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Technical Report

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ABSTRACT

This report is a summary of a workshop on shellfish disease issues of current concern to the shellfishing industry of the northeastern United States. The workshop, sponsored by the Woods Hole Oceanographic Institution Sea Grant Program, was held on February 26, 1987, at the Woods Hole Oceanographic Institution. Its principal aim was to keep the shellfishing community abreast of the latest information on diseases of importance to wild and cultured shellfish stocks in the area. Topics addressed by invited speakers (scientists, managers, and growers) included 1) MSX oyster disease, which has recently caused a high incidence of oyster mortality at one location on Cape Cod, 2) tumors of soft-shell clams, 3) "brown tide," a new problem with recent dramatic effects on scallops in New York and mussels in Rhode Island, and 4) shellfish hatcheries and shellfish importation in relation to disease concerns. The workshop was attended by more than 100 people, primarily shellfishermen, shellfish officers, members of town shellfish commissions, and shellfish biologists from Massachusetts.

THE HISTORY OF MSX OYSTER DISEASE

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Oyster production on the East Coast of the United States has been generally declining since the early 1880s when the first Federal statistics were gathered. The New Jersey industry in Delaware Bay from about the middle of the last century was largely based on the transplanting of seed oysters from the upper part of the Bay to leased grounds in the lower Bay. Average annual harvest from the early 1880s to 1930 was approximately two million bushels. This dropped to about one million bushels from 1930-1950 and the early 50s saw a general decline in seed oysters available from the upper Bay natural beds. In the spring of 1957 there was an unprecedented kill of planted oysters in the lower Bay and within three years 90-95% of the oysters on planted grounds were killed. Severe mortalities also occurred on the lower two-thirds of the seed beds. The new microscopic parasite causing these mortalities was first called MSX in 1958. Its scientific name is now Haplosporidium nelsoni. In these earliest MSX experiences it was noted that mortalities were reduced in freshened areas at the mouths of tributary creeks and rivers as well as on the uppermost seed beds.

Through a program of bay-wide study of the activity of MSX throughout the years since its appearance, we know that the pressure of this disease on the oyster population has not decreased. There have been four periods of relatively light pressure interspersed with periods of relatively high disease prevalence accompanied by higher-than-average mortalities. The last two years have been unusually severe.

How has the industry managed to survive in Delaware Bay? Principally for two reasons: 1) Delaware Bay oysters have been under continuing disease pressure since 1957 and the surviving natives are measurably more resistant to MSX kill than those in the Bay in 1957; 2) there have been significant changes in industry practices designed to reduce MSX losses.

Through trial and error, oyster planters have found that, in general, their oysters have better survival if they avoid the lowermost high salinity planting areas and concentrate their oysters on grounds as far up Bay as they are permitted to plant. A second change in practice is to reduce the period of time over which planted oysters are exposed to MSX in the lower Bay. This is done by a shift to planting larger seed oysters that can usually be brought to market size within a single growing season. In pre-MSX years, oysters were on planting grounds from two to four or five years. This shift in practice puts a premium on larger seed and more dredging pressure on the lower seed beds where growth is generally more rapid than on the upper seed beds.

An attempt to increase survival of oysters by extending the planting area upbay has not been generally successful because much of the upbay bottom available, i.e. between the established seed beds, is unstable.

No one has yet been able to infect oysters experimentally with MSX though many groups of investigators have tried. This failure in direct transfer of MSX, coupled with rarity of MSX spores (a stage in the life cycle, which has been found in only about two dozen oysters out of several hundred thousand examined in Delaware Bay) and the fact that, in nature, oysters can become infected when placed in areas where other oysters are rare or absent, has led us to the speculation that MSX may have an alternate or reservoir host in its life cycle. A reservoir host, releasing variable numbers of infective MSX particles, could also plausibly account for the great differences in MSX prevalences and in oyster mortalities from year to year.

Within two years after its appearance in Delaware Bay, MSX was found by the Virginia Institute of Marine Science Laboratory to be the major cause of serious oyster mortalities in Virginia. Major planting areas in the lower Chesapeake, e.g. Mobjack Bay, are out of production and the Virginia oyster industry is largely confined to the major river estuaries such as the Rappahannock and the James. Also in the early 1960s, the VIMS laboratory found another serious haplosporidian parasite in the oysters of its coastal bays and sounds. This was called "SSO" for "Seaside Organism" and has since been found in coastal bays up as far as Maine. In its northern areas, it has not generally been associated with significant mortalities.

During the drought of the mid 1960s, MSX was associated with mortalities in oysters in Maryland waters (e.g. Marumsco Bar and Tangier Sound). After that drought, MSX retreated but in statewide sampling in recent years has reappeared farther upbay in several locations. Currently, workers at the Oxford Laboratory (Farley and Kern) are reporting MSX farther up tributary rivers than ever before.

As early as 1965 MSX was found in oyster plantings throughout Great South Bay, Long Island, but with little associated mortality. Although MSX has been found consistently here, and in other Long Island and New England regions, until recently there has been little evidence for kills approaching in severity those of the Chesapeake and Delaware Bay areas. Oysters brought from Long Island Sound and Great South Bay for testing for resistance to MSX disease have been found highly susceptible to kill under the MSX pressure of Lower Delaware Bay. An interesting and potentially very important question for oyster resource management is why for twenty five years or more, MSX was consistently present in this region with little evidence for significant mortality and why is it now apparently causing severe oyster kills?

ACCOUNT OF A RECENT, SEVERE INCIDENCE OF MSX OYSTER DISEASE
ON CAPE COD

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Cotuit Bay has been famous for oysters since the mid 1800s. Traditionally, the planters, who numbered several dozen in early times, would procure their 'seed' each season from producers in the Long Island Sound area. This was necessary as Cotuit Bay had a poor record of natural setting. This was probably due to the vast exchange of water between the Bay and Nantucket Sound. The 'seed' that the planters brought in was semi-mature oysters of 2 or 3 year old class which would count about 350 to 375 per bushel. These would be planted in the spring of each year, allowed to grow over the summer and were harvested in the fall and winter. By mid-fall the stock had usually grown to count between 225 and 250 per bushel, which was about a 50% increase in volume.

The planters seldom realized more than a bushel harvested for each bushel planted. This translated to a mortality rate of approximately one third. Some of this was due to natural predators such as the whelk (both varieties) and the oyster drill, with an occasional starfish being present. Human predation was also present at times. The remaining unidentified losses were termed "background mortality" which could account for approximately one-half of the total mortality. There are a limitless number of possibilities contributing to these "background losses"...an area which is beginning to receive more attention.

In the 1950s and 60s seed became in short supply, forcing many planters both on Cape Cod and in Connecticut out of business. When George Matthiessen and I took over Cotuit Oyster Co., Inc. in 1973 it was nearly defunct and was the last planting operation remaining in Cotuit. Seed oysters were obtained from a rack culture operation in Wareham, Massachusetts, placed on the bottom for one growing season and then were successfully marketed. In succeeding seasons seed was obtained from various parts of Connecticut and Rhode Island with minimal results, as much of the available supply was of poor shape, had

brittle shell, and often showed slow growth. In all areas of transplanting, an annual test on 50 oysters from each area was performed to determine any presence of MSX. Until the fall of 1984 all results were 'negative'.

We began to supplement the sporadic supply of natural seed with hatchery stock in the late 70s and were encouraged by the early results, although some mortality did appear between the ages of 2 and 3 years, a few months before market size was attained. The hatchery seed planted was usually about one inch long.

In the fall of 1984 we took a routine sample of 50 oysters from a load of natural oysters originating from the Hammonasset River in Clinton, Connecticut to do our annual MSX test, so that permits could be obtained for the transplants anticipated from spring, summer and fall of 1985. This had been the accepted procedure in prior years, with all 'negative' test results. This 1984 sample, however, proved positive with heavy infection in several animals.

When we surveyed our crops in early March of 1985, mortalities of up to 50% were found in the 2+ year old hatchery oysters. (When surveyed in late December of 1984 this crop was about 90% alive). The summer of 1985 brought another round of mortality to the crop, after a late spring/early summer evidence of minimal kill. August was the peak time. The end result of this was about 85% total mortality on that crop. The following year's crop showed little mortality in 1985 other than about a 10% predation. In 1986, however, the same pattern occurred on that crop, with an end result of 85-90% total mortality.

Now, in 1987 we have gone back to the drawing board and intend to continue planting only hatchery oysters of a larger size thus decreasing the exposure time. It is our belief that by doing this, while concurrently attempting to breed MSX-resistant strains, our business can have a substantial future.

We wish to express our deep appreciation and gratitude to Dr. Louis Leibovitz for conducting a monthly monitoring program of our test plantings last season. This program was instrumental in helping us determine our future course.

The future of aquaculture is bright...but there is much to be learned. There has to be a dialogue and understanding between the planters, the scientists, the hatchery operators, and the regulators in order for the industry to succeed. Regulation and mutual trust are necessary in any industry. In aquaculture, any future regulations governing seed production and transplanting to other areas must be carefully thought out, so that they will be effective and at the same time will allow the legitimate operators to proceed and conduct their businesses without undue hardship.

We look forward to the future!

A PRELIMINARY STUDY OF DISEASES OF
CULTURED AMERICAN OYSTERS (CRASSOSTREA VIRGINICA)
DURING AN ANNUAL GROWING CYCLE AT THE COTUIT OYSTER COMPANY
IN SOUTHEASTERN MASSACHUSETTS

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A study was undertaken to determine the patterns and causes of oyster mortality on a commercial oyster farm known to be infected with MSX (Haplosporidium nelsoni) during a normal growing season. Sibling MSX-free seed oysters, approximately two years of age, were obtained from a hatchery at Fishers Island, New York and planted monthly at the Cotuit Oyster Company in trays, containing 100 seed oysters each, from April to November, 1986.

Individual trays of each month's planting were raised and sampled each month and examined to determine the number alive and dead. The valves were examined for signs of predation.

During the monthly examinations of the April-planted trays, twenty-five oysters were removed. Each of these oysters was examined grossly and microscopically at the laboratory. The height and width of each oyster was measured. The individual fouling organisms found on or in the shell and lesions of disease were recorded. Transverse sections of the soft tissues of the upper and lower third of the body were taken for histopathological examination. The former sections included the mantle, visceral mass, gills and palps. The latter sections included the mantle, gills, rectum, portion of the visceral mass, heart, and adductor muscle. The stained processed

histological sections were examined microscopically, and pathological findings were recorded. The results of the study indicated that the greatest causes of mortality were MSX and predation. Although MSX was noted in older stocks planted in previous years, the earliest MSX mortalities in oysters planted during this study were first noted in July in the April-planted oysters, three months following their planting. A minimum incubation period of two months was noted in oysters planted afterwards. Salinity remained relatively constant during the period of observation and was not considered as a factor influencing the course of MSX during this study. ~~Although the incubation~~ period remained relatively constant in newly planted oysters, higher peak mortalities were related to higher seawater temperatures. The highest mortalities were noted from July through September. The persistent presence of MSX infection in infected older resident oysters and newly infected oysters was evident throughout the entire study.

Gross examinations of monthly samples of the April-planted oysters revealed the presence of many common fouling agents, including slipper shells (Crepidula), barnacles, bryozoa, tubeworms, micro and macroalgae, jingle shells, limpets, and others. "Mud Blisters" (containing viable Polydora) were common in oysters prior to planting and persisted in newly planted oysters until June, after which, only remaining scars without worms were in evidence. "Hinge-Rot," a degenerative disease of the valvular ligament, was noted in September. "Lip-Bill," a deformation of the new shell growth, was detected two months after planting. A previously undescribed disease resulting in death of ovarian tissues (Ovarian Necrosis) was first detected in May. The disease persisted through the following months until September when reproductive activity ceased. Bacterial infections of the visceral mass were first detected in the May-collected specimens and continued until August when MSX dominated detectable disease alterations.

Microscopic examinations of stained tissue sections demonstrated that marked gonadal development occurred in June. Release of eggs and sperm in the gonadal ducts was noted in the July and August samples. Approximate sexual parity ratios were maintained until September, when the majority of oysters

were not, detectably, sexually differentiated. "Amorphous Blue Bodies" (round blue inclusions in the lining cells of the digestive tract) were detected at a low level from May through August. An abundance of algal foods was present in the digestive tract from April until, and including, the month of July. After that time, the digestive tract contained limited quantities of complete food particles consisting principally of fragments of diatom frustules.

MSX appeared suddenly and almost uniformly in the August specimens, resulting in infection of seventy-two percent of the oysters, of which forty-four percent represented localized infection and twenty-eight percent represented generalized infection. The apparent portal of entry for the infective organisms was the surface cells covering the inner food-collecting surfaces of the oral palps and the terminal food-collecting grooves at the extremities of the gills. The single cell MSX infecting unit was first contained in a vacuole within the surface cell, where rapid proliferation of the organism occurred, yielding progressively two, four, and eight nuclei within the extended cell wall of the infected cell. The continued invasion of the surface cells of the oyster's tissues and the great increase in size of the parasites resulted in the destruction of the protective outer layer of oyster tissues. Some of the parasites entered into blood vessels of the rich vascular gill structure and were carried to other organs of the oyster's body, resulting in the more fatal generalized infection. Most of the parasites were discharged from the infected tissues into the environment.

The percentages of MSX infection gradually increased from seventy-two percent in August to ninety-six percent in November. During periods of increase in the percentages of new infection, there were alternate increases and decreases of the percentages of generalized infections, as compared to localized infections. It is assumed that this inverse relationship resulted in mortality differences between months when the less fatal localized infections progressed to generalized, more fatal infections in the next monthly period. Thus the initial sudden increase in percentage mortality was followed by fluctuations in mortality resulting from the alternate increases and decreases of new infections and older generalized mortalities. The persistent high increasing percentage of infection and the sustained additive mortality rates suggest that once an oyster becomes infected, it remains infected and ultimately dies of the disease.

Although some experts consider the oyster to be an accidental host for MSX, the senior author believes that the enormous number of parasites discharged from the diseased tissue of the infected oyster must contribute to the pool of infective material either directly or indirectly (intermediate hosts). Such massive discharge of the agent must contribute to establishing reservoirs of new infections.

This is a preliminary study, based upon limited sampling during a single season of production. It is an initial examination of important problems for the oyster industry, which like all shellfish industries throughout the world requires the prevention, control, and possible eradication of devastating, economically important diseases. Since each oyster-culture environment is different, each location must examine and understand its own disease problems before health programs can be implemented. This study represents such an examination.

The results of this study suggest that the introduction of infected stocks into disease-free areas poses a real and dangerous problem for the oyster industry. The repeated introduction of completely susceptible oysters into infected areas fails to select for natural resistance to disease and results in greater economic losses each year. Resistant disease-free replacements are apparently needed to reduce economic losses. Management should develop methods of cultivation that would reduce disease losses. Local propagation (shellfish hatcheries) is required to avoid the introduction of new disease or to eliminate established diseases.

In addition, the study indicates some basic scientific needs to confront such serious diseases as MSX and others found in this study. These include a basic understanding of the disease process, accurate and sensitive methods of disease recognition (both in the field and in the laboratory), an understanding of the life cycles of disease organisms; and the development of disease-resistant, disease-free oyster strains and methods of management that prevent disease. It is unfortunate that support for study of diseases of marine animals is very limited and lags far behind that of other medical sciences. Much of this indifference relates to failure to develop our understanding of such disease processes, thereby failing to demonstrate the economic value of such knowledge.

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PROGRESS ON THE DEVELOPMENT OF MSX-RESISTANT OYSTER STRAINS

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Epizootic mortalities caused by the parasite Haplosporidium nelsoni (MSX) in Delaware Bay between 1957 and 1959 were followed by signs that resistance was beginning to develop in the native population. The evidence included a ~~lowering of mortalities of planted seed oysters beginning in 1961~~, better survival of Delaware Bay stocks compared to oysters imported from areas without MSX, and steadily decreasing mortality of successive year classes setting in the lower bay between 1957 and 1959. In the early 1960s, a hatchery breeding project was begun at Rutgers Oyster Research Laboratory in New Jersey to determine whether resistance to MSX was really heritable and, if so, to develop laboratory-reared strains of oysters resistant to the parasite.

Oysters that survived heavy mortality (90-95%) were selected as broodstock. In addition to Delaware Bay natives, lines were begun from groups originating in James River, Virginia; Navesink River, New Jersey; and Long Island Sound. Offspring were placed in trays and exposed to MSX during a standard 33-month testing period in lower Delaware Bay. This is approximately the time required for oysters to reach market size in this location. In each generation, survivors of the standard exposure period were selected as parents for the next generation. To ensure that better survival in the offspring of selected stocks was not due to lessened MSX abundance or virulence, control groups were produced from imported oysters, which had not experienced selective mortality. These were exposed to infection in exactly the same manner as the selected offspring.

Average mortalities for control and selected groups after 33 months of exposure is shown below.

<u>Generation</u>	<u>Number of Groups</u>	<u>Average Total Mortality</u>
Control	35	92
First	12	64
Second	13	59
Third	9	40
Fourth	5	37
Fifth	4	30
Del. Bay Natives	14	68

The 92% mortality of unselected control groups is about the same as the initial mortality of Delaware Bay oysters during a comparable period between 1957 and 1959, indicating that the parasite is still as abundant and virulent as it ever was. Selection and breeding of oysters for resistance to MSX under these conditions has continuously improved average survival for five generations. There are about 10 times as many survivors in the fifth generation as in the unselected controls.

Delaware Bay native stocks, tested under the same conditions as the hatchery-reared groups, have an average mortality that is about the same as first generation offspring. The reasons that their survival has not improved as much as the hatchery groups is because there are so many unselected (susceptible) oysters in low-salinity areas of the bay that are protected from MSX. Their larvae mix with and dilute larvae from selected (resistant) oysters, producing a moderately resistant oyster, but one that is considerably less resistant than the hatchery-bred oysters.

Unfortunately, the picture is not quite as simple as described above. Selected (resistant) oysters still become infected by MSX, but infections remain localized in the surface layers of the gill for considerable periods of time. This contrasts with susceptible oysters, in which parasites quickly

move from initial sites of infection in the gill into the circulatory system and then throughout the body of the oyster, killing a large percentage within 6 to 8 weeks. As long as parasites remain localized, resistant oysters show relatively few ill effects from the infections. Eventually, however, the stress of chronic infection and/or annual reinfection proves too much for even the most "resistant" strains and a large proportion die from MSX after 5 or 6 years of continued exposure. It is important to remember, though, that most of the highly selected oysters reach market size before significant mortalities occur.

Although survival has improved, on the average, with each generation, the performance of individual strains has been more variable. In fact, some of the strains have shown unexpectedly high MSX-caused mortalities in the 4th, 5th, and 6th generations. We are now conducting a study to determine whether inbreeding has caused a loss of hybrid-vigor. Preliminary evidence indicates that this is not the case. Additional research is underway with the aim of increasing the reliability and predictability of the resistant strains, and of improving growth and meat yields.

Over the past year, we have supplied selected brood stock to hatcheries in several states. This collaboration will provide information on the performance of the resistant strains in areas other than Delaware Bay. Also, progeny have been returned to us for testing under "standard" conditions in Delaware Bay. We consider the MSX-resistant strains to be still in the developmental state. Relatively little research has been done on selective breeding of bivalve molluscs so that we are working in an uncharted area. Much more research must be done before the breeding of oysters reaches the level of farm animal breeding, but we have already made major strides in raising the level of MSX-resistance in selected strains.

TUMORS OF SOFT-SHELL CLAMS

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There is one tumor of significance in soft-shell clams. Much controversy exists concerning the naming of this tumor. Three terms are currently in usage. They are Hematopoietic Neoplasia, Sarcoma, and Proliferative Disease.

Detection of the tumor is accomplished by either examination of histological sections of body tissues or by examination of the clam's hemolymph (blood). Examination of the hemolymph provides the fastest answers and additionally can detect the tumor in earlier stages than histological examination. In order to examine hemolymph, it is withdrawn from the posterior adductor muscle sinus and a hemolymph preparation is made (analogous to a blood smear). Traditionally the hemolymph preparations were stained with a common cytological stain then examined for neoplastic cells. Dr. Reinisch's laboratory has developed monoclonal antibodies which recognize epitopes specific for these neoplastic cells. Using these antibodies which attach only to neoplastic cells and not normal ones, I have developed a diagnostic test termed the IP test, which can be used to more easily and accurately detect and evaluate the neoplastic cells in hemolymph preparations. Using this test, clams can be diagnosed and staged by technicians untrained in cytological evaluation.

The neoplasia progresses in the clam from only a few neoplastic cells in the circulation to a point at which greater than 99% of the cells in the circulation are neoplastic. In concert with the increase of cells in the hemolymph, the neoplastic cells can be observed to percolate throughout the animal's body and proliferate in every tissue. Our observations demonstrate that death of affected clams occurs in the final stages of the disease.

If only a few clams developed this tumor it would be of relatively little importance or concern to us. However, our laboratory and other laboratories have found the neoplasia is endemic in soft-shell clams of the east coast, including all of Massachusetts. Furthermore, there have been reports of possible epidemics of this neoplasia in recent studies.

The cause of this neoplasia has not yet been determined. Possibilities include virus, pollution or a combination of these two.

What are the implications of this tumor for us? First, we should keep in mind it may be contagious for other soft-shell clams. Second, it may be that observation of this disease in clams will provide information in pollution monitoring. Finally, the economic implications could be significant due both to possible epidemics and to constant mortality of clams in endemic populations.

EFFECTS OF "BROWN TIDE" ON SCALLOPS IN NEW YORK

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A small (2 μm diameter) chrysophyte alga, Aureococcus anorexefferens, bloomed in Long Island's southern and eastern embayments throughout the summers of 1985 and 1986. The "brown tide" attained peak, mid-summer concentrations exceeding 1 million cells per ml. A bloom of similar characteristics was described during the summer of 1985 in Narragansett Bay, Rhode Island, where it caused catastrophic mortalities of blue mussel populations. In New York State, the bay scallop, Argopecten irradians irradians, has been the commercial shellfish species most severely impacted by the "brown tide" phenomenon. The 1985 bloom coincided with the scallops' June-July spawning period, causing massive recruitment failure of the 1985 year class, and 76% reduction in mean muscle weights of adults (1984 year class) in the Peconic estuary. Mortality rates of the adult year class were not documented. Post-spawning survivors of the 1985 bloom showed remarkable recovery in tissue weight after the bloom subsided in the fall. They experienced a 3-fold increase in adductor muscle weight during September, so that the mean weight surpassed that of populations in 1984. Adult scallops also showed an approximate two-month delay in the winter period of mass natural mortality, so that it is estimated that 30% of the population could have potentially survived to a second spawning in 1986. Natural recovery of stocks was precluded, however, by reappearance of the bloom in the summer of 1986. Transplant programs of hatchery-reared seed into Long Island's bays began in the fall of 1986, in an attempt to rehabilitate New York State's bay scallop fishery. A total of about 1.7 million scallop seed was distributed among five major sites within Peconic and Gardiner's Bay estuaries.

Potential mechanisms explaining the impact of the bloom on shellfish include: poor retention of small (less than 5 μm) particles by the bivalves' feeding apparatus, toxicity effects, poor nutritional quality of Aureococcus, and/or inefficient feeding at high algal densities. Laboratory grazing studies using field collected water samples demonstrate that bay scallops retain the small alga with low efficiency (about 36%) relative to blue mussels

(about 59% retention efficiency). Low retention efficiency is, however, insufficient to entirely account for the starvation effects observed, given the high algal densities present during the bloom. Other hypotheses are currently being tested using scallops and mussels as test organisms, and laboratory cultures of A. anorexefferens. Results of laboratory feeding studies can be used to predict the age-specific effects (e.g. rate of weight loss) experienced by shellfish at field algal concentrations. This information should be useful to hatchery operators and fishery managers, e.g. in assessing the need and benefit of temporarily transferring stocks from impacted to unaffected areas, or in selecting the optimum size of animals for transplant programs.

SHELLFISH MANAGEMENT STRATEGIES TO CONTROL
THE INTRODUCTION OF UNDESIRABLE ORGANISMS

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Mortalities in shellfish populations from many locations throughout the world are being reported with alarming frequency. The scientific literature records cases of inadvertent or establishment by design of populations of non-indigenous species of shellfish in many locations around the world. Documentation is increasing on concomitant transfer and establishment of shellfish diseases, parasites, predators, pests, and other entities through introduction of shellfish to new environments.

In North America there are at least two well documented lethal molluscan disease epizootics that have resulted from introductions of infected animals into previously unaffected ecosystems. Perhaps the best example is Malpeque Bay disease reported from Prince Edward Island, Canada, whereby a thus far unidentified infectious agent of unknown origin was transported to mainland maritime locations through introductions of infected oysters. Also the pathogen MSX (Haplosporidium nelsoni) was introduced with oysters from Virginia into the previously uninfected stocks at Wellfleet, Massachusetts. Other information would indicate that molluscan diseases and parasites in North America have been spread from one ecosystem to another by the introduction of native and exotic species of shellfish. The recent reports of viral diseases of shrimp and their introduction into the aquaculture systems of Hawaii and the spread to Europe of the North American crawfish plague fungal disease are only the latest tragedies to be reported.

In Europe, introductions of at least three infectious disease entities from sources which at present can only be speculated upon have been responsible for mass mortalities of oysters. These include the viral "gill disease" and two diseases caused by the protozoans Bonamia ostreae and Marteilia refringens. Similar diseases have now been reported in Australia.

International guidelines to control or prevent the spread of infectious diseases in North America and northern Europe have been developed by the International Council for the Exploration of the Sea (ICES) and the United Nations-sponsored European Inland Fisheries Advisory Commission (EIFAC). Other international groups are considering similar types of recommendations.

Individual countries such as Great Britain and Canada have also developed guidelines for the management of shellfish transports to minimize the risks of the introduction of exotic species, including disease agents.

In the United States, although there are many laws and regulations particularly at the state level, there are no compatible and coherent programs to address disease control and prevention in molluscan and crustacean populations. However, development of such programs is in progress on the federal/state level through the Marine Fisheries Commissions of the Gulf, East, and West Coasts of the United States working cooperatively with the National Marine Fisheries Service, various state fishery and conservation agencies, and industrial organizations.

OFF-BOTTOM CULTURE OF SEED OYSTERS TO LARGE SIZE
FOR PLANTING IN MSX-INFECTED AREAS

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Ocean Pond Corporation has been engaged in the production of seed oysters (Crassostrea virginica) since 1962. Located on Fishers Island, New York, the company raises all of its oysters in a 35-acre brackish water pond. For the past ten years, Ocean Pond Corporation has been closely affiliated with the Cotuit Oyster Company, providing this company with a large percentage of its oysters.

Until recently, the practice was to harvest the oysters from the pond when they were 30-40 mm in size and not more than a year old. These would be packed in burlap bags and trucked to the Cape, where they would be spread on the bottom to mature to market size. A minimum of two more growing seasons would be required for these oysters to become marketable.

The occurrence of MSX on the Cotuit beds has necessitated a change in this system, since oysters remaining for more than one year on the beds may experience unacceptably high mortalities. The two options available to us were to 1) produce seed oysters with high resistance to this disease, or 2) raise the seed to a much larger size on Fishers Island before moving them to Cotuit, thereby minimizing the period of exposure to MSX. We concentrated primarily on the latter option during this past year, and the results are reported in this paper.

Ocean Pond Corporation presently produces all of its oysters initially from a small hatchery facility located on the shore of the pond. The larvae are set on small chips of shell or dolomite that can be transferred immediately to floating fly-screen trays as soon as the larvae have attached. Four to six weeks after setting, the juvenile oysters have reached 10 mm in size and are transferred at reduced densities to floating trays having a coarser mesh. By late fall, the oysters have reached 25 mm or so in size and are transferred to Nestier trays, assembled in tiers and suspended in deeper water to avoid ice.

During the following spring, after another period of growth, the oysters ordinarily would be harvested from the pond and moved to Cotuit. Under the present system, however, they are transferred to 5-tiered lantern nets, at densities of 200 oysters per tier. These nets are suspended from long-lines moored either in the pond or in Fishers Island Sound. The oysters will remain in the nets for an additional year or so, the objective being to delay their transfer to Cotuit until the majority of the oysters have reached 60 mm or more in size and can be planted on the Cotuit beds with the minimum risk of infection and mortality.

This system is intended to produce about one million oysters of this size each year. A total of one thousand lantern nets are required to do this, resulting in a considerable amount of handling and maintenance. Once each month during the growing season, each net is removed from the water and transferred to shore, where it is air-dried for four to six hours. This has been found to be more effective in discouraging biofouling than hosing by pump. A major nuisance is the tendency of the smaller oysters to grow into the mesh of the net. Also, we have found a very high incidence of Polydora among oysters grown in suspension. Finally, by the end of their second growing season, many of the oysters weigh 40 grams or more, adding to the problems of net handling.

The major advantages to this system include a very high rate of survival - nearly 100% - during the first two years of growth; a satisfactory growth rate under highly crowded conditions; and flexibility in terms of selection of a culture site. It is believed that these factors combined with the high price of high-quality oysters will tend to justify the additional labor and expense involved in off-bottom culture.

REGULATORY ASPECTS CONCERNING THE IMPORTATION OF SHELLFISH

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The Division of Marine Fisheries (DMF) clearly has the authority and a vested interest to control the transport, transplanting, and introduction of shellfish into the waters of the Commonwealth. This authority can be found in various sections (2, 17, 17B, 20, 28, 69, 75) of Chapter 130, MGL and is both both direct and indirect.

In effect, the Division is directed to "...assist and cooperate with coastal cities and towns for the purpose of increasing the supply of shellfish and exterminating the enemies thereof..." (Section 20). The Division also has general regulatory authority (Sec. 17, 17B), and permitting authority (Sec. 2, 69, 75) regarding seed and contaminated shellfish and can determine that certain "fish", including shellfish, are "injurious".

Currently, the transport and introduction of shellfish is controlled by statute, regulation and policy.

State statute (Sec. 69) requires a permit from the Director of DMF to take or possess seed shellfish for replanting in waters of the Commonwealth, while Sec. 75 authorizes permits to take shellfish from contaminated areas for purposes of transplanting for natural depuration.

The code of Massachusetts Regulations (322 CMR) Section 3.03 requires a special permit from the Director of DMF to ". . . plant, transplant or introduce for the purpose of transplanting seed or adult oysters, into any waters or into any shellfish areas within the Commonwealth . . ."

The introduction of exotic or non-indigenous species is prohibited as a matter of policy under authority emanating from the various statutes allowing the Division to issue permits and set permit conditions which have the full force of regulation.

Any violation of the above is subject to fines up to \$1,000, confiscation of any shellfish, revocation of any permits or licenses issued by the Division and possible imprisonment.

The primary reason for regulating the planting of shellfish is conservation, that is, the regulations are ultimately designed to protect the Commonwealth's shellfish resources from diseases, predators, competitors, and nuisance organisms. In so doing, these measures also help to protect the planter's investment no matter if the planter happens to be a private grower or a municipality.

The Division has tried to avoid creating a regulatory morass making the transplantation of shellfish so difficult that it discourages individuals altogether or worse, encourages circumvention of the permitting process. The unfortunate reality is that, as with many other things, life may get more complicated as concern increases regarding the spread of shellfish diseases like MSX and Neoplasia as well as the possible introduction of various pests and competitors associated with the transplantation of shellfish.

Oyster Relays

Permits have been required to transplant oysters into the Commonwealth and/or across town lines since April 1, 1970, as a result of the discovery of MSX in Wellfleet Harbor in 1969 and an assessment of the shellfish disease situation along the east coast of the United States.

For one to obtain a permit, oysters must be certified free of all known serious oyster diseases and parasites such as: MSX (Haplosporidium nelsoni), SSO (Haplosporidium costalis), Oyster Fungus Disease ("Dermo"), and Herpes-type Virus Disease of Oysters. Examination and certification must be conducted by a recognized laboratory such as the National Marine Fisheries Service Laboratory, Oxford, Maryland or another laboratory acceptable to the Division prior to issuance of a permit by the Division.

If oysters are found to be infected with any of these diseases or other diseases and parasites considered to be a serious threat to oyster stocks, transplants will not be allowed except under certain conditions in the case of SSO.

Since SSO has been declared "endemic throughout the northeast" by the National Marine Fisheries Service, relay of oysters infected with H. costalis will be allowed; however, only into those areas within the Commonwealth which are now known to be infected with this pathogen or can be demonstrated to be infected prior to any new introduction of oyster stocks.

Procedure:

- a) Not less than 50 oysters shall be examined from each major source site and examination must have been made within 12 months prior to the date of the relay. These oysters shall be obtained from mid-August - mid-October.
- b) Division biologists will also take into consideration the prior history of the source area in addition to the examination results prior to recommending transplants.
- c) If oysters which are intended for transplant are found to be infected with H. costalis, the transplant will only be allowed provided that oyster stocks at the final relay site are known to be infected with this pathogen or can be demonstrated to be infected by examination of not less than 50 oysters at that site prior to any new introduction of oyster stocks.
- d) Introduction of infected oysters into areas traditionally considered non-oyster areas shall not be allowed.
- e) As a matter of policy, only oysters free of H. costalis should be transplanted whenever possible.
- f) A bond of \$500.00 written specifically for diseased oysters must accompany the application for a permit to transplant oysters (waived on municipal applications).
- g) Nothing in this policy presently affects the transplant of oysters within the corporate boundaries of any city or town. However, in practice, most municipalities have adhered to the above policy within their municipal limits.

Import Policy

At the present time, the Division has a general embargo in place, regarding sources of indigenous shellstock. In effect, no shellstock can be brought into the Commonwealth for planting from areas south of New York State as a routine matter. Shellstock from other areas of the country or from outside of the country may be allowed subject to certification that they are free of disease and various pests and parasites and under other conditions deemed appropriate.

For example, from the West Coast we would be concerned about the presence of the parasite Mytilicola or the possible inadvertent introduction of unwanted species like the Pacific oyster, C. gigas, or the Manila clam Tapes semidecussata. Obviously, on the East Coast our primary concern would be with MSX and "Dermo" in oysters but not necessarily limited to these diseases of the oyster. The State of Maine recently restricted the importation of blue mussels from Massachusetts to Maine based upon concern over the possible introduction of Codium and pea crabs.

If Neoplasia in soft-shell clams is the result of viral infection as it appears to be, then transplanting clams without prior disease certification could result in spreading the disease into uninfected areas along the East Coast. Presently, it appears to be endemic in much of the Northeast.

Exotic Species.

Regarding exotic or non-indigenous species there is also a general prohibition concerning introduction into the wild. However, in 1977 the Commonwealth allowed limited introduction of European oysters, Ostrea edulis, into historically non-oyster producing areas. These oysters originated from Maine hatcheries using Maine parent stock from long established local populations that were determined to be free of diseases and parasites.

Also, under suitable controls such as those described in the International Council for the Exploration of the Sea's (ICES) "Report . . . on the Introduction of Non-Indigenous Marine Organisms", exotics may be allowed in hatcheries or marine laboratories for scientific studies or as parent stock

for production and shipment to an approved destination outside the Commonwealth. Such controls would include, but not be limited to, disease certification, quarantine and control or treatment of effluents. Presently, one hatchery in the state is rearing the Black Pearl oyster Pinctada umbricata in a separate closed system for shipment to the Bahamas for field grow-out.

Major problems facing regulation of shellfish importation and disease control are the difficulty in obtaining disease certifications, the reliability of such certifications, introduction by people who are unaware of or disregard the significance of indiscriminate transplants, and wet storage of market shellstock not being held for grow-out.

SUMMARY OF RESPONSES TO WORKSHOP EVALUATION QUESTIONNAIRE

WHOI Sea Grant Shellfish Disease Workshop

February 26, 1987

Woods Hole Oceanographic Institution

More than 100 people attended the workshop. Forty-one workshop evaluation questionnaires were returned, the results of which are summarized below.

QUESTIONNAIRE

Occupation? Audience was about equally comprised of shellfishermen, shellfish officers, members of town shellfish commissions, and shellfish biologists.

How did you learn about this workshop? Mailed Announcement 30
Newspaper 2
Radio 0
Other 9

How much of the information presented was new to you? Lots 22
Some 19
Little 0

Which of the workshop topics interested you most?
MSX 30
Tumors 15
Brown Tide 13
Hatcheries/Importation 13

What did you think of the workshop organization? Good 38
Fair 3
Poor 0

How could the workshop have been improved?
Wide range of opinions here, with no general consensus.

Did you find the workshop worthwhile? Yes 40 No 1
If "no," what was wrong?

One person felt that the scientific information should have been better geared to non-scientific attendees.

What was your overall evaluation of the workshop? Excellent 20
Good 20
Fair 1
Poor

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