of 2,000 meters. By 1989, the *Shinkai 2000* had made 630 dives, collecting organisms that live near hydrothermal vents on the sea bottom, including horse mussels and tube worms. In 1987, the unmanned submersible system, *Dolphin 3K*, capable of operating at a depth of 3,300 meters, was commissioned. It provides backup support for the *Shinkai 2000*. In 1990, the *Shinkai 6500* and its support ship, the 4,500 ton *Yokosuka*, became operational. This submarine, the world's deepest-diving research submersible, enables its operators to study and collect marine organisms to a maximum depth of 6,500 meters, potentially permitting scientists access to 98% of the world's ocean floor and 96% of the ocean floor within Japan's 200-mile limit (Karube, 1989; Arai, 1989; Anonymous, 1991c). The last addition to this submersible fleet, *Kaiko* ("trench"), was completed in early 1993, and finished performance tests in 1993. It is a unmanned submersible, capable of reaching a depth of 10,000 meters (Anonymous, 1993h). The *Kaiko* is scheduled to dive the bottom of the Marianna Trench sometime during 1994, and hopes to better the underwater record of 10,920 meters set by the manned submersible *Trieste* in 1960 (Anonymous, 1994c). Taking into account this range of manned and unmanned submersibles, JAMSTEC most probably possesses the best deep-ocean exploring capability in the world. This capability may soon be

augmented; it is reported that a new submersible, *Shinkai 11,000*, is under planning and may be placed in service by the end of the century (Broad, 1994).

In 1993, JAMSTEC spent \$47.40 million to support deep-sea submersible research (Anonymous, 1993f).

In addition to its submersible fleet, JAMSTEC has established a 300 meter saturation diving station, which allows scientists to study deep sea life directly. The diving station is supported by the research ship *Kaiyo Maru* (see below), equipped with a towing type underwater television system and able to command an unmanned research vehicle (Anonymous, 1989g).

JAMSTEC supports two major marine biotechnology projects. In 1990, it began a 15-year project, "Deep-Sea Environment Exploration Program: Suboceanic Terrain Animalcule Retrieval (DEEPSTAR)," which is being executed by a team of 30 scientists led by H. Horikoshi of RIKEN. DEEPSTAR is directed toward discovery, recovery, and culture of marine microorganisms that live under conditions of high pressure and low temperature present at depths of 6,500 meters (Anonymous, 1993; Anonymous, 1993u). JAMSTEC has committed \$43 million to support DEEPSTAR's activities during 1990-1997 (Myers and Anderson, 1992).

DEEPSTAR recently reported 22 new strains of oil-degrading microorganisms recovered from the sea floor or ocean water proximal to Japan's coast. Despite heavy shipping traffic, little oil pollution has been recorded on or near the coasts of Japan. To explain this finding, Japanese scientists hypothesized that the oil is degraded by microorganisms. Support for this hypothesis came in the late 1991 when a DEEPSTAR group reported isolating a bacterium from samples collected at a depth of 1,600 meters in Suruga Bay. The bacterium produces a surfactant that enhances oil degradation. Aside from obvious applications in oil spills, this microorganism might also find use in tertiary oil recovery from terrestrial wells. While interesting, these findings are not new, since many others have reported similar findings previously (Leahy and Colwell, 1990). DEEPSTAR researchers have also recovered marine microorganisms that have the

ability to oxidize sulfur and degrade steroids (Anonymous, 1993c). Much of this work is still at an early stage.

The second project is called "R&D Related to the Utilization of Oceanic Deep Sea Resources," headed by T. Hirano from Tokai University (Anonymous, 1989g). The project has three research directions. Two of them are indirectly related to marine biotechnology—one aims to investigate the effectiveness of deep seawater for culture of various organisms, and the second is to study the ability of algae to degrade pollutants in seawater. However, the third is directly relevant, aiming to develop a system for sampling microorganisms from the sea bottom, at depths of between 2,000 and 6,000 meters and to grow these bacteria in the laboratory under high pressure. This project will enable scientists to study various aspects of these extremophiles, including cell membranes, enzymes, and secondary metabolites. Also, the investigators hope to classify the extremophile isolates, using fundamental biochemical components, e.g., fatty acids and nucleic acids.

Ministry of International Trade and Industry

MITI's main responsibility is to foster Japan's industrial economic growth (Zaborsky et al., 1989). It is Japan's leading sponsor of private sector R&D programs. MITI strives to provide optimum conditions for development of the private sector and shapes industrial technology policies to promote and encourage those fields that are difficult for the private sector to implement on its own. These include high-risk areas of research that are essential for industrial advancement, fields necessitating cooperation between industry, government, and universities, and requiring large-scale development investments, fields having high socio-economic priority, such as energy, and fields that fulfill community needs, such as medical treatment, public welfare, and the environment. Because of their risky character (in terms of return on investment), and because their applications, if any, are likely to be distant in time, industry typically has been unwilling to fund research to develop these uncertain technologies. In 1993, MITI's budget for supporting R&D was \$2.56 billion (Gener-

al Coordination Division, 1993). MITI's funding for general biotechnology is not known for 1993, but in 1992 it was \$79 million (Anonymous, 1992c).

Two subordinate agencies have been established by MITI to promote fields deemed to have high priority. The Agency of Industrial Science and Technology (AIST) fosters large-scale, high-risk research and development for improvement of basic technology in areas of exceptional promise to Japan. In 1993, AIST spent \$251 million to support 16 national laboratories that employed 3,567 researchers (Agency of Industrial Science and Technology, 1993a). AIST also provides technological leadership to the second MITI agency, the New Energy Development Organization (NEDO), whose primary functions are to fund the research and development of industrial technology, equip base facilities for this research, and promote international collaborative research (Anjo, 1989). MITI also oversees Japan's Patent Office.

One of the mechanisms that MITI employs to advance fields it deems important is to institute large-scale, long-term national programs addressing R&D required to develop those fields. Typically, national programs run for five to ten years and are constituted by many interlocking smaller projects, each focussed on some facet of the field of interest. Until 1993, MITI supported six such national programs. Broadly speaking, three were focussed on industrial technologies and three on energy and environmental technologies.

Referring to the first group of the three, the oldest national program was the "Large-Scale Project," which commenced in 1966 and was budgeted at \$3 billion. Its aim was to support research to develop technologies that appeared futuristic at the time of their introduction, but believed to hold future promise for Japanese industry. Eventually, the Project supported 30 projects, whose topics included manganese nodule mining systems, underground space development, supersonic transport propulsion, and human sensory measurement application technology. The second national program, "The Medical and Welfare Equipment Technology Project," started in 1976 and was budgeted at \$118 million. It sought to develop a series of technologies to assist handicapped and older persons. The third national program, begun in 1981, was named "The Next Generation

Project." Its budget was \$600 million, and it focussed on industrial technologies of the future, e.g., photo-active materials, superconductive materials, and bioactive devices.

The oldest national program in the second group was "The Sunshine Project," which began in 1974 and concentrated on developing new, environmentally benign energy systems based on coal, solar power, and geothermal energy. The related "Moonlight Project," started in 1978, stressed developments related to more efficient methods for transferring and storing power. MITI allocated \$5.4 billion to fund these two national programs. The third program, "Global Environmental Industrial Technology Program," which began in 1990, had as its dual aim the development of technologies that would help Japan meet its international obligations with respect to decreasing emissions of greenhouse gases and CO₂ from Japanese industry and technologies of use to developing countries. Its funding was \$180 million.

In 1993, MITI appears to have redirected its mission, possibly in order for it to be more responsive to the heightened interest of Japanese citizens in matters related to the quality of their lives and concerns about environmental degradation. As part of MITI's realignment, the six national programs were consolidated into two new programs. The first three programs, related to industrial technologies, were transformed into "The Industrial Science and Technology Frontier Program" (Frontier Program). The Frontier Program, in general, focusses on basic research and industrial technologies aimed at improving human welfare and quality of life (Anonymous, 1994h). In 1993, the Program's budget was \$228 million.

Similarly, the three national programs in the second grouping were unified in "The New Sunshine Program" (Anonymous, 1994h). Specifically, "The New Sunshine Program" promotes and funds research that leads to the development of technologies required to meet the government's goals for decreasing CO₂ from Japanese sources; increases collaborative international research projects that aim to reduce emissions of greenhouse gases; and leads to the development of environmentally benign, but effective, technologies that may be transferred to Asian developing countries. Much of the research to be undertaken under this program will be carried out

at the Research Institute of Innovative Technology (RITE), which is a private institute established with funding provided by MITI and private sources. MITI has allocated \$7.1 billion through 2000 to fund "The New Sunshine Program."

MITI recognized the importance of biotechnology to Japan's future in the late 1970s and, since then, plays a major role in establishing biotechnology as a key technology for future industries. In 1981, it declared biotechnology an area of special interest, giving it the recognition it previously lacked, and, thereby, facilitating financing of research and development in this field. Several large-scale biotechnology projects form part of the "Frontier Program," including "Fine Chemicals From Marine Organisms" (1988-1996), "Bioelectric Devices" (1986-1995), "Molecular Assemblies for Functional Protein Systems" (1989-1998), "Production and Utilization Technologies of Complex Carbohydrates" (1991-2000), and "Technology for Evaluating Functions of Tropical Organisms" (1993-1994). Under "The New Sunshine Project," biotechnology-related projects include "Biodegradable Plastics" (1990-1997), "High-Performance Bioreactor for Production of Biochemicals" (1990-1999), "Carbon Dioxide Fixation and Efficient Utilization Technology" (1990-1999), and "Environmentally Friendly Technology for the Production of Hydrogen" (1991-1998) (Agency of Industrial Science and Technology, 1993b).

AIST also is the major funder of five national institutes where significant biotechnology research is performed and a sixth that is in the process of being established. They are:

- **National Institute of Bioscience and Human Technology.** This institute was founded in 1993 in Tsukuba Science City when the Fermentation Research Institute was combined with elements from the National Chemical Laboratory for Industry, Industrial Products Research Institute, and Research Institute for Polymers and Textiles. It is dedicated to performing original basic research in the fields of biotechnology, biomedical engineering, and human engineering (medicine and welfare, lifestyle assets, etc.). Its first Director-General is Dr.

Osamu Suzuki, who was the former chief of the Fermentation Research Institute. The National Institute of Bioscience and Human Technology is Japan's only repository for organisms under the terms of the 1980 Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. In 1993, the institute employed 221 persons and its total budget was \$13.8 million (Agency of Industrial Science and Technology, 1993a).

The institute is taking part in three biotechnology-related national programs — “Fine Chemicals From Marine Organisms” (see below), “Molecular Assemblies for Functional Protein Systems,” and “Production and Utilization of Complex Carbohydrates.” In marine biotechnology-related areas, institute scientists also are investigating algal species for their potential in CO₂ fixation, microecosystems with respect to solidification and clean-up of oil spills at sea by microorganisms, bioabsorbent and biodegradable chemicals produced by marine microorganisms, and oxidation biocatalysts in microaqueous systems (Agency of Industrial Science and Technology, 1993a).

Joint research with both public and private institutions, as well as with industry, is given much attention by the institute. Thus, the oil spill project is being done in cooperation with the Governmental Industrial Research Center, Shikoku (see below), research on breakdown of sulphur compounds by marine bacteria with Marine Biotechnology Institute (MBI) (see below), R&D on basic marine life technologies with MBI, and R&D on biodegradable plastics with the non-profit corporation Research Institute of Innovative Technologies for the Earth (Agency of Industrial Science and Technology, 1993a). Cooperation with industry includes joint projects with Asahi Chemical Industry Company and Eisai Company, a leading pharmaceutical company; a marine biotechnology-related project is being done in cooperation with the Taiyo Fishery Company, Ltd., and involves research on new lectin in American crab species that specifically bind sialic acid.

In August 1993, STA designated the institute as one of Japan's first three Centers of Excellence. The objective of STA's Centers of Excellence program is to provide the designated institutes with extraordinary support to enable them to rise to the highest level in the world in their respective fields. Thus, the Institute will receive an additional \$3.64 million annually for five years, which will be used to develop its Biosignalling Department. The Department will use the new funding to form a 27-person research team to investigate signals that trigger cell aging and other phenomena within cells (Anonymous, 1993z).

- Government Industrial Development Laboratory, Hokkaido. The laboratory conducts research in three areas—resources and energy, applied chemistry, and new materials. Its biotechnology-related research aims to develop new catalysis technology using enzymes, including enzymes from marine extremophiles, the functional analysis of enzymes having commercial possibilities, and deep-sea CO₂ fixing. In 1993, the Hokkaido laboratory employed 98 staff and its total budget was \$4.9 million (Agency of Industrial Science and Technology, 1993a).
- Government Industrial Research Institute (GIRI) at Chugod. The institute was set up in 1971 to conduct pollution control research in the Seto Inland Sea, but has expanded its research focus to encompass the open oceans. Its biotechnology-related research is focussed on investigating the production and utilization of bioactive substances by photosynthetic microorganisms and characterization of membranes of extremophile microorganisms. In 1993, the institute employed 51 persons and its total budget was \$3.1 million (Agency of Industrial Science and Technology, 1993a).
- GIRI at Osaka. This GIRI is the oldest of the GIRIs, having been established in 1918 to support the chemical industry. Its

major focus now is on new materials of three types: energy-related; optical; and functional surface material. The institute is part of the national program "Fine Chemicals from Marine Organisms," discussed below, and is collaborating with Israeli and Russian institutions in marine biotechnology research (see below). In 1993, the Institute employed 207 persons and its budget was \$11 million (Agency of Industrial Science and Technology, 1993a).

- GIRI at Shikoku. The institute was established in 1967, among other objectives, to develop the marine resources of the Shikoku region. Thus, among the six GIRIs (Research Development Corporation of Japan, 1993), GIRI at Shikoku is most heavily involved in marine biotechnology. Its marine biotechnology research program has three directions. First, it aims to develop manufacturing processes to produce bioactive substances recovered from aqueous microorganisms. Second, it develops production technologies for fibre and biodegradable plastic utilizing polysaccharides from marine organisms, particularly locally available macroalgal species. Third, its researchers cooperate with colleagues at the National Institute of Bioscience and Human Technology in the special research project to analyze microecosystems with respect to solidification and clean-up of oil spills at sea by microorganisms. A project touching on marine biotechnology is to develop hollow fiber-shaped chitin derived from microorganisms (Agency of Industrial Science and Technology, 1993a). In 1993, the institute employed 47 persons and its budget was \$2.54 million (Agency of Industrial Science and Technology, 1993a).
- Biotechnology Inspection Center. The center, which was established in August 1993, will be located at the International Trade and Inspection Institute in Tokyo. Its research objectives are to investigate heat-resistant fungi, to clarify the heat-resistant mechanisms at the genetic level, and to analyze the

DNA of cyanobacteria and other photosynthetic microorganisms with the aim of developing environmentally benign production processes utilizing these organisms (Anonymous, 1993e). The center will be funded out of MITI's supplementary budget, but neither the amount, nor staffing requirements are known to us.

In 1988, marine biotechnology was accorded high priority status by MITI, indicated by the formulation of two new national programs. The first, "Fine Chemicals From Marine Organisms," is funded at over \$110 million for the nine year period 1988-1996 (Anonymous, 1989g; Agency of Industrial Science and Technology, 1989). Its general objective is to develop marine organisms or their products for industrial purposes (Anonymous, 1989b). Since this program is executed by the MBI, it is explained in the section below where the MBI is discussed. The second program, "R&D on High Performance Chemicals Manufacturing," has two phases—a basic research phase (1990-1994) and a phase during which applications are developed from basic research findings (1994-1997). The aim of the second program is to develop supporting technologies, such as a marine natural environment reproduction facility, a mass culturing facility, and a database containing information about marine organisms. The six AIST research institutes that participate in the basic research phase are the National Institute of Bioscience and Human Technology, National Institute of Materials and Chemical Research, GIRI at Osaka, GIRI at Shikoku, and GIRI at Chugoku.

Beginning in 1994, MITI is committing \$270 million over ten years under the "The New Sunshine Program" to fund research aimed at developing biotechnology for environmental protection, which will include marine biotechnology projects. Several universities and about 60 chemical and construction companies are expected to be involved in this national program (Anonymous, 1993r). Japan recognizes that it is behind the U.S. and Europe in environmental biotechnology, so it expects that the new program will begin to redress this imbalance.

Ministry of Education, Science and Culture

MESC funds basic research in universities. MESC's 1993 budget for supporting R&D was \$9.5 billion (Science and International Affairs Bureau, 1993). Of this, MESC's budget for biotechnology R&D was \$181.8 million (Anonymous, 1993 |). As the major supporter of basic research in universities, MESC undoubtedly funds marine biotechnology research, but the amount spent for this purpose is not known.

Ministry of Health and Welfare

MHW's purview includes public health, development of drugs and vaccines, and regulation of medicinals. MHW supports basic and applied research on health-related topics, with a R&D budget for 1993 of approximately \$585 million (Minister's Secretariat, 1993). In 1992, MHW disbursed \$343 million to support biotechnology (Anonymous, 1992c).

Research programs supported by the MHW included the Anti-AIDS Research Promotion Funds, the Research Fund on Circulatory Diseases, the Psychoneurosis Research Fund, Cancer Research Subsidies, the 10-year Comprehensive Anti-cancer Strategic Research Project, Promotion of Research on Psychosomatic Disorders, the Therapy and Research Fund for Specific Chronic Pediatric Diseases, Human Science Basic Research Expenditures, and the Drug Side Effect Victims Relief and Research Promotional Fund (Anonymous, 1992f). Potentially any of these programs could support investigations that may lead to the discovery of new marine natural products having anti-cancer, anti-inflammatory, and anti-viral properties. However, in 1992, the MHW had no programs or funding directed toward marine biotechnology.

Ministry of Agriculture, Forestry and Fisheries

MAFF promotes and supports research that aims to improve Japanese agriculture, forestry, fisheries, and the food industry

(Zaborsky et al., 1989). MAFF's 1993 R&D budget was approximately \$731 million (General Affairs Division, 1993), including \$91.9 million designated for biotechnology (Anonymous, 1993[]).

In Japan, MAFF is the major supporter of research related to aquaculture. MAFF's Fisheries Agency supervises the National Research Institute of Aquaculture, the National Research Institute of Fisheries Engineering, and seven regional fisheries research laboratories (Gibor, 1991; Research Development Corporation of Japan, 1993). The National Research Institute of Aquaculture was established in 1979. In 1992, its budget was \$9.54 million and it employed 92 persons, including 59 researchers. Its main facility is located in Nansai, with two large branches in Nikko and Ohmura (Research Development Corporation of Japan, 1993). MAFF supports many programs for improving species for aquaculture, including "Project to Develop Cultivation Technology for the Generation of Sexuality (Female) in Fish and Shellfish," "R&D on Generation of Superior Strains of Livestock and Fishes," "Project for Domestication by Nuclear Transplantation and Successful Individual Creation of Fish," and MAFF's Fisheries Agency's "Project for Projection of Regional Biotechnology Research and Development" (Ministry of Agriculture, 1989). These projects largely are aimed at improving or perfecting mass processing, artificial fertilization, and hatching techniques, as well as polyploidization techniques such as temperature shock and high pressure processing, chromosomal manipulation in fish, and other applications. In addition, MAFF is funding the "Biomedica Project," which has important aquaculture and marine biotechnology components (Ministry of Agriculture, 1989) (see below).

MAFF has been sponsoring biotechnology-related research since the early 1980s. In particular, the ministry has supported important research related to mapping the rice genome, plant breeding using biotechnology, and biotechnology to control and utilize agricultural, forestry, and fishery gene resources. Some of this research has been performed at the Bio-Oriented Technology Research Advancement Institution (BRAIN), which was set up by MAFF in 1986 to investigate new agricultural, forestry, and fishery technolo-

gies. BRAIN also administers certain R&D investment and loan programs on behalf of MAFF.

Under the auspices of MAFF, several marine biotechnology-related projects have been formulated; some of which currently are operational. These include the "Biomass Conversion Program" (a comprehensive study of the development of technologies for efficient utilization of biological resources), "Biocosmos Project" (a comprehensive program for the elucidation and control of ecosystems in agriculture, forestry, and fisheries), "Marine Frontier Project" (to promote the utilization of novel and uncommon marine resources), "Seed Cultivation Project for Promoting Biotechnology," and the "Biomedica Project" (Matsusato, 1989). Of these projects, perhaps of most interest to this report are the "Seed Cultivation Project" and "Biomedica Project."

The "Seed Cultivation Project," which began in 1983, had three components, one of which was a five-year program on the application of marine microorganisms. The program was led by Dr. U. Simidu, University of Tokyo, but scientists from five universities participated. Investigations carried out during the project included marine bacteria that resist viruses, aerobic phototrophic bacteria, production of marine animal toxin by bacteria, algal products that stimulate fish growth, and bacteria that produce anti-cancer compounds.

The "Biomedica Project" supports research that seeks to improve genetic analysis techniques that needs to be done before rDNA techniques and other genetic manipulations can be used to their fullest capacity in research. The primary objective of the "Biomedica Project" is to clarify the mechanism, with regard to maturation and egg production in fish, of *in vivo* information transmission at the cellular level (Ministry of Agriculture, 1989). MAFF's budget for the "Biomedica Project" was \$4.1 million in 1992 (Anonymous, 1992h).

MAFF supports marine biotechnology R&D related to marine viruses, bacteria, microalgae, macroalgae, invertebrates, and vertebrates. Marine virus research is funded under the program "Analysis and Development of Utilization Technology of Animal DNA," which seeks to prevent epidemics among aquacultured fish and to develop healthy seedling cultivation techniques. MAFF-supported

R&D on marine bacteria investigates bacteria in special environments, marine nitrogen-fixing bacteria, bacteria useful in food processing, parasitic bacteria, and pathogenic bacteria. Microalgal R&D includes intensive study of organisms that cause red tides, such as *Dinophyta* (see below). With reference to macroalgae, the species *Porphyra yezoensis* is under intensive study, to clarify its breeding mechanism and to optimize culture conditions.

MAFF's "Marine Ranching Project" serves as an extension service, spreading newly developed techniques throughout Japan. The invertebrates that are being investigated by MAFF researchers include rotifers (to develop culture methods), sponges (for natural products), corals (to develop culture methods, especially to supply the jewelry industry), urchins (to develop culture techniques for improved food production), lug worms (annelids that burrow in beach sand and produce unique toxins and adhesives), shrimp and crabs (to develop culture techniques and to investigate information processing systems of these animals), cuttle fish and octopus (the neurological systems of these animals are being studied for possible applications in future generations of computers), and ascidians (tunicates, such as sea squirts, which can accumulate metals). In addition, MAFF is supporting basic research in preparation for employing genetic engineering techniques to develop transgenic fish (Anonymous, 1989b).

The Fisheries Agency provided \$54.54 million in 1988 to construct a 2,849 ton research vessel *Kaiyo Manu*, which became operational in 1989 (Matsusato, 1989). The vessel's purpose is to assist scientists in performing research projects that promote efficient utilization of Japan's marine bioresources. Recently, *Kaiyo Manu* supported two marine-biotechnology related projects—one aims to perform physicochemical characterization of marine products and the second to clarify metabolic processes in marine organisms.

Environment Agency

EA, a relative newcomer among the Japanese government agencies, was established in 1979. It sponsors research projects aimed at developing pollution prevention technologies to curb the emission of pollutants into air and water, appropriate waste disposal and recy-

cling, assessing the impact of pollutants on animal and plants, behavior of pollutants in the environment, and preservation of natural environments (Anonymous, 1989c; Anonymous, 1989d; Environment Agency, 1992). EA's 1993 budget for R&D was \$114.3 million (Planning and Coordination Bureau, 1993b). In 1992, EA designated \$1.2 million for biotechnology (Anonymous, 1992c).

The involvement of EA in general and marine biotechnology has been minor, but is likely to increase in the future. In particular, EA's National Institute for Environmental Studies opened the Environmental Genetic Engineering Laboratory in November, 1993, the major aim of which is to produce microorganisms and plants that can be applied to preserving the environment (Anonymous, 1993m). In reference to marine biotechnology, a joint R&D project between the Fishery Agency and EA is the "Project to Develop Techniques for the Prediction of Red Tide Occurrence," which aims to search for substances that inhibit the growth and reproduction of red tide organisms. In 1993, EA, in cooperation with MITI, initiated a five-year marine bioremediation program to develop improved bioremediation techniques suitable for Japanese conditions (Anonymous, 1993o). As part of that program, EA's National Research Institute for Pollution Prevention will develop technologies to immobilize oil remediating microorganisms on mats, the Life Engineering Industrial Research Laboratory will investigate various microorganisms for their oil-degrading capabilities and develop those that are most efficient, and GIRI at Shikoku will research biological dispersants and nutrients required by oil-degrading microorganisms (Anonymous, 1993g). The program's budget in 1993 was \$296,000.

EA is charged to address environmental safety. In 1993, it drafted guidelines to protect inland seas and bays from land-source pollutants. Until then, Japan had no regulation controlling the volume of nitrogen and phosphorous being dumped in the ocean by factories and waste disposal plants. As a result, high concentrations of these chemicals had stimulated red tide blooms and led to large-scale fish kills (Anonymous, 1993i). The new guidelines, which took effect October 1, 1993, controls the discharge of effluences into Tokyo Bay, Seto Inland Sea, and 86 other inland seas and bays.

EA is responsible for handling environmental problems arising from biotechnology. However, Japan's regulatory situation in regards to biotechnology is unclear, as is discussed on page 271.

Ministry of Construction (MOC)

In 1993, the MOC did not support biotechnology R&D directly. However, in 1989, the ministry allocated \$900,000 to develop a new wastewater treatment system utilizing marine organisms to purify wastewater. The advantages of the system, which was functional by 1991, includes a higher degree of efficiency, compared to existing systems and causes less environmental impact (Anonymous, 1989c; Anonymous, 1989d).

Inter-ministry Programs

Of relevance to this report are two large scale interministerial programs, Key Technology Centers (KTC) and Human Frontier Science Program (HFSP).

- **Key Technology Centers.** This program, cosponsored by MITI and the Ministry of Posts and Telecommunications, promotes establishment of research centers undertaking research in areas such as biotechnology, electronics, information processing, telecommunications, and new materials. The funding for KTC in 1993 was circa \$236 million.
- **Human Frontier Science Program.** The HFSP is an international program, the administration of which is headquartered in Strasbourg, France. The Program aims to promote international cooperation in basic research focussed on superior functions of organisms (Anonymous, 1991a). Thus, the Program funds basic research carried out by international teams, fellowships for scientists wishing to conduct research in foreign countries, and meetings where information from HFSP research is discussed and disseminated. In 1992, STA and MITI, together, furnished approximately \$37.8 million to the

Program (Science and Technology Agency, 1993b; Anonymous, 1994h).

In summary, direct federal government support for general and marine biotechnology is estimated as follows. The total amount the government spent in 1992 to support science and technology was \$21.26 billion, including approximately \$917 million for biotechnology (4%). It was more difficult to estimate how much of this funding was used to support marine biotechnology. To determine this sum, we used the following four-step approach. First, we calculated funding for projects or programs that were undoubtedly marine biotechnology, for example, MITI's "Fine Chemicals from Marine Organisms," MBI's CO₂ utilization project, ERATO's Fusetani project, and MAFF's new technologies for marine industry. The amount of this funding was \$36.47 million.

Second, as described above, there were many programs and projects that have marine biotechnology components, but clearcut, descriptive information is not available with respect to funding allocation. Examples are MITI's CO₂ fixation and hydrogen generation, STA's physiological functions, EA's red tide, and MAFF's "Biomedica" and new fishery technologies projects. With respect to funding for these programs and projects, we made an assumption about the probable range of funding. Thus, we assumed that, on the low side, 20% of a program or project funding was spent on marine biotechnology research and, on the high side, 80%. Accordingly, total funding for these programs and projects was estimated to range between \$8.64 million and \$34.6 million in 1992.

Third, although the MESC funding for general biotechnology is known (\$149 million in 1992), there is no information on distribution of these funds. However, as is clearly demonstrated in the section beginning on page 287, most marine biotechnology basic research has been, and is being, done by universities, with funding largely from MESC. Therefore, the conservative assumption is that 10% of MESC biotechnology funding supports marine biotechnology research, i.e., \$14.9 million. Remaining conservative, we assume that other ministries, e.g., MHW and MOC, did not fund marine

biotechnology projects in 1992, although they probably did to a minor extent.

Thus, the grand total of Japanese federal support for marine biotechnology research is determined to have been between \$59.51 million and \$86.47 million in 1992 (see Figure 21), a reasonable range of estimated funding, considering all of the caveats stated above.

Indirect Federal Activity to Support Science and Technology

Indirect federal activity influencing development of biotechnology and marine biotechnology in Japan includes credit allocation for industry, tax policies that favor investments in biotechnology, and regulations that control biotechnology research, testing and application.

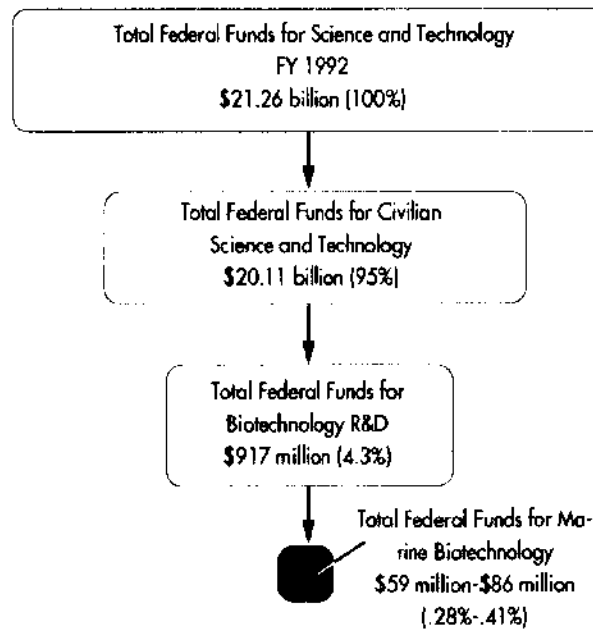


Figure 21. Trickle-down effect in Japanese biotechnology, 1992.

Credit Allocation

To promote biotechnology, the Japanese government provides a number of incentives for industry. Several programs exist to support companies searching for initial capital for ventures in biotechnology. Programs include the "Basic Technology Center Financing System," "Financing System to Promote Biotechnology Industrialization," "Financing System to Promote Regional Commercialization in Biotechnology," and "Financing System to Promote Small and Medium Biotechnology Industrialization" (Anonymous, 1989c; Anonymous, 1989d). The first program is aimed specifically toward industry interested in biological research and development and finances 70% of basic research costs, with conditional non-interest bearing financing. The second program, "Promotion of Biotechnological Industrialization," administered and financed by the Japan Development Bank Fund, assists enterprises and non-profit organizations, with loans repayable within 15 years at an interest rate of 5.05%. However, each loan is limited to 40% of the total expenditures of the targeted research program (Biotechnology Division, 1990). In 1989, the program was capitalized at \$540 million. The third program, also aimed toward enterprises and nonprofit organizations, involves the Hokkaido-Tohoku Development Bank and is capitalized at \$300 million. The conditions are similar to those of the Japan Development Bank, with the exception that a company can apply for up to 80% of the targeted program's expenditures (Biotechnology Division, 1990). The last program of program for which information is available involves the Small Business Finance Corporation, funded at \$180 million in 1989 and is aimed at the industrialization stage (Anonymous, 1989c; Anonymous, 1989d).

Tax Incentives

As can be seen in Table 2, there are seven tax incentive programs aimed at promoting R&D activities. At the national level, the most important probably is the special three-year exemption for biotechnology R&D, which has been in effect since 1985. This tax exemption refunds part of the cost of machines and facilities ac-

quired, manufactured, or constructed for basic technology research and development, by allowing individuals and corporations to add an additional 7% to their tax credit. In addition, the 7% is deducted from individual and corporate income taxes (Biotechnology Division, 1990). A detailed explanation of Japan's tax structure related to promoting the acquisition of research equipment can be found in a publication by the Agency of Industrial Science and Technology (1992b).

At the local level, a two-year exemption has been in effect since 1985 that covers research equipment and facilities acquired, manufactured, or constructed for the purpose of safe execution of research in gene recombination technology and its application technologies. The incentive adjusts the fixed asset tax rate for an individual or corporate entity to two-thirds of the standard taxable value for three years from the year the equipment was acquired or construction costs paid (Science and Technology Agency, 1993a).

Table 2. Major Tax Incentive Programs for Promotion of R&D Activities.

	Year Implemented	Description
1. Special Tax Program for Promotion of Basic R&D	1967	A combined total of the following expenditures may be deducted from a company's income. The total deductions, however, must not exceed 10 percent of the taxable corporate income. If program 1(b) or 1(c) are used, the total must not exceed 15 percent of the taxable corporate income.
(a) Deduction for increased R&D expenses	1967	If R&D expenses exceed those of previous years, up to 20 percent of the excess amount may be deducted.
(b) Promotion of basic technology R&D	1985	Up to 7 percent of acquisition costs may be deducted if a corporation acquires depreciable assets for R&D activities in specified cutting-edge technology areas.
(c) Strengthening basic research at small and medium-size enterprises	1985	Small and medium-size corporations may deduct up to 6 percent of their R&D expenses. If program 1(a) is used, this program is not applicable.
(d) R&D expenses incurred in joint research with government research institutes	1993	Up to 6 percent of R&D expenses may be deducted if a corporation conducts joint research with a national research institute.
(e) R&D expenses incurred in environmental research technologies to be defined in the energy conservation and recycling law	1993	Up to 6 percent of R&D expenses may be deducted if a corporation conducts environmental technology research.
2. Special Prefectural and Municipal Municipal Tax Program for R&D Expenses	1985	All the deductions allowed under program 1, except program 1(a) may be deducted from taxable corporate income. Total deductions must not exceed 15 percent of the taxable income.

(Compiled from Japanese Government Reports)

Regulations

Until 1979, Japan had no regulations specifically addressing biotechnology research, field testing, or application. In view of this regulatory vacuum, scientists followed the NIH guidelines of the U.S., while companies were mainly "self-regulated." However, in 1979, MESC issued a set of guidelines pertaining to rDNA research in universities and, shortly thereafter, STA issued another set of guidelines for industry and national research institutions (Tomizuka, 1993). In 1987, MITI formulated "Recombinant DNA Engineering Guidelines," which were derived from those adopted in July 1986 by the Organization for Economic Cooperation and Development (OECD), of which Japan is a member (Biotechnology Division, 1990). Since that time, MHW and MAFF have promulgated similar guidelines. Thus, in April 1989, MAFF published "Guidelines Toward the Utilization of Recombinant Products in the Fields of Agriculture, Forestry, and Fishery," which permitted non-closed system testing (Anonymous, 1989e; Anonymous, 1989f; Ministry of Agriculture, 1991b). In general, the MAFF guidelines are based on estimating a level of risk for each organism and performing a case-by-case review of environmental and agricultural applications of that organism. They outline a control standard based on extending experience with that organism through a step-wise progression of experiments, from the laboratory, to the greenhouse, to the small-scale field-test, and then to the large-scale field test (United States Congress, 1991).

In regard to field testing, in December 1990, EA submitted to the Japanese Diet an encompassing proposal to regulate field applications of new varieties of living organisms (Anonymous, 1990c). If approved, this proposal will give the EA authority beyond that of other ministries over all of field testing. It is our understanding that the EA initiative has triggered a jurisdictional battle between EA and MAFF that may delay legislative action on regulation of field testing. In addition, EA's action has raised concern among researchers and industry involved in biotechnology. Citing their safety record, oppo-

nents of EA's proposal argue that the proposed regulation will make it difficult for industry and academia to respond to technological progress, that the worldwide trend is towards a relaxation of regulation, so adopting new restrictive regulations will go against the worldwide trend. They also argue that the proposal will send a one-sided message to the citizenry that biotechnology is a dangerous technology. During the past ten years of self-regulation by industry, no hazards have been generated and EA is considered a newcomer, with virtually no administrative experience in bio-related areas. EA counters that if problems arise, ambiguity exists on the assignment of responsibility (Anonymous, 1989a; Anonymous, 1990a; Anonymous, 1990c).

Japan lags behind Europe and the U.S. in large-scale field testing. In fact, it was not until January, 1991 that MAFF approved the first environmental testing of a genetically engineered organism, a transgenic tomato. In early 1993, developers of four other types of transgenic plants received permission to test their products in secure nurseries (Anonymous, 1993~). By June 1993, a total of five transgenic plants were undergoing field testing and an addition five were being prepared for imminent field tests. In comparison, more than 1,000 field tests have been completed or are underway in the U.S., and in excess of 400 in the rest of the world. The Japanese government's hesitancy in developing a unified set of national biosafety guidelines probably stems from a combination of factors, including protests that have been voiced by the public in reference to several biotechnology projects in the past (Tomizuka, 1993), which may indicate a negative attitude by the Japanese public towards environmental release of genetically engineered organisms, and the propensity of Japanese researchers to focus their attention on research potentially useful for the pharmaceutical and food industries, rather than for agriculture, where most field testing presently is being done.

The debate over field testing continues in the Diet and future decisions about regulations that seek to ensure biosafety will affect the future, not only of marine biotechnology, but of all biotechnology in Japan. We believe that the many uncertainties associated with Japanese regulation of biotechnology activities, for the present, repre-

sents an unfavorable legal environment for industry because it hinders long-term strategic planning pertaining to product testing. However, it is unclear how Japan's regulatory structure eventually will shape up. Without doubt, national regulations can have a significant effect on how rapidly or slowly biotechnology progresses (United States Congress, 1984). If the Japanese Diet adopts onerous or harsh regulations, experimentation and field testing will be hindered. Conversely, if it passes weak or unclear regulations, industry may be precluded from undertaking long-term, strategic planning.

In 1992, MHW issued "Guidelines for Foods and Food Additives Produced by Recombinant DNA Techniques." According to a knowledgeable observer, the ministry "has imposed a strict regulatory regime specific to foods and food additives manufactured with recombinant DNA techniques" (Miller, 1993).

Local Government Activity in Biotechnology

Japan comprises 47 prefectures, which are roughly equivalent to states in the U.S., although politically less independent. Most prefectural governments have initiated wide-ranging, well-supported programs to promote scientific endeavors within their jurisdictions. The totality of their efforts is meaningful; in 1992 prefectural and city governments provided \$5.20 billion to fund science and technology, which is 26.7% of the national science and technology budget (National Institute of Science and Technology Policy, 1993). The reason why prefectures are spending so much money in this way is explained in a STA-sponsored study (National Institute of Science and Technology Policy, 1993):

As well as contributing to the formation of a multipolar and decentralized society by acting as a driving force for the stimulation of regional activities, regional S&T should respond precisely to various regional requirements and improve the lives of people living in such regions.

Prefectures use three mechanisms to support science and technology: science councils, *kosetsushis*, and third-sector centers.

- Science councils. Eleven prefectures have established science councils. Generally, science councils have three aims: to formulate science and technology policies for prefectural governments, identify important R&D that should be performed by local research centers, and disburse funds from local sources to prefectural research organizations. Some science councils also act as advisory bodies to governors and prefectural government departments.
- *Kosetsushi* centers. These centers, the name of which may be translated as “technology upgrading centers” (*ko*=public; *setsu*=establish or organize; *shi*=examine, as in *shi-kenjo* or research institute) are established by local governments to provide special scientific services, such as applied research, technology assistance, testing, training, and information dissemination, to local small and medium-sized enterprises. They are often supported by federal agencies through their local offices. In 1992, *kosetsushi* centers employed approximately 15,000 scientific workers, which is more than 1.5 times the number employed by national research institutions (Anonymous, 1993k).

Several *kosetsushi* centers are concentrating their efforts in general and marine biotechnology. An example of a *kosetsushi* project in marine biotechnology is one between the Industrial Technology Development Center in Amori City, Amori Prefecture, and the University of Hirosaki that aims to identify an glycogen poly-sugar anti-cancer agent in scallop stock (Anonymous, 1994a); another example is the Algae Resource Center, Kochi Prefecture, that extracts and purifies lectin from seaweed (*Eucheuma serra*) growing off Tokushima (Anonymous, 1994d). Other centers are also active in marine biotechnology, for example, a center located in Chiba Prefecture concentrates on analyzing DNA in blue-green algae (Anonymous, 1994e), one in Ehime Prefecture promotes biotechnology applications in fisheries, a center in Kumamoto Prefecture is

developing fish growth hormones for local aquaculture, and a center in Kagoshima Prefecture is utilizing biotechnology techniques to counter red tides (National Institute of Science and Technology Policy, 1993).

- “Third-sector” centers. These are R&D institutions set up on the prefectural level, with funding from three sources: federal government; prefectural government; and industry. Their main function is to perform research that may be applied by the industries that have invested in them (Science and Technology Agency, 1993a).

MAFF, MITI, MESC, and STA all strongly support science at the prefectural level. Their regional funding programs have assisted various marine biotechnology-related projects, such as one being undertaken by *kosetsushi* and third-sector centers in Aichi, Fukushima, and Saga Prefectures to develop new varieties of seaweed suitable for the specific region by development of protoplast seedlings. Another project, the development of technology for production of large fishes, is being undertaken by research units in the Prefectures of Aomori, Fukuoka, Gifu, Ishikawa, Kumamoto, Miyagi, Nagano, Nagasaki, Shiga, Tokushima, Tottori, Toyama, Yamagata, and Yamaguchi (Ministry of Agriculture, 1991a). The major approach is to apply sterilizing technology and technology to extend the reproductive life and the production of high value fishes through establishment of sex control technology. In a different area of marine biotechnology, a joint project is underway between cooperatives and research units in the Prefectures of Iwate and Shizuoka, largely funded by NEDO, to discover and develop useful natural products from marine animals.

In accordance with the provisions of a law adopted by the Diet in 1988, a special type of third-sector centers, called “Basic Facility for R&D,” are to be established in Japan with funding from NEDO, local government and private firms. A “Basic Facility” is defined as “a large R&D facility for advanced industrial technology to be used by many researchers” (Anonymous, 1989g). The first was the Center for

Industrial Use of Marine Organisms, which is part of MBI and is discussed below (the other Basic Facilities will include the Underground Microgravity Environment Experiment Center in Hokkaido and the Ion Engineering Center in Kinki).

International Cooperative Projects

The government of Japan supports several well-funded programs aimed at the development of international collaboration and technology transfer in biotechnology. Two of these have been described above—STA's ERATO and MITI's and STA's HFSP. MITI also funds the program "Promotion of Comprehensive International Research Cooperation" at the level of \$16.36 million in 1992 (Anonymous, 1994h). Further, several programs have been set up to promote international exchanges of researchers. These include the "Fellowship Program" funded by STA, the "Special Foreign Researcher Program" funded by the Japan Society for the Promotion of Science, and the "Research Exchange Program" initiated in 1988 by MITI's AIST. In 1989, these programs funded travel and living expenses of 300 foreign researchers in Japan, who represented approximately 30 countries (International Cooperation Division, 1990).

With respect to international ties, in 1987, a five-year joint project, named "STARMER," between France and Japan commenced. The objective of the project was to study the South Pacific Ocean plate, including hydrothermal vents located in the North Fiji Ocean Basin at a depth of 1,980 meters. Project investigators have collected a variety of interesting extremophiles, including sea horse mussels, blind crabs, previously unknown deep sea shrimp, and several types of microorganisms that use hydrogen sulfite and methane as energy sources (Anonymous, 1989g), which are now being investigated in French and Japanese laboratories. In 1993, the Japanese Biotechnology Association (JBA) entered into a collaboration in marine biotechnology with the French Association for the Development of Bioindustry and the French Ministry of Foreign Affairs. The French coordinator is Y. Le Gal (Pardo, 1993).

Wide-ranging cooperation between Russia and Japan was discussed during the second Russo-Japan Science and Technology Co-

operation Committee meeting held in May 1994. By the conclusion of the meeting, the two governments had agreed to set up cooperative arrangements among their research organizations in 34 research themes. One of these are in marine biotechnology, namely a project that aims to genetically engineer microalgae for the production of useful materials (Anonymous 1994k).

Preceding the general agreement between the two countries, in 1993, MITI's AIST dispatched a team to Vladivostok, Russia, to discuss cooperative R&D with the Pacific Ocean Biology and Organic Chemistry Research Institute in that city (Anonymous, 1993t). The Japanese seek to access the Institute's vast collection of marine microorganisms originating from seas under Russian jurisdiction. Samples will be analyzed by GIRI at Osaka, to determine if any produce biochemicals having a potential for industrial application (Anonymous, 1993f). A cooperative arrangement of this type appears to be worthwhile for both sides. The Russian institute is in dire financial straits so if it will be able to procure funding from the Japanese, its survival would be ensured. The Japanese will have access to many organisms that they otherwise might not have been able to screen, thus encouraging their quest for natural products having pharmaceutical and industrial properties. It is worth noting that Vladivostok and a large marine area surrounding it was closed to all foreigners until the Soviet Union collapsed in 1991. This immense area, which probably contains a treasure chest of marine biological diversity, is mostly virgin territory in terms of natural product prospecting.

Also in 1993, MITI came to an agreement with the government of Israel to enter into cooperative research in biotechnology (Anonymous, 1993y). It is following in the tracks of Japanese industry and eight companies already have constructed plants in Israel. One of the aims of cooperative research to be undertaken between GIRI at Osaka and the Hebrew University is to develop high-performing microalgae (Anonymous, 1993s). Experiments, utilizing genetic engineering techniques, will be done on microalgal species provided by the Israelis (see below).

Another area containing marine biological resources of vast number and variety is the South Pacific. Japanese researchers are ac-

tive here, also, searching for marine natural products. For example, the Japanese have been able to negotiate an agreement with the Federated States of Micronesia (FSM) that allows them to collect biological specimens from its reefs, which contain about 60% of the world's coral species (in comparison, the Caribbean has about 20%). Similar activities are being conducted by Japan at Palau. The Japanese scientists, who are not required to recompense FSM, reportedly are looking for products having anti-fouling, bioremediating, and pharmacological properties.

THE ROLE OF THE PRIVATE SECTOR IN SUPPORTING SCIENTIFIC RESEARCH

More than any other industrialized country, the Japanese private sector plays a vital role in science and technology, a role that is likely to continue in marine biotechnology. This section, which seeks to clarify how important that role is, has two parts. First, we discuss the Japanese industry's support of science generally, and marine biotechnology specifically. Second, the less important but nevertheless significant role of joint ventures and non-governmental organizations in marine biotechnology endeavors is clarified.

Japanese Industry and Support of Scientific Research

A wide-ranging study was performed by the STA in 1992 to assess the state of research in Japan (Agency of Industrial Science and Technology, 1992a). One of its major findings was that Japanese industry is the most important component in Japan's science and technology infrastructure. To demonstrate, of all research done in Japan in 1992, industry performed 80.6%, universities were responsible for 11.6%, and government institutions 7.8%. Further, the trend since 1980 is one of industry continuously increasing its share, while that of the government decreases. Among the principal industrialized countries the share of industry's research expenses provided by government is the lowest in Japan, standing at 2.7%, compared to 31.2% in the U.S. Another revealing trend can be seen in the percentage of research expenses received by universities from various sources. In 1983, the government funded 82.2% of all research performed in

universities, while industry supported 17.6%. However, in 1990, the government's share had dropped to 65%, while industry's had increased to 35%. The type of research that industry supports is overwhelmingly applied or developmental research (90%). The study demonstrated a well known fact, namely that Japan's basic research ratio of 12.6% (compared to 15.1% in the U.S.) is the lowest among principal industrial countries. It can be seen that the findings of the study supports the generally accepted notion that Japan tends to neglect basic research in favor of goal-oriented research, a tendency that is likely to continue.

Until fairly recently, there was little cooperation between industry and universities. It is only since 1983 that this situation began changing, after new MESIC guidelines defining university-industry relations came into effect (Koizumi, 1992). Among others, the guidelines allow university laboratories to undertake proprietary research that companies may develop into products. Further, researchers from industry are allowed to work in university laboratories while still employees of the companies. Over the last ten years close collaborations have been developed between academic researchers and companies through mechanisms such as joint appointments, joint publications, consultancy agreements, and contract research. For example, in 1983 there were just 56 joint university-industry research projects, but in 1992 this had increased to 1,241 projects with 1,398 researchers participating in them (Ministry of Education, 1993). The total funding of university-industry projects was approximately \$41 million for 1992 (Normile, 1993). However, many academic scientists still harbor a bias against industries and applied research (Koizumi, 1992), while industry "view Japan's universities as little more than a filter for sifting out the brightest of the next crop of employees" (Normile, 1993).

Most academic research sponsored by Japanese industry, as noted above, is applications oriented. However, recently it was reported that Japanese companies are shifting their strategies from low-profit, large-scale products to high value-added products, such as new materials and bioactive substances. For this reason, companies were investing in basic research facilities in order to understand basic phenomena underlying the development and production of these prod-

ucts (Hirano, 1992). Our observations from having monitored biotechnology developments in Japan for over four years supports this observation. Specifically, many Japanese companies are investing in biotechnology, even companies that one would not usually associate with the life sciences (e.g., companies involved with the manufacture of automobiles, machinery, mining equipment, etc.). Further, as Japanese companies deplete the possibilities of current knowledge and as more companies move into biotechnology, many of them are recognizing the importance of basic research. A significant number of these companies are funding basic research projects in academic institutes, thus, the tendency of industry to fund applied research, noted above, may not hold true in biotechnology.

In addition to needing to explore new marketing possibilities, incentives offered by the Japanese government to industry have encouraged Japanese companies to enter biotechnology. What is noteworthy in this regard is how incentives are designed to ensure that investments by industry are for the long-term. The Japanese companies therefore do not expect to turn a profit in the short term. Rather, Japanese industry is anticipating reaping profits and other benefits in five, ten, or even fifteen years.

Companies of course know that the Japanese biotechnology market is already large, and will grow much larger. To illustrate, the JBA conducted a survey of 134 companies in 1992. It found that the biotechnology market had increased in size from \$900 million in 1987 to \$5.45 billion in 1992, and is estimated to reach \$28 billion in 2000 and \$90.9 billion in 2010 (Anonymous, 1993d)! The present market derives an income of \$2.92 billion from medical products, \$800 million from chemical products, \$636 million from biotechnology-supporting industry, \$364 million from agricultural products, and \$273 million from food products (Anonymous, 1993d). The average amount each company spent on biotechnology R&D annually was \$6.63 million.

As can be seen in the next section, Japanese industry is heavily involved in marine biotechnology research. Many of the companies that are investing in marine biotechnology projects (see Table 3) also are encouraging their scientists to enter into collaborative research

with academic investigators. For example, a joint project between Hokkaido University and Tamazukuri Ltd. investigates the fungistatic action of chitosan oligomers; two projects between Hokkaido University and Hokkaido Prefecture focus on developing sea urchin resources; Tohoku University and the Shizugawa-machi Company are trying to rectify pollution from Shizugawa Bay aquafarms; University of Tokyo is working with the Research Institute for Innovative Technology for the Earth to improve the efficiency of photosynthesis of algae and bacteria; in another project supported by MAFF, University of Tokyo researchers are working with colleagues from the Asahi Chemical Industry Company to extract peptides from skipjack (bonito) viscera that evidence anti-hypertensive activity (Anonymous, 1994i); Tokyo University of Agriculture and Technology has several industrial partners, including Tensei Fisheries Ltd. (development of physio-active substances from mackerel extract), Simadzu Corporation and Onoda Cement Ltd. (to develop algae-based bioreactors for CO₂ fixation and production of useful substances), Pentaru Ltd. (develop plant physiology-activating substances from cyanobacteria and measures the effect of these on plants), Nippon Kokan Ltd. (investigate CO₂ fixation by algae), Shuseido Company Ltd. (to search for useful substances from marine organisms), and Kanegafuchi Chemical Industry Company Ltd. (cultivation of blue marine algae); between Tokyo University of Fisheries and Institute of Pearl Science Ltd. for searching for useful fish genes; Mie University and the Mikimoto Pharmaceutical Company Ltd. are isolating active components from fishery organisms; Osaka University is cooperating with Yatoron Ltd. to develop reagents for testing fish toxins; Hiroshima University is working with the Chugoku Electric Power Company Inc. to convert CO₂ into resources by using and modifying algae and with the Hiroshima Prefecture to develop techniques for controlling oyster shell ligaments and muscles; Yamaguchi University together with Rengo Ltd. are developing chlorella culturing in fermentation vats; and Ehime University and Katakura Chikkarin Company Ltd. are researching methods to remove pectinesterase by means of a chitosan-pectin compound (Ministry of Education, 1993).

Table 3. Japanese companies investing in marine biotechnology.

Aicello Chemical Company	Nichiro Company
Asahi Chemical Industry Company, Ltd.	Nippongene Company
Asahi Glass Company	Nippon Kokan, Ltd.
Chugoku Electric Power Company, Inc.	Nippon Mining-Kyodo Oil Corporation
Cosmo Development	
Dainippon Ink and Chemical Company	Nippon Paint Company
Dowa Mining Company, Ltd.	Nippon Steel Corporation
Ebara Research Company, Ltd.	Nippon Suisan Kaisha
Institute of Pearl Science, Ltd.	Nissan Oil Mills
Fujitsu	Onoda Cement, Ltd.
Hitachi Zosen Corporation	Pentaru, Ltd.
Idemitsu Kosan Company	Rengo, Ltd.
Ihara Chemical Industries	Sapporo Breweries Company
Ishihara Sangyo Kaisha, Ltd.	Sekisui Chemical Company
Japan Tobacco, Inc.	Shimadzu Corporation
Hugoromo Foods	Shimizu Construction Company
Hazama-Gumi Ltd.	Shiseido Company, Ltd.
Kajima Corporation	Shizugawa-machi
Kanegafuchi Chemical Industry Company, Ltd.	Suminoe Kikaku Company
Kansai Paint Company	Sumitomo Chemical Company, Ltd.
Katakura Chikkarin Company, Ltd.	Sumitomo Metal Mining Company, Ltd.
Katokichi Company	Suntory Ltd.
Kawasaki Steel Company	Suzuyo and Company Ltd.
Kirin Brewery Company	Taisei Corporation
Kyowa Hakko Kogyo Company	Taiyo Fisheries
Kyowa Hakko Nippon Steel	Tamazukuri, Ltd.
Kumagai Gumi Company, Ltd.	Tensei Fisheries, Ltd.
Maruha Group	Tokyo Electric Power Company
Meiji Seika Kaisha, Ltd.	Tonen Sekiyu Kagaku, K.K.
Mikimoto Pharmaceutical Company, Ltd.	Toray Industries, Inc.
Mitsubishi Gas Chemical Company, Ltd.	Tosoh
Mitsubishi Life Sciences	Toyo Soda Manufacturing Corporation
Mitsubishi Rayon Company	Unitika Company
Nichirei Corporation	Yatoron, Ltd.

After having followed the development of marine biotechnology in Japan from 1989 to the present, it is our impression that similar to the present situation in the U.S. and other countries, marine biotechnology is an emerging field in science in Japan. Although

many Japanese companies are making financial and manpower investments in marine biotechnology (see Table 3), these probably are not done according to strategic planning. More likely, the reason companies have made these investments is to be in good entry position should opportunities in the field develop. Further, collaborations between academia and industry in Japan generally are successful in terms of both sides gaining benefits, therefore, it is likely that industry will continue increasing its support of marine biotechnology research in the academic sector for the foreseeable future.

From the information that was compiled for this analysis, it was not possible to estimate directly how much investment Japanese industry is making in marine biotechnology. However, analysis of official Japanese government statistics from past years confirms that industry provides funding for circa 80% of all research in Japan (Agency of Industrial Science and Technology, 1992a). If this holds true for marine biotechnology, by using the funding figures derived from federal agencies (see page 187, above), we deduce that industry funding for marine biotechnology research in 1992 was in the range of between \$297.55 million and \$432.35 million.

We also surveyed the literature for information about research activities of Japanese companies. While much less exact information was available on this subject than on academic research (see below), it was clear that the companies whose major business lie in the food and pharmaceuticals areas are at the forefront of supporting marine biotechnology research; the types of research they favor tend to be focussed on aquaculture and marine natural products.

Joint Ventures and Non-governmental Organizations Active in Marine Biotechnology

Joint Ventures

The MBI is the principal joint venture in marine biotechnology between Japan's government and industry (Anjo, 1989). The main objective of MBI is to perform bioengineering research utilizing marine organisms. Ultimately, its function is to transfer and license results from that research to its supporting companies. Accordingly,

activities of the MBI are concentrated on basic technologies for utilization of marine organisms, technologies for producing useful substances, technologies for utilization of useful biological functions, and support technologies. Specific targets of the company include: techniques for producing novel surfactants, dyes, viscous polysaccharides, and coatings; bioreactors; bioremediation; and techniques for utilizing useful biological functions or marine organisms, such as the ability of some algae to accumulate and concentrate rare metals. Organisms that MBI scientists investigate include marine microorganisms, microscopic algae, and other algae, protochordata, sponges, and coelenterates.

MBI has three components: MBI, Center for Industrial Use of Marine Organisms (CIUMO), and the research vessel *Sohgen Maru* (Miyachi, 1993b). The first, MBI, is the parent organization, which sets policy and disburses funds to support research undertaken at the institute. It was established in December 1988 as a cooperative venture between MITI and 24 private companies. Funding for the MBI in 1991 was \$19.6 million, of which MITI supplied \$10.2 million, the 24 companies furnished \$7.8 million and RITE provided \$1.6 million (Miyachi, 1993b). MBI's funding level increased slightly in 1992 to \$22.27 million (Miyachi, 1993b), although most of this increase may reflect a lower value of the dollar versus the yen. The MBI is headed by Director General S. Miyachi, who is well known for his research on the physiology of photosynthesis. He also has carried out basic research on methods to minimize the release of carbon dioxide into the environment (Gibor, 1990a).

Most research being carried out at MBI is performed at CIUMO, which was established in 1989 and became operational in late 1991. Major funding to establish CIUMO was provided by NEDO and the same 24 companies involved with MBI. CIUMO is comprised of two research centers, located in Kamaishi and Shimizu, each of which cost approximately \$27.27 million to construct and equip. Each has about 5,000 square meters of floor space and each is exceedingly well equipped. At present, each CIUMO center is staffed by approximately 30 doctoral-level researchers, most of whom are on loan from the 24 investing companies (Miyachi, 1993b). In general, the Kamaishi center has a more biological out-

look, while that of the Shimizu is more chemically directed, but there are overlaps.

The *Sohgen Maru* is a 3,205 ton dedicated research ship, which previously was the University of Tokyo's research vessel. It has a crew of 27 and can accommodate up to 58 scientists. As presently configured, it has seven laboratories, dark room, and two deep sea winches capable of reaching depths of 14,000 and 6,000 meters. By the end of 1992, it had made six expeditions, venturing as far as Palau, Yap, and Australia's Great Barrier Reef (Miyachi, 1993a).

The MBI presently is carrying out three research programs. The first comprises the nine-year (1988-1996) national program "Fine Chemicals From Marine Organisms," which was supported by MITI to the extent of \$11.9 million in 1992. The program's general objective is to develop marine organisms or their products for industrial purposes (Anonymous, 1989b). Research has been carried out along four parallel paths at CIUMO (Miyachi, 1993b). The first path is the development of basic technology for utilization of marine organisms, which supports studies to clarify symbiosis in giant clams and corals, investigate picoplankton in oceans, the development of cell culture systems for macroalgae, and preservation of marine organisms. The second, called the "Biofouling" project, aims to discover substances produced by marine organisms that can prevent adhesion of biofouling organisms to marine structures. The third, called "Bioremediation of Oil Spill," seeks to discover new marine bacteria useful for bioremediation of oil spilled into the ocean. The fourth, termed supporting technologies, develops information processing techniques, including a database dedicated to recording characteristics of collected marine organisms.

The second program, "Fixation of Carbon Dioxide," began in 1990 and is being done in cooperation with RITE. Its major activity is to screen microalgal species in order to discover strains that fix CO₂ efficiently. Already this work has resulted in the isolation of a novel organism able to grow in 60% CO₂ (Agency of Industrial Science and Technology, 1993a). "The New Sunshine Program" provided \$1.82 million to this program in 1992 (Miyachi, 1993b).

The third programmatic area is a set of projects supported by MBI's 24 investing companies, which are mainly focussed on screen-

ing of marine organisms for bioactive substances and developing methods for recovering them. According to MBI's annual report, substances of special interest include "docosahexaenoic acid, xanthine oxidase inhibitors, singlet oxygen quenchers, and carotenoids" (Miyachi, 1993b). In 1992, MBI received \$8.82 million to pay for this set of projects.

In early 1993, the MBI's Executive Director, O. Imada, listed the institute's four major research accomplishments (Imada, 1993). First, MBI researchers discovered a new group of prokaryotic green microalgae (picoplankton) that is widely distributed in the ocean between Japan and Australia at a depth of 100 meters. This finding, which was accomplished with the help of a cell sorter aboard the *Sohgen Maru*, helps explain certain evolutionary relationships and may aid in explaining why the oceans store more carbon dioxide than expected. Second, extremely thermophilic sulfur bacteria were recovered from deep-sea hydrothermal deposits and these are being investigated as to their nutritional needs and the proteolytic enzymes they contain, which function at 105° C. Third, MBI scientists have discovered and identified a substance, called tribromomethylgramine, secreted by a bryozoan that repels fouling organisms. Fourth, MBI scientists are screening the waters in the area between Japan and Australia for microalgae that recover CO₂ with high efficiency. In the course of this project, scientists based on the *Sohgen Maru* discovered a new type of small, green microalgae, picoplankton, that live at a depth of circa 100 meters. MBI scientists theorized that these microalgae are responsible for absorbing a large proportion of the CO₂ that is believed to be stored in some unknown manner in the earth's environment.

It is clear that outstanding scientists of MBI are carrying out basic research in marine biology, microbiology, toxicology, and molecular genetics. A review of MBI will be carried out in 1996, at which time the contributions of MBI to marine biotechnology will be assessed. At that time, some questions may be raised whether there is an appropriate balance between the basic research and applied research that is being performed at MBI.

Non-governmental Organizations

In general biotechnology, JBA, which formerly was called the Bioindustry Development Center (BIDEC), is a non-profit organization whose primary purpose is to promote the growth of bioindustry in Japan. Its members include several hundred companies and over 1,600 private individuals (Zaborsky et al., 1989). In regard to marine biotechnology, in September 1987, academic researchers formed the Japanese Society of Marine Biotechnology (Anjo, 1989). Since its inception, the Society has served as a forum for the exchange of ideas and information between academics and industrialists and has promoted the growth of marine biotechnology in Japan through meetings and conferences. For example, the Society sponsored the first International Marine Biotechnology Conference, held in Tokyo in 1989. In 1992, the Society began publishing the *Journal of Marine Biotechnology* in English, which is the first scientific publication to focus on the applications of marine resources. In the journal's first editorial, the three editors claim that the journal is filling a new niche created by the rapid growth in marine biotechnology research (Miyachi et al., 1993).

MARINE BIOTECHNOLOGY RESEARCH AND DEVELOPMENT IN JAPAN

The total R&D program in Japan for marine biotechnology is larger and more diverse than that of the U.S. While the survey that follows is less detailed than presented in Chapter 1, sufficient information is given to convey the richness, depth, and variety of Japanese activities in marine biotechnology. As is seen below, Japanese R&D in the six areas of marine biotechnology delineated in Chapter 1 is substantial. Adhering to the format of Chapter 1, where some important contributions by Japanese scientists to marine biotechnology are noted, we describe important marine biotechnology research being done related to aquaculture, marine animal health, marine natural products, biofilms and bioadhesion, bioremediation, and marine ecology and biological oceanography.

Aquaculture and Biotechnology

Since interest in aquaculture is very strong in Japan, it is understandable that much attention is being devoted to R&D aimed at enhancing the performance of the aquaculture industry. Aquaculture-related marine biotechnology R&D in Japan, similar to work being carried out elsewhere, aims to improve the economically important characteristics of finfish, marine invertebrates, and micro- and macro-algae through direct genetic manipulation and hormonal control of reproduction.

Genetic Manipulation of Marine Animals

Japanese researchers seek to improve the growth and development of fish through greater understanding of physiological and biochemical principles, with an emphasis on the function of growth hormones. M. Maeda of the University of Tokyo has appraised the value of bacterial flora for larval fish, including rate of growth and development, and contribution to larval health, while H. Sugita of Nihon University in Tokyo has done similar studies on adult fish (Gibor, 1990a). In studies on fish growth hormones, investigators have isolated and sequenced the genes coding for growth hormones in tuna, flounder, red sea bream, salmon, carp, cod, yellowtail, and trout (Environment Agency, 1992). S. Itoh of Kyowa Hakko Company is studying the salmon growth hormone (Seto, 1990) and S. Moriyama of Kitasato University has demonstrated the efficient uptake of intestinally-administered salmon growth hormone by rainbow trout (Gibor, 1990a). Systems that already have been developed include the production of tuna growth hormone by *E. coli*, production of eel growth hormone by *E. coli*, and development of two fibroblast-like cell lines from medaka (Environment Agency, 1992).

Studies are underway to clarify how certain marine organisms to survive in extreme environments (Matsusato, 1989). An example is the winter flounder, which thrives in waters at near freezing temperature. If the ability of the flounder to resist cold can be transferred to other varieties of fish, aquaculture could be established in the waters of Japan's northern islands.

The Japanese research strength in both aquaculture and marine natural products has laid the basis for the development of sophisticated fish cell culture systems for producing pharmaceuticals. For example, R. Higuchi of Kyushu University in Fukuoka is studying the structure and biological activity of the gangliosides in starfish, *Asterina pectinifera*, and has discovered that one ganglioside fraction supports the survival of cultured cerebral cortex cells (Schmitz and Yasumoto, 1991).

Hormonal Control of Reproduction in Marine Animals

At MAFF's National Research Institute of Aquaculture, scientists have successfully cloned rainbow trout, cherry trout, and amago, using the "gynogenesis" technology, where only female fish are produced (Anonymous, 1990d). By early 1991, research that was focussed on sexuality (male and female), polyploids, cell fusion, etc., has been done on 30 types of fish (Anonymous, 1990d). For example, T. Onozato at the National Research Institute of Aquaculture is developing transgenic fish (Seto, 1990). Through direct injection of cloned genes into the nuclei of Medaka eggs, E. Tamiya of the University of Tokyo, K. Inoue of the company Nippon Suisan Kaisha, and K. Ozato of Kyoto University have developed a system which serves as a model for genetic manipulation of fishes (Gibor, 1990a). Scientists at the Nippon Suisan Kaisha company are testing a transgenic trout with an additional growth hormone gene. The company claims that the transgenic trout grows 1.2 times as large as normal trout (Anonymous, 1993u).

In addition to finfish, much research is focussed on rotifera, coral, sea urchins, nereids, shellfish, ascidians, squid, and octopus (Matsusato, 1989). For example, M. Morisawa at the Misaki Marine Station of Tokyo University is studying the fertilization and development of invertebrates (Gibor, 1991). A mass cultivation method has been developed for micro-cellular Rotifera. Cultivation of this zooplankton is important because of its role as the first biological feed for marine fish larvae (Matsusato, 1989). When fed with rotifers, shellfish larva grow larger and are more disease resistant than if artificial feed is used.

Advances in cultivation methods of sea urchins have been achieved with funding provided by MAFF's "Marine Ranching Project" (Matsusato, 1989). MAFF's "Biocosmos Project" is providing funding for investigations aimed at clarifying interactions between sea urchins and seaweeds at the physical level. Thus, the work of S. Kamura is focussed on the effect of sea urchin grazing on algal biomass and composition (Gibor, 1990b) and that of N. Suzuki of Kanazawa University's Noto Marine Laboratory is on the biochemical aspects of fertilization of sea urchin eggs (Gibor, 1991). Suzuki is studying biologically active peptides associated with the extracellular matrix around the eggs, which demonstrate activating effects on sperm cells (Gibor, 1991).

Algal Aquaculture and Biotechnology

In Japan, significant R&D is being performed on both microalgae and macroalgae (Matsusato, 1989; Anonymous, 1989h). Algal culture is one of Japan marine biotechnology program's strengths. For example, GIRI at Osaka is a leading Japanese research institute in genetic manipulation of algae. A GIRI team has inserted a gene coding for beta-carotene into *Spirulina*. The gene was obtained from scientists in Israel, but GIRI scientists constructed the vector which successfully transferred the gene into the host. GIRI claims that as a result the beta-carotene production capability of the transformed *Spirulina* has increased greatly (Anonymous, 1992i).

Another GIRI team, headed by H. Kojima, claims to be the first in the world to have successfully transformed *Spirulina* using electroporation. The introduction of genes coding for chloramphenicol acetyltransferase (CAT) was proven when the acetylated chloramphenicol products were detected via chemical means in the transformed *Spirulina* cells. Gene transduction was confirmed by PCR analysis (Anonymous, 1992b). The Kojima team is also attempting genetic engineering of the algae *Porphyridium*, to produce human prostaglandins. This alga naturally produces the chemical arachidonate, which is a precursor of prostaglandins. Prostaglandins have im-

portant functions related to control of blood pressure, muscle function, and blood clotting.

Scientists from GIRI at Osaka also are attempting to develop large-scale culture systems for the green alga, *Botryococcus*, an organism that is very difficult to culture because of its susceptibility to contamination (Anonymous, 1992i). *Botryococcus* produces high quality hydrocarbons that could be used as an alternate source for fuel. S. Okada and K. Yamaguchi of the University of Tokyo and H. Iwamoto of the Meiji University in Tokyo are employing genetic engineering in an attempt to increase the capacity of this alga to produce hydrocarbons.

At AIST's Fermentation Research Institute (now incorporated in the National Institute of Bioscience and Human Technology), investigators have used electroporation to insert a gene coding for the hydrogenase enzyme into a thermophilic cyanobacterium. When the genetically modified bacterium is exposed to illumination, it responds by producing and releasing hydrogen (Anonymous, 1993n). At the same time, Y. Fujita at the Okazaki National Research Institutes is studying development of the photosynthetic membranes of cyanobacteria (Gibor, 1991).

Research on large seaweeds presently is concentrated on production of laver and kelp, e.g., N. Saga of the Hokkaido Fish Institute is developing improved methods for culturing giant kelp (Seto, 1990). T. Kajiwara of Yamaguchi University is improving cultivation of the green alga, *Ulva*, and studying algal-gamete attracting substances (Gibor, 1990a; Kitagawa, 1988). A. Miura and J.-A. Shin of the Tokyo University of Fisheries have improved techniques for nori production through the hybridization of *Porphyra* (Gibor, 1990a). Investigation of laver cell protoplast formation and cell fusion techniques are funded by MAFF's "Integrated Research on Biotechnology and Plant Cultivation," while kelp production is supported by the "Biomass Project."

Due to the growing importance of environmentally-related research in Japan, companies are finding it worthwhile to enter this field. For example, at the Tokyo Electric Power Company, which es-

tablished its biotechnology research laboratory in 1990, scientists claim to have discovered a blue-green algal species in the Janata hot spring on Shikine Island that absorbs and fixes CO₂ four times more efficiently than an equivalent mass of tropical rain forest. Even so, a cultivation area of 40 square kilometers is required to absorb and fix the amount of CO₂ emitted by one 600,000 kilowatt liquefied natural gas thermal power plant (Anonymous, 1994j).

Marine Animal Health

Japan's large aquaculture industry is continuously challenged by infectious diseases, yet, marine animal health so far has received relatively little attention by Japanese scientists. Viral diseases appear to be especially problematic to the Japanese (Kimura and Yoshimizu, 1991). Some research aims to detect viral infections and vaccines against selected viruses causing fish diseases are under development. For example, Y. Kamei of Sapporo Breweries Company in Tokyo has constructed vaccines to immunize cultured fish against viral infections and also for diagnostic purposes (Gibor, 1990a). Recently, antibodies have been developed for three types of pathogenic viruses infecting fish salmonids, the Infectious Hematopoietic Necrosis virus (IHNV), the Infectious Pancreatic Necrosis virus (IPNV), and the Halibut Rhabdo virus (HRV; Anonymous, 1989i). At Kyushu University, monoclonal antibodies for fish disease viruses have been developed by H. Murakami (Seto, 1990). However, research related to developing inexpensive but efficient delivery system for vaccines is lagging, therefore, Japanese-made vaccines have not yet been applied in the field.

MAFF is attempting to strengthen this area of marine biotechnology. In 1993, it set up a new three-year project to develop vaccines that protect cultured fish from viral diseases (Anonymous, 1993j). The research is being conducted at the National Research Institute of Aquaculture and the National Institute of Health, but scientists from Nagasaki and Hiroshima universities are collaborators. Reportedly, the first step will be to identify which viruses should be targeted for investigation.

Marine Natural Products

The types of marine natural products being investigated by Japanese scientists include antibiotics, agents showing anti-inflammatory, anti-tumor or anti-viral properties, toxins, enzymes, and agents having insecticidal or herbicidal properties. A brief discussion of each of these subjects is provided. In addition, we include a miscellaneous grouping, which consists of natural products that do not fit in any of the foregoing categories.

Antibiotics

Marine invertebrates and microalgae, in particular, are being investigated as important sources of antimicrobial and antibiotic compounds (Gibor, 1990a; Kitagawa, 1988). Examples of notable research in this area include that of H. Kamiya at the Kitasato University School of Fisheries, who is screening marine invertebrates, such as the sea hare and abalone, for antibiotic and antitumor compounds (Gibor, 1991). He has identified and purified a polypeptide that inhibits the biosynthesis of macromolecules *in vitro* by tumor cells within two hours after application. Other researchers are screening substances recovered from organisms, ranging from marine invertebrates to phytoplankton and macroalgae, in a search for compounds with antifungal properties. For instance, Y. Miura at Osaka University and T. Matsunaga at the Tokyo University of Agriculture and Technology are screening microalgae for antibiotic compounds and have discovered two strains which produce yeast-inhibiting substances (Gibor, 1990a). At the University of Tokyo, M. Murakami and K. Yamaguchi have isolated an antifungal polyether macrolide produced by the dinoflagellate *Alexandrium hiranoi* that prevents fungal growth at a concentration of 0.5 ug/ml (Gibor, 1990a).

Anti-inflammatory, Anti-tumor, and Anti-viral Agents

Anti-tumor substances include anti-tumor polyether macrolides, cell-division inhibiting substances, antileukemic substances, cardiotoxic peptides, anti-clotting substances, and marine al-

kaloids with antiviral properties (Gibor, 1990a; Kitagawa, 1988; Matsusato, 1989). Examples of notable work in the area include that being done by N. Fusetani (Seto, 1990; Schmitz and Yasumoto, 1991) (see above). From the sponge *Mycale adhaerens*, Fusetani has isolated a cytotoxic compound, 13-deoxytedanolide, which has showed good anti-tumor activity. J. Kobayashi of Mitsubishi Life Sciences and the Hokkaido University in Sapporo is searching for bioactive metabolites from Okinawan marine organisms and has recovered a variety of antineoplastic and antileukemic substances from marine sponges, tunicates, and dinoflagellates (Seto, 1990; Schmitz and Yasumoto, 1991). M. Fujiwara of Kyoto University has isolated a cardiotoxic peptide, *Goniopora* toxin, from a stony coral species, *Goniopora* (Kitagawa, 1988). And T. Kusumi of the University of Tsukuba has isolated a cytotoxic, antiviral, and antifungal marine alkaloid from the Caribbean sponge *Ptilocaulis spiculifer*, and from the Red Sea sponge, a *Hemimycale* species (Schmitz and Yasumoto, 1991). M. Yamasaki at Teikyo University has discovered a glycoprotein in a local sea hare (*Aplysia kurodai*) and shellfish (called "tatsunamigai") that have powerful antineoplastic properties, while manifesting few adverse effects on normal cells (Anonymous, 1993u). The substances work in a new way, by causing the DNA in cancer cells to unwind, which results in replication errors and dysfunctional genes. Similarly, the substance acts against DNA in fungi, raising the possibility that it can be developed as a fungal antibiotic (Anonymous, 1993p). D. Uemura at Shizuoka University has isolated cytotoxic alkaloids from the sponge, *Halichondria okadai*, from which okadaic acid and potent antitumor polyether macrolides have been isolated (Kitagawa, 1988; Schmitz and Yasumoto, 1991). Y. Kamei, Hokkaido University, collects bacteria from aquaculture ponds, estuaries, and beaches and screens them for anti-viral properties. He has found that a very high percentage (more than 60%) of bacterial species recovered from estuaries produce anti-viral substances that inhibit fish pathogenic viruses.

A marine natural product with proven anti-inflammatory property is sodium scymnol sulphate, which was first discovered by T. Kosuge at the Shizuoka Pharmacy College in the mid-1980s. He

had heard that shark fishermen applied extracts from shark bile on their faces to clear up their skin. After testing the substance, and getting positive results, he contracted with the McFarlane Laboratories in Australia to develop it. Five years of testing in Australia, England, and France have demonstrated that the substance, whose commercial name is "Isolutrol," controls excessive oiliness in skin and cures facial acne without negative side effects (Anonymous, 1993a).

An antitumor compound has been found in the ink secreted by squid and octopus. In addition, squid ink is being utilized as a raw material in liquid crystal. The squid nervous system provides the research basis for fifth-generation computer development. These R&D projects are supported as part of the MAFF "Project to Develop Cultivation Techniques for the Generation of Sexuality (Female), etc. in Fish and Shellfish" and "Development of Cultivation Techniques for Shellfish Such As Abalone and Clams," as well as the Fisheries Agency "Project for the Promotion of Regional Cooperation of Research and Development for New Technologies such as Biotechnology" (Matusato, 1989).

Scientists working for Ishihara Sangyo Kaisha, Ltd. have screened 59 marine macroalgal species and found that extracts from 38 of these species suppress the proliferation of T cells, while 16 species provide extracts that affect lymphocytes and macrophages (Anonymous, 1993b). These substances are undergoing further investigation with the aim of developing drugs to treat autoimmune diseases.

Many Japanese scientists from Kyushu University, Kagoshima University, Kyoto University, Kochi University, and Nansei Regional Fisheries Research Institute are working together to discover bacteria that kill or inhibit the microalgae that constitute "red tide." Their investigations have shown that anti-microalgal bacteria are widely distributed in the seawater and can be recovered from algal surfaces. Dr. Fukami, Kochi University, has found that at the beginning of the bloom of a particular microalga, the bacteria that promote the growth of the alga proliferate and, in reverse, anti-algal bacteria increase in number during the declining phase of the bloom. The work of the group has led to the isolation of many anti-algal strains

of bacteria that inhibit *Chattonella*, *Heterosigma*, and *Chaetoceros*. However, so far few strains have been found that inhibit *Alexandrium* species (Simidu, 1994).

Marine Toxins

Most Japanese studies seek to elucidate the structure and mechanism of marine toxins, while a smaller number concentrate on the discovery or comparison of toxins in various marine animals and organisms. This research appears to be concentrated on phytoplankton toxins, paralytic shellfish toxins, venoms from cone shells, and ecological origins and distribution of tetrodotoxins (TTX) (Gibor, 1990a; Kitagawa, 1988; Matsusato, 1989). Examples of important work includes investigations on red tide toxins being done at the Tokyo University of Fisheries by K. Shiomi, who is extracting toxins and venoms from marine animals. In the course of his work, he has isolated and purified hemolytic venoms of six different species of fish (Gibor, 1991). At the University of Tokyo, K. Kogure has clarified the origins of TTX (Gibor, 1990a). Using a sensitive *in vitro* bioassay, it was shown that a large number of bacterial species isolated from sea water, sea sediments, and marine animals synthesized TTX or related sodium-channel blocking agents. These toxins accumulate in various marine animals that are at the end-point of several complex food chains. M. Isobe of Nagoya University has partially synthesized okadaic acid and an optically active TTX (Schmitz and Yasumoto, 1991). Y. Ohizumi of Tohoku University in Sendai has shown the effects of maitoxin, the principal toxin of ciguatera seafood poisoning, on calcium channels (Schmitz and Yasumoto, 1991). T. Yasumoto, also at Tohoku University, has studied phytoplankton toxins in relation to diarrhetic shellfish poisoning and identified four polyethers from the dinoflagellates, *Dinophysis fortii* and *D. acuminata* (Gibor, 1990a; Kitagawa, 1988). K. Tachibana of the Marine Biological Institute of the University of Tokyo, who is studying the mode of action of paradaxins secreted by the sole, *Pardachinus* species, reputed to possess a shark-repelling property (Seto, 1990; Schmitz and Yasumoto, 1991).

Enzymes

The types of enzymes being investigated include enzymes important in synthesis of invertebrate bioactive polymers, sterols, algal metabolites, carotenoids, algal terpenoids, shark-repelling pavoninins and mosesins, and UV absorbing substances, as well as enzymes involved in bioluminescence and enzymes in magnetotactic bacteria (Gibor, 1990a; Kitagawa, 1988; Matsusato, 1989; Anonymous, 1990e; Kobayashi et al., 1988; Nakanishi, 1988). Notable research is being done by K. Horikoshi of RIKEN, who is searching for novel microorganisms from the deep ocean (Myers and Anderson, 1992) (see page 178, above), T. Goto of Nagoya University who is studying bioluminescence (Kitagawa, 1988), and Y. Yamada at the Tokyo College of Pharmacy who is synthesizing cyclopentanoids (Kitagawa, 1988; Schmitz and Yasumoto, 1991).

One of Japan's most successful projects, the "Superbugs" project, was completed in 1991 (Myers and Anderson, 1992). This five-year, \$15 million study, which was led by K. Horikoshi (now head of the DEEPSTAR project), sought to discover new thermophilic, alkaliphilic, and psychotropic organisms in the deep oceans (Gibor, 1991). A notable discovery was a mix of marine bacterial-derived enzymes, including cellulases and proteases, that can hydrolyze cellulose in a high pH environment. The enzyme mixture is being used in a detergent, "Attack," and now garners 60% of the Japanese laundry detergent market. Exaggerating somewhat, a Japanese publication claims that when this product was marketed, Japan became the first country in the world to use "genetically engineered detergent" (Anonymous, 1993m). Another RIKEN discovery, an alkaline amylase, breaks down starch and, in the process, forms as an end product, the cyclic molecule cyclodextrin. This substance can be used to manufacture capsules useful for the slow, controlled release of drugs and fragrances.

At AIST's Life Sciences Engineering Research Institute (now the National Institute of Bioscience and Human Technology) a group headed by Y. Asada is seeking to transform a species of thermophilic alga, *Synechococcus elongatus*, by introducing by electropora-

tion the genes from the bacterial species *Clostridium* that code for enzymes producing hydrogen gas. The aim of this research is to convert the alga, which uses only sunlight as an energy source, to the production of practically unlimited amounts of hydrogen as an inexpensive, clean source of energy (Anonymous, 1993i).

Investigators at the Seawater Research Institute of Japan Tobacco, Inc., report discovering a new type of agarase (Anonymous, 1992e). After having screened over 1,000 samples of Japanese coastal water and sea bottom sediment for organisms containing possible useful substances, they found a new *Vibrio* species that produces the agarase in question. The agarase can be used to decompose agar, which is a polysaccharide, in order to produce degradation products consisting of monosaccharides and oligosaccharides. These substances are useful in helping preserve rice cakes, bean paste and other starch-containing foods. Agarase may also be used by researchers to dissolve the walls of red algae, a development that may enable researchers to perform protoplast fusion using different species of alga.

Duong van Qua, University of Tokyo, has isolated marine bacteria that produce halophilic protease. The purified protease requires an astounding 18% NaCl concentration to exhibit maximum activity. He has applied the enzyme for the production of "nukmum," which is a fermented fish sauce popular in South East Asian countries. The product, named Marinage, is produced commercially by Mitsubishi Gas Chemical Company Ltd. (Simidu, 1994).

Work in a similar vein is being done by C. Imada, also at the University of Tokyo, who aims to discover marine bacteria that produce protease inhibitors. After having screened approximately 3,000 strains of marine bacteria, he obtained three strains that were inhibitor producers. The amino acid sequence of one of the smaller-molecule inhibitors, named Marinostatin, showed no similarity to inhibitors of terrestrial origin, suggesting that the phylogenic development of the two occurred independent of one another.

Miscellaneous Marine Natural Products

Remarkable research is proceeding at the Tokyo University of Agriculture and Technology on genetically engineering freshwater

and marine cyanobacteria to produce a variety of products, including amino acids and plant promoters (Matsunaga, 1992). The transformation of cyanobacter species has been achieved using shuttle vectors, electroporation, "biolistics" (transformation by high-speed particles coated with DNA), and conjugation. Since microalgal production systems tend to be inefficient because the organisms grow in low density, the Tokyo University researchers are developing high density culture methods for the cyanobacteria.

Microalgal species are being screened by scientists from the Ebara Research Company to discover strains that produce large amounts of the bioactive compound docosahexaenoic acid, reputed to have health improving effects. A species has been found that grows well at 15° C and is easy to culture. The company claims that there is a large market for the microalgae-producing docosahexaenoic acid as food for fish and shellfish raised in aquaculture (Anonymous, 1993b).

Another fatty acid with reputed health effects, eicosapentaenoic acid (EPA), which is discussed in Chapter 1, is the focus of K. Yazawa's work at the Sagami Central Research Institute. He screens marine bacteria that inhabit pelagic marine fish, such as horse mackerel, mackerel, and sardines, for strains that produce EPA. Since previously only eukariotic organisms were known to produce EPA, Yazawa's approach is rather unique (Simidu, 1994).

A group headed by T. Matsunaga at the Tokyo University of Agriculture and Technology is studying magnetite found in the freshwater bacterium, *Aquaspirillum*. Magnetite is thought to have an important role in how migratory species, including birds and fish, recognize direction and location. The Japanese researchers have been able to identify and isolate the genes that code for the production of biogenic magnetite. There is industrial interest in this work. Magnetotactic bacteria, which use magnetite to orient themselves in magnetic fields, are being investigated by scientists at Meiji Seika Kaisha Ltd. for use in targeted drug-delivery systems. Injections of drugs encapsulated by magnetite are coordinated with the placement of a magnet on the targeted body part, e.g., a tumor, which results in the drug being concentrated at the site of the tumor.

Investigators at MBI's laboratory located at Shimysu have discovered a marine bacterium that produces a UV light absorbing substance. The microorganism, tentatively named *Micrococcus* strain AK-334, was collected from the ocean surface layer off Palau Island in the South Pacific. Previously, it was known that some marine plants protected themselves from the sun by producing UV light absorbing substances (Rhineheimer, 1980). H. Larsen (Larsen, 1962) suggested such a role for the pigment produced by halobacteria. This more recent follow-up of Larsen's work with the newly isolated *Micrococcus* strain is interesting. *Micrococcus* strain AK-334 has been cultured in the Shimysu laboratory, where researchers were able to extract the active substance, using chromatography. When analyzed by NMR, the substance was identified as imino-mycosporine amino acid shinorine, which absorbs light at the 334 nm wavelength (Anonymous, 1993v). These findings indicate the possible application of this substance as sunscreen, suggesting a follow-up of Larson's earlier studies with the halobacterium is merited.

Chitin and its chemical derivative chitosan have been the objects of much R&D throughout the world. The Japanese are leaders in this area. Chitin extracted from crustacean shells and minerals produced by *Spirulina* forms the basis of a liquid manure called "Chitoleana," which is produced by the Dainippon Ink and Chemical Company. This specialty product is used to grow a high quality turf for golf courses and parks (Anonymous, 1993n). T. Tsugita of Kato-kichi Company has successfully used the shell constituents, chitin and chitosan, as a material in pharmaceutical products. K. Kifune of Unitika Company, Kyoto, has developed an artificial skin made of chitin (Seto, 1990; Anonymous, 1993w). The artificial skin, called "Beschitin W," when used to treat 657 patients suffering from "normal" wounds and thermal burns proved to promote healing and healed surfaces had excellent cosmetic appearance. Chitin is also used in biosensors (*vide infra*).

Because of environmental concerns, much attention is being focused in Japan on biodegradable plastics, which includes biodegradable films and foams. These are substances used in wrapping food and in packaging materials that are strong enough to serve as well as

conventional plastics, but decay readily when exposed to the sun or other natural physical and biological forces to residual substances that do not harm or burden the environment. The market for biodegradable plastics in Japan in 1992 was \$3.63 million and this market will grow to an estimated \$13.63 million in 1994 (Anonymous, 1993x).

While several types of biodegradable plastics are varieties of chemical synthetic plastics, Japanese researchers, in both the public and private sectors, have developed biodegradable films and foams, using natural substances from marine organisms as the starting material. The Aicello Chemical Company has developed a biodegradable film, using chitosan derived from crab and shrimp shells. The company claims that the film is as strong as ordinary plastic film, but will decompose completely into harmless endproducts within two weeks of being buried in the soil (Anonymous, 1989j). Similarly, Mitsubishi Rayon Company is producing a biodegradable film, called "Soafil," used for packaging by the food and cosmetics industries. In this case, the basic material is carrageenan from macroalgae (Anonymous, 1993q).

Nereids are being cultured for a number of physiologically active substances, including fish attractants and poisons active against higher animals, as well as new adhesives for use in underwater construction (Matsusato, 1989). Research on the internal systems of crustaceans is proceeding with funding from MAFF's "Biomedica Project" (Matsusato, 1989). Y. Naya of the Suntory Institute for Bioorganic Research in Osaka has been studying the regulation of ecdysteroidogenesis in crustaceans, especially in relation to *in vivo* exhibition of molt-inhibiting effects (Schmitz and Yasumoto, 1991).

Japan is the only nation whose research workers cultivate ascidians. This organism has a capability to bioconcentrate microquantities of certain metals, such as vanadium. Research is presently focussed on understanding the mechanisms of such bioconcentration (Matsusato, 1989).

Biofilms/Biofouling

Reportedly some good results related to antifouling substances has been achieved in the past by K. Ina at Shizuoka University (Kita-

gawa, 1988). Ina is studying attractants for shellfish that will allow farmers to control such behaviors as embedding and breeding (Seto, 1990).

Bioremediation

One of Japan's most striking projects is the "Tokyo Bay Restoration Project," which aims to clean and restore Tokyo Bay by creation of artificial tidelands. Heading the project are T. Okabe and H. Nakahara of the Research Institute for Ocean Economics (RIOE) (Gibor, 1991). It is based on the premise that tidelands serve an essential role in the ecology of bays. A second project was the MOC's "Wastewater Treatment Project." An important initiative for developing new methods to bioremediate polluted soils and natural waterways is being supported by a nine-company consortium, led by the Japan Research Institute Ltd. (JRI). Participants include Ebara Research Company, Ltd., Kumagai Gumi Company, Ltd., Sumitomo Chemical Company, Ltd., Toray Industries, Inc., Sumitomo Metal Mining Company, Ltd., Dowa Mining Company, Ltd., and Hitachi Zosen Corporation. JRI has also reached an accord with the U.S.-based Ecova Corporation on the use of Ecova's expertise in soil remediation (Anonymous, 1991d). Notable research in bioremediation is being done by Y. Ishida of Kyoto University who is isolating organisms from oligotrophic lakes, i.e., lakes containing very pure water, and investigating them for special properties useful in the purification of waste waters and bioconcentration of rare elements (Gibor, 1991).

Marine Ecology and Biological Oceanography

Research in marine ecology and biological oceanography, much of which has important implications for public health, has high priority in Japan. Two general types of research in this area may be distinguished, applications of molecular techniques to marine ecology and the development of biosensors.

Molecular Techniques in Marine Ecology

Much research is focussed on attempting to understand, predict, and prevent red tides, which through the ages have been responsible for massive fish kills and serious damage to laver cultivation. In 1990, MAFF's Fisheries Agency and EA launched a five-year project aimed to prevent red tides. As part of this project, monoclonal antibodies against each red tide plankton will be developed, effective control methods against red tides will be formulated, micro-plankton will be speciated by characterizing their DNA restriction patterns, and various environmental improvement strategies will be evaluated (Anonymous, 1990b). Other research on red tides attempts to purify and characterize the various toxins responsible for massive fish kills. Significant research includes that being done by K. Yamaguchi of the University of Tokyo on the ecological origins and distribution of TTX (Gibor, 1991), and Y. Oshima of Tohoku University in Sendai on mixtures of saxitoxin derivatives detected in strains of *Gymnodinium catenatum* (Schmitz and Yasumoto, 1991).

M. Kodama of the Kitasato University School of Fisheries is studying the origins of paralytic shell fish poisons (Gibor, 1991). One of Kodama's recent findings was that the degree of toxicity of the dinoflagellate *Protogonyaulax* is related to the presence of intracellular bacteria. Another example of remarkable research in public health is being performed at the Suminoe Kikaku Company where researchers have isolated a marine plant extract that is reported as being almost 100% effective in killing vibrios and *Salmonella* species in sea water.

R&D focussing on Anthozoa (or coral) is mainly related to jewelry products and coral reef construction (Matsusato, 1989). Supplies of high-quality coral for jewelry is steadily decreasing, stimulating interest in coral propagation. Furthermore, the inverse relationship between reef-building coral activity and global carbon dioxide levels has generated further interest in coral reef construction. Research in understanding those ecosystems surrounding reef-building corals is essential for developing replacements for reef areas. Such

work is being performed at the University of Ryukyus in Okinawa by T. Higa who is working to identify organisms related to, and dependent on, coral reefs (Gibor, 1991; Kitagawa, 1988). K. Yamazato, a leading expert on the biology and ecology of coral reefs, is studying the physicochemical and morphological characteristics of reef-related organisms (Gibor, 1990b; Gibor, 1991).

Biosensors

Some Japanese analysts believe that, of all developments in biotechnology, biosensors are likely to have the greatest economic impact in the shorter term. By the year 1995, the biosensor market is estimated to reach \$200 million per year by 2000 and \$1 billion per year by 2010 (Anonymous, 1991b; Technology Forecast Study Committee, 1991).

Biosensor research in Japan appears to be focussed on improving sensitivity, increasing the range of applications, and microminiaturization. Key breakthroughs in this area have included development of more stable and sensitive devices and chemiluminescent materials. Biosensors are being developed for many purposes, including environmental pollution monitoring, health and medical monitoring (improvement of diagnostic and treatment methods for diseases), the highly sensitive measurement of meat and fish freshness, immune-system monitoring, measuring fatigue, and development of biosensors as components of artificial organs.

I. Karube at the Research Center for Advanced Science and Technology at the University of Tokyo is developing biosensors that can be emplaced within the human body to measure glucose continually for up to three months. The new biosensor was constructed by using chitin from cuttlefish cartilage in combination with glucose oxidase. The chitin/enzyme mixture, which overlays a thin layer of gold, dissolves over a three-month time period and, in the process, generates an electric signal that is transmitted by the gold to a transducer. Animal experimentation with the device is now underway. Unlike most sensors that when placed in the blood stream elicit a destructive immune response, this sensor is inert. This work is being done in cooperation with the company Nippon Suisan Kaisha,

which is funding the major part of this research (Anonymous, 1993j).

An interesting approach is being taken by scientists at the Toray's Medical Systems Institute to incorporate light-emitting enzyme extract containing luciferase in diagnostic assay kits (Anonymous, 1992g). The extract is collected from *Cypridina*, which is a small, plankton-like organism living in the coastal water off Chiba Prefecture. When the animal releases luciferase into the water, the subsequent reaction produces blue-white light. At Toray investigators have been able to extract the gene coding for the luciferase from *Cypridina*, clone it in *E. coli*, and produce large quantities of the enzyme. Once they have a pure product, they bind the enzyme to an antibody that has been designed for specific biological substances including, for example, interleukin, myoglobin, and creatinine kinase. When the diagnostic agent containing the enzyme encounters the target substance, it emits light, the quantity of which is proportional to the amount of the target agent. Toray expects to be able to market several different diagnostic kits based on luciferase in one to two years.

EMPHASIS OF JAPANESE RESEARCH IN MARINE BIOTECHNOLOGY

In order to ascertain which areas of marine biotechnology are being emphasized in Japan, we searched the Life Sciences Collection database, 1982-1991, and issues of *Marine Biotechnology Abstracts*, 1989-1991, for publications of Japanese origin. Eventually, 350 were found. Each was scrutinized and the work that was reported was classified according to the marine biotechnology area it addressed. We found that 37% of the Japanese publications reported on marine biotechnology research was related to natural products (mostly marine-derived toxins), 27% addressed problems or needs in aquaculture (mostly to enhance survival rates and improve reproduction of marine organisms raised in culture), 18% concerned biological oceanography, 6% addressed marine animal health, 4% were related to the development of marine cell culture systems, 3 % reported on biosensors, 2% were dedicated to bioremediation, and less than 1%

concerned biofilm/biofouling (see Figure 22). There were some overlaps, for example, between biological oceanography and biosensors, and between aquaculture and animal health. Nevertheless, the results presented here clearly indicate the areas of marine biotechnology to which Japanese scientists pay most attention.

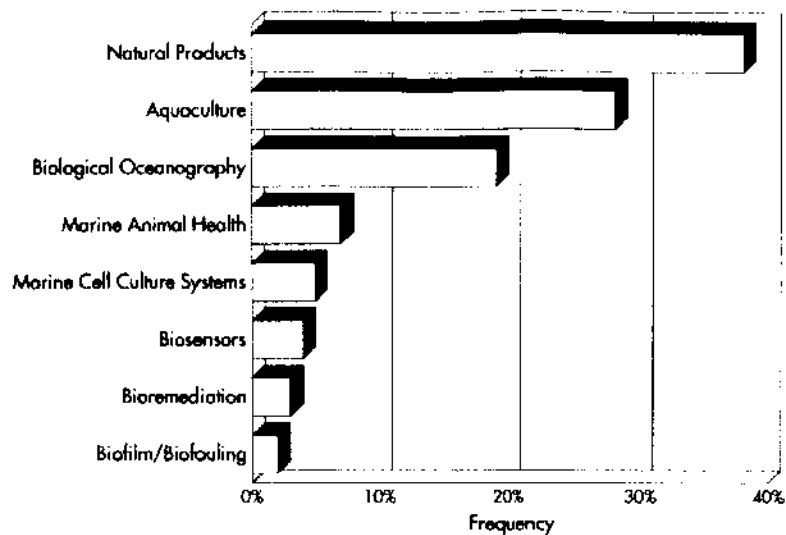


Figure 22. Application of Japanese marine biotechnology research by publications, 1982-1991.

CONCLUSION

Modern biotechnology in Japan appears to be entering a second phase of development. The first phase began in the early 1980s and largely was stimulated by the concern that U.S. researchers and industry would take a commanding position in the field by patenting new life forms and genetically engineered products. During this time, the Japanese government supported programs whose aim was to build a strong base for biotechnology industry and rice agriculture. Although some support was given for basic research projects in mostly academic laboratories, this aspect of biotechnology largely was neglected.

In the late 1980s, this applied, industry-directed approach was broadened, to include programs that were more environmentally directed and basic research was strengthened. These events came about because of two forces; the Japanese public was indicating a heightened concern about environmental problems and Japanese decision-makers and scientists recognized, for many reasons, that they needed to strengthen basic research and contribute more to international science. About this time, marine biotechnology became well funded (about 1988), as did several national environmental research programs (in 1989 and 1990), and most ministries designed and implemented wide-ranging international programs in which foreign research groups were encouraged to partake.

However, the real change occurred in 1993, and this may signal the initiation of the second phase. On the one hand, for the first time in twenty or more years Japan was facing economic hardship, which constrained the ability of ministries to act. On the other hand, the Japanese public strongly indicated that the way politics and economics had functioned in the past was no longer adequate or, indeed, appropriate. The response of the ministries to public pressure was dramatic, as indicated by MITI's reorganization of its national programs described above. What has happened in biotechnology is a reflection of the wider change, namely, there is more emphasis on science as it pertains to human wellbeing and the health of the environment. Further, despite economic hardship, the Japanese government unequivocally has indicated that it will continue to support science strongly by increasing funding for almost all areas of scientific research. This, then, is the setting for the second phase of biotechnology in Japan.

The second phase has four major characteristics. First, biotechnology research for industry continues to be well supported although, relatively speaking, less so when compared to other fields. Therefore, the national program "Fine Chemicals from Marine Organisms" continues and is supplemented by other programs. Since many or most of the "fine chemicals" that will result from this program are likely to be used for pharmaceutical purposes, the program is directly related to human wellbeing and can be strongly defended on that basis.

Second, biotechnology research for industry increasingly will be performed at the local level and will be locally directed. This trend results from the need to decentralize economic activity in Japan, combined with the realization of prefectural governments that biotechnology is a strong force for local economic development. The initiatives of prefectural governments and the strong federal support for these initiatives, described and discussed above, will continue and grow, with implications for marine biotechnology because so many economic activities are aqueous, marine-based, or similarly oriented. Therefore, it is our sense that prefectures increasingly will be important for the promotion and maintenance of marine biotechnology in Japan by, for instance, prefectural governments taking the initiative to set up *new kosetsushis* and third sector centers whose aim will be to develop a special area of marine biotechnology in which they perceive to have a competitive advantage.

Third, there will be enormous growth in environmentally-directed biotechnology research. Having recognized the global scope of such research, the Japanese are likely to invite an ever growing number of foreign researchers to take part in environmentally-directed research. In the first instance, scientists from developing countries of the Asia-Pacific region will be invited to Japan, to receive training in specialized techniques and to take part in collaborative research projects of regional significance. Much of this research will, perforce, be related to biological oceanography, bioremediation, and marine organisms. We can expect that, as a result of these activities, within ten years marine biotechnology will flourish in the Asia-Pacific region generally.

The growing emphasis on environmentally-directed biotechnology research will impact heavily on marine biotechnology. Already large projects are aimed at discovering and developing marine microalgae, plankton, and bacteria to fix CO₂, cleanly produce hydrogen as an energy source, and utilize biomass of marine origin. Giving the context of Japan as a island country with limited natural terrestrial resources, these types of programs are likely to continue and grow.

Fourth, basic research in biotechnology will be given more emphasis and, at the same time, more international cooperation will be encouraged in basic research projects. There are clear signs of this development. Japan was the initiator of the Human Science Frontier Program and remains its major funder. Just this year, 1994, two projects in the U.S. were approved for funding by ERATO (Normile, 1994). While neither project involves biotechnology, they do indicate the outward direction of Japanese science. It is reasonable to believe that Japanese agencies soon will wholly or partially fund marine biotechnology basic research projects involving U.S. scientists.

On the applied side of marine biotechnology, Japanese companies seem to be concentrating on making and improving basic research discoveries, an area which underscores Japan's strength in product development and its doggedly persevering researchers, both of which are essential for utilization of marine biotechnology now and in the future. In particular, Japanese industry and government agencies are focussing a great deal of attention on marine natural products. It appears as if Japan's pharmaceutical companies have recognized that this area of marine science has immense economic potential. This interest stems from Japan's voracious appetite for health and medical products, i.e., the Japanese pharmaceutical market was the second largest in the world, with \$25 billion in sales in 1987 (Yuan and Hsu, 1990).

Japan's traditional strengths in bioprocessing technology, e.g., its prowess in the fermentation and bioprocessing industry, strong applied research base, and robust direct and indirect government support, more than balance out any weaknesses that might be cited by critics; i.e., the emphasis on applied research over basic research, lack of venture capital, lack of cooperation between ministries, and an under-developed regulatory structure. Recognizing where their strengths lie, large companies are likely to support research in areas where they can advance rapidly; i.e., in areas where they traditionally have been strong and where weak or uncertain regulations are not likely to stop progress. Thus, we are likely to see the Japanese make tremendous progress in marine natural products development and development of productive cell culture systems.

Marine biotechnology related to aquaculture is rapidly growing in Japan, based in large part on the technology of processing natural products, finfish, shellfish and other invertebrates, and algae that has been part of the long tradition of Japanese scientists in marine research. Japan, and to some extent, Taiwan and the People's Republic of China, have had a leading position in aquaculture since before World War II. Japanese scientists have done significant, advanced research on chromosome manipulation of fish, hormonal control of growth, and hatchery culture of finfish and shellfish, fish vaccine development and production, and development of cell culture systems for algae.

Conversely, marine biotechnology developments that could generate public concerns, such as the application of transgenic finfish, shellfish, and microalgae to aquaculture or transgenic bacteria to bioremediation, are likely to be hindered, as in other countries discussed in this report. Partially, this is due to the Japanese public's distrust of biotechnology and partially because of the uncertain federal regulatory situation in Japan. Similarly, overly strict regulations are likely to form barriers to the development of cell culture systems based on transgenic marine organisms to produce specialty chemicals for human or animal consumption.

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Chapter 9

CONCLUDING THOUGHTS ON THE FUTURE OF MARINE BIOTECHNOLOGY

The world's oceans cover more than 70% of the earth, but knowledge of this significant component of the environment is relatively incomplete, compared to that available for the terrestrial sphere. Water, vital for life on the planet, drives many important processes, both geological and biological. The oceans, for example, moderate climate, and store CO₂. The oceans also are important sources of food, minerals, and natural products. Unfortunately, it is often assumed that the oceans have an unlimited capacity to absorb the wastes of civilization, an assumption proving faulty as data are gathered on the extent and effects of pollution, especially on the world fisheries. In view of the need for information on the marine environment, it is not surprising that the age of biotechnology, which began in the early 1970s and, now, twenty years later, has reached revolutionary proportions, stems from experiments done with terrestrial microorganisms, plants and animals, leaving the marine aspects of biotechnology unexplored and under-exploited.

Reflecting the diversity of science, marine microbiologists applied some molecular genetics to the research in the early 1970s, but marine biologists in the early 1980s began to apply the methods of molecular biology more extensively. By the late 1980s, interest in biotechnology applied to marine organisms reached a critical level, measured by the number of publications in scientific journals on natural products alone. Simultaneously, a number of investigators lo-

cated primarily in the U.S. and Japan began to describe their work as "marine biotechnology," following the seminal publications of Colwell (1983, 1984a,b). In retrospect, the interest of scientists in marine biotechnology was sparked primarily by the following characteristics of this new field:

- the unique physiology and metabolism of many marine organisms, notably extremophiles, from hypothermal vents on the ocean floor,
- the fascinating and potentially commercially valuable compounds produced by marine organisms, some of which exhibit highly unusual chemical structure and intriguing bioactive properties,
- the ease with which eggs of fish and shellfish can be manipulated and the results of manipulation observed, especially for commercially important species,
- the potential for exploiting the world oceans, notably diverse biological habitats, such as the coral reefs, without detrimental environmental effect, i.e., by utilizing molecular genetic methods to tap the genetic diversity through gene cloning, tissue culture, and cell manipulation methods.

Thus, marine biotechnology is experiencing significant growth in many countries of the world, especially those that traditionally have depended on the sea for food and food products. Besides work in the U.S., exciting marine biotechnology research and development is taking place in Australia, Canada, China, France, Germany, Israel, Italy, Norway, Sweden, Taiwan, Thailand, the United Kingdom, and other countries. However, both time and funding restraints allowed detailed coverage of developments only for a few foreign countries and, of these, the most important is Japan.

Japan, with its limited land mass and terrestrial resources, very naturally has directed its impressive scientific assets to the investiga-

tion of the seas that surround the country, to seek out new resources that may be profitably exploited under environmentally sound conditions, and to remediate the effects of land-source pollution. The effort that the Japanese are investing in marine biotechnology is admirable and the results they have achieved to date are impressive.

Both the Japanese government and the industrial sector of Japan realized the importance of the marine environment for economic progress and understood very quickly its value, which goes beyond simply promoting aquaculture and fisheries. The data indicate that Japan spent circa \$357 million to \$519 million in 1992 on marine biotechnology research and development; a sum that has increased every year since then. About 80% of this funding is supplied by industry, in contrast to the current U.S. investment pattern where government funding predominates. However, the Japanese government provides significant indirect support of industry in the form of special tax advantages, loan programs, well-funded schemes for industry-university cooperation in R&D, and regional promotional activities. The major areas of emphasis selected by the Japanese for research and development are aquaculture, marine natural products, and biosensors, although investments in environmental applications are increasing rapidly.

As a consequence of the significant scientific research and development that the Japanese have accomplished to date, with a sharp focus on discovering new marine natural products, significant discoveries have already been made. We predict that 10-15 years from now, results from these discoveries will include a cascade of new drugs derived from marine organisms and developed by Japanese scientists. These will appear in Western pharmacies and will be used to treat a wide range of infectious and non-infectious diseases, including cardio-vascular diseases, cancers, immunological disorders, and bacterial, fungal and viral diseases. In addition, it is highly probable that Japan will be the world's major source of biosensors for medicine and environmental monitoring. A smaller commercial market, in comparison to pharmaceuticals, but, nevertheless, of importance to the U.S. in terms of balance of trade, Japanese aquaculture will benefit significantly from marine biotechnology applications, espe-

cially those related to preventing and treating diseases of finfish and shellfish and marketing genetically improved finfish and shellfish species. In addition to fisheries biotechnology, Japanese scientists are focussing on advances in biological oceanography, especially the role of plankton and picoplankton in the world oceans and their effect on global climate. This work is significant and will offer benefits by the 21st century, particularly to international efforts aimed at improving the environment.

Based on our study of marine biotechnology in Japan, we believe that nation will continue promoting its marine potential. Furthermore, investment of capital from private industry will be a major factor in its successful development of new products. The Japanese effort is to be applauded because it will generate valuable scientific information and new knowledge, which will assist the Japanese in combatting their own marine pollution problems, and also elucidate oceanographic and atmospheric phenomena related to global problems, such as the greenhouse effect and global climate. It is possible that, as a result of the resources that Japanese business and government sectors are investing in marine biotechnology, by 2010 Japanese scientists will be among the world leaders in marine biological and physical scientific research and development.

In addition to basic research advances, the return on the investment in marine biotechnology will yield products of genuine value for Japanese aquaculture and their pharmaceutical and chemical industries. These successes can be predicted to occur in the mid-term, i.e., within five or ten years, and they will enhance Japan's industrial output, increasing the power of that country's already impressive and highly competitive commercial prowess.

To sum up the status of marine biotechnology in Japan, during the past decade Japan has ardently encouraged marine biotechnology at both the national and local levels. The approaches of Japan and the U.S. towards promoting marine biotechnology are dissimilar. Whereas the United States' approach has been to support basic research in areas of marine biotechnology, Japan uses a more focussed, developmental approach. Since one of the primary tenants of marine biotechnology is potential utilization, and not just exploration and research for knowledge, Japanese industry will possess the world's

most advanced capabilities in many marine biotechnology applications, particularly marine natural products development, marine biotechnology to enhance aquaculture, biological oceanography, and biosensors. As has been observed: "The Japanese are now repeating their successful accomplishments in the field of electronics in the novel field of marine biotechnology" (Gibor, 1991).

Marine biotechnology in Australia and Norway can be predicted to provide advances that are likely to have significant national economic effect and will incrementally increase scientific knowledge, in general. For example, Australian investigators are probing their spectacular coastal zone, including the coral reefs, and can be expected to discover a range of marine species capable of producing chemically unique, biologically active substances. It can be predicted that some of these compounds will be useful as medicinal agents, generating profits for their developers. However, the commercial climate in Australia, which tends to be risk averse, does not appear to be conducive to the type of imaginative, long-term programs required to bring the results from marine biotechnology research to the market. Therefore, most such products are likely to be exploited with the aid of affluent foreign companies.

Based on their achievements to date, Norwegian scientists will join the front ranks of research and development in targeted areas, e.g., transforming wastes from aquaculture and fisheries into useful products, such as animal feed, industrial enzymes, and specialty chemicals. The aquaculture industry in Norway, already the world's largest, will be positioned to utilize marine biotechnology-derived diagnostics, therapeutics and vaccines to improve its fisheries output, thereby becoming even more efficient and competitive in seafood markets worldwide. However, in view of predictable market conditions from increasing supply, as well as increasing competition from the growing aquaculture industry in developing countries, improvements to Norway's aquaculture will have to become more cost effective and technologically efficient to remain at the forefront of seafood production.

An ancillary effect of the growth and development of Norwegian aquaculture is growth of other types of companies, i.e., those that offer services and products useful to aquaculture. These will be

in a powerful position to compete in sectors of the international market comprising marine animal feed, diagnostics and therapeutics. These sectors are relatively small, but are potentially lucrative and serve as entry-points for smaller biotechnology-based companies, which are likely to be a base of major economic development in the 21st century.

This study was not initiated with the intent of providing complete details of international progress in marine biotechnology, a difficult task, in view of the many research units throughout the world that now are active in this field (see Appendix 4). Instead, selected important components of the international marine biotechnology community have been highlighted. But we would be remiss if we did not mention two developments in international science promoting marine biotechnology by informing a wider audience of scientists about exciting research taking place within the field and clarifying to the public and its representatives its benefits. First, a series of major international marine biotechnology conferences have been convened, the first in Tokyo, in 1989, and the second in Baltimore, Maryland, in 1991. The third international marine biotechnology conference was held in Bergen, Norway in 1994. Second, as a sequel to a World Bank report on marine biotechnology and the developing countries (Zilinskas and Lundin, 1993), the World Bank, United Nations Development Program and United Nations Industrial Development Organization sponsored the first of what will be a series of regional conferences, which was held during November 1993 in Bangkok, Thailand. Its focus was on the possibilities offered by marine biotechnology for the Asian-Pacific nations. The greatest impact is expected in the short to medium-term to be on aquaculture and natural products development.

Emergence of marine biotechnology in the U.S. has occurred in two phases, with a third on the horizon. Initiating the first phase, a small number of U.S. scientists, working largely in isolation and supported by only a few funding agencies, recognized the importance of marine biotechnology in the late 1970s and early 1980s. A significant contribution, which led to exciting scientific achievements, was to adopt the then recently developed molecular biology techniques

to marine biology. Soon a larger number of bioscientists recognized the many research possibilities the marine environment presented and proceeded to take advantage of these opportunities, which led to an initial spurt of growth in marine biotechnology R&D. During the first phase, a small marine biotechnology center was founded in North Carolina, but focussed on data storage and dissemination. Two research centers dedicated to marine biotechnology subsequently were established, the first in Maryland and, shortly afterwards, a second in California. Both of these centers flourished immediately, with rapid growth ensuing.

In the late 1980s, a transition from the first phase into the second occurred. The term "marine biotechnology" began to appear, not only in scientific publications, but also in policy-related documents and government publications. The number of publications in the field of marine biotechnology increased significantly, in some areas eight to ten-fold from the early 1980s to the early 1990s. *Marine Biotechnology Abstracts* was first published in 1989 and has doubled in size of the publication. Two additional journals dedicated to marine biotechnology were launched, one in the U.S. and the other in Japan. Several other journals covering closely related fields, e.g., marine biodiversity and biological oceanography, and biotechnology applications have been founded in the last five years. Diverse books with marine or aquatic biotechnology included in their content or wholly or partially devoted to marine biotechnology have been published recently.

Despite these developments, as evidenced by MARBIO data, funding for marine biotechnology in general remained essentially level during 1991 and several of the preceding years. Furthermore, most of the academic and industry scientists interviewed during the time MARBIO was in development expressed a belief that the funding situation would not improve in the immediate future. Industry interest and, more importantly, investment in marine biotechnology in the United States was meager, compared to U.S. investment in biotechnology over-all. As indicated by MARBIO data, total funding for marine biotechnology research by the federal government, state governments, and industry was circa \$40 million in 1992.

For purposes of comparison, this total equaled circa 7% to 11% of what the Japanese spent on research in this field. Shortage of funds in the U.S. hindered marine biotechnology from achieving the explosive growth as occurred in other areas of biotechnology.

During the second phase of development in marine biotechnology, some observers of science voiced concerns about the safety of marine biotechnology. However, after careful analysis and taking into account controversies associated with release of genetically engineered organisms to the environment, we conclude that issues related to biosafety have not been a barrier to the advancement of marine biotechnology. In fact, to the contrary, procedures already developed to ensure safety in other biotechnology research can be applied directly to marine biotechnology. As indicated by concerns expressed by environmental groups in 1990, prior to testing of transgenic carp in closed facilities in Alabama, the prospect of open field testing of transgenic marine organisms is expected to result in public debate. As occurred in other areas of biotechnology, some industries will defer from making investments in marine biotechnology, especially in development of transgenic marine organisms (see below), pending resolution of safety issues.

Several states began making major investments in marine biotechnology during the second phase. The two first significant marine biotechnology research centers, in Maryland and California, have grown significantly in size and importance. The North Carolina center, which languished for some years, finally developed into a full-fledged, state-wide marine biotechnology program. A fourth major center, concentrating on marine natural products development, was established in 1993 at the University of California at San Diego, and is active in promoting marine biotechnology in that state. Long established marine research centers, such as Harbor Branch Oceanographic Institution in Florida and the Marine Biological Laboratory in Massachusetts, have expanded their programs to include marine biotechnology and related areas. Other coastal states are augmenting their investments in the marine biological sciences, either by promoting the growth of departments in universities that already are active in the marine field, expanding the scope of existing

traditional ocean sciences centers, or establishing new marine biotechnology centers.

Despite significant advances in marine biotechnology research, some of which has produced results leading to significant applications, U.S. industry has not played a major role in marine biotechnology in the second phase. In fact, the survey of U.S. companies conducted as part of our analysis revealed that, by 1992, about 80 companies were dedicated to marine biotechnology or sponsored marine biotechnology R&D, either in-house or extramurally. The analysis of data stored in MARBIO showed that most of the effort by industry was devoted to natural products development. Nevertheless, it is a notable achievement that three pharmacological agents derived from marine biotechnology are in clinical trial, in one case, showing activity against tumors, in another, an ability to inhibit viruses and, in third, efficacy in the treatment of psoriasis. Approximately five additional compounds have shown good promise and are in pre-clinical trial. In contrast to natural products development, the aquaculture industry in the U.S. is essentially nascent, with a need for significant technological advancement to develop to its fullest capacity.

It can be postulated that at least three reasons explain the apparent indifference of U.S. industry towards marine biotechnology. First, marine biotechnology is unknown, or nearly so, to many firms. Consequences of this lack of knowledge are that these companies are not in a position to consider entering this new field and, for most companies, marine biotechnology, at best, is relatively unknown, with the perception of the working environment of marine biotechnology as being fraught with difficulties, since ocean resources have historically been viewed as being difficult to discover and, even when located, requiring strenuous efforts to exploit on a dependable and sustainable basis. Further, companies may be aware only of the relative low technological level of marine biology that existed previously, before the advanced techniques made possible by molecular biology and genetics were applied to marine-related biological research. Therefore, as explained in the body of this report, only a comparatively few companies appear to be comfortable in marine-

related research and development. Even these companies often are unaware of the truly rich commercial potential of marine biotechnology, especially in new product development.

Second, most applications within the six areas of marine biotechnology identified in this report will come to fruition only in the mid-term, at best, and, more likely, in the longer term. U.S. companies, especially the smaller bioindustries, tend to do their planning in three to five year cycles, thereby excluding many research directions, especially in the long range, high risk category.

Third, while we noted that the biosafety issue has not affected the advance of marine biotechnology research significantly, uncertainties remain with respect to development and production, especially for future field testing related to transgenic marine fish, plants and microorganisms and the safety of such products in human nutrition. Until these uncertainties are eliminated, most companies will hesitate to make a long-term commitment to bring findings from marine biotechnology to the market. However, at the same time, it would be unwise and unproductive to revisit all the issues addressed by the National Research Council report (United States National Research Council, 1989) and OECD (Directorate for Science, 1992) (Cantley, 1994).

Marine biotechnology in the U.S. appears primed to enter a third phase, likely to be characterized by significant and rapid growth. This conclusion is based, in part, on the expectation that the U.S. Senate is likely to join the U.S. House of Representatives and adopt the Marine Biotechnology Investment Act of 1993, described in Chapter 3, thus making available significant new funding (\$20 million per year for the first two years) to scientists in both private and public research institutes and laboratories. This will, without doubt, correct in part the problem of under-funding of this field as reported by FCCSET and revealed by MARBIO data. We can expect new initiatives in marine biotechnology R&D, much of which will be of value to industry. However, the intent of the Marine Biotechnology Investment Act would be greatly enhanced if a national effort designed to produce marine biotechnology products and processes were launched. Such an effort will produce a far greater return to

society than the uncoordinated process currently operating. Funding by Congress of the 1980 National Aquaculture Act would promote such a national effort.

From the study of marine biotechnology-related industry described in this report, four major developments can be predicted in the third phase. First, aquaculture will become increasingly important in the U.S. as technological advancement, particularly application of molecular techniques, allows this industry to expand significantly. Both basic research and industrial development of marine biotechnology, will be needed if innovations, such as closed system production for domestic aquaculture, is to be economically feasible, which it certainly can be, as demonstrated by profitable systems operating in Israel, Japan, and Norway.

Second, the most significant impact, in the short term, may prove to be marine bioremediation. Chemical and physical methods presently are methods of choice, but only for the short term. Bioremediation companies will rely on naturally occurring microorganisms, some of which will be developed for greater efficiency using classical methods for breeding and selection, in the initial phase of development of this industry. Availability of genetically engineered marine microorganisms designed specifically for bioremediation of estuaries, near shore, open coast and deep water areas of the world oceans will open new vistas for pollution remediation of the marine environment, not only oil spills, but also other toxic pollutants reaching the open ocean. As noted in Chapter 5, risk assessments schemes applicable to the marine environment and field tests of transgenic marine microorganisms are required before applications useful to the remediation industry can become reality.

Third, with development of improved screening methods for detecting a wider variety of potentially useful properties of marine organisms, more companies will be attracted to invest in R&D derived from basic research in marine biology and molecular marine biology, as well as natural products chemistry. It is envisioned that cooperative projects that partner industry and universities will increasingly be undertaken. Companies other than those interested solely in human drug development will enter into new endeavors, for exam-

ple, chemical companies interested in natural products useful as pesticides, food preservation agents, fertilizers, and other properties.

Fourth, the foregoing three practical developments will be accompanied by an equally important, if a less tangible advance, namely, the present knowledge of marine ecosystems, and their complexity and biodiversity, will expand greatly as the techniques of marine biotechnology are applied to the study of marine ecology and biological oceanography. As a result, we will gain a better understanding of how the oceans influence the weather, of the systemic effects of pollutants on the environment, of the life cycles and movements of pelagic fish, of the complex communities of marine populations such as those existing on and about coral reefs and estuaries, and of other important marine phenomena.

There is no question but that marine biotechnology has great potential. Perhaps the full flowering of that potential will be realized when its techniques are seamlessly integrated in the host of activities that constitute coastal zone development and marine resource utilization. A pictorial representation of such an integration is reflected in Figure 23, where employment of biosensors to complement remote sensing instrumentation installed on buoys and satellites, the use of bioremediation to restore waters in and around sensitive coastal environs and valuable man-made structures; application of diagnostics and therapeutics made possible by molecular biology to promote largely non-polluting aquaculture; aquaculturing of a larger variety marine animals and plants than is now possible or practicable, at times integrated with the operation of ocean thermal energy conversion (OTEC) systems, to make available for markets a wider variety of tasty and nutritious foods; and growth of pharmaceutical and specialty chemical industries based on compounds and chemicals of marine origin. Fortunately, this potential inherent to marine biotechnology is now beginning to be realized—with society the benefactor and the recipient of the wealth of the ocean's resources.

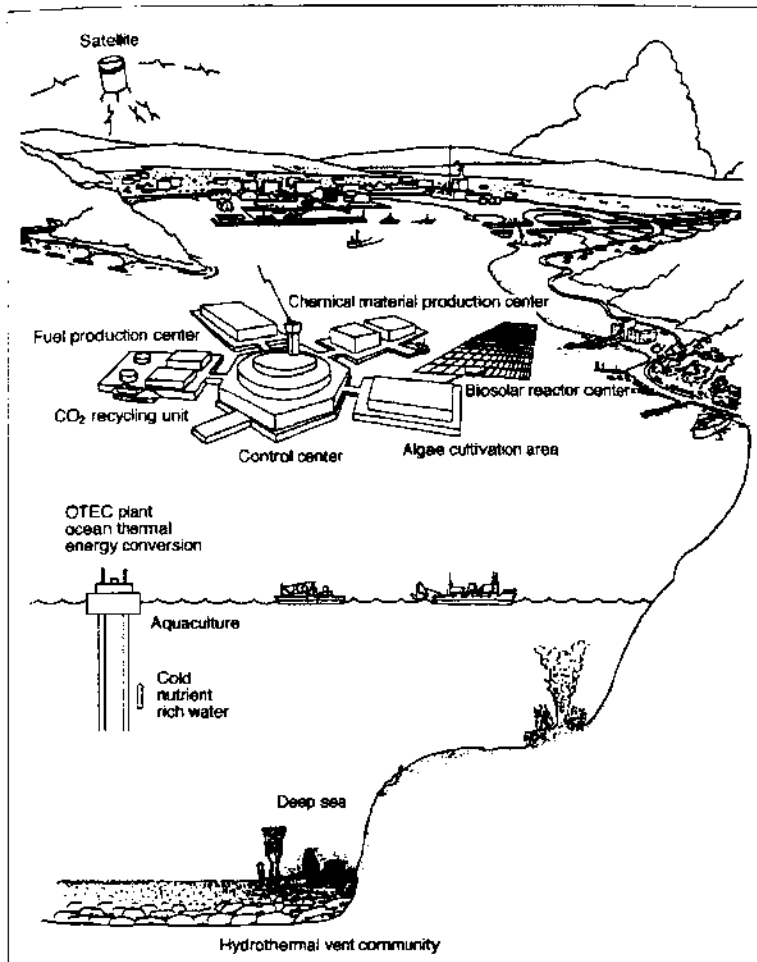


Figure 23. Integrated ocean utilization system.

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Appendix 1

DEFINITIONS OF MARINE BIOTECHNOLOGY

In 1991, the World Bank commissioned a study on marine biotechnology for the developing countries (Zilinskas and Lundin, 1993). As part of that study, scientists in many countries were asked how they defined the term "marine biotechnology." The following list of definitions, reproduced with permission from the World Bank, contains their replies.

"Given the fact that biotechnology is any aspect of biological system that makes money, I would say that marine biotechnology is any aspect of biotechnology that either directly concerns aquatic (marine and freshwater) systems or had as its origin an aquatic biological system."

(Dr. Joseph Bonavenatura, Director Marine Biomedical Center, Duke University Marine Laboratory, North Carolina)

"Marine biotechnology as the application of molecular biological techniques/methods to the production or modification of potential commercial products. This might include the use of marine species for the application, or the use of molecular bio-techniques in the marine environment."

(Dr. David L. Nebert, Assistant Director for Research and Administration, Institute of Marine Science, University of Alaska-Fairbanks, Alaska)

"Our definition of marine biotechnology is the use of biotechnology for studies of marine organisms or the use of marine organisms for applications in the field of biotechnology."

(Dr. Bert Ely, Director Institute for Biological Research and Technology, University of South Carolina, S.C.)

"I would say that marine biotechnology is the use of all the tools and knowledge in the life sciences to produce a desired effect on or for mankind."

(Dr. Robert S. Jones, Director Marine Science Institute, University of Texas at Austin, Texas)

"Marine biotechnology is the manipulation of marine organisms to produce a beneficial product for humankind."

(Dr. Kent S. Price, Associate Dean, College of Marine Studies, Lewes, Delaware)

"The manipulation and/or use of all or part of a specific marine biological system to generate a desired product or products."

(Dr. Donald W. Renn, Senior Research Fellow, FMC Corporation, Maine)

"I would define marine biotechnology simply as the application of the techniques of modern molecular biology to marine biology. It covers the use of these techniques to study the biology of marine organisms as well as exploit practical applications of molecules derived from marine organisms."

(Dr. Norman R. Wainwright, Director of Research, Associates of Cape Cod, Inc., Massachusetts)

"Marine biotechnology can be defined as the efficient utilization of marine living resources or their components to provide desirable products and services."

(Dr. M. Chandrasekaran, Microbiology Laboratory, Department of Applied Chemistry, Cochin University of Science and Technology, Kochi 682022, India)

"Marine biotechnology, an extension of marine biology, blends science and technology to develop the methods for mass production and processing of marine organisms for a wide range of industrial and commercial uses."

(Dr. Saipin Chaiyanan, Department of Microbiology, Faculty of Science, King Mongkut's Institute of Technology Thonburi, Bangmod, Rasburana Bangkok 10140, Thailand)

"Marine biotechnology is a branch of marine science dealing with marine organisms to enhance the production of food, feed and chemicals for the betterment of mankind."

(Dr. N.B. Bhole, National Institute of Oceanography, Dona Paula, Goa-403 004, India)

"I consider that any proven technology, which is aided by the biological systems, can be called biotechnology, so one can apply this definition to marine biotechnology."

(Dr. M.S. Andhale, Department of Microbiology, Government Institute of Science, Nipatnirajan, A'bad Caves Road, Aurangabad 431 004, India)

"I would like to define marine biotechnology from my understanding that: any marine biological knowledge which could be applied to increase yield or marine products is marine biotechnology. Marine biotechnology is very wide in the sense, there are a lot of things to be done in the field of marine biotechnology. For example, only marine bacteria and marine plankton can play very important role in marine biotechnology."

(Dr. Twee Hormchong, Director Institute of Marine Science, Burpha University, Bangsaen, Chonburi 20131, Thailand)

"The term biotechnology generally implies the application of technology to organisms. In other words, we try to mould the organisms or its function to achieve our target. Nonetheless, to say better exploration and exploitation of the ocean and the organisms there in for the transmogrification of mankind. We would like to define marine biotechnology as the application of genetic engineering to marine sciences i.e. to utilize the untapped gene pool in:

1. The transport of minerals (nutrient cycle)
2. Novel photosynthetic system (primary production)
3. Utilization of H₂S, NH₃, H₂ etc (chemosynthesis)
4. Production of fish, mollusks, crustaceans in natural and hatchery system (secondary and tertiary production)
5. Marine pheromones, toxins, and pharmacological compounds."

(Dr. Shanta Achuthankutty, National Institute of Oceanography, Dona Paula, Goa 403 004, India)

"Marine biotechnology is the application of marine organisms including their systems or processes for the manufacture of industrial products and for the practical solution of problems created by human activity."

(Milagrosa R. Martinez, Associate professor and Director, Learning Resources Center, University of Philippines at Los Banos, College, Laguna 3720, Philippines).

“The application of biological sciences which utilizes living marine organisms, their cells or parts of cells to produce good and services.”

(Dr. S.T. Chang, Department of Biology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong)

“I shall define marine biotechnology as the commercial exploitation of living marine organisms or their components. The organisms will include microbes, and also plants as well as animals; the later will encompass the application of molecular biology and cell culture techniques.”

(P.M. Sateesh Seshaiya, Post Graduate Lecturer in Microbiology, Post Graduate Department of Microbiology, Sri Paramakalyani College, 29 West Car Street, Kallidaikurichi 627 416, Tamilnadu, India)

“The definition about marine biotechnology managed by the Institute is the same used in other Latin America countries and in Europe, any technology used to increase production where the final product has commercial importance. In this sense, in USA and Canada this concept is much more restricted and its use has been applied to technology where only DNA is manipulated.”

(Dr. Patricio Bernal Ponce, Executive Director, Instituto de Fomento Pesquero, Jose Donningo Canas 2277, Casilla 1287, Santiago, Chile)

“I define marine biotechnology as: The use of marine organisms or their genetic information, for applications on aquaculture, pharmacology, and pollution control.”

(Dr. M.L. Lizarraga-Partida, Centro de Investigacion Cientifica y de Educacion Superior de Ensenada, Av. Espinoza No. 843, Apartado Postal 2732, Ensenada, Baja California, Mexico)

“Marine biotechnology is the science dealing with the study of marine organisms (preferentially microorganisms and plants) at a molecular level, specially on their genetic structure and on the techniques that could be used to modify or improve their genomes in order to produce substances (food, medicines, etc) at a high quality and quantity level or to degrade debris and undesirable substances in by-products useful to mankind.”

(Dr. Enrique C. Mateo, Fondo de Reactivacion del Sector Pesqueria, German Schereiber 198, Francia 726-Miraflores, Lima, Peru)

“Marine biotechnology is the integration of advances in marine microbiology, marine biochemistry (including cell biology, molecular biology, and molecular genetics), marine biology and process engineering, for application in such areas as food and feed industry, pharmaceutical industry, environmental pollution and energy, medical diagnostics, fermentation industry, and chemical industry.”

(Dr. Gideon Abu, Department of Microbiology, Box 274, University of Port Harcourt, Port Harcourt, Nigeria)

“I would want to define marine biotechnology as studies and development of marine (aquatic) resources for human welfare using the available biomolecular tools as well as developing newer and better research tools for application and improvement- enhancement of our understanding of marine (aquatic) life in general.”

(Dr. S.O. Emejuaiwe, Imo State University, P.M.B. 2000, Okigwe, Imo State, Nigeria)

“In my opinion, the task of biotechnology is to synthesize the modern theory and methods of engineering and biology, to research the variations of biological structure and function on different level and artificially to control these variations by using engineering and technique, in order to develop some new types of industry or new biological products on a large scale, such as genetic engineering, cell engineering, enzyme engineering, microbial engineering, biochemical engineering and the technique of comprehensive utilization for biological resources.”

(Dr. Chen Dou, Institute of Oceanology, Academia Sinica, 7 Nan-Hai Road, Qingdao, Shandong, Peoples Republic of China)

More recently, the U.S. House of Representatives has represented marine biotechnology to mean “the application of molecular and cellular biology to marine and fresh water organisms for the purpose of identifying, developing, and enhancing products derived from these organisms.”

(Marine Biotechnology Investment Act of 1993--H.R. 1916).

Appendix 2

MARINE BIOTECHNOLOGY QUESTIONNAIRE FOR RESEARCH UNITS

For this questionnaire, we define marine biotechnology as any scientific investigation that focuses on marine organisms and that utilizes new cell, protein and nucleic acid technologies such as recombinant DNA, hybridoma/monoclonal production, protein engineering, polymerase chain reaction, DNA hybridization, and other related technologies.

Areas related to marine biotechnology include aquaculture/mariculture research, fisheries research, marine biology, biochemistry/physiology of marine organisms, phycology, fermentation processes that utilize marine organisms, and marine natural products chemistry.

I. Respondent Criteria

Are you a researcher engaged in full-time research in marine biotechnology or a marine biotechnology-related area?

- Marine biotechnology
- Marine biotechnology-related area
- Neither (If this is checked, please complete only the next section; i.e., Section II: Respondent Information).

II. Respondent Information

(Please answer all questions. Type or print clearly. Do not use home address or telephone number.)

Last name: _____

First name: _____ Middle initial: _____

Your position: _____

Department: _____

Institution: _____

Address: _____

Telephone: _____

Facsimile (FAX): _____

Time for interview :

III. Information about Research, Personnel and Facilities

A. Please circle the type of institution that your laboratory or unit is affiliated with.

1. University or similar institution of higher learning.
2. Research unit or center associated with a university.
3. National research center.
4. State research center (please specify state).
5. Industry-connected basic research laboratory.
6. Industry-connected applied or developmental laboratory.

B. Please consider the following research areas

1. Aquaculture
16. Immunology

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- | | |
|--------------------------------|-----------------------|
| 2. Biochemistry | 17. Macroalgae |
| 3. Biometabolites/Toxins | 18. Marine biology |
| 4. Bioremediation | 19. Microalgae |
| 5. Cell Biology | 20. Microbiology |
| 6. Chemistry, Natural Products | 21. Molecular Biology |
| 7. Chemistry, Polymer | 22. Neurobiology |
| 8. Developmental Biology | 23. Oceanography |
| 9. Ecology | 24. Oncology |
| 10. Engineering, Bioprocess | 25. Pharmacology |
| 11. Engineering, Chemical | 26. Physiology |
| 12. Enzymology | 27. Toxicology |
| 13. Fisheries | 28. Transgenic Fish |
| 14. Food Science | 29. Other (specify) |
| 15. Genetics | |

Please enter by number the two areas listed above that best describe your research focus:

C. Please consider the following applications areas:

- | | |
|---------------------------------|-------------------------------------|
| 1. Agrochemicals | 8. Environment/Bioremediation |
| 2. Aquaculture/mariculture | 9. Fermentation Processes |
| 3. Basic research | 10. Food products |
| 4. Bioadhesion/Biofouling | 11. Human health care/Public health |
| 5. Bulk chemicals | 12. Naval Defense/Warfare |
| 6. Diseases of marine organisms | 13. Pharmaceuticals/Fine chemicals |
| 7. Energy/Biomass | 14. Other (specify) |
| 8. Environment/Bioremediation | |

From the above list, please indicate by number the one or two marine biotechnology application(s) that fits your research most closely:

D. Has any of the research done at your laboratory in the last three years been applied commercially?

Yes

No

If YES, please list the commercial product(s) or process(es):

E. Has any discoveries made by your laboratory led to patents or patent applications?

Yes

No

If YES, please indicate:

1. Number of patent(s) received:
2. Patent number(s) (registry of patents):

3. Application number(s) of patents applied for:

F. Please indicate by circling the organisms that you use most often in your research or that your research is focussed on (up to three organisms).

- | | |
|---------------|-----------------------------|
| 1. Finfish | 9. Zooplankton |
| 2. Shellfish | 10. Phytoplankton |
| 3. Molluscs | 11. Marine macrosymbionts |
| 4. Macroalgae | 12. Marine microsymbionts |
| 5. Microalgae | 13. Protozoa |
| 6. Virus | 14. Nematodes (worms, etc.) |
| 7. Bacteria | 15. Insects |
| 8. Fungi | 16. Other (please specify) |

G. Please consider the following list of advanced biotechnology techniques:

- | | |
|--------------------------------------|---|
| 1. Cell Culture | 10. Transcription Amplification System |
| 2. DNA Library | 11. Gene cloning |
| 3. DNA Probe Construction | 12. Restriction enzyme analysis |
| 4. Hybridoma/Monoclonal Construction | 13. Restriction fragment analysis |
| 5. In Situ hybridization | 14. Cell fusion |
| 6. Polymerase Chain Reaction | 15. Microinjection |
| 7. Protein Engineering | 16. Hybrid analysis |
| | 17. Classical genetics (breeding)—animals |

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8. Protein Sequencing
9. Recombinant DNA
18. Classical genetics (breeding)—plants
19. Other (please specify):

Please list by number, and in decreasing order of priority, up to three of the advanced biotechnology techniques in which your laboratory has strong expertise:

H. Please provide the number of full-time personnel in your laboratory:

1. Scientists:
2. Post-docs:
3. Graduate students:
4. Technicians:
5. Other faculty (including visiting):
6. Undergraduate students:

I. Please indicate by circling the range which best represents that total annual budget for marine biotechnology for your laboratory:

1. \$1-10,000
2. \$10,001-50,000
3. \$50,001-100,000
4. \$100,001-250,000
5. \$250,001-500,000
6. \$500,001-1 million
7. \$1-5 million
8. Over \$5 million

J. Has this budget increased or decreased over the previous three year period?

Increased Decreased

By approximately what %?

K. Would you estimate that this budget will increase or decrease in the next three years?

Increase Decrease

By approximately what %?

L. What are the sources of research funding for your laboratory?
(Total should equal 100%.)

- 1. Sea Grant _____%
- 1a. NOAA but not Sea Grant _____%
- 2. ONR/U.S. Navy _____%
- 3. NIH/NCI _____%
- 4. NSF _____%
- 5. USDA _____%
- 6. FDA _____%
- 7. DARPA _____%
- 8. US Army/USAMRIID _____%
- 9. Other federal (please list) _____%
- 10. State source(s) (please name program) _____%
- 11. University _____%
- 12. Private foundation(s) (please name) _____%
- 13. Industry (please name company) _____%
- 14. International source(s) (such as FAO, UNDP, WHO,
UNESCO, etc.—please specify agency)] _____%
- 15. Other (specify) _____%

M. Does your laboratory have:

- | | | |
|--------------------------------|-----|----|
| 1. MacArthur fellow(s)? | Yes | No |
| 2. NSF predoctoral fellow(s)? | Yes | No |
| 3. NSF postdoctoral fellow(s)? | Yes | No |
| 4. NIH Career Awardee(s)? | Yes | No |

[If YES], please indicate which category and how many?

N. Briefly describe your marine biotechnology or marine-biotechnology-related research focus:

O. Does your laboratory have collaboration(s) with industry?

Yes

No

[If YES], briefly describe this collaboration and name the industry.

P. Does your laboratory have collaboration(s) with a laboratory or laboratories on other countries?

Yes

No

[If YES], briefly describe this collaboration and name the collaborating laboratories:

In order to get a better idea of the scope of marine biotechnology research and development in the U.S., an additional question has been added to this survey. We apologize for the additional time.

Q. Are there additional laboratories doing research in marine biotechnology or marine biotechnology-related area in your institution?

Yes

No

[If YES]: Of the total amount of funds spent on marine biotechnology research in your institution, please estimate the percentage which your laboratory receives.

Approximately what %? _____

Appendix 3

MARINE BIOTECHNOLOGY QUESTIONNAIRE FOR INDUSTRY

For this questionnaire, we define marine biotechnology as any scientific investigation that focusses on marine organisms and that utilizes new cell, protein and nucleic acid technologies such as recombinant DNA, hybridoma/monoclonal production, protein engineering, polymerase chain reaction, DNA hybridization, and other related technologies.

Areas related to marine biotechnology include aquaculture/mariculture research, fisheries research, marine biology, biochemistry/physiology of marine organisms, phycology, fermentation processes that utilize marine organisms, and marine natural products chemistry.

I. Respondent Criteria

Is your firm engaged in research in marine biotechnology or a marine biotechnology-related area?

- Marine biotechnology
- Marine biotechnology-related area
- Neither (If this is checked, please complete only the next section; i.e., Section II: Respondent Information.)

II. Respondent Information

(Please answer all questions. Type or print clearly. Do not use home address or telephone number.)

Last name: _____

First name: _____ Middle initial: _____

Your position: _____

Department: _____

Institution: _____

Address: _____

Telephone: _____

Facsimile (FAX): _____

III. Information about Research, Personnel and Facilities

A. Please circle the type of business (**Choices like public sector/private, etc.).

1. Multi-National Corporation.
2. U.S. Corporation.
3. Partnership.
4. Joint Venture.
5. Family Owned.
6. Non-Profit Research Institute.
7. Other (please specify).

B. Year Firm Established: _____

The following questions refer only to marine biotechnology or marine biotechnology-related area:

C. Please consider the following research areas:

- | | |
|--------------------------------|-----------------------|
| 1. Aquaculture | 16. Immunology |
| 2. Biochemistry | 17. Macroalgae |
| 3. Biometabolites/Toxins | 18. Marine biology |
| 4. Bioremediation | 19. Microalgae |
| 5. Cell Biology | 20. Microbiology |
| 6. Chemistry, Natural Products | 21. Molecular Biology |
| 7. Chemistry, Polymer | 22. Neurobiology |
| 8. Developmental Biology | 23. Oceanography |
| 9. Ecology | 24. Oncology |
| 10. Engineering, Bioprocess | 25. Pharmacology |
| 11. Engineering, Chemical | 26. Physiology |
| 12. Enzymology | 27. Toxicology |
| 13. Fisheries | 28. Transgenic Fish |
| 14. Food Science | 29. Other (specify) |
| 15. Genetics | |

Please enter by number the two areas listed above that best describe your firm's research focus: _____

D. Please consider the following applications areas:

- | | |
|---------------------------------|-------------------------------------|
| 1. Agrochemicals | 8. Environment/Bioremediation |
| 2. Aquaculture/mariculture | 9. Fermentation Processes |
| 3. Basic research | 10. Food products |
| 4. Bioadhesion/Biofouling | 11. Human health care/Public health |
| 5. Bulk chemicals | 12. Naval Defense/Warfare |
| 6. Diseases of marine organisms | 13. Pharmaceuticals/Fine chemicals |
| 7. Energy/Biomass | 14. Other (specify) |
| 8. Environment/Bioremediation | |

From the above list, please indicate by number the one or two marine biotechnology application(s) that fits your firm's research program most closely: _____

E. Has any of the research done at your firm in the last three years led to marketable products?

Yes

No

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If YES, please list the commercial product(s) or process(es):

Product Annual Sales Volume (if available)

F Has any discoveries made by your firm led to patents or patent applications?

Yes

No

If YES, please indicate:

1. Number of patent(s) received: _____

2. Patent number(s) (registry of patents):

3. Application number(s) of patents applied for:

G. Please indicate by circling the organisms that your firm uses most often in its research program or that its research program is focussed on (up to three organisms).

- | | |
|---------------|-----------------------------|
| 1. Finfish | 9. Zooplankton |
| 2. Shellfish | 10. Phytoplankton |
| 3. Molluscs | 11. Marine macrosymbionts |
| 4. Macroalgae | 12. Marine microsymbionts |
| 5. Microalgae | 13. Protozoa |
| 6. Virus | 14. Nematodes (worms, etc.) |
| 7. Bacteria | 15. Insects |
| 8. Fungi | 16. Other (please specify) |

H. Please consider the following list of advanced biotechnology techniques:

- | | |
|-----------------|--|
| 1. Cell Culture | 10. Transcription Amplification System |
| 2. DNA Library | 11. Gene cloning |

- | | |
|--------------------------------------|---|
| 3. DNA Probe Construction | 12. Restriction enzyme analysis |
| 4. Hybridoma/Monoclonal Construction | 13. Restriction fragment analysis |
| 5. In Situ hybridization | 14. Cell fusion |
| 6. Polymerase Chain Reaction | 15. Microinjection |
| 7. Protein Engineering | 16. Hybrid analysis |
| 8. Protein Sequencing | 17. Classical genetics (breeding)—animals |
| 9. Recombinant DNA | 18. Classical genetics (breeding)—plants |
| | 19. Other (please specify) |

Please list by number, and in decreasing order of priority, up to three of the advanced biotechnology techniques in which your firm's research laboratory or laboratories have strong expertise:

I. Please provide the number of personnel in your firm's research laboratory or laboratories:

1. Senior Scientists/Researchers: _____
2. Junior Scientists: _____ 3. Post-docs: _____
4. Technicians: _____
5. Other Scientists (including visiting): _____

J. Please indicate by circling the range which best represents the total annual budget for marine biotechnology or marine biotechnology-related research at your firm:

- | | |
|----------------------|------------------------|
| 1. \$1-10,000 | 5. \$250,001-500,000 |
| 2. \$10,001-50,000 | 6. \$500,001-1 million |
| 3. \$50,001-100,000 | 7. \$1-5 million |
| 4. \$100,001-250,000 | 8. Over \$5 million |

K. Has this budget increased or decreased over the previous three year period?

Increased

Decreased

By approximately what %?

L. Would you estimate that this budget will increase or decrease in the next three years?

Increased

Decreased

By approximately what %?

M. What are the sources of funding for your firm's research program? (Total should equal 100%.)

- 1. Sea Grant%
- 1a. NOAA but not Sea Grant%
- 2. ONR/U.S. Navy%
- 3. NIH/NCI%
- 4. NSF%
- 5. USDA%
- 6. FDA%
- 7. DARPA%
- 8. US Army/USAMRIID%
- 9. Other federal (please list)%
- 10. State source(s) (please name program)%
- 11. University%
- 12. Private foundation(s) (please name)%
- 13. Industry (please name company)%
- 14. International source(s) (such as FAO, UNDP, WHO, UNESCO, etc.—please specify agency)]%
- 15. Other (specify)%

N. Briefly describe your firm's marine biotechnology or marine biotechnology-related research focus:



O. Does your firm have collaboration(s) with a university or universities?

Yes

No

If YES, briefly describe this collaboration and name the university or universities:

P. Does your firm's researchers have collaboration(s) with a laboratory or laboratories in other countries?

Yes

No

If YES, briefly describe this collaboration and name the collaborating laboratories:

Q. Are there other firms doing research in marine biotechnology or marine biotechnology-related area that you are aware of?

If YES, please name.

R. Of the total amount of funds spent on research and development in your firm, please estimate the percentage which marine biotechnology or marine biotechnology-related research receives.

Approximately what %

Appendix 4

FOREIGN MARINE BIOTECHNOLOGY-RELATED RESEARCH INSTITUTES

The following list of foreign marine biotechnology or marine biotechnology-related research institutes is not comprehensive; the list merely reflects the names of institutes and laboratories that we have come across while undertaking the present study. Obviously, many countries, and many institutes in listed countries, are missing from the list. The list is placed in this report to give the reader an idea of the number and variety of scientific institutes that there are in the world dedicated to performing marine biology and marine biotechnology-related research.

ARGENTINA

University of Buenos Aires
Faculty of Sciences
Department of Biological Sciences

AUSTRALIA

Brackish Water Fishery Culture Research Station (Salamander Bay)
Commonwealth Scientific and Industrial Research Organization
Division of Chemistry and Wood Technology
Division of Entomology
Division of Fishery
Division of Water Resources Research
James Cook University, North Queensland
Department of Chemistry and Biochemistry
Murdoch University
Algal Biotechnology Laboratory
School of Environment and Life Sciences
Queensland Institute of Medical Research (Brisbane)
University of Adelaide
Department of Organic Chemistry
University of Melbourne

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Department of Organic Chemistry
University of New South Wales (Kensington)
School of Microbiology
School of Biochemistry
University of Queensland
Departments of Chemistry and Biochemistry
Department of Zoology
University of Tasmania
Department of Agricultural Sciences
University of Wollongong
Department of Chemistry

AUSTRIA

University of Vienna
Institute of Physical Chemistry

BELGIUM

Belgium Nucleic Center
Department of Biology
I.I.F.-I.M.C.-C.E.R.I.A
Unite de Biotechnologie (Bruxelles)
State University of Ghent (Ghent)
Laboratory of Mariculture
Laboratory of Pharmacological Microbiology and Hygiene
University of Liege
Laboratory Centre Genetique

BRAZIL

Fishery Institute (Sao Paulo)
Division of Freshwater Fish
University of Sao Paulo
Bioscience Institute and Marine Biology Center

CANADA

Atlantic Salmon Federation (St. Andrews)
Salmon Genetic Research Program
Atlantic Veterinary College
Department of Pathology and Microbiology
Biological Sciences Branch (West Vancouver, B.C.)
Department of Fisheries and Oceans
Canadian Institute of Fishery Technology (Halifax)
Department of Fishery and Oceans
Development Division (Newfoundland)

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Freshwater Institute (Manitoba)
Dalhousie University (Halifax)
 Department of Biochemistry
 Department of Biology
McMaster University
 Health Sciences Center
 Department of Biochemistry
Huntsman Marine Sciences Center (St. Andrews, New Brunswick)
Macdonald College, McGill University (St. Anne de Bellevue, Quebec)
 Department of Food Sciences and Agricultural Chemistry
Maurice Lamontagne Institute (Mont-Joli, Quebec)
 Department of Fisheries and Oceans
 Biology Oceanography Division
Memorial University of Newfoundland
 Ocean Sciences Center
 Marine Laboratory
 Department of Biochemistry
Montreal Neurology Institute
National Research Council of Canada
 Institute of Marine Biosciences
New Brunswick Department of Agriculture (Fredericton)
Pacific Biological Station (Nanaimo, British Columbia)
 Biological Sciences Branch
 Department of Fisheries and Oceans
Queen's University (Kingston)
 Department of Biochemistry
Research Branch (Ottawa)
 Plant Research Center
St. Francis Xavier University
 Department of Nutrition and Consumption Studies
Technical University of Nova Scotia
 Canadian Institute of Fisheries Technology
Universite Laval (Quebec)
 Pavillon Paul Comtois
 Centre de Recherches Nutrition
University of Alberta (Edmonton)
 Department of Pharmacology
University of British Columbia (Vancouver)
 Department of Botany
 Department of Chemistry
 Department of Food Sciences
 Department of Oceanography

University of Calgary (Alberta)
 Department of Microbiology
 Department of Biological Sciences
University of Guelph
 Department of Zoology
University of Manitoba
 Department of Zoology
University of New Brunswick
 Department of Biology
 Department of Chemistry
University of Ottawa
 Department of Chemistry
 Ottawa-Carleton Chemistry Institute
University of Toronto (Ontario)
 Department of Microbiology
University of Victoria
 Department of Biology
West Vancouver Laboratory

CHILE

Pontificia University Catolica of Chile
 Faculty of Biological Science
 Department of Ecology
Universidad Catolica de Valparaiso
 Escuela de Ingenieria Bioquimica
University of Austral Chile (Valdivia)
 Centro Investigaciones Marinas
University of Santiago of Chile
 Faculty of Sciences
 Department of Chemistry

CHINA

Academia Sinica
 Guangzhou Institute of Chemistry
 Institute of Genetics
 Institute of Hydrobiology (Wuhan)
 Institute of Oceanology
 Shanghai Institute of Organic Chemistry
 South China Sea Institute of Oceanology
Beijing Norm. University
 Department of Chemistry
Chinese Academy of Fishery Sciences
 Freshwater Fishery Research Center (Jiangsu)
 Heilongjiang Fish Research Institute (Harbin)
 Pearl River Fishery Institute (Guangzhou)

South China Sea Fishery Institute (Guangzhou)
Yellow Sea Fishery Research Institute (Qingdao)
Dalian Fishery College
Aquaculture Department
Fishery Research Institute Hebei Province (Qinhuangdao)
Fujian Institute of Oceanology (Xiamen)
Hainan University (Haikou)
Fishery Department
Liaoning Norm. University (Dalian)
Department of Biology
Nanjing University
Department of Biology
Office of Yantai (Shandong Province)
Water Conservation
Qingdao Medical College
Department of Neurology
Shandong Institute of Marine Mat. Medicine (Qingdao)
Shandong Marine Cultivation Institute (Qingdao)
Shantou University
Department of Biology
Shenyang Pharmacy College
Animal Chemistry Department
South China Normal University (Guangzhou)
Department of Biology
Ichthyology Laboratory
Xiamen University
Department of Oceanography
Zhongshan University (Guangzhou)
Department of Biology
Laboratory of Pharmacology
Department of Chemistry

CZECHOSLOVAKIA

Akad. Vlastimil Barus
Ustav Syst. Ekol. Biol. CSAV (Brno)
Czechoslovakia Academy of Sciences (Brno)
Institute of Biophysics
Pracoviste Dol (Vltavou)
Vyzk. Ustav Ryb. Hydrobiol. Vodnany

DENMARK

University of Aarhus
Institute of Ecology and Genetics

Kemisk Institute
University of Copenhagen
H.C. Oersted Institute
Department of Genetic and Organic Chemistry
Marine Chemistry Section

ETHIOPIA

Asmara University
Marine Biology Unit

FINLAND

Aabo Akademi
Department of Biochemistry and Pharmacology
Department of Biology
University of Helsinki
Department of Microbiology
Water and Environment Research Institute (Helsinki)

FEDERAL REPUBLIC OF GERMANY

Albert-Ludwigs Universität (Freiburg)
Institut für Biologie und Mikrobiologie
Alfred-Wegener-Institute for Polar and Marine Research (Bremerhaven)
Deutsche Krebsforschungszentral
Institute of Experimental Pathology
Genzentrum/Max-Planck-Institut für Biochemie
Humboldt University
Department of Genetics
Kernforschungsanlage Juelich
Institut für Biotechnologie
Lehrstuhl für Mikrobiologie der Universität München
Universität Köln
Zoologie Institut
Lehrstuhl Tierphysiologie
Max-Planck-Institut für Biochemie
Philipps-Universität
Department of Molecular and Genetic Biology
Rühr Universität
Lehrstuhl Allgemeine Botanik
Universität Bonn
Abt. Futtermittelkd.
Institut Tierernähr.
Entwicklungsgeschichtliche Abteilung des Zoologischen Instituts
Universität Duesbergweg

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Abteilung Angewandte Molekularbiologie
Institut für Physiologische Chemie
Institut für Physiologie und Pathophysiologie
Universität Freiburg
Zellbiologie
Institut für Biologie
Universität Göttingen
Institut Tierz. und Haustiergenet.
Universität Heidelberg
Physiologie Institut
Universität Kiel
Institut für Allgemeine Mikrobiologie
Biologiezentrum
Universität Regensburg
Lehrstuhl für Genetik
Lehrstuhl für Mikrobiologie
University of Bremen
Department of Marine Botany
University of Hamburg
Institute of Cell Biochemistry and Clinical Neurobiology
University of Tuebingen
Institut für Chemie und Pflanzenphysiologie

FRANCE

Association Universite de Joseph Fourier (Grenoble)
Laboratoire Propre CNRS
Centre d'Etudes et de Valorisation des Algues
Centre d'Etudes Nucleiques Saclay (Gif-sur-Yvette)
Service Biochimique
Centre de Brest (Brest)
IFREMER
CERMAV-CNRS (Grenoble)
CNRS (Gif-sur-Yvette)
Departement de Neurochimie
Laboratoire de Neurobiologie Cellulaire et Moleculaire
CNRS (Marseille)
UPR 223 Faculte de Sciences Luminy
Microbiologie Marine
College de France (Concarneau)
Laboratoire de Biologie Marine
CREMA-l'Houmeau (CNRS-IFREMER) (Nieul-sur-Mer)
Ecole Nationale Superieure de Chimie (Paris)
Faculte des Sciences et Techniques de Saint Jerome

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Laboratoire de Phytochimie
IFREMER (Nantes)
IFREMER Centre Brest (Plouzane)
INRA (Rennes)
Laboratoire d'Ecologie et d'Hydrobiologie
I.N.S.E.R.M. Unite 303 'Mer et Sante' (Villefranche-sur-Mer)
Institut National de Sante et Recherches Medicales
Institut Limnologique-INRA (Thonon-les-Bains)
Laboratoire National Ecosysteme (La Temblade)
Musee Nationale d'Histoire Naturelle (Paris)
Association CNRS
Laboratoire de Chimie
Station Biologique (Roscoff)
C.N.R.S.
Centre d'Etudes Oceanologiques et de Biology Marine
Station Marine d'Endoume
Ecole Pratique des Hautes Etudes
UA-CNRS 648 (Brest)
Faculte de Medecine
Laboratoire de Physiology
Unite de Metabolism Energetique-LCB (Marseille)
Universite Angers
Universite de Grenoble
CERMAV-CNRS
Laboratoire de Biochimie Macromoleculaire Vegetale
Universite de Limoges
Unite de Biotechnologie
Universite de Maine
Faculte de Sciences
Laboratoire de Biologie et de Physiology Vegetale
Universite de Montpellier II
Laboratoire Genetique Institut de Science d'Evolution
Universite de Montpellier III
Laboratoire de Zoogeography Genetique
Universite de Rennes
Laboratoire de Biologie de Reproduction
Universite Paris XI (Orsay)
URA CNRS 1121
Laboratoire de Physiology Comparative
Universite Pierre-et-Marie-Curie
Laboratoire de Biologie et Physiology Marines
Station Zoologique (Villefranche-sur-Mer)

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USTL Laboratoire d'Hydrobiologie Marine

GHANA

University of Cape Coast
Department of Zoology

GREECE

Aristotelian University of Thessaloniki
Science School
Laboratory of Animal Physiology

GUATEMALA

Laboratorio Unificado de Control de Alimentos y Medicamentos (Guatemala City)

HUNGARY

Hungary Academy of Sciences (Budapest)
Veterinary Medicine Research Institute

ICELAND

Institute of Freshwater Fishery (Reykjavik)
University of Iceland (Reykjavik)
Science Institute

INDIA

Andhra University (Visakhapatnam)
School of Chemistry
Anna University
Center for Environmental Studies
Annamalai University (Parangipettai)
Center for Advanced Studies in Marine Biology
Banaras Hindu University (Varanasi)
Center for Advanced Studies in Zoology
Fish Endocrinology Laboratory
Central Institute of Medicinal and Aromatic Plants (Lucknow)
Central Drug Research Institute (Lucknow)
Indian Institute of Technology
Center of Rural Development and Appropriate Technology
Department of Chemistry
Konka Agriculture University (Bombay)
Taraporevala Marine Biology Research Station
Kumaun University

Faculty of Science
Department of Zoology
Madurai Kamaraj University
School of Biological Sciences
National Environmental Engineering Research Institute (Nagpur)
Punjab Agriculture University (Ludhiana)
Department of Zoology
Fishery Research Complex
University of Agricultural Sciences (Mangalore)
College of Fishery
University of Gorakhpur
Department of Zoology

ISRAEL

Bar-Ilan University (Ramat-Gan)
Department of Life Science
Laboratory of Fish Immunology and Genetics
Ben-Gurion University Negev (Beer-Sheva)
Department of Electrical and Computer Engineering
Institute of Applied Research
Jacob Blaustein Desert Research Institute
Laboratory of Environmental and Applied Microbiology
Laboratory of Applied Hydrobiology
Microalgal Biotechnology Laboratory
Fish and Aquaculture Research Station (Mobile Post of Hacarmel)
Agriculture Research Organization
Hebrew University (Jerusalem)
School of Applied Sciences and Technology
Oceanography Program
Institute of Life Sciences
Division of Microbiology and Molecular Ecology
Hebrew University of Jerusalem (Eilat)
Department of Biological Chemistry
Biotechnology Unit
H. Steinitz Marine Biology Laboratory
Institute of Life Sciences
Department of Zoology
National Institute of Oceanography (Haifa)
Israel Oceanographic and Limnological Research
Marine Biology Department
Tel-Aviv University
George S. Wise Faculty of Life Sciences
Institute for Nature Conservation Research

Department of Microbiology
School of Chemistry
University of Haifa
Department of Maritime Civilization
Recanati Center for Maritime Studies
Weizmann Institute of Science
Biochemistry department
Yigal Allon Kinneret Limnology Laboratory

ITALY

CNR (Naples)
International Institute of Genetics and Biophysics
Consiglio Nazionale delle Ricerche
Institute of Protein Biochemistry and Enzymology
Istituto di Biochimica delle Macromolecole
Istituto di Biochimica delle Proteine ed Enzimologia
Istituto per la Chimica di Molecole di Interesse Biologico del C.N.R.
Istituto Super. Sanita (Roma)
Dipartimento di Environmental Hygiene
Villa Comunale
Stazione Zoologica
Universita di Napoli
Dipartimento di Chimica
Dipartimento di Chimica delle Sostanze Naturali
Dipartimento di Chimica Organica e Biologica
University of Camerino
Department of Cell Biology
Laboratory of Genetics
University of Lecce
Department of Biology
University of Naples
Department of Zoology
University of Roma
Department of Animal and Human Biology
Department of Biochemical Sciences
Department of Bio-Pathology

JAPAN

Azabu University
School of Veterinary Medicine
Department of Veterinary Pharmacology
Chiba University

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Research Center for Pathology of Fungi and Microbial Toxicoses
Developmental and Reproductive Biology Center
Gifu Pharmacy University
 Department of Public Health
Gifu University School of Medicine
 First Department of Internal Medicine
Gumma University (Maebashi)
 College of Medical Care and Technology
Hiroshima University
 Faculty of Applied Biological Science
 Department of Applied Biochemistry
Hokkaido Regular Fishery Research Laboratory
Hokkaido University
 Faculty of Pharmaceutical Sciences
 Department of Biochemistry
 Faculty of Fisheries
 Department of Food Science and Technology
 Laboratory of Embryology and Genetics
 Laboratory of Microbiology
Ibaraki University
 Department of Agricultural Chemistry
Institute of Physical and Chemical Research (Wako-Shi, Saitama)
Japan Fish Bioscience Institute (Ashiya)
Japan Marine Sciences and Technology Center (Yokosuka)
Kagoshima University
 Faculty of Fishery
Kanazawa University
 Cancer Research Institute
Keio University
 Faculty of Science and Technology
Kitasato University
 School of Fishery Sciences (Iwate)
 Laboratory of Marine Biological Chemistry
 Laboratory of Molecular Endocrinology
 School of Hygiene Science (Kanagawa)
 Department of Biophysics
 School of Medicine (Kanagawa)
 Department of Physiology
Kochi University
 Faculty of Agriculture
 Department of Culture and Fisheries
Kyorin University (Hachioji)

School of Health Sciences
Kyoto Pharmacy University
Department of Natural Product Research
Kyoto University
Yoshida College
Biology Laboratory
Kyushu University
Faculty of Agriculture
Department of Agricultural Chemistry
Faculty of Pharmaceutical Sciences
Meiji Institute of Health Sciences (Kanagawa)
Meiji University (Kanagawa)
Experimental Animal Research Laboratory
Meijo University (Nagoya)
Faculty of Pharmacy
Mitsubishi Kasei Institute of Life Sciences
Miyazaki Medical College
Department of Microbiology
Nagoya City University
Faculty of Pharmaceutical Science
Nagoya University
Department of Chemistry
National Institute of Environmental Studies (Ibaraki)
National Research Institute of Aquaculture (Nansei)
Osaka City Institute of Public Health and Environmental Sciences
Department of Epidemiology
Osaka University
Department of Biology
Faculty of Pharmaceutical Sciences
Research Institute for Microbial Diseases
Department of Bacteriology and Serology
Rakuno Gakuen University (Hokkaido)
Department of Food Sciences
Shimonoseki University of Fishery
Department of Food Sciences and Technology
Laboratory of Microbiology
Shinshu University (Ueda)
Faculty of Textile Sciences and Technology
Institute of High Polymers Research
Toho University
Faculty of Science
Tohoku University (Sendai)

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Chemistry Research Institute of Nonaqueous Solutions
Faculty of Agriculture
Faculty of Sciences
 Department of Chemistry
 Pharmaceutical Institute
Tokushima Bunri University
 Faculty of Pharmaceutical Sciences
Tokyo College of Pharmacy
Tokyo Institute of Technology
 Department of Life Science
Tokyo Metropolitan Institute of Gerontology
 Department of Biochemistry
Tokyo University of Fishery
 Department of Food Sciences and Technology
University of Osaka Prefecture
 College of Agriculture
 Department of Agricultural Chemistry
University of Tokyo
 Department of Agricultural Chemistry
 Department of Botany
 Department of Chemistry
 Faculty of Agriculture
 Laboratory of Marine Biochemistry
 Laboratory of Fish Physiology
 Faculty of Sciences
 Zoology Institute
 Laboratory of Radiation Biology
 Institute of Applied Microbiology
 Ocean Research Institute
 Research Center for Advanced Science and Technology
University of Tsukuba (Ibaraki)
 Department of Chemistry
 Institute of Biological Sciences
Yamaguchi University
 Department of Agricultural Chemistry

KOREA

Hanyang University (Seoul)
 College of Natural Sciences
 Department of Biology
National Fishery University at Pusan
 Department of Food Sciences and Technology

KUWAIT

Kuwait Institute of Scientific Research (Salmiya)
Food Resources Division
Mariculture and Fishery Department
Kuwait University (Safat)
Department of Biochemistry
Department of Botany and Microbiology

MALAYSIA

University Pertanian Malaysia
IDRC-sponsored Asian Fish Health Network

MEXICO

Centro de Investigaciones Cientificas y de Educacion Superior de Ensenada,
B.C.
Division of Oceanography
Center for Genetic Engineering and Biotechnology Research (Morelos)

NETHERLANDS

University of Amsterdam
Laboratory of Microbiology
University of Groningen (Haren)
Department of Microbiology
Vrije University (Amsterdam)
Biology Laboratory
Wageningen Agriculture University
Department of Fish Culture and Fishery
Department of Water Pollution Control

NEW ZEALAND

Fishery Research Center (Wellington)
University of Auckland
Department of Chemistry
Department of Zoology
Leigh Marine Laboratory
University of Canterbury (Christchurch)
Department of Chemistry
Department of Zoology
University of Otago (Dunedin)
Department of Microbiology
Seaweed Research group

NIGERIA

Rivers State University of Sciences and Technology
Department of Biological Sciences
Marine Biology Unit

NORWAY

Austevoll Marine Aquaculture Station (Storeboe)
Institute of Marine Research
Institute of Aquaculture Research (Aas-NLH)
Agriculture Research Council of Norway
Institute of Fishery Technology Research (Bergen)
Institute of Fishery Technology Research (Tromsø)
Institute of Marine Research (Bergen)
Division of Aquaculture
National Veterinary Institute (Oslo)
Norwegian Herring Oil and Meal Industry Research Institute (Bergen)
Norwegian Institute of Natural Resources (Trondheim)
Norwegian Institute of Technology (Trondheim)
Laboratory of Marine Biochemistry
Division of Biotechnology
Organic Chemistry Laboratory
University of Bergen
Department of Biochemistry
Department of Fishery Biology
Department of Microbiology and Plant Physiology
Laboratory of Biotechnology
Marine Genetics A/S
University of Oslo
Department of Biology
Institute of Medical Biochemistry
University of Trondheim
Biology Station
Department of Zoology

PAKISTAN

University of Karachi
H.E.J. Research Institute of Chemistry

PHILIPPINES

International Center for Living Aquatic Resources Management (ICLARM)
(Metro Manila)
Southeast Asian Fisheries Development Center
Aquaculture Department

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University of the Philippines at Diliman
College of Science
Marine Science Institute

POLAND

Agriculture Academy of Krakow
Department of Ichthyobiology and Fishery
Polish Academy of Sciences
Institute of Oceanology

PORTUGAL

University of Azores
Department of Geosciences

RUSSIA

Russian Academy of Sciences
Far East Branch (Vladivostok)
Institute of Marine Biology
Institute of Cytology
Institute of Molecular Biology and Genetics
N.D. Zelinsky Institute of Organic Chemistry
Pacific Institute of Bioorganic Chemistry
Moscow State University
Melozersky Laboratory of Molecular Biology and Bioorganic Chemistry

SENEGAL

Universite Cheikh Anta Diop de Dakar
Departement de Biologie Vegetale
Departement de Chimie

SINGAPORE

Ministry of National Development
Freshwater Fishery Sector
National University of Singapore
Department of Zoology

SOUTH AFRICA

Rand Afrikaans University
Department of Zoology
Rhodes University (Grahamstown)
Department of Chemistry and Biochemistry
University of Natal (Pietermaritzburg)

Department of Botany
UN/FRD Research Unit of Plant Growth and Development

SPAIN

Autonoma University (Bellaterra, Barcelona)
Department of Genetics and Microbiology
Institute of Aquaculture-Torre de la Sal (Ribera de Cabanes)
Universitat de Barcelona
Facultat de Biologia
Department d'Ecologia
Universitat de la Laguna
Instituto Universitario de Bio-Organica
Universidad de la Sevilla
Facultat de Quimica
Department de Bioquimica
University of Cadiz
Faculty of Sciences
Department of Chemical Engineering
University of Las Palmas G.C. (Islas Canarias)
Faculty of Marine Sciences
Department of Biology
University of Malaga
Faculty of Sciences
Department of Microbiology and Histology
University of Pais Vasco (Bilbao)
Department of Cellular Biology And Morphology
Cytology-Histology Laboratory
Department of Microbiology
University of Santiago (Santiago de Compostela)
Department of Microbiology and Parasitology

SWEDEN

Linköping University
Department of Physics and Measurement Technology
National Veterinary Institute (Uppsala)
Royal Swedish Academy of Sciences (Stockholm)
Swedish Institute for Food Research (Göteborg)
Tjärnö Marine Biology Laboratory (Strömstad)
University of Göteborg
Department of Genetic and Marine Microbiology
Department of Zoophysiology
University of Stockholm
Wennergren Institute

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University of Umeå
Department of Microbiology
Uppsala University
Uppsala Biomedical Center
Department of Pharmacy
Institute of Limnology

SWITZERLAND

Friedrich Miescher-Institut (Basel)
University of Zurich
Department of Pediatrics
Division of Clinical Chemistry

TAIWAN

Academia Sinica (Taipei)
Institute of Biology and Chemistry
Institute of Zoology
Division of Biochemistry and Molecular Sciences
National Taiwan Ocean University (Keelung)
College of Fishery
Department of Marine Food Sciences
National Taiwan University
Academia Sinica
Institute of Biology and Chemistry
College of Medicine
Department of Physiology
Institute of Fishery Sciences
Tungkang Marine Laboratory (Pingtung)

TRANSKEI

University of Transkei
Department of Zoology

UNITED KINGDOM

British Antarctic Survey (Cambridge)
Hannah Research Institute (Ayr, Scotland)
Hatfield Polytechnic
Department of Biological Sciences
Division of Biological and Environmental Sciences
Algal Biotechnology Unit
Institute of Oceanographic Sciences Deacon Laboratory (Surrey)
Imperial College of Science, Technology, and Medicine
Department of Chemistry
Lancashire Polytechnic (Preston)

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Department of Applied Biology
London School of Hygiene and Tropical Medicine
Department of Tropical Hygiene
Marine Laboratory (Aberdeen)
Paisley College of Technology
Biology Department
Polytechnic South West (Plymouth)
Department of Biological Sciences
Sonning Aquatic Research Center (Reading)
Aquatic Weeds Research Unit
Southampton University
Department of Biology
University College (Swansea)
Department of Biochemistry
University College of North Wales
School of Biological Sciences (Bangor)
School of Ocean Sciences (Gwynedd)
Marine Sciences Laboratory
University College of Wales
Department of Botany and Microbiology
University of Aberdeen
Department of Clinical Biochemistry
Marischal College
Department of Biochemistry
Department of Cell and Molecular Biology
University of Birmingham
Department of Plant Biology
School of Biochemistry
University of Cambridge
Department of Biochemistry
University of Dundee
AFRC Research Group Cyanobacteria
Department of Biological Sciences
University of East Anglia (Norwich)
School of Biological Sciences
University of Edinburgh
Medical School
Department of Bacteriology
University of Essex (Colchester)
Department of Biology
University of Exeter
Department of Microbiology

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- University of Glasgow
 - Department of Biochemistry
- University of Lancaster
 - Department of Biological Sciences
- University of Leeds
 - Department of Chemical Engineering
 - Department of Pure and Applied Biology
- University of Leicester
 - Department of Microbiology
- University of Liverpool
 - Biochemistry Department
 - Department of Evolution and Biology
 - Department of Genetics and Microbiology
- University of London
 - Queen Mary and Westfield College
 - School of Biological Sciences
 - King's College
 - Department of Biology
 - Royal Holloway and Bedford New College (Surrey)
 - Department of Biochemistry
 - Department of Botany
- University of Sheffield
 - Wolfson Institute of Biotechnology
- University of Stirling
 - Department of Biological Sciences
 - NERC Unit Aquacultural Biochemistry
 - Institute of Aquaculture
- University of Surrey
 - Department of Microbiology

VIETNAM

- National Center for Scientific Research of Vietnam (Hanoi)
 - Institute of Biology