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 in-
creased to 35%. The type of research that industry supports is over-
whelmingly 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 ne-
glect basic research in favor of goal-oriented research, a tendency
that is likely to continue.

Until fairly recently, there was little cooperation between indus-
try and universities. It is only since 1983 that this situation began
changing, after new MESC guidelines defining university-industry
relations came into effect (Koizumi, 1992). Among others, the
guidelines allow university laboratories to undertake proprietary re-
search that companies may develop into products. Further, re-
searchers from industry are allowed to work in university laborato-
ries while still employees of the companies. Over the last ten years
close collaborations have been developed between academic re-
searchers and companies through mechanisms such as joint appoint-
ments, joint publications, consultancy agreements, and contract re-
search. For example, in 1983 there were just 56 joint university-indus-
try research projects, but in 1992 this had increased to 1,241 pro-
jects with 1,398 researchers participating in them (Ministry of Edu-
cation, 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 ap-
plicated research (Koizumi, 1992), while industry "view Japan's univ-
ersities 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 not-
ed 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 ma-
terials and bioactive substances. For this reason, companies were in-
vesting in basic research facilities in order to understand basic phe-
nomena 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-nachi 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, 1994a); 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 bio-reactors 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), Shiseido 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 Chikkairin 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

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Company Name</th>
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<tbody>
<tr>
<td>Actelio Chemical Company</td>
<td>Nichin Company</td>
</tr>
<tr>
<td>Asahi Chemical Industry Company, Ltd.</td>
<td>Nipponge Chemical Company</td>
</tr>
<tr>
<td>Asahi Glass Company</td>
<td>Nippon Kokan, Ltd.</td>
</tr>
<tr>
<td>Chugoku Electric Power Company, Inc.</td>
<td>Nippon Mining-Kyodo Oil Corporation</td>
</tr>
<tr>
<td>Cosmo Development</td>
<td>Nippon Paint Company</td>
</tr>
<tr>
<td>Daihachi Ink and Chemical Company</td>
<td>Nippon Steel Corporation</td>
</tr>
<tr>
<td>Dowa Mining Company, Ltd.</td>
<td>Nippon Sanso Kaisha</td>
</tr>
<tr>
<td>Ebisu Research Company, Ltd.</td>
<td>Nisshin Oil Mills</td>
</tr>
<tr>
<td>Institute of Pearl Science, Ltd.</td>
<td>Omoto Cement, Ltd.</td>
</tr>
<tr>
<td>Fujisa</td>
<td>Pentran, Ltd.</td>
</tr>
<tr>
<td>Hitachi Zosen Corporation</td>
<td>Riko, Ltd.</td>
</tr>
<tr>
<td>Idenmune Kosen Company</td>
<td>Sapporo Breweries Company</td>
</tr>
<tr>
<td>Ibara Chemical Industries</td>
<td>Sekisui Chemical Company</td>
</tr>
<tr>
<td>Ibaraki Sangyo Kaisha, Ltd.</td>
<td>Shimizu Corporation</td>
</tr>
<tr>
<td>Japan Tobacco, Inc.</td>
<td>Shimizu Construction Company</td>
</tr>
<tr>
<td>Hugonome Foods</td>
<td>Shoco Company, Ltd.</td>
</tr>
<tr>
<td>Hazama-Gumi Ltd.</td>
<td>Shinkage-machi</td>
</tr>
<tr>
<td>Kajima Corporation</td>
<td>Sumitomo Kakaku Company</td>
</tr>
<tr>
<td>Kamegashira Chemical Industry Company, Ltd.</td>
<td>Sumitomo Chemical Company, Ltd.</td>
</tr>
<tr>
<td>Kansai Paint Company</td>
<td>Sumitomo Metal Mining Company, Ltd.</td>
</tr>
<tr>
<td>Katakuric Chikkarin Company, Ltd.</td>
<td>Sunory Ltd.</td>
</tr>
<tr>
<td>Katokidai Company</td>
<td>Suzuki and Company Ltd.</td>
</tr>
<tr>
<td>Kawasaki Steel Company</td>
<td>Tasei Corporation</td>
</tr>
<tr>
<td>Kirin Brewery Company</td>
<td>Taisei Fisheries</td>
</tr>
<tr>
<td>Kurobe Hakko Kogyo Company</td>
<td>Tanazakura, Ltd.</td>
</tr>
<tr>
<td>Kyowa Hakko Nippon Steel</td>
<td>Tensei Fisheries, Ltd.</td>
</tr>
<tr>
<td>Kumagawa Gumi Company, Ltd.</td>
<td>Tokyo Electric Power Company</td>
</tr>
<tr>
<td>Maruhana Group</td>
<td>Tonen Sekiyu Kagaku, K.K.</td>
</tr>
<tr>
<td>Merji Seiko Kaisha, Ltd.</td>
<td>Toray Industries, Inc.</td>
</tr>
<tr>
<td>Mikamato Pharmaceutical Company, Ltd.</td>
<td>Toyosh</td>
</tr>
<tr>
<td>Mitsubishi Gas Chemical Company, Ltd.</td>
<td>Toyosoda Manufacturing Corporation</td>
</tr>
<tr>
<td>Mitsubishi Life Sciences</td>
<td>Utsuka Company</td>
</tr>
<tr>
<td>Mitsubishi Rayon Company</td>
<td>Yacoza, Ltd.</td>
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</table>

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 focused 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 colelenterates.

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 <i>Sohgen Maru</i> 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, 1988b). Research has been carried out along four parallel paths at CIUMO (Miyachi, 1993a). 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 focused 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 tribromomethylamine, 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 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 academicians 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 fish, 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 cell 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 (Matsuoto, 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 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 (Schnitz 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 "gynogenics" technology, where only female fish are produced (Anonymous, 1990d). By early 1991, research that was focused 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. Tanuya 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, 1993a).

In addition to finfish, much research is focused on rotifers, coral, sea urchins, mussels, 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 larvae 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 focused 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, 1989b). 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 *Spirodina*. 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 *Spirodina* has increased greatly (Anonymous, 1992). Another GIRI team, headed by H. Kojima, claims to be the first in the world to have successfully transformed *Spirodina* 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 *Spirodina* 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-
important 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, 1992b). 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, 1991). 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 ex-
established its biotechnology research laboratory in 1990, scientists claim to have discovered a blue-green algal species in the Janata hot spring on Shukine Island that absorbs and fixes CO$_2$ 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 CO$_2$ 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. Karnei 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, 1993i). 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 in-
vestigated as important sources of antimicrobial and antibiotic com-
pounds (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 in-
hibits 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 inver-
tebrates to phytoplankton and microalgae, in a search for compounds
with antifungal properties. For instance, Y. Miura at Osaka Univer-
sity and T. Matsumura at the Tokyo University of Agriculture and
Technology are screening microalgae for antibiotic compounds and
have discovered two strains which produce yeast-inhibiting sub-
stances (Gibor, 1990a). At the University of Tokyo, M. Murakami
and K. Yamaguchi have isolated an antifungal polyether macrolide
produced by the dinoflagellate _Alexandrium lituratus_ that prevents fun-
gal growth at a concentration of 0.5 ng/ml (Gibor, 1990a).

Anti-inflammatory, Anti-tumor, and Anti-viral Agents

Anti-tumor substances include anti-tumor polyether
macrolides, cell-division inhibiting substances, antileukemic sub-
stances, cardiotonic 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-deoxytetanolide, 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 cardiotonic 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 _Pilosarcus speculifer_, and from the Red Sea sponge, a _Hemimycete_ species (Schmitz and Yasumoto, 1991). M. Yamasaki at Teikyo University has discovered a glycoprotein in a local sea hare (_Aplysia kurodai_ and shellfish (called "tatsumamigai") that have powerful antineoplastic properties, while manifesting few adverse effects on normal cells (Anonymous, 1993a). 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 okadae_, 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 scytoxol 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 "isololol," 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" (Matsusato, 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 *Chatoceros*. 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. Shiono, 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 paralytic toxins secreted by the sole, *Pandalina* 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 pavonmins and mosescins, and UV absorbing substances, as well as enzymes involved in bioluminescence and enzymes in magnetotactic bacteria (Gibor, 1990a; Kitagawa, 1988; Matisato, 1989; Anonymous, 1990a; 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 “Superbug” 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, alkalophilic, and psychrotropic 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 anylase, breaks down starch and, in the process, forms an end product, the cyclic molecule cycloextrin. 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, 1993).

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 “nukumuri,” 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. (Simdu, 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 phylogenetic 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 eukaryotic 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 Shimyu 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 Shimyu 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 shiborine, 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 Katakichi 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 (more 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, 1993b).

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, 1989a). Similarly, Mitsubishi Rayon Company is producing a biodegradable film, called “Soal,” used for packaging by the food and cosmetics industries. In this case, the basic material is carrageenan from macroalgae (Anonymous, 1993a).

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 “Biomedia 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 exhibiting of molting-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 focused 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. Nakaharo 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 focused on attempting to understand, predict, and prevent red tides, which throughout the ages have been responsible for massive fish kills and serious damage to larval 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 speculated 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. Oshimura 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 Protagonyaulax 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 Ryukyu 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 focused on improving sensitivity, increasing the range of applications, and miniaturization. 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 bloodstream 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, 1992j).

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 Cypardina, 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 Cypardina, 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.

![Bar chart showing frequency of research topics](image)

*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 kōsetsushis 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 focusing 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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