Virginia

MARINE RESOURCE

BULLETIN

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Cover photo (from top, clockwise): *Crassostrea virginica, Crassostrea rivularis, Crassostrea gigas.*

The Experiment

With the continuing decline of Virginia's oyster resource, the state legislature directed the Virginia Institute of Marine Science (VIMS) to develop a plan for the restoration of this vital ecological and economic resource.

VIMS will be testing a promising native strain *and* experimenting with non-native oysters to ascertain whether they could survive in the Chesapeake Bay, an estuary which is considered a high stress environment with pervasive oyster diseases. Fundamentally, VIMS will be providing the scientific information necessary for any restoration efforts by the Commonwealth. The experiments are being conducted with scientific assistance from the Shellfish Genetics and Breeding-Technology Transfer Services at the Haskin Shellfish Research Laboratory, Rutgers University.

This issue of the *Virginia Marine Resource Bulletin* is intended as an informational tool, to provide an overview of the current situation, and to explain the experiments which will be conducted by the Institute.

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Virginia's Native Oyster

The Complicated Demise of a Filter Feeder Extraordinaire

The status of the native oyster, *Crassostrea virginica*, is bleak. In less than a century, the Chesapeake Bay's abundant resource dwindled to almost non-existent levels. The Bay bivalve which prompted the oyster wars, the bivalve which was so popular that it was shipped as far as Europe and Australia during the 19th century—for all practical purposes vanished.

The culprit, or culprits? The cause is bandied about, and is usually diplomatically attributed to a combination of overfishing, disease, and environmental stress. While it is true that the resource's demise was the result of *factors*, it is probably more accurate to say that one factor led to another until it was too late.

Overfishing, Disease, Environmental Stress

The Chesapeake Bay, as we know it now, is far different than it was 30 or more years ago. Habitat characteristics have changed. At one point in time, the oyster reefs were a prominent topographical feature. Even though oysters on or near the bottom of the reef may have had to filter through a great deal of non-nutrients (sediments, for instance) to obtain their sustenance, oysters higher in the water column usually had better access to favorable food conditions. Because of overfishing and shell mining,* the oyster reefs of yesteryear do not exist. Without these structures, it would be difficult for oysters to feed, fight off diseases *and* develop a resistance to pathogens.

Unfortunately, the already beleaguered oyster population faced another challenge, in the form of two pervasive diseases, *Perkinsus marinus* and *Haplosporidum nelsoni*. The diseases, which do not harm humans, cause early mortality in the bivalves, leaving few oysters to harvest. Environmental conditions during some of the crucial years favored the disease, were years of high salinity in the Bay. Despite more than 30 years of disease activity, the native oyster developed neither tolerance nor absolute resistance to the diseases. In disease endemic

*See the "Oyster Shell Use" article on page 7 for a few ways shells were utilized.

On the right:

in former days of plenty.

areas of Virginia, *Crassostrea virginica* did not show any recovery. In addition, repletion programs failed to restore permanent production to areas lost to disease.

Environmental degradation caused by the growth in population and in land use in the Chesapeake Bay region further complicates the picture. The problems are not limited to sediments and nutrients; many other elements, and some toxicants, are part of the mix. Toxicants are suspected of making oysters more susceptible to disease at certain stages, and may also interfere with growth and survival.**

In the best of all possible estuarine worlds, there are many environmental factors—such as nutrient availability and temperature—which can have an impact on the bivalve during any one life stage. A whole host of predators are also part of the system. *Crassostrea virginica* has been a resilient animal, surviving at least 3.5-4 or more million years.*** Yet it may be that too many natural and man-made factors may have constituted an overwhelming set of obstacles for the animal's continued level of abundance in the Chesapeake Bay.

Filter Feeders Extraordinaire, Habitat Creators

An oyster is a filter feeder. Stationary in its adult life, the manner in which it obtains necessary nutrients is by filtering them out of the water. At the same time an oyster obtains sustenance, it may filter out other substances and, depending on the substance, may concentrate it. This is basic biology, yet it was not until recent times that the benefits of the oyster's feeding mechanism were more fully appreciated. It became evident, after the oysters had been depleted in the Chesapeake Bay, that the bivalves had been a substantial player in improving water quality. A number of estimates have been quoted calculating the amount of time it would take the 1870s stock and that of a few years ago to filter the Bay. The much quoted figure for filtering potential contrasts a capacity to filter the entire volume of the Chesapeake Bay of a few days in the 1870s, to almost a year in the very recent past. Debate exists about the estimates, yet there is no doubt that filtering the Bay today takes much, much longer than before.

Not only is an oyster an excellent filterer, it is a habitat creator, providing niches for all sorts of life, forms that will feed some animals higher in the food chain. Barnacles, sea anemones, fan worms, hooked mussels, oyster spat, cockles, mud crabs, skilletfish, gobies, blennies, nudibranches, bryozoans, small hydroids, sponges, and sea squirts are some of the inhabitors of oyster reefs in the Chesapeake Bay. Benthic, or bottom dwelling life, is needed in the Bay to sustain resident or migratory fish and crustaceans which rely on this part of the Bay for food.

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On the right:

Oyster shells outside a shucking house. If the oysters were to be used for reshelling a bed (young oysters prefer to settle upon adult shells), they would be loaded onto a barge.

**The exact relationship of pollutants to the onset of disease in the Virginia oyster has not been fully established. In most cases, toxic substances in low doses do not cause the immediate death of an organism. Instead, the substances may stress biota by interfering with normal physiological functions or by depleting energy or other crucial reserves. Experiments conducted at various times at the Virginia Institute of Marine Science indicated that the more the oyster was stressed, that is exposed to toxins, the more susceptible the animal was to disease.

***C. virginica* may be older than this. While it is found in the Pliocene, some 3.5 to 4 million years ago, occurrences have been located elsewhere in the geologic record.

Oyster Shell Use

When people think about an oyster reef as a resource, they usually associate its value with the animal and its use as food. True enough, but reefs were mined for oyster seed (to be planted elsewhere) and for reshelling oyster grounds (larvae normally settle on oyster shell).

About 98 percent of an oyster shell is made up of calcium carbonate, a substance with numerous applications, of which only a few, obvious ones are mentioned here. Calcium carbonate is utilized in medicine, in antacid, and as a dietary calcium supplement. It can be found in food in the form of baking soda. It is employed in the manufacture of toothpaste, rubber tires and paint! It is also an anti-caking medium for table salt.

Historically, oyster shell was used extensively as grit for poultry; in a chicken's gizzard food is ground up with the aid of ingested pebbles or, in this case, oyster shell. Calcium carbonate is used in poultry feed to enhance bone growth and the production of eggshells. In the past, oyster shell was abundantly used for road construction material and as a component of building materials.

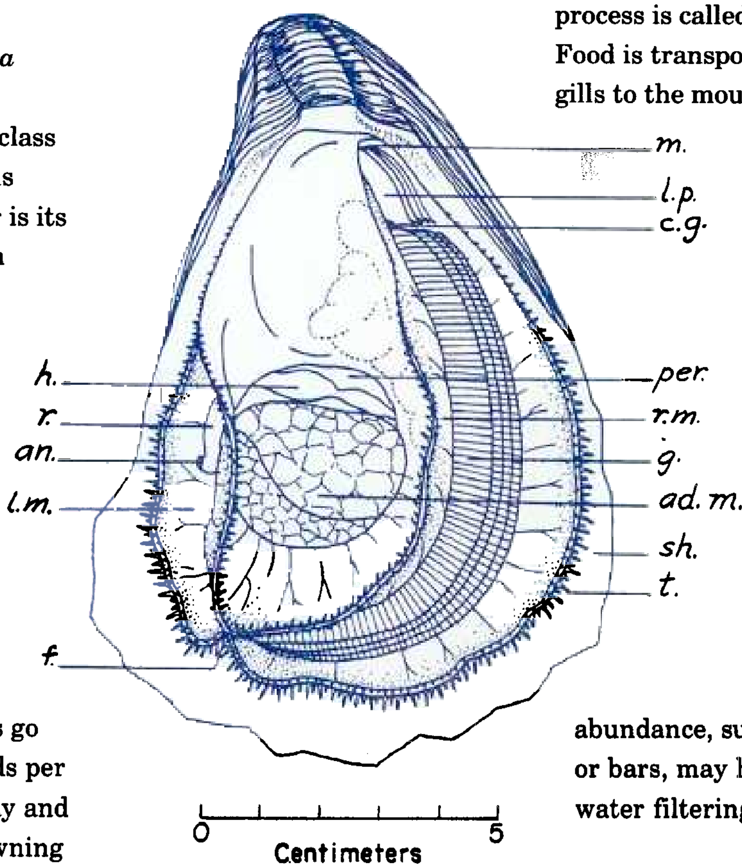
Oyster Biology

The following article summarizes some of the biological aspects of the Eastern oyster, Crassostrea virginica. Most of the information also pertains to the Pacific oyster, Crassostrea gigas. However, variations may exist, for instance, in fecundity levels, sexual maturation, and growth rates.—ed.

Crassostrea virginica is a member of the phylum Mollusca, class Bivalvia. The most obvious visual feature of the oyster is its shell, which is laid down in growth rings and varies in length depending on age. Elements necessary for shell construction are extracted from the water and converted into shell material by the mantle, an internal organ. Approximately 98% of the shell material is calcium carbonate.

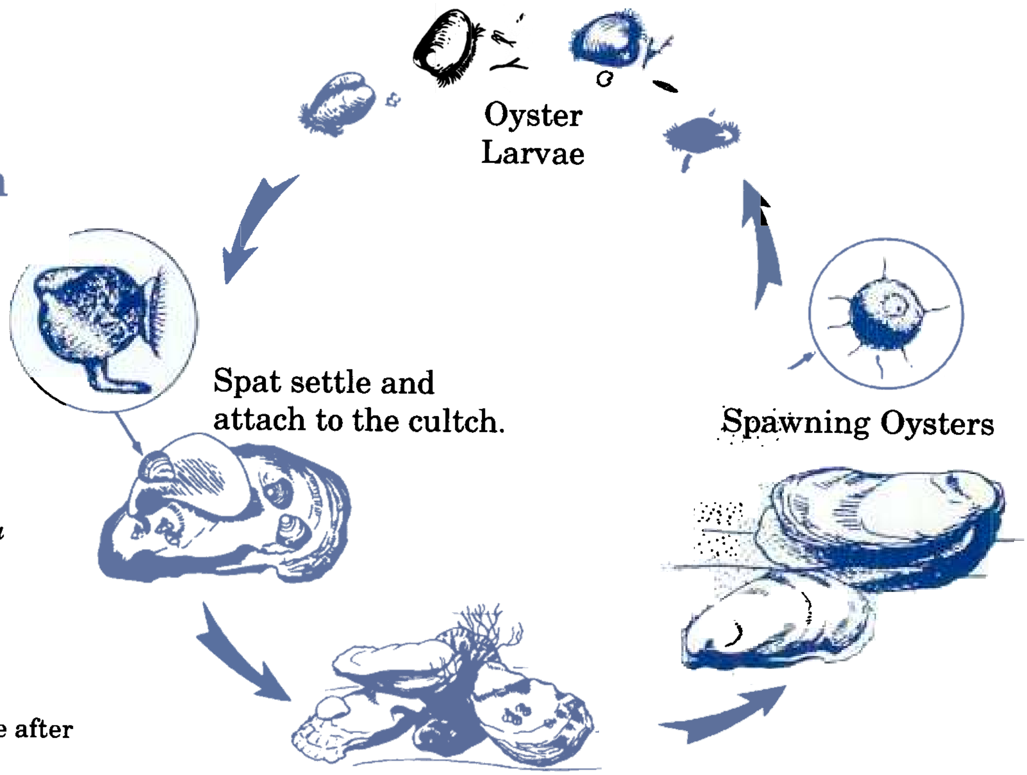
Chesapeake Bay oysters go through two growth periods per year, one from April to May and a second after the fall spawning season, sometime in October. Shell growth is somewhat temperature dependent, as no shell growth occurs in waters colder than 40°F. Shell growth does take place in summer, but to a far lesser extent since the oyster expends most of its energy on reproduction at this time. In many places in Chesapeake Bay, *C. virginica* grows approximately one inch per year.

The oyster feeds by using its gills. Small, hairlike projections on the gills called cilia beat and draw water into the shell and also act to expel it. The water passes through the oyster's partially open shell carrying with it food suspended in the water: small microscopic plants and animals (plankton) along with other small pieces of organic material and microorganisms such as bacteria. The food material filtered out of the circulating water is trapped by the mucus that is secreted by the gland cells on the gills. This process is called filter feeding. Food is transported from the gills to the mouth as a mucus thread moves via the cilia. This filtering action is rather efficient and large oysters have been known to pump up to 10.6 gallons of water per day through their gills. Consequently, areas of significant oyster abundance, such as oyster reefs or bars, may have significant water filtering capacity.



Organs of C. virginica seen after the removal of right valve. ad.m.—adductor muscle; an.—anus; c.g.—cerebral ganglion; f.—fusion of two mantle lobes and gills; g.—gills; h.—heart; l.m.—left mantle; l.p.—labial palps; m.—mouth; per.—pericardium; r.—rectum; r.m.—right mantle, sh.—shell; t.—tentacles. The right mantle contracted and curled up after the removal of the right valve, exposing the gills. Portion of the mantle over the heart region and the pericardial wall were removed.

Illustration from Paul Galtsoff's book, The American Oyster: Crassostrea virginica.



Oyster Life Cycle

Reproduction

Oysters are protandrous hermaphrodites*; they are capable of being either male or female, changing from one sex to the other at different times during their life cycle. *C. virginica* usually becomes reproductively viable within a year. When first mature, the *Crassostrea* species usually function as males. Anytime after this and between spawning season, the sex organs of the male transform into functional ovaries. Therefore, by the time an oyster is legally harvestable, i.e. three inches long and approximately three years old, it has usually made the change from male to female. Females spawn several times during one season with estimates of fecundity ranging from 15-115 million eggs per spawn.

The *Crassostrea* species reproduce by expelling eggs and sperm directly into the water column where fertilization occurs. Within four to six hours after fertilization, the embryonic bivalve has developed a rudimentary shell and a row of cilia, and is considered a larva. The cilia enables the larva to swim to a limited degree in the water column. During the next 15-20 days the oyster larva feeds on plankton in the water column and searches for an appropriate settling location. The term "setting" is used to describe the process of settling and attaching to a firm substrate via an excreted chemical cement. Larvae that have recently set are called "spat." During this sensitive time in the animal's life cycle, many larvae are lost to predation or inhospitable environmental conditions. The Virginia Marine Resources Commission estimates that, "approximately one of every three million eggs survive to become spat."

Spat have been called gregarious because of their tendency to settle in places where there are already other newly-settled spat. It is generally believed that oyster larvae are induced to settle by the release of a chemical substance by larger oysters on the bottom. This factor, plus the observation that oysters prefer a hard, stable substrate to set on, is one explanation for the natural propagation of oyster reefs. *—The Sea Grant advisory which contained this segment was authored by Dave Smith and Michelle Monti.*

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*A true hermaphrodite possesses fully functioning male and female sex organs simultaneously. A potandrous hermaphrodite is an animal which changes from one sex to another.

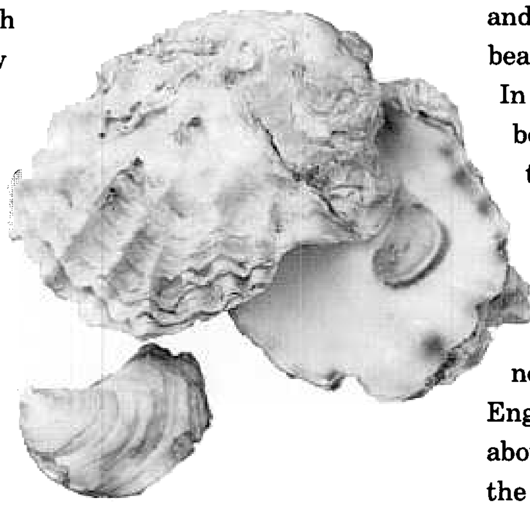
In Some Sort of Regard

It might seem prosaic to marvel at the adaptability of an oyster to changes in the environment since this is a feature of most, if not all life on Earth. Yet, it seems appropriate to at least pause in some sort of regard.

Among animals, a number of options exist for growing *and* protecting internal organs. Humans contain an internal skeleton, which grows along with the internal organs. Crabs grow by shedding an outer protective shell; in optimal conditions the new, soft shell will harden fairly quickly into an armor-like covering. Bivalves, such as oysters, protect internal organs via an external shell. Growth is achieved by the accretion of material secreted near the edge of the shell.

A substantial external shell proves a successful strategy for a stationary animal which lives in a highly variable environment. Faced with adverse conditions, such as cold temperatures, low salinity, or low dissolved oxygen, the oyster will close its shell, and, if necessary, begin a temporary state of hibernation. An oyster can even live without dissolved oxygen for a few days if it is able to keep its valves closed. Some predators are evaded by the healthy oyster's ability to keep its shell securely clamped shut.

Stationary, an adult oyster is literally glued to the bottom or to other shells in a reef. What, then, about reproduction, and the need to produce prolific amounts of larvae to ensure the existence of future generations? No problem. Reproduce all at once and as broadcast spawners, releasing great quantities of eggs and sperm into the water column



so fertilization can take place.* The cues for the oysters' synchronous spawning are believed to be a combination of environmental conditions—including water temperature and food availability—conditions which would be conducive for survival.

Some oyster species are hermaphroditic and some are either male or female, with sex changes taking place several times within the animal's lifetime. In the case of the Chesapeake Bay's *C. virginica*, oysters are bisexual when only a few months old. They will become males by their first winter. In another year they will change into females.

Conclusive information about the rhyme and reason behind an oyster's sex changes, and the sex ratio when oysters mature does not appear to exist—not exactly. A few substantial question marks can be found in the literature. It may be that local conditions, such as temperature and food availability, have a bearing on the sex of the animal. In Paul Galtsoff's encyclopedic book on *C. virginica* he reports that, "In the warmer waters of Beaufort, N.C. young oysters are more apt to develop directly into females than in the northern cold waters of New England." The common wisdom about the oyster is that most of the older ones are female. This would make some sense, since the larger animal could produce far greater amounts of eggs. However, exceptions have been reported.

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*Not all oysters reproduce in exactly this fashion. Oysters fall into two categories: nonincubatory and incubatory. In the first case, the eggs are discharged into the water and are fertilized outside the female. In the second case, the fertilization takes place in the gill cavity. The larvae are incubated and discharged after they reach an advanced state of development.

The Geographical Distribution Of *Crassostrea virginica, C. gigas, and C. rivularis*

Crassostrea virginica

The eastern oyster's natural range is from the Gulf of St. Lawrence, in Canada, southward to the Gulf of Mexico, Panama, and the Caribbean Islands. However, introductions of this popular bivalve to regions outside of its range have been persistent in history.

As early as the mid-19th century, records indicate that attempts were made to introduce the eastern oyster to France. Later in the 19th century, and in the 20th, similar efforts to introduce the eastern oyster were made in England, Wales, Ireland, the Netherlands, and Denmark. The populations failed to become established.

Introductions to the Pacific Coast of North America were slightly more successful. In British Columbia, a small population still exists. During the late 1890s, freight trains hauled carloads of eastern oysters to Washington state; however, World War I disrupted the industry and a red tide killed most of the rest of the population. Introduced oysters in Oregon demonstrated some reproduction, but ultimately the bivalve did not become established. For a while, introductions into California had limited initial success, but apparently environmental conditions proved unfavorable to the establishment of an industry.

Thousands of miles away from the natural range of the eastern oyster, in the middle of the Pacific Ocean, the introduction was successful in Hawaii. A large population exists there today.

Researchers believe that the failure of *C. virginica* introductions to become established may be due to a number of factors, and are mainly site dependent. The factors range from not high enough temperatures for successful spawning and a lack of suitable substrate for spat settlement, to poor water quality, a want of suitable food, and not enough effort by fishermen to husband the resource.

Crassostrea gigas

The Pacific oyster is found throughout much of the world. It is located in the Indo-West Pacific, from Pakistan to Japan and Korea, and the Philippine Islands, Borneo, and Sumatra, and along the Chinese coast. It has been introduced into many counties, including the west coast of Canada, United States, Mexico, as well as to Chile, Korea, Taiwan, New Zealand, Australia, and coastal European countries. The Pacific oyster is common in shallow protected waters in optimal salinities of 23 to 28ppt. *Crassostrea gigas* is one of the most important food oysters in many parts of the world and is widely cultivated, especially in Japan, Korea, the west coast of the United States, Canada, and Europe. It also is being produced commercially in Brazil, Chile, and Ecuador.

Crassostrea rivularis

This bivalve can be found from the Philippines and Taiwan to Formosa, Thailand and North Borneo. An occasional specimen may occur at low tide, but most seem to have been dredged from few to 100 m. Some debate exists about its classification. It has been placed in *Ostrea*, *Crassostrea* and under the new genus *Planostrea*.

—The last two entries about C. gigas and C. rivularis are derived from the new, extensive book, The Eastern Oyster: Crassostrea virginica, produced by the Maryland Sea Grant Program. *See page 23 for an overview of the books's contents.*

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Common Names

Although a species is properly known by its one scientific name, it may have a number of common names. The following is a short list, giving the scientific and common names of the oysters being experimented upon.

Crassostrea virginica, eastern oyster.

Crassostrea gigas, Pacific oyster, Japanese oyster.

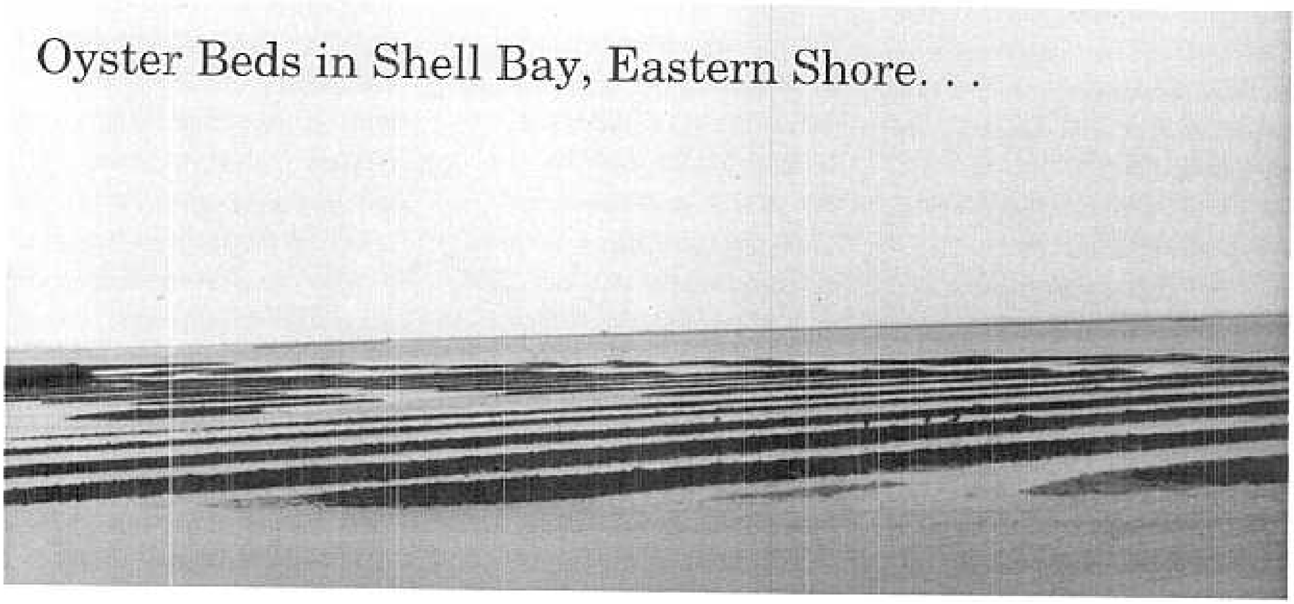
Crassostrea rivularis, palm-rooted oyster, talabang, pulid-pulid.

Change, Change, Change—
State of the Chesapeake Bay and Eastern Shore Seaside Lagoons as Shellfish Growing Environments

For many years the Chesapeake Bay and coastal lagoons of the Virginia Eastern Shore were prime growing areas for oysters, clams and other shellfish species of commercial and ecological interest. Within recent memory, however, there has been a significant decline in the shellfish stocks in both locations. In developing a plan to reverse this trend it is important to understand the long term (recent geological and within recorded human settlement) history of the region in order to develop a picture of the environment before human impact. Oysters, clams, and other mollusks are members of a very old lineage that is well represented in the fossil record. The Chesapeake Bay and seaside lagoons as known today are very geologically young—approximately 10,000 years old. The Bay filled with sea level rise, conditions became saltier, and oysters and other mollusks invaded the Bay. With increasing sea level, oyster reefs grew as three dimensional structures. The Bay has an enormous watershed, extending as far north as New York State. Prior to colonial settlement this region was predominantly forested with a sparse native American population. Dense forests and their complex ecosystems were such that seasonal runoff was controlled by forest cover and beaver dams, and large influxes of silt laden water or freshets were probably rare, even in extended rainfall periods. Water entering the Bay was cleaner with lower nutrient levels. This was probably the case well after the establishment of early settlements. Ships' logs comment on mariners being able to see the bottom of the James River. In such an environment, the filter-feeding activity of oysters and clams would have been optimal.

With time, colonists settled much of the Chesapeake Bay watershed and began to remove the forest cover and develop agriculture. Important natural flood controls were eliminated (notably beaver dams). In combination with poor soil management practices, increased sediment runoff was inevitable. This process continued at an increasing pace with urbanization and use of Bay tributaries as convenient disposal conduits. Imagine the progression to a watershed that is now home to more than 14 million people—all immersed in an energy intensive lifestyle, supported by intensive farming, involved in numerous industrial pursuits, with surface water infiltration inhibited by residential developments and satellite shopping malls—and it is not difficult to understand the magnitude of the forces that have changed and shaped the Bay, as the recipient of the cumulative impacts of an evolving society. Add to this the historic development of commercial marine activity, including some of the largest ports in the world and accessory maintenance activity, such as dredging, and the continually changing pressures for freshwater diversion and control, and the result is an environment that bears little resemblance to that encountered by the first colonists only a few hundred years ago. The cumulative impact of human activity is marked, and changes in the ecosystem should not be viewed with surprise. The once clear waters are no longer clear, and a regime of increased silt and nutrient loads prevails. Neither is it ideal for filter feeding shellfish. Consequently, it should not be surprising that shellfish populations have diminished, even in the absence of disease or fishing pressure. Against this background of environmental change there remains the problem of optimizing conditions for growth of native shellfish species or, alternatively, seeking to restore the Bay's badly degraded ecosystem using filter feeding shellfish from other geographic locations. The importance of environmental reparation cannot be underscored enough; without commensurate and parallel reparative efforts, any attempts to rejuvenate shellfish species have limited chances of success.

Why should an attempt be made to restore or rejuvenate the oyster resource of Chesapeake Bay? An initial, and perfectly defensible response to this question would probably be because it supports a commercially valuable industry. It can as well be argued, however, that direct commercial exploitation is of secondary importance. Benthic communities of Chesapeake Bay in pre-colonial times were highly influenced by intertidal oyster reefs. Oyster reefs were important geological as well as biological structures. They supported extensive associated communities that, in turn, provided the base levels of food webs that eventually support commercially important finfish and crab species. These important food-web interactions often are underestimated in current attempts to "manage" finfish and crab stocks on a species-specific basis. Further, the filtering role of the oyster in controlling primary productivity* in Chesapeake Bay should not be minimized. The calculations offered by Newell in 1989 are illuminating—a two order of magnitude decrease in filtration capacity compared to pre-1870 oyster stocks! Whereas the pre-1870 oyster population had the *potential* to filter all the waters of the Bay in approximately three days, the present stocks only manage that task in approximately 325—and stocks are still declining. A healthy and substantial oyster stock in Chesapeake Bay may be a most effective mechanism of simultaneously harvesting microplankton, reducing the impact of excess nutrients, sustaining a directly harvestable resource, improving water quality, and maintaining a diverse and stable food web. Unfortunately, four centuries of exploitation and wholesale mining of the oyster resource, both living and shell (the latter for industrial purposes), has resulted in the present situation, in which sparse populations survive in disparate, low salinity sanctuaries from endemic diseases as subtidal crusts of living material overlaying a base of reef material. Ecologically and economically, the importance of the oyster as a cornerstone species in Chesapeake Bay likely surpasses that of the directed fishery.



—From the Virginia Institute of Marine Science report to Virginia's General Assembly

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* Primary production is a term used to describe the first level of a food chain. In an aquatic system, the primary producers are phytoplankton, minute plants.

Crassostrea gigas Under Scrutiny:
Previous VIMS Tests With The Pacific Oyster

After a thorough literature review of the environmental requirements of various oyster species around the world, the Pacific oyster (*Crassostrea gigas*) was chosen as the species whose requirements reasonably matched those of the lower Chesapeake Bay. Importantly, the Pacific oyster has no significant diseases in its native range, and it has been resistant to local diseases wherever it has been introduced for aquaculture purposes.

The first investigation with *C. gigas* involved disease susceptibility. If it were as susceptible to local diseases as the native oyster, then it would be of no value to aquaculture or to the rehabilitation of the public fishery. Initial disease challenge experiments involved diploids and triploids.* *C. gigas* and *C. virginica* were held side-by-side in quarantine flumes and exposed to *Perkinsus marinus* over one summer. In these experiments, 64% of the *C. gigas* became infected with *P. marinus*, but all the infections remained low in intensity and there was no disease-associated mortality. All *C. virginica* in the experiments died from heavy infections of *P. marinus*. These results demonstrate that *C. gigas* can acquire *P. marinus* infections, but there is no adverse effect.

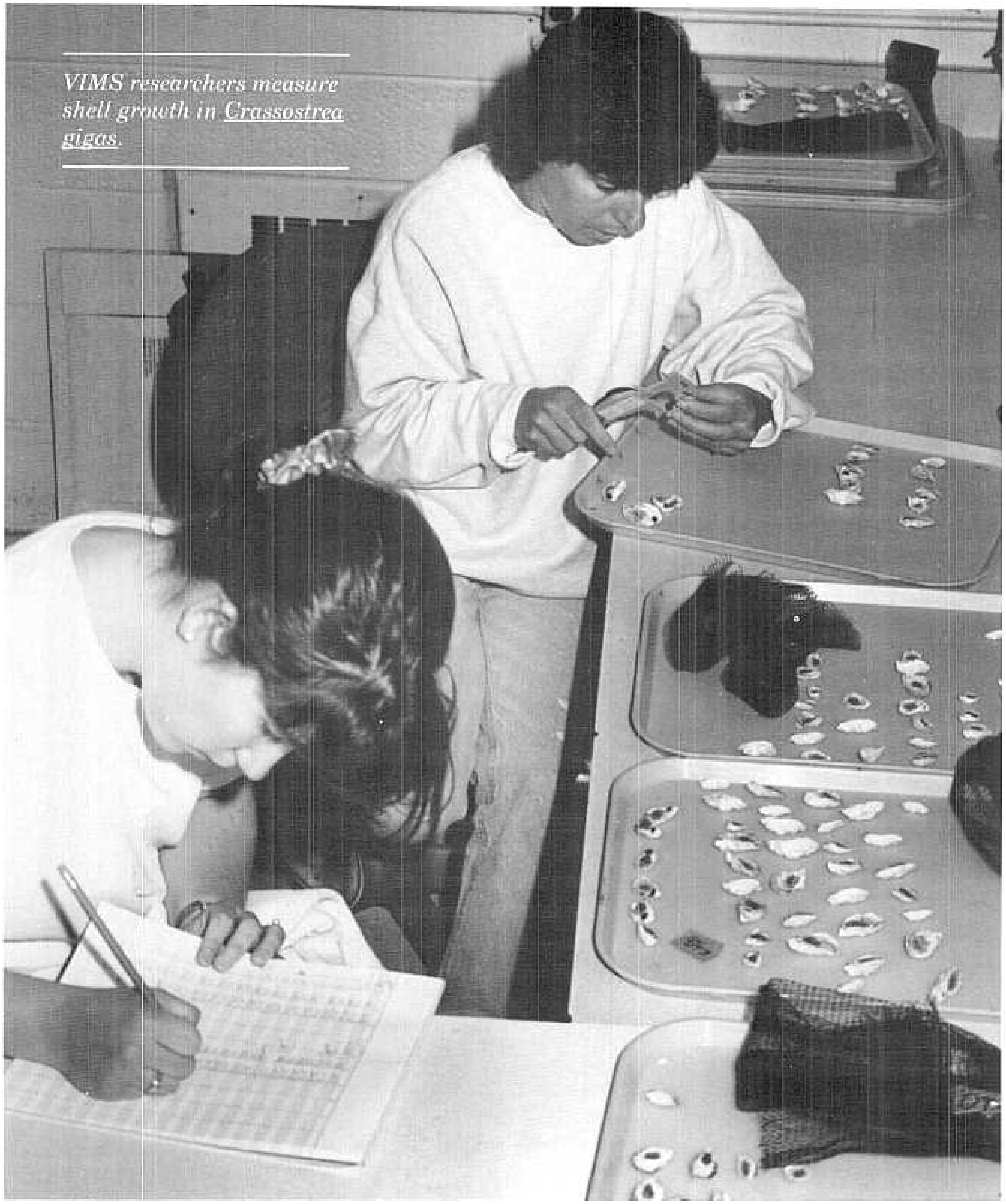
The next step was to investigate the susceptibility of *C. gigas* to *Haplosporidium nelsoni* (MSX). Researchers have not been able to infect oysters with this disease under laboratory conditions.** After appropriate debate about the risks and benefits of a limited introduction of *C. gigas* for disease challenge, the experiment was approved for the summer, 1993. Only triploid *C. gigas* were used to minimize the risk of spawning. Two hundred triploid *C. gigas* and 400 *C. virginica* control oysters were deployed at the Virginia Institute of Marine Science dock, lower York River, Virginia. The *C. virginica* were from the Rappahannock River in Virginia, and from Wye River, Maryland.

Weekly tests were made for disease diagnoses and mortality estimates. The experiment was terminated in February of 1994 after confirmation that some *C. gigas* individuals were mosaics, that is possessing both diploid and triploid cells (see footnote). *H. nelsoni* proved to be a problem in the Virginia and Maryland oysters. The maximum prevalence of the disease was 84 percent in the Virginia oysters, and 92 percent in the Maryland controls, with a high proportion of moderate and heavy infections. None of the *C. gigas* were infected. The other oyster disease, *Perkinsus marinus*, took its toll among the native oyster; the maximum prevalence was 96 percent in the Virginia oysters, 100 percent in the Maryland controls. *C. gigas* fared better; *P. marinus* infected 24 percent of the Pacific oysters. A high proportion of heavy and moderate infections occurred in the Virginia and Maryland control groups, but all *P. marinus* infections in *C. gigas* were of low intensity. Mortality rates among the Virginia and Maryland oysters were of plague-like dimensions: 90 percent. In the Pacific oyster, the rate was 25 percent and was not attributable to disease.

The growth rate of the Pacific oyster was also an aspect of this experiment. The *C. gigas* increased in size and weight during summer, but did not grow

*Very tersely, with the case of this animal, a diploid is sexually viable, a triploid is sterile. A mosaic possesses both diploid and triploid cells. Whether it is able to reproduce is thought to be unlikely, but is unknown to date. See page 16 for a more in-depth explanation.

**The entire life cycle of *H. nelsoni* is unknown. An intermediate host apparently plays a role in the transmission of the disease. If the intermediate host, or hosts, were known, it might be possible to infect oysters in the lab. This is why in-field testing is a necessity in determining whether *C. gigas* is resistant to the disease.



during the fall, as expected. Because of the short duration of the experiment, no spring growth data are available for the Pacific oyster.

Results from this short experiment suggest that the Pacific oyster of this size are not susceptible to the major oyster diseases of the Chesapeake Bay.

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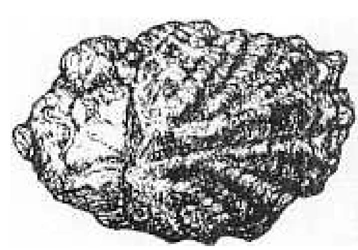


C. gigas, by Susanna Musick

Mosaics

The jargon and complexity of genetics tend to confuse those outside that scientific realm. No wonder. . .advances in the field have been lightning fast, and what is being observed on the molecular level does not belong to the commonplace language of the uninitiated. As a consequence, perhaps, terms like "reversion" have been used to convey what happened to the first Pacific oysters tested in the Chesapeake Bay. What actually took place is a bit more complicated and the word "reversion" is misleading in a strict scientific sense.

Diploidy, Triploidy

Organisms which reproduce sexually are usually diploid, meaning they possess two sets of chromosomes, the structures which contain DNA. The offspring of a diploid receives one set from the female parent and one from the male.*

When a diploid is manipulated to have three sets of chromosomes—the state of triploidy—this is anomalous, is abnormal. This triploid is normally sterile.

It may be that the natural world has something akin to a genetic insurance policy; when the number of chromosome sets is aberrant, the organism is often not fertile. When a deletion or a deficiency exists within individual chromosomes, as opposed to the number of complete sets, the result is a disorder which is often fatal, which frequently takes place before the organism can reproduce.

> Polyploidy is the state of possessing more than an organisms's (or cell's) normal number of chromosome sets. This type of genetic approach has long been used in agriculture.

The Pacific oysters initially tested by the Virginia Institute of Marine Science (VIMS) in 1992 were made triploids through a chemical process, through the use of an antibiotic, cytochalasin B. Simply put, and not including all the steps, this is how an oyster triploid is created: during the dance of division (mitosis), the time when a germ cell is rearranging itself to create a new cell, the female egg is manipulated. Under normal circumstances, the egg and sperm cells each ultimately reduce their two chromosome sets to one before sexual union. During this time cytochalasin B suppresses this process and the egg retains two sets of chromosomes. The male contribution, the sperm, adds a third chromosome set, making a triploid.

What was observed in the Pacific oysters tested was not a reversion. A reversion in a triploid means that the animal actually becomes diploid again. Instead, what hap-

*For simplicity's sake, the article only addresses diploids. In the natural world all manner of reproduction takes place. For instance, some insects, like male bees, only have one set of chromosomes (are monoploid) since they develop from unfertilized eggs. The female bees are diploid, the result of fertilized eggs. When it comes to plants, no hold seems barred reproductively. A rose contains both sex organs on the same flower. Corn, on the hand, contains both sex organs but they are located on different flowers.

pened was the occurrence of "mosaics." Some of the oysters began to exhibit both diploid (normal) as well as triploid cells. Whether these animals were capable of reproduction has not been determined. Because the animals were manipulated into triploids, the probability was high that they were sterile. Even so, VIMS stopped the tests.

When the mosaics were detected, Standish Allen, researcher at the Haskin Shellfish Research Laboratory at Rutgers University, searched the literature and did not find a case where a triploid became a mosaic after a short period of time. Whether this is a new occurrence, or whether this often happens but has never been looked at carefully, is still to be determined.

The Uses of Polyploidy

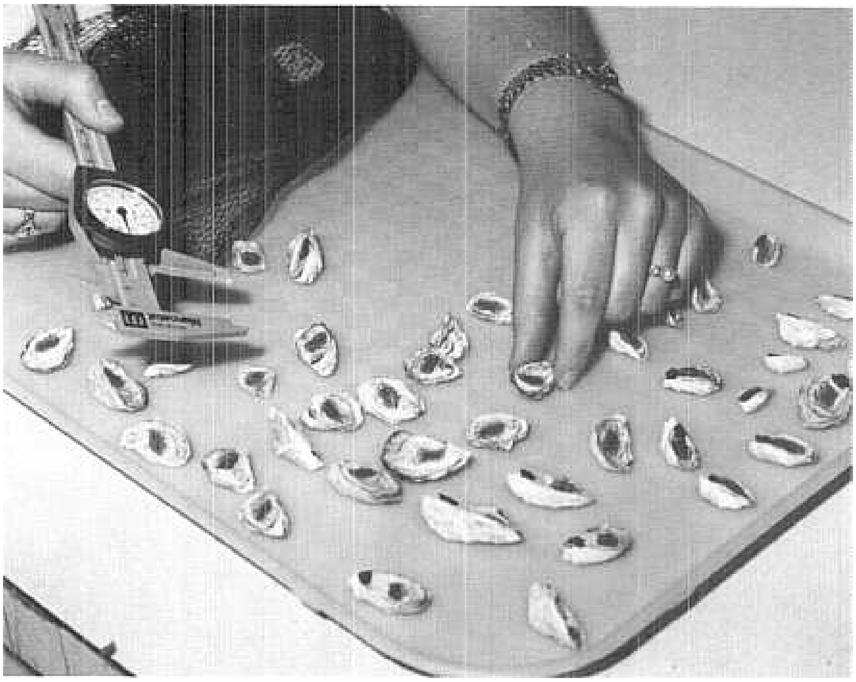
The bananas you buy in the supermarket are assuredly triploids. An odd number of chromosome sets, in this case, three, usually makes for a sterile animal or plant. If you were to cut open a supermarket banana, you would find ovules, a structure in a seed plant's ovary that would have developed into a seed if it had been fertilized. You could not plant these seed vestiges and expect a plant to grow. The reason bananas are engineered to be triploid is because a sterile plant or animal does not expend much energy in reproduction; this translates into a greater yield. In the case of the banana, the absence of large viable seeds makes it much more palatable to humans.

Reproduction can cost quite a bit, energy wise, and the amount of energy expended can differ from species to species. The Pacific oyster can be a prolific spawner. This results in the bivalve basically being unavailable to the market when spawning takes place; they are considered unsuitable, unmarketable. A sterile animal circumvents this problem. Plus, triploids are often meatier.

Creating Triploids

Although triploid oysters can be produced through various means—through heat, pressure and chemicals—the success rate of these methods varies. Up until recently, the chemical process appeared the best; however, the appearance of mosaics (animals with both diploid and triploid cells) brought unwanted variables into the earlier Virginia Institute of Marine Science experiment.

Triploids will now be produced by mating normal diploids with engineered tetraploids (the oyster is manipulated so it contains four sets of chromosomes). The lead scientist in this project is Standish Allen, from the Haskin Shellfish Lab at Rutgers. To date, Allen's results indicate a virtual non-incidence of "mosaics" using this strategy.



Measuring shell growth.

The Plan

The role of the Virginia Institute of Marine Science (VIMS) in the possible introduction of a non-native oyster is *not* one of policy making. Rather, VIMS' role is to provide a science-based foundation from which decisions can be made by state agencies and legislative entities.

Even though researchers are testing a non-native species, they have not given up on the native oysters, *Crassostrea virginica*. The intention is not to outright supplant the local bivalve. Instead, in light of the oyster's economic and ecological importance to the Chesapeake Bay region, scientists are exploring several research avenues.

Selective breeding of the native oyster, that is crossing animals with disease-resistant traits for stronger individuals, has been part of the VIMS plan to revive the fishery. This is not a new venture. Attempts were made in the past. However, at that time the oyster fishery was still viable, even if sometimes marginally, and support for the prior programs was minimal.

Almost ten years ago, a new selective breeding program began at VIMS, with funding from the Virginia Sea Grant College Program until 1992. A number of strains have been evaluated and discarded because of high mortality. Presently, three strains are being examined, and one, a third generation Delaware Bay oyster,* shows promise. The Delaware Bay strain reached market size in 18 months with mortality less than 15 percent in the presence of high pressure from both *Haplosporidum nelsoni* and *Perkinsus marinus*.** After two summers of exposure, mortality in this group was 50 percent, much less than the mortality in the other two groups under evaluation. However, 50 percent may be too high for this strain to be given consideration.

A related project is being regionally conducted by Rutgers University, the University of Maryland, and VIMS, with support from the National Oceanic and Atmospheric Administration. This breeding program will utilize *H. nelsoni*-resistant oysters developed by Rutgers and the Delaware Bay strain that is apparently resistant to both *H. nelsoni* and *P. marinus*. Disease resistant oysters still become infected with the parasites, but intensity remains low and mortality is greatly reduced.

A central question in the test plan is resistance to disease. The tests will compare results between the disease resistant strains of the native oyster and the non-native species. Three

*The Delaware Bay oyster is obviously the same as the native bivalve. It is just found in a different location and is considered a different strain.

**The diseases can not be transmitted to humans. They are harmful to oysters alone.

strains of the species *Crassostrea gigas*, and the species *Crassostrea rivularis* will be tested for their resemblance to the native species as reef-forming species which are tolerant of mid- to sub-tropical latitude and high stress environments, and are resistant to Chesapeake Bay diseases.

The purpose of the plan is to first screen for the candidate species most likely to succeed in the local estuarine environment. Second, the results from the tests will enable an assessment of environmental risk. That is, the geographic range of likely reproductive success will be estimated.

Quarantined, hatchery-raised progeny from imported broodstock will be used. This last procedure ensures that the offspring are free of parasites and disease which the parent may have carried.

To the extent possible, all field tests will utilize natural triploid stocks of the non-native species.*** Current results indicate that this strategy minimizes risks for reproductive capacity. As well, utilization of triploid stocks in field challenge allows testing under a range of more natural conditions not available in a laboratory setting. For example, researchers have been unable to infect oysters with *H. nelsoni* in a laboratory.

The following constitutes the strategy which was approved by the Virginia Marine Resources Commission and which would be conducted by VIMS.

- A series of comparative studies in laboratory quarantine to evaluate larval and post-settlement response to a range of environmental conditions.
- A series of field challenges, under secured conditions, of native oysters and triploid non-native species to compare disease resistance, growth characteristics, and susceptibility to invasion of macro organisms under a range of environmental conditions.
- Evaluation of the likely success of candidate species and an assessment of likely geographic range of reproduction of non-native species if introduced in substantial numbers.
- Upon review, and if additional tests are needed to settle ambiguities, limited in-water testing of diploid hatchery-reared stock with small lots under secure conditions.

Specifically, each species will be examined for the following qualities:

- Growth rate and longevity comparable to the native, Chesapeake Bay species.
- Resistance to endemic disease.
- Growth and survival in local conditions of temperature, salinity, and suspended sediments.

(Continued on Page 22. . .)

***Triploidy is explained in depth on page 16.

The VIMS Plan

Much of this issue of the *Bulletin* is devoted to explaining a facet of the Virginia Institute of Marine Science's strategic plan for molluscan shellfish research. The plan, detailed in the *Strategic Plan For Molluscan Shellfish Research, Including A Rational Plan For Testing Application Of Non-Native Oyster Species*, is an ambitious strategy for the restoration of the oyster resource to the Chesapeake Bay. **A great deal of credit for the plan should be given to the former Director of Research and Advisory Services, Dr. Robert Byrne.** It was under his leadership—with the work of numerous scientists at the Institute—that the plan took form and became an actuality.

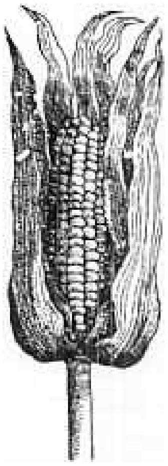
Over the years, the Institute has conducted a multitude of important programs, from the monitoring of the oyster resource, to disease research. A former Dean, Dr. William Hargis, along with his fellow scientists at the Virginia Institute of Marine Science, are responsible for the encyclopedic knowledge we possess about the native oyster and the industry which surrounded it.

Introductions

It is fairly modern thinking to question the introduction of a plant or animal. Right or wrong, introductions have been an important part of human history. Many of the species we associate with a country, or part of a country, originated elsewhere. In fact, the foundation of the U.S.'s food base is made up of non-indigenous crops and livestock—in the form of soybeans, wheat, and cattle. At least 4,500* species of foreign origin have established populations within the United States, according to the federal Office of Technology Assessment.

The re-inventing of the natural ecosystem started early in the New World. When colonists arrived, they brought the plants and animals they knew, with full intentions of establishing these life forms in their new home. World-wide, this type of behavior was the norm, and not just on continents. Even islands, including the then remote Pacific islands, were not left untouched. Sailors oftentimes left animals with the assumption that the animals would proliferate and provide food for their, or another sailor's next visit.

It wasn't until the last half of the 20th century that introductions were sometimes viewed askance. Unexpected results undermined confidence in even managed introductions. Kudzu is a prime example. A vine native to Japan, kudzu literally took over landscapes in the South. Sometimes the introductions were virtual escapees from human cultivation; loosestrife, an attractive nursery plant, ended up a major wetland weed. In recent history, a disastrous introduction arrived in the Great Lakes via ballast water: zebra mussels. In an amazingly short time, the bivalve spread, wreaking expensive damage as it colonized.



Ear of corn, from G. B. Ramusio, Navigationi et Viaggi, Venice, 1556.

"Man has literally remade the green face of the earth."

—Melville Bell Grosvenor

It was not an exaggeration when Grosvenor made that statement almost 40 years ago in a book about plant discovery and introductions. Worldwide, the extent of introductions is overwhelming. Before the widespread manufacture and use of synthetic materials, some natural materials were at a premium. Explorers collecting plants were often held in high regard for their "discoveries" could be crucial to a country or a region. Example: in the 1870s, Henry Wickham smuggled 70,000 rubber seeds out of the Amazon. These seeds were germinated in England, transported to Ceylon where they became the basis of the southeast Asia rubber industry. In a fairly short time, the southeast Asia industry would supply 90 percent of the world's natural rubber. The advent and popularity of the automobile made rubber, until a synthetic was found, a valuable commodity. How valuable? The World War II war machine was highly dependent on rubber for its vehicles. When the Japanese conquered southeast Asia, the United States was cut off from 98 percent of its rubber source. The race to find a synthetic substance intensified.

*This number includes plants, terrestrial vertebrates, insects and arachnids, fish, mollusks, and plant pathogens. Insects and arachnids (spiders, scorpion, mites, ticks), and plants predominate, each accounting for approximately 2,000. Many unintended plant introductions were literal escapees from cultivated gardens.

An exhaustive study of the introduction and spread of harmful non-indigenous species in the U.S. was conducted by the Technology Assessment Board of the 103rd Congress. The book, *Harmful Non-Indigenous Species in the United States* (OTA-F-565) was published in 1993.

Non-Intentional, Intentional Introductions

Increasingly, the general public seems to make little distinction between different types of introductions. Instead, there is a perception that they all are detrimental. Perhaps this is due to the notoriety of some non-intentional, harmful introductions like the zebra mussel. In actuality, introductions should fall into two categories: intentional and non-intentional. Both kinds have resulted in good and harm. This is because our understanding of ecosystems, and of the potential to upset a system by introducing or removing a species, is relatively new. Plus, never before in human history have distances been so short. World transportation is constant and often rapid, opening new, and sometimes unsuspected avenues. Who would have ever thought that the Asian tiger mosquito would arrive in the U.S. via a containerized shipment of used tires? The insect, a carrier of dengue fever and encephalitis, breeds in small, protected pools of water, the kind that can form inside tires.

Today, a number of national and international agencies are involved in the process of planned introductions, and in the prevention of harmful non-indigenous introductions. Now it requires extensive research and planning for an intentional introduction. Ironically, while the awareness of the potential danger of non-indigenous organisms has increased, little thought is given, by at least a good portion of the general public, to the spread of non-indigenous species from one area to another within the United States. According to the federal Office of Technology Assessment, "The current popular interest in 'wildflowers' for ornamental uses and 'native grasses' for livestock and wildlife forage may inadvertently be fueling widespread plants of non-indigenous species in natural and semi-natural areas." Even if an introduction is not overt, organisms can find ingenious pathways. Take the garlic mustard, first reported in 1918 in Illinois. Via flood waters, mowers, trains, cars, and on the boots, clothes and hair of hikers, the weed made its way across 42 counties in Illinois. Boats, vans, and motor homes can be vectors for organisms, too.

International Guidelines for Marine Non-Indigenous Species

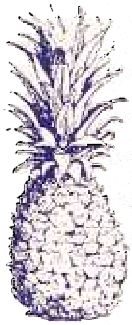
The International Council for the Exploration of the Seas (ICES) developed a "code of practice" to reduce the risks associated with marine non-indigenous species. The ICES Working Group on Introductions and Transfers of Marine Organisms works in an advisory capacity with the country considering an introduction. The guidelines developed by the ICES Working Group pertaining to intentional introductions have been recognized in the scientific community and were suggested as guidelines when the introduction of *Crassostrea gigas* was first proposed in Virginia.

Very briefly, the protocol requires the following:

- a clear rationale for introduction;
- selection of candidate species, including a consideration of associated pests, parasites and diseases;
- testing, utilizing quarantine systems, before a decision to proceed with introduction; and,
- introduction using quarantine procedures and monitoring after release to provide data for subsequent considerations for introductions.

To ensure that fellow travelers—that is, pests, parasites and diseases—are not brought into the Bay, the parents will be destroyed. Only offspring will be used in the experiments. Also, when researchers perform the initial in-field testing, sterile animals will be used to eliminate the possibility of an unintentional introduction.

'The apples of love' (the tomato plant), from John Gerard, The Herball of generall historie of plantes, London, 1633.



Introductions, A Global Phenomena

The following is a short, short list of some plant origins—meant to give an idea of how global introductions have been historically. The list represents the common knowledge about origins and does not reflect any recent scientific or archeological findings. Also, a plant might occur naturally in two areas, but the introduction is from a specific place.

Brazil—rubber, pineapple (the pineapple is not native to Hawaii)
Mexico—poinsettia, zinnia
The Americas—sweet potato, potato, maize, common bean, lima bean, tomato, garden peppers, tobacco
Africa—geranium, okra, gladiolus, African violet, coffee (through the Arabian port of Mocha)
Asia Minor—tulip
Persia (and to a far lesser extent India)—muskmelon
Afghanistan (and adjacent areas)—carrot
India—cucumber, eggplant, cowpea (the last, the cowpea, came to the Americas through Africa aboard slave ships)
Southern Asia—banana
Northern Burma (and possibly eastern India)—lemon, lime
China—chrysanthemum, camellia, clematis, azalea, rhododendron (the last two were also in the New World), hollyhock, forsythia, peach, apricot, orange (the last also in Indochina)
Japan—wisteria
Australia—strawflowers

The Plan. *(Continued from Page 19.)*

- Ability to reproduce in local waters (this indicates that they would develop self-sustaining populations).
- Lack of ability to reproduce in local waters (this would be an advantage if it were determined that a non-native oyster would be used, but only in hatcheries.
- Reef-forming habits (the ability to form reefs would be necessary in the Chesapeake Bay).
- Lower susceptibility to predators.
- Suitability as a commercial product.

Whether or not a non-native species should be introduced to Bay waters on a large scale is for policy makers to decide. VIMS will conduct the science and evaluate risks, and deliver that information to the governmental groups which oversee fishery regulations and natural resources in the Commonwealth.

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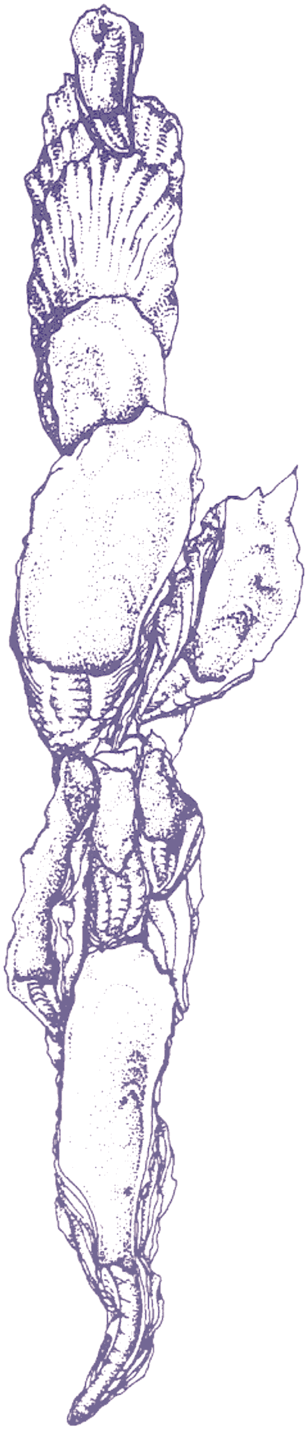
Where Do The Test Oysters Come From?

The National Oyster Broodstock Facility at Hatfield Marine Science Center, Oregon State University. The Oregon facility maintains a source of oysters with known lineages. Without this knowledge an experiment would have limited value. Known lineages provide this type of information: the exact geographical location where it exists, which in turn, is an indicator of tolerances to salinity and temperature; and its resistance to various pests and pathogens.



Recent Publication

The Eastern Oyster: *Crassostrea virginica*

Albert F. Eble, Victor S. Kennedy and Roger I.E. Newell, editors
$95.00

This comprehensive volume of research on many aspects of the eastern oyster was published by Maryland Sea Grant College in July 1996. The book is aimed at the specialist, but should be of value to scientists, managers and commercial aquaculturists. Chapters in the book include: A Catalogue of Selected Species of Living Oysters (*Ostracea*); General Anatomy; The Shell and Ligament; Adductor and Mantle Musculature; Mechanisms and Physiology of Larval and Adult Feeding; Digestion and Nutrition of Larvae and Adults; the Circulatory System; Hemocytes: Form and Function; Reproduction and Early Development; Larval Biology; Biochemical and Population Genetics; Cytogenetics and Evolution; Environmental Factors: Response to Metals; The Bioaccumulation and Biological Effects of Lipophilic Organic Contaminants; Predators and Pests; Diseases and Defense Mechanisms; Culture: Application; Transfers and World-Wide Introductions; and Management of Natural Populations.

This book can be ordered from the Maryland Sea Grant Program, 0112 Skinner Hall, University of Maryland, College Park, Maryland 20742.

Oysters, long part of human fare, appear in all sorts of art forms, from painting to poetry. Lewis Carroll's oysters fell prey to the fast-talking Walrus, a character who, along with the Carpenter, lured the bivalves away from the oyster bed only to feast upon them.

—Drawing by John Tenniel, from *Alice's Adventures in Wonderland*, 1865.

"O Oysters, come and walk with us!"
The Walrus did beseech.
"A pleasant walk, a pleasant talk,
Along the briny beach. . ."
–Lewis Carroll

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