NATIONAL SEA GRANT COLLEGE PROGRAM

MARINE BIOTECHNOLOGY

ANNUAL REPORT FOR FISCAL YEAR 1988

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Abstract

Biotechnology may be defined as the application of scientific and engineering principles to provide goods and services through mediation of biological agents. By this broad definition it is not new. It has been applied for centuries in using micro-organisms to make bread, beer, wine, cheese and other products. However, recent developments in DNA technology indicate that exploitation has not come far compared to its potential. In fact, some authorities expect that high technology, especially biotechnology, will be America's economic panacea and save it from the types of losses suffered by countries like England and France during earlier industrial revolutions. Although marine organisms, like terrestrial organisms, have enormous potential for production of food, chemicals, pharmaceuticals, and services, they have been the subjects of relatively little research geared to their biotechnological exploitation.

In addition to aquacultural research on animal and algal food species, the National Sea Grant College Program funds a small program of research directed toward developing part of the basis for marine biotechnology. In fiscal year 1988 the program supported 55 projects in the following overlapping categories with $2,126,000 in federal funds and $1,625,000 in matching funds:

- biochemistry and pharmacology,
- DNA technology,
- biochemical engineering, and
- microbiology and phycology.

The level of support and number of projects are down sharply (15%) from fiscal year 1987. This reflects in part the drop in federal support for Sea Grant overall, and may indicate a more conservative attitude toward research among state and regional Sea Grant programs at a time of severely inadequate funding. It suggests that the United States will make only a weak effort to be an international competitor in marine biotechnology.

Sea Grant researchers in marine biotechnology as usual have been quite effective over the past year in advancing science and technology. This report provides examples and Appendix A lists 70 of their recent publications.

Introduction and Discussion

Previous annual reports on marine biotechnology in the National Sea Grant Program have defined this field of research, but it is useful to repeat it because it is a broad definition, one encompassing more than DNA technology and genetic engineering. Biotechnology can be termed the use of living organisms or their components to provide goods and services. By this definition its application in sewage treatment and water purification now comprises the largest sector in volume. Production of beer and spirits, cheese and other dairy products, baker's yeast, organic acids, and antibiotics follow in order of decreasing value. These traditional applications of biotechnology, which are based on use of microorganisms, are enormously important to the economy as well as human health and nutrition.

Recent developments in DNA technology have greatly heightened public and commercial interest in and expectations of biotechnology as a means to provide valuable new services and products, particularly complex biochemicals useful in medicine. The potential of biotechnology with terrestrial organisms is far from fully realized. Application of biotechnology in exploitation of marine plants, animals, and microorganisms has been largely unexplored. Sea Grant's small program shows that research advances marine science while producing results with strong commercial potential for providing new biotechnological products and processes. This is significant at a time when international competitiveness is such an important issue. Some experts[1] expect high technology, especially biotechnology, to be America's panacea and to save it from the types of losses suffered by countries like England and France during earlier industrial revolutions.

Japan is taking an aggressive role in biotechnology, including marine biotechnology, through its Ministry of International Trade and Industry. In collaboration with major industrial firms the Japanese government will invest over $200 million over the next ten years to establish and operate a Marine Biotechnology Institute (MBI). The objective of the MBI is to be the first full-scale marine biotechnology research and development base, not only nationally, but globally. It is based on the premise that marine biotechnology is the "greatest remaining technological and industrial frontier."[2]

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[1]Johnston, R.F. and C. G. Edwards, 1987. **Entrepreneurial Science: New Links Between Corporations, Universities, and Government**, Quorum Books, New York, 157pp, p.2.

[2]Committee for the Marine Biotechnology Institute, 1987. **Prospectus for Establishment of Marine Biotechnology Institute**, Ministry for International Trade and Industry, Tokyo.

It is interesting to note that Japanese researchers at the Tokyo University of Agriculture and Technology recently developed methods that induce marine algae to produce large amounts of the enzyme superoxide dismutase (SOD). SOD is a natural antioxidant that converts oxygen radicals into ordinary oxygen molecules. It can be used in a range of medical, cosmetic, and food applications. In December, 1987 **Genetic Technology News** reported a potential annual market for SOD in the United States of $700 million.

The United States takes marine biotechnology less seriously than Japan. However, the Office of Naval Research and the National Cancer Institute support some marine biotechnological research. The National Science Foundation promotes the use of biotechnology in studying oceanic processes. For the past several years the National Sea Grant College Program has invested a small but significant share of its budget in research that will aid in the development of marine biotechnology. Research of this type in fiscal year 1988 is the subject of this report. Aquaculture of food species is not included in this report although a few projects directly relevant to it are.

Tables 1 and 2 below divide the research projects into four categories which are not precisely exclusive of one another. They show the number of projects in each and their levels of funding in fiscal years 1980 through 1988. Total federal and matching funding for these projects was over $3.8 million in fiscal year 1988. This level of support and the number of projects in marine biotechnology are down alarmingly from fiscal year 1987. This 15 percent decrease in total funding, 20 percent in federal funding, reflects in part the drop in federal support for Sea Grant overall, and may indicate a more conservative attitude toward research among state and regional Sea Grant programs at a time of severely inadequate funding. It suggests the United States will make only a weak effort to be an international competitor in marine biotechnology.

TABLE 1
Funding for Sea Grant Projects in Marine Biotechnology in Fiscal Years 1987 and 1988
(in thousands of dollars)

Category	FY 87 No. of Projects	FY 87 Funding Fed	FY 87 Funding Match	FY 88 No. of Projects	FY 88 Funding Fed	FY 88 Funding Match
Biochemistry & Pharmacology	23	916	574	22	810	522
Genetic Engineering	17	778	464	15	730	513
Biochemical Engineering and Industrial Chemicals	11	393	327	8	237	287
Microbiology and Phycology	15	593	424	10	349	303
TOTALS	66	2,680	1,789	55	2,126	1,625

TABLE 2
Federal Funding for Sea Grant Projects in Biotechnology in Fiscal Years 1979 - 1988
(in thousands of dollars)

Category	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988
	FISCAL YEAR									
Biochemistry and Pharmacology	465	440	402	525	440	671	820	865	916	810
Genetic Engineering	---*	---*	100*	266*	419*	487	537	624	778	730
Biochemical Engineering & Industrial Chemicals	246	349	285	454	515	540	581	384	393	237
Microbiology and Phycology	---*	---*	50*	100*	284	248	206	342	593	349
TOTALS	711	789	837	1,345	1,658	1,946	2,144	2,215	2,680	2,126

*Estimate

Appendix C provides a listing of all projects funded in fiscal year 1988 with principal investigators and their institutional affiliations. The funding levels shown are not comparable because some are for more or less than a one year period and some do not include funding for the students and postdoctoral associates assigned to the projects.

Appendix A provides a partial listing of recent publications of the students, professors and postdoctoral associates that conduct the research in these projects. Following each bibliographic citation in parenthesis is a code that identifies a Sea Grant program from which the publications can be ordered. Appendix B provides a key to the codes and addresses for the programs.

Examples of Progress

In general the research is fundamental and long-range in terms of commercial application. However, a good record of commercial development of research results already is accumulating. The general character of the research program in marine biotechnology has changed little over the last year. It continues to advance science and technology. A few examples are presented briefly below.

Several investigators focused on determining the role of microorganisms in **marine fouling and corrosion** as a basis for developing measures to control them. For example, Ralph Mitchell and his students at Harvard University studied two bacteria in the genus **Pedomicrobium** because of their ability to rapidly corrode iron and manganese alloys. Related bacteria have fouled hydroelectric systems by mediating the deposition of manganese encrustations. They hypothesized that exopolymers of the microorganisms play a key role in corrosion. Their initial data show that these bacterial polymers strongly bind iron and manganese, an interaction that appears to correlate with metal oxidation. They are using polarization and electrical resistance probes to study the effect of the exopolymers on oxidative corrosion. (J. P. Black, T. E. Ford, and R. Mitchell, 1988. Corrosion Behavior of Metal-Binding Exopolymers from Iron- and Manganese-Depositing Bacteria, **Corrosion 88**, St. Louis, Missouri, NACE Pub., Houston, TX, Paper No. 94.)

As a result of work with other collaborators, Mitchell also [illegible] the symbiosis of **methylotrophic bacteria and deep-sea** presented evidence that bacteria capable of using reduced C-1 compounds as their carbon and energy sources, occur as intracellular symbionts of the seep mussels. This would represent a symbiosis different from that based on sulfur-oxidizing bacteria at hydrothermal vents in the deep ocean and may suggest additional biochemical abilities that can be exploited for biotechnological processing. (Cavanaugh, C.M. et al., 1987. **Nature** 325, 346-348.)

Nick Anast and John Smit working through the Washington Sea Grant Program studied marine caulobacters, a group of bacteria with a distinctive organelle for attachment. Although one reason for studying them was their possible role in biofouling, the results of their work may have more importance in **manipulation and expression of genes** from the marine world or in introducing foreign genes to the caulobacters to effect useful activities, like metabolism of pollutants, not normal to native strains. They showed the caulobacters have conjugally transferred plasmids that are good candidates for stable cloning vectors. They demonstrated the transfer of plasmids between freshwater and marine caulobacters. (Anast, N. and J. Smit, 1988. Isolation and Characterization of Marine Caulobacters and Assessment of Their Potential for Genetic Experimentation, **Appl. Environ. Microbiol.** 54, 809-817.)

In extension of earlier studies of **biomineralization** and calcium carbonate crystallization, A.P. Wheeler of Clemson University and C. S. Sikes of the University of South Alabama studied synthetic polypeptides modelled after natural materials forming the matrices of molluskan shells. These natural materials inhibit crystallization of calcium carbonate which is a primary component of precipitates that form in and damage a variety of industrial equipment. They demonstrated that a hydrophobic terminus enhanced inhibition and that a polyaspartate molecule of 40 residues appeared to be the optimal size for inhibition. Their work also led them to hypothesize that crystal binding, rather than calcium binding, by soluble components of the shell matrix is responsible for initiation of biomineralization in living mollusks. (Wheeler, A. P. et al., 1987. Evaluation of Calcium Binding by Molluscan Shell Organic Matrix and its Relevance to Biomineralization, **Comp. Biochem. Physiol.** 87B, 963-960.)

Dietrich Knorr and co-workers at the University of Delaware developed a method to form capsules with liquid cores whose permeability can be modified without sacrificing membrane strength. The capsule is formed by dropwise addition of a chitosan solution in to a alginate (polyanion) solution. The resultant capsule consists of a liquid chitosan core with a hard alginate coating. The method is one result of Knorr's program to improve **technology for immobilization of plant, animal, and microbial cells.** (Daly, M.M. and D. Knorr, 1988. Chitosan-Alginate Complex Coacervate Capsules: Effects of Calcium Chloride, Plasticizers, and Polyelectrolytes on Mechanical Stability, **Biotech. Prog.** 4, 76-81.)

William Gerwick and associates at Oregon State University showed a red alga to be a rich source of the **icosanoid immunohormone** 12-(S)-HEPE that is physiologically potent in mammals. The biochemical, which is a potent inhibitor of platelet aggregation and mediator of inflammation, had been previously reported only from animal tissues and cells. (Bernart, M. and W. H. Gerwick, 1988. Isolation of 12-(S)-HEPE from the Red Marine Alga **Murrayella periclados** and Revision of Structure of an Acyclic Icosanoid from **Laurencia hybrida**. Implications to the Biosynthesis of the Marine Prostanoid Hybridalactone, **Tetrahedron Letters**, 29(17), 2015-2018.)

In further **pharmacological studies of manoalide**, a marine natural product with powerful anti-inflammatory activity, Robert Jacobs and associates at the University of California, Santa Barbara, showed that manoalide inhibits the release of arachidonic acid and prostaglandin E_2 in cultured mouse cells and **in vivo**. Their results suggest that the analgesic properties of manoalide are partially correlated with reduced eicosanoid production. (A.M.S. Mayer, K.B. Glaser and R. S. Jacobs, 1988. Regulation of Eicosanoid Biosynthesis **in Vitro** and **in Vivo** by the Marine Natural Product Manoalide: A Potent Inactivator of Venom Phospholipases, **J. Pharm. Exper.Therap.** 244, 871-878.)

William Fenical and his students at the Scripps Institution of Oceanography isolated six new diterpenoid lactones, solenolides, from a previously undescribed Indopacific sea whip, an octocoral from the shallow waters of Palau. The solenolides are modifications of the well-known briarein class of marine metabolites, and several possess potent **antiinflammatory and antiviral properties**. The structures of the new compounds were assigned on the basis of extensive spectral analyses aided by selective chemical modifications. (Groweiss, A., S.A. Look, and W. Fenical, 1988. Solenolides, New Antiinflammatory and Antiviral Diterpenoids from a Marine Octocoral of the Genus **Solenopodium**, **J. Org. Chem.** 53, 2401-2406.)

Boudewijn Brinkhuis and students at the State University of New York, Stony Brook, reported on their investigations to determine whether sporogenesis in the Spring contributes to autumnal recruitment of a marine alga that is a source of **industrial polysaccharides** used widely in the food and pharmaceutical industries. Their studies indicated that only sporophytes prepared from winter and autumn plants could survive summer conditions and that the recruitment observed in autumn can only be the result of the previous autumn's sporogenesis. The phenology and distribution of the alga at the southern limit of its distribution could not be explained by growth response to light and temperature, but may be governed by other factors such as predation. (Lee, J. A. and H. Brinkhuis, 1988. Seasonal Light and Temperature Interaction Effects on Development of **Laminaria saccharina** (Phaeophyta) Gametophytes and Juvenile Sporophytes, **J. Phycol.** 24, 181-191.)

At the University of Maine B. L. Nicholson, Paul Reno and associates have developed a simple and rapid **immunodot assay** for the identification of certain aquatic birnaviruses. The birnaviruses constitute a newly recognized family of viruses, the Birnaviridae. The birnaviruses cause infectious pancreatic necrosis and other diseases in salmonid fishes and branchionephritis in eels. The new assay uses monoclonal antibodies and does not require sophisticated instrumentation. It is expected to find widespread application in the diagnosis of viral diseases of fish. This work represents one aspect of an ongoing program of research to develop diagnostic tools and vaccines against a variety of diseases affecting aqualcultural species of fish. (Lipipun, V. et al., 1989. Enzyme Immunoassay Utilizing Monoclonal Antibodies for Identification of European Eel Virus (EEV), an Aquatic Birnavirus, In **Immunological Approaches to Coastal, Estaurine, and Oceanographic Questions**, Springer-Verlag Pub., C. Yentch, Ed., in press.)

At Oregon State University J.-A. Leong, John Fryer and associates have determined the complete **nucleotide sequence** of the nucleocapsid gene of the infectious hematopoietic necrosis virus(IHNV), which is responsible for a serious disease in salmon, and developed neutralizing **monoclonal antibodies** to several strains of the virus. The antibodies recognized antigenic variants and could be used to separate the viruses into four groups that tended to be related by geographic area. These latter results suggest it may be necessary to develop IHNV vaccines for different geographic areas. (Gilmore, R. D., Jr. and J.-A. Leong, 1989. The Nucleocapsid Gene of Infectious Hematopoietic Necrosis Virus, a Fish Rhabdovirus, **Virology**, in press; Winton, J. R. et al., 1988. Neutralizing Monoclonal Antibodies Recognize Antigenic Variants among Isolates of Infectious Hematopoietic Necrosis Virus, **Diseases of Aquatic Organisms**, 4, 199-204.)

Opportunities for the Future

The results of Sea Grant's investment in marine biotechnology show that academic research can advance science while also providing the technical basis for new research tools and new commercial products and processes. This research has been limited to only a few of the many current and potential areas of marine biotechnology and suggests broader and heavier investment would benefit U.S. science and technology. Some of the possible topics for greater investment and new directions are mentioned below. Common to most areas of biotechnological research is the need to meld chemical and biological sciences and often engineering science as well, yet by and large most projects are conducted from the point of view of a single academic discipline. Few have combinations of investigators representing more than one discipline. It is the kind of disconnection that caused Arthur Kornberg to call for bridging the gap between biological and chemical sciences and the membership of Sigma Xi to express angst in a recent survey over the lack of interdisciplinary training of scientists. Four of five respondents to Sigma Xi's survey agreed that "government agencies [that] make awards for scientific research are dominated by some methodological paradigm that virtually excludes the funding of non-mainstream research." To a significant degree Sea Grant is looking for non-mainstream research.

Table 3 below shows the Sea Grant federal investment in several categories of biotechnological research. Because marine biotechnology is a broad topic and the investment is small, most of the research categories have insignificant or no funding--a situation suggesting many opportunities for the future. The total level of effort is approximately 12 full-time equivalents exclusive of approximately 50 half-time graduate research assistants. This low level of effort will maintain progress at only a slow pace and may tend to make for investment in conservative research related primarily to urgent issues or payoff in the short-term. While some research of this character is required, the long-term advancement of the field in a way that will advance the competitive economic position of the United States requires fundamental research and forays in new directions and high risk areas and sustained development in some. None of the categories is funded near optimal level. Considerable expansion of effort is needed in DNA technology for genetic engineering, particularly of algae, microorganisms, fish and invertebrates and in development of modern diagnostic and assay reagents.

The whole field of biochemical engineering and related biological research as they relate to fermentation, photobioreaction, bioreactor design, processing, and bioprocess instrumentation have had very little attention that relates to exploitation of marine organisms. Research on use of marine organisms in development of biosensors and biogenerators for marine applications also has had little research attention and may offer important opportunities for advancing technology.

The biosensor is an analytical tool that uses an immobilized biological material in contact with a transducer to convert biochemical signals into quantifiable electrical signals. Among other uses biosensors might be used in monitoring environmental pollutants including heavy metals, bacteria, and organic carcinogens. Biogenerators might be used to power remotely controlled devices for which conventional power is inappropriate.

Cell culture of marine invertebrates and associated physiology is another subject of potential importance to biotechnology in the long-term and in which little investment has been made. Many marine organisms especially tropical invertebrates harbor a wide range of useful or potentially useful secondary metabolites. Some are not amenable to synthesis on a practical scale and harvest or aquaculture of the animals from which they come is normally not possible because of environmental concern or slow rate of growth. However, the scientific issues in regard to cell culture are complex, particularly in those animals which harbor endocellular symbionts. Fundamental research in cell culture may set the stage for the use in bioreactors of cells with a wide and fabulous array of metabolic capabilities.

In summary, all of the categories of marine biotechnology warrant increased attention. In some categories there is little or no Sea Grant research. They include the following:

- culture of marine microorganisms, cell culture of invertebrates and associated physiology and nutrition,

- biochemical engineering, including fermentation and photobioreaction, bioreactor design, biosensors, and biogenerators, biocatalyst reactors,

- biochemistry of enzymes, and

- DNA technology for genetic engineering of marine species, including algae, aquacultural finfish, mollusks, and crustaceans, lower invertebrates and microorganisms that may be exploited for specialty chemicals or pharmaceuticals.

Research in all requires or could benefit from interdisciplinary efforts.

TABLE 3
Funding for Marine Biotechnology
by subcategory

	FY 88 Funding (thousands of $'s)
BIOCHEMISTRY & PHARMACOLOGY	
Chemistry of Lipids & Other Small Molecules	500
Biochemistry of Enzymes	45
Chemistry & Physics of Biopolymers	12
Pharmacological & Biochemical Mechanisms of Biological Activity	253
	810
GENETIC ENGINEERING	
DNA Technology for Microorganisms	64
DNA Technology for Algae	132
DNA Technology for Finfish	141
DNA Technology for Molluscs, Crustaceans and Other Lower Animals	190
Diagnostic & Quality Control Reagents	134
Vaccines	69
	730
BIOCHEMICAL ENGINEERING	
Immobilized Biocatalyst Reactors	12
Product Separation & Purification	28
Processing, Bioprocess Instrumentation and Equipment Purchase	197
Fermentation & Photobioreaction	—
Bioreactor Design	—
Biosensors & Biogenerators	—
	237
MICROBIOLOGY, PHYCOLOGY & PHYSIOLOGY	
Physiology & Nutrition of Bacteria, Yeasts, & Fungi	81
Physiology & Nutrition of Micro- & Macroalgae, and Vascular Plants	152
Enhancement of Reference Collections	—
Cell Culture of Higher Organisms & Associated Physiology	—
Biofouling and Corrosion	116
	349
TOTAL FUNDING	2,126

APPENDIX A

RECENT PUBLICATIONS

Keeran, W. S. and R. F. Lee, 1987. The Purification and Characterization of Glutathione s-transferase from the Hepatopancreas of the Blue Crab, **Callinectes sapidus, Arch. Biochem. Biophys.** 255(2):233-243. (GAUS-R-87-004)

Crowe, J. H. et al., 1987. Stabilization of Dry Phospholipid Bilayers and Proteins by Sugars, **Biochem. J.** 242:1-10. (CUIMR-R-87-18)

Anchordoguy, T. J. et al., 1987. Modes of Interaction of Cryoprotectants with membrane Phospholipids during Freezing, **Cryobiology** 24:324-331. (CUIMR-R-87-026)

Quinoa, E. and P. Crews, 1987. Phenolic Constituents of **Psmmaplysilla, Tetrahedr. Ltrs.** 28(28):3229-3232. (CUIMR-R-87-019)

Kernan, M. R. and D. J. Faulkner, 1987. The Luffariellins, Novel Anti-inflammatory Sesterterpenes of Chemotaxonomic Importance from the Marine Sponge **Luffariella variabilis, J. Org. Chem.** 52(14):3081-3083. (CUIMR-R-87-032)

Gleason, F. K. and J. M. Wood, 1987. Secondary Metabolism in the Cyanobacteria, in **The Cyanobacteria**, P. Fay and C. Van Baalan, Eds., pp. 437-452. (MINNU-R-87-004)

Kakau, Y. and P. Crews, 1987. Dendrolasin and Latrunculin A from the Fijian Sponge **Spongia mycofijiensis** and an associated Nudibranch **Chromodoris lochi**, **J. Nat. Prod.** 50(3):482-484. (CUIMR)

Gerwick, W. H., S. Reyes and B. Alvarado, 1987. Two Malyngamides form the Caribbean Cyanobacterium **Lyngbya majuscula, Phytochem.** 26(6):1701-1704. (ORESU-R-87-011)

Colon, M. et al., 1987. 5'-Hydroxyisoavrainvilleol, a New Diphenylmethane Derivative from the tropical Green Alga **Avrainvillea nigricans, J. Nat. Prod.** 50(3):368-374. (PRU-R-87-008)

Lopez, A. W. H. Gerwick, 1988. Ptilodene, a Novel Icosanoid Inhibitor of 5-Lipoxygenase, and Na^{+}/K^{+}-ATPase from the Red Marine Alga **Ptilota filicina, Tetrahedr. Letrs.** 29(13):1505-1506. (ORESU-R-88-004)

Bernart, M. and W. H. Gerwick, 1988. Isolation of 12-(S)-HEPE from the Red Marine Alga **Murrayella periclados** and Revision of Structure of an Acyclic Icosanoid from **Laurencia hybrida, Tetrahedr. Lets.** 29(17):2015-2018. (ORESU-R-88-005)

Mayer, A. M., K. B. Glaser and R. S. Jacobs, 1988. Regulation of Eicosanoid Biosynthesis in-vitro and in-vivo by the Marine Natural Product Manoalide: a Potent Inactivator of Venom Phospholipases, **J. Pharm. Exper. Therap.** 244(3):871-878. (CUIMR-R-88-010)

Baker, B. J., P. J. Scheuer and J. N. Shoolery, 1988. Papuamine, an Antifungal Pentacyclic Alkaloid from a Marine Sponge **Haliclona** sp., **J. Am. Chem. Soc.** 110:965-966. (HAWAU-R-88-002)

Adamczeski, M., E. Quinoa and P. Crews, 1988. Unusual Anthelminthic Oxazoles from a Marine Sponge, **J. Am. Chem. Soc.**110:1598-1602. (CUIMR-R-88-009)

Molinski, T. F. et al., 1988. Petrosamine, a Novel Pigment from the Marine Sponte **Petrosia** sp., **J. Org. Chem.** 53:1340-1341. (CUIMR-88-008)

Rodriguez, A. D., R. K. Akee and P. J. Scheuer, 1987. Two Bromotyrosine-cysteine Derived Metabolites from a Sponge, **Tetrahedr. Letrs.** 28(42):4989-4992. (HAWAU-R- 87-011)

Karuso, P. and P. J. Scheuer, 1987. Long-chain , - bisisothiocyanates from a Marine Sponge, **Tetrahedr. Letrs.** 28940):4633-4636. (HAWAU-R- 87-010)

Manes, L. V. et al., 1988. Chemistry and Revised Structure of Suvanine, **J. Org. Chem.** 53(3):570-575. (CUIMR-R-88-003)

Glaser, K . B. and R. S. Jacobs, 1987. Inactivation of Bee Venom Phospholipase A_2 by Manoalide. A Model Based on the reactivity of Manoalide with Amino Acids and Peptide Sequences, **Biochem. Pharm.** 36(13):2079-2086. (CUIMR-R-87-043)

Carlson, J. L., T. A. Leaf and F. K. Gleason, 1987. Synthesis and Activity of Analogs of the Natural Herbicide Cyanobacterin, **Synthesis and Chemistry of Agrochemicals** 13:141-150. (MINNU-SG-JR208)

Chang, C. W. J., et al., 1987. Kalihinols, Multifunctional Diterpenoid Antibiotics from Marine Sponges **Acanthella** spp., **J. Am. Chem. Soc.** 109(20):6119-6123. (HAWAU-R-87-007)

Albizati, K. F. et al., 1987. Luffariellolide, an Anti-inflammatory Sesterterpene form the Marine Sponge **Luffariella** sp., **Experientia** 43:949-950. (CUIMR-r-87-047)

Molinski, R. F. et al., 1987. Three New Diterpene Isonitriles form a Palauan sponge of the Genus **Halichondria**, **J. Org. Chem.** 53(15):3334-3337. (CUIMR-R-87-038)

Fenical, W., 1987. Marine Soft Corals of the Genus **Pseudopterogorgia**: A Resource for Novel Anti-inflammatory Diterpenoids, **J. Nat. Prod.** 50(6):1001-1008. (CUIMR)

Arabshahi, L. and F. J. Schmitz, 1987. Brominated Tyrosine Metabolites from an Unidentified Sponge, **J. Org. Chem.** 52(16): 3584-3586. (OKLAU-R-87-003)

Bloor, S. J. and F. J. Schmitz, 1987. A Novel Pentacyclic Aromatic Alkaloid from an Ascidian, **J. Am. Chem. Soc.** 109(20): 6134-6136. (OKLAU-R-87-001)

Molinski, T. F. and D. J. Faulkner, 1988. An Antibacterial Pigment from the Sponge **Dendrilla membranosa**, **Tetrahedr.** 29(18): 2137-2138. (CUIMR)

Ettouati, W. S. and R. S. Jacobs, 1987. Effect of Pseudopterosin A on Cell 'Division, cell cycle Progression, DNA, and Protein Synthesis in Cultured Sea Urchin Embryos, **Molec. Pharm.** 31:500-505. (CUIMR-R-87-044)

Ksebati, M. B. and F. J. Schmitz, 1987. New Spongiane Diterpenes form an Australian Nudibranch, **J. Org. Chem.** 52(17):3766-3773. (OKLAU-R-87-002)

Ballantine, D. L. et al., 1987. Antibiotic Activity of Lipid-soluble Extracts form Caribbean Marine Algae, **Hydrobiolog.** 151/152:463-469. (PRU-R-87-007)

Cimino, G. et al., 1987. Revised Structure or Bursatellin. **J. Org. Chem.** 52(11):2301-2303. (OKLAU-R-87-004)

Hwang, S.-P. L., S. L. Williams, and B. H. Brinkhuis, 1987. Changes in Internal Dissolved Nitrogen Pools as Related to Nitrate Uptake and Assimilation in **Gracilaria tikvahiae** McLachlan (Rhodophyta), **Bot. Mar.** 30:11-19. (NYSGI-R-87-005)

Polne-Fuller, M. and A. Gibor, 1987. Microorganisms as Digestors of Seaweed Cell Walls, **Hydrobiolog.** 151/152:405-409. (CUIMR-R-87-041)

Knorr, D. and S. M. Miazga, 1987. Production of Protease from Cell Cultures of Common Milkweed (**Asclepias syriaca** L.), **J. Agri. Food Chem.** 35:621-624. (DELU-R-87-003)

Jannatipour, M. et al., 1987. Translocation of **Vibrio harveyi** N,N'-diacetylchitobiase to the Outer Membrane of **Escherichia coli**, **J. Bact.** 169(8):3785-3791. (CUIMR-R-87-050)

Vreeland, V. et al., 1987. Molecular Markers for Marine algal Polysaccharides, **Hydrobiolog.** 151/152:155-160. (CUIMR-R-87-045)

Downing, S. L. and S. K. Allen, Jr., 1987. Induced Triploidy in the Pacific Oyster, **Crassostrea gigas**: Optimal Treatments with Cytochalasin B Depend on Temperature, **Aquaculture** 61:1-15. (WASHU-R-87-016)

Brinkhuis, B. H., et al., 1987. **Laminaria** Cultivation in the Far East and North America, in **Seaweed Cultivation for Renewable Resources**, K. T. Bird and P. H. Benson, Eds., pp. 107-146, Elsevier. (NYSGI-R-87-011)

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APPENDIX B

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APPENDIX C

MARINE BIOTECHNOLOGY PROJECTS

TITLE/INVES./INST		FED FUNDS	MATCHING FUNDS
I. BIOCHEMISTRY AND PHARMACOLOGY			
AGRICULTURAL PEST CONTROL AGENTS FROM MARINE ORGANISMS ISAO KUBO UNIVERSITY OF CALIFORNIA, BERKELEY	(13)	$ 20,777	$ 22,390
MARINE CHEMISTRY AND PHARMACOLOGY PROGRAM: DEVELOPMENT OF NEW PHARMACEUTICAL AGENTS FROM MARINE INVERTEBRATES D. JOHN FAULKNER UNIVERSITY OF CALIFORNIA, SAN DIEGO	(12)	$ 56,692	$ 17,743
MARINE CHEMISTRY AND PHARMACOLOGY PROGRAM: DEVELOPMENT OF NEW DRUG LEADS FROM MARINE PLANTS AND GORGONIAN CORALS WILLIAM H. FENICAL UNIVERSITY OF CALIFORNIA, SAN DIEGO	(12)	$ 48,196	$ 23,924
MARINE CHEMISTRY AND PHARMACOLOGY PROGRAM: PHARMACOLOGICAL SCREENING AND EVALUATION ROBERT S. JACOBS UNIVERSITY OF CALIFORNIA, SANTA BARBARA	(12)	$ 84,716	$ 18,459
GABA-MIMETIC PEPTIDES FROM MARINE ALGAE AND BACTERIA: A NEW CLASS OF POTENTIAL DIAGNOSTIC AND THERAPEUTIC AGENTS DANIEL E. MORSE UNIVERSITY OF CALIFORNIA, SANTA BARBARA	(12)	$ 18,401	$ 18,459
MARINE NATURAL PRODUCTS IN PHARMACOLOGY: DEVELOPMENT OF LEADS FROM MARINE ANIMALS PHILLIP CREWS UNIVERSITY OF CALIFORNIA, SANTA CRUZ	(12)	$ 25,980	$ 20,985
DEVELOPMENT OF A POTENTIAL ANTI-TUMOR DRUG FROM MARINE WASTE BY-PRODUCT: ANGIOGENESIS INHIBITOR FROM CARTILAGE OF ELASMOBRANCH KIN-PING WONG CALIFORNIA STATE UNIVERSITY, FRESNO	(12)	$ 39,441	$ 24,528

TITLE/INVES./INST			FED FUNDS		MATCHING FUNDS
BIOPOLYMERS FROM MARINE SOURCES JOHN E. CASTLE DELAWARE SEA GRANT COLLEGE PROGRAM	(13)	$	11,700	$	33,024
MENHADEN OIL AS A SUPPRESSOR OF THE GENETIC TRAIT FOR DIABETES AND HEART DISEASE CAROLYN D. BERDANIER GEORGIA SEA GRANT COLLEGE PROGRAM	(12)	$	16,400	$	0
NEW PHARMACEUTICALS AND AGROCHEMICALS FROM MARINE ANIMALS THAT POSSESS SYMBIOTIC MICROORGANISMS MOORE, RICHARD E. UNIVERSITY OF HAWAII SEA GRANT COLLEGE PROGRAM	(13)	$	29,711	$	51,686
NEW IMMUNOMODULATING NATURAL PRODUCTS FROM HAWAIIAN MARINE ALGAE AND TUNICATES BRUENING, REIMAR C. UNIVERSITY OF HAWAII SEA GRANT COLLEGE PROGRAM	(13)	$	3,906	$	16,642
MARINE METABOLITES FOR MEDICINE AND AGRICULTURE SCHEUER, PAUL J. UNIVERSITY OF HAWAII SEA GRANT COLLEGE PROGRAM	(13)	$	31,366	$	16,067
MARINE ORGANISMS AS SOURCES OF AGROCHEMICALLY SIGNIFICANT COMPOUNDS CARDELLINA, JOHN H. MONTANA STATE UNIVERSITY	(13)	$	46,000	$	31,000
MODIFIED NUCLEOSIDES OF MARINE ORGANISMS G. SHARMA WILLIAM PATERSON COLLEGE	(12)	$	5,000	$	2,700
STRUCTURAL AND SYNTHETIC STUDIES ON MARINE NATURAL PRODUCTS JON CLARDY CORNELL UNIVERSITY	(12)	$	62,345	$	37,829
PREPARATION AND ELUCIDATION OF BENEFICIAL BIOLOGICAL EFFECTS OF N-3 POLYUNSATURATED FATTY ACIDS FROM MARINE SOURCES JOHN E. KINSELLA CORNELL UNIVERSITY	(12)	$	39,620	$	65,010

TITLE/INVES./INST		FED FUNDS	MATCHING FUNDS
SYNTHESIS OF PHARMACOLOGICALLY ACTIVE SAPONINS BASED ON SHARK REPELLENTS KOJI NAKANISHI COLUMBIA UNIVERSITY	(12)	$ 13,808	$ 24,865
BIOACTIVE COMPOUNDS FROM MARINE ORGANISMS SCHMITZ, FRANCIS J. UNIVERSITY OF OKLAHOMA, NORMAN	(12)	$ 70,200	$ 35,496
BIOMEDICINALS FROM MARINE ALGAE GERWICK, WILLIAM H. OREGON STATE UNIVERSITY, CORVALLIS	(12)	$ 58,200	$ 19,000
COLD-ACTIVE TRYPSIN PROTEASES FROM COD (GADUS MORHUA) FOX, JAY W. UNIVERSITY OF VIRGINIA, CHARLOTTESVILLE	(11)	$ 45,342	$ 24,944
EFFECTS OF FISH OIL FEEDING AND EPA OR DHA IN HYPERLIPIDEMIA ROBERT H. KNOPP UNIVERSITY OF WASHINGTON, SEATTLE	(13)	$ 50,747	$ 27,000
HERBIVORE DETERRENCE AS AN INDICATOR OF THE PHARMACOLOGICAL ACTIVITIES OF TROPICAL ALGAE PAUL, VALERIE J. UNIVERSITY OF GUAM--MARINE LABORATORY	(13)	$ 31,784	$ 20,200
TOTAL -----------------------------		$ 810,332	$ 551,951

II. MOLECULAR BIOLOGY

VECTORS OF GENETIC ENGINEERING IN MARINE ALGAE: THE TI PLASMID TAYLOR, KENNETH B. UNIVERSITY OF ALABAMA, BIRMINGHAM	(5)	$ 49,812	$ 24,905
RAPID IDENTIFICATION OF BACTERIAL FISH PATHOGENS BY ANTI H COAGGLUTINATION RONALD J. SIEBELING LOUISIANA STATE UNIVERSITY	(8)	$ 40,273	$ 22,346

TITLE/INVES./INST			FED FUNDS		MATCHING FUNDS
SUPERIOR SHELLFISH STOCKS BY POLYPLOIDIZATION HIDU, H. UNIVERSITY OF MAINE	(3)	$	14,558	$	20,111
NEW DIAGNOSTIC TESTS AND ANTIGENIC CHARACTERIZATION OF AQUATIC BIRNAVIRUSES UTILIZING MONOCLONAL ANTIBODIES NICHOLSON, B. UNIVERSITY OF MAINE	(8)	$	68,661	$	73,206
GENETIC ENGINEERING OF FISH AND THE USE OF GH HORMONE TO ENHANCE FISH GROWTH POWERS, DENNIS A. JOHNS HOPKINS UNIVERSITY	(2)	$	78,020	$	43,400
IMMUNOLOGICAL DETECTION OF THE BROWN TIDE ANDERSON, DONALD M. WOODS HOLE OCEANOGRAPHIC INSTITUTION	(7)	$	25,300	$	15,672
DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF THE OYSTER PARASITE HAPLOSPORIDIUM NELSONI (MSX). S.E.FORD RUTGERS UNIVERSITY	(8)	$	0	$	37,100
REGULATION OF LUX GENES IN VIBRIO FISCHERI: CONTROL OF A HIGH-LEVEL GENE EXPRESSION SYSTEM IN A MARINE BACTERIUM E. PETER GREENBERG CORNELL UNIVERSITY	(11)	$	22,671	$	38,542
CREATION OF GENETIC CLONES FOR INCREASED COMMERCIAL PRODUCTION OF BAY SCALLOPS, ARGOPECTEN IRRADIANS RICHARD K. KOEHN STATE UNIVERSITY OF NEW YORK AT STONY BROOK	(3)	$	44,870	$	36,980
CONTROL OF VIRUS DISEASES IN FISH LEONG, JO ANN C. OREGON STATE UNIVERSITY, CORVALLIS	(8)	$	68,500	$	21,800
MOLECULAR CLONING AND CHARACTERIZATION OF PENAEUS VANNAMEI VITELLOGENIN GENE SEQUENCES BRADFIELD, JAMES Y. TEXAS A&M UNIVERSITY, COLLEGE STATION	(1)	$	37,774	$	18,887

TITLE/INVES./INST		FED FUNDS		MATCHING FUNDS
BIOTECHNICAL APPROACHES TO GROWTH IMPROVEMENT IN PACIFIC OYSTERS KENNETH K. CHEW (3) UNIVERSITY OF WASHINGTON, SEATTLE	$	93,114	$	67,800
CELLULAR LOCALIZATION AND MOLECULAR BIOLOGY OF DINOFLAGELLATE TOXINS BARBARA A. BOCZAR (8) UNIVERSITY OF WASHINGTON, SEATTLE	$	40,700	$	14,100
GENE ANALYSIS AND TRANSFORMATION IN MARINE ALGAE R.A. CATTOLICO (5) UNIVERSITY OF WASHINGTON, SEATTLE	$	82,381	$	36,300
GENETIC MANIPULATION OF GROWTH AND PRODUCTION OF SELECTED GREAT LAKES COOLWATER FISHES AMUNDSON, CLYDE H. (2) UNIVERSITY OF WISCONSIN, MADISON	$	63,481	$	41,416
TOTAL ----------------------------	$	730,115	$	512,565

III. BIOCHEMICAL ENGINEERING AND INDUSTRIAL CHEMICALS

MARINE MICROALGAL METABOLITES: THEIR INFLUENCE ON BIVALVE FEEDING RATES AND THEIR IMPLICATIONS FOR BIVALVE AQUACULTURE NANCY M. TARGETT (8) DELAWARE SEA GRANT COLLEGE PROGRAM	$	8,618	$	11,200
USE OF CHITOSAN FOR PLANT BIOTECHNOLOGY PROCESSES DIETRICH W. KNORR (1) DELAWARE SEA GRANT COLLEGE PROGRAM	$	12,266	$	49,529
A NOVEL TECHNOLOGY FOR THE MANIPULATION OF FISH REPRODUCTIVE CYCLES: CONTROLLED RELEASE OF GONADOTROPIN RELEASING HORMONES. ROBERT LANGER (6) MASSACHUSETTS INSTITUTE OF TECHNOLOGY	$	43,000	$	30,984
BIOTECHNOLOGICAL APPLICATIONS OF MARINE BIOPOLYMERS MARCUS KAREL (6) MASSACHUSETTS INSTITUTE OF TECHNOLOGY	$	57,000	$	34,382

TITLE/INVES./INST		FED FUNDS	MATCHING FUNDS
METHODS FOR IMPROVING THE EFFICIENCY OF SUPERCRITICAL EXTRACTION FOR THE FRACTIONATION OF FATTY ACIDS FROM MARINE OILS SYED S.H. RIZVI CORNELL UNIVERSITY	(35)	$ 9,750	$ 18,680
DEVELOPMENT OF COASTAL FISH OIL RESOURCES FOR POTENTIAL U. S. HEALTH INDUSTRY TURCOTTE, J. G. UNIVERSITY OF RHODE ISLAND	(13)	$ 9,000	$ 48,758
ADVANCES IN ANTI-SCALING AND ANTI-FOULING TECHNOLOGY BASED ON THE PROPERTIES OF NATURAL INHIBITORS OF MINERALIZATION A.P. WHEELER CLEMSON UNIVERSITY	(13)	$ 57,000	$ 35,300
CHITIN-CHITOSAN COATED FIBERS G.G. ALLAN UNIVERSITY OF WASHINGTON, SEATTLE	(13)	$ 40,300	$ 28,700
TOTAL ----------------------------		$ 236,934	$ 257,533

IV. MICROBIOLOGY AND PHYCOLOGY

GENETIC ANALYSIS OF BACTERIAL ADHESION IN MARINE BIOFOULING HEATH, HARRY E. UNIVERSITY OF ALABAMA, TUSCALOOSA	(13)	$ 50,699	$ 25,611
GENETICS OF MORPHOLOGY AND GROWTH IN LAMINARIA FROM THE NORTH ATLANTIC OCEAN CHARLES YARISH UNIVERSITY OF CONNECTICUT SEA GRANT PROGRAM	(5)	$ 34,380	$ 18,874
INFLUENCE OF MARINE BACTERIAL FILMS ON THE OXYGEN REDUCTION REACTION AND CATHODIC PROTECTION S. C. DEXTER DELAWARE SEA GRANT COLLEGE PROGRAM	(28)	$ 9,695	$ 14,310
EXPLOITATION OF THE GENETIC RESOURCES OF HALOPHYTES: SOMACLONAL VARIANTS FOR SALT TOLERANT CROP DEVELOPMENT JOHN L. GALLAGHER DELAWARE SEA GRANT COLLEGE PROGRAM	(5)	$ 26,579	$ 99,806

TITLE/INVES./INST		FED FUNDS	MATCHING FUNDS
EXPERIMENTAL TERRESTRIAL FARMING OF SEAWEEDS DOTY, MAXWELL S. UNIVERSITY OF HAWAII	(5)	$ 16,529	$ 42,742
THE ROLE OF IRON AND MANGANESE OXIDIZING BACTERIA IN MARINE CORROSION PROCESSES MITCHELL, RALPH HARVARD UNIVERSITY	(7)	$ 55,000	$ 27,500
MANIPULATION OF SEAWEED-MICROBE ASSOCIATIONS VALRIE A. GERARD STATE UNIVERSITY OF NEW YORK AT STONY BROOK	(5)	$ 32,316	$ 23,459
MARICULTURE AND PHYSIOLOGY OF COMMERCIALLY VALUABLE RED SEAWEEDS FROM BAJA CALIFORNIA, MEXICO BOUDEWIJN H. BRINKHUIS STATE UNIVERSITY OF NEW YORK AT STONY BROOK	(5)	$ 26,137	$ 12,481
STUDIES OF HYDROCARBON SEEP COMMUNITIES ON THE TEXAS/LOUISIANA CONTINENTAL SLOPE BROOKS, JAMES M. TEXAS A&M UNIVERSITY, COLLEGE STATION	(40)	$ 48,629	$ 20,697
CRAB SHELL CHITOSAN, ITS MODE OF GENE ACTIVATION LEE A. HADWIGER WASHINGTON STATE UNIVERSITY	(13)	$ 48,581	$ 17,600
TOTAL		$ 348,545	$ 303,080
GRAND TOTAL		$ 2,125,926	$ 1,625,129