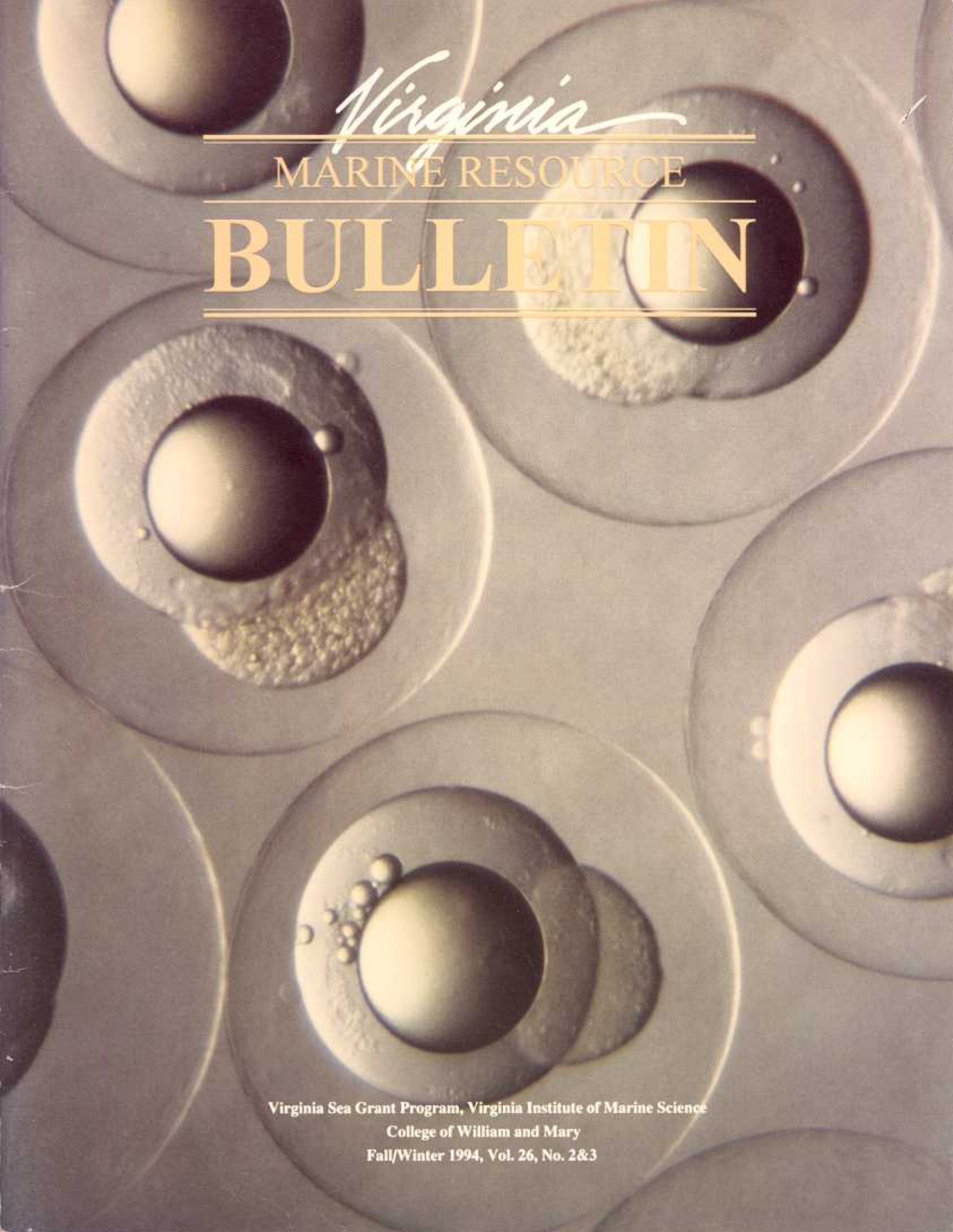

Virginia MARINE RESOURCE BULLETIN

Virginia Sea Grant Program, Virginia Institute of Marine Science
College of William and Mary
Fall/Winter 1994, Vol. 26, No. 2&3

Virginia
MARINE RESOURCE
BULLETIN

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Fall / Winter 1994 **Vol. 26, No. 2 & 3**

Contents

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The Marine Resource BULLETIN is a quarterly publication of Marine Advisory Services of the Virginia Sea Grant College Program which is administered by the Virginia Graduate Marine Science Consortium with members at the College of William and Mary, Old Dominion University, University of Virginia and Virginia Polytechnic Institute and State University. Subscriptions are available without charge upon written request.

The BULLETIN is intended as an open forum for ideas. The views expressed do not imply endorsement, nor do they necessarily reflect the official position of Sea Grant or the Virginia Institute of Marine Science.

Sea Grant is a partnership of university, government and industry focusing on marine research, education and advisory service. Nationally, Sea Grant began in 1966 with passage of the Sea Grant Program and College Act.

The BULLETIN is printed on recycled paper.

Photo on opposite page represents hydrogel chitosan beads, made from crab shell, in aqueous medium. The solutions tend to form fibers instead of "beads" at higher solution concentration; this produces the stringy protrusions seen on some beads. The beads are useful for ion exchange of heavy metal ions.

For centuries, the most manifest, the most visible exploration involved great physical distances—across oceans, continents and even into the depths of submarine canyons. Perhaps the apex of this type of discovery was when humans traveled through the Earth's atmosphere, through space, to the heavenly body which orbits us at a distance of about 237,275 miles, the moon.

In this century and part of last, a counterpart to the outward exploration took place except this one was the inverse of the other: on a minute, if not molecular level, remarkable distances were being traveled. Advances in chemistry meant that substances could be made to replace what is found in the natural world and could be designed and altered for different applications. Synthetic materials are ubiquitous in our society, and are used from cradle to grave.

Progress in biology was no less remarkable. The mechanisms of DNA, the blueprint for life, were elucidated, unveiling a powerhouse of information about ourselves and our surroundings. Findings in biology and chemistry would even unlock some of the mysteries within *Homo sapiens'* very own brain, the mysteries behind normal functions, moods, and even disorders.

Breakthroughs in electronics made it possible at first to see visual images within the home on what was once novel, the T.V. Later, via computer technology, it would make it possible to process astronomical amounts of information and to send or access information all over the world, within minutes or seconds.

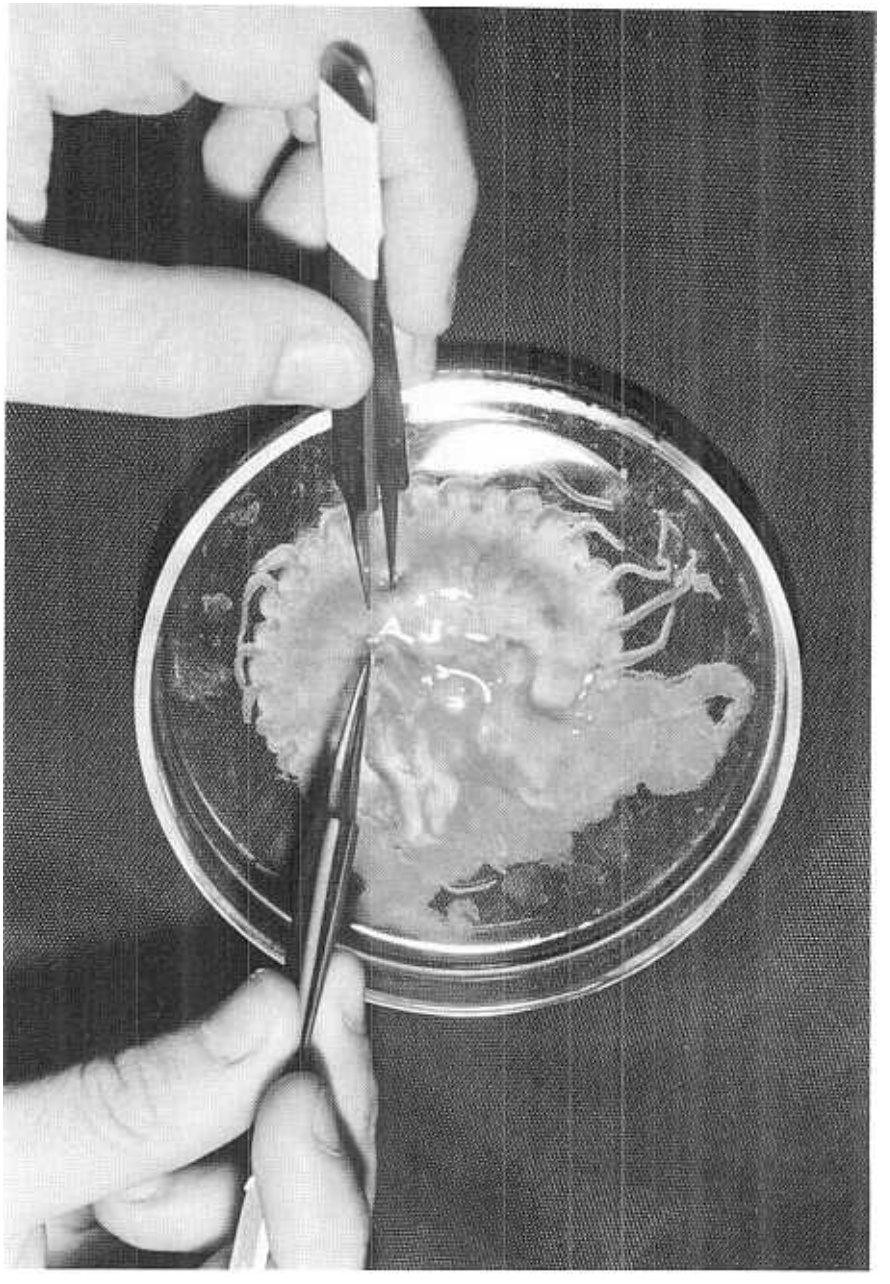
While the sciences moved forward at an astounding pace, the lines between disciplines became less rigid; scientists were utilizing information from different areas for work in their specialties. At the same time, subsets of the traditional scientific areas emerged. Biotechnology is one such area, a science which employs information from practically every sector. Marine biotechnology is subset of a subset, a grouping which is fairly new.

(Continued on the next page)

Marine biotechnology is an uncertain but exciting realm of exploration. Oceanic life forms can be pronouncedly different than terrestrial ones. It is this difference that interests researchers. The variety of organisms, and their chemical uniqueness may be a storehouse of information for the future.

The extent of marine biotech research can only be hinted upon in the following pages, but it ranges from using the neurological systems of cuttlefish and octopus as a research basis for fifth-generation computer development, to finding a way to convert an alga—which only uses sunlight as an energy source—to the production of unlimited amounts of hydrogen for a source of power.*

The intent of this issue of the *Bulletin* is to provide readers with a sampler of marine biotech possibilities, and to highlight a few Virginia projects.



Marine Biotechnology . . . What Exactly Is it?

The definition of marine biotechnology appears to be as general or specific as an individual author's personal bias. Even so, one way of explaining what marine biotechnology can encompass is to divide it into three broad and oftentimes overlapping categories.

The first category is somewhat obvious, though at times surprising in its results: a product from the marine environment is used as it naturally occurs, or is changed into another form for human use. Examples: the discovery of oil-degrading microorganisms in the marine world has led some researchers to believe that a new means has been found for environmental remediation. Crab shells—an environmental headache for processors—can be altered into a number of forms, ranging from beads which absorb

*Specifically, a Japanese research group is seeking to transform a species of thermophilic (an organism that thrives at high temperatures) alga, *Synechococcus elongatus*, by introducing the genes from the bacterial species *Clostridium* that code for enzymes producing hydrogen gas.

heavy metals, to artificial skin for burn victims.

The second division is a natural outcome of the last. Instead of *using* a specific marine product, scientists enlist the information they have obtained from the natural world and utilize it for a human application. To develop drugs which treat inflammatory diseases such as arthritis, California Sea Grant researchers have been examining marine organisms, searching for a modern-day elixir—pain inhibiting compounds which could be the basis for synthetic versions.

The third category entails using biology as a tool toward an objective, one which can range from genetic manipulation to stock identification. Growth enhancement of fish has been an active field, with scientists working both with growth hormones and with transgenics, the transferal of genetic traits from one species to another. Another example of biology as a tool would be the DNA "fingerprinting" of a stock to determine if the species represents one, two or more genetic groups.

Even though marine biotechnology is a promising frontier, it is an uncertain one. The degree to which any area is developed is subject to the availability of funding and the practicality of applications. Case in point: crab processors in Virginia will probably continue to process crab shells into meal for livestock. The crab shells are now considered waste, with an inherently low value. The supply of shells is seasonal and uncertain. A plant to reprocess shells for medicinal or other purposes is too far removed from local blue crab processors. As it currently stands, it is more feasible to process crab shells into meal or for it to be taken to a landfill. This, however, could change. ✥ ✥

Marine biotechnology is not necessarily new. Innovative people have always looked to the resources around them for new products. A particularly inventive soul thought to weave byssal threads from a bivalve into what has been called cloth-of-gold, an exceptionally sheer fabric with a metallic sheen. A byssal thread is a fine thread that a bivalve uses to attach itself to a stationary object. The source of the byssal threads for cloth-of-gold was the pen shell *Pinna nobilis*, a Mediterranean bivalve which can grow to a length of two feet and has a tuft of byssal threads, gold-bronze in color. A tuft of threads (as opposed to a singular thread for each bivalve) made collection easier. Even so, the amount of threads needed for three ounces of fabric is sizeable—in the neighborhood of a pound.

Cloth-of-gold is believed by some historians to be the Golden Fleece of Greek mythology. Even if that were not the case, it has been used for the raiment of royalty right up to fairly modern times. Even Queen Victoria was said to have worn this fabric from the sea.

Marine Biotechnology, The Possibilities

The National Sea Grant College Program sponsors research in support of marine biotechnology. The Sea Grant role is expected to increase and may be given a substantial boost through an act which is pending congressional consideration, the Marine Biotechnology Investment Act.

With adequate funding, research could potentially open new avenues for monitoring health and treating disease; provide innovative techniques to restore and protect aquatic ecosystems; increase the food supply through aquaculture; enhance seafood quality and safety; develop new types and sources of industrial materials and processes; and expand knowledge of biological and geochemical processes in the world ocean.

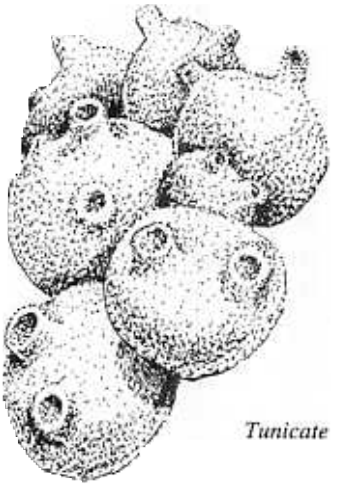
In the following list are a few examples of recent marine biotech developments. The categories are general groupings which the National Sea Grant College Program has identified as areas worth pursuing. The projects cited are not necessarily the product of Sea Grant research, but illustrate areas of interest.

Molecular Genetics

- Certain Antarctic fish produce a natural antifreeze, called glycoproteins, which inhibit ice crystal growth in their tissues; use of molecular techniques may permit their large-scale production for research and practical application. It may be possible to transfer the genes coding for this natural antifreeze to other species in order to improve their growth and survival in cold environments.
- DNA-fingerprinting techniques demonstrated that traditional stock assessments of Atlantic salmon were erroneous, resulting in a large share of valuable United States stocks being given over to European fishing interests.

Bio-Organic Chemistry and Pharmacology

- Manoalide, an anti-inflammatory and analgesic agent isolated from a Pacific sponge, is now in clinical trials. Its action differs from that of standard drugs and it appears free of the side-effects of steroids.
- A substance isolated from shark cartilage inhibits blood supply to tumors, thus restricting their growth. Bryozoans and tunicates have also yielded novel compounds with highly specific antitumor activity; some are now undergoing clinical trials.
- Halenquinone, isolated from a sponge, is a powerful new antibiotic, while didemnin, from a tunicate, exhibits antiviral and anticancer activity.



Tunicate

Immunobiology and Pathology

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

Endocrinology and Developmental and Reproductive Biology

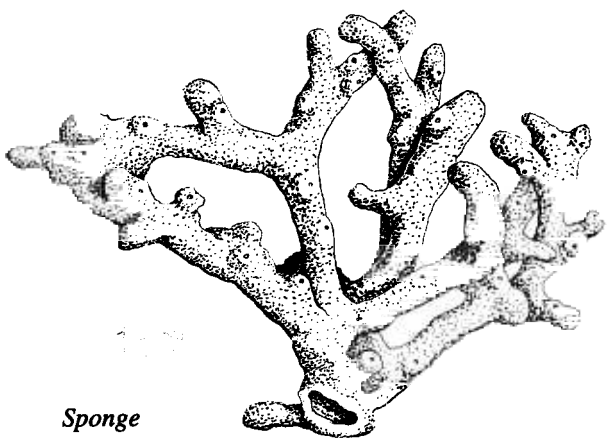
- Cloning of growth hormone and growth promoting factor genes of some commercially important fish has led to production of rapidly-growing stocks. Injections of growth hormone biosynthesized by bacteria containing this gene increase the growth rate of trout, while transgenic carp and catfish containing copies of this gene grow up to 50% faster than controls.
- Identification of factors controlling spawning and settlement of abalone and oysters has allowed synchronized spawning in captivity, leading to the development of commercial hatcheries for these valuable shellfish.

Environmental and Evolutionary Biology

- Use of gene probes to rapidly identify and enumerate marine organisms, particularly small but ecologically important forms such as phytoplankton and zooplankton. This work is currently extremely labor intensive, and can be a major bottleneck in oceanographic research.
- Investigation of harmful effects of the ozone "hole" in the Antarctic are being carried out by examination of DNA of marine organisms exposed to increased UV radiation. UV-tolerate forms are also being examined for potential natural sunscreens.

Aquaculture

- Development of a triploid oyster that makes possible year-round rather than seasonal oyster production has contributed greatly to the revival of the West Coast oyster industry.
- Development of vaccines against two major diseases of salmon, IPN and IHN virus sets the stage for commercial development of improved vaccines to increase survival in cultured trout and salmon.

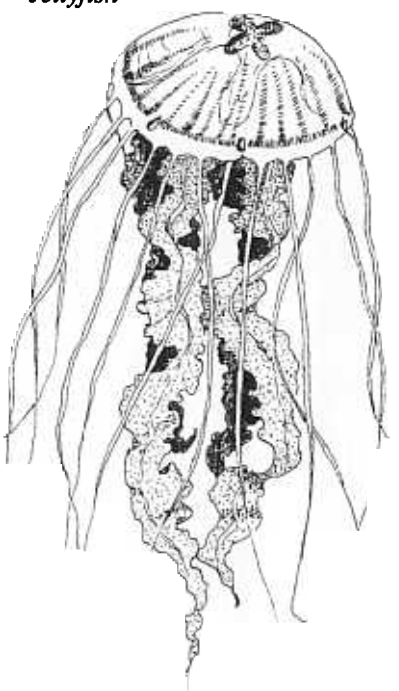


Sponge

Environmental Remediation

- Oceanic bacteria have been discovered that directly oxidize and precipitate iron, manganese, cobalt, nickel and other valuable and strategic metals. The genes and enzymes of these bacteria may be the key to separating these metals from low-grade ores, bypassing more expensive and environmentally-damaging industrial processes now employed.
- A test plant employing naturally occurring bacteria which degrade phenols has demonstrated a 99% drop in the concentration of chlorinated phenols, from 100 to 1 part per million in a bioreactor system. These procedures were tested successfully by the Environmental Protection Agency at a Superfund site, and two firms have contracted to use these procedures in commercial applications.

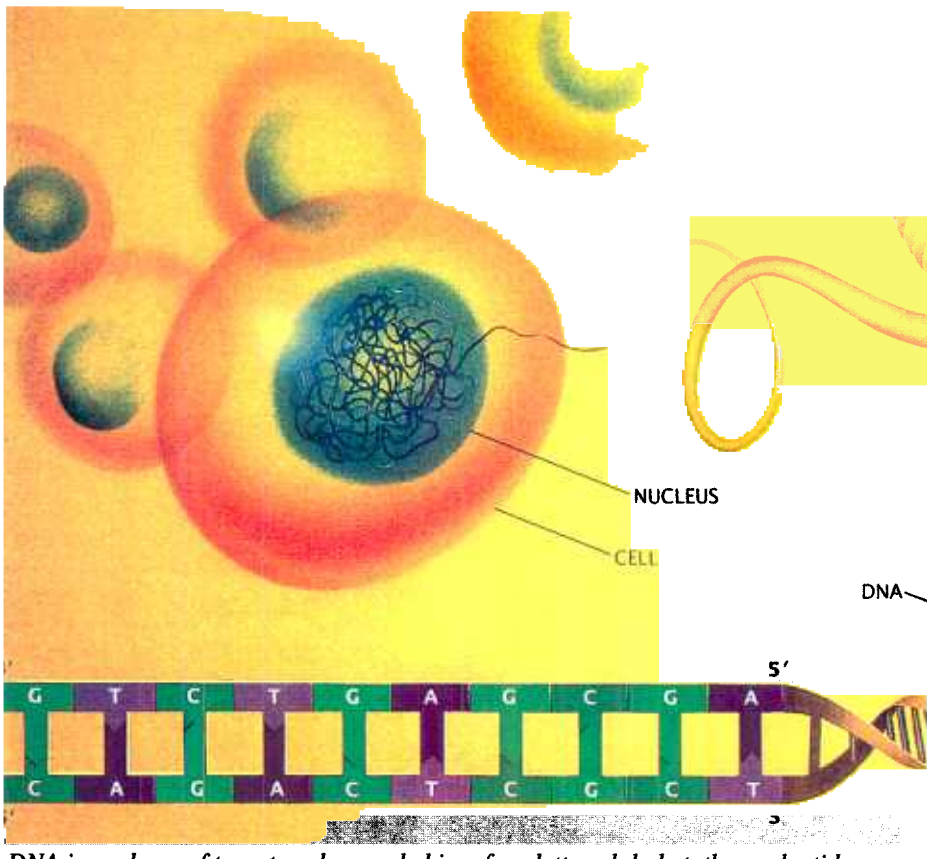
Jellyfish



DNA Analysis, A Powerful Molecular Tool

The article in the April 1953 issue of *Nature* was little more than a page long, in between a confluence of scientific, anthropologic and agricultural studies. Yet, the "Molecular Structure of Nucleic Acids," in which authors James D. Watson and Francis Crick postulated the structure of deoxyribonucleic acid (DNA) was one of the most influential scientific papers of the century; the double helix structure was a substantial clue to understanding how the DNA molecule encodes and transmits genetic information.

Scientists Watson and Crick no doubt realized the importance of what they were describing, but they may not have anticipated how widespread and even pragmatic the eventual applications of DNA analysis would be. DNA analysis as a molecular tool is being utilized in many scientific disciplines—including marine science.



DNA is made up of two strands encoded in a four letter alphabet, the nucleotides thymine (T), adenine (A), cytosine (C), and guanine (G). A's are always opposite T's and G's are opposite C's. Illustration courtesy of Scientific American *Inc., George V. Kelvin©.*

DNA is the blueprint for life. Both in animals and plants, the majority of DNA is found in the chromosomes of a cell's nucleus. The information is encoded in DNA's four letter alphabet, its nucleotides— thymine (t), adenine (a), cytosine (c), and guanine (g). A specific sequence of these "letters," is called a gene, and has part of the information necessary for constructing components of an organism.

To say that the information within a chromosome's DNA is massive, is only to hint at the magnitude of information; the DNA chain can contain millions or billions of nucleotides.

For some DNA research, it proved easier to work with mitochondrial DNA. Although 99.9% of DNA is found in an animal cell's nucleus, the remaining is located in the mitochondrion, a specialized subcellular structure, the "powerhouse" of the cell. The number of nucleotides in this location is far less daunting; in a fish the mitochondrial DNA comprises approximately 16,000-20,000 nucleotides, as opposed to information in the nucleus which can number a staggering three billion units.

Another factor which advanced DNA-aided research was the discovery of restriction endonucleases, enzymes which cut strands of DNA at specific points— molecular scissors of a sort. With the aid of these enzyme tools, scientists could more easily focus on a specific DNA sequence. Researchers could use the restriction endonucleases for genetic engineering (see article on page 18), or to develop a "probe," a molecular tool used to identify a genetic sequence in the DNA chain.

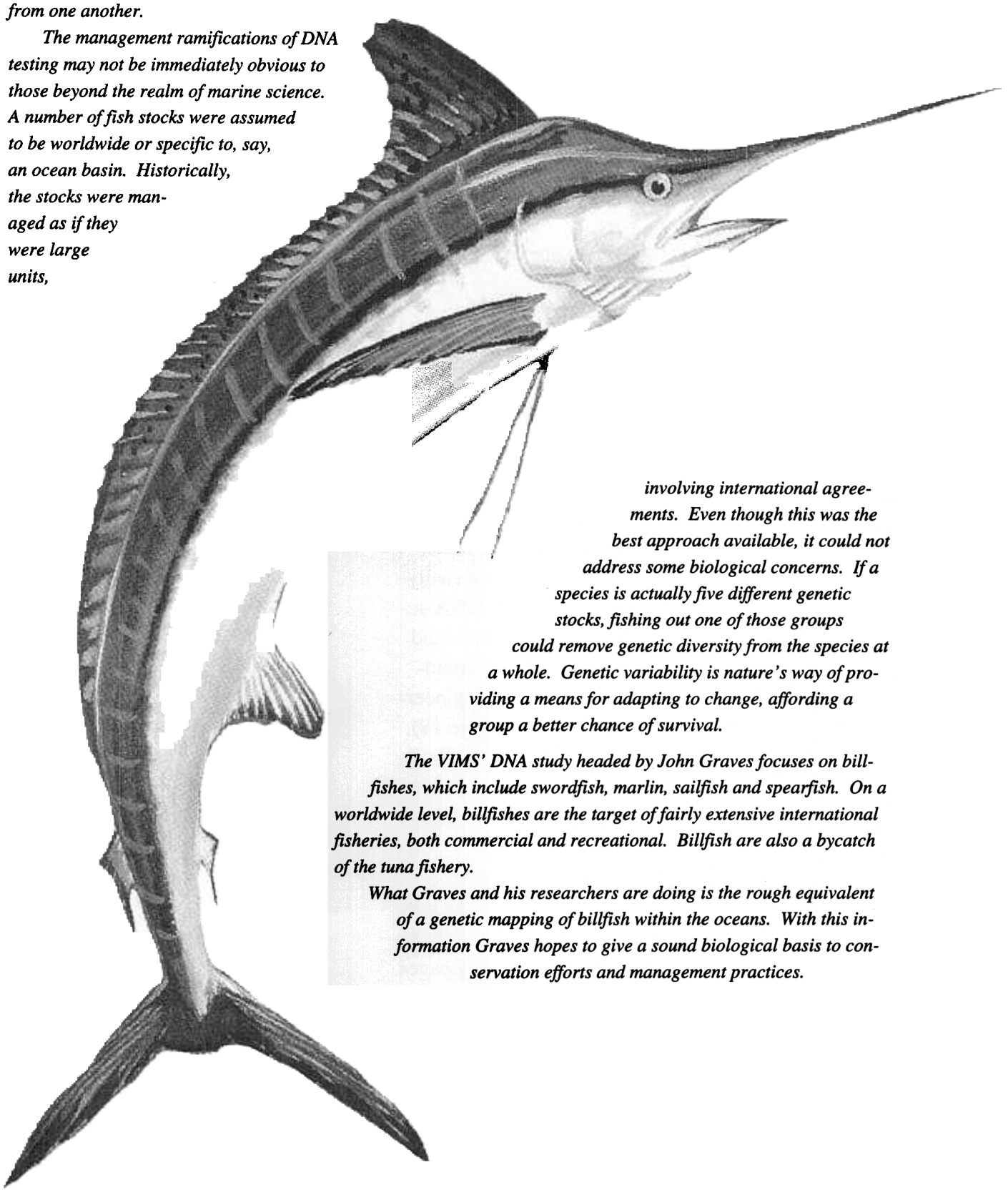
At VIMS, a number of DNA projects are being conducted, two of which are featured on the following pages.

✣ ✣ ✣

Molecular Mapping

The oceanic world can pose seemingly intractable challenges for fishery managers and scientists. Prior to DNA testing, it was difficult to know if a worldwide species like blue marlin was made up of one stock or more. Traditional tools like tagging could ascertain movements, but they could not determine if groups were genetically homogeneous or if they were distinct from one another.

The management ramifications of DNA testing may not be immediately obvious to those beyond the realm of marine science. A number of fish stocks were assumed to be worldwide or specific to, say, an ocean basin. Historically, the stocks were managed as if they were large units, involving international agreements. Even though this was the best approach available, it could not address some biological concerns. If a species is actually five different genetic stocks, fishing out one of those groups could remove genetic diversity from the species at a whole. Genetic variability is nature's way of providing a means for adapting to change, affording a group a better chance of survival.



The VIMS' DNA study headed by John Graves focuses on billfishes, which include swordfish, marlin, sailfish and spearfish. On a worldwide level, billfishes are the target of fairly extensive international fisheries, both commercial and recreational. Billfish are also a bycatch of the tuna fishery.

What Graves and his researchers are doing is the rough equivalent of a genetic mapping of billfish within the oceans. With this information Graves hopes to give a sound biological basis to conservation efforts and management practices.

Genetic Analyses of Billfishes

By
John Graves and Jan McDowell
Virginia Institute of Marine Science

Why Genetic Studies?

Effective management and conservation of the billfishes will require a thorough understanding of each species' population genetic (stock) structure. Without such information it is not possible to infer the ranges and composition of management units (do all Pacific striped marlin comprise a single stock or are several genetically distinct stocks present?), or to determine if unique genetic variation is restricted to specific regions (will a fishery collapse in one area remove genetic variation from the species?). Unfortunately, little is known of the population genetics of billfishes, even though several recent scientific and management panels have identified the delineation of billfish stock structure as a major management need. To address this issue, researchers at the Virginia Institute of Marine Science (VIMS) have been applying new advances in biotechnology to investigate the population genetic structure of several species of billfish.

Biotechnology and Billfishes

To investigate the population genetics of billfishes, scientists study a small, rapidly evolving piece of genetic material known as mitochondrial DNA (mtDNA). Researchers purify mtDNA from heart tissue and analyze the molecule with restriction endonucleases—enzymes that recognize specific DNA sequences and break the molecule at that site, generating fragments. Differences in the DNA sequence between individuals can result in different fragment patterns. By employing a dozen or so enzymes to analyze the DNA, researchers produce a composite genotype or "genetic fingerprint" for each individual. If the distribution of genetic fingerprints is different among fish from different areas, then one can infer that very little mixing is occurring. On the other hand, if the distributions are similar, one can assume that there is *at least* sufficient exchange (on the order of 10 individuals per generation) to prevent the accumulation of genetic differences.

What Has Been Learned?

For most species of tuna, little genetic differences exist between samples from within an ocean or even between oceans. For example, yellowfin tuna from five samples across the Pacific Ocean and one from the Atlantic Ocean had virtually the same distribution of mtDNA fingerprints. Each sample contained several different types of mtDNA fingerprints, but they occurred in similar frequencies across all samples. These results suggest that there is some movement (and subsequent mating) of yellowfin tuna within and between oceans. Genetically they represent a single stock.

It was generally assumed that billfishes, like tunas, would also show little genetic divergence among fish from distant areas. Surprisingly, this is not the case. Striped marlin from four sites within the Pacific Ocean were genetically distinct. The distribution of mtDNA fingerprints of each sample was unique, indicating that there is very limited exchange between areas. These results indicate that Pacific Ocean striped marlin do not comprise a single genetic stock and that significant within-ocean population structure exists. To conserve the unique genetic variation, current management models need to be revised to account for the population structure.

Blue marlin, like striped marlin, show stock structure within ocean basins as well as between oceans. VIMS' results indicate that the Atlantic and Pacific blue marlin are the same species. Several Atlantic blue marlin have the same or similar genetic fingerprints as all Pacific blue marlin, representing a family of DNA fingerprints scientists call "ubiquitous." However, about 45% of Atlantic blue marlin have mtDNA fingerprints not found in

Pacific fish (see Figure 1). This difference has provided a means to positively identify Atlantic blue marlin, and consequently to enforce the U.S. Fishery Management Plan for Billfishes which allows the sale of Pacific blue marlin but not Atlantic blue marlin.

Within the Atlantic Ocean, blue marlin appear to have population structure. Several blue marlin collected in Jamaica during 1991 and 1992 had a genetic fingerprint not found outside the Caribbean. Although preliminary, these results suggest limited mixing of Caribbean fish.

In addition to blue marlin and striped marlin, the VIMS laboratory is studying white marlin and sailfish. Samples of white marlin from the U.S. Atlantic coast in 1992 and 1993 are quite similar to one another, as are samples of white marlin from Brazil in 1992 and 1993. However, some differences are apparent between U.S. and Brazil samples. To determine the significance of these differences more Caribbean samples will need to be examined.

Sailfish show the most population structuring of any billfish, but like the blue marlin, they represent a single, circumtropical species. About 15% of Atlantic sailfish have mtDNA fingerprints identical to those found in Pacific fish, but the remaining 85% are unique. To find out what happens throughout the rest of the world's oceans VIMS researchers are collecting samples for a comprehensive, global analysis of sailfish population structure.

Added Benefits

Scientists at VIMS have now analyzed more than 750 billfish. While this data set has allowed researchers to determine the stock structure of several billfish species, it also provides an extremely useful resource for identifying unknown specimens. Last fall VIMS researchers determined that a 1,635 lb. marlin, tentatively identified as a black marlin, was actually a blue marlin.

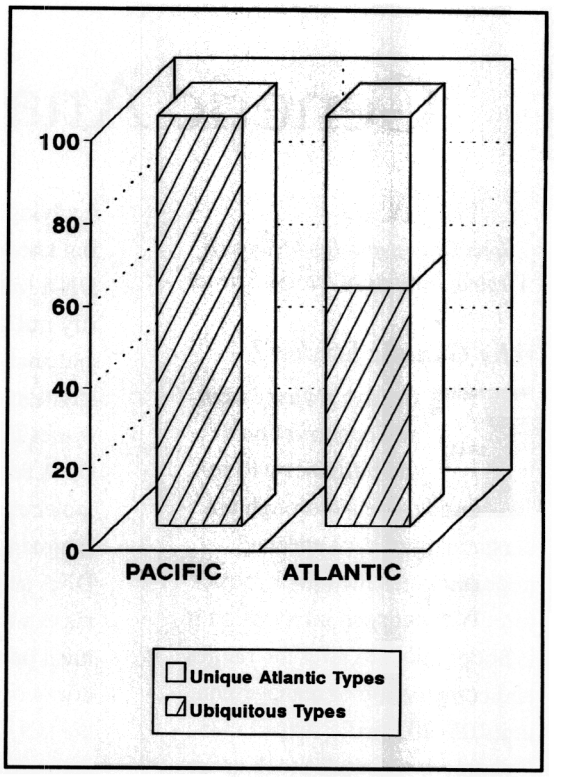


Figure 1. Blue Marlin Population Structure. A unique family of DNA fingerprints occurs in almost one-half of blue marlin from the Atlantic Ocean, while the other half of the Atlantic fish have genetic fingerprints similar to their Pacific relatives.

Closer to home, researchers also identify marlin tissues from seafood markets. Last year VIMS researchers were asked to analyze fish marketed in Virginia as "striped marlin from Ecuador." Fortunately, VIMS just happened to have a reference sample of 42 striped marlin from Ecuador. While researchers could have identified the geographic location of the striped marlin samples, they didn't need to. The fish fillets were Atlantic sailfish, a product that cannot legally be sold in the U.S. . . Busted . . .

Management and Conservation

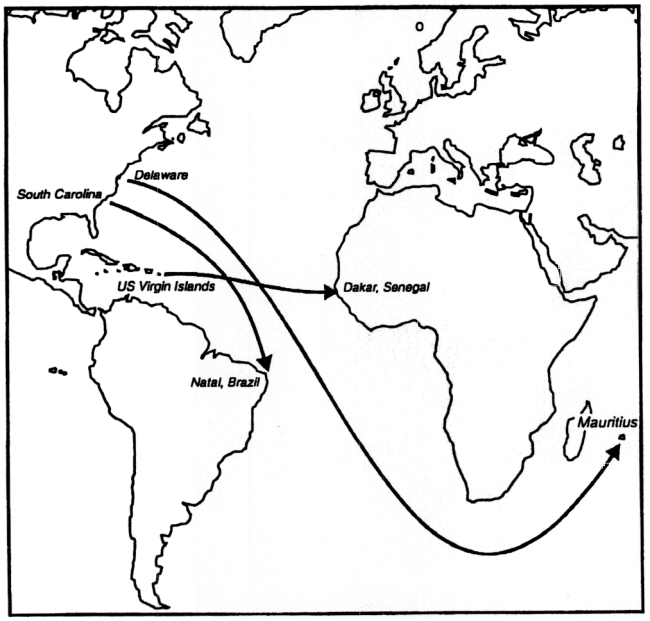
A major goal of VIMS research is to produce good science that will result in better fishery management and conservation. VIMS researchers typically communicate their results through peer-reviewed articles in international fishery journals. While they have published several papers on billfish population structure, management agencies are not always current with the scientific literature. So, VIMS researchers take their results to them.

In addition, VIMS scientists presented their results at a special International Commission for the Conservation of Atlantic Tunas (ICCAT) billfish workshop in 1992. ✣ ✣

Release That Fish—But Tag It First!

Tagging and genetic studies go hand-in-hand. Neither analysis alone provides information on both short-term and long-term movements of fish, data that is essential for effective management. Genetic studies give an evolutionary overview of the movement of fishes between areas (migration and spawning). They are effective in delineating stock structure and revealing the spatial distribution of genetic variation. Why genetic studies fail to do is provide short-term information on the interaction of fish from different areas. In contrast to the historical perspective provided by genetic analysis, tagging studies provide a "snapshot" of the movements a fish makes during part of its life. Tagging data quickly and effectively demonstrate interactions between fish from different geographic areas, as well as fish taken by different types of fisheries (for example, fish taken by recreational and commercial fisheries). Tagging data also provide critical information on growth rates and longevity of billfishes.

So while geneticists greatly appreciate the tissue sample afforded by boated fish, important scientific information can be gained from those that are tagged and released.



Although tag returns indicate that blue marlin are capable of trans-Atlantic and even transoceanic movement, most animals are recaptured close to the site of tagging even after long periods at liberty.

Area and season tagged	Days free	Area returned	Season returned
Caribbean Sea			
Summer	1,453	Caribbean	Summer
Summer	427	Bahamas	Fall
Summer	187	W. Africa	Winter
Fall	153	W. Africa	Winter
Fall	55	Mid-Atlantic	Fall
Fall	636	Caribbean	Summer
Fall	426	Caribbean	Fall
Winter	2,906	Caribbean	Winter
Gulf of Mexico			
Summer	492	Gulf of Mexico	Fall
Summer	208	Bahamas	Winter
Fall	134	Bahamas	Winter
Winter	63	Gulf of Mexico	Spring
Bahamas			
Summer	315	Caribbean	Spring
Summer	357	Bahamas	Summer
Summer	130	Bahamas	Winter
Winter	911	Gulf of Mexico	Summer
U.S. East Coast			
Summer	1,042	Bahamas	Summer
Summer	412	Caribbean	Fall
Fall	263	U.S. East Coast	Summer

Summary of Atlantic blue marlin tag/recapture data by geographic location and season (calendar), 1954-88. (From "Blue Marlin, Makaira nigricans, *Movements in the Western North Atlantic: Results of a Cooperative Game Fish Tagging Program, 1954-88," by W. N. Witzell and E. L. Scott.)*

On the Trail of MSX

Ordinarily, a species is able to keep a disease at bay, with only individuals—not a large group—falling prey to a pathogen. For any number of reasons, occasionally a disease garners an advantage. These reasons can range from favorable environmental conditions, to what must be serendipity for a pathogen: introduction into a new system with plentiful hosts that have weakened or undeveloped defenses. The latter may be part of the plight of *Crassostrea virginica,* the Virginia oyster, in the Chesapeake Bay.

The collapse of the Chesapeake Bay oyster fishery is currently believed to be the combined result of overfishing, environmental stresses and two persistent diseases, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo), pathogens which harm oysters— not humans. Even if overfishing and environmental stresses could be kept in control, MSX has been especially troublesome since its life cycle is unknown. Containing a disease, or at least minimizing its impact, is improbable if the life cycle of the pathogen and its mechanism(s) for transmission are not understood. On a human level, it would be like trying to control malaria without knowing it is transmitted by mosquitoes.

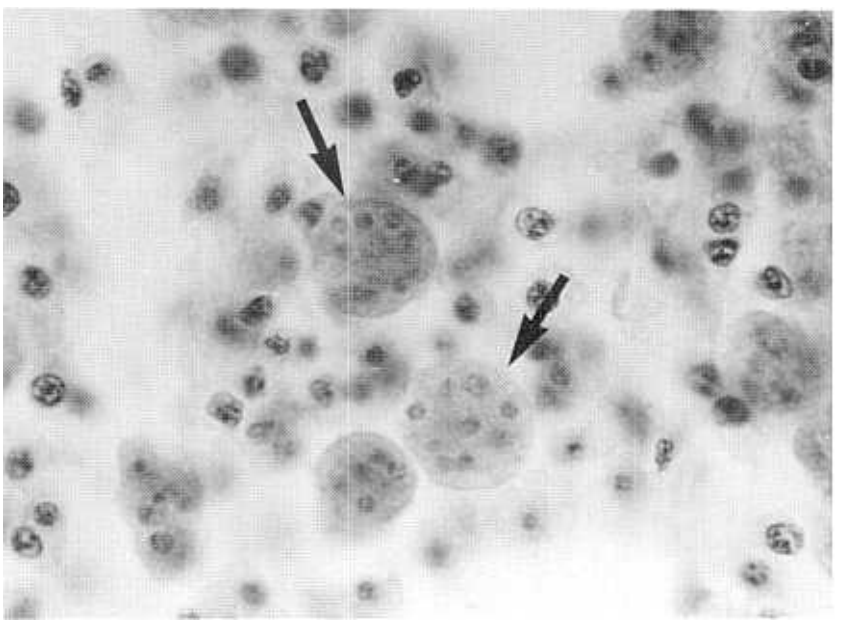
In a collaborative effort, scientists from the Virginia Institute of Marine Science (VIMS) and Rutgers University have reached a milestone in MSX work, one which could facilitate elucidating MSX's life cycle. The researchers developed a DNA probe—a sequence of DNA material specific to MSX—and a primer pair—a DNA tool to detect even minute amounts of the disease. The researchers who made these important findings are Nancy Stokes and Gene Burreson at VIMS, and Dunne Fong and Susan Ford at Rutgers.

DNA Probe

At Rutgers' Haskin Shellfish Research Laboratory, scientists sequenced a gene of MSX, and pinpointed a portion of that sequence which may be specific to the pathogen. VIMS researchers independently confirmed this sequence, and then sequenced the same gene from a parasite related to MSX. By comparing the two, VIMS researchers were able to locate two regions of the MSX DNA sequence which were unique to MSX, and not characteristic of related forms of the parasite. One of these regions was used as a DNA probe and tested against Dermo, and against other parasites similar to MSX. Results indicated that the probe was, in fact, very sensitive and very specific for MSX.

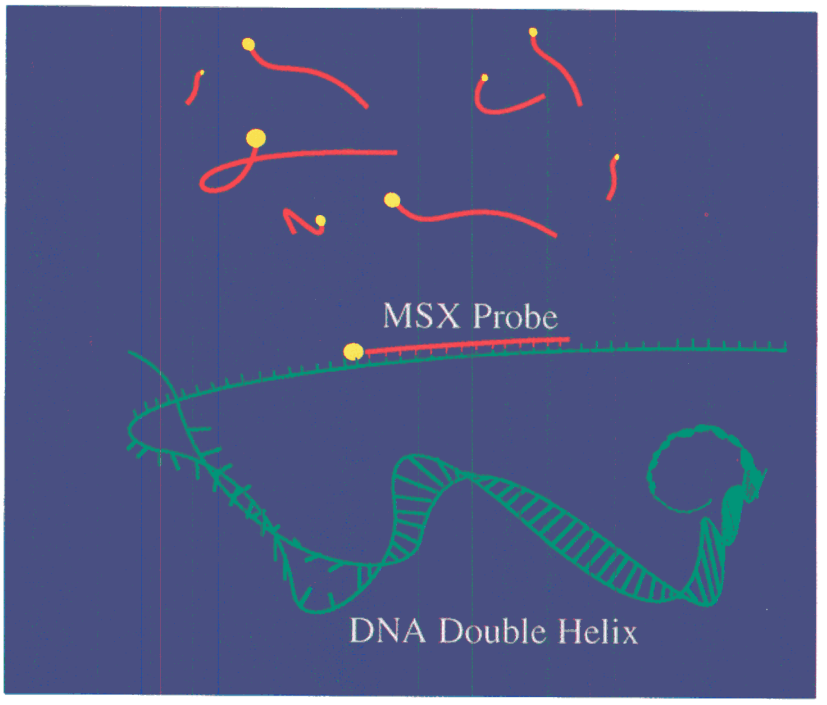
How the probe operates may be routine for DNA researchers, but may constitute modern-day alchemy to outsiders. Very simply, a very thin slice of tissue is placed on a slide, and treated with an enzyme to poke minuscule holes in the tissue (to allow the probe entry into the cells). The tissue is heated, causing the

(Continued on page 17)



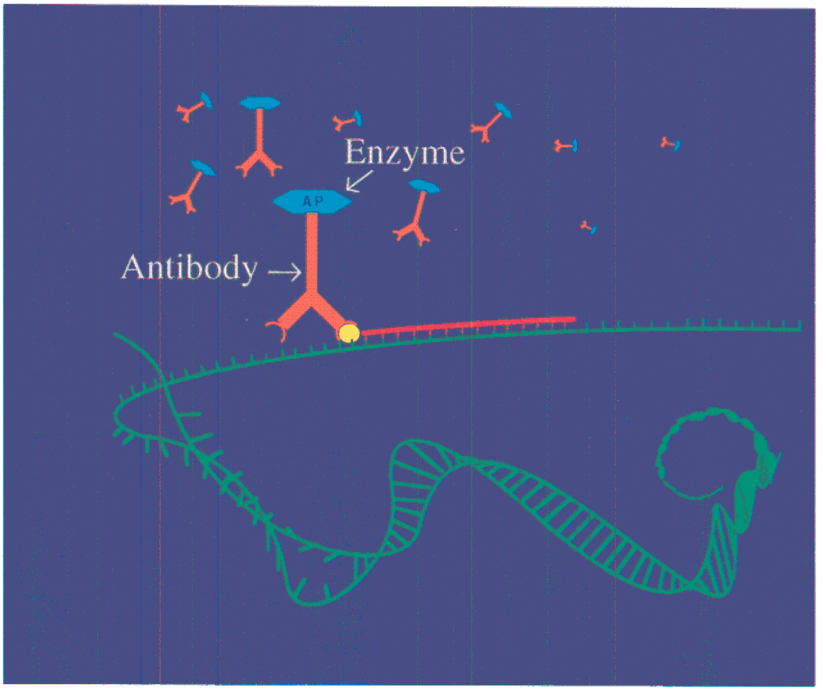
MSX.

Opposite page: Researchers Nancy Stokes and Gene Burreson.



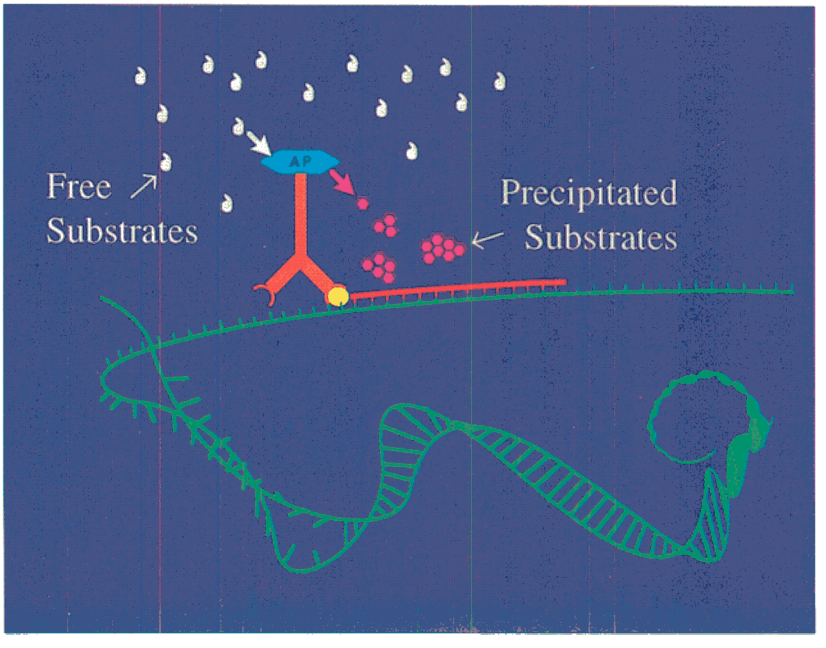
Basics of the MSX probe.

From top to bottom:
1) The DNA double helix separates and the probe attaches to the complementary section of DNA. Any unbound probe is washed away.



2) An antibody with an enzyme binds to the label attached to the end of the probe.

3) Free substrates are processed by the enzyme, resulting in precipitated substrates. The color change, caused when the enzyme processed the substrates, indicates to researchers that MSX is present.



DNA double helix to separate (see illustration on adjacent page). The probe is added, and if MSX DNA is present in a tissue, the probe will attach to the complementary section of DNA in the host tissue. Any unattached probe is washed away, and an antibody with an enzyme is added. If any probe has attached, the antibody will bind to it. Then a substrate (a substance with which an enzyme reacts) is added. The substrate will change color as it is processed by the enzyme. Detection of the colored product means MSX is present.

Polymerase Chain Reaction Primers

The polymerase chain reaction (PCR) is little more than a decade old, but has been responsible for monumental advances in DNA work. PCR can be utilized for many applications, including, in this case, the detection of minute amounts of MSX.

The DNA double helix is long and extremely complex. Plus, every cell contains all the genetic information to construct the entire organism. Extracting a specific genetic sequence from a DNA strand would be like locating a single volume in a large but uncatalogued library. Instead of the nearly impossible task of sorting through massive amounts of DNA, a scientist could have access to an unlimited number of a sequence or a gene through PCR. To use PCR a scientist needs two primers, short segments of DNA which flank the portion of the DNA to be copied. The two regions of the MSX DNA that the VIMS researchers found to be unique for the parasite work as very specific and sensitive primers for PCR.

Very basically, this is what the PCR process entails: cells are broken open and the DNA is isolated. Some of this DNA is placed in a small tube containing the primers, an enzyme (DNA polymerase) and nucleotides. When heated, the DNA double helix will separate, exposing the nucleotides (T,A,C,G), the bases encoded with genetic information. The temperature is then decreased and the primers attach to the complementary sequence of the DNA. DNA polymerase causes the "free" nucleotides—ones that are not part of the two strands—to attach to the two strands in predictable DNA fashion—A's opposite T's, and G's opposite C's. Two new, complete strands have been formed, each containing the information in the original DNA strand. The process of separation, primer attachment, and making new DNA is repeated 30 to 40 times, a procedure which takes about four hours. If no MSX DNA is present, the primers will not attach and no copies will be made. If only one copy of the MSX gene is present, billions of copies can be made in four hours.

Interim Hosts

Determining if an oyster is infected with MSX is not the main intent behind the MSX probe and PCR primers. Researchers are keenly interested in determining if MSX utilizes an intermediate host before it infects oysters. This scenario seems plausible since, to date, scientists have not been able to infect oysters by exposing them to MSX in the lab. In contrast, Dermo is readily transmitted from oyster to oyster. Another reason that scientists suspect an intermediate host is that despite the current, low number of oysters in the lower Chesapeake Bay, MSX is still pervasive.

Currently, researchers do not know the structure and form of MSX at every point in its life history. An organism may or may not appear the same in its different life stages, or for that matter, in different hosts. This lack of knowledge has an obvious disadvantage; how would a scientist recognize MSX in a different form? The MSX probe and primers nicely solve this human problem, since on a molecular level the two DNA tools *always* recognize MSX, with a rapidity and certitude of which *Homo sapiens* is incapable—at least not without these subcellular components. No matter what the form of the organism, its DNA is always the same. ✥ ✥

Early portions of this research were funded by the Virginia Sea Grant Consortium and the New Jersey Sea Grant program. Recently, the research has been funded by the National Oceanic and Atmospheric Administration.

The Fray

Biotechnology which employs genetic manipulation is a powerful tool. And, as the case with many potent tools, it has both positive and negative capacities. When scientists began unraveling the mechanisms of DNA, they discovered that the DNA strand can be severed and new material inserted, creating altered or new genetic material. The possibilities of recombinant DNA seemed endless. For some, the potential for creating havoc was beyond imagination; for them, it was the biological equivalent of opening Pandora's box. For others, the commotion about recombinant DNA was unfounded; genetic manipulation employing classical methods—crossbreeding, for instance—has been in use for thousands of years.

A great debate about recombinant DNA ensued, world conferences were conducted, and the basis for countless sci-fi movie scripts was established. After a while the furor began to diminish. Established were guidelines for working with recombinant DNA, record-keeping mechanisms, and laws governing the release of genetically engineered organisms. Risk assessment experiments indicated that the probability of wreaking havoc with recombinant DNA were slight for a number of reasons. At this point, the laws governing these areas will obviously evolve, as they do in any realm.

There is some irony in the recombinant DNA debate. While this new type of genetic manipulation was creating a tempest, few people seemed cognizant of a hazard that has been the unintended result of traditional genetic manipulation. Many important world food crops are monocultures, meaning that one strain is cultivated instead of many varieties. In a worst case scenario, a disease could decimate an important crop like rice. Some researchers believe that the human and economic cost could be devastating.*

Beyond the furor about recombinant DNA are more subtle questions about biotechnology. Patents on substances from less developed countries might enrich the country that has the patent and create an inequity for the less developed country. For example, if an important drug were found in the rainforest—a substance developed by the indigenous people— and if another country re-engineered and then patented the substance, which group should profit and to what extent? Some researchers would question the ethics of any patent (or Intellectual Property Rights) of this sort.

Space limitations preclude a lengthy discussion about all aspects of the debate about biotechnology. As with any revolution, and it is fair to place biotechnology in that category, it will take time before both the benefits and drawbacks are obvious and fully understood. ✣ ✣

*To protect biodiversity, "Gene Banks" have been established all over the world. Germ plasm is collected, evaluated and preserved. In the face of a massive crop failure caused by disease, researchers would— ideally—be able to find genetic material resistant to the disease. This would not avert the immediate impact of the crop failure.

Journeying Toward the Future

A segment of scientific inquiry is really an adventure in time travel, in which researchers are working with concepts that are futuristic. This is not to say that the ideas are improbable. Rather, all that is lacking is a catalyst in whatever form it might appear—a political event (oftentimes a war), an economic need, an environmental concern, a societal problem or opportunity.

Chitin—a principal component of crustacean shells and insect exoskeletons—is a resource whose time has not quite arrived in the U.S. Articles appear about the substance's promise and a small chitin/chitosan* operation occasionally appears and sometimes disappears. Still, chitin research continues for the substance has a number of desirable qualities, including its ability to attract, adsorb, or absorb water; its biocompactability and biodegradability; and its capacity to absorb some heavy metals and even proteins. Ion exchangers made from chitosan have been used in food and pharmaceutical processes, medical and agricultural drugs, and in wastewater treatment.

*Chitin is a component of crustacean shells and insect exoskeletons. Chitin can be regenerated into a fiber form or into chitosan, a powderous substance soluble in dilute acetic acid.

Light-Weight Biological Armor?

When the Gulf War was at its peak, a few people in the military were reportedly looking for fabric made of chitin. The thinking was this: since chitin in some forms is highly absorbent, and can serve as an environmental sink for heavy metal ions as well as pesticides, it might provide protection against toxic agents.

Fabric from crab shells might sound far-fetched, but not if one thinks of the natural sources of fabric: silk (a worm); wool (an animal); cotton (a plant). Even if a fabric were not the intended end result, the way in which the chitin resource can be altered for human use is far-reaching.

It can be made into sutures, gauze bandages, and into artificial skin for burn victims. It can be used for paper coating, antistatic fabric finishing, water clarification and even topsoil preservation when added to water used for irrigation, by preventing the run-off of organic matter. Chitosan is also useful in removing hazardous metal contaminants from wastewater and proteins from processing effluents.

Since chitin is non-toxic and biologically degradable, much research with chitosan has also focused on its use in edible coatings for food, and for the preparation of cosmetics and pharmaceuticals.

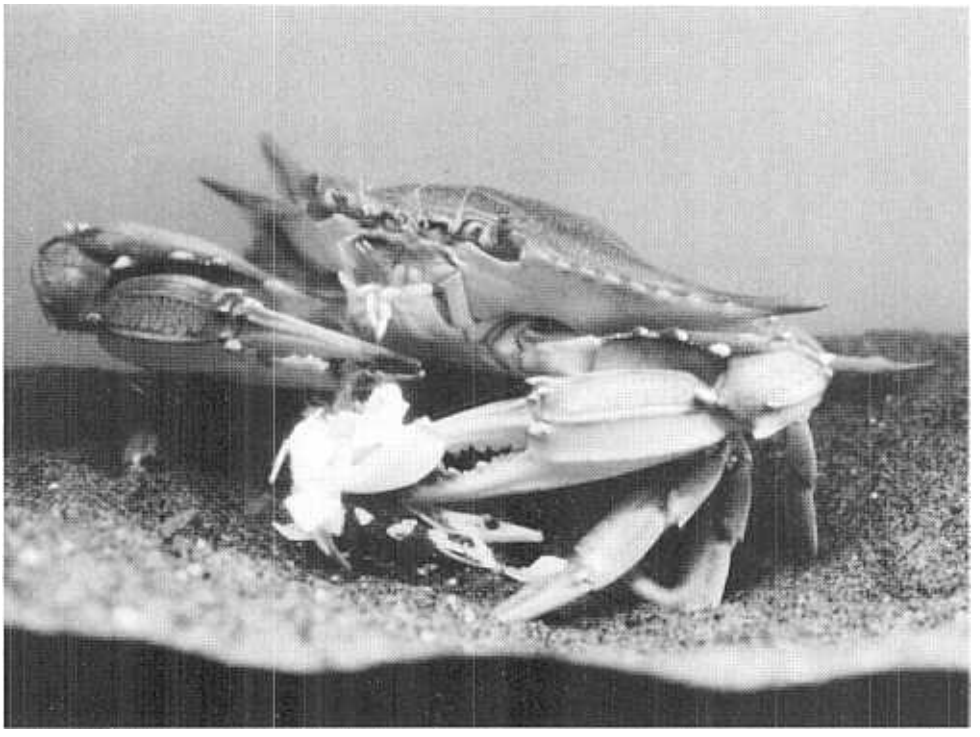
Delaware Sea Grant was at the forefront of U.S. chitin research. More than a decade ago, researchers developed a method to extract chitin, and contributed to the development of several major applications. Fourteen chitin-related patents were issued to Delaware Sea Grant researchers and licensed by companies in the United States and abroad. In the United States, DuPont and ConAgra created a subsidiary called Vanson, a company which is reportedly seeing success in several chitin applications.

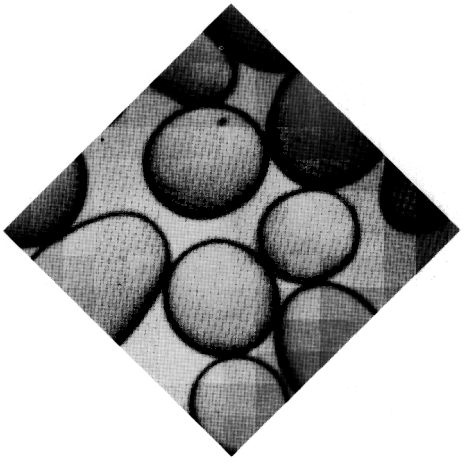
Crab Waste— Bane or Bounty?

Chitin is found abundantly in crustaceans, notably crabs and shrimp, and the amount of shell waste from crab processing operations is sizable. In Virginia the approximate landings of hard blue crabs average 40 million pounds yearly. That translates into only four million pounds of crab meat—the rest is considered waste. . . 36 million pounds. Yet that waste can be broken down into (on a dry basis) approximately 50% calcium of one form or another; 35% of protein; and 15% chitin.

Until recently, the easiest way to deal with crab waste was to simply bury it. In recent times

(Continued on page 21)





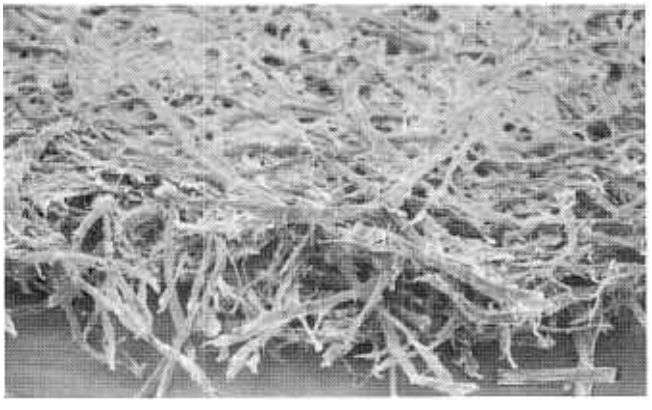
From top left, clockwise:

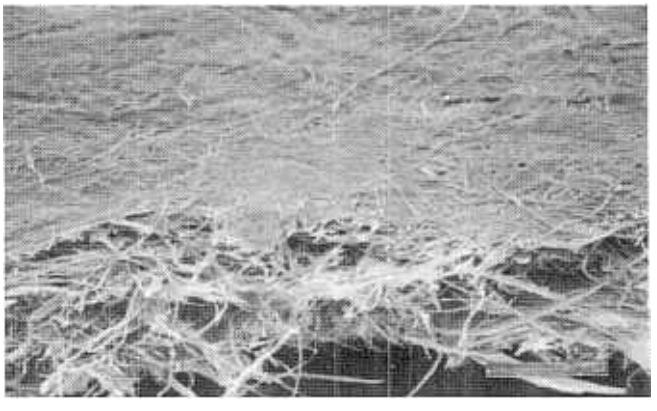
1) *Callinectes sapidus*, the blue crab, is an ample source of chitin, a substance which in certain forms has significant human applications, from artificial skin to beads which absorb heavy metal ions.

2) Fabric (non-woven) made from chitin.

3) Hydrogel beads from chitosan taken through an optical microscope, in aqueous medium. The beads are useful for ion exchange of heavy metal ions. They are formed by adding a chitosan solution in dilute acetic acid into weak alkali.

4&5) Photomicrographs of the surfaces and torn edges of 100% chitosan fibrid paper, and 100% cellulose paper.





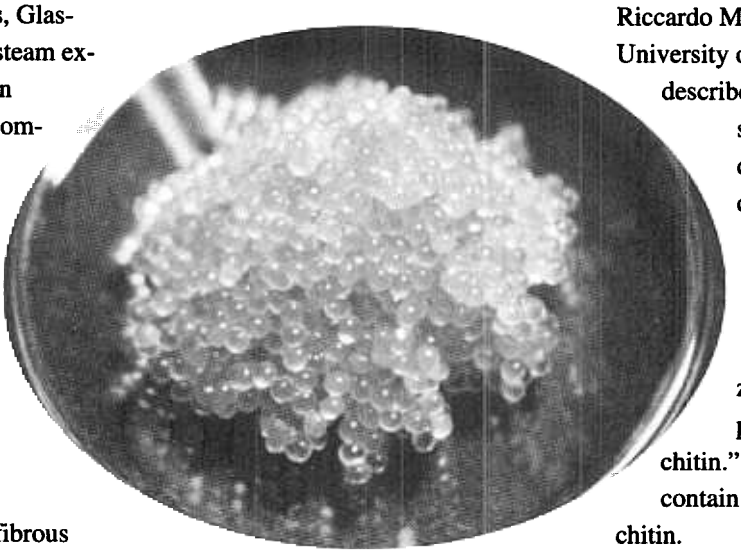
landfill regulations have changed; in Virginia, the waste must be low in moisture. So, many processors convert the shells into dried crab meal, a feed supplement.

Seconds Instead of Hours

At the Virginia Polytechnic Institute (VPI) in Blacksburg, researcher Wolfgang Glasser believes he has a means to readily isolate chitin, a process which traditionally uses large amounts of acid to demineralize the shells. With research associates Bob Wright, Willer de Oliveira, and several graduate students, Glasser examined the use of steam explosion to isolate chitin in hundreds of seconds as compared to hours using conventional methods. Steam explosion is akin to pressure cooking; the materials are subjected to high pressure steam for a period of time. With steam explosion, the release of steam causes the matter to explode into a fibrous mass. This work was performed at the VPI demonstration steam explosion facility, the only one of its kind in North America, a facility which has the capacity to treat up to one ton of organic waste per hour.

Glasser and his associates are keenly interested in this new means for extracting chitin, seeing it as a major step toward utilizing a plentiful and very much renewable resource, envisioning the extensive use of what some call a "wonder macromolecule." These researchers may be ahead of their time—at least in the U.S.

While the U.S. utilizes relatively little of the chitin resource, Japan is reportedly producing in the neighborhood of 700-1,000 tons a year.* Japan is now considered the world leader in chitin research and is actively pursuing applications. Why the difference? Undoubtedly, many reasons exist, but two seem readily apparent. Bounded by the sea and with a land mass that is mostly mountainous (74%) and not suitable for agriculture, Japan views the oceanic world differently than a good portion of the U.S. population. Case in point: Japan is the world's largest fishing nation, the world's largest consumer of fish products, and is the world leader in aquaculture. Japan owns the world's deepest-diving submersible, enabling scientists access to an estimated 98 percent of the world's ocean floor. In addition to a different world view, the Japanese appear more likely to invest in research which will not show an immediate financial return, but may have a long term benefit.



Chitosan beads

Chitin Brewing?

An obstacle to more U.S. chitin use, as mentioned before, could partially be the seasonal supply of, say crab shells. Yet there are other sources of chitin. In an extensive book on chitin, Riccardo Muzzarelli, with the University of Ancona, Italy, describes an intriguing source: ". . . large quantities of fungi currently grown in fermentation systems producing organic acids, antibiotics and enzymes, constitute a potential source of chitin." Some fungal species contain high quantities of chitin.

Beyond the means for extraction and the accessibility of a large source of raw material is the biggest obstacle to U.S. chitin production: a market. Ten years from now a substantial U.S. market might exist, or chitin/chitosan might still be on the verge. . . of being utilized.

*Sources varied in their estimates. The amount of chitin/chitosan might be more. One source indicated that the University of Tokyo reported 1,270 tons in 1986; most of that amount was chitosan.

End Notes

Biotechnology. . . Continued

There are simply too many biotechnology projects to cover, and it seems a shame not to be able to devote more space to them. In the future issues of the *Bulletin*, a column will be devoted to bringing readers more news about work in this field.

New STORM Observation Center Established

This year when a powerful hurricane or northeaster hits the East Coast, information about the condition of beaches and beach property will be available almost immediately through the Storm Tracking and Observational Reports to Media (STORM) Center.

Recently established by the University of Maryland's Laboratory for Coastal Research through a grant from the National Weather Service of the National Oceanic and Atmospheric Administration, STORM will operate through a network of up to 300 volunteers in major coastal towns from Maine to Florida. Stephen Leatherman, director of the Laboratory for Coastal Research and STORM, currently is recruiting volunteers.

"We need people who live or work on the beach, who walk it every day, who know their beach," said Leatherman. Although formal training in science is not necessary, volunteers should "have a genuine interest in learning about beaches and the impact that storms have on them," he said. "STORM will study the relationship between a storm's characteristics, such as central pressure, wind speed and wave height, and its impact in terms of coastal flooding, beach and dune erosion and overall property damage," said Leatherman. People who are interested in becoming volunteers should call the STORM Center at the University of Maryland at (301) 405-4074.

New Publications

To order the following publications, write Virginia Sea Grant, Marine Advisory Program, Virginia Institute of Marine Science, Gloucester Point, VA 23062. Please make checks out to VIMS.

The Economic Impact of Marine Aquaculture on Virginia's Eastern Shore

Author: Sayra Thacker

Aquaculture is the fastest growing agricultural industry in the United States. During the 1980s, United States aquaculture production quadrupled. By 1990, the estimated U.S. production was 860 million pounds with a farm gate (dockside) value of 760 million dollars. For coastal regions where traditional fisheries and related employment may be in decline, development of marine aquaculture provides employment opportunities that maintain links to traditional lifestyles. Marine aquaculture may also provide a basis for rejuvenating the local seafood industry.

Virginia's Eastern Shore represents an area with potential for marine aquaculture development primarily because of the abundance of its coastal areas and because the Eastern Shore lacks many of the man-made influences of more developed coastal regions. Designated as a United Nations Biosphere Preserve, the Eastern Shore's Atlantic coastline has one of the longest chains of barrier islands on the East Coast.

In *The Economic Impact of Marine Aquaculture on Virginia's Eastern Shore,* Business Specialist Sayra Thacker examines the current status of marine aquaculture on the Eastern Shore, and the potential impact of increased production. Specifically, the author surveys blue crab shedding, and culturing efforts with clams, oyster, and other bivalves.

The first copy of this advisory is free to Virginia residents, and additional copies cost $2.00. The cost of the publication for out-of-state residents is $2.00

The Crab Industry in Venezuela, Ecuador, and Mexico.
Implications for the Chesapeake Bay Blue Crab Industry

Authors: Michael J. Oesterling and Charles Petrocci

In recent years, Chesapeake Bay crabmeat processors have been besieged by imports into their traditional domestic marketplaces. This comes at a time of rising production costs and local resource fluctuations. Asian and Latin American countries have been significantly contributing to this influx of picked crabmeat. Without some grasp of foreign crab production (present and potential), marketing strategies, and biological parameters of the exploited species, Chesapeake Bay crabmeat producers will continue to lose their share of the domestic crabmeat market.

With the completion of a 1992 overview of a portion of the Asian crab industry (*The Warmwater Crab Fishery in Asia*, Petrocci and Lipton), the first step has been taken in providing the Chesapeake Bay crabmeat industry with a better understanding of their global competition. However, more than any other region, Latin America has the longest history of exporting crabmeat to the United States, and yet very little is known about the crab industries in those areas.

In *The Crab Industry in Venezuela, Ecuador, and Mexico*, the authors characterize existing crab fisheries/production facilities, and identify potential areas of expansion. The authors examine the industry's harvesting and marketing strategies and its production capabilities. Additionally, the authors give reasons why successful crabmeat industries have developed in some areas but not in others.

The Crab Industry in Venezuela, Ecuador, and Mexico is a joint Virginia/Maryland Sea Grant project The cost of the publication is $3.00.

On the cover: Fertilized fish embryos

Sea Grant Communications
Virginia Institute of Marine Science
Gloucester Point, Virginia 23062

Address correction requested