Chapter 5

MARINE BIOTECHNOLOGY AND BIOSAFETY

INTRODUCTION

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

In view of its short history and because its activities have so far been limited chiefly to research, marine biotechnology itself offers few examples that may be used to clarify questions about its safety or risk. We instead must scrutinize the history and experience of the two fields from whence marine biotechnology stems—aquatic biology and general biotechnology—and draw lessons from them. Accordingly, in the following sections we: (1) examine aquatic biology, to evaluate the effects of past introductions of exotic marine organisms into new environments and consider national and international policy responses to them; (2) examine general biotechnology, to identify problems that its activities have generated pertaining to biosafety and consider measures that were taken to alleviate them; (3)
use lessons from the preceding two subject areas to analyze marine biotechnology activities in terms of salinity; and (4) state some concluding thoughts on the advisability of testing transgenic marine organisms in the open environment.

**DISPERALS OF MARINE SPECIES TO NEW ENVIRONS**

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

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

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

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

Mediated dispersals of marine species occur when human activities cause them to be deliberately or accidentally introduced in or transferred to an area where they have never existed before. Since the time when humankind took up sailing, by chance or accident people have affected aqueous habitats throughout the world's oceans, rivers, and lakes, just as people have influenced terrestrial habitats. Ships have carried organisms from one place to another in their ballasts, encrusted on their hulls, and bored in their wooden hulls (Carlton, 1992a; Carlton and Geller, 1993). The ever expanding global network of interoceanic and inter-lake canals have given organisms many new routes for migration. Owners of personal aquariums have released ornamental fish and other animals into local waterways (An-
Traders have carried crustaceans, fish, and mollusks long distances from fishing grounds to market places. Pathogens that afflict these fishery products have been swept along (Carlton, 1989). Thus, since transoceanic trading commenced humans have moved marine species numbering in the thousands across the globe in innumerable patterns.

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

The zebra mussel’s faculty to inflict severe damage stems from its high reproduction rate and ability to settle on, and colonize, a wide variety of surfaces, including stone, steel, concrete, wood, plastic and glass. Under favorable circumstances, its population density can be astounding—over 100,000 individuals per square meter. As a result of these attributes, its biofouling capabilities are high. Zebra mussels have fouled water intake pipes at electric power plants, public water supply facilities, and conventional and nuclear fuel power plants. The weight of colonizing animals has sunk marker buoys and mussel larva have been drawn into the cooling systems of ships, where they settle and grow, causing overheating that damages engines. Mussel biofouling also has damaged harbor structures, canal locks, and flood control mechanisms. Unless control measures can be developed that slow the spread of the animal into new territories, as well as limit or eliminate present infestations, the cost to pay for
damage caused by the zebra mussel will rise to astronomical heights. The economic damage that the zebra mussel will cause by the year 2000 is estimated at $5 billion (Aquatic Nuisance Species Task Force, 1992). Having recognized the peril the country faces, in 1991 the U.S. Congress passed a public law that seeks to meet the dangers posed by the zebra mussel (see below).

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

In the late 1970s and early 1980s, large-scale introductions included striped bass (Morone saxatilis) to the U.S. west coast, the Pacific oyster (Crassostrea gigas) to the U.S. and Canadian west coasts and to France, Pacific salmon (Oncorhynchus species) to Atlantic waters, the pink salmon (O. gorbuscha) to the Arctic Sea coast of the former USSR, a shrimp species from Panama (P. styliodactylus) to Hawaii; and the Pacific seaweed (Undaria pinnatifida) to France (Welcombe,
1986; Sindermann, 1986). More recently, in 1989, the macroalgal species *Eucheuma spinosum* was transported from the Philippines to Zanzibar, where it is now cultured, and the dried product is exported to Europe where polysaccharide is extracted for use as a food conditioner (Zilinskius and Lundin, 1993).

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

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

Sometimes it appears initially as if an introduction is successful, but in the longer term it proves to be detrimental. This may be illustrated by two examples of deliberate introductions that were initially successful, but which ended up disastrously. First, after several attempts had been made during the 1950s to introduce the Nile perch (*Lates niloticus*) into Lake Victoria, the fish became established in the middle 1960s. For a few years local fishermen benefitted as they
were able to garner an average of 60,000 tons of the fish per annum. But in the 1970s harvests declined and, coincidentally, scientists discovered that as *L. niloticus* colonized Lake Victoria waters, it eliminated native cichlid fish stocks found nowhere else. In addition, the only practical way of preserving perch harvests proved to be smoking, which demanded great quantities of wood, spurring the cutting of bushes and trees, and leading to deforestation. It is now clear that the deliberate introduction of the Nile perch caused extensive damage to aquatic and terrestrial biodiversity, while the initial bountiful harvests could not be sustained (Baskin, 1992).

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

A rapidly growing body of literature addresses the causes and effects of damaging dispersals. Some authors discuss and analyze problems pertaining to aquaculture in general (Carlton, 1992b; Courtenay Jr. and Williams, 1992; Davidson et al. 1992). Others write about more specific problem areas, including those pertaining to marine plants (Neushul et al. 1992), mollusks (Farley, 1992), shellfish (Kern and Rosenfield, 1992; Lightner et al. 1992), and finfish (Ganzhorn et al. 1992; Thorgaard and Allen, 1992). Analysis of the effects of past mediated dispersals, whether accidental or incautious deliberate introductions, yields six lessons:
• An introduced animal may disrupt local fauna through competition or predation. In the worst case, the introduction of an exotic species may lead to the extinction of one or more wild species.

• Genetic degradation of the host stock may result from its introduction into a new locale. When an introduced species breeds with wild species, some of its advantageous genetic characteristics may be lost or degraded.

• Genetic degradation of wild species inhabiting the locale where the alien species is introduced may take place. For example, if the introduced species breeds with indigenous wild species, adaptations for survival that the wild species have evolved may become diluted or disappear in hybrid progeny. Even worse, important genes may be lost if the exotic species replaces the wild species.

• When an exotic stock is intentionally transferred to a new locale, exotic disease agents infecting members of the introduced stock may be accidentally introduced at the same time, and these pathogens could attack susceptible indigenous species.

• The exotic species may damage or disrupt some aspect of the habitat into which it is introduced, thereby upsetting natural balances, leading to degradation or destruction of the local environment.

• Once an introduced species colonizes a locale, it may become endemic and impossible to eliminate.

From the foregoing it is clear that the extent of long-term damage of accidental or planned introductions in the marine environment of exotic animal species usually cannot be reliably assessed at the time of introduction, nor is it possible to determine with cer-
tany whether benefits stemming from deliberate introductions ultimately will outweigh costs.

**U.S. and International Measures to Control Dispersal**

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

**U.S. Measures to Control Introductions**

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

Attempts by the U.S. government to prevent damage to the natural environment by the deliberate introduction of exotic species has a short history. In the 19th century, the Supreme Court enunciated the principle that wildlife is the property of all the people and that government is the public trustee in wildlife conservation. Until 1900, state governments exclusively fulfilled this role. However, that year Congress adopted the Lacey Act, which seeks to preserve wildlife through the regulation of interstate commerce. As originally intended, it had three objectives. First, it sought to strengthen and supplement the wildlife conservation laws of states. Second, it allows states to prohibit the importation of wildlife killed legally or illegally in other states. Third, it empowers the Secretary of Agriculture to adopt whatever measures are necessary to prevent importation of birds or animals that have the potential of harming U.S. agriculture or horticulture (Bean, 1983).
The original objectives have been considerably expanded during the 94-year-old history of the Act. Important additions were the Black Bass Act of 1926, which extended the Act's coverage to include fish, and an 1960 amendment that banned the import of animals that not only could injure U.S. agriculture and horticulture, but also U.S. wildlife and wildlife resources. However, the most important augmentation was the Lacey Act Amendment of 1981.

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

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

In 1990, Congress passed Public Law 101-646, also known as the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (hereafter the 1990 Act). Although the primary impetus for the 1990 Act was congressional concern about the zebra mussel infestation of the Great Lakes, it provides a framework for addressing
accidental introductions generally. It has five objectives: (1) to prevent the introduction and dispersal of exotic species into U.S. waters through, for example, ballast water management; (2) to coordinate federally supported research and prevention activities on aquatic nuisance species, especially the zebra mussel; (3) to institute control measures to prevent and control non-intentional introductions of exotic species through means other than ballast water; (4) to minimize impacts when exotic species become established; and (5) to establish a national program for assisting states to control zebra mussels (Kern and Rosenfield, 1992).

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

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

Although it would seem that the federal regulatory structure seeking to prevent and control accidental or deliberate introductions of exotic aquatic species into the U.S. is sufficiently comprehensive
to accomplish its intent, in fact severe problems have impeded its operation. In particular, as explained by Peoples et al. (Peoples Jr. et al. 1992), the FWS has been thwarted in its attempts to formulate policies for enforcing wildlife regulations by a combination of national politics and inertia by the U.S. executive.

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

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

The FWS supports research at the National Fishery Research Center in Gainesville, FL, and two field stations at Stuttgart, AK and Marion, AL. This research encompasses studies on 43 exotic species
that have been introduced into U.S. waters and have become established (Peoples Jr. et al. 1992). The Center also serves as an important information exchange center on exotic fish. It is interesting to note that in the early 1980s, the FWS was asked by the American Fisheries Society to identify exotic fish that might have commercial possibilities for the U.S. Over 2,000 species were so identified (Peoples Jr. et al. 1992)!

This means that the U.S. aquaculture industry has a vast number of possibilities for developing new products to satisfy the growing demand for seafood in the U.S. Of course, as new seafood products are developed, the number of problems associated with introductions will proliferate, potentially creating a nightmare for agencies that regulate these matters.

**International Measures**

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

Due to the uneven implementation of the ICES code by nations, Dr. Carl Sindermann has suggested strategies for dealing with future proposals for introductions (Sindermann, 1986; Sindermann, 1992). The overriding strategy is for UN agencies and non-governmental organizations to educate the public, policy-makers and national regulatory agency personnel about the potential damage that the importation of a non-indigenous species can do to native stocks.
and the local environment. This education, it is hoped, will lead to
the understanding among politicians that it is in the best economic
interest of a country to have a strong regulatory regime in place to
prevent unauthorized introductions and delineate the conditions un-
der which authorized introductions may proceed.

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

SAFETY AND GENERAL BIOTECHNOLOGY

Putative risks inherent to biotechnology research, testing and
applications have been enunciated but never demonstrated. Howev-
er, the history of general biotechnology research, the safety concerns
it generated, and attempts by regulatory agencies to meet these con-
cerns are useful to review. The potential risks posed by the field test-
ing of inanimate and animate products generated by general
biotechnology research and the evolving regulatory regimes that
seeks to lower or contain possible hazards also can be productively assessed.

**General Biotechnology Research and Biosafety**

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

Initially, the NIH guidelines:

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

- created a national Recombinant DNA Advisory Committee (RAC) with headquarters at the NIH, which reviews proposals for projects requiring the use of BL3 and BL4 facilities and adjusts the NIH guidelines in view of new scientific knowledge.

- required each institution receiving government funds to set up and maintain an Institutional Biosafety Committee (IBC), which has initial responsibility for reviewing research proposals involving rDNA experiments presented by local researchers and specifying the conditions under which these should take place. Difficult problems that require policy decisions are referred to the RAC.

With the enactment of the NIH guidelines, scientists began to perform a series of risk assessment experiments to deliberately try to create pathogens. These confirmed that the possibility of accidentally creating pathogens in the laboratory was infinitesimal. There are three major reasons for the safety of research employing genetic engineering. First, the successful invasion, colonization and infection by
a parasite that causes disease in the host is a complex process. Not only is the number of genes involved on both sides large, but also the interactions between these many genes are to a considerable extent dependent on their locations in a three-dimensional space. The probability of recreating this complex milieu by accident when manipulating only one or a few genes is minute. Second, genes include regulatory DNA sequences, called operons, that control in a positive or negative way the expression of genes in each particular cell. It is unlikely that complex regulatory operons would be created by accident in the laboratory. Third, the insertion of foreign genes in a microorganism usually weakens that organism in some way, diminishing its ability to compete with wild organisms or to survive the many stresses of natural conditions outside the laboratory.

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

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

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

As data accumulated proving the safety of biotechnology research, the RAC progressively relaxed the NIH guidelines. Since the
late 1970s, public fears about rDNA research in contained situations, such as laboratories, have largely disappeared.

**Biosafety in Reference to Field Testing**

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

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

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

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

The second concern, the so-called deliberate release issue, is currently receiving much attention by the scientific community and the public. The two possible major risks of deliberate release are: can
the introduced organism directly harm the environment or any of its inhabitants, and can any of the genes that the introduced organism carries disperse and become integrated in the genomes of non-target organisms? The U.S. National Research Council (NRC) has scrutinized the issues related to the field testing of genetically engineered microbes or plants in terrestrial situations and concluded that there are three essential criteria for evaluating the risks associated with a proposed release (United States National Research Council, 1989):

- Are we familiar with the properties of the organism and the environment into which it may be introduced?

- Can we confine or control the organism effectively?

- What are the probable effects on the environment should the introduced organism, or a genetic trait it carries, persist longer than intended or spread to non-target organisms?

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

**Plants.** When evaluating possible risks associated with the field testing of a plant species, the following characteristics must be considered:
• the reproductive potential and biology of the plant, such as its flowers, pollination requirements and seed characteristics, and the history of the plant’s controlled reproduction in an environment similar to the test site;

• the mode of action, persistence, and degradation of any newly acquired toxic property;

• the characteristics of the biological vector used to transfer DNA to the plant;

• the possible interactions with other species and biological systems.

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

• the organism’s capability for dispersal, survival, and multiplication;

• the organism’s interactions with other species and biological systems;

• the organism’s potential for gene transfer;

• the mode of action, persistence and degradation of any newly acquired toxic property.

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

• significant ecological and environmental considerations related to the site that might bear on the safe performance of the field test, such as the water run-off pattern, water table, wind
patterns, and other meteorological and geophysical phenomena peculiar to the test site;

- the size of the site, including a possible safety zone;

- the site's geographic location as related to the nearby or distant presence of biota that could be affected by the organism being tested.

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

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

The first activity inherent to any proposal considered by the USDA is the development of an environmental assessment. The assessment addresses health and environmental concerns by considering both direct and indirect effects stemming from the proposed release. It must convincingly evidence a conclusion that the proposed release would probably not significantly alter or harm any aspect of the environment or its biota. Permission for testing probably would not be forthcoming if the organism to be tested was likely to present high risk to non-target animals or plants; for example, because it possessed characteristics such as enhanced fitness, increased pathogenicity, or contained novel phenotypes. If the USDA assesses a project as having negligible impact on the environment, this finding is published in the Federal Register before a final decision is made in order to give the opportunity to the public and its representatives to scrutinize the assessment report and to comment on it. The agency
must take these comments, as well as statements made by other interested parties, into account before it makes its decision. By the start of 1994, the EPA and USDA has given final approval to over 1,000 applications for field trials of genetically engineered organisms, mostly plants but including two types of transgenic fish (see below). No negative effects have so far been observed, indicating that the scheme seems to be working, at least in the short term (Miller et al. 1991).

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

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

It bears noting that some persons worry about biotechnology industry manufacturing products that could replace natural products. For example, cell culture systems have been developed that mass produce products, such as agar, saffron and vanilla, important to the economies of developing countries that grow the plants from which the natural products are processed for export. Similarly, European farmers have protested against the use of recombinant bovine somatotropin in animal husbandry, claiming that it would result in an
over-production of milk. While these are important socio-economic problems that need to be addressed by governments when they formulate policies for biotechnology research, development and applications, they have nothing to do with the safety of these activities.

BIOSAFETY AND MARINE BIOTECHNOLOGY

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

Experience of General Biotechnology Relevant to Safety of Marine Biotechnology Research

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

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

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

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

Similar to natural inanimate products, when a cell culture system using genetically modified microorganism is developed by marine biotechnology, it will not create an unusual situation demanding extraordinary control measures or regulations. For example, the development of a recombinant killed vaccine against a viral fish disease
would most probably be done using procedures similar to those used to develop vaccines for other animals; the field testing of the fish vaccine would most likely follow established animal vaccine testing procedures; and the developmental and testing process would be monitored adequately by existing national regulatory authorities. Consequently, present protocols for testing products produced by conventional or advanced biotechniques are appropriate for use in the testing of marine biotechnology products.

Field Testing of Transgenic Marine Organisms

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

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

Several authors have already considered biosafety aspects of the future field testing of various transgenic marine organisms (see Table 1). We draw much information from this body of work to summarize the most important biosafety concerns related to the field testing of marine organisms in general, and the more specific concerns
in regard to macroorganisms (including transgenic finfish, shellfish, mollusks and other invertebrates, and plants) and to microorganisms (marine bacteria and microalgae).

**General Marine Biotechnology Safety Concerns**

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

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

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

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

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

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

Macroorganisms

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

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

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

The test proposal was strenuously opposed by various environmental groups, including the Foundation on Economic Trends and the National Wildlife Federation, on grounds that the tested carp could escape from the testing ponds and reach nearby watersheds,
where it could damage insects, plants and other fish in fresh water habitats. In view of these concerns, which had been inadequately addressed in the initial testing proposal, the proposal was remanded and its drafters were asked by the USDA to provide more information about possible environmental impacts.

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

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

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

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

Duplicating the contained system used in the mirror carp experiment in the marine environment would be extremely difficult. As noted by Medley and Brown (1992):
In marine systems the affected environment could be broadly
defined, which would increase substantially the issues and
concerns that would need to be addressed in environmental
documents such as an environmental impact statement.
Therefore, outdoor testing of genetically engineered marine
organisms, other than in contained ponds, would require a
complex environmental analysis.

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

- Improved metabolism—for the purpose of speeding up mat-
  uration, attaining larger adult growth, increasing reproduction
  rates, lowering the amount of fat in body tissues, and/or im-
  proving food utilization. Examples of relevant research are the
  development of transgenic carp and catfish that contain
  growth hormone genes from trout.

- Improved tolerance to physical factors—to make it possible
  for the target organism to better tolerate colder or warmer
  water, water of different salinity contents, higher concentra-
  tions of metals or pollutants, and/or lower concentrations of
dissolved oxygen. An example of relevant research is the at-
tempt in Canada to develop transgenic salmon containing
genes from flounder coding for an antifreeze protein.

- Improved chemical constituents—a marine plant may be en-
gineered to overproduce substances that are valuable as phar-
naceuticals or specialty chemicals. For example, several projects are underway to develop macroalgae that produce commercial quantities of carrageenans.

- Improved control over settling—the spat from most molluskan species require specific chemical signals before they will settle and grow on a surface. If species valuable to aquaculture could be engineered to settle in a controlled manner, the efficiency of aquaculture operations would increase significantly. Although research on larval settling is in progress (see Chapter 1), practical applications are far from realization.

- Improved resistance to diseases—an organism’s ability to resist pathogens may be increased by engineering it to produce more or different cytokines, higher concentrations of antibodies against common bacterial, viral and fungal pathogens, and/or immune enhancers. Research on the application of marine biotechnology to animal health is discussed in Chapter 1.

- Improved food quality characteristics—traditionally, conventional breeding has been done to improve the characteristics of animals and plants used for human food, such as texture, color, fillet size, and nutritive content. In this vein, thought is being given to modifying edible Pacific seaweed for improved use as sushi and transferring food properties from Pacific seaweed to Atlantic seaweed that presently cannot be used as food. Although research in this area is not yet being undertaken, as far as we know, it could commence at any time since sufficient knowledge and appropriate techniques are available.

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

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

No one has yet proposed the testing of a transgenic marine macroorganism in unsecured facilities or the open marine environment. Before this can occur, technical barriers must be overcome and environmental risks reduced. The technical barriers relate to the cost-effective transfer of valuable genes and promoters into large number of fish; the ready identification of transformed individuals among the treated group; and the selective breeding of transformed fish to develop superior progeny. In 1990, it was estimated that it will take a minimum of ten years to overcome these technical barriers (Kapuscinski, 1990).
The major means whereby risks related to transgenic fish may be reduced could be to sterilize all fish to be used for outgrowth in culture. Fish may be sterilized by two methods. First, hormones can be administered to fish embryos, which render them sterile. Researchers do not favor this method since it cannot achieve 100% sterilization and hormonal residues may contaminate food fish. Second, fish eggs can be treated so the progeny are triploid; i.e., each fish carries three sets of chromosomes rather than two (see Chapter 1). Triploids are sterile. For added safety, triploid induction can be combined with further treatment that produces an all female progeny. Triploid females are 100% non-fertile.

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

**Microorganisms**

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

In view of the technical and environmental difficulties that have to be overcome before a transgenic macroorganism is field tested, it may be more likely that a microorganism will be the first candidate for field testing. Since much research is being done to genetically engineered bacteria (for bioremediation) and microalgae (for increased production of food additives and enhanced CO₂ uptake to counter the "greenhouse effect"), one of these should be considered as the primary candidates for the first marine field testing. Further support for this contention comes from the report that the firm Envirogen
Inc. in New Jersey is preparing a proposal for the field testing of a bacterium that has been genetically engineered to improve its ability to degrade the industrial pollutant trichlorethylene (Anonymous, 1991b).

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

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

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

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

The second gene exchange mechanism is transduction, where a vector transfers genetic material from one cell to another. For example, viruses that infect bacteria, called bacteriophages (or phages), may transfer genes between bacterial cells. In general, phages are specific, one type of phage will attack only a single bacterial species. Results from recent research demonstrates that an immense number of viruses and viral particles populate the ocean surface layer (Proctor and Fuhrman, 1990; Suttle et al. 1990; Wommack et al. 1992).
These data indicate that one milliliter of surface water can contain between \(10^7\) and \(10^9\) viruses, which means that the one millimeter thick surface layer of the world's oceans would contain a total of \(3.6 \times 10^{18}\) viruses (Anonymous, 1990b). The role of viruses in the marine environment is unknown, although it is believed that most of them are phages, attacking species of marine bacteria, microalgae, plankton and other organisms. Whether transduction occurs in nature between marine bacteria and cyanobacteria—or between different cyanobacteria—is not known, but may be possible and is under active study (Ciferri et al. 1989).

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

**Terrestrial Versus Marine Biotechnology**

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

The difference, then, is the field testing of genetically engineered organisms. We have noted that the USDA has given permission for the testing in contained, open-air ponds of two genetically engineered aquatic organisms—a transgenic carp and a transgenic catfish (see above for a more detailed description of one of these experiments). These tests are likely to generate much data on the growth patterns of the transgenic species, their behavior, and so forth, but since they are being carried out in closed systems, they cannot be considered more than prototypes to the future field test-
ing of transgenic marine animals in the open marine environment. Here the researcher and the regulator would face special problems not encountered in contained field trials. Similar to field tests in the atmosphere (Stetzenbach et al. 1992), in the open marine environment it may be impossible to ensure the biological isolation of the organisms being tested. Biological isolation cannot be guaranteed because of the continuity and movement of the ocean waters and the existence of potentially many unfamiliar biological modes for gene dispersion. The lesson from dispersals is that after release, the subsequent dispersal via natural mechanisms cannot be predicted, so the consequences of escapes also are unforeseeable.

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

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

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

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

In summary, the present regulatory situation does not favor the field testing of transgenic marine macro- or microorganisms in the aquatic environment. Due to the many uncertainties that would accompany the field testing of transgenic marine organisms regulatory agencies should not allow the field testing of organisms in the marine environment until research in biological oceanography, microbial ecology, and environmental toxicology has clarified the details of the mechanisms of dispersal of organisms and genes in the marine environment and a satisfactory risk assessment methodology for field testing in the oceans has been developed. It can be seen that the greatest need at the present time is for research to be done that would lay a scientifically-sound basis for the safe and effective field testing of genetically engineered organisms in the marine environment.
### Table 1. Articles Related to Transgenic Marine Organism and Safety.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Reference Number(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenic finfish</td>
<td>(Office of Agricultural Biotechnology, 1990) (Parker, 1992)</td>
</tr>
<tr>
<td>Transgenic shellfish</td>
<td>(Thorngard and Allen, 1992)</td>
</tr>
<tr>
<td>Transgenic mollusks</td>
<td>(Cembella and Sharkey, 1994)</td>
</tr>
<tr>
<td>Transgenic invertebrates (other)</td>
<td>(Anonymous, 1992)</td>
</tr>
<tr>
<td>Transgenic plants</td>
<td>(Mens and Van Euten, 1992)</td>
</tr>
<tr>
<td>Transgenic microalgae</td>
<td>(Ciferni et al., 1989) (Skujins, 1992)</td>
</tr>
<tr>
<td>Transgenic marine bacteria</td>
<td>(Colwell, 1992) (Crane and Pritchard, 1992) (Geall, 1992) (Save and O'Morchoe, 1992)</td>
</tr>
</tbody>
</table>

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