REPORT OF THE
VIRGINIA INSTITUTE OF MARINE SCIENCE

STRATEGIC PLAN FOR MOLLUSCAN SHELLFISH RESEARCH; INCLUDING A RATIONAL PLAN FOR TESTING APPLICATION OF NON-NATIVE OYSTER SPECIES

TO THE GOVERNOR AND
THE GENERAL ASSEMBLY OF VIRGINIA

HOUSE DOCUMENT NO. 16

COMMONWEALTH OF VIRGINIA
RICHMOND
1996

PREFACE

This report is submitted pursuant to House Joint Resolution No. 450 of the 1995 Session of the General Assembly (see Appendix A). HJR 450 derives from House Document No. 56, a report directed towards development of a strategic plan for the revitalization of the shellfish industry in Virginia. Reading of HJR No. 450 indicates two charges to the Virginia Institute of Marine Science, to which this report responds:

1. To develop a strategic ten-year plan for molluscan shellfish research, and

2. To initiate the process of seeking approval, in conformance with state, federal, and international laws and protocols, for in-water testing of oyster species not native to Virginia waters.

✠ ✠ ✠ ✠

Acknowledgments: The report preparation was coordinated by Robert J. Byrne via the efforts of staff experts at the Virginia Institute of Marine Science; Eugene M. Burreson, William D. DuPaul, Howard I. Kator, James E. Kirkley, Mark W. Luckenbach, Roger L. Mann, Michael J. Oesterling, and N. Bartlett Theberge, Jr. with incorporation of contributions of Fu-Lin Chu, Mohammed Faisal, and Stephen Kaattari. Mr. Theberge and Gwynne D. Brown prepared the materials addressing current laws, policies, and regulations on introduction of non-indigenous species.

In addition, our planning efforts were assisted by members of the shellfish industry and interested public; T. Robins Buck, Lake S. Cowart, Jr., A. Thomas Leggett, Jr., Dr. William M.Peirson, Dr. William L. Rickards, Peter W. Rowe, Benjamin G. Smith, Kenneth "Pete" Terry, Dr. Jane C. Webb, and Dr. James A. Wesson. The staff and administration are deeply indebted to these individuals for their contributions to this effort. Their participation should, however, not be interpreted as endorsement of the report offered.

Grateful acknowledgment is due to Lorrie Andrew-Spear for assistance in all phases of report preparation as compiler and editor. Finally we gratefully acknowledge the Chesapeake Bay Commission through which the Virginia Delegation provided funds to partially defray costs.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

PURPOSE OF REPORT. This report addresses a plan of research and monitoring to support the following:

1. Restoration of native oyster resources and continued viability of other shellfish resources,
2. Continued development of molluscan shellfish aquaculture, and
3. Elucidation of the significance of shellfish and shellfish habitat in ecosystem function and the natural benefits of ecosystem and economic development which result from a fully functional resource.

One component of this plan involves testing, in quarantine and in the field, the response of non-native oyster species to the two oyster pathogens endemic to the Chesapeake Bay and other regions on the East Coast. Identification of oyster species with superior natural resistance is pivotal to any alternative species strategy to rejuvenate oyster resources. In-field testing of non-native species constitutes an "intentional introduction" of non-indigenous species. Hence, those components of the plan requiring exposure to non-quarantine conditions must conform with international, national, regional, and Commonwealth terms of approval and associated protocols.

STATUS OF RESOURCE AND DEPENDENT FISHERIES. Once the leading producer of *Crassostrea virginica*, the oyster species extant on the East and Gulf Coasts, the Virginia fishery has diminished to crisis level. Three factors appear to be responsible. First, historical evidence clearly documents over-harvesting and habitat depletion. Second, since the late 1950s, two oyster pathogens have decimated oyster populations in higher salinity zones. More recently the pathogens have spread into most growing areas. Third, over time land uses in the watershed of the Chesapeake Bay system have evolved with resulting increased loads of suspended sediment and other loadings which compromise water quality. Thus, resource restoration efforts are being pursued in the face of challenges not prevailing during past times.

Today, shellfish resources other than oysters—most notably, the hard clam—support very significant native fisheries. Other fisheries, including whelks and ark shell clams, are active.

Aquaculture, the controlled husbandry of various species, has flourished in Virginia. Already a leading producer of cultured hard calms, significant aquaculture efforts are underway with the native oyster, and research and development is near completion for husbandry of the bay scallop. As well, there are additional shellfish species—the softshell clam and others—offering significant potential.

CURRENT PROGRAMS IN RESEARCH AND MONITORING. The Virginia Institute of Marine Science (VIMS) has a long and distinguished record of research, monitoring, and advisory service in support of resource conservation, native fisheries, and development of shellfish aquaculture. The efforts continue as a principal thrust of Institute programs. In addition to receiving state funds, the staff has been successful in competing for extramural funds directly applicable to Commonwealth needs. In brief, the recent and current efforts include:

- In support of the native fisheries and related industry
  - formal assessment of oyster stock
  - monitoring surveys of oyster spatfall and post-settlement mortality
  - monitoring the distribution and intensity of oyster diseases
  - intensive research on the dynamics of oyster diseases endemic to the Chesapeake Bay
  - limited life history studies on shellfish species having developing fisheries
  - assessments of human health threats associated with shellfish
  - assessment of oyster reef restoration
  - limited economic assessments of the shellfish industry

- In support of shellfish aquaculture
  - developing hatchery, nursery and grow-out technology and training for the private sector
  - providing seed oysters, clams, and bay scallops to private sector collaborators to demonstrate and revise grow-out strategies
  - developing methods for predicting oyster growth rates to assist in selection of grow-out rates for oyster aquaculture
  - research in testing triploid oysters, algal diet formulations, and evaluation of toxic algal blooms

RECOMMENDATIONS. The Virginia Institute of Marine Science has provided essential research and advice to resource managers as well as industries associated with both the native fisheries and shellfish aquaculture. Development of the Ten-Year Strategic Research Plan has enabled the Institute to critically examine current activities and to identify areas where program enhancements are essential. Those program elements include:

- In support of the native fisheries and related industry, highest priority should be placed on continuation of the fisheries independent stock assessment program for oysters which was initiated in 1993 via federal funding. Federal funding ends in 1996. This program provides the only firm foundation for ongoing management and long-term resource restoration. In addition to surveys of the public oyster bottoms, the program should be expanded to include hard clams.

- The ongoing program to monitor oyster spatfall and post-settlement success provides guidance to managers and industry members as to the levels of potential recruitment to the stocks. Modest expansion is required to include assessments on the seaside of the Eastern Shore.

- Oyster disease monitoring provides information on the abundance and distributions of disease for resource managers, industry members, and scientists. The program should be expanded to include the Eastern Shore and aquaculture sites. In addition, sampling and evaluation should include parasites of the hard clam.

- Results from oyster disease research help maintain the native fishery and also benefit aquaculture development. Five priority research thrusts have been identified:
  - Developing a disease resistant native oyster offers substantial promise for aquaculture. The objective is to provide strains that will reach market size with very low mortality from disease.
  - Determining the life cycle of MSX (*Haplosporidium nelsoni*) is crucial to developing disease avoidance strategies and potential control measures.
  - Determining the mechanisms by which pathogens invade susceptible oysters, survive the host/parasite interaction, and cause infection may lead to control methods for both pathogens.
  - Development of chemical treatments for disease would be useful in aquaculture applications.
  - Enhancing the ability to predict changes in oyster pathogen abundance in response to environmental conditions such as salinity and temperature is critical to estimating impacts on native stocks and aquaculture.

- Determining oyster reef structure and function is an essential component of habitat restoration. Priority should be given to determination of ways reefs support higher levels of the food chain, including finfish. It is also important to evaluate alternative substrates for reef construction, given that shell material is in short supply.

- In support of shellfish aquaculture it is critical that the VIMS aquaculture facilities at the Eastern Shore be expanded and upgraded. Recommended is construction of a new facility, an Aquaculture Research Center. This expansion is required to assist in the economic development of the growing industry. Particularly relevant is strategically-directed research toward diversification to species not currently cultured. In addition, expanded effort will be required for broodstock selection and maintenance of new species in addition to current hard clam, bay scallop, and oyster broodstock.

- Determining the impacts of human and natural pathogens in shellfish growing waters and the means to alleviate impacts is necessary for success in the marketplace. Program expansion is required.

- Economic assessments must have a high priority. Long-range economic studies are needed because cultured and wild mollusk species have overlapping markets. Emphasis should be placed on understanding how wild and cultured products contribute to coastal economies and how to mitigate competition between the two product sources in order to optimize Virginia's position in the regional, national, and international marketplace.

- Communicating research findings and providing hands-on advice to industry is essential to advance both aquaculture and the native fisheries. In addition, there is an urgent need to better integrate research and monitoring results. To this end, use of a geographic information system, compatible with that in Maryland, is recommended.

RATIONAL PLAN FOR TESTING APPLICATION OF NON-NATIVE OYSTER SPECIES. The proposed plan is intended to provide resource managers with a science-based foundation from which public policy decisions may be made regarding use of non-native oyster species for restoration of oyster stocks in the Commonwealth. The program of study requires in-water testing to assess resistance to the oyster pathogen *Haplosporidium nelsoni* (MSX) and, in the final stages, further in-water testing to confirm response to environmental conditions. Such in-water testing constitutes an intentional introduction of non-indigenous species. The plan was submitted in December 1995 to the Virginia Marine Resources Commission for endorsement and permission for in-water testing.

The proposed program has two objectives. First, the test series will serve to screen for the candidate species, or strains, most likely to succeed in the local estuarine environment. Second, the test results will enable an assessment of environmental risk. Specifically, the geographic range over which non-native species may successfully reproduce will be estimated.

The plan, which will require four years, adopts guidelines of the International Council for the Exploration of the Seas (ICES), wherein quarantined hatchery-raised progeny from imported broodstock are utilized. Three strains of the species *Crassostrea gigas*, and the species *Crassostrea rivularis* are proposed for testing, based upon their close resemblance to the Eastern oyster as reef-forming species tolerant of mid to sub-tropical latitude, high stress environments. The proposed strategy includes:

1. A series of comparative studies in quarantine systems to evaluate larval and post-settlement response to a range of environmental conditions.

2. A challenge, in quarantine, with the oyster disease *Perkinsus marinus* (Dermo).

3. A field challenge with triploid (functionally sterile) animals for the oyster disease *Haplosporidium nelsoni* (MSX).

4. Via 1 through 3, evaluation of likely success of candidate species and assessment of likely geographic range of reproduction if introduced in substantial numbers.

5. Given acceptable risk, limited in-water testing of normal hatchery-reared stock with small lots under secure conditions.

Substantial additional resources will be required to conduct the plan proposed.

PART 1. STRATEGIC PLAN FOR MOLLUSCAN SHELLFISH RESEARCH

I. Introduction: Purpose of the Plan

Formulation of this ten-year strategic plan of shellfish research at the Virginia Institute of Marine Science (VIMS) derives directly from the findings of the Shellfish Industry Study Committee (HJR 95, 1994, and reported in House Document 56, 1995) and earlier results of the Blue Ribbon Oyster Panel. The emphasis of these legislatively or executively driven pursuits derived from, and initially focused upon, the desperate state of Virginia's native oyster fishery. This current situation resulted from the stresses of harvest pressure compounded by high mortalities induced by oyster diseases and other stress factors. The scope of interest enlarged with the realization that shellfish aquaculture* in the Commonwealth is a thriving and growing industry. Contemporaneous with these developments has been the growing understanding that oyster beds and associated benthic communities play a highly significant role in the Bay's ecosystem. The function of oysters and other shellfish in the filtration process that removes phytoplankton and suspended sediments, although long known to marine scientists, has taken on increased importance as policy-makers grapple with the problem of restoring water quality in the Chesapeake Bay system. This, in turn, has focused attention on the natural habitat conditions for oysters—that is, oyster reefs—and the restoration of that habitat. The vital role of healthy oyster habitat in the Chesapeake Bay ecosystem has modified the previous view of oyster population restoration goals, which until recently, have been harvest-oriented. Fishery and ecosystem function goals are not *de facto* in conflict; rather the management strategy required is fishery management within resource management.

Another factor influencing harvest potential in both the native fishery and in aquaculture is that of minimizing human health risks—e.g. providing shellfish safe for consumption. No single factor has a greater influence on the marketability of shellfish products. This latter problem immediately brings into focus issues associated with land-use in proximity to growing-waters, and with delineating appropriate indicators for consumption risk.

With the virtual collapse of the native oyster fishery, attention has been drawn to the potential of using non-indigenous, potentially more disease resistant, oyster species. Such introductions require an assessment of risk to the ecosystem and, if admissible, implementation of management strategies that minimize ecosystem risk. As well, such introductions also should be evaluated in terms of both market potential and value with respect to achieving water quality and/or habitat restoration goals.

The goals of the ten-year strategic plan for shellfish research are necessarily multifaceted, as three important facets interconnect; the native fishery, both public and leased grounds; aquaculture pursuits; and the ecosystem role of shellfish. As well, the plan addresses shellfish other than

---

*The Aquaculture Act of 1992, Chapter 9.1, Sections 3.1-73.8 (Code) defines aquaculture as the propagation, rearing, enhancement, and harvest of aquatic organisms in controlled or selected environments, conducted in marine, estuarine, brackish or freshwater.

oysters, because both the native fisheries and aquaculture potential of other native species have great economic significance.

Goals: The goals of the ten-year strategic plan are

1. To provide a framework for research and monitoring which supports the restoration of native oyster resources and the viability of the native hard clam stocks;

2. To provide a program of research which will elucidate the significance of shellfish and shellfish habitat in ecosystem function, and the natural benefits of ecosystem and economic development which support the restoration of a fully functional resource, and

3. To provide a framework for research and technology transfer to assist in the development of molluscan shellfish aquaculture.

II. State of 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 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 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 understated—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 cannot be understated. The calculations offered by Newell (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 3 days, the present stocks can only manage that task in approximately 325 days—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—see Haven, Hargis and Kendall, 1978; Kennedy and Breisch, 1981) 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.

III. Reviews of Current Fisheries Status and Research and Monitoring Efforts

A. Native Fisheries

1. **Oysters**. The Eastern oyster (*Crassostrea virginica*) resource of Chesapeake Bay has been in continuing decline since well before the turn of the century (Haven, Hargis and Kendall, 1978; Kennedy and Breisch, 1981; Hargis and Haven, 1988). During the mid 1800s Chesapeake Bay oysters were consumed throughout the United States and exported to England. Nearly 7,000,000 bushels were harvested from the Bay in 1865 with approximately 2,000,000 of these coming from Virginia. Although records have some inadequacies there is good reason to believe that oyster production in the Bay approached 20,000,000 bushels per year for the 1875-1885 period. The Commonwealth of Virginia surpassed the State of Maryland in total oyster landings in the early 1900s and remained the largest producer of oysters on the Atlantic seaboard until the advent of disease-related losses in 1959 (Hargis and Haven 1988). Prior to 1960, average annual oyster production was 3.5 million bushels in Virginia and 2.2 million bushels in Maryland. Virginia oyster production in the 1980s decreased from 1,172,000 bushels in 1981 to 273,000 bushels in 1989. This decline has continued over the past decade as illustrated landings from the James River—the site of predominant oystering activity in Virginia (Table 1).

Table 1: Oyster production in the James River, Virginia for the period 1982-1994 by season.

SEASON	SEED OYSTERS	MARKET OYSTERS
82/83	445,193	16,131
83/84	346,134	48,746
84/85	409,867	21,467
85/86	276,503	28,756
86/87	202,406	342,784
87/88	134,453	297,774
88/89	41,303	146,956
89/90	51,383	68,542
90/91	55,010	43,406
91/92	53,537	25,584
92/93	94,658	19,986
93/94	75,477	5,474

The continuing decline in commercial fishing yield also reflects the impact of two diseases, *Haplosporidium nelsoni* (commonly known as MSX) and *Perkinsus marinus* (commonly known as "Dermo"). *Haplosporidium nelsoni* and *Perkinsus marinus* were at record high levels of abundance during 1986 and 1987 as a result of continuing drought conditions over the Chesapeake Bay watershed (Burreson and Andrews, 1988). During 1986 and 1987, estimated overall mortality on public beds in Virginia was between 70% and 90% each year, the highest values recorded in 28 years

of continuous monitoring (E. M. Burreson, unpublished data). During 1988 *Perkinsus marinus* spread to all monitored oyster beds in the Virginia portion of Chesapeake Bay. Since that time some abatement has occurred in low salinity areas (Burreson, unpublished data, May 1991) but the disease remains endemic to the majority of formerly productive oyster bottom. The combined effect of both oyster diseases has been the recent elimination of commercial oyster production from essentially all waters in the Virginia portion of the Bay with the exception of a few oyster bars in the upper James River and very limited areas in the upper Rappahannock River. Many oyster bars in the Maryland portion of the Bay have also been denuded by the diseases. The remaining locations in Virginia, about 5% of the total public oyster grounds, are the subject of continuing, intense fishing pressure. Between 1987 and 1989 approximately 90% of the entire Virginia harvest came from the upper James River, although this declined to approximately 68% in the 1990-91 public oyster season. The magnitude of the problem and the economic implications are obvious.

2. **Hard Clams**. The range of the hard clam (quahog), *Mercenaria mercenaria*, extends along the Atlantic Coast from the Gulf of St. Lawrence to Florida and along the Gulf of Mexico Coast to the Yucatan Peninsula. It is found in bays, coves and inlets over a wide range of bottom types. The hard clam grows best in salinities over 20 parts per thousand. Hard clam distribution in Virginia is limited to the lower portion of Chesapeake Bay (from the mouth of the Rappahannock River southward), the lower James River/Hampton Roads, portions of the Bay-side of the Eastern Shore and all along the seaside of the Eastern Shore.

   The hard clam resource supports a major wild fishery in Virginia's waters. In addition, there is a large aquaculture industry. The record year for commercial wild fishery landings was 1938, when 2.8 million pounds of meat were landed (Virginia Marine Resources Commission, 1983). Over the past two decades, wild fishery landings have fluctuated from an all-time low of 497,238 pounds of meat in 1978 to landings of approximately 1.5 million pounds of meat in 1989-1990 (Table 2). The wild hard clam fishery experienced a resurgence in the mid-1980s as many watermen entered the fishery because of the decline of the public oyster fishery. On a price per pound basis, the hard clam has been one of the most valuable commercial species landed in Virginia, with dockside prices as high as $3.98 per pound in 1987, and more than $3.50 per pound since that time.

Table 2. Reported landings of hard clams from the wild fishery, 1976-1992

YEAR	MEAT (LB)	VALUE	$/LB
1976	839,304	868,191	0.97
1977	843,020	992,549	1.18
1978	497,238	963,884	1.94
1979	619,712	1,255,175	2.03
1980	753,200	1,700,000	2.26
1981	1,110,530	1,862,835	1.68
1982	711,170	1,657,635	2.33
1983	1,207,165	2,287,872	1.90
1984	739,191	1,837,068	2.49
1985	613,254	1,518,525	2.48
1986	905,177	2,472,365	2.73
1987	1,004,580	4,000,415	3.98
1988	1,307,863	4,772,063	3.65
1989	1,519,483	5,903,619	3.88
1990	1,559,108	5,695,741	3.65
1991	1,068,243	4,063,696	3.80
1992	1,094,391	4,025,129	3.68

(Data extracted from Virginia Marine Resources Commission, Commercial Fisheries Statistics, Virginia Landings, Annual Summaries 1976-1992.)

3. Other Species with Historical Fisheries

a. Bay Scallops. The bay scallop, *Argopecten irradians*, was once native to the lower Chesapeake Bay and seaside of the Virginia Eastern Shore. It appeared in concentrations sufficient to support commercial harvesting during the 1920s, but virtually disappeared in the early 1930s coincidental with widespread loss of its eelgrass habitat. Historical landings information is difficult to obtain; however, some commercial landings for Virginia bay scallops are presented in Table 3.

Table 3: Bay scallop landings

YEAR	POUNDS	VALUE
1925	360,732	74,272
1926	Landings records not available	
1927	Landings records not available	
1928	Landings records not available	
1929	1,145,598	207,883
1930	1,824,948	147,564
1931	1,226,478	78,990
1932	658,584	80,090
1933	No reported landings	
1934	No reported landings	
1935	No reported landings	

Unless there is substantial resurgence of submerged aquatic vegetation and seeding efforts, the bay scallop is not likely to return to harvestable levels in the near future.

b. **Ribbed Mussels**. The ribbed mussel *(Geukensia demissa)* is distributed throughout Virginia's portion of Chesapeake Bay and the seaside of the Eastern Shore. Many local inhabitants harvest ribbed mussels for personal consumption and there has been some very small-scale commercial harvesting, primarily on the seaside of the Eastern Shore. In the late 1930s and early 1940s there was an emerging fishery for ribbed mussels as a source for vitamin B, which could be extracted and concentrated from its soft tissues. This was a very short-lived fishery when a method for synthesizing this vitamin was discovered. A United States fishery statistics publication first reported Virginia commercial landings of 23,200 pounds of ribbed mussels, valued at $776, for 1935; 1936 production increased to 77,400 pounds, worth $2,257. Given the widespread production and marketing of the more familiar blue mussel (*Mytilus edulis*), it is unlikely that the ribbed mussel will soon find commercial market potential.

c. **Ark Shells**. In recent years a fishery has developed for the blood ark (*Anadara ovalis*) and the ponderous ark (*Noetia ponderosa*), primarily for sale to the U.S. West Coast and Asian markets. Prior to 1991, ark shells were considered a useless incidental bycatch in the harvest of other clams. Since the inception of the food-fishery, ark clam demand has been erratic. At this time no official data on landings are available. There is some concern that the resource could be over-harvested because the market size for ark shells includes animals six years of age and older. This fishery will likely continue at a very low level in the near future.

d. **Soft Shell Clams.** The soft shell clam *(Mya arenaria)* resource in Virginia does not generally occur in exploitable populations. While Maryland has a thriving soft

shell clam fishery, there has been no commercial fishery in Virginia since the mid-1960s. Prior to 1965 there were few reports of commercial harvests of soft clams from Virginia. In 1965, landings of 18,333 bushels (approximately 220,000 pounds of meat), valued at $46,495 were reported from Virginia. This prompted the following statement in Fishery Statistics of the United States: "Virginia entered this fishery for the first time and had 220,000 pounds of meats. This small harvest may be important because Virginia has a large, untapped supply of soft clams and market demand for soft clam meats is increasing." The next year (1966), 33,175 bushels of soft clams ($86,715) were reported as coming from leased grounds in Virginia, and Fisheries Statistics reported, "The producing areas of Virginia are located in certain tributaries of the Potomac River and in some sections of the Rappahannock River." By contrast, in 1967, only 2,567 bushels of soft clams were landed from leased grounds, again from Fisheries Statistics, that, "The small Virginia landings reflected the indifference of the packing industry to soft clam processing and led the Virginia Fisheries Commission to grant $21,000 for a survey of soft clam resources of the State ..." After minuscule landings in 1968 and no reported landings in 1969, the 1970 fishery statistics report stated, "Few stocks of soft clams exist in Virginia, which had no commercial clam fishery in 1970." Since that time there has been no commercial fishery, although on several occasions individuals have attempted experimental harvesting on leased grounds, only to discontinue because of poor resource abundance or harvesting difficulties.

A notable event in the soft clam fishery of Chesapeake Bay (primarily Maryland) occurred in 1972 when Hurricane Agnes dropped unprecedented amounts of freshwater into the Bay watershed. Subsequently it was reported (Fishery Statistics of the United States) that, "The soft clam fishery had the highest number of mortalities (around 90%), because the influx of freshwater, together with some extremely warm weather immediately following the hurricane, reduced the oxygen in the water." There is no reason to expect a resurgence in commercial harvesting of soft clams in Virginia; however, the soft shell clam may be a significant candidate for future aquaculture pursuit.

e. **Surf Clams**. The surf clam *(Spisula solidissima)* is found from the Gulf of St. Lawrence to the northern Gulf of Mexico; however, areas of abundance occur in the mid-Atlantic coastal region from Long Island, New York, to the offshore zone of Virginia's coast. Virginia entered the commercial fishery for surf clams in the mid-1960s. At that time, declining harvests from the traditional fishing grounds off New Jersey forced the fishing fleet to shift to beds off the Delmarva Peninsula and Virginia. All harvesting of surf clams is done outside of state waters. Virginia landings reached a peak in 1974 at 58.2 million pounds. This represented 60% of the total U.S. harvest for 1974. Since then, Virginia landings have steadily declined to 5.6 million pounds in 1990 (most recent data). During the mid-1970s, stringent federal regulations were imposed upon the surf clam fishery following years of increasing fishing pressure and declining harvests. In the late 1980s and

early 1990s, the Virginia surf clam processing industry came under increasing regulatory scrutiny, forcing many processing plants to close because of water quality issues.

f. **Whelks**. The whelks—locally known as "conchs"—are distributed from New England southward to Florida. Within Virginia waters three different species of whelks are utilized in the commercial fisheries, *Busycotypus canaliculatus* (channeled whelk), *Busycon carica* (knobbed whelk) and *Busycon sinistrum* (lightning whelk)(Turgeon, et al., 1988). Of these, the channeled and knobbed whelk are by far the more important species. The whelk fishery occurs both within the Chesapeake Bay and in offshore Atlantic waters. Harvesting gears include trawl nets, crab pots, whelk (conch) pots, crab dredges, whelk (conch) dredges, surf clam dredges, and hand harvest. Until 1993, dredges directed at harvesting primarily *B. carica* comprised the major fishery. Recently, a whelk pot fishery targeting *Busycotypus canaliculatus* has developed within Virginia territorial waters and in the adjacent federally-controlled waters.

Commercial landings of whelks from Virginia were first reported by Fishery Statistics of the U.S. in 1940 (DiCosimo, 1986). Landings averaged approximately 63,000 pounds of meat per year for the period 1940-59 (exclusive of the war years 1942-43), 335,000 pounds of meat per year from 1960 to 1979, and less than half the 1960-79 figure with 125,000 pounds of meat per year for 1980-84 (DiCosimo, 1986). The increased landings during 1960-79 coincided with an expanding surf clam fishery and as a result of by-catch from the crab dredge fishery. Since 1985, landings of whelks have increased dramatically (Table 4), with a high of 644,000 pounds of meat in 1988.

TABLE 4. Virginia whelk landings by gear type, 1973-1994
All landings are expressed in pounds of meat.
Data provided by the Virginia Marine Resources Commission.

YEAR	TRAWL	CRAB POT	CLAM DREDGE	CRAB DREDGE	WHELK DREDGE	WHELK POT
1973	44124	3100	233797	13840	0	0
1974	20160	4653	1006111	6320	0	0
1975	27815	27500	7548	6320	0	0
1976	4750	1500	241380	66875	0	0
1977	55087	80477	45138	20433	0	0
1978	92234	15000	54735	172497	16667	0
1979	35681	7204	41651	100498	13635	0
1980	28716	73	8246	53163	81971	0
1981	14137	5199	15738	33222	44611	0
1982	28239	2341	8010	70145	4230	0
1983	45144	200	7802	3477	0	0
1984	68696	0	7927	41021	23360	0
1985	101224	6674	365	9331	25315	0
1986	73376	28792	671	222930	203182	0
1987	58346	6936	110	25656	223040	0
1988	157414	35483	0	40709	39958	0
1989	51913	14084	0	23142	261782	0
1990	59442	23419	0	75527	113455	0
1991	90045	44189	0	123056	289184	0
1992	71270	763	0	33112	22888	0
1993*	78048	20110	0	38823	217599	11506
1994*	N/A	7757	11	100562	326058	83807

* Preliminary
N/A = Data not available yet.
NOTE: 1978 through 1982, 76239 pounds of meat landed by "hand";
1986 through 1994, 41675 pounds of meat landed by "hand";
1978 through 1994, 21135 pounds of meat landed by "other".

B. Aquaculture

1. **Historical Development**. The origins of shellfish culture in Virginia can be traced to the mid-1800s when Virginians began cultivating the Eastern oyster, initially in a frontier-style without regulation and then in a managed framework. Controversies over early planting practices led the Virginia General Assembly in 1892 to pass "An Act to Protect the Oyster Industry of the Commonwealth." This marked the beginning of the dual management system for the public oyster fishery and the private oyster culture industry.

   As a result of the 1892 Act, the naturally-producing oyster grounds of the time were delineated and set aside for the public trust. Named after their surveyor, these "Baylor Grounds" comprise 243,000 acres of public oyster harvesting grounds. Any areas not

included within the Baylor Grounds are potentially available for private leasing. It did not take long for leasing to become a major factor in the oyster industry. By 1900 almost 48,000 acres were already under lease. Subsequent years showed steady increases: 1927, 59,500 acres; 1944, 70,600 acres; 1955, 127,000 acres; and in the record year of 1967, 134,500 acres. Since 1967 there has been a decline of acres under lease to 1990 when 108,500 acres were leased for shellfish production (oyster and hard clam). The Virginia Marine Resources Commission is charged with administering the leasing system and is responsible for rent collection.

Exclusion of an area from the original Baylor Survey meant that at the time oysters did not occur there naturally. Thus, leaseholders had to manipulate their grounds in some manner to make them productive. This usually meant one of two things. If the lease was in an area where a natural strike of oysters could be expected, but had not occurred, most likely the bottom was too soft to support the weight of oysters. As a consequence, the leaseholder needed to stabilize the bottom, usually with oyster shells, to encourage naturally-occurring oyster larvae to settle. Areas with bottoms solid enough to support the weight of oysters, but where no natural settlement occurred, offered the opportunity to plant seed oysters from other locations. From this latter strategy developed the most prevalent method of oyster culture in Virginia, harvesting seed oysters from one area and transplanting them for growth in another area. The primary source for seed oysters in Virginia has been the James River. The amount of seed planted per acre depends on bottom stability and growth characteristics of the area. Private planters now monitor the condition of their growing grounds more closely because of disease activity and may routinely have their oysters tested for disease.

Other methods for growing oysters have been attempted. During the late 1930s the Chesapeake Corporation investigated using a tray and rack system to grow oysters in the York River. Trays containing approximately ½ bushel of oysters were supported off the bottom on short wooden stakes. At one time more than 11,000 trays stretched for 3 miles along the shore of the York River. This project was discontinued in 1942, presumably because of the war effort and high labor costs of maintaining the trays/racks and handling the oysters.

Sometime in the early 1950s oyster planters began placing wire mesh bags of oyster shell on the bottom in hopes of receiving a good set. After the onset of MSX (*Haplosporidium nelsoni*) and a decline in setting intensity, the use of shell bags increased. An estimated 100,000 shell bags—each holding about ½ bushel of shells—were set in the Great Wicomico and other Virginia rivers by 1971. The use of shell bags today has all but disappeared, again presumably because of the cost in constructing and handling them.

2. **More Recent Aquaculture Pursuits**. Tracing the history of oystering in Virginia discloses the sequence of harvesting the natural habitat, relocation of resources to external grow-out areas, to manipulation of placement for growth and harvest

advantage. The most recent step is modern aquaculture, wherein the process is vertically integrated from broodstock to hatchery process, to nursery, and grow-out to harvest. VIMS has played a significant role in research and outreach leading to commercial development.

a. **Oysters.** The lack of consistent production of natural seed supplied the impetus for investigations into the development of hatcheries to supplement natural seed production. By the late 1960s VIMS actively researched alternative methods of oyster culture and had established an oyster hatchery at its Gloucester Point campus.

   Three drought periods occurred during the 1980s, further crippling the oyster industry. This resulted in renewed VIMS' efforts on both the hatchery production of seed oysters and more innovative methods for growing oysters. One research project focused on the use of off-bottom culture as a means of augmenting production and possibly circumventing the problems of oyster pathogens by "out-growing" the diseases. Through cooperative projects involving private culturists, VIMS scientists sought to improve off-bottom culture techniques designed for the production of single oysters (cultchless) destined for the half-shell market. Coupled to this aspect of the project were investigations regarding broodstock selection for desirable traits (i.e. fast growth, proper shell shape, disease resistance), the potential for genetically manipulated oysters (triploids, etc.) and descriptors for predicting best growth.

   Intensive, off-bottom oyster culture is developing as an approach to grow oysters in the presence of the oyster diseases Dermo and MSX. Though off-bottom culture is not new, an integrated approach predicated on avoiding early disease exposure and rapid growth to market size has been developed at VIMS over the past several years.

   At the present time, approximately 150 people within Virginia are engaged in this form of aquaculture. Most are involved in "gardening" oysters (growing several thousand for personal consumption), but a growing number are engaged in commercial aquaculture. The present size of the industry component is difficult to estimate, but at least 1 million oysters grown in off-bottom culture have been harvested and approximately another 2 million are presently in culture.

   VIMS continues to play an active role in all phases of oyster aquaculture from seed production to outreach. The Institute is working to shift from being the primary supplier of oyster seed to a new phase in which private sector hatcheries produce and sell seed to the industry. At present, VIMS staff are working directly with several private hatcheries to develop these capabilities.

b. **Hard Clams, *Mercenaria mercenaria*.** It is noteworthy that Virginia's Eastern Shore was the site of the first commercial clam hatchery in the U.S. In 1956, using

methods developed by the U.S. Bureau of Commercial Fisheries (now the National Marine Fisheries Service), Richard L. Kelly set up a clam hatchery in an oyster house in Atlantic, Virginia. Production from this hatchery/nursery was reasonably successful, but sporadic. Unfortunately, field plantings were complete failures, most likely because of predation. As attempts were being made to improve field planting success, Mr. Kelly died and his work was not continued until the efforts advanced by VIMS.

Research and advisory activities at VIMS have played a central role in the development of clam aquaculture and the Institute is widely recognized as a national leader in this area. Research conducted at the VIMS Eastern Shore Laboratory during the past 35 years has led to the development of practical approaches to the hatchery, nursery and grow-out phases of clam culture. This work has been widely published in the scientific literature (e.g., Castagna 1984; Kraeuter and Castagna 1985a & b), and these technologies have also actively been transferred to the industry through advisory publications (Castagna et al. 1970; Castagna and Kraeuter 1981; Oesterling 1995), seminars and one-on-one contacts, through significant support of the Sea Grant Program.

VIMS presently maintains six selected stocks of clams derived from 30 years of breeding experiments. These selected stocks will continue to be important components in future breeding programs to develop improved strains of hard clams. Recent advances in the nursery system include the use of bacterial additives to improve survival (Castagna et al. 1990) and the use of systems to improve growth (Castagna and Luckenbach, unpublished data). The grow-out methods currently used by the industry are based on those developed by Castagna and colleagues.

According to a 1994 survey by the Virginia Agricultural Statistics Service, the value of the cultured clam industry in 1993 was more than $11,000,000—more than twice the value of the wild fishery for hard clams.

c. **Bay Scallops, *Argopecten irradians*.** During the early 1970s, VIMS scientists initiated culture activities on the bay scallop. The bay scallop was considered suitable for marine aquaculture for a number of reasons: 1) it has potential for a high market value; 2) there is a high level of consumer recognition and acceptance; 3) natural populations experience fluctuating stock abundance; 4) they grow rapidly to market-size; and 5) hatchery techniques for spawning and rearing larvae/juveniles have been successfully demonstrated. Early VIMS research was instrumental in documenting the growth and hatchery production aspects of bay scallop culture; however, two major impediments were identified as constraints to further development. One was the need for better grow-out methods. The second was that the economics of producing bay scallops for the shucked meat market did not look favorable. At that time, only the scallop adductor muscle was utilized. Recently, however, interest has developed in using the entire animal, as is done

with oysters or hard clams. This product may command a premium price in the market, making the economics of culture more favorable.

More recent VIMS research in the early 1990s addressed the problem of field grow-out and marketing of whole bay scallops. Successful grow-out methods were demonstrated and marketing strategies developed. A private shellfish hatchery initiated the production of bay scallop seed for sale to culturists, and a small, but growing, bay scallop industry has developed on Virginia's Eastern Shore. As better storage and marketing technology is developed, this portion of Virginia's shellfish aquaculture industry might be expected to expand.

C. Economics and Markets

1. **Information Available**. Presently, there exists little information about broad or general issues relating to the economics and marketing of products of wild fisheries and aquacultured enterprises. There are, however, numerous studies that tend to be species and/or geographical specific. For example, the text Economics of Aquaculture (Jolly and Clonts 1993) offers more than 150 case studies on aquaculture. The chapter on marketing presents 27 references on marketing aquacultured product; only one reference, Glude (1983) "Marketing and Economics in Relation to U.S. Bivalve Aquaculture" deals explicitly with mollusks. Yet, the underlying economics and marketing of product will eventually determine the success of aquaculture ventures.

   Relative to the wild fisheries and aquacultured molluscan products of Virginia, several key questions remain to be answered. For example, what are the costs and profits of harvesting or producing hard clams? How much employment would be generated if the state spent revenue on advertising to increase the consumption of oysters, hard clams or bay scallops? Investment in studies of resource economics is essential to address these questions of potential industry growth.

2. **Major Bivalve Species**. Both the hard clam and Eastern oyster are commercially harvested and produced by aquacultural enterprises. Other species with some promise for commercial fisheries or aquacultural production are bay scallops, soft shell clams, ribbed mussels, and surf clams.

   a. **Hard Clams**. In 1992, fishermen landed 1,094,391 pounds of hard clam meats with a dockside value of $4,025,129. Relative to all other species harvested within the Chesapeake Bay and its tributaries, the landed value of hard clams was surpassed only by blue crabs. In comparison, the producer value of aquacultured clams, just from the eastern shore of Virginia, was in excess of $4,500,000 (Thacker 1994). Oesterling (1995) reports that gross sales of cultured hard clams exceeded $11,400,000 from production of approximately 72 million pounds of littleneck clams in 1993. A direct comparison of these values, however, may be inappropriate because fishermen primarily sell to wholesalers and dealers, while growers or aquaculture producers are primarily wholesalers or distributors.

In general, the harvest levels of clams from the Virginia commercial fishery have been declining during the past several years. For example, the landed value of hard clams from the Virginia commercial fishery exceeded $5,500,000 in 1989 and 1990. Landings in each year were, respectively, 1,519,483 and 1,559,108 pounds. Relative to U.S. landings from the capture fisheries for hard clams, landings declined a modest 6.5% between 1985 and 1993. In contrast, U.S. aquaculture production of clams increased 114.2% between 1985 and 1992 (the most recent year for which data on total U.S. production of aquacultured product are available).

It is becoming increasingly obvious to fishery managers and the seafood industry that satisfying a growing U.S. demand for hard clams is likely dependant upon aquaculture. Between 1992 and 1993, total consumption of domestic hard clams (wild harvest plus aquacultured production) increased four percent. In order to satisfy this growing demand, the U.S. has increasingly relied on imports. The U.S. runs a trade deficit in both quantity and value terms; the U.S. imports approximately 2.4 million more pounds than it exports with a value difference of approximately $2.3 million. The U.S. Department of Agriculture forecasts demand, and particularly aquaculture production of clams, to continue to increase over the next few years.

The growing demand for hard clams and the apparent full utilization of the wild resources creates substantial opportunities for Virginia aquaculture producers of hard clams. Virginia producers have an excellent competitive advantage relative to those in other states. Virginia is centrally located, with easy assess to major metropolitan markets such as Washington D.C., Baltimore, and New York. In addition, the length of time from clam spawning to obtainment of market size is 24 to 36 months. This is a relatively short time period, which permits producers to easily adjust production and inventory schedules.

b. Oysters. In contrast to clams, the economic situation for Virginia oysters is less promising. Not only has the wild harvest dramatically declined in recent years, there is also evidence to indicate that consumer demand in the United States for oysters has substantially declined. The decline in demand is, however, mostly in response to negative media publicity about the dangers of consuming raw oysters, the apparent preferred product form. In some locations many traditional market outlets remain strong. With proper attention to reviving demand for Virginia oysters, markets could possibly be restored or sales enhanced.

In 1989, total landings of market oysters was 284,180 bushels, with a dockside value of $6,186,112. By 1992, landings of market oysters declined to 105,235 bushels, with a dockside value of $2,171,129. Depending upon how aquaculture production is defined, production was 1,500 or 28,847 bushels in 1992; the difference is due to the fact that the Virginia Agricultural Statistics Service

Aquaculture Survey only considers off-bottom culture as aquaculture (1,500 bushels), whereas the VMRC views production from leased grounds as culture production (28,847 bushels). The 28,847 bushels is included in the reported total of 105,235 bushels for 1992. Regardless of how viewed, commercial landings and culture production have both declined in recent years.

More importantly, there are indicators that the overall demand for oysters is declining. If total per capita consumption or demand for oysters in the United States is considered, domestic demand for all types of oysters declined 54.4% between 1985 and 1993. In addition, domestic wholesalers appeared to be unconcerned about the decline in demand or domestic supply; they, in fact, decreased their demand for imports by 66.7% between 1985 and 1993.

On a positive note, there appears to be localized renewed interest in raw bars and consumption of raw oysters. This is especially prevalent in the metropolitan coastal areas of Virginia such as Hampton, Virginia Beach, Norfolk, and Newport News. It is not known whether the same trend is occurring across the United States or in other nearby cities such as Baltimore, Washington D.C., and New York. In addition, there is increasing demand by foreign nations—especially Canada, Korea, and Taiwan—for oysters; the foreign demand, however, is mostly for West Coast oysters.

c. **Ribbed Mussels**. There is no commercial fishery for the ribbed mussel in Virginia. Small quantities are, however, recreationally harvested for personal consumption. The ribbed mussel is widely available in Virginia's marine waters and has many characteristics which make it a potential candidate for aquaculture production. However, the blue mussel currently controls the market.

The ribbed mussel has a wide range of tolerance for salinity, and thus may be grown in many locations around the state. It also may benefit from the growing demand for blue mussels; market promotion could be relatively limited and take advantage of the demand for blue mussels.

d. **Bay Scallops and Other Potential Aquaculture Species**. Other species that have the potential to be produced by aquaculture include the bay scallop, surf clams, and soft shell clams. Of the various species, the bay scallop, and possibly the soft shell clam, probably have the greatest potential for aquaculture production. This is because of extensive work already completed on culturing and marketing bay scallops and soft shell clams. Work has only recently been initiated on culturing soft shell clams in Virginia, but considerable information is available from other states. Moreover, soft clams are widely available along the east coast of the United States and there are already well established markets into which Virginia producers would have to gain entry. Surf clams pose another vexing problem, in that substantial processing is required and most of the processing companies are located outside Virginia.

The bay scallop, like the soft shell clam, is also widely marketed outside of Virginia. Consumers readily accept bay scallops, and the domestic demand appears to be quite strong. Presently, the economics of raising bay scallops in Virginia, however, appear to be unfavorable. If demand for particular product forms such as whole and half-shell can be increased, it is likely that prices will be adequate to cover production costs.

3. **Economic Importance**. Of critical concern to the Commonwealth is the current and potential economic importance of the commercial fisheries. Presently, data necessary for a detailed assessment of the economic importance of the bivalve species are unavailable. Information available on aquacultured products indicates there is considerable variation in the relationship between production and individuals employed. For clam aquaculture, available information suggests that for every $102,243 of sales of clams from the producer, one full-time job is created. For off-bottom oyster culture, one job is created for every $4,778.33 in sales by the producer. Relative to other bivalve aquaculture, information suggests that one job is created for every $4,000 in producer sales.

   Similar information is not available for the commercial fisheries. It is, in fact, the lack of economic information that has complicated the management and regulation of the commercial fisheries of Virginia. In 1994, the VMRC issued more than 14,000 commercial licenses to approximately 3,200 individuals. Based on a current survey of the commercial seafood industry by VIMS, it appears as though more than one-third of the 3,200 individuals obtained licenses strictly for recreational and subsistence purposes. Of the approximately 2,200 individuals that were active commercial watermen, 25-40% were part-time fishermen.

D. Shellfish Pathogens. Only two shellfish pathogens warrant consideration, and they are both pathogens of the native Eastern oyster. There are no important pathogens known for hard clams, or other commercially-important mollusks. This section considers the oyster pathogens of concern, *Haplosporidium nelsoni* (MSX), and *Perkinsus marinus* (Dermo).

Haplosporidium nelsoni. Large-scale oyster mortality attributable to *H. nelsoni* was first observed in Delaware Bay in 1957 and in the lower Chesapeake Bay in 1959. Since 1959, *H. nelsoni* has spread all along the East Coast and is presently known from Maine to Florida, although it causes significant oyster mortality only in the middle Atlantic region. The pathogen multiplies and causes oyster mortality at salinities between 15 and 34 ppt, but is very sensitive to low salinity and cannot tolerate salinity below 10 ppt for more than about one week. Thus, during wet years *H. nelsoni* is less abundant and causes less oyster mortality than during dry years. Since continuous monitoring for *H. nelsoni* began in the lower York River in 1960 there has only been one year, 1990, in which the pathogen was not detected in oysters (Figure 1). During the last four years, 1992-1995, *H. nelsoni* has been at record high levels in the York River with maximum annual prevalence of more than 80%. There are few oysters remaining in the lower Chesapeake

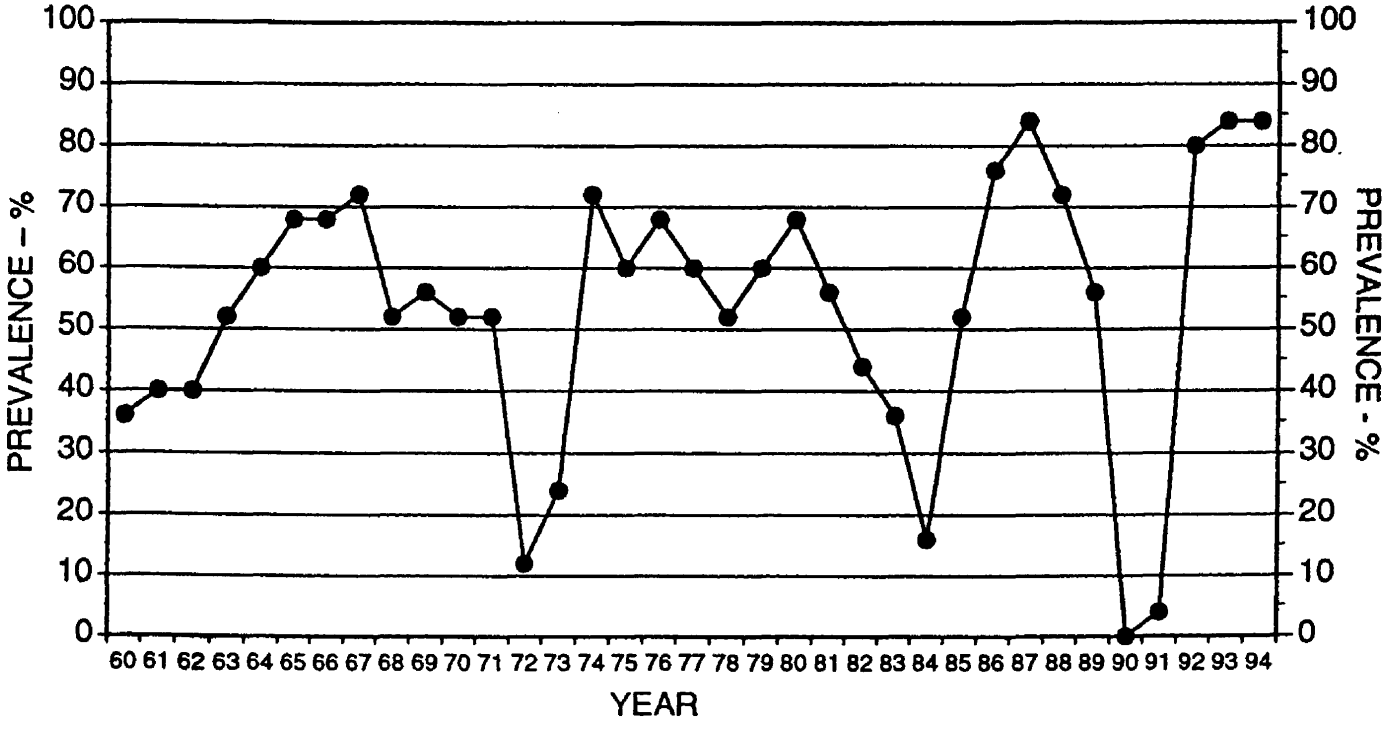


Figure 1. Prevalence of *Haplosporidium nelsoni* (MSX) in oysters at VIMS, 1960–94.

Bay where *H. nelsoni* is most abundant; so oyster mortality attributable to the pathogen has not been particularly high since 1990, but the monitoring results demonstrate that *H. nelsoni* has not decreased in abundance or pathogenicity since it first began causing oyster mortality in 1957. Clearly, *H. nelsoni* will threaten development of aquaculture or recovery of public oyster beds in the lower Bay.

The unusually warm and dry winter and spring of 1995 allowed *H. nelsoni* to overwinter at high levels and there was significant oyster mortality during June, 1995 at Wreck Shoal in the James River and in the lower reaches of the Rappahannock, Piankatank and Great Wicomico rivers. These results reinforce the important role of environmental conditions in the seasonal dynamics of *H. nelsoni*.

Perkinsus marinus. This pathogen has probably always been an associate of oysters. It was first reported in the Chesapeake Bay in 1947—the first time oysters were examined for *P. marinus* using the now-routine fluid thioglycollate culture technique. Historically, *P. marinus* was restricted to the lower Chesapeake Bay areas of Hampton Roads, Mobjack Bay, the bayside of the Eastern Shore and to the mouths of the major tributaries south of the Potomac River. The pathogen was responsible for some oyster mortality each year in these areas, but it was usually less than 20% except during very dry years when it increased somewhat. This level of mortality was tolerable by industry and oyster landings were not significantly affected most years. The four consecutive drought years from 1985 through 1988 resulted in sustained increased salinity throughout Chesapeake Bay and allowed *P. marinus* to increase in abundance and to spread throughout all oyster beds in both Virginia and Maryland. The spread occurred both naturally and by the movement of infected oysters. Oyster mortality was high in all areas, especially during 1987 and 1988, except in the uppermost reaches of the major tributaries. *Perkinsus marinus* has declined slightly in abundance since its peak in 1991, but it is still present on all oyster beds in Virginia. The tenacious persistence of *P. marinus* in the upper reaches of major tributaries where salinity has returned to normal levels suggests that the spread of *P. marinus* to these areas was more or less a permanent acquisition. Whenever salinity increases because of drought conditions the pathogen will increase in abundance and oyster mortality will result. The ramifications of the present widespread distribution of *P. marinus* are perhaps best exemplified by the situation in 1995. The unusually dry and warm winter and spring in 1995 allowed higher than normal overwintering levels of *P. marinus*, abundance of the pathogen increased unusually rapidly in June, and oyster mortality is predicted to be high during the summer.

E. Shellfish Safety. Harvesting of shellfish from growing waters is carefully regulated because shellfish can concentrate human pathogens, toxins, and chemicals. Transmission of diseases, such as typhoid and cholera, through consumption of contaminated bivalve shellfish is well recognized, and national regulatory controls to prevent health problems have been in place since the 1920s. Nevertheless, recent occurrences of viral disease in humans transmitted by shellfish from waters approved for direct harvesting suggest that the existing controls are inadequate to protect the public health. For instance, nearly 1,400 documented cases of shellfish-associated hepatitis A were reported between 1961

and 1990. Diseases can also be transmitted by shellfish containing chemicals or infective agents that are naturally occurring components of the shellfish habitat. These include biotoxins produced by phytoplankton and the human pathogen *Vibrio vulnificus*. The latter has become a significant concern because of recent deaths traced to shellfish harvested from Gulf of Mexico coastal waters.

1. **Coliform Bacteria as Indicators of Gastrointestinal Diseases**. Shellfish become contaminated with pathogens causing enteric disease (e.g. viruses, parasites and bacteria) when sewage or feces are introduced into growing waters. Population growth and development in Virginia's coastal areas, sewage effluent discharges, stormwater runoff, and runoff from other nonpoint sources have contributed to an increase in closures of productive molluscan shellfish growing waters. Coliform bacteria found in the intestines of humans and warm blooded animals are used as surrogates for the presence of fecal contamination and are the approved indicators used to assess sanitary water quality. Numerical standards based on coliform indicators are one of the primary tools used to classify and prohibit shellfish harvesting in growing areas.

   Historically, methods to detect coliforms in water have been refined to improve the detection of contamination from feces or sewage, i.e., shifting from total coliforms to fecal coliforms (a subset of total coliforms) to *Escherichia coli* (the dominant fecal coliform in feces and sewage). A number of problems surround the use of total and fecal coliform indicators. Among these are concerns that either may indicate the need for closures in areas affected by nonpoint sources which are not expected to cause a significant human health risk; and conversely, that the indicator may fail to indicate the risk of viral illness from human contamination in other environments. Moreover, approved growing area numerical coliform standards are arbitrary values that were not derived using techniques of risk assessment. Other concerns are imprecise methods of indicator enumeration and inadequate characterization of growing areas based on indicator data.

   Efforts to resolve the inadequacies of current indicators and methods have been exceedingly slow and have lacked a cohesive and uniform federal policy. Because of the deficiencies of the current coliform indicator/standard as a measure of human fecal contamination, major issues still requiring resolution include: (1) the identification and validation of improved indicators and methods, (2) verification of indicator applicability to all major regions of the country, under a variety of point and nonpoint source pollution conditions, (3) the need to establish numerical relationships between selected indicators and risk of disease from consumption of uncooked shellfish on the basis of epidemiological studies, and (4) ultimately, the derivation of health effects criteria on which to base a growing area classification system for point and nonpoint affected shellfish growing waters. Although many of these issues require resources that exceed state capabilities, as a state with a major future stake in the production of "safe and wholesome shellfish," it is in Virginia's best interest to promote research and implementation programs supporting this goal.

2. **Diseases Caused by Biotoxins**. The National Shellfish Sanitation Program (NSSP) provides recommended procedures to protect shellfish consumers from marine biotoxins that periodically and unpredictably affect certain regional areas. Biotoxins include paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), and most recently, domoic acid. NSSP recommendations include a contingency plan for emergency response, and monitoring sufficient to detect background levels of toxigenic organisms and to recognize actual or potential changes that could result in public health problems. Although bloom species associated with biotoxins have been reportedly found in the Chesapeake Bay, fortunately there have been no recorded incidents of these diseases in Virginia waters at this writing.

3. **The National Indicator Study**. Realizing the need for improved methodology for evaluating the safety of molluscan shellfish for raw consumption, a coalition composed of the Interstate Shellfish Sanitation Conference, university researchers, industry, state and federal agencies, approached Congress to establish a national study to address problems associated with the current use of coliform indicators. In 1989, Congress appropriated funds to initiate the National Indicator Study (NIS). Three major objectives of the NIS were the identification and development of laboratory methods for evaluating fecal risk indicators, field testing of the new indicator methods, and verification of indicators on the basis of epidemiological (risk assessment) studies. The overall goal of the study was to establish quantitative relationships between health risk (incidence of enteric illnesses among a volunteer population ingesting raw shellfish from specific waters) and the sanitary quality of the shellfish harvest waters as measured by traditional and new indicators of pollution. Unfortunately, after an initial period of productive design and preliminary research stages, the program lost its political funding base in 1993 and in the current regulatory climate reinstatement in the near future is very uncertain.

4. **Shellfish Aquaculture**. The production of molluscan shellfish through small scale or intensive aquaculture practices where product is intended for human consumption in an uncooked state must be carried out in conformance with NSSP regulations. Therefore, the same limitations and uncertainties of the current regulatory system will also apply to molluscan aquaculture. It is in Virginia's best interest to maintain or reclaim growing area water quality to conditions that permit harvesting of shellfish for direct consumption produced through aquaculture practices. Because there is considerable evidence that certain growing area closures are related to deficiencies characteristic of the current indicator system, rather than reflecting a true health risk, in a general sense research to address and resolve these problems should be supported at state and federal levels.

   The proposed use of floating containers in Virginia for the culture and possible relay of oysters requires that the shellfish be grown and harvested in waters suitable for that purpose and allowed to purify under conditions required under Virginia regulatory

authority. Because it is anticipated that initial culture may occur in growing waters classified as restricted or conditionally-approved, the public and industry users must be educated about relaying, the principles involved, and the pertinent health concerns. Similarly, approaches to produce shellfish in simultaneous culture (coculture) with other organisms must be evaluated with regard to potential public health concerns. Aquaculture performed in approved growing areas depauperate in harvestable shellfish is of great potential economic benefit but is similarly bound by NSSP regulations if product is to be harvested for raw consumption.

Shellfish placed in approved waters are considered able to purify themselves of certain contaminants. This ability is used successfully by industry to process shellfish harvested from partially contaminated (restricted) areas, and is known as relaying. In controlled relaying, shellfish harvested from restricted areas are held in approved or conditionally approved areas, with or without a container, for a period of time demonstrated as sufficient for reduction of fecal or other contaminants in accordance with procedures described in the NSSP manual. The time required depends on many factors, particularly original contaminant level and water temperature. Of the contaminants of concern, bacteria are the most effectively purged by relaying, often within a 14 day period. Some members of the Virginia shellfish industry have embraced the use of containers for the significant improvements in process efficiency and reduced mortalities provided when compared with traditional on-bottom methods.

5. **Indigenous Bacterial Pathogens Causing Disease.** *Vibrio vulnificus* is a naturally occurring estuarine microorganism associated with the Eastern oyster. *V. vulnificus*-caused disease has been consistently associated with consumption of raw oysters harvested from Gulf of Mexico coastal waters. Studies have shown that although the microorganism is widely distributed—having been isolated from both water and shellfish worldwide, including Virginia waters—incidents of disease have been traced to oysters harvested from the Gulf states during the warmer months. There have been no reported cases of *V. vulnificus* traced to shellfish harvested from either Washington or Oregon nor are there documented reports traced to consumption of raw *Crassostrea gigas*. Cases that have been recorded for California are suspected as being caused by oysters harvested from Gulf of Mexico waters. There have been no reported cases transmitted by oysters harvested from the Chesapeake Bay. A recent death in the Midwest from Virginia oysters was subsequently attributed to Gulf of Mexico oysters originally mislabeled as Virginia oysters.

Previously called the "lactose-positive" vibrio, *V. vulnificus* is an opportunistic pathogen that can cause blood poisoning and death in susceptible humans. The microorganism is transmitted primarily through ingestion of raw shellfish but also through wounds encountered during contact with estuarine environments. Significant numbers of mortalities attributed to consumption of raw shellfish have led consumer groups to condemn the Food and Drug Administration (FDA) and the ISSC, and to criticize the shellfish industry. There are no current standards to regulate the harvest or sale of Gulf coast oysters based on *V. vulnificus* numbers. Gulf states have

reported 138 cases of the disease since 1988 with a 42% fatality rate. The National Shellfish Sanitation Program (NSSP), which as noted, protects the consumer from the still relevant threat of disease caused by enteric pathogens in shellfish contaminated with human waste or sewage, was not designed with naturally-occurring pathogens in mind. Workshops jointly sponsored by the FDA, ISSC and the National Oceanic and Atmospheric Administration (NOAA) to review the status of available information and develop recommendations to address the vibrio problem have been held in 1988 and 1994. A basic problem facing the regulatory community and the shellfish industry is to develop a strategy or strategies that can minimize the impact of this microorganism on that portion of the shellfish consuming public at risk, while not devastating an already marginal industry. The ISSC recently deliberated the issue following an FDA proposal that would limit seasonal use of Gulf coast shellfish to shucked product only and specifically labeled not to be consumed uncooked. Consequently, Gulf states have agreed to implement control measures designed to reduce *V. vulnificus* growth after harvesting, to establish a shelf life limit, and to educate consumers who eat raw shellfish.

Although there have been no reported cases of *V. vulnificus* disease attributed to consumption of oysters harvested from Virginia waters, a limited number of oyster packers located in the Chesapeake region have been importing oysters from the Gulf of Mexico for the purposes of reselling. These oysters are harvested from Gulf of Mexico waters and shipped intact (unshucked) to Virginia where they can be immediately shucked and sold as oysters labeled from the Gulf of Mexico. Another option is to place the oysters in Virginia waters for 6 months, after which, according to regulation, they can be sold as Virginia oysters. These designations are important because the market may exhibit preferences based on origin.

F. Research and Monitoring

1. Stock Assessment

a. Oysters: *The Virginia oyster resource and the need for stock assessment.* Estimates of standing stock size and basic population descriptors—such as growth rate, age at first reproduction, and fecundity (egg production) in relation to size—are fundamental to sound resource management. Oysters are ideal candidates for such estimation: they are sedentary, relatively easy to collect, and their life history is well documented. It is difficult, then, to understand why there has been an historical lack of attention to stock size estimation for Virginia oysters. Perhaps it is because the resource was, for an extended period, considered limitless. Indeed, no statistically defensible fishery independent assessments were made until 1993! Extensive descriptions of the Virginia oyster resource and history of its utilization have been given by Haven, Hargis and Kendall (1981), and more recently reviewed by Hargis and Haven (1988). These contributions describe a fishery in a state of continuing decline. To facilitate resource management, a fishery independent survey was proposed to and subsequently supported by the

Chesapeake Bay Stock Assessment Committee of the National Oceanic and Atmospheric Administration in 1993. This three-year collaborative effort between VIMS and the Shellfish Replenishment Program of the VMRC began in the fall of 1993.

Fishery independent data treats the resource as a sampling problem without regard for any prior fishery data. It allows development of statistically sound sampling with an estimable degree of confidence in the final values. It is not dependent on reporting or record keeping of third parties. It is, however, labor intensive and expensive. Fishery dependent data uses catch and effort data from fishery management and enforcement agencies. It is second hand and subject to all the quality limitations of data whose origin cannot be controlled. By contrast with fishery independent data it is a "free" byproduct of regular fishery management activity. Both approaches have been examined for the Virginia oyster fishery.

The fishery independent survey program has focused predominantly on the James and Rappahannock rivers. Limited surveys have been initiated on the seaside of the Eastern Shore; however, these data are still under analysis at the time of writing this report.

A quantitative sampling program was utilized in the James River using quadrants located in a random grid placed over a map of the known oyster resources. This is known as a stratified random grid, with the documented oyster reefs or rocks forming the strata. The area examined is described in extensive surveys made by VIMS and reported by Haven and Whitcomb (1983). These same areas have been subjected to regular survey by VMRC and VIMS personnel for at least two decades by dredge. The limits of the known oyster reef were mapped by the Surveying Engineering Department at VMRC and the grids for sampling set with Loran coordinates (Loran was checked daily when in the field from known markers at both the beginning and end of the day). Sampling sites were picked by random numbers within the grids and oysters were sampled with a hydraulically operated patent tong. In this manner a total of 823 stations on 19 major oyster reefs were occupied in the James River in 1993, and 786 stations on 23 major reefs were occupied in 1994. The sampling protocol for the Rappahannock River was the same as for the James River and employed a quantitative stratified random sampling program using quadrants located in a random grid placed over a map of the known oyster resources. Although once extensive, oyster resources are now limited to the upper part of the Rappahannock above Bowlers Rock and Morattico Bar. The only commercially exploited reef of any consequence is Ross Rock. A more general description of the Rappahannock oyster reefs is given in Haven and Whitcomb (1989).

General summary of population sizes. A summary of standing stock estimates for 1993 and for 1994 for the James River is given in Table 5 and Table 6, respectively. Data are presented on a per reef basis. This form of data

Table 5: James River Stock Assessment: Fall 1993

Small oyster and market oyster density (per sq. meter, bushels on reef and bushels per acre) for each reef
n = number of samples collected for identified reef

JAMES RIVER

REEF #	REEF NAME	n	SMALL per sq. m	MARKET per sq. m	SML+MKT per sq. m	SMALL bushels	bu /acre	MARKET bushels	bu/acre	SML+MKT bushels	bu/acre
1	Up D Wtr Shl	99	49	20	69	46472	199	37359	161	83831	360
2	Low D Wtr Shl	20	9.9	8.5	18.4	798	40	1371	69	2169	109
3	Up Horsehead	3	294.7	55.3	350	3588	1192	1348	448	4936	1640
4	Mid Horsehead	20	214.3	7.4	221.6	16877	867	1158	59	18035	926
5	Low Horsehead	20	253.4	18.8	272.2	19963	1025	2954	152	22917	1177
6	Moon Rock	4	247	24.8	271.8	3948	999	791	200	4739	1199
7	V-Rock	36	157.6	20.3	177.9	45950	638	11842	164	57792	802
8	Pt of Shoals	35	104.9	23.9	128.6	55906	424	25463	193	81369	617
9	Cross Rock	19	75.1	7.8	82.9	11151	303.9	2329	63.5	13480	367.4
10	Shanty Rock	4	32.5	2.3	34.8	471	132	65	18	536	150
11	Dry Lump	6	15	0.8	15.8	360	60.7	40	6.7	400	67.4
12	Mulberry Point	45	18.3	6	23.9	6436	74	3937	45	10373	119
13	Swash	19	3.5	1	4.8	2355	14	1687	10	4042	24
14	Upper Jail Is	65	5.5	5.6	11	13560	22	27578	45	41138	67
15	Swash Mud	134	20.8	5.6	26.4	104703	84	56092	45	160795	129
16	Offshore Swash	67	24.5	5.7	30.2	62175	99	28911	46	91086	145
17	Lower Jail Is	69	9.3	4.9	14.2	23571	37	24936	40	48507	77
18	Offsh.Jail Island	105	7.8	2.4	10.2	32109	32	20151	20	52260	52
19	Wreck Shoal	55	6.4	2.3	8.7	15188	26	10671	18	25859	44

Table 6: James River and Rappahannock River Stock Assessment: Fall 1994

Oyster spat (per sq. meter), small oyster and market oyster density (per sq. meter, bushels on reef and bushels per acre) for each reef
n = number of samples collected for identified reef

REEF #	REEF NAME	n	SPAT per sq. m	SMALL per sq. m	MARKET per sq. m	SML+MKT per sq. m	SMALL bushels	bu /acre	MARKET bushels	bu/acre	SML+MKT bushels	bu/acre
JAMES RIVER												
1	Up D Wtr Shl	72	5.32	40.51	9.21	49.72	34866	149	24214	104	59080	253
2	Low D Wtr Shl	8	2.8	11.9	8	19.9	871	44	1792	90	2663	134
3	Up Horsehead	6	30	192.17	26.17	218.33	2127	707	885	294	3012	1001
4	Mid Horsehead	11	53.8	194.6	11.9	206.5	13938	716	2606	134	16544	850
5	Low Horsehead	10	104.5	280.2	26.7	306.9	20068	1031	5843	300	25911	1331
6	Moon Rock	7	187.3	310	26.7	336.7	4505	1140	1186	300	5691	1441
7	V-Rock	20	79.17	154.55	15.35	169.9	40969	569	12433	173	53402	741
8	Pt of Shoals	32	61.05	138.69	25.31	164	73923	561	26984	205	100907	766
9	Cross Rock	10	77	69.3	8.4	77.7	255	9354	94	3464	349	12818
10	Shanty Rock	7	44	102.9	3.6	106.4	1490	416	103	29	1594	445
11	Dry Lump	7	35.7	54.9	0.4	55.3	1197	202	29	5	1225	207
12	Mulberry:upriver	10	8	30	3.4	33.4	10544	121	2390	28	12934	149
13	Mulberry & Swash	28	2	12.7	3.3	15.9	8466	51	4340	26	12806	78
14	Upper Jail Is	62	1.17	13.68	4.31	17.98	30785	50	29618	48	60403	99
15	Swash Mud	122	5.85	24.25	3.03	27.23	122151	98	30558	25	152709	123
16	Offshore Swash	64	12.92	31.55	1.97	33.52	72713	116	13866	22	86579	138
17	Lower Jail Is	63	1.05	8.73	2.38	11.11	20200	32	16833	27	37034	59
18	Offsh.Jail Island	101	7.12	14.33	2.23	16.55	53615	53	25473	25	79088	78
19	Wreck Shoal	52	8.43	8.98	0.94	9.92	19321	33	6194	11	25515	44
20	Days Point	73	7.73	21.04	0.32	21.36	23223	77	1063	4	24286	81
21	Hotel Rock	7	25.86	40.43	1.57	42	2012	149	239	18	2251	166
22	Snyders	7	14.71	19.86	0.86	20.71	770	73	102	10	872	83
23	Triangle Rock	7	23	114.43	17.14	131.57	3086	421	1413	193	4498	614
RAPPAHANNOCK RIVER												
1	Ross Rock	8	0	3	0.4	3.4	387	12	97	3	484	15
2	Carters Rock	7	0	0	0	0	0	0	0	0	0	0
3	Bowlers Rock	7	0.1	2.4	1.9	4.3	58	10	88	15	146	25
4	Long Rock	7	0	1.9	2.7	4.6	116	8	338	22	454	29
5	Sharps Inshore	7	0.6	7.9	2	9.9	78	32	40	66	118	48
6	Morattico Bar	42	0	0	0.2	0.2	222	0	1773	2	1995	2
7	Mouth	115	0.9	1.8	0.3	2.1	8443	7	2586	2	11029	10

presentation is appropriate because such resources can be managed by reef, and the reefs are by no means uniform in stock character. The stock estimates were developed from direct oyster counts assuming conversion figures for numbers of small and market oysters per bushel at 1000 and 500 respectively. These correspond to below and above 2½ inches (62.5 mm) height (maximum dimension). These summaries do not include young of the year (also commonly termed spat) oysters which are very small and occupy a comparatively negligible volume. Note that absolute densities of oysters are highly variable, with 1993 data exhibiting a range from high values of 350, 272, 271, 222, 173 and 129 per square meter at Upper Horsehead, Lower Horsehead, Moon Rock, Middle Horsehead, V Rock and Point of Shoals respectively, to low values of 14, 11, 10, 9, and 5 at Lower Jail Island, Upper Jail Island, Offshore Jail Island, Wreck Shoal, and Swash respectively. Mean estimates of standing stocks of seed (small) and market oysters are 465,356 and 258,869 bushels respectively, for a total of approximately 724,225 bushels in the surveyed section of James River. The confidence interval around these values gives upper and lower values of 318,542 and 612,169 bushels for seed (small), and 155,582 and 365,078 bushels for market oysters. A limited number of individual rocks had lower estimates of zero for market oysters—these reflect analysis of data that include a large number of samples with zero market size oysters present.

Substantial seed (small) oyster resources are present in a number of locations: Upper Deep Water Shoal, the components of Horsehead Rock, V Rock, Point of Shoals, Cross Rock, and the large areas of Swash and Jail Island. The bulk of market oysters are located on the same rocks.

In the Rappahannock River, 1993 standing stock estimates were made for Carters Rock, Ross's Rock, Bowlers Rock, Long Rock, and Sharps Rock (inshore). These are all very small rocks and of limited commercial importance. The estimated seed oyster resources on these rocks were 126, 637, 36, 78, and 13 bushels respectively. The estimated market oyster resources were 69, 371, 79, 202 and 0 bushels respectively. Only Ross's Rock supported any commercial activity in the public oyster season of 1993-94.

Like 1993 data, 1994 surveys again illustrated the variability in oyster density by reef, varying from 337 per square meter at Moon Rock to only 10 per square meter at Wreck Shoal. More importantly from a management standpoint the total standing stock remained reasonably stable, suggesting that the combined effects of harvesting and disease were offset by growth and recruitment. This is encouraging, and suggests that catch can be regulated by employment of stock assessment information with the long term goal of rebuilding stocks to former levels. Unfortunately, there are always uncontrolled factors in natural systems, and the freshets of June and July in the pivotal James River, associated with runoff from earlier periods of extensive heavy rain in the James watershed, caused significant mortality in the upriver sections of the James. At prevailing summer

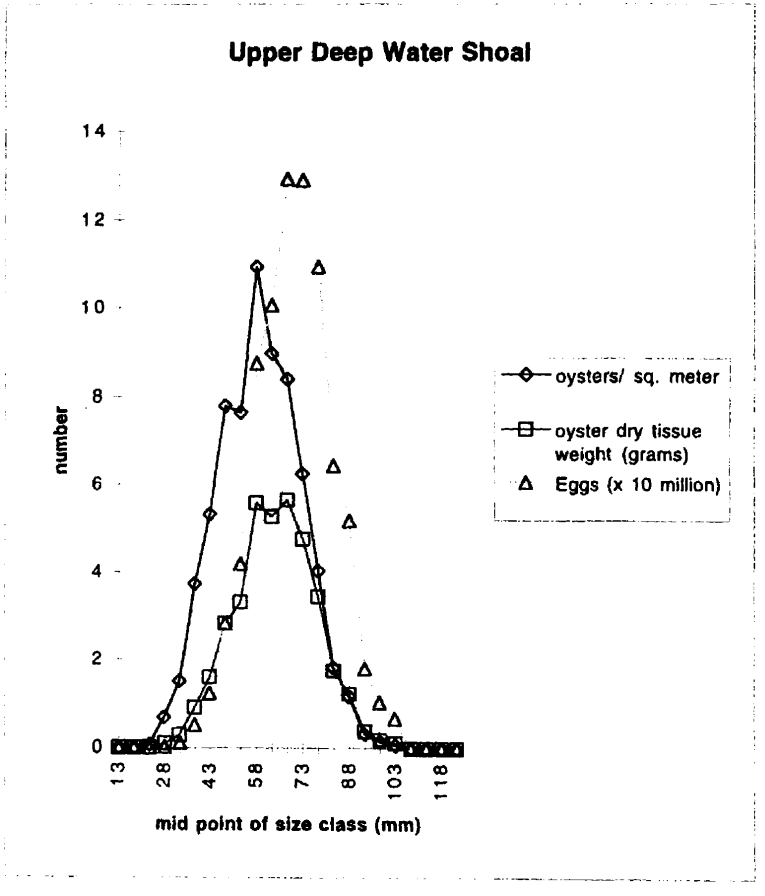
temperatures oysters have limited ability to survive extended periods at low salinities (this is unlike the situation at winter or early spring temperatures) and losses on upriver reefs such as Deep Water Shoal may take some years to recover. The full extent of the losses will be evaluated in planned stock assessment in the fall of 1995.

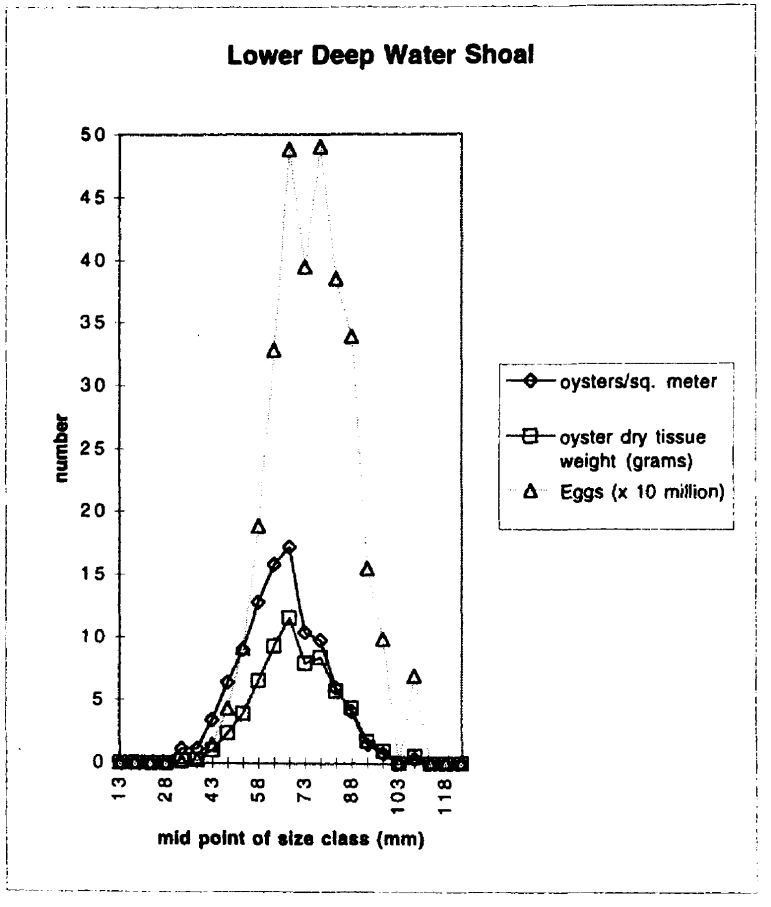
Size distribution data, by numbers of individual oysters present within each 5 mm height size class interval, for all sampled areas, also was collected. This is important because it illustrates both the current market resource and the future resources in oysters that are currently below market size. An example is given in Figure 2. For convenience, young of the year (spat) oysters are not included in this illustration. The dominant feature of all plots is the rapid decrease in number of individuals in all locations above the 60-65 mm (midpoint 63 mm on Figure 2) size class. This corresponds closely with the 2½-inch (62.5 mm) minimum size for market oyster harvest, suggesting efficient harvesting above the size limit. Despite this, individual oysters of over 100 mm maximum dimension were found in very limited numbers in the majority of locations. The size distribution data illustrate how an increase in minimum size for market oyster exploitation to three inches (76 mm), as employed for the 1994-1995 public oyster season, may result in some hardship to watermen because large numbers of individuals were at or below 60 mm in the fall of 1993, and would have to grow substantially through the spring and summer of 1994 to attain a 76 mm size and become available to the fishery in the fall of 1994.

Size distribution is also important because it provides valuable information concerning reproduction in the population. Contribution to reproductive potential is not equal among individuals, and larger oysters are disproportionately more important. When size distribution data, by individual numbers, is replotted by either biomass or potential contribution to egg production these other facets of stock management become obvious.

Size significance is illustrated for 1993 data from Upper Deep Water Shoal in Figure 2. In the instance of both live and dry tissue weight the mode moves above the 60-65 mm size class, illustrating the importance of the less abundant but larger oysters in the ecologically important process of filtration. The fecundity issue is critical, because the basis for setting minimum harvest size is to maximize reproductive output prior to harvest (although this is somewhat questionable in the James River where, until the 1994-1995 season, seed harvest procedures allowed removal of essentially all oysters from the majority of public oyster ground). When considering contribution to egg production 76% and 65% of production for Lower and Upper Horsehead is in the 60-65 mm size class and above—that is, the majority of egg production is in the few large oysters that are available for harvest; they produce more eggs than all of the smaller oysters considered together! Harvesting these larger oysters, despite their limited numbers, can clearly have major impact on egg production. Note that these percentages are calculated giving

Figure 2. A comparison of oyster size class distribution by number per square meter, dry tissue weight, and egg production per square meter





equal weighting to sex ratio with increasing oyster size. Although a matter of debate in the scientific literature, many scientists consider there to be a predominance of females with larger size classes. If the latter were the case then the 76% and 65% values are conservative estimates! An increase of minimum harvest size to 76 mm decreases the percentage egg production in harvestable oysters considerably; 48% and 32% respectively of estimated egg production comes from individuals in the 75-80 mm size interval and larger in the two locations. Increasing the minimum size limit for market oysters from 2½ inches to 3 inches (62.5 to 76 mm) effectively doubles the available egg production from the resource.

Fishery Dependent Methods. Barber and Mann (1991), supported by Chesapeake Bay Stock Assessment Committee (CBSAC) funds, employed Leslie-DeLury analysis of commercial fishery data (daily and weekly boat count data to estimate effort and landing data to estimate catch—both data sets collected and provided by the VMRC) to estimate recent decline in standing stock of oysters in the James River. A secondary objective of the more recent study was to compare, where possible, fishery independent and fishery dependent estimates of standing stock.

The analysis founded on fishery dependent data suggested a rapidly diminishing resource with stock values well below those suggested by the fishery independent estimates. The problem is traceable to the fact that the data used for catch per unit effort do not distinguish between effort devoted to market oyster harvest and that devoted to seed oyster harvest. On any particular boat at any time in the period studied, attention may have been devoted to market oysters or seed oysters in isolation, or to the peculiar (to this river, and again a product of the regulations allowing both seed and market oyster harvest from the same location) activity of "two piling"—retaining both seed and market oysters as separate catches for inspection purposes on the same vessel. Summary data that does not distinguish effort between the two resources should not be used to generate fishery independent estimates of standing stock.

Conclusions from stock assessment activities to date. The James River will remain the only substantial source of both seed and market oysters for the public fishery for the immediate future. The resource in the Rappahannock will remain of minor importance to the total fishery production. The James River market oyster harvest for the 1993-94 public season of 5,173 bushels represents approximately 2.2% of the estimated standing stock; however, the seed harvest of 72,470 bushels for the same period represents approximately 15.6% of the estimated standing stock. These are the first ever fishery independent estimates, so long term comparisons of harvest versus standing stock are not possible, although such levels of exploitation appear reasonable at this time. There is considerable spatial variability in oyster density, and harvesting will probably continue to focus on those areas with high density such as Horsehead, Moon Rock, V Rock and Point

of Shoals. The seed resource is still substantial, but its utilization will probably be controlled by factors other than availability to the watermen. Lease holding planters are reluctant to purchase seed oysters that may have already been exposed to disease. While mortalities associated with disease are limited in the upper part of the James, oysters may have contracted infective particles. When transferred to higher salinity grow-out sites, infected oysters essentially participate in a race between the progressing disease and growth to market size. The financial consequences to the planter of disease-related loss in this instance has prompted caution in seed sales.

The recent increase in minimum size of market oysters will continue to have potential impact for one or two seasons as the majority of large oysters grow into the larger size class before being available for harvest. From an ecological perspective the increase in minimum size is to be applauded. More importantly, accompanying calculations suggest that the modest increase in minimum size will double the available egg production from remaining oysters—a clear bonus in a long term plan to rebuild the resource. Offset against this must be the temporary hardship to watermen and the as yet due to the impact of summer freshets in 1995.

The nature of fishery dependent data records is such that they do not adequately distinguish between market and seed oyster fishing activity, and changes in emphasis from one to the other cause major variability in catch per unit effort data. In turn this compromises the value of standing stock estimates obtained by Leslie-DeLury analysis. In summary:

(1) Fishery dependent methods of stock assessment cannot be used with the current database.

(2) Fishery independent estimates provide a stable method of stock estimate for management purposes.

b. Hard Clams. The most recent stock assessment survey is that by Wesson (1995) using fishery independent methods in a survey of Hampton Roads and the lower James River. Comparing these results with previous results from limited VIMS studies over the past 25 years led Wesson to conclude that the standing stock of clams in the lower James/Hampton Roads appears similar to levels previously reported. However, the size and age structure over a 20 year period appears to have changed, with fewer larger, older clams in the current clam population.

2. Monitoring

a. Spatfall Monitoring. VIMS conducts surveys of oyster spatfall (or "setting") in Virginia waters throughout the summer reproductive period. This survey provides an estimate of the potential of a particular area for receiving a "strike" or set of oysters on the bottom and helps define the timing of setting events. Information

obtained from this effort is valuable to the VMRC for its shell repletion program and to private oyster growers, both of whom are interested in maximizing the timing of shell planting. In addition, by maintaining a long-term data base, trends in spatfall throughout the lower Chesapeake Bay can be monitored. This in turn provides an index of the general "health" of the Bay.

Shellstrings are the standard monitoring tool. A shellstring consists of 12 oyster shells of similar size (about 3") drilled through the center and strung (inside of shell down) on a piece of heavy gauge wire. Throughout the study period shellstrings were deployed 0.5 m off the bottom at each station. Shellstrings were replaced after a one-week exposure, and the number of spat that attached to the smooth surface (underside) of the center 10 shells was counted with the aid of a dissecting microscope. This number was then divided by 10 to get the number of spat per shell for that time interval. A computer program was used to calculate the number of spat per shell per week. These values were interpreted as follows: <0.1= "none"; 0.1-1.0="light"; 1.1-10.0="moderate"; and >10.1="heavy."

Weekly sampling allowed setting trends over the course of the summer to be compared among the various locations. Comparisons of setting intensity among years are made by adding the weekly values of spat per shell for the entire setting season. The number of spatfall on shellstrings is an indicator of relative numbers of larvae (ready to set) in a particular location at a particular time. Subsequent spat settlement and survival on nearby shoal areas is variable and dependent on a number of factors. High spat counts on shellstrings may not be accompanied by a good set on bottom shell if it is not plentiful or clean enough to attract the metamorphosing larvae. Conversely, for unknown reasons, good setting on bottom shell may occur even though setting on shellstrings was light. It is not known what level of setting on shellstrings is indicative of good setting on bottom cultch, if conditions on the bottom are optimal. Also, it is not known whether recruitment is more readily effected by continuous, light setting, or intense setting of short duration.

Spatfall in 1993 and 1994 was monitored from June through the first week of October at a total of 32 stations in the Virginia tributaries of the Chesapeake Bay, 12 stations in the Potomac River, and 15 stations on the Eastern Shore of Virginia. This is typical of the distribution of effort over the history of this program, the roots of which can be traced back to studies beginning in the 1930s. The number of stations can be modified as required for any particular purpose. For example, four stations were added in 1992 to the Potomac River portion of the survey—Nomini Bay, Currioman Bay, Lower Machodoc River, and Ragged Point—and have been included from that time because these low salinity areas have become the focus of recent seed oyster plantings. A significant number of new stations have also been added recently on the Eastern Shore, these approximating inshore to offshore transects from Chincoteague, Wachapreague, Quinby, and Hog Island.

Recent trends in oyster spatfall throughout the waters of the Commonwealth have been discouraging. Consider just the 1991 through 1994 period. Overall, spatfall potential in Virginia in 1992 was very poor. Of the 40 locations for which comparisons could be made, 39 had lower spat/shell totals in 1992 than in 1991. The only location for which total spatfall in 1992 exceeded that in 1991 was Wilson Creek in Mobjack Bay. Similar 1991 and 1992 values were observed at a limited number of sites in the lower James River (Dog Shoal, Dry Shoal, Days Point, and Rock Wharf), at Palace Bar in the Piankatank River, Fleeton Point in the Great Wicomico River, and on the Eastern Shore at Wachapreague. Spat/shell totals for 1992 were lower than the long-term average (up to 10 years) at all but the station at Wilson Creek in Mobjack Bay (and this was not particularly high). The James River data are particularly distressing because 1992 was the fifth year in a row that spat/shell totals in the upper James River—the critical seed bed area around Point of Shoals, Horsehead, and Deepwater Shoal—have been below the 10-year average.

b. **Post Settlement Monitoring**. Twice a year VIMS conducts a survey of selected public oyster bars (shoals) in Virginia waters for the purpose of assessing the status of the resource. Surveys conducted in the spring provide information about over-winter mortality and relative fishing pressure from the current harvesting season. Surveys conducted in the fall provide information about spatfall or recruitment, summer (disease) mortality, and the status of each shoal as a source of seed or market oysters before the beginning of the harvesting season.

   The following data are obtained for each sample: number of market (>3" in shell height) oysters, number of small (submarket sized) oysters, number of spat (1991 recruits), number of recent boxes (inside of shells clean; dead a month or less), and number of old boxes (inside of shells dirty; dead a month or more). Surface water samples are obtained at each location for temperature (°C) and salinity (ppt) determination. Where possible, 20-25 oysters are collected for disease analysis (prevalence of *Perkinsus marinus*). In addition, observations are made regarding the condition of the bottom at each shoal: bottom material, predators, and fouling organisms. Data are summarized for each shoal as the average number of market, small, spat, and total oysters per bushel and percent recent mortality.

c. **Oyster Disease Monitoring**. The oyster disease monitoring program is designed to determine the annual abundance and distribution of the major oyster pathogens *H. nelsoni* and *P. marinus*. The information is supplied in the form of an annual monitoring report that is circulated to VMRC and interested industry members. Since 1987, oyster samples have been collected each month from four locations in the James River. These locations, Wreck Shoal, Point of Shoals, Horsehead Rock and Deepwater Shoal, are the only beds left in Virginia that harbor sufficient oysters for routine sampling. Oysters at all four locations are examined for *P. marinus*, but *H. nelsoni* is diagnosed only at Wreck Shoal and Horsehead Rock. High sampling frequency of this region allows correlation of oyster disease

abundance with environmental parameters such as temperature and salinity which are collected at the time of sampling and also obtained from other sources. Most traditional oyster rocks on the western side of the Bay are sampled during spring and fall for standing stock estimates, and samples for disease diagnoses are collected wherever possible. This sampling regime allows determination of the broad-scale distribution of both disease agents throughout Virginia. In addition, VMRC personnel submit oyster samples for disease analyses at other times of the year and from areas not routinely sampled by VIMS. The annual abundance of *H. nelsoni* is monitored in trays of susceptible oysters placed in the lower York River. Oysters are obtained from the upper James River or the upper Rappahannock River and deployed at VIMS on 1 May each year. Mortality and disease status are monitored monthly until 1 December. Monitoring of MSX abundance in trays has been ongoing since 1960 and this 35-year record has allowed correlation of *H. nelsoni* abundance with environmental parameters and has yielded much information on the environmental control of seasonal dynamics of the pathogen.

The monitoring program has disclosed that *H. nelsoni* is highly susceptible to salinity below 10 ppt such that the pathogen will not survive for more than 10 days. Typical spring runoff usually eliminates or greatly reduces this pathogen in oysters in the James River and other major tributaries. The parasite usually re-invades an area during late summer but causes little mortality because development of the pathogen is curtailed as water temperature declines during fall. However, if spring runoff is low, the pathogen can persist at high levels throughout the year and cause significant oyster mortality the following summer. In high salinity areas of the lower Bay, *H. nelsoni* is usually present and, unless there has been unusually high rainfall, significant oyster mortality will, in most years, result.

In contrast to *H. nelsoni*, *P. marinus* has been shown to be highly tolerant of low salinity and persists tenaciously even in the upper portions of major tributaries where the water may be entirely fresh for short periods during the spring. This pathogen has declined in abundance somewhat since its peak in 1991, but it is still present on all oysters beds in Virginia and causes significant mortality in most areas during dry summers. Cold winters have little effect on subsequent summer abundance of *P. marinus*, but warm winters allow higher overwintering levels and result in higher abundance the following summer.

3. **Oyster Disease Research**. Because of intermittent funding, oyster disease research has progressed slowly from the descriptive phase to more sophisticated studies on host/parasite interactions. Only with the funding provided by the NOAA Oyster Disease Research Program since 1990 has the pace of acquisition of new knowledge accelerated significantly. Early studies on both *H. nelsoni* and *P. marinus* conducted in the 1950s and 1960s elucidated the morphology of both pathogens, their distribution in Chesapeake Bay and the seasonal pattern of infection and oyster mortality. The causal relationship between the presence of the pathogen and oyster mortality was firmly established for both parasites. Direct transmission of *P. marinus*

from oyster to oyster was established and a general understanding of the environmental requirements of both pathogens developed. A reasonably good understanding of the annual abundance cycle of *H. nelsoni* and of its environmental requirements has been achieved, but understanding the life cycle of the parasite is yet incomplete. Because of failure of direct transmission experiments, speculation persists that a host other than oysters carries stages of the parasite and releases stages that are infective to oysters. However, direct transmission of the parasite from oyster to oyster cannot be ruled out.

Two significant breakthroughs in oyster disease research have been made by VIMS scientists within the past two years. The first was the continuous laboratory culture of *P. marinus*. Continuous culture has, for the first time, provided a steady supply of pure pathogen cells for establishing experimental infections in oysters and for other research that was impossible to conduct previously. For example, the salinity tolerance of cultured *P. marinus* cells, in the absence of host influences, has been determined. Continuous cultures have now been established at various low salinities confirming that the pathogen can acclimate to a wide variety of environmental conditions. These studies have helped to explain the persistence of *P. marinus* in low salinity areas of the Chesapeake Bay such as the upper James River. Continuous cultures have also allowed the investigation of some of the factors involved in *P. marinus*-induced mortality of oysters. It has been learned that *P. marinus* cells secrete proteolytic enzymes, and these enzymes may be at least in part responsible for the pathogenicity of *P. marinus*. Several studies are being conducted at VIMS to determine the role of these enzymes in *P. marinus* pathogenicity.

The second significant breakthrough has been the development of a DNA probe and Polymerase Chain Reaction (PCR) primers specific for *H. nelsoni*. (PCR is a molecular amplification technique that results in an exponential increase in the amount of the target DNA sequence over a period of a few hours.) In addition to rapid and specific pathogen diagnosis, these molecular tools provide, for the first time, a means for rapidly screening potential intermediate hosts for *H. nelsoni* and should greatly facilitate elucidation of the life cycle of *H. nelsoni*. The PCR primers will amplify only *H. nelsoni* DNA, so even if only one cell is present in another organism, it will be detectable. There is no need to know the form of the life-cycle stage or its location in the host, one just looks for the presence of parasite DNA. Once an intermediate host is identified, subsequent studies using the DNA probe can determine the site of the parasite in the host and its morphology.

Another significant result made possible by the molecular tools developed for *H. nelsoni* has been to verify that *H. nelsoni* was introduced to the East Coast; it is not a natural pathogen of *C. virginica*. Oyster mortality attributable to *H. nelsoni* appeared rather suddenly in Delaware Bay in 1957 and in Chesapeake Bay in 1959 and there is good evidence that the pathogen was not present before that time. An unnamed parasite morphologically similar to *H. nelsoni* has been known for some time in the Pacific oyster, *Crassostrea gigas*, in Japan, Korea and California. This parasite is rare

and apparently causes little if any mortality in *C. gigas*. The *H. nelsoni*-specific DNA probe developed at VIMS reacts very strongly with the parasite in *C. gigas* from all three areas, suggesting but not confirming that they are one and the same. The *H. nelsoni*-specific PCR primers were used to amplify DNA from the parasite in *C. gigas* obtained from California, and its DNA sequence was determined to be exactly the same as that of *H. nelsoni*. DNA sequence equivalence confirms that the parasite in *C. gigas* is *H. nelsoni*. These results indicate that *H. nelsoni* was introduced to the east coast of the United States, probably with trial plantings of *C. gigas* that are known to have occurred, but possibly with the as yet unknown intermediate host. Although no populations of *C. gigas* have survived along the East Coast, one of its parasites was apparently able to infect the native oyster, *C. virginica*, in which it develops to pathogenic levels.

Over the past few years, oyster disease research at VIMS has focused on six main areas:

1. Elucidation of the life cycle of *H. nelsoni* using molecular techniques. As discussed above, most research during the last few years involved developing the molecular tools necessary to be able to make significant progress in solving the life cycle of *H. nelsoni*. With these tools, potential intermediate hosts are being screened for the presence of *H. nelsoni* DNA; given appropriate funding, such screening will continue until the intermediate host is identified.

2. Defense mechanisms of oysters in relation to oyster pathogens. Oysters have only a limited defense capacity compared with higher organisms and it is relatively poorly studied. Much valuable information has been generated at VIMS on the correlation between various defense components and environmental temperature and salinity, but a definitive link between defense components and *P. marinus* abundance has not been established. If oyster immune defense mechanisms play any role in regulating *P. marinus* abundance the mechanisms have not been identified.

3. Environmental control of *P. marinus* dynamics. Laboratory studies have determined that the critical salinity below which *P. marinus* infections will not develop and cause oyster mortality is about 9 ppt. Field experiments using sensitive diagnostic techniques have demonstrated that *P. marinus* overwinters in a much higher percentage of oysters than previously believed. New infections of *P. marinus* can be initiated in the laboratory at temperatures as low as 5°C and at salinities as low as 3 ppt. Other studies have determined that meronts are more infective to oysters than prezoosporangia. Field experiments have determined that oysters acquire new *P. marinus* infections primarily during late August and early September, a period that correlates closely with oyster mortality. This suggests that infective cells are released from dying oysters, as hypothesized in the 1960s by Dr. Jay Andrews at VIMS. These types of studies are continuing, and will, hopefully, greatly increase the ability to predict the response of *P. marinus* to changing environmental conditions.

4. Virulence factors in *P. marinus*. *Perkinsus marinus* produces potent extracellular serine proteolytic enzymes (proteases) which degrade oyster plasma and tissue. These proteases may be responsible for the extensive tissue degradation observed in heavily infected oysters and they may play an important role in parasite invasion and spread throughout an oyster. The proteases may also play a role in counteracting oyster defense mechanisms. Inhibitors of these proteases have been found to stop growth and cause mortality of cultured *P. marinus* cells. Studies are ongoing to further elucidate the role of proteases and protease inhibitors in the dynamics of *P. marinus* infections.

5. Effects of toxicants on the progression of *P. marinus* disease in oysters. Although the increase in oyster diseases over the last decade is clearly related to drought conditions of the late 1980s, a common assumption by the lay public is that pollution is responsible for the increase in oyster diseases. There is no evidence to support this assumption because both *H. nelsoni* and *P. marinus* are abundant in relatively pristine areas with favorable salinity, but the role of pollutants has received little attention from oyster disease researchers until recently. Research at VIMS has demonstrated that exposure to high concentrations of creosote-contaminated sediments from the Elizabeth River accelerates the development of *P. marinus* infection in oysters. Another study at VIMS showed that exposure to environmentally relevant concentrations of tributyltin (TBT) accelerated *P. marinus* infections in oysters and resulted in increased oyster mortality. These studies demonstrate that pollution may enhance the effects of oyster diseases. It is clear, however, that increased salinity, not pollution, is the primary reason for the increase in abundance and distribution of oyster pathogens.

6. Development of disease-resistant oysters. A selective breeding program to develop disease resistant oysters has been underway at VIMS since 1987. Basically, surviving oysters in disease endemic areas are spawned in the hatchery and the offspring are exposed in the lower York River for a period of two or three years. Survivors are returned to the hatchery and spawned, and the process is repeated. A number of strains have been evaluated and discarded because of high disease-induced mortality in the second generation, but a few have been continued through three generations. Presently four strains are under evaluation at VIMS: 1) a Delaware Bay native strain (third generation), 2) a lower James River strain (third generation), 3) a Louisiana strain (first generation) and 4) a lower James River/Mobjack Bay hybrid strain (first generation). The Delaware Bay strain is demonstrating significantly higher survival than any of the other selected strains or susceptible controls. It also has significantly lower levels of both *H. nelsoni* and *P. marinus* midway through the second summer of exposure. This strain will be used as broodstock for the oyster aquaculture program during 1996 to see if it performs similarly using actual industry grow-out techniques.

4. **Habitat and Ecosystem Function**. There is growing evidence that oyster reefs are (or were historically) important components of the coastal ecosystem. As habitat for a

diverse array of organisms, including many commercially important species, oyster reefs play a functional role similar to that of corals in tropical reef systems. Oyster filtration capacities suggest that they are capable of reducing the effects of nutrient enrichment, structuring food webs, and driving carbon cycling in many estuaries. The ecosystem level functions of oysters have been proffered as a justification for habitat restoration, but not widely incorporated into either the construction criteria or the management practices.

Over the past three years VIMS has worked closely with the VMRC Repletion Program to initiate a program of reef restoration. Using a combination of state, federal and private dollars, joint efforts have been undertaken to construct the foundations of oyster reefs which, if properly managed, should develop viable reef communities. These reefs should serve as broodstock sanctuaries for oysters and as habitat for other species. This is in sharp contrast to the prior repletion practice of thinly spreading shells onto oyster seed beds.

Research and monitoring at these reef sites have clearly indicated the importance of (1) placement, construction material and tidal height in reef construction and (2) reef habitats for other commercially important species (e.g. clams, blue crabs, finfish). VIMS' monitoring efforts on these reefs are generally associated with the previously described programs and thus not specifically designed to address many important aspects of habitat. Better knowledge of the spatial patterns of settlement, growth and disease progression will be fundamental to the success of future restoration efforts.

5. **Aquaculture**. As noted earlier, VIMS has played a highly significant role extending from generic science through commercial development. These efforts continue through methods development and continuing outreach to clients. Having led hard clam aquaculture to commercialization, VIMS, in 1984 directed increased attention to oysters. A hatchery was expanded at Gloucester Point to develop and improve hatchery, nursery, and grow-out techniques. Following these developments, collaborators in the private sector—individuals to small businesses—were enlisted to test the products. These efforts proved successful at small scales with provision of seed oysters from the VIMS hatchery. The more recent phase has concentrated on shifting oyster seed supply to private hatcheries with VIMS' redirection to technology development and problem solving for those interested in grow-out applications, "gardeners," and commercial enterprise. Research has been directed to six main objectives:

   a. **Development of Disease Resistant Oysters.** Section III.G.3.6 describes efforts to develop disease resistant strains of oysters. This line of research is yielding promising results and VIMS expects to have improved broodstocks available to industry within a few years.

   b. **Development and Testing of Triploid Oysters.** VIMS developed protocols for producing triploid oysters which have limited reproductive capacity. Thus, during

the summer, these oysters continue to grow and fatten rather than divert energy to reproduction. While triploidy did not confer any disease resistance to oysters, triploids grew faster and produced better meat qualities than diploids. Triploid oysters may ultimately prove to be useful to aquaculture in Virginia, but at the present time the induction process is very costly in terms of reduced hatchery production.

c. **Predicting Oyster Growth Rates in the Field.** VIMS has worked to develop a quantitative model for prediction of oyster growth rates based upon the quantity, quality and supply rate of food. By combining hydrographic data with measures of chlorophyll, particulate organic carbon, particulate organic nitrogen and suspended sediments, VIMS has been able to calculate estimates of *potential* growth rates at various field locations. This approach may then be used as a "soil test analog" in the selection of grow-out sites for oyster aquaculture.

d. **Interactions Between Dinoflagellate Blooms and Oysters.** Although the species of dinoflagellates responsible for blooms in Virginia have been considered non-toxic (from the human health perspective), their impacts on shellfish are not necessarily benign. The effects of four bloom-forming species have been investigated: *Prorocentrum minimum, Gyrodinium uncatenum, Cochlodinium heterolobatum* and *Katodinum rotundatum,* with respect to grazing, growth and mortality rates of juvenile oysters. Results indicate that some species can have very pronounced effects on the production and survival of oysters. Further work is required to improve upon remedial measures for hatcheries and nurseries.

e. **Improvements to Hatchery Techniques.** Ongoing efforts to improve larval culture techniques are an important part of VIMS' hatchery operations. Testing of various nutrient additions, algal diets and feeding regimes has led to improvements in larval growth rates over the past few years. Modifications to setting procedures and testing for optimal setting density have led to significant increases in the percent of oyster larvae which successfully metamorphose and set to become spat. These improvements in procedures not only enhance hatchery operations, but are being transferred to private shellfish hatcheries to increase their productivity .

f. **Refinements to Grow-out Techniques.** In collaboration with private sector culturists VIMS has been working to improve handling techniques in the field. This has led to the development of improved containment systems. One such system, the Taylor float, has proven to be the most cost effect method for growing oyster off-bottom in relatively protected creeks and embayments. It is now widely used by most oyster culturists in Virginia. Improved procedures for reducing fouling and eliminating some predators have provided valuable cost savings to the culture process.

6. **Non-Indigenous Species**. After a thorough literature review on the environmental requirements of various oyster species around the world (Mann et al., 1991), the

Pacific oyster, *Crassostrea gigas* was chosen as the species whose requirements reasonably matched those of the lower Chesapeake Bay. In addition, the Pacific oyster has no important diseases in its native range, and it has been resistant to local diseases wherever it has been introduced for aquaculture purposes.

This first investigation with *C. gigas* involved disease susceptibility. If it was 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 diploid and triploid *C. gigas* and *C. virginica* held side-by-side in quarantine flumes and exposed to *Perkinsus marinus* over one summer (Meyers et al., 1991). *Perkinsus marinus* challenge was by addition of homogenated infected oyster tissue to the flumes twice a week for six weeks. In these experiments, 64% of the *C. gigas* became infected with *P. marinus*, but all infections remained very 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). Because the life cycle of *H. nelsoni* is unknown it was not possible to infect oysters with this pathogen under experimental conditions; challenge required field deployment. After appropriate debate on the risks and benefits of a limited introduction of *C. gigas* for disease challenge, the field experiment was approved for the summer of 1993. Conditions imposed were that only individually-typed triploid *C. gigas* could be used in order to minimize spawning risk. In June 1993, 200 triploid *C. gigas* (45 mm) and 400 diploid *C. virginica* control oysters were deployed in double mesh bags at the VIMS dock in the lower York River, VA. The *C. virginica* consisted of two separate groups—200 from the upper Rappahannock River, VA and 200 from the Wye River, MD. Samples of 25 oysters from each group were removed for disease diagnoses in August, September and October. Counts of live and dead oysters for mortality estimates were made weekly. The experiment was terminated in February 1994 following confirmation that some *C. gigas* individuals reverted to diploid status. Maximum prevalence of *H. nelsoni* was 84% in the Virginia controls and 92% in the Maryland controls with a high proportion of moderate and heavy infections. No *C. gigas* was infected with *H. nelsoni*. Maximum prevalence of *P. marinus* was 96% in the Virginia controls, 100% in the Maryland controls and 24% in the *C. gigas*. A high proportion of heavy and moderate infections occurred in both control groups, but all *P. marinus* infections in *C. gigas* were of low intensity. Mortality was greater than 90% in both control groups by 1 November 1993; mortality was 25% in *C. gigas* and was not attributable to disease. There was a more or less continuous low-level mortality in the *C. gigas* through late October, with a few oysters dying each week. There was no mortality of *C. gigas* after about 21 October. The *C. gigas* increased in size and weight during summer, but did not grow during the fall as expected. Because of the short duration of the experiment, no spring growth data are available for *C. gigas*.

Shells of *C. gigas* were heavily infested with the polychaete *Polydora ligni.* These small worms produce a u-shaped mud tube on the inner surface of the oyster shell. The oyster deposits shell material over the tube to produce what is called a mud-blister. The entire inner shell surface of all *C. gigas* examined for disease was covered with mud blisters produced in response to the worm. These worm infestations may have been responsible for the limited growth during fall. Although the meat quality is not affected, blisters make shucking difficult and would probably affect marketability of *C. gigas*, especially for the half-shell trade.

These results suggest that *C. gigas* of the size range tested are not susceptible to the major oyster diseases of the Chesapeake Bay. The tests demonstrated that *H. nelsoni* can infect *C. gigas* since DNA work has demonstrated that the haplosporidian parasite occurring naturally in *C. gigas* in California and in the Orient is *H. nelsoni.* Failure to find infections of *H. nelsoni* in *C. gigas* during this experiment can be explained by the very low prevalence typical of *H. nelsoni* infections in *C. gigas* in California and the Orient. Prevalence is usually only 1% to 2%, so it is not surprising that no infections were found in the 75 *C. gigas* examined during this experiment.

IV. Ten Year Strategic Research Plan

A. Research and Monitoring Needs

1. **Native Fisheries**. Current policy adopts a minimal management goal of no net loss for oyster stocks; that is, no net loss from an already devastated resource. The purpose of the stock assessment and monitoring programs is to provide scientifically derived evaluations for use in prudent management of the resource, and to support, through those management decisions, the fishing industry. Substantial financial outlay is required to provide this information, and to be cost effective, the information must be utilized in resource management decisions. The recently initiated fishery independent stock assessments for oysters, for example, must be considered a foundation for decisions, rather than *ad hoc* assessments provided by harvesters. The current funding from the Chesapeake Bay Stock Assessment Program (NOAA) ends in spring 1996. It is imperative that state funding be provided to continue the program.

   a. **Stock Assessment**. Stock assessment is the basis of sound resource management. Preceding sections have described current activities, including the recent, federally funded project examining both fisheries dependent and fisheries independent oyster stock assessment. Only the fishery independent survey provides a firm foundation for critical application in management and long-term resource restoration.

   Continuation of the fishery independent stock assessment for oysters is essential. As well, it is prudent to continue and extend, probably at lower frequency, the clam stock assessments using the same methodology. The oyster stock assessment has been a collaborative effort between the VMRC and VIMS, and it should continue as a collaboration. VMRC operates the R/U Baylor designed for the purpose, and VIMS has participated in the analysis and provided additional personnel for execution of the surveys. As important, the collaboration provides a continuing liaison between the agencies in a mutually recognized effort of fundamental importance.

   The following stock assessment survey frequency is recommended:

   Oysters:
   - James River: annual, because of the critical nature of the resource
   - Rappahannock and Piankatank rivers: three-year frequency
   - Eastern Shore: three-year frequency

   Clams:
   - An initial assessment of Virginia's waters, followed by
   - Sectional surveys at a three to five year interval

Database management is a critical element to program effectiveness. Database evolution should proceed from a map database for generating survey positions (GPS controlled), to data analysis, to GIS presentation. Partial steps in this process have been achieved. Full integration assumes the combined efforts of VMRC and VIMS.

b. Monitoring

i. **Spatfall.** Victor Loosanoff initiated the spatfall survey in the 1930s to document oyster settlement in the James and other Virginia tributaries. The purpose was to develop "maps" of temporal and spatial distribution of competent to settle oyster larvae in the Virginia tributaries for each spawning and associated settling season. This program has been effected with consistency for a number of years with limited resources and with essential collaboration with the VMRC. The end product is compiled as a newsletter interim report and distributed with the cooperation of the VIMS Advisory Services Department. The mailing list is extensive. The number of monitoring stations occupied has recently increased substantially on the Eastern Shore at the request of VMRC because they have increased repletion efforts in this region. This increased effort will remain for at least another two years.

The shellstring/spatfall program has utility as a measure of potential substrate settlement; however, the surveys do not resolve important questions relating the abundance of spat set on water column shellstrings to successful bottom settlement. All parties are best served by leaving the program to serve the purpose for which it was originally designed—as a semi-quantitative survey to cover large areas at modest expense. In this respect the program is very cost effective in meeting its goals. The long term record illustrates decreasing oyster settlement associated with harvest pressure and disease impact. Given the diminished state of the current resource, it is obvious that continued monitoring is critical.

ii. **Post-Settlement Mortality.** Fall surveys measure the survivors of the past summer's settlement plus the recruitment survivorship from previous years. Spring surveys measure overwintering survival. These surveys are essential. These efforts must continue; however, cost-effectiveness can be achieved if the aforementioned stock assessment surveys are funded.

c. Emergent Molluscan Fisheries. Substantial information has been developed concerning the biology and stocks for oysters and hard clams; however, relatively little attention has been focused on other commercially exploited molluscan species including the whelks and ark clams. Life history, growth characteristics, and especially recruitment information is essential for management of developing fisheries. In addition, a program to acquire fisheries independent stock assessment data should be implemented and integrated with fisheries dependent information.

d. Baylor Ground Reexamination

Restoration of oyster production in the Commonwealth to former levels is strongly dependent upon availability of suitable bottom substrate for oyster settlement and growth. The original surveys of Baylor encompassed such grounds within the waters of the Commonwealth and set them aside for public use. The noted decline in oyster resources over the past three decades in the higher salinity regions of the Bay and tributaries has resulted in very substantial portions of the designated Baylor bottoms devoid of oysters. Under these conditions silt accumulation and eventual burial of the shell substrate results and the bottom becomes functionally useless for oyster settlement and growth. Rehabilitation can be effected in a limited number of cases through surficial application of suitable substrate (optimally shell, but other substrates should not be excluded from consideration). Assurance of maintenance suitable for settlement is only attained with the presence of healthy oyster populations. The question therefore arises as to the current status of Baylor bottom with respect to immediate utility as oyster bottom and/or the need to rehabilitate degraded or buried substrate at sites of former production. The rehabilitation option must be pursued through a series of questions including the quantitative need for rehabilitation (can seed be supplied at a rate commensurate with substrate disease losses?), and costs associated with restoration. While stock assessment and other activities described elsewhere in this document address the current and immediate future of the oyster, the current and immediate future of the substrates remains unaddressed to this juncture. A resurvey of the Baylor bottom for the purposes described is recommended.

Timeline: The resurvey effort will require three years, using acoustical scanning methods and associated bottom sampling.

2. Oyster Diseases

a. Monitoring. Monitoring for *H. nelsoni* (MSX) and *P. marinus* (Dermo) should be continued throughout the ten-year period. The oyster disease monitoring program provides information for resource managers, industry members and scientists on the annual abundance and distribution of both diseases. As noted earlier, information is provided in the form of an annual report published in February of each year. The report presents results of all sample analyses and also discusses results in relation to temperature and salinity patterns for the current year and the previous four years. Disease abundance and distribution data have been critical in decisions related to shell transplant repletion efforts, where to obtain uninfected seed oysters, where to plant seed oysters and when to harvest to minimize losses from disease. In addition, the monitoring program has provided critical information for understanding the relationship between environmental factors and distribution and abundance of both pathogens and, thus, has increased predictive capability as environmental conditions change. It is absolutely imperative that disease monitoring efforts be continued, but some changes in the present program are warranted and additional changes may be needed if the oyster resource begins to recover. The following program should be implemented:

i. **Continue monthly sampling at four stations in the James River—Wreck Shoal, Point of Shoals, Horsehead Rock and Deepwater Shoal.** These oyster rocks represent the only locations in Virginia with sufficient oysters for monthly sampling and they are also the major sources of seed oysters for private planters. It is important to monitor disease status on these rocks because it is critical that seed oysters infected with *P. marinus* not be moved from the James River. In addition, these oyster rocks are located along a strong salinity gradient and they provide important data on the effect of salinity changes on dynamics of *P. marinus*. In addition, emplacement of a permanent salinity monitoring station at Wreck Shoal to obtain continuous salinity data for correlation with pathogen abundance is strongly recommended in order to trace infection levels with salinity. Monthly measurements do not provide the needed resolution .

ii. **Continue spring and fall monitoring at selected oysters rocks throughout Virginia.** These samples are presently collected in conjunction with ongoing stock assessment surveys, and they provide a good picture of the Bay-wide distribution and abundance of both *H. nelsoni* and *P. marinus*. The fall survey is especially important because both pathogens are near maximum abundance at this time and the samples provide a good indication of the severity of the diseases during that year.

iii. **Expand sampling to include late summer/fall samples of native oysters from the seaside of the Eastern Shore and also from aquaculture grow-out areas.** Seaside oysters are becoming an increasingly important component of the industry and little information is available about pathogen abundance in grow-out areas. As aquaculture develops, it will be important to develop a database on pathogen abundance at various potential or actual grow-out sites.

iv. **Expand sampling to include monitoring of hard clam parasites once each year during summer from natural populations and as mortality occurs in cultured clams.**

v. **Develop GIS mapping capability to facilitate preparation of pathogen abundance maps for incorporation with other parameters to identify optimal grow-out areas and potential user conflict areas.**

b. **Research**. Support of a continuous research effort on oyster diseases is imperative if effective mitigating measures or management strategies to avoid losses from disease are to be developed. Although one or both pathogens may abate for periods ranging from a few months to more than a year, experience has shown that when favorable environmental conditions return the pathogens will rapidly increase in abundance and cause oyster mortality. This is especially true now that *P. marinus* is present on all oyster beds in Chesapeake Bay.

An oyster disease research plan should have as its goal a sustained increase in the oyster harvest from the natural fishery and a viable aquaculture industry. To accomplish these goals it will be necessary to accomplish one or more of the following: 1) develop (or identify) an oyster that can survive in the continuous presence of both *H. nelsoni* and *P. marinus*, 2) develop effective disease control measures applicable to aquaculture, 3) develop effective disease avoidance strategies for aquaculture, and 4) develop effective management strategies to preserve the oyster resource and increase harvest from the public and private on-bottom fishery in the continuous presence of disease. Research projects should be directed, ultimately, toward these goals. The following research projects are proposed as being the most critical at the present time; they are listed in order of importance.

i. **Develop a disease resistant native oyster through a cooperative, regional, selective breeding program.** A selective breeding program has been underway at VIMS since 1987 with funding from the Sea Grant College Program until 1992. As discussed earlier in this document, a number of strains of oysters have been evaluated and discarded because of high mortality. Presently, three strains are being evaluated, and one strain, third generation (F_3) Delaware Bay native oysters, is showing particular promise. The Delaware Bay strain reached market size in 18 months with mortality less than 15% in the presence of high pressure from both *H. nelsoni* and *P. marinus*. After two summers of exposure mortality in this group is 50%, much less than mortality in the other two groups under evaluation.

   The NOAA Oyster Disease Research Program has funded the first year (FY96) of a regional selective breeding program involving Rutgers University, the University of Maryland and VIMS. The 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. Continued federal funding for this effort is in jeopardy. The Commonwealth must view this effort as a priority.

   Disease resistant oysters will be used to promote oyster aquaculture as they will be most useful in an aquaculture situation. Resistant oysters will allow culture operations in the continuous presence of both diseases and will permit aquaculture throughout the lower Chesapeake Bay wherever growing conditions are appropriate. Resistant oysters cannot be expected to repopulate public beds, because the genetic basis for the resistance will be diluted by hybridization with disease-susceptible oysters from natural populations.

   Timeline: The first year will be devoted to spawning pure lines of the Rutgers and VIMS resistant strains and one hybrid cross between the two lines. Spat

will be deployed in three areas—lower Delaware Bay, upper Chesapeake Bay (MD) and lower Chesapeake Bay (VA). Oysters will be placed in grow-out conditions actually in use in the various regions and will be monitored for two or three years depending on mortality. In Virginia, four sites will be utilized. Survivors will be returned to the hatchery and spawned to produce a second generation that will be deployed at the same sites for an additional two to three years. Broodstock will then be released to industry if disease susceptibility has decreased significantly in any of the strains.

ii. **Elucidate the life cycle of *Haplosporidium nelsoni* (MSX) using PCR technology.** To improve predictive capabilities for disease abundance and distribution, disease avoidance strategies for aquaculture and other management strategies, it is critical to gain as complete an understanding as possible of parasite biology and ecology. In addition, experience suggests unanticipated control measures may become evident with increased knowledge.

An understanding of the complete life cycle of *H. nelsoni* is one of the critical gaps in the knowledge of the biology of this pathogen and this lack of knowledge has greatly hindered research progress. All efforts to infect oysters with *H. nelsoni* in the laboratory have failed. To conduct controlled experiments on a wide variety of topics, it is essential that the life cycle be understood. This inability to conduct controlled experiments has greatly hindered research on host/parasite interactions, control methods, and pathogenicity to name just a few areas. In addition, the predictive capability is limited because the life cycle stages of the parasite and its habitat or environmental requirements are not known. Thus, it is difficult to propose effective disease avoidance strategies for aquaculture or management strategies for the public fishery.

Repeated failure of direct transmission experiments suggests that the parasite is not transmitted directly from oyster to oyster, but requires a host other than oysters to complete its life cycle. Other evidence for an intermediate host is the continuing high abundance of *H. nelsoni* in the lower Chesapeake Bay even though oyster abundance is greatly reduced. The number of organisms that could potentially serve as the intermediate host for *H. nelsoni* is extremely high, and previous attempts to identify the host have not been successful. However, the molecular tools developed at VIMS coupled with the use of PCR technology provide an extremely sensitive, rapid screening technique that will greatly increase the probability of identifying the intermediate host.

Elucidation of the life cycle of *H. nelsoni* will not assure a method for controlling the parasite, but successful control methods cannot be developed without knowing the life cycle. Knowledge of the life cycle will greatly enhance the ability to explain and predict how the abundance and distribution of the parasite change with changing environmental conditions. It will also

enhance development of effective disease avoidance and management strategies. At present, elucidation of the life cycle is the most important research need for *H. nelsoni.*

Timeline: Genomic DNA from potential intermediate hosts will be screened using the *H. nelsoni*-specific primers in the PCR reaction continuously throughout the year until the intermediate host is identified. This may take up to three years. Once the intermediate host is identified, the DNA probe specific for *H. nelsoni* will be used to determine the location of the parasite within the intermediate host. The morphology and developmental cycle of the life cycle stage in the intermediate host will be determined. Spores of *H. nelsoni* from oyster spat will be fed to intermediate host individuals to verify transmission from oyster to intermediate host. The cell type that is the end product of parasite development in the intermediate host will be exposed to uninfected oysters to verify transmission from intermediate host to oyster. These studies will take an additional three years.

iii. ***C. gigas/C. virginica* comparison to determine why *C. gigas* is not susceptible to either *H. nelsoni* or *P. marinus*.** It has recently been shown at VIMS that *H. nelsoni* and *P. marinus* are pathogenic in *Crassostrea virginica*, the native eastern oyster, but not in *Crassostrea gigas*, the Pacific oyster. These data suggest some innovative studies to elucidate basic mechanisms of pathogenicity. It is not clear whether the Pacific oyster is more resistant (active defense mechanisms are involved) or less susceptible (the pathogen cannot live in *C. gigas* for reasons that are not defense mechanism-related). Characterization of hemolymph composition of the two oyster species, concurrent experiments to culture *P. marinus* in the presence of *C. gigas* and *C. virginica* hemolymph or tissue extracts, and characterization of specific defense-related capabilities may shed some light on the relative susceptibility/resistance of the two host species. Of particular importance are identification of the virulence factors or mechanisms of pathogenicity for both *H. nelsoni* and *P. marinus* and determination of how *C. gigas* evades or counteracts virulence mechanisms of the pathogens. Increased knowledge in these areas may lead to control methods for both pathogens.

Efforts should be directed to understanding the mechanisms by which *P. marinus* and *H. nelsoni* invade susceptible oysters, survive the host/parasite interaction and cause a generalized infection. In particular, the following studies are needed:

1. Determine the role of extracellular proteins secreted by *P. marinus* cells. Some of these proteins were identified by VIMS researchers as proteolytic enzymes that are capable of lysing a wide variety of proteins including oyster hemolymph. Therefore, there is a need to determine the exact target of these protozoal proteases, the role of these proteins in counteracting

host defense mechanisms, and their importance for parasite nutrition and survival within infected oysters.

Timeline: The first three years will be devoted to characterization of *P. marinus* proteases and to determination of their target compounds and their pathogenicity in oysters. Subsequent studies will focus on the role of these proteases in counteracting host defense mechanisms.

2. Determine how *P. marinus* cells evade intracellular killing inside phagocytic oyster blood cells. *P. marinus* cells are recognized as foreign by oyster blood cells and they are readily phagocytosed. However, there appears to be only limited intracellular killing (if any) and the infection level is not diminished, at least during summer and fall. The failure of oyster hemocytes to produce reactive oxygen intermediates (ROI) suggests that intracellular killing of *P. marinus*, if it occurs at all, may not be mediated by toxic oxygen metabolites and/or that certain evasive mechanisms may exist in *P. marinus*. In addition, recent studies at VIMS discovered that live *P. marinus* and extracellular products from this parasite are able to suppress ROI production by hemocytes. Significantly higher acid phosphatase (AP) activity exists in the parasite than in the host hemocytes and plasma. Acid phosphatase is an enzyme hypothesized to be responsible for blocking host superoxide production in the molluscan parasite, *Bonamia ostreae*, and the human parasite, *Leishmania donovani*. Therefore, it is important to determine whether *P. marinus* possess antioxidant enzymes which scavenge or suppress the host superoxide/reactive oxygen intermediate production.

Timeline: Experiments will be conducted to determine whether *P. marinus* has antioxidant enzymes other than AP and the role of the antioxidant enzymes in the pathogenicity of the parasite. Acid phosphatase and other antioxidant enzymes will be isolated and purified, their specificity and effect on chemiluminescence suppression/inhibition will be characterized *in vitro*. The effect of environmental temperature and salinity on *P. marinus*' AP and other antioxidant enzymes will be determined. Drugs and natural inhibitors from other molluscan species will be identified in particular to target the parasite's antioxidant enzymes. Four to five years are estimated to be required to accomplish the above activities.

3. Oyster serum molecules (defensins) have been hypothesized to play a role in defense against *P. marinus*, but no mechanism has been elucidated and the role of these putative defensins remains speculative. Three general types of molecules could be protective: 1) lectin-like molecules that bind specifically to target molecules on the parasite surface, 2) non-specific enzymatic molecules such as lysozyme or oyster-derived proteases, and 3) defensins, such as anti-proteases, that interfere with parasite virulence factors. Definitive demonstration of the prophylactic capabilities of oyster defensins requires their

isolation, purification and ultimately either their specific exposure to the disease agent or graded introduction into susceptible hosts for demonstration of prophylaxis. These studies will use both *C. gigas* and *C. virginica*. Once candidate defensins are identified, monoclonal antibodies will be produced to the molecules. This will initially serve a two-fold purpose: 1) production of immunoadsorbents for the large-scale and rapid purification of the defensins and 2) conclusive experiments demonstrating that removal of these molecules exacerbates the disease process. In the latter case, monoclonal antibodies will be injected prior to or simultaneously with the parasites. The antibodies will block or neutralize the host defense molecules resulting in a much more rapid proliferation of the parasite.

Timeline: It is anticipated that within the first four years the critical defensin molecules will be identified and characterized by the above procedures. In the following years the monoclonal antibodies will be produced and used in studies to determine how to promote the expression of prophylactic quantities of these molecules. A better understanding of the role of defense mechanisms may lead to methods for enhancing defense mechanisms or otherwise controlling infections.

iv. **Development of chemotherapeutants for both *H. nelsoni* and *P. marinus*.** Although chemotherapy is impractical as a disease control method on public oyster beds in Chesapeake Bay, it may be useful in aquaculture applications where groups of oysters in trays can be bath treated. A number of anti-coccidial compounds have been assayed for control of *P. marinus*, but no effective compound has been found. Additional compounds should be evaluated, emphasizing those of known antiprotozoal efficacy and trials should continue until a suitable drug is identified. Possible antiprotozoal candidates are suramin, pentamidine, pentostam, nifurtimox, melarsoprol, metronidazole enzymes, and lipolytic enzymes.

Timeline: Trials will continue over the ten year period until a suitable compound is identified.

v. **Determine the role of environmental factors in disease dynamics for both *H. nelsoni* and *P. marinus*.** Substantial information has been generated at VIMS and elsewhere on the effect of temperature and salinity on abundance, distribution, infectivity and pathogenicity of both pathogens. Much of the information has been obtained from field observations, although some experimental work has been accomplished, usually addressing salinity or temperature effects alone. Carefully planned laboratory experiments that evaluate the combined effects of temperature and salinity on disease development or regression are needed for a full understanding of the role of the environment in disease dynamics. Now that *P. marinus* is permanently established in low salinity areas, especially the upper James River, it is

important to determine if transmission between oysters is occurring in these regions. Understanding the role of environmental factors in disease dynamics is essential for prediction of changes in parasite abundance as environmental conditions fluctuate and development of disease avoidance and management strategies.

Timeline: Four years will be devoted to laboratory experiments designed to assess the combined effects of various winter temperature and salinity combinations on subsequent development of *P. marinus* infections, and to field experiments in the upper James River using sentinel oysters to determine infection acquisition. For the laboratory experiments, critical combinations of warm and cold, and wet and dry winters will be evaluated.

3. Ecosystem Function and Habitat

a. Oyster Reef Structure and Function. There has long been recognition of the fact that oysters were, and probably still are, keystone organisms in the bay system—filtering primary production, forming food for organisms in higher trophic levels, and creating a physical habitat that is used by a multitude of species either in their early life history (e.g. juvenile fish) or throughout much of their adult life (e.g. predatory crabs). As well, it is accepted that oysters formed three dimensional structures in normal situations. Thus, the two above noted premises are accepted in the scientific community. How did the reefs function in this connection? There are large and important gaps in understanding because most of the habitat was ravaged before systematic study. Restoration of oysters as part of habitat and ecosystem rehabilitation is now an accepted goal, and significant federal funds have already been committed to this end in reef building programs. While general concepts serve to guide such activity, there is a decided lack of knowledge of the nature of specific interactions in reef systems, whether they be behind the barrier marshes of the Eastern Shore or constructed on historical footprints of formerly productive intertidal reefs in the Chesapeake tributaries. Given that investment in environmental rehabilitation will continue, and that the sums of money involved will be very substantial in this cumulative effort, it is sensible to insure these actions are based on sound scientific knowledge. Limited studies on constructed reefs in the Piankatank River have already altered current concepts of recruitment and survival dynamics of early life history stages of oysters. Settlement at or near the mid tide level can be substantial—a situation not considered in current repletion practices involving application of monolayers of shell over wide areas. Small scale processes are very important, and settlement below the reef surface provides considerable refuge from predators and physical stress. A knowledge base of recruitment dynamics in three dimensional structures needs to be secured for not only the oysters but also the associated crab and finfish communities. Observations of finfish activity at the Piankatank site show it to be a thriving community for small fishes that form a major portion of the food resource for commercially and recreationally important finfish such as spotted trout and striped

bass. In short, oyster habitat rehabilitation has beneficial effects on crab and finfish populations in addition to oysters, and the multi-species impact is probably much more significant than immediately evident. Finally, earlier discussions concerning current standing stock and size distribution of oysters in the James River (see section III.F.1) underscore the need to protect some segment of the larger oysters to optimize egg production. In addition, large oysters are efficient filtering agents. The development of reef communities as broodstock sanctuaries must be a priority goal, but it can only work in areas where disease impacts are minimal. This will, unfortunately, place them in regions of commercial oyster exploitation, but this cannot be avoided. Reasonably well-developed models of oyster egg production, recruitment and growth on the current two dimensional submerged reef systems in the James have been developed. These need to be extended to evaluate three dimensional, rehabilitated reefs against two dimensional models to assist further rehabilitation efforts.

Historical oyster reefs consisted of live oysters in the surface region overlying oyster shell resulting from the vertical growth of the structure. Oyster shell for reef construction is in short supply. This immediately raises the question of alternative materials for reef construction such that they may be either capped with shell, or themselves serve as settlement substrate. A penetrating analysis and study is needed to address alternative materials.

Timeline: Given that the construction and monitoring of ecosystem function requires several years at each site, this effort is envisioned to be ongoing throughout and beyond the ten year period.

b. Application of Hydrodynamic Models in Habitat Planning and Utilization. In recent decades substantial advances have been made in development and application of three-dimensional hydrodynamic models for depiction of flow fields and salinity distributions in the Bay and tributaries. VIMS now has the capability for high spatial resolution, three dimensional hydrodynamic modeling. First configured for the James River, it is currently being adapted to the York River, to be followed by the Rappahannock River. Upon calibration, the models depict with reasonable fidelity the tidal flows, the residual (net) circulation arising from the mixing of fresh water and saltwater, and the distribution of salinity. Hydrodynamic models could also be useful in planning sites for oyster or habitat restoration. Predicted flow patterns and salinity distributions would serve as a guide for selection of sites partially favorable for larval dispersal and/or setting.

These tributary models should be utilized to depict, in map atlas form, salinity distributions for various stages of fresh water inflow. Such products could then be used by managers and fishing industry members to guide responses to normal versus abnormal conditions. In addition, other model products depicting net circulation patterns would be useful in planning locations for habitat restoration efforts.

Timeline: Both products, maps and salinity regimes as function of fresh water inflow and net circulation, should be accomplished within five years.

4. **Aquaculture**. While declines in wild shellfisheries within the Commonwealth continue, clear short-term solutions are not apparent. Conversely, shellfish aquaculture has experienced a rapid growth and is now a multi-million dollar industry. The full extent of the economic potential for shellfish aquaculture is unknown, but it is apparent that this industry has the capability of expanding into a very significant portion of the total seafood industry within Virginia. VIMS has played the central role in the development of shellfish aquaculture in Virginia and will need to continue to lead with active research, extension and educational programs if shellfish aquaculture is to reach its full potential.

   Currently, the vast majority of production in the shellfish aquaculture industry is that of the hard clam (*Mercenaria mercenaria*). Hatchery and grow-out capabilities are rapidly expanding, and because of the continual drop in the wild harvest, market potential is high. There appears to be few if any market constraints that would impact the increasing production of aquaculture hard clams for the next 3-5 years. However, many hold the belief that industry diversification is an essential evolutionary objective because in many case studies single species aquaculture has experienced disruptive events. As the wild harvests of many species of shellfish experience declines in the face of growing marketing opportunities, it is logical to predict the emergence of a species-diversified aquaculture industry in Virginia. In fact, this very process is happening in several states that have an established aquaculture industry. There is currently a modest commercial aquaculture activity for the American oyster (*Crassostrea virginica*) and, to a lesser extent, the bay scallop (*Argopecten irradians*). This activity should expand with the continued aid of research and outreach programs.

   In Virginia, several shellfish species are candidates for industry diversification. These include the soft shell clam (*Mya arenaria*), the surf clam (*Spisula solidissima*), the ribbed mussel (*Geukensia demissa*) and the blood ark (*Anadara ovalis*). The selection of these species are based on the fact that: (1) they are native to the area, (2) they offer the potential for both high and low salinity regimes, (3) they have established or potential marketability for both seed and market sized animals, and (4) hatchery and nursery technology is, by in large extant, compatible with existing research and commercial hatcheries. Strategically-directed research will be crucial to the development of practical and economically sound strategies for culturing and marketing other shellfish species.

   In order for VIMS to assist in significant further advances in the economic development of shellfish aquaculture, additional facilities are required. Current and projected shellfish aquaculture in Virginia indicates the Eastern Shore will be the centroid of growth due to environmental suitability. VIMS has serviced industry needs through very modest facilities at its Eastern Shore Laboratory at Wachapreague, and with a larger production facility at Gloucester Point. However, the Gloucester

Point facility has experienced recurrent problems due to salinity range, troublesome algal blooms, and other water quality problems.

Proposed is a capital outlay project for expansion of the facilities at Wachapreague, an Aquaculture Research Center. This facility would allow progression of effort to alternative species. The facility proposed is included in VIMS' 1998-2000 Capital Program. Acceleration to an earlier date would enable more rapid response to industry needs.

a. Research

i. **Improvements to hatchery and nursery techniques.** Some improvements in hatchery and nursery technology are needed in the area of alternative algal diets. As hatchery managers attempt to "close off" their systems from the external environment, more research will be needed to improve algal diets and produce energy (cost) efficient food sources. Ongoing efforts to improve culture practices will be required for all species and all stages of the culture operations. New algal isolates hold promise for improving larval growth rates within hatcheries, but will require further experimentation before they can be passed on to industry. Increasingly, private hatcheries are looking for methods to reduce the impacts of variable water quality on their nursery and hatchery operations. Additionally, naturally occurring toxic algal blooms have adversely affected operations of bivalve hatcheries. The development of predictive capabilities to identify conditions for toxic algal growth would reduce potential negative impacts. These needs place new emphasis on recent research into artificial diets and closed system aquaculture. Advances in these areas should be carefully evaluated as VIMS directs its research efforts for the next decade.

ii. **Broodstock selection and maintenance.** Conditioning and maintenance of broodstock to coincide with hatchery availability and capacity will be necessary. If the principal production species is hard clams, for example, then other species must be conditioned to spawn when facilities are available. Independent of the species being cultured, the shellfish culture industry will likely be dependent upon VIMS for the development and maintenance of vigorous broodstock lines. For hard clams this need has been apparent over the past three years because industry members have sought from VIMS fast growing stocks. Present efforts to develop selected stocks of oysters with resistance to MSX and acceptable tolerances to Dermo are likely to produce the broodstocks on which oyster culture will depend. This research and development role for broodstocks is similar to that filled by agricultural experiment stations for terrestrial agriculture.

iii. **Grow-out techniques.** Practical grow-out strategies must be developed and refined for traditional and alternative species. Experimentation is needed to test various grow-out strategies related to disease, predators and fouling

control. Working in close cooperation with industry, culture techniques must be continually improved in the framework of environmental and economic conditions. Again, the analogy to ongoing improvements in agriculture is appropriate.

b. **Education**. Most of the present hatchery managers within the state (indeed throughout the U.S. Atlantic and Gulf coasts) hold four-year degrees, and several have graduate training. Two of the present hatchery managers within the state are former VIMS hatchery managers. Clearly VIMS has a role to play in the training of professionals to fill the technically complicated job of running shellfish hatcheries. Through the development of graduate and advanced undergraduate courses in shellfish aquaculture, and the use of summer internship training programs and on-the-job training, VIMS should seek to expand its role in providing technical skills required for effective hatchery operations and concomitant industry expansion.

c. **Marketing and Economic Analyses**. With each species, as commercial production becomes a reality, marketing strategies must be examined to insure a reasonable potential for success. Product forms and transportation to markets must be examined in the face of necessary and reasonable profit margins for producers. Market analysis must be accompanied by the full understanding of business plans detailing cost, earnings and return on investment and labor. Outreach and educational programs must be developed to insure proper financial management and rewards in the face of changing market conditions and consumer expectations. Eventually, product promotion would become the responsibility of a marketing agency, industry cooperators or an individual company. These elements are essential to assist the diversification of the existing aquaculture industry in Virginia.

Timeline: These activities will continue throughout the ten-year period.

5. **Human and Naturally Occurring Pathogens**. Many of the human pathogen research concerns raised in Section III.E., although extremely important to public health and effective management of shellfish and recreational marine waters, consider issues that are national in scope and can only effectively proceed at this level from both technical and funding perspectives. Accordingly, the following list of research needs addresses particular issues that are important to the Commonwealth in terms of maintaining public confidence in the safety of bivalve shellfish products, which ultimately provides an atmosphere for continued economic development. It is assumed that the list below may be augmented in response to developments that may take place in shellfish aquaculture.

a. **Relaying of Aquaculture-Raised Oysters Through use of Floating Containers**. Considerable interest has surfaced in recent years promoting the use of floating containers for oyster culture in Virginia waters. Consequently, concerns have

arisen that oysters cultured in this manner to be sold for raw consumption are grown in waters classified appropriate to this purpose and that relaying (if required) conform to VMRC regulations. An analysis of relaying based on elimination of fecal coliform organisms in floating containers is currently being performed under VIMS guidance in concert with the Shellfish Division of the Virginia Department of Health. Upon completion of this study, it may be necessary to append existing VMRC regulations. Accordingly, educating the public and industry to these concerns will be necessary and should be accomplished through dissemination of a practical publication describing all aspects of the containerized relaying process. This document should justify the technical basis for relaying, the microbiological considerations involved, consider the physical mechanics of the relaying process in terms of container design, mortality reduction, container maintenance, and describe VMRC's regulatory responsibilities.

Timeline: Completion of VIMS evaluation of floating containerized relaying and production of the relaying manual can be completed in 1996.

Finally, these studies if slightly expanded could provide a significant benefit relative to concerns centered around viral pathogens and the inability of coliform indicators to reflect viral presence. As previously noted viral pathogens account for the overwhelming majority of shellfish-borne gastroenteritis in this country. As yet there are few if any data illustrating elimination of viral indicators (FRNA coliphage) or the most common viral pathogen, Norwalk type virus. Accordingly, within the context of these container studies, it would be advantageous to naturally contaminate and follow elimination under conditions of floating container relaying. This may be of some significance because floating container grow-out areas may be in waters classified as restricted, and viral contamination through runoff is a possibility. The program will require natural contamination of shellfish proximate to a source of FRNA coliphage followed by relaying to a clean area and analysis of shellfish FRNA coliphage concentrations at 0, 7, and 14 day relay intervals.

Timeline: Completion of the study will require one year to complete.

b. ***Vibrio vulnificus*** **Issues; "Dip and Ship" and Ecological Concerns**. The focus of this program is to assist industry with studies related to the fate of *Vibrio vulnificus* in Virginia waters and shellfish wet stored or relaid in Virginia waters. Transferring molluscan shellfish from the Gulf of Mexico to the Chesapeake Bay for economic purposes presents two potentially adverse consequences that relate to *V. vulnificus*. In the first case shellfish shucked and packed in Virginia, and therefore perceived as Virginia oysters, may be introduced into interstate commerce and cause this unfortunate disease.

The second concern relates to the introduction of Gulf of Mexico shellfish to Virginia waters prior to harvesting and shipping to other states. Regulations now

require that oysters from the Gulf be resident in Virginia waters for six months before they "become" Virginia oysters. This law was conceived before *V. vulnificus* was identified as a human health problem. Importation of Gulf oysters, which can contain high densities of *V. vulnificus* when harvested during the warmer months, could be a source of undesirable strains to the Chesapeake Bay. Conditions in the Chesapeake may favor multiplication of the pathogen in stressed oysters. Establishment of Gulf strains could lead to health concerns and have unfortunate consequences for an already marginal shellfish industry. On the other hand it is possible that Gulf strains would not be as persistent as indigenous strains or other factors such as predation and survival would preclude this problem. If importation and reselling of Gulf oysters is allowed there is a need to study aspects of the ecology and persistence of strains of *V. vulnificus* obtained from oysters and water in the Gulf of Mexico in the waters of Chesapeake Bay.

Various investigators have examined depuration or controlled purification as a possible means to reduce levels of *V. vulnificus* in Gulf of Mexico oysters. In general, investigators have found depuration ineffective, noting a lack of elimination and even enhanced multiplication and persistence of naturally occurring *V. vulnificus* at 23°C in oysters (*C. virginica*) held in depuration tanks. There is little information available on the use of long term relaying or transplanting in non-indigenous waters on the fate of *V. vulnificus* in oysters harvested from Gulf states. There have been no studies of this nature in Virginia shellfish growing waters. Studies of this kind are therefore necessary to make decisions allowing importation of Gulf of Mexico oysters to Virginia waters and to assess the efficacy of relaying on the reduction of *V. vulnificus* levels. VIMS is now assisting the shellfish industry through monitoring levels of *V. vulnificus* in Gulf of Mexico shellfish relaid to the Eastern Shore. Results of this study should provide basic information concerning the purification process and may lead to studies designed to promote relaying as a "polishing" process to reduce *V. vulnificus* levels below those commonly encountered in Gulf shellfish received through interstate shipment. An approach considered is the use of relaying into colder northern growing waters to achieve significant reductions in the levels of *V. vulnificus*. Basic questions related to the survival of Gulf of Mexico *V. vulnificus* strains in Virginia waters are also important to address. VIMS microbiologists have developed a research plan to address *V. vulnificus* concerns in Virginia waters. The purpose of this program is to (a) develop expertise required for detection and enumeration of *Vibrio vulnificus* in shellfish and waters of the Chesapeake Bay, (b) to apply this expertise to problems related to the introduction of potentially virulent non-indigenous *V. vulnificus* strains to Virginia waters of the Chesapeake Bay, (c) to provide information to appropriate regulatory and industry representatives in an advisory capacity to further the continued production of safe shellfish from Virginia, and (d) to contribute to the body of technical information describing the ecology of *V. vulnificus*.

The positive nature of the preliminary experiment suggests that pursuit of this work on a more comprehensive scale with harvesting and analysis controlled by VIMS is an appropriate research goal. To this end a dialog has been initiated with the FDA laboratory in Dauphin Island, Alabama, as a source of Gulf oysters. Furthermore, the laboratory has agreed to analyze the oysters and water samples at harvest. A study is proposed to examine the effects of relaying of Gulf oysters into Virginia waters on concentrations of *V. vulnificus* over a range of seasons and to perform persistence experiments to evaluate the ability of Gulf strains of the organism to survive in Chesapeake Bay waters under similar seasonal conditions.

Timeline: Completion of the study will require two years.

c. **Develop an Integrated Approach to Sampling Shellfish Growing Waters.** Conventional water quality sampling is accomplished through discrete or grab sampling. This means a finite sample of small volume is collected over a very short time interval, generally measured in seconds. In the case of shellfish growing waters, a comparatively small number of samples/year are collected to characterize a given location. Given the variability of indicator microorganisms in dynamic estuarine systems on both temporal and spatial scales, and the poor to moderate analytical precision of viable count methods, sampling approaches that integrate large volumes of water over large time intervals (days) would be less susceptible to variability as well as provide increased analytical sensitivity because of larger material flow. VIMS researchers seek to develop and validate new sampling devices, analytical methods and technical requirements that would permit extended integrated sampling.

Timeline: Completion of the study will require two years.

d. **Develop an Approach to Empirically Establish the Dimensions of STP Buffer Zones.** Discharges from sewage treatment plants (STP) represent the largest potential inputs of pathogenic human enteroviruses to shellfish growing waters. Municipal treatment facilities that discharge into growing areas will always pose a health risk because of the possibility of plant breakdown or treatment that is ineffective against enteroviruses. When discharging near a shellfish growing area such as in the James River, contiguous buffer zones must be established to prevent harvesting shellfish, and presumably to provide for dilution of effluent and time sufficient for accidents to be noted and implementation of administrative procedures for halting shellfish harvesting.

Buffer zones contiguous with STP discharges represent the largest proportion of harvest-limited acreage, ranging from 67 percent in the Northeast to 44 percent in the Middle Atlantic. Despite the significance of this closure type, the size and shape of buffer zones is usually not based on either physical processes or microbiological data, and certainly not on health risk. These facts and the inadequacies of the fecal coliform indicator, should provoke methods and

approaches designed to predict the distribution of the pollutant field from an STP outfall, and to verify this using field data. VIMS microbiologists have reported that FRNA coliphages appear to be discharged at relatively consistent and high densities from secondarily treated and chlorinated STP outfalls to marine waters. Using FRNA coliphages as a viral indicator, effluent fate could be evaluated and used to develop a method to determine buffer zone size on the basis of empirical observations and computer modeling. VIMS has performed field research to evaluate the use of FRNA coliphages for this purpose.

The availability of a 3-D computer model at VIMS provides an opportunity to evaluate its use as a tool to empirically establish buffer zones around STP outfalls based on a viral indicator. Work performed by VIMS at one STP system and supported by NOAA/Sea Grant lends validity to this approach. What is now required is fine tuning of the model to match field data and to extend use of the approach to other STP estuarine buffer zones. Successful demonstration of general applicability will lead to adoption of this approach, improve the safety of shellfish and thereby improve public confidence in bivalve shellfish as a food.

Timeline: A two-year effort is proposed.

6. Economic Assessments

a. Introduction. As the wild fisheries of Virginia and the nation become over or fully-exploited in the face of increasing consumer demand, aquaculture will become increasingly important in terms of supplying local, national, and international markets for selected mollusks. Aquaculture thus offers substantive opportunities for economic development in many coastal communities of Virginia. Without an appropriate long-run plan to deal with the economic and marketing issues, development of aquaculture may be haphazard and fail to maximize its full potential.

It is essential that a long-range economic research agenda for aquacultured and wild molluscan species in Virginia be developed since market forces are not mutually exclusive. Emphasis of this agenda is on understanding how the wild and cultured products contribute to coastal economies and how to mitigate competition between the two sources of product, discovering opportunities for expanding either production or increasing value added, determining production methods and scales of operation that maximize profits to harvesters and culture producers, gaining market share, defining the costs and benefits of regulatory compliance, and expanding markets throughout the world.

b. Essential Data. An essential economic component of the ten-year plan is the routine collection of necessary social and economic data or information. Without basic data on such things as production activities, costs of production and marketing, economic returns, prices, and community structure, it will not be

possible to maximize the joint economic opportunities for culture and wild fisheries production. Successful expansion of aquaculture and wild-capture fishery opportunities will require knowledge of bottlenecks or limitations to production and marketing.

Three types of information collection activities are recommended. First, data from secondary sources will be collected. These types of data include information from trade magazines and newspapers and various fishery and development agencies. Second, it is proposed that VIMS staff work with culture operators and producers, wholesalers and dealers, and restaurants and retailers to collect primary data about costs, earnings, prices, and market opportunities. Third, it is recommended that VIMS staff work with culture operators and watermen to develop engineering information. This latter type of data collection permits VIMS to obtain information based on controlled experimentation (e.g., examine growth, survivability, and economic returns given different blends and volumes of food).

Another essential ingredient of the plan is routine monitoring of production activities and legislation that may limit culture and wild fishery production. Monitoring activities will involve mostly secondary sources but will also include VIMS' monitoring activities. Parameters of interest include water temperature, salinity, dissolved oxygen, U.S. and world market demand, pollution problems around the U.S. and world that affect the demand for mollusks, and local, state, and federal legislation that may limit production and sales activities of culture operators, watermen, wholesalers, dealers, shippers, exporters, importers, restaurants, and retail outlets. Information learned from these monitoring activities will be readily communicated to members of the seafood industry and state and community planners.

c. **Plan Elements.** In essence, the plan focuses on the product flow of cultured and wild-captured products. There are basically three sources of product: (1) local wild-capture fishery, (2) local cultured product, and (3) product imported from either foreign nations or other states of the United States. Production or supply levels of any given product depend on production costs, prices received or market demand, and regulations. All of these, however, are driven mostly by final user demand. Under some possibly very specific instances, it may be possible to create demand (e.g., advertising and flooding the market with local product at very low prices).

The research plan seeks to understand and explain the product flow. Within any given sector of the product flow, however, methods would be explored for improving production, adding value to products, expanding markets, reorganizing production scale and activities, and mitigating bottlenecks and limitations to production. The research plan will also explore the possibility of defining optimum scales of production and output levels from the culture and wild-capture producers.

Initially, hard clams (*Mercenaria mercenaria*), the American oyster (*Crassostrea virginica*), and the bay scallop (*Argopecten irradians*) will be emphasized. Regarding data collection and monitoring activities, it will be necessary, however, to include all species of fish and shellfish which may compete with the three major candidates; for example, what might happen to the demand for hard clams or quahogs if the supply of soft clams, oysters, or calico scallops increased? Presently, all that can be offered is an educated guess about the potential interactions in demand among the various species. If other states are also attempting to increase production for the same species Virginia is targeting for aquaculture, it may be to Virginia's advantage to focus on other shellfish species, particularly if Virginia has a competitive advantage in production or marketing. While the market for some shellfish is growing, it is not unbounded. It is important that the research plan attempt to estimate market choke levels—combinations of supply, demand, and prices at which consumer demand changes to a level that makes it unprofitable for Virginia culture producers and wild-capture harvesters.

Optimum scales of production will also be examined. That is, research will attempt to determine the size, production level, workforce, and use of other factors of production that maximize profit to culture operators. This will require extensive on-site analysis and mathematical programming.

The on-site studies will also be combined with financial feasibility analyses. That is, the economic returns and financial feasibility of alternative scales of production will be examined. As part of this long-run plan, financial analysis will be routinely prepared for present and potential culture operators. Similar analyses will be conducted for wild-fishery producers.

Another major area of investigation will be determining the potential economic returns and marketability of alternative product forms. For example, market saturation may occur for shellfish such as hard clams. Expansion and growth opportunities may become restricted to new product forms or value added forms.

Alternative marketing strategies will also be explored as an option for expanding markets. A considerable amount of market research has been done around the world on ways to increase market share or increase sales. For example, selling hard clams in onion bags with a cooking recipe on the bag has helped sales of hard clams. Panel testing and demonstration projects are also usually quite successful in expanding sales or generating interest in new product forms and different species. For example, VIMS' work with restaurants on the bay scallop could be expanded to ribbed mussels and different product lines. In addition, conjoint analysis will be explored as a method for determining optimum product forms and sizes of the various mollusks; the optimum is defined as the product form yielding the greatest profit to the producer.

The economics of polyculture, culturing of more than one species, and multiple product production, producing more than one product form of the same species, will also be examined. Emphasis will be on determining an optimum mix of species, products, output levels, and input usage; the optimum will focus on profit maximization to culture operators and capture operators. Thus far, there appears to have been little or no research that actually attempts to determine an optimum production plan for polyculture and multiproduct production.

Of particular concern to the Commonwealth is the benefit of expanding aquaculture production and the value of the wild fisheries for the state and coastal communities. Moreover, it is recommended that an input-output or economic impact model be developed for aquaculture. This analytical framework will permit state and local planners and agencies to understand how employment opportunities and tax revenues will change in response to fishery development and expansion activities. Important bottlenecks which limit production activities can be readily identified before they occur, and planners and producers can take steps to mitigate the potential problems. Because aquaculture is a relatively environmentally friendly industry, the input-output framework will permit state and local officials to evaluate the economic trade-offs between aquaculture, commercial fisheries, and industries that are less friendly to the environment.

The demand for various fish and shellfish must be regularly estimated and assessed for changes in patterns. This will be necessary to assess the financial feasibility of aquaculture products, and to better develop markets and new product forms. This work will also be necessary to inform present culture and wild-fish producers about potential market problems so that they may then take corrective action to avoid market problems. In addition, banks and other financial institutions often request this information when considering loans for businesses.

As aquaculture grows, a critical problem for producers will be regulatory compliance. Data must be collected and analyzed to determine the costs and benefits of regulatory compliance. For example, changes in water quality regulations may require culture operators to purchase expensive equipment or make changes in the ways culture product is raised and harvested. As a consequence, production or marketing costs may increase and make culture operations unprofitable. Changes in costs and profitability associated with new or changing regulations will be assessed. Information from these assessments will be communicated to regulatory agencies and industry.

The plan will also be concerned with monitoring production activities around the world. Numerous nations have the potential for aquaculture production or are presently producing cultured product. Many of the nations are less developed countries desperately in need of foreign currency, particularly U.S. dollars. Vietnam is one nation with substantial potential for aquaculture production of prawns, as is most of southeast Asia. As part of the overall research plan, nations

with the potential to produce competitive products or close substitutes will be identified and their culture production activities and scales will be assessed. This information will be made available to industry, banks, and state and local planners to better facilitate culture initiatives in Virginia.

The final component of the plan will be capital budgeting and risk assessment. Capital budgeting to a large extent will involve financial feasibility analysis. That is, an assessment of the profitability of investments in an activity whose returns are typically expected to extend beyond one year. Emphasis of this research will be to identify projects, species, and product forms with relatively short pay-back periods in which all costs are covered. This research will also, however, include an assessment of species, production activities, and product forms that have longer pay-back periods; this is necessary because the risks of short and long cost-recovery projects may be quite variable. For example, a project with a short pay-back period may have high risks of failure, while some other project may require several years before costs are recovered but the risks are quite low.

Risk assessment will be included as a routine analysis. For the most part, this will focus on several types of risk: (1) social risks such as possible changes in consumer tastes, attitudes, and preferences, (2) economic risks such as possible changes in prices of inputs and output, the inflation rate, and other important economic variables, (3) marketing risks such as uncertainty in demand and timing of product flow and production, (4) production risks such as when to purchase seed or stocking densities, (5) financial risks such as changes in the supply of funds, (6) physical risks such as weather and facility problems, and (7) governmental regulation.

In response to concerns about risk assessment, options for risk management will be investigated. Managing risks is likely to be one of the most important steps necessary for advancing the aquaculture industry. Risk management will basically involve seven possible strategies: (1) diversification or the production of more than one species or product, (2) continuous or sequential marketing or timing the production and sales of product, (3) formal insurance against losses, (4) future market and production contracts which specify prices and quantities for buyers and sellers and time of delivery, (5) government programs such as financial assistance programs, (6) third party equity capital or the use of outside equity capital which is a form of risk sharing or pooling, and (7) safety devices such as automation of warning signals.

Timeline: The tasks to be completed over the ten-year period are prioritized as follows:

i. **Data collection, monitoring, and analysis**. The collection of data and monitoring of important economic variables is of the highest priority and

absolutely critical to the successful and efficient development of aquaculture within the state. This will be on-going over the entire ten-year period.

ii. **Development of engineering and optimization models to assess the feasibility of proposed aquaculture projects**. Utilizing data obtained from the collection and monitoring program, engineering and optimization models will be developed and used to assess the likely financial outcomes of various types and scales or sizes of aquaculture operations. This will require concentrated effort over the first three years. After the models are completed, it will be possible to assess the economic feasibility of any potential scale or size of aquaculture operations.

iii. **Development of market analyses and assessments of market opportunities**. Market models of demand and supply will be developed and used to assess how much the market can sustain of a given price and quantity for given regions of the U.S. and international markets. As part of this activity, however, routine monitoring of production and market activities in other states and countries will be necessary. This activity will be concentrated in the first two years. Monitoring activities, however, will continue throughout the ten-year period.

iv. **Development of a community program to develop small scale aquaculture businesses in coastal areas**. This is a very high priority even though it is listed as fourth. Without the development of a community program to develop small scale aquaculture, it will be extremely difficult to promote aquaculture as an economic development tool. This activity will be intense during the initial phases but extend as consultation service throughout the period.

v. **Development of financial outreach program to determine sources and availability of capital funding**. This activity will be intense during the first two years and thereafter available as a consultative service.

vi. **Assessments of the interactions between the potential aquacultural production activities and the wild fishery sectors**. This is a critical component of the aquaculture program. If natural stocks recover and allow commercial watermen to have competitive advantage over culture operators, culture activities will experience severe economic problems. This component will be ongoing throughout the plan period.

7. **Communication of Results and Outreach**. Communications and outreach associated with the VIMS Ten-Year Strategic Plan for Shellfish Research and Monitoring must include activities associated with both the wild fishery and aquaculture components. Clearly both are closely related and exhibit certain interdependencies. Information and research advances pertaining to oyster diseases are essential to both the wild fishery and aquaculture interests. In addition, the growth

and diversification of the shellfish aquaculture industry is directly related to the state of the wild fisheries and market conditions. Both components are impacted by regulatory constraints associated with leased bottoms and human pathogens.

The outreach programs associated with shellfish aquaculture will be more clearly defined and articulated. Aquaculture advisory specialists must be knowledgeable in a wide array of hatchery and grow-out technologies and must be able to apply research results to immediate problems. The success of a hatchery in a given season may depend upon rapidly diagnosing and treating problems related to microbes, changes in water quality, and the introduction of harmful contaminants. As important, an effective outreach program must be able to close the "technology transfer loop" and identify new research needs and priorities.

Information gathered through the oyster disease monitoring program has provided and will continue to provide important information on disease prevalence to managers and industry. This service should continue to be offered to both the wild harvest and aquaculture industry because vital business and management decisions must be made in the context of oyster disease prevalence.

Mechanisms to deliver or communicate information are varied. Information transfer through one-on-one contact and educational outreach (workshops, classes, etc.) are common tools used by outreach personnel. These personal contacts are important in providing a sense of reality to the process. Clients become familiar with outreach staff and gain a sense of confidence in the individual and in the information they convey. It will be important to have a cadre of trained professionals skilled in the practice of extension and knowledgeable about the subject matter.

Written communications are another important tool for information transfer. Here, is it important for the scientist, outreach specialist and a skilled communicator to cooperate in developing a technology or information transfer instrument. It would be important to develop a regular (two to three times per year) newsletter or similar device targeted to a specified client base associated with the interests of the shellfish industry. Information pertaining to the progress or elements within the Strategic Plan, workshops, available publications and other pertinent information could easily be delivered at a modest cost.

Some of VIMS' most successful information transfer projects have been the development and delivery of "how to" manuals. The importance of the "Manual for Growing the Hard Clam (*Mercenaria mercenaria*)" and the "Manual for Handling and Shedding Blue Crabs (*Callinectes sapidus*)" can not be understated nor underestimated. Opportunities for similar manuals exist and are needed for oysters. In addition, a revised version of the hard clam manual is in order. As important, communications with the general public in the form of public relations efforts, and to Virginia's Legislature in the form of briefing documents, such as a biennial report focusing on the success and needs of the shellfish program, should be developed.

Current monitoring and assessment programs and the proposed enhancement of those programs generate information of enormous value to managers and members of the industry and to members of the science community. These products need to be better integrated in order to improve the effectiveness of communication and to promote synthesis of the results. It is recommended that the Geographic Information System, ARC/INFO, currently in place at VIMS, be utilized. To the extent practicable, the format should be consistent with similar pursuits in the state of Maryland.

B. Timeline and Resources Required

1. **Current Funding Status.** The Commonwealth currently provides principal support for monitoring oyster diseases, oyster spatfall and post-settlement evaluation, and for hatchery and outreach activities in support of aquaculture. The Commonwealth also provides to VIMS partial funding for current activities in oyster stock assessment, oyster disease research, habitat and ecosystem studies, human pathogen studies, economic assessments, and communications and outreach. Very substantial funding is obtained through extramural grants and contracts. For illustration, the expenditures for FY96 are expected to be approximately as follows:

Activity	General Funds	Grants/ Contracts
Oyster Stock Assessment	44,000	77,000
Oyster Disease Research	116,000	588,000
Habitat and Ecosystem Studies	42,000	166,000
Human Pathogen Studies	36,000	34,000
Economic Assessments	40,000	40,000
Communication/Outreach	70,000	150,000

2. **Extramural Funding Opportunities.** VIMS staff have been aggressive and successful in competing for extramural funds to support Commonwealth interests related to the native fisheries, shellfish aquaculture, and habitat restoration. Of the awarding agencies, various programs of the National Oceanic and Atmospheric Administration (NOAA, U.S. Department of Commerce) have provided most of the support. Those programs include the National Sea Grant Program, Oyster Disease Research Program, Chesapeake Bay Stock Assessment Program, and the Virginia Coastal Resource Management Program. Some funding has been very substantial; for example, the Oyster Disease Research Program has funded $2.2M to VIMS since the program's inception in 1989. The U.S. Environmental Protection Agency has supported, via the Chesapeake Bay Program, relevant efforts through the Chesapeake Bay Environmental Effects Program and the Habitat Restoration Program.

   VIMS staff will continue aggressive pursuit of extramural funds to support the activities identified. However, it is clear that those federal agencies most supportive in the past are likely to experience substantial budget reductions. Even with more aggressive pursuit, the yield is likely to decrease.

3. **Additional Resources Required**. The additional resources required to support the program elements, fully addressed in the report text, are arranged in Table 7. As well, the timeline for the various elements is shown. For purposes of comparison, the approximate expenditures for FY96 are shown for both General Funds (GF) and Grants/Contracts (G/C). It is important to understand that long-term monitoring programs are generally non-supportable via extramural funds. Federal sources such as the Chesapeake Bay Stock Assessment (CBSAC) Program may launch a new effort, but after proof of merit, they expect the state to sustain the program. There has also been reluctance on the part of federal agencies to fund studies involving intentional introduction of non-indigenous species. Finally, and least surprising, there are only very limited federal funds available, via peer review programs, for capital construction.

As indicated in Table 7, very significant resources will be required to accomplish the programs envisioned for the ten-year period. Some success in gaining extramural support can be assumed. However, substantial increases in General Fund support will also be required. As a guide to those needs, the following commentary briefly addresses function and purpose, and relative priority.

1. Native Fisheries

a. HIGH PRIORITY: Stock Assessment; $120,000 per year, 2.75 FTE, 10 years. This activity, currently funded by NOAA-CBSAC, must be considered as the essential foundation for management of the remaining oysters, and the hard clam. The current funding, limited to oyster assessment, ends August 1996. General fund support is vital for program continuation with modest expansion to include hard clams.

b. Monitor Oyster Spatfall/Post-Settlement; $53,000 per year, 1 FTE, 10 years. This monitoring program provides ongoing guidance to managers and industry members as to the levels of potential oyster spat recruitment and subsequent settlement success. The surveys are indispensable and modest expansion is required to include activities on the seaside of the Eastern Shore.

c. Baylor Ground Reexamination; $248,000, first year; $113,000 per year thereafter, 3 FTE, 3 years. The purpose is to assess substrate conditions with respect to suitability for oyster settlement. Very substantial areas of the Baylor grounds are devoid of oysters and siltation may have altered substrate.

2. Oyster Disease

a. HIGH PRIORITY: Monitoring Oyster Disease; $49,000, first year; $35,000 per year thereafter, 1 FTE, 10 years. This program provides information for resource managers, industry members and scientists on the annual abundance and distribution of both oyster diseases. Program expansion is needed to include the seaside of the Eastern Shore and aquaculture grow-out areas.

b. Oyster Disease Research. Of a broad spectrum of potentially important research, five topics have been identified as highest priority:

i. HIGH PRIORITY: Develop Disease Resistant Native Oyster; $160,000, first year, $130,000 per year, years 2 through 7, 2.5 FTE, 7 years. This program is vital to aquaculture interests in order to provide broodstocks which attain market size with low mortality. Substantial progress is being made through a cooperative, regional, selective breeding program. Federal funds supported the activity through 1992; since then the project has been maintained at a low level.

ii. HIGH PRIORITY: Determine Life Cycle of MSX; $47,000 per year, 1 FTE, 6 years. Understanding the life cycle is crucial to development of disease avoidance strategies and potential control measures. Due to the inability to infect oysters with MSX in the laboratory, all tests for resistance require in-field exposure, including tests on non-indigenous species.

iii. Comparison of *C. gigas/C. virginica*; $221,000 per year, 5 FTE, 7 years. The two endemic diseases are not pathogenic in *C. gigas*, but are in the native oyster. The objective is to determine the mechanisms by which the diseases invade susceptible oysters, survive the host/parasite interaction, and cause a generalized infection. Such knowledge may lead to control methods for both pathogens.

iv. Chemotherapeutants for Diseases; $35,000 per year, 1 FTE, 10 years. Although chemotherapy is impractical as a disease control method in public oyster beds, it could be useful in aquaculture applications. Compounds with known antiprotozoal efficacy should be tested until a suitable drug is identified.

v. Environmental Factors in Disease Dynamics; $37,000 per year, 1 FTE, 4 years. Understanding the environmental factors of salinity and temperature is essential for prediction of changes in parasite abundance as environmental conditions fluctuate.

3. HIGH PRIORITY: Ecosystem Function and Habitat; Oyster Reef Structure and Function; $45,000 per year, 1FTE, 7 years. Given that investment in habitat restoration will continue, it is essential that the benefits are understood and documented. Particularly important is determination of community structure of the trophic levels. Also important is evaluation of alternative substrate, given that shell material is in short supply.

4. HIGH PRIORITY: Aquaculture Support Programs

a. Broodstock Selection and Maintenance; $21,000 per year, 0.5 FTE, 10 years. Broodstock selection and maintenance provides critical support for the aquaculture industry. Currently maintained are stocks of hard clam, bay scallop and

some oyster species. This activity will increase, and a modest increase in resources will be required.

b. Aquaculture Research Center at the Eastern Shore Laboratory: Facility Construction in FY98-99 at $1.1M, with ongoing costs of $90,000 per year. A new facility in support of shellfish aquaculture is essential to assist in economic development of the growing industry, particularly to assist in diversification to shellfish species not currently cultured. Strategically-directed research will be critical to development of practical and economically sound strategies for culturing and marketing additional species. Current and projected shellfish aquaculture indicates the Eastern Shore will be the centroid of growth due to environmental suitability.

c. Hatchery and Nursery Technology; $15,000 per year, 0.5 FTE, 10 years. Practical hatchery techniques and grow-out strategies have been developed by VIMS and adopted by industry. These efforts are central to industry support and require modest expansion.

5. HIGH PRIORITY: Human and Natural Pathogens; $91,000 per year, 2 FTE, 4 years. Of the issues identified, some are national in scope and must be addressed at that scale. However, there are important local needs that should be addressed. Included in these are means to relay aquaculture stocks grown in marginal waters for depuration in fully approved waters. Another program of significance is investigation of the fate of the pathogen *Vibrio vulnificus* in Virginia waters when imported oysters are wet-stoned or relaid in Virginia waters.

6. HIGH PRIORITY: Economic Assessment; $113,000 per year, 2 FTE, 10 years. It is imperative that long-range economic research be undertaken for aquacultured and wild molluscan species since market forces are not mutually exclusive. Emphasis is on understanding how wild and cultured products contribute to coastal economies and how to mitigate competition between the two sources of product, and to define strategies to optimize Virginia's position in the regional, national, and international marketplace.

7. Communication of Results and Outreach; $77,000 per year, 2 FTE, 10 years. Communication of research findings and hands-on advice to industry is a central component to advances in both aquaculture and the native fisheries. In addition, there is an urgent need to better integrate research and monitoring results. To this end, utilization of a geographic information system, compatible with that in Maryland, is recommended.

8. HIGH PRIORITY: Non-Indigenous Species Research; $293,000, first year; $253,000 per year, second and third years; $240,000 per year, fourth and fifth years, 3 FTE, 5 years. As discussed in Part 2 of this report, a series of tests is proposed, including in-water testing of non-native species *C. gigas* and *C. rivularis*. The program of study is fundamental to provide the scientific framework for species selection and risk assessment associated with consideration of large-scale utilization of non-native species.

Table 7. TEN-YEAR PLAN FUNDING REQUIREMENTS

	Current Resources Applied State + G/C	1. FY97	2. FY98	3. FY99	4. FY00	5. FY01	6. FY02	7. FY03	8. FY04	9. FY05	10. FY06	TOTAL
1. Native Fisheries a. Stock Assessment	GF 44,000 1 FTE G/C 77,000 1 FTE	120,000 2.75 FTE	120,000	120,000	120,000	120,000	120,000	120,000	120,000	120,000	120,000	1,200,000
b. Monitoring Spatfall Post-Settlement	GF 65,000 1.35 FTE	68,000 1 FTE	53,000	53,000	53,000	53,000	53,000	53,000	53,000	53,000	53,000	545,000
c. Baylor Grounds Re-Survey				248,000 3 FTE								474,000
2. Oyster Disease a. Monitoring	GF 54,000 1.15 FTE	49,000 1 FTE	35,000	35,000	35,000	35,000	35,000	35,000	35,000	35,000	35,000	364,000
b. Research i. Selective Breeding Program	GF 115,627 2.3 FTE G/C 588,512	160,000 2.5 FTE	130,000	130,000	130,000	130,000	130,000	130,000				940,000
ii. Lifecycle (MSX)		47,000 1 FTE	47,000	47,000	47,000	47,000	47,000					282,000
iii. *C. Gigas/C. Virginica* Host-Parasite Interaction		221,000 5 FTE	221,000	221,000	221,000	221,000	221,000	221,000				1,547,000
iv. Chemothera-peutants for disease		35,000 1 FTE	35,000	35,000	35,000	35,000	35,000	35,000	35,000	35,000	35,000	350,000

	Current Resources Applied State + G/C	1. FY97	2. FY98	3. FY99	4. FY00	5. FY01	6. FY02	7. FY03	8. FY04	9. FY05	10. FY06	TOTAL
v. Environmental Factors in Disease Dynamics	(see previous cell entry)	37,000 1 FTE →	37,000	37,000	37,000							148,000
3. Ecosystem Function and Habitat	GF 42,000 0.5 FTE G/C 165,917	45,000 1 FTE →	45,000	45,000	45,000	45,000	45,000	45,000				315,000
4. Aquaculture a. Broodstock Selection and Maintenance	GF 18,000 0.5 FTE	21,000 0.5 FTE →	21,000	21,000	21,000	21,000	21,000	21,000	21,000	21,000	21,000	210,000
b. Aquaculture Research Center-Eastern Shore Lab	ES GF 60,000 2 FTE		1,100,000 1 FTE →		90,000	90,000	90,000	90,000	90,000	90,000	90,000	1,730,000
c. Hatchery and Nursery Technology, Additional Species		15, 000 0.5 FTE →	15, 000	15, 000	15, 000	15, 000	15, 000	15, 000	15, 000	15, 000	15, 000	150, 000
Gloucester Point Hatchery	GF 177,135 3 FTE G/C 62,000											
5. Human and Natural Pathogens	GF 36,000 0.4 FTE G/C 34,000	91,000 2 FTE →	91,000	91,000	91,000							364,000
6. Economic Assessments	GF 40,000 0.5 FTE G/C 40,000	113,000 2 FTE →	113,000	113,000	113,000	113,000	113,000	113,000	113,000	113,000	113,000	1,130,000

	Current Resources Applied State + G/C	1. FY97	2. FY98	3. FY99	4. FY00	5. FY01	6. FY02	7. FY03	8. FY04	9. FY05	10. FY06	TOTAL
7. Communication & Outreach, Including GIS	GF 70,000 1.5 FTE G/C 150,000	77,000 2 FTE	77,000	77,000	77,000	77,000	77,000	77,000	77,000	77,000	77,000	770,000
8. Non-Indigenous Species Research		275,000 4 FTE	235,000	210,000	185,000	185,000						1,090,000
TOTAL		1,374,000	2,375,000	1,498,000	1,428,000	1,300,000	1,002,000	955,000	559,000	559,000	559,000	11,609,000

REFERENCES, PART 1

Barber, B.J. and R. Mann. 1991. Estimation of Standing Stock of Oysters in the James River, Virginia, Using Commercial Fishing Records. Virginia Institute of Marine Science, Special Report in Applied Marine Science and Ocean Engineering, No 310.

Burreson. E.M. and J.D. Andrews. 1988. Unusual intensification of Chesapeake Bay oyster diseases during recent drought conditions. Proc. Oceans 88: 799-802.

Castagna, M.A. 1984. Methods of growing *Mercenaria mercenaria* from postlarval- to preferred-size seed for field planting. Aquaculture 39: 355-359.

Castagna, M.A. and J.N. Kraeuter. 1981. Manual for Growing the Hard Clam *Mercenaria.* VIMS Special Report in Marine Science and Ocean Engineering No. 249. Virginia Institute of Marine Science, Gloucester Point, VA. 110 pp.

Castagna, M.A., M. Luckenbach and P. Kelly. 1990. Use of concentrated bacteria to enhance survival of early post set *Mercenaria mercenaria* in a flowing seawater nursery. J. Shellfish Res. 8: 444.

Castagna, M.A., L.W. Mason and F.C. Biggs. 1970. Hard clam culture method developed at VIMS. Aggregates on bottom protect seed clams from predators. Va. Inst. Marine Sci., Mar. Resourc. Adv. Ser. 4, 3 p.

DiCosimo, Jane. 1986. Biological review and commercial whelk fisheries analysis of *Busycon carica* with comments on *B. canaliculatum* and *B. contrarium* in Virginia. College of William and Mary, Virginia Institute of Marine Science, Master of Arts Thesis, 125 pp.

Glude. J.B. 1983. Marketing and Economics in relation to U.S. Bivalve Aquaculture. Journal of World Mariculture Society. 14: 576-586.

Hargis. W.J. Jr. and D.S. Haven. 1988. Rehabilitation of the troubled oyster industry of the lower Chesapeake Bay. J. Shellfish Res. 7: 271-279.

Hargis, W.J. Jr. and D.S. Haven. 1988. The imperilled oyster industry of Virginia. VIMS Special Report Number 290 in Applied Marine Science and Ocean Engineering. 130p.

Haven, D.S., W.J. Hargis. Jr. and P.C. Kendall. 1978. The oyster industry of Virginia: Its status, problems and promise. VIMS Spec. Pap. Mar. Sci. No. 4. 1024p.

Haven, D.S., W.J. Hargis, Jr. and P. Kendall. 1981. The present and potential productivity of the Baylor Grounds in Virginia. Va. Inst. Mar. Sci., Spec. Rep. Appl. Mar. Sci. Ocean. Eng. No. 243: 1-154.

Haven, D.S. and J.P. Whitcomb. 1983. The Origin and Extent of Oyster Reefs in the James River, Virginia. J. Shellfish Res. 3(2):141-151.

Haven, D.S. and J.P. Whitcomb. 1989. The Location and Topography of oyster reefs in the Rappahannock River Estuary, Virginia. J. Shellfish Res. 8:105-116.
Jolly, C.M. and H.A. Clonts. 1983. Economics of Aquaculture. Food Products Press, The Haworth Press, Inc. New York. 319 pp.

Kennedy, V.S. and L.L. Breisch. 1981. Maryland's oysters: research and management. Maryland Sea Grant, University of Maryland, College Park, MD. 286p.

Kraeuter J.N. and M. Castagna. 1985. The effect of clam size, net size, and poisoned bait treatments on survival of hard clam, *Mercenaria mercenaria*, seed in field plots. J. World Maricul. Soc. 16: 377-385.

Kraeuter J.N. and M. Castagna. 1985. The effects of seed size, shell bags, crab traps, and netting on the survival of the northern hard clam, *Mercenaria mercenaria* (Linne). J. Shellfish Research 5: 69-72.

Mann, R., E.M. Burreson and P.K. Baker. 1991. The decline of the Virginia oyster fishery in Chesapeake Bay: considerations for introduction of a non-endemic species, *Crassostrea gigas* (Thunberg). J. Shellfish Res. 10(2): 379-388.

Meyers, J.A., E.M. Burreson, B.J. Barber and R. Mann. (in review). Susceptibility of diploid and triploid pacific oysters, *Crassostrea gigas*, to *Perkinsus marinus*. J. Shellfish Res.

Newell, R.I.E. 1989. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the American Oyster (*Crassostrea virginica*)? in: Understanding the Estuary: Advances in Chesapeake Bay Research. Chesapeake Research Consortium Publication No. 129: 536-546.

Oesterling, Michael. 1993. Marine Aquaculture in the Commonwealth of Virginia. VIMS Educational Series No. 39. Virginia Institute of Marine Science, Gloucester Point, VA. 24 pp.

Oesterling, Michael J. 1984. Manual for Handling and Shedding Blue Crabs (Callinectes sapidus). VIMS Special Report in Applied Marine Science and Ocean Engineering No. 271. Virginia Institute of Marine Science, Gloucester Point, VA. 76 pp.

Thacker, Sayra G. 1994. The Economic Impact of Marine Aquaculture on Virginia's Eastern Shore. Virginia Sea Grant Marine Resource Advisory No. 55. Virginia Institute of Marine Science, Gloucester Point, VA. 14 pp.

Turgeon, D.D., A.E. Bogan, E.V. Coan, W.K. Emerson, W.G. Lyons, W.L. Pratt, C.F.E. Roper, A. Scheltema, F.G. Thompson, and J.D. Williams. 1988. Common and scientific names of aquatic invertebrates from the United States and Canada: Mollusks. American Fisheries Society Special Publication 16.

Virginia General Assembly, House Document No. 56 (1995). House Joint Resolution No. 95 (1994) - A Strategic Plan for the Revitalization of the Shellfish Industry in Virginia. 12 pp.

Virginia Marine Resources Commission. 1982. Eighty-third and eighty-fourth annual reports of the Virginia Marine Resources Commission for the fiscal years ending June 30, 1981 and June 30, 1982. 65 pp.

Virginia Marine Resources Commission, Commercial Fisheries Statistics, Virginia Landings, Annual Summaries 1976-1992.

Wesson, James A. 1995. Fishery Independent Stock Assessment of Virginia's Hard Clam Population of the Chesapeake Bay. Final Report to Virginia Coastal Resources Management Program, Department of Environmental Quality. 20 pp.

PART 2. RATIONAL PLAN FOR TESTING APPLICATION OF NON-INDIGENOUS OYSTER SPECIES

I. Current Laws, Policies and Regulations

STATE

- Control Agency: Virginia Marine Resources Commission
  Virginia Code Annotated, Sec. 28.2-825
  VMRC Regulation 450-01-0102

The state code prohibits the importation of fish, shellfish or crustacea for introduction unless
1) the species under examination for introduction is on the Commission's "approved list" of species and originates from an "approved" state or water, or
2) the applicant received the written permission from the Commissioner of the Virginia Marine Resources Commission.

Even though a list exists, it does not contain any reference to non-indigenous oyster species. An applicant wishing to introduce a species not listed or not from an approved state or waters must receive permission from the Commissioner. Under such circumstances, it is ultimately the decision of the Commissioner whether a non-indigenous species can be introduced. The Commissioner does not need the concurrence of the Director of the Virginia Institute of Marine Science; the Director's concurrence only applies to the removal or addition of waters (or states) and species to the "approved lists."

REGIONAL

- Chesapeake Bay Program
  Chesapeake Bay Policy for the Introduction of Non-indigenous Species

"It shall be the policy of the Jurisdictions of the Chesapeake Bay Basin to oppose the first-time introduction of any non-indigenous aquatic species into the unconfined waters of the Chesapeake Bay and its tributaries for any reason unless environmental and economic evaluations are conducted and reviewed in order to ensure that the risks associated with the first-time introduction are acceptably low."

An applicant wishing to introduce a non-indigenous species must first submit an application to the appropriate agency, in this instance, the VMRC. If after evaluating the application, the VMRC considers it adequate for possible approval, the VMRC would forward all pertinent documents to the Living Resources Subcommittee. An *ad hoc* panel would then review the proposed introduction and provide advice to the VMRC. The VMRC is the ultimate authority and can choose to oppose the majority opinion of the panel. In such cases, the VMRC should 1) provide an explanation of their decision ("particularly in relation to potential threats to adjoining

jurisdictions") and 2) delay implementation for three weeks to allow other jurisdictions the time to notify "affected parties" of the decision.

- Atlantic States Marine Fisheries Commission
  (LaPointe, G., Director, Interstate Fishery Management Program, ASMFC, personal communication, September 14, 1995)

The information available from the Atlantic States Marine Fisheries Commission indicates that it is their general policy to oppose the introduction of non-indigenous species, including *Crassostrea gigas.*

FEDERAL

- The Lacey Act, 16 U.S.C. Sec. 3371-3378
  "Injurious Wildlife," 50 CFR Sec. 16

The Lacey Act is often cited as a federal statute affecting introductions of non-indigenous species. The Lacey Act, however, only prohibits importation of "injurious" animals. Very few animals receive this distinction and no known oyster species are currently listed. Federal regulation promulgated in response to the Lacey Act states that introductions of non-injurious animals (which would include *C. gigas*) are prohibited "except by the State wildlife conservation agency having jurisdiction over the area of release or by persons having prior written permission for release from such agency." In the state of Virginia and in the case of a non-indigenous oyster, the VMRC would be "such [an] agency."

- The National Environmental Policy Act of 1969,
  42 U.S.C. Sec. 4321-4347

The National Environmental Policy Act requires that federal agencies file environmental impact statements with "proposals for legislation and other major federal actions significantly affecting the quality of the human environment." NEPA, however, only addresses federal actions. An introduction of a non-indigenous species would not raise the possibility of NEPA applicability and a federal impact study unless an introduction occurred on federal lands, federal monies were requested to aid in the introduction, or some major federal action or decision was involved in the introduction.

- The Endangered Species Act, 16 U.S.C. Sec. 1531-1534

The Endangered Species Act was designed to prevent the extinction of endangered or threatened species by joint preservation of the species and its habitat. In regards to non-indigenous species, it is enacted when an introduction affects an endangered species or its habitat. Those species not yet listed, but awaiting evaluation, are also protected. A threatened or endangered aquatic species would have to exist and be threatened by a possible introduction of a non-indigenous oyster in order for the Endangered Species Act to be applicable.

- Executive Order No. 11987, "Exotic Organisms"

Executive Order No. 11987 directs executive agencies to restrict the importation, exportation and introduction of "exotic species." It also instructs executive agencies to "encourage the States, local governments, and private citizens to prevent the introduction of exotic species into natural ecosystems of the United States." The order's effect on introductions occurring in Virginia waters is limited unless a species is introduced on federal lands. In addition, by the order's definition of "exotic organisms," species currently established within the territorial United States are not considered exotic. Because *Crassostrea gigas* exists naturally in small pockets on the west coast of the United States, it is not, by definition, exotic.

- Non-indigenous Aquatic Nuisance Prevention and Control Act of 1990, 16 U.S.C. Sec. 4701-4751

The intent of the Non-indigenous Aquatic Nuisance Prevention and Control Act is to prevent unintentional introductions of aquatic nuisance species, promote research, disseminate information regarding all aquatic introductions, and minimize the impact caused by established non-indigenous aquatic nuisance species. The Act recognizes the risk of intentional introductions, but does not currently regulate them. Section 4727, entitled "Intentional Introductions Policy Review," required that a Task Force "identify and evaluate approaches for reducing the risk of adverse consequences associated with intentional introductions of aquatic organisms." The report entitled, "Findings, Conclusions, and Recommendations of the Intentional Introductions Policy Review," was sent to Congress, but it is unlikely that any action will be taken in response to the report in the foreseeable future. Although the Act is under re-authorization this year, no changes that would relate to intentional introductions are anticipated. (Troxel, J., Fish and Wildlife Service, U.S. Department of the Interior, personal communication, August 7, 1995).

Because the Act currently only addresses unintentional introductions, it would not affect the intentional introduction of a non-indigenous oyster, especially one that has not been declared a "nuisance."

INTERNATIONAL

- The International Council for the Exploration of the Seas

  The ICES Working Group on Introductions and Transfers of Marine Organisms

The International Council for the Exploration of the Seas 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.

II. Rationale for Introduction

BACKGROUND

The Chesapeake Bay oyster fishery for *Crassostrea virginica* is in a state of continuing decline. In Virginia the situation of the public fishery is accurately described as in crisis. Two oyster pathogens, *Haplosporidium nelsoni* and *Perkinsus marinus* have virtually eliminated oysters from the Virginia tributaries, and more recently, disease has spread into most growing areas of the Bay. Despite more than 30 years of disease activity, the native oyster has developed neither tolerance nor absolute resistance to these diseases, and does not exhibit any recovery in disease endemic areas of Virginia. Repletion programs have completely failed to restore permanent production areas lost to disease. Present fishery management activities are limited to retreat areas, yet vulnerable to climate variations with periodic incursions of disease.

The habitat characteristics of the once flourishing oyster resource have also changed. Past harvesting practices have reduced the original intertidal oyster reef habitat to topographic obscurity. As well, the watershed of the Chesapeake Bay has evolved in land-use with increased loads of suspended sediment, and other loadings which compromise water quality. Thus, oyster resource efforts are pursued in the face of challenges not prevailing during past times.

In recent times there is growing realization that the once flourishing oyster resource in its natural reef habitat may have played a significant role in maintaining water quality in the bay system through the filtering action in feeding. Moreover, the reef habitat itself is argued to have supported higher trophic levels. The ecosystem function of healthy oyster habitat has modified the view of oyster restoration goals. Once oriented toward harvest of the resource, the role of oysters and other shellfish in water quality maintenance and/or restoration enlarges the dimensions of that resource.

The mixed role of the oyster and associated habitat, a resource targeted to a fishery and a resource with significant ecosystem value, presents to all concerned a serious dilemma. Can the dual roles be satisfied? Is there any realistic likelihood that either or both roles can be realized through management of the native species, given its susceptibility to the endemic diseases? Given these questions, recent efforts toward restoration, aside from harvest management, need assessment.

With respect to oyster resource restoration, two programs in particular are germane. Over a number of years there has been a collaborative effort on the part of research institutions, including VIMS, in selective breeding of the native oyster for reduced susceptibility to diseases. These pursuits have shown promise and must continue with vigor. Current results indicate these strains have very significant potential for off-bottom aquaculture. These strains are unlikely candidates for widespread stock restoration, however, since interbreeding with remnant disease susceptible oysters would dilute the trait sought.

In recent years efforts have been undertaken to restore oyster reef habitat through construction of three-dimensional structures. Intended as broodstock sanctuaries, success is dependent upon

reduced mortalities due to disease. In order to enhance the likelihood of success, placement of structures has been restricted to areas where disease challenge is relatively low and periodic.

Given the joint goals for restoration of oyster stocks and reef habitat, and that the native, disease susceptible, oyster is not likely to repopulate once-productive areas, the case for examining the feasibility of alternative oyster species is compelling. Disease resistance is the pivotal issue.

RATIONALE FOR TESTING NON-INDIGENOUS SPECIES

The plan proposed is intended to provide a science-based foundation from which public policy decisions may be made regarding utilization of non-native oyster species for restoration of oyster stocks. Most of the test series would be performed in quarantine systems. However, two elements require in-water testing (intentional introductions). In-water testing is required to assess resistance to the oyster pathogen *Haplosporidium nelsoni*, and in the final stages further confirmatory in-water testing is proposed to gauge response to actual environmental conditions.

The program proposed has two objectives. In the first instance, the test series will serve to screen for the candidate species, or strains, 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.

The plan, which will require four years, adopts ICES guidelines, wherein quarantined hatchery-raised progeny from imported broodstock are utilized. Three strains of the species *Crassostrea gigas*, and the species *Crassostrea rivularis* are advanced for testing, based upon their close resemblance to the native species as reef-forming species tolerant of mid to sub-tropical latitude, high stress environments. The strategy advanced includes the following:

1. a series of comparative studies in quarantine systems to evaluate larval and post-settlement response to a range of environmental conditions
2. a challenge, in quarantine, with the oyster disease *Perkinsus marinus* (Dermo)
3. a field challenge with triploid animals for the oyster disease *Haplosporidium nelsoni* (MSX)
4. evaluation, via 1 through 3, of likely success of candidate species and assessment of likely geographic range of reproduction if introduced in substantial numbers
5. given acceptable risk, limited in-water testing of diploid hatchery-reared stock with small lots under secure conditions

It is appropriate to begin a search for an alternate species within the genus *Crassostrea*—reef forming species tolerant of mid to subtropical latitude, high stress environments. Tables 8-10 summarize species in the genus *Crassostrea*, and compare published data describing their temperature and salinity tolerances as both larval and adult forms. Caution must be applied in literature review in determining the geographic origin of *Crassostrea virginica* under examination (see comments in Hedgecock and Okazaki, 1984 and Reeb and Avise, 1990 concerning lack of genetic uniformity throughout the zoogeographic range of this species), and, where possible, which geographic type of *Crassostrea gigas* (there are four, named by prefecture of origin, Hokkaido, Myagi, Hiroshima and Kumamoto, see comments in Torigoe, 1981; Quayle, 1989;

Kusuki, 1990) is being described. Geographic types of *Crassostrea gigas* are characterized by distinct growth rates and forms (so much so that they serve quite different commercial markets) that may have different temperature and salinity optima and tolerances. Such information on geographic type is rarely given, therefore data in tables 8-10 encompasses all types. For the present comparative purpose this is acceptable in that it may overestimate rather than underestimate possible ranges of *Crassostrea gigas* in the Chesapeake Bay. In general, the Myagi strain has been the focus of work in the hatchery-based fishery of the Pacific coast of North America; however, there has been much intentional interbreeding of introduced stocks, and precise pedigrees are lacking. The predominant oyster of that and the European fisheries can better be described as Myagi-like. Several other species lack adequate documentation for complete comparison; however, it is evident that strong similarities exist between *Crassostrea virginica* and *Crassostrea gigas*.

Table 8. ***Crassostrea*** **species: distribution and synonyms**

Source material:
1. Ahmed, 1971; 2. Boffi, 1979; 3. Carreon, 1969; 4. Chen, 1972; 5. Dang, 1972; 6. Durve, 1967; 7. Kamara et al., 1976; 8. Kong and Luh, 1977; 9. Langdon and Robinson, in press; 10. Mann, 1981: 11. Menzel, 1974; 12. Newball and Carriker, 1983; 13. Shafee and Sabatie, 1986: 14. Tebble, 1966; 15. Torigoe, 1981; 16. Zenkevitch, 1963.

Atlantic coast of North America: **virginica (= rhizophorae)**, 12.
Brazil: **brasiliensis (= rhizophorae = virginica?)**, 2, 7
Western Europe, English Channel to Morocco (now rare): **angulata**, 11, 14.
Europe, North Sea through Mediterranean to Morocco: **gigas**, 10, 13.
Pacific coast of North America: **gigas**, 10, 13.
Japan, Korean Peninsula through Vietnam: **gigas, araikensis (= rivularis), nippona**, 5, 15.
Pakistan, China: **araikensis (=rivularis)**, 9
India: **gryphoides, madrasensis, rivularis (= araikensis)**, 1, 6.
Thailand / Malaysia: **belcheri (= nippona?)**, 4, 8.
Phillippines: **iredali (= madrasensis or even = rivularis?)**, 3.
West Africa: **gasar (= tulipa)**, 7.
Black Sea: **taurica**, 16.

Table 9. Temperature and salinity ranges of adults of *Crassostrea* species
(Optimum ranges given in parentheses.)

	temperature °C		salinity (ppt)		reference
species	growth	spawning	growth	spawning	
virginica	5-34(28-32)	18-25 (23)	>5 (12-27)	>8	7,8,21, 22,23,32
angulata	20-30	20	21-43	<33	3,4,16
araikensis	15-30(20-35)	7-40 (30-40)	15-30(20-35)		5,11,16, 19
gasar	25-30	5-34	14-20		1,29,30
gigas	3-35(11-34)	16-30(20-25)	10-42(35)	10-30(20-30)	2,4,15,18, 20,25,26
gryphoides	19-33	27-31	4-40 (30-40)	13-29	11,13,24
iredali	30-33	<45	>15		4
madrasensis	26 (30)	1-41 (8-25)	17-35(20-35)		16,17,27,28,31
nippona	no data				
rhizophorae			22-40(26-37)		4,5,12
taurica	3-28	17-18			33

References:

1. Ajana, 1980; 2. Allen at al, 1988; 3. Amemiya, 1926; 4. Bardach et al., 1972; 5. Boveda and Rodriguez, 1967; 6. Breese and Malouf, 1977; 7. Butler, 1949; 8. Chanley, 1958; 9. Davis, 1958; 10. Davis and Calabrese, 1964; 11. Desai et al., 1982; 12. Dos Santos and Nascimento, 1985; 13. Durve, 1965; 14. His et al., 1989; 15. Hughes-Games, 1977; 16. Jhingran and Gopalakrishnan, 1974; 17. Joseph and Madhyastha, 1984; 18. King, 1977; 19. Langdon and Robinson, in press; 20. Le Gall and Raillard, 1988; 21. Loosanoff, 1958; 22. Loosanoff, 1969; 23. Loosanoff and Davis, 1952; 24. Mane, 1978; 25. Muranaka and Lanna, 1984; 26. Nell and Holliday, 1988; 27. Rao, 1951; 28. Rao and Nayor, 1956; 29. Sandison, 1966; 30. Sandison and Hill, 1966; 31. Stephen, 1980; 32. Wells, 1961; 33. Zenkevitch, 1963.

Table 10: Temperature and salinity ranges of *Crassostrea* larvae
(Optimum ranges given in parentheses.) Reference material as in Table 9.

species	temperature °C	salinity (ppt)	reference
virginica	20-33	8-39 (10-29)	3,9,10
angulata		21-43 (28-35)	3,4,16
araikensis	20-28 (25-28)	10-35 (15-20)	5, 19
gigas	18-35 (30)	19-35	2,14,15
rhizophorae	<30 (25)	20-40 (28)	12

no data available for **gasar**, **gryphoides**, **iredali**, **madrasensis**, **nippona** and **taurica**.

The end product of this data compilation suggest *Crassostrea gigas* and *Crassostrea rivularis* as good candidate species based on temperature tolerances and what is known of other oyster pathogens which are not endemic to the Chesapeake Bay. The analysis is, however, far from complete because it does not give guidance on susceptibility to other oyster predators present within the Chesapeake but absent from the native or source range of a candidate species. For example, recent studies at VIMS have found *Crassostrea gigas* to be susceptible to infestation by the shell boring worm *Polydora* in quarantine systems. This questions its suitability for extensive introduction. It also underscores the need for caution when deciding whether to proceed to in-water testing. Tests in quarantine provided a variety of new knowledge developments without any environmental risk.

III. Overview of Protocol for Studies of Non-Indigenous Oyster Species as Candidates for Establishment in the Chesapeake Bay

Introduction: Based upon current knowledge and understanding, two species of the genus *Crassostrea* have been selected for trials. *Crassostrea gigas* (the Pacific or Japanese oyster, hereafter *C. gigas*) and *Crassostrea araikensis* (=*rivularis*) (the Suminoe oyster, hereafter *C. rivularis*) are selected because of close resemblance to native Eastern or American oyster, *Crassostrea virginica* (hereafter *C. virginica*) as reef-forming species tolerant of mid to sub-tropical latitude, high stress environments.

The strategy advanced includes the following:

1. A series of comparative studies in a quarantine system to evaluate larval and post-settlement response to a range of environmental conditions.
2. A challenge, in quarantine, with *Perkinsus marinus*.
3. A field challenge with triploid animals for *Haplosporidium nelsoni*.
4. Evaluation, via 1 through 3, of likely success of candidate species and assessment of likely geographic range of reproduction.
5. Given acceptable risk, in-water testing of diploid hatchery-reared stock in limited lots under secure conditions.

Overview of protocol: The protocol begins with broad adoption of ICES guidelines and the establishment of an F1 and subsequent population in a quarantine system. The ICES guidelines require:

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

The established F1 and subsequent generations are the individuals subjected to testing before open introduction, and are the genetic source material for an introduction should it be deemed

reasonable in terms of likely success and acceptable risk. After establishment of the broodstock, the protocol proceeds to a disease and environmental parameter matrix challenge as appropriate. The cumulative data from these challenges dictates the nature (or abandonment) of further studies. If there is a move to limited exposure in the natural environment, then testing focuses on appropriate employment of triploidy, as required for disease challenge. Eventual deployment of diploid animals should consider quantitative issues in population development in deployment site selection.

The Protocol: This is presented in a step by step manner. Where appropriate, the objectives are sequential in chronology. Table 11 further summarizes the sequence timeline. Each **Objective** includes an assessment of the **Status of Knowledge** with respect to a *Candidate Species*. Where knowledge is available it is summarized or the relevant literature cited. Where appropriate a brief **Scenario** for continuing or *de novo* developing of knowledge is given together with a **Resources and Timeline** estimate. At any point in the sequence a lack of appropriate data or developed data illustrating unacceptable species characteristics can be used in **Data Evaluation** to promote or terminate the sequence. Candidate species surviving the sequence without illustrating unacceptable characteristics could be promoted for introduction.

Objective 1. Identify the need: what are desirable characteristics?
Growth rate and longevity comparable to native species.
Resistance to endemic disease.
Growth and survival in local conditions of temperature, salinity, suspended sediments, etc.
Ability to reproduce in receiving waters (develop self-sustaining populations).
Lack of ability to reproduce in receiving waters (controlled cultured product only).
Reef-forming habits (for self-sustaining populations).
Low susceptibility to predators.
Suitability as a commercial product.
N.B. This is not considered an exclusive list, just one of predominant characteristics.

Objective 2. Literature survey of described species

Status of knowledge: In theory the database is never too large, although the nature of reported data can compromise its value in the current application.

Scenario: Start with taxonomically related species and diversify if suitable candidates do not appear.
Identify candidate species group—the genus *Crassostrea*.
Identified candidates, based on items listed in Objective 1: *C. gigas, C. rivularis*. Note that *C. gigas* exhibits distinct geographical variation in its native oriental range with oysters from the localities of Hokkaido, Myagi, Hiroshima, and Kumamoto presenting distinctive growth characteristics and forms. The Kumamoto form is the subject of continuing taxonomic debate and probably unsuitable for consideration for the Chesapeake Bay environment. The remaining three have rarely been examined or reported on as "pure" forms in the literature. Thus the literature summary provided elsewhere in this document is a summary of all data. Further, most data developed from *C. gigas* stocks from Pacific Northwest hatcheries are from animals that are best

described as Myagi like, but of unknown precise lineage with probable contribution from other geographical forms. Limited previous work at VIMS in collaboration with Rutgers University researchers with triploid *C. gigas* was with Myagi like individuals, and a substantial case is made for repeating *C. gigas* work with animals of known lineage and defined relation to the geographical forms. The current listing of *C. gigas* therefore includes Myagi, Hokkaido, and Hiroshima forms as separate study items.

Resources and Timeline: Continuing development of this database can be made at minimal cost.

Objective 3. Examine database to facilitate quarantine studies

Status of knowledge: Does VIMS have the necessary experience in and facilities for quarantine culture of non-indigenous oyster species? Yes. Is the facility currently operating? No.

Scenario: Re-establish and improve active quarantine capability at VIMS.

Resources and Timeline: Some refurbishments would be required to initiate studies. Continuing costs include labor and the need to temporally isolate all hatchery operations for all non-indigenous species from native species work—that is, the hatchery will not be available for any other purposes while working with non-native species. This has a cost in the timetables of other shellfish service activities.

Status of knowledge: A sequence of baseline questions:
(a) Have the candidate species been cultured through the larval stages to settlement in the laboratory?
C. gigas: yes, including at VIMS.
C. rivularis: yes, in extensive studies at the Hatfield Laboratory of Oregon State University, but not at VIMS.

(b) Are settlement and juvenile requirements understood?
C. gigas: yes.
C. rivularis: yes.

(c) Has the species been cultured in large (pilot or commercial) scale?
C. gigas: yes, but only in laboratory scale at VIMS.
C. rivularis: yes, but not at such a large scale as for *C. virginica* at VIMS. The Hatfield studies were in 500L larval culture containers.

(d) Can VIMS identify possible broodstock sources?
C. gigas: Natural range throughout Japan, introduced populations in the Pacific Northwest of the United States and Canada, introduced populations in Europe, experimental (quarantine) populations in laboratories (e.g. Rutgers University). The introduced populations are nearly all Myagi-like and of poorly documented lineage. Some "pure" lines have been obtained and maintained at the Hatfield Laboratory and at Rutgers University.

C. rivularis: Natural populations in Asia, experimental populations at the National Oyster Broodstock facility at Hatfield Marine Science Center, Oregon State University, Newport, Oregon.

(e) Consider the history of possible broodstock sources to minimize genetic problems—does this further limit options?
C. gigas: Yes, to either Japanese (direct collections) or Pacific Northwest stocks (still some potential lack of lineage) or Rutgers University or Hatfield Laboratory (see above).
C. rivularis: No adequate data are available, but stocks from the Hatfield Laboratory would be adequate for most of the subsequent quarantine tests.

(f) Has there been an exhaustive study of potential movement of disease, pest and parasites with source broodstock?
C. gigas: This has been addressed in the previous section for Pacific Northwest hatchery stocks.
C. rivularis: Data search has disclosed no references for Asian populations, prompting choice of quarantined stocks at the Hatfield Laboratory.

(g) Identify the **preferred** broodstock source:
C. gigas: National Oyster Broodstock facility at Hatfield Marine Science Center, Oregon State University, Newport, or Rutgers University. If geographically distinct lineages are not available for all three forms then direct collections would be the only resort, with the associated time, expense and logistic constraints.
C. rivularis: Hatfield Laboratory quarantine stock.

Scenario: Establish broodstock at VIMS.

Resources and Timeline: Broodstocks for both species could be obtained at modest cost, but *C. rivularis* would be in limited number. Appropriate regulatory bodies must be notified of intent to move broodstock to quarantine systems. In Virginia this requires a letter to the Commissioner of the Marine Resources Commission from the Director of the Virginia Institute of Marine Science. Prior precedent with movement of *C. gigas* to VIMS did not require further action by the Marine Resources Commission, in that the movement was to a secured quarantine system and not to the open waters of the Commonwealth.

Objective 4. Develop a spawning capability in quarantine system

Status of knowledge: Prior experience throughout the world with non-endemic studies indicates the "safest" approach is to insure no mixing with native OR other non-native species in system.

Scenario: *Both species*: Establish a requirement for temporal and spatial isolation from native species, and spatial isolation from other non-native species. This is feasible with the current hatchery facilities at VIMS.

Import only the required number of adults for spawning. Some geographical forms of *C. gigas* can be obtained in limited numbers essentially on demand, others may be more limited. Typical

importations would be of the order of 50-200 animals depending on size. *C. rivularis* would be in limited numbers, probably <50 given the proposed sources.

Condition and spawn adults as soon as feasible. Note temperature and salinity requirements for spawning as predictors for spawning potential in receiving waters. The two candidate species have some differences:

C. gigas: Prior experience at VIMS, Gloucester Point, where salinity can be marginally low for optimum spawning illustrates feasibility, but broodstock can only be spawned once. A serious option would be to perform *C. gigas* spawning in quarantine at Eastern Shore Laboratory at Wachapreague. Support services in algae culture will be considerable for any conditioning effort at either location.

C. rivularis: This species does not develop gonad as quickly as *C. gigas* under identical field conditions and is slower to condition in the laboratory. There may be a learning period here in that published guidelines are for 20°C and 30-35 ppt salinity (even though the larvae grow well at 20 ppt). Estimate one season to develop spawning protocols for estuarine salinities. Support services could be intensive. Terminate parental stock with appropriate archiving for histological and possible genetic analysis.

Both species: Standard protocols for archiving for histological and genetics purposes have been established. Creating the archive is of minimal expense; examining it can be very time consuming if the intent is to search for possible parasites and pathogens that may be available for vertical transmission, or developing large libraries of genetic data.

Resources and Timeline: For the development of spawning protocols and sustained larval cultures estimate one season (3 months of dedicated hatchery) to fix protocols for *C. gigas* and establish larval culture for F1 and subsequent generations. Estimate two seasons (3 months each in successive years) for similar results in *C. rivularis*. Note that the comparative tardiness of development of gonad in *C. rivularis* may dictate that the species are worked on in different time frames within a year. Additional personnel support will be required.

Objective 5. Culture of larval forms

Status of knowledge: Although both have been cultured before elsewhere and at higher than typical estuarine salinities the database for **estuarine** situations is limited. For *C. gigas* this is mostly unpublished data from previous studies at VIMS. These studies are still less than adequate for *C. gigas* in that they do not address the issue of geographical form, although they are a good start. The data for *C. rivularis* are quite comprehensive, via recent work by Langdon and Robinson at the Hatfield laboratory. Growth and settlement of larvae was greatest at 15-20ppt, with settlement occurring at up to 30 ppt. Spat grew best at 25°C and 25-35 ppt but were tolerant of less than optimal salinities and temperatures.

Scenario: Examine in matrix design the temperature and salinity requirements for growth and survival of larvae in a direct comparison with native *C. virginica* in local ambient (estuarine

through the lower bay) conditions. Water quality issues are considerable in the lower sub-estuaries of the Chesapeake Bay, and while the literature data from the Hatfield studies are very useful the extension of data from diluted, higher salinity Oregon estuary water to local conditions has some limitations. The end product would be a database for predicting potential range of distribution of larvae of both *C. gigas* and *C. rivularis* in receiving waters in comparisons with the native species.

Resources and Timeline:
C. gigas: Assuming broodstock are available for all geographical forms, this could probably be complete by the end of the second year. Additional personnel and material support will be required.

C. rivularis: Assuming broodstock conditioning could be mastered at lower salinities, this could be complete by end of the second year. Additional personnel and material support will be required.

Scenario: Examine in matrix design the temperature, salinity, and substrate requirements for metamorphosis of larvae—these are the basis for comparison with local ambient (estuarine) conditions and the database for predicting potential spatial range of survival of settling larvae in receiving waters. Include a "control" treatment with metamorphosing larvae of the native *C. virginica* in the matrix.

Resources and Timeline: *Both species*: Assuming this activity will run concurrently with larval studies, these should be completed by the second year.

Data evaluation: Species or geographical forms not illustrating tolerance to local conditions can be eliminated at this time. Note that these tolerances may not be consistent in consideration of self-sustaining populations versus culture only product in that larval culture requirements may differ from post larval requirements. The latter may be more tolerant with a hatchery-based culture product being grown in areas which would not support larval growth and settlement.

Objective 6. Culture of early post-settlement forms: physical requirements.

This is contingent on positive data from Objective 5 above.

Status of knowledge: With respect to culture at typical mid-Atlantic estuarine physical conditions the database is again limited for *C. gigas* to unpublished data from previous studies at VIMS. *C. rivularis* data can be taken from Langdon and Robinson's studies with growth observed at 15-35 ppt, increasing with increasing salinity. No growth was observed at 5 ppt salinity. An expansion of the salinities tested between 5 and 25 ppt would be critical in the proposed context.

Scenario: Examine in a matrix design the temperature, salinity, and suspended sediment tolerances of post-settlement forms in comparison to local ambient conditions, again with a

control of native *C. virginica*. This is, again, a section of the database for predicting potential range of survival of juveniles in receiving waters.

Resources and Timeline: *Both species*: Assuming this activity will run sequentially with larval studies, these should be completed by second year.

Objective 7. Culture of early post-settlement forms: macro organism interactions.

Status of knowledge: The database for typical mid-Atlantic estuarine situations is very poor—limited to *C. gigas* and unpublished data from previous flume studies at VIMS. Such studies have not been performed with *C. rivularis*.

Scenario: Examine in a matrix design the susceptibility of post-settlement forms to a limited set of local predator guilds (i.e. flatworms and boring sponge, including size and density dependencies) in comparison to local ambient conditions, again with a control of native *C. virginica*. This is a further section of the database for predicting potential range of survival of early post-settlement juveniles in receiving waters.

Resources and Timeline: *Both species*: Assuming this activity will run sequentially with larval studies, these should be completed by (optimistically) the second or (realistically) the third year.

Objective 8. Culture of early post-settlement forms; disease organism interactions.

Status of knowledge: Controlled challenges of Myagi like *C. gigas* in parallel with *C. virginica* for *Perkinsus marinus* have been effected. While infection occurs, progression to mortality in *C. gigas* is limited. No such studies have been made with pure geographical forms of *C. gigas* or *C. rivularis*. One challenge of Myagi like *C. gigas* by MSX was effected in a limited environmental exposure. Resistance to MSX was demonstrated but the experiment was limited in scope.

Scenario: *Perkinsus marinus* challenge. Examine in a matrix design the susceptibility of post-settlement forms to disease organisms, in this instance *Perkinsus marinus*, under quarantine conditions, again with a control of native *C. virginica*. This is a further section of the database for predicting potential range of survival of juveniles in receiving waters.

Resources and Timeline: *Both species*: Assuming this activity will run sequentially with larval studies, these should be completed by second (optimistic if all larval work proceeds well) or third (allowing for problems with broodstock or larval culture) years respectively. Methods for *Perkinsus* inoculation to laboratory maintained populations have been established, although this is a subject of continuing improvement in methods with time. These time frames may be liberal estimates.

Haplosporidium nelsoni (MSX) Challenge. To this time there is no reliable method for consistent infection of oysters in laboratory systems. Until such time that this technical hurdle is overcome the only tractable approach to this challenge is direct environmental exposure in a location of

known MSX challenge. A prerequisite for direct exposure is development of triploid individuals. This gives rise to Objective 9.

Objective 9. Triploidy induction in remaining candidate species.

Status of knowledge: Triploid induction from diploid parents using cytochalasin B is now a standard protocol in hatcheries. VIMS has such experience with *C. virginica*, but not with the candidate species. Colleagues at Rutgers University have these protocols well established. Tetraploid - diploid crosses to produce triploid offspring is a very new tool in oyster studies, and is still under development. Again, colleagues at Rutgers are in the forefront of this research. A sub-contact to Rutgers University is envisioned.

Scenario: Two approaches are possible.

(1) Effect matrix design experiments to optimize protocols for triploidy induction in candidate species using cytochalasin B inducer and flow cytometry assay. Culture through juvenile form with regular testing to assure triploidy (blood or mantle assays by flow cytometry). Identify individual triploid animals for in-water testing and proceed to comparative studies with native species.

(2) Employ only tetraploid females and diploid males in spawning efforts. The result will be assured triploid offspring. Tetraploid adults are rare and the result of manipulative breeding in prior efforts. They are a recently mastered addition to the arsenal of oyster geneticists and tetraploid broodstock populations are still being established. At this time VIMS has had verbal reports of tetraploid *C. rivularis* from colleagues at Rutgers University. VIMS continues to support their efforts to develop this technology as a powerful tool to provide assured triploid individuals of *both C. rivularis* and *C. gigas* for direct environmental challenges by endemic diseases with minimal environmental risk.

Resources and Timeline: Optimistically one year after optimizing larval culture.

Objective 10. In-water challenge for *Haplosporidium nelsoni* (MSX)

Status of Knowledge: Limited to one challenge with *C. gigas* at VIMS.

Scenario: Examine in a matrix design the susceptibility of post-settlement forms to disease organism. This is a further section of the database for predicting potential range of survival of juveniles in receiving waters.

Resources and Timeline: Additional personnel and materials will be required. Field challenge would be scheduled to begin in late spring of the second year.

Objective 11. Spawning and Egg Viability of Adult Oysters

Previous objectives focus on early life history. This objective addresses the influence of salinity on the ability of spawning oysters to provide viable eggs and larvae. Should oysters be introduced to the Chesapeake Bay, this objective addresses what segment of the population resulting from larval dispersal and settlement will successfully contribute to the maintenance of the species in the new environment.

Status of Knowledge: No test results known.

Scenario: Oysters will be approximately two years old at time of investigation. Examine in matrix design spawning success in a wide range of salinities sufficient to include ranges occupied by oysters in Chesapeake Bay.

Data Evaluation for Objectives 5 through 11

Results would be effected in continuing temporal sequence, or in parallel to a limited extent, in comparison with native species with appropriate statistical design. Representative sampling and archiving should occur throughout for histological examinations investigating possible vertical transmission of disease-related organisms from parental broodstock, and genetic typing.

Completion of objectives 5 through 11 will constitute a thorough examination of response to environmental conditions in quarantine. With respect to disease challenge both species will have tested in quarantine for *Perkinsus marinus,* and in-water tested for response to both *Haplosporidium nelsoni* and *Perkinsus marinus.* This ensemble will determine whether either species would succeed in establishing populations. As importantly, the studies enable completion of a risk assessment on the likely geographic range for reproductive success.

Objective 12. Initial in-water studies of diploid stocks for growth, survival, and disease resistance.

Status of knowledge: None for the Chesapeake.

Scenario: Limited introduction of experimental lots at various secure locations. Observations to include environmental parameters (salinity, temperature, suspended sediment, etc.)

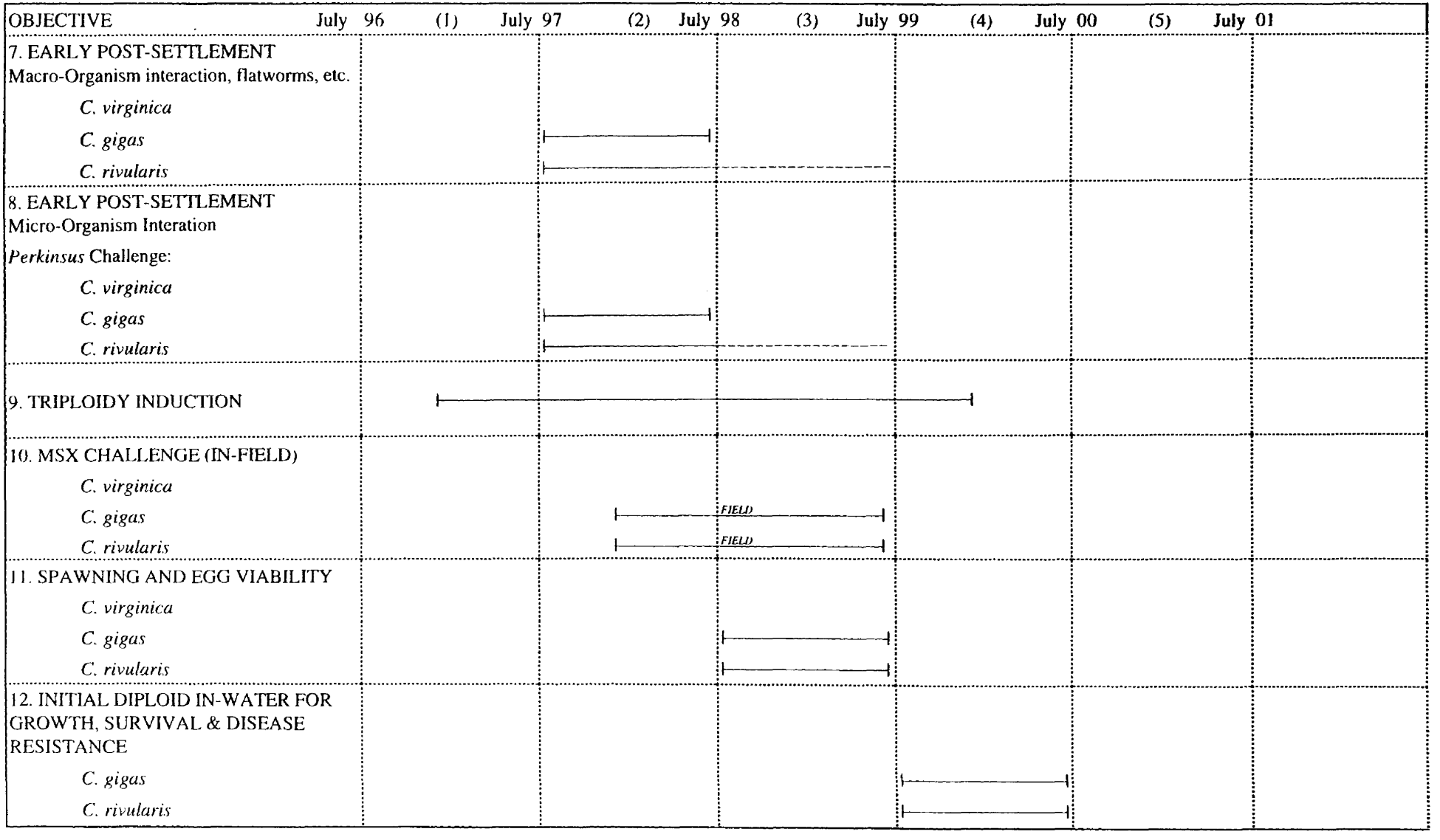
Resources and Timeline: Field studies initiated in the early summer of the third year and continue for one full year. Additional personnel and material resources required.

Data Evaluation: A summary evaluation of all data to this point would be completed at this time. Sufficient data should be available to make reasonable estimates of some aspects of ecological impact of large scale introduction and, by extension, economic impact in the commercial sector.

Table 11. TIMELINE

A Protocol for Studies of Non-Indigenous Oyster Species as Candidates for Establishment in the Chesapeake Bay

OBJECTIVE	July 96 (1)	July 97 (2)	July 98 (3)	July 99 (4)	July 00 (5)	July 01
(1. & 2. Not Included.)						
3. ESTABLISH BROODSTOCK IN QUARANTINE AT VIMS (*C. gigas and C. rivularis*)	⊢——⊣					
4. SPAWNING IN QUARANTINE						
C. gigas	⊢——	———	———	———	———	
C. rivularis	⊢——	———	———	———	———	
5. CULTURE OF LARVAL FORMS						
Larval Growth and Survival: Temperature/Salinity Matrix:						
C. virginica						
C. gigas		⊢——⊣				
C. rivularis		⊢———	———	⊣		
Larval Metamorph: Temp/Salinity/TSS:						
C. virginica						
C. gigas		⊢——⊣				
C. rivularis		⊢———	———	⊣		
6. EARLY POST-SETTLEMENT Temp/Salinity/TSS:						
C. virginica						
C. gigas		⊢——⊣				
C. rivularis		⊢———	———	⊣		


OBJECTIVE
July 96
(1)
July 97
(2)
July 98
(3)
July 99
(4)
July 00
(5)
July 01
7. EARLY POST-SETTLEMENT
Macro-Organism interaction, flatworms, etc.
C. virginica
C. gigas
C. rivularis
8. EARLY POST-SETTLEMENT
Micro-Organism Interation
Perkinsus Challenge:
C. virginica
C. gigas
C. rivularis
9. TRIPLOIDY INDUCTION
10. MSX CHALLENGE (IN-FIELD)
C. virginica
C. gigas
FIELD
C. rivularis
FIELD
11. SPAWNING AND EGG VIABILITY
C. virginica
C. gigas
C. rivularis
12. INITIAL DIPLOID IN-WATER FOR GROWTH, SURVIVAL & DISEASE RESISTANCE
C. gigas
C. rivularis

IV. Risk Analysis for Introduction of Diseases with a Non-Indigenous Species

Crassostrea gigas. The argument in support of possible use of *Crassostrea gigas* in restoration of the presently unproductive areas of the Bay has some positive aspects. Questions of diseases associated with *Crassostrea gigas* in its native and introduced range remain—are there such diseases and could they be transferred to the Bay with an introduction? *Crassostrea gigas* has, in its native range, no known diseases that have been associated with large-scale mortalities (Koganezawa, 1975). In addition, it has been used successfully as an introduced species in areas where native oysters have been decimated by diseases. *Crassostrea gigas* has been resistant to the local diseases. For example, *Crassostrea gigas* is not susceptible to *Bonamia ostreae* and *Marteilia refringens*, diseases that have caused massive mortalities in *Ostrea edulis*, the native species in western Europe. It has also been resistant to similar protozoan diseases where it has been introduced in Australia and New Zealand. In addition, *Crassostrea gigas* is resistant to the viral diseases that caused mass mortalities of the Portuguese oyster in France, and is not susceptible to *Perkinsus marinus* and *Haplosporidium nelsoni*, pathogens that have decimated eastern oyster populations in the middle Atlantic region (Burreson et al., 1994). The Japanese oyster is the basis for the hatchery-based industry in the Pacific Northwest and no new diseases (that cause measurable mortality) have been introduced into that region (Glude, 1975), even though there have been periodic importations of *Crassostrea gigas* since 1902, and early introductions were effected without any control measures. Recently Friedman (1996) documented the introduction of an unidentified haplosporidian to California from infected *C. gigas* spat from Japan; however, prevalence of the parasite in resident *C. gigas* populations is very low (<3%). Andrews (1980) reviewed oyster introductions around the world and discussed potential problems with such importations and precautions necessary to avoid disease introductions.

The extensive movement of *Crassostrea gigas* has provided, in addition to the native range, many potential sources for broodstock for a proposed introduction. For the present discussion consideration of source broodstock is limited to that from the state of Washington. The pedigrees of these stocks are not definitively documented; the stocks are mostly of Myagi Prefecture origin, but many years of hatchery breeding may have resulted in some limited crossing with stocks from other sources. They do, however, have a known and documented history concerning associated pests, parasites, and diseases. The listing below includes only those organisms reported from *Crassostrea gigas* that are actual or potential disease agents in oysters or other bivalve mollusks. It does not include the numerous parasites, mostly metazoan, found in oysters world-wide that have never been implicated in host mortality. Infectious diseases of *C. gigas* have recently been reviewed by Elston (1993).

1. Diseases of Unknown Etiology.

Hematopoietic Neoplasia. This disease results in a massive tissue invasion of abnormal blood cells and is analogous to leukemia in vertebrates. It has been implicated in large-scale mortalities of mussels in the state of Washington and of soft-shell clams in Chesapeake Bay. The syndrome has been reported in *Crassostrea gigas*, *Crassostrea virginica*, and *Ostrea lurida*, but has not

been associated with mortality in these species. A virus has been suggested as the cause for this disease, but the evidence is weak.

Potential implications: This syndrome is already present in Chesapeake Bay and has been observed occasionally in *Crassostrea virginica*.

2. *Viral Diseases.*

a. Oyster Velar Virus. This disease affects oyster larvae and has been reported from two hatcheries in the state of Washington (Elston and Wilkinson, 1985). It has been observed occasionally in hatcheries from March to August in larvae greater than 150 mm in shell height. Infection results in loss of motility and death of larvae. Measured losses of hatchery production up to 50% have been recorded, but there is no established link between the disease and mortality because it has not been experimentally transmitted. There have been no outbreaks of the disease in recent years (R.A. Elston, Battelle Center for Marine Disease Control, Sequim, WA, personal communication). The virus is thought to be an iridovirus.

Potential implications: This virus is primarily a hatchery problem where larvae are held at high density in tanks, but even in hatcheries the virus has never caused mortality of more than 50%. It is not expected to be a problem in nature where density of larvae is much lower than in hatcheries, and transmission of viral particles between larvae is greatly reduced.

b. Herpes-like virus. Herpes-like viral infections of hatchery-reared *C. gigas* larvae have been reported in New Zealand (Hine et al., 1992) and France (Nicolas et al., 1992; Le Deuff et al., 1994). Mortality of larvae may reach 100% by day 6.

Potential implications: As with oyster velar virus, this virus is a hatchery problem where larvae are held at high density in tanks. It is not expected to be a problem in nature where density of larvae is much lower than in hatcheries and transmission of viral particles between larvae is greatly reduced.

c. Hemocytic Infection Virus (HIV) and Gill Necrosis Virus (GNV). These iridoviruses have been reported from adult *Crassostrea gigas* in France. Both viruses were implicated in mass mortalities of the Portuguese oyster *Crassostrea angulata* in France during the 1970s (Comps and Bonami, 1977), but neither virus causes mortality in *Crassostrea gigas* in the same area (Comps, 1988). In fact, Comps (1988) states that the ability of *Crassostrea gigas* to resist mortality from these viruses resolved a very serious economic problem associated with the total elimination of the Portuguese oyster.

There has been some speculation that *Crassostrea gigas* is a carrier for these viruses and that one or both of them was introduced into France with importations of *Crassostrea gigas* from Japan. According to Henri Grizel, IFREMER, France, (personal communication, 12 March 1990) the lesions characteristic of the viral infections were observed in *Crassostrea angulata* prior to introduction of *Crassostrea gigas*, which suggests that the viruses were already present in France.

Unfortunately, no attempt was made to isolate viruses at that time, so it will never be known with certainty if the viruses were already present.

Potential implications: GNV and HIV have not been observed in *Crassostrea gigas* from the Pacific Northwest. In addition, the very characteristic gill lesion caused by GNV has not been observed (R.A. Elston, personal communication, 14 March 1990).

There are many reports in the literature about other viruses in oysters and other marine mollusks, including five different viruses from the eastern oyster, *Crassostrea virginica* (Johnson, 1984). There is no firm evidence that any of these viruses (other than HIV and GNV) can be pathogenic to their hosts.

3. Bacterial Diseases.

a. Bacillary Necrosis. Many species of bacteria in the genus *Vibrio* are present naturally in seawater. They are not normally pathogenic, but can become so because of adverse environmental conditions, usually high temperature. These bacteria have been implicated in often complete mortality of larvae in hatcheries from various regions of the world. Juvenile oysters have also been reported to be affected in hatcheries in Maine. Affected oyster species include *Crassostrea gigas*, *Crassostrea virginica* and *Ostrea edulis* (Elston, 1984; Sindermann and Lightner, 1988).

Potential implications: Vibrios and other bacteria that may cause this problem are present naturally in seawater. Rigorous hatchery sanitation measures usually are sufficient to prevent mortalities. The Virginia Institute of Marine Science oyster hatchery has experienced no problem of this type.

b. Nocardiosis. This disease is caused by the actinomycete bacterium *Nocardia*, and often results in raised green to yellow nodules on the mantle. It is apparently at least partially responsible for the historically reported phenomenon of summer mortality in adult *Crassostrea gigas* in the Pacific Northwest (see Friedman, Beattie, Elston and Hedrick, 1991), even though prevalence of the condition is only about 18%. Similar nodules have been observed in other oysters from other areas, including *Crassostrea virginica* (Elston, Beattie, Friedman, Hedrick and Kent, 1987), but the cause of the nodules has not been determined in those cases.

Potential implications: This is a husbandry disease with local environmental sources of the bacterium in Washington. It is not a disease of major concern in that area.

c. Ligament disease. This is a common disease of many species of juvenile bivalves (< 1 cm shell height), including *C. gigas* (Elston, 1993), but it is known only from cultured oysters. The disease results in destruction of the hinge ligament; mortality may be high, but has not been quantified. The disease appears to be caused by bacteria that occur naturally in temperate marine environments.

Potential implications. This is a husbandry disease with local sources of bacteria. There is no potential for introduction.

d. Rickettsiae. Rickettsia are obligate intracellular organisms and have been reported from digestive diverticula epithelial cells in *C. gigas* and *C. virginica* and many other bivalve mollusks (Kinne, 1983) and from vesicular connective tissue cells in *C. gigas* (Meyers and Short, 1990). Rickettsiae have not been implicated in mortality of bivalves. Extracellular giant rickettsia have been reported in *C. gigas* in Spain (Azevedo and Villalba, 1991), but rickettsia, by definition, are intracellular so the organism is probably not a rickettsia. However, it does appear to be a prokaryote (Elston, 1993). No prevalence or mortality data were provided.

Potential implications: Rickettsiae have already been reported from *Crassostrea virginica* in Chesapeake Bay.

4. Protozoan Diseases.

a. Marteilia refringens. This parasite has been responsible for massive mortality of the native oyster *Ostrea edulis* in France. *Marteilia refringens* has also been reported in *Crassostrea gigas* in France (Cahour, 1979), but prevalence and intensity were low and only early stages of development were observed. The infections were considered to be transient and no mortality has been observed in *Crassostrea gigas.*

Potential implications: This parasite is known only from Europe and does not develop normally in *Crassostrea gigas.* There is little chance of importing this parasite if the broodstock is limited to *Crassostrea gigas* from the state of Washington, and ICES guidelines for quarantine of broodstock are followed.

b. Haplosporidium spp. A parasite that is morphologically similar to *Haplosporidium nelsoni* (MSX) has been observed in *Crassostrea gigas* in Korea (Kern, 1976), Japan and California (Friedman et al., 1991; Friedman, 1996). Prevalence is low (<3%) at all sites and no mortality has been reported. Sporulation is restricted to the epithelium of the digestive diverticula as in *Haplosporidium nelsoni.* Another haplosporidan was reported in a single *Crassostrea gigas* from California (Katkansky and Warner, 1970) and from three *C. gigas* in France (Comps and Pichot, 1991). Spores were observed throughout the connective tissue, similar to *Haplosporidium costale* (SSO) in *Crassostrea virginica*, but spore size was intermediate between *Haplosporidium nelsoni* and *Haplosporidium costale.* Plasmodial stages of a haplosporidan were observed in a single *Crassostrea gigas* from Washington (Pereya, 1964).

Potential implications: There has been speculation that the two haplosporidans from Korea and California are *Haplosporidium nelsoni* and *Haplosporidium costale,* respectively, and that they were introduced to Chesapeake Bay region with unauthorized private plantings of *Crassostrea gigas* during the 1950s. Recent molecular evidence (Burreson, unpublished data) supports the introduction of *H. nelsoni* with infected *C. gigas* or with an infected intermediate host. There is little danger of importing parasites with *Crassostrea gigas* if initial broodstock are kept in

quarantine and only uninfected progeny from the hatchery are used in susceptibility studies or possible introductions.

c. Marteilioides chungmuensis. This parasite infects eggs of *Crassostrea gigas* in Japan and Korea (Comps, Park and Desportes, 1986). It is related taxonomically to important oyster pathogens such as *Marteilia refringens* discussed above, but *M. chungmuensis* is not known to cause mortality. This parasite may be what Becker and Pauley (1968) observed in eggs of *Crassostrea gigas* in California. Less than 10% of the eggs were infected in any one female oyster and there was no evidence of oyster mortality.

Potential implications: Transmission studies have never been attempted with this parasite, and the life cycle is unknown; however, this parasite infects eggs suggesting that quarantine of broodstock may not provide sufficient control. This parasite is apparently not pathogenic and it has never been reported from the Pacific Northwest.

d. Mikrocytos mackini. This parasite infects vesicular connective tissue cells and causes abscess-type focal inflammatory lesions in the mantle and gonad of *Crassostrea gigas.* It is known only from British Columbia, Canada, although a similar parasite has been observed in *Crassostrea gigas* from Hawaii (Farley, Wolf and Elston, 1988). Average mortality of 34% was observed during early occurrences of the disease before growers learned proper management techniques to avoid mortality (Bower, 1988). Oysters less than two years of age are not affected and mortality of older oysters is reduced when held high in the intertidal zone.

Potential implications: This parasite is not known from the state of Washington. Quarantine of broodstock and use of progeny for field studies would prevent introduction of the parasite even if it were present.

5. Metazoan Parasites.

Mytilicola orientalis. This highly modified copepod inhabits the digestive tract of *Crassostrea gigas* in Japan. It was introduced to the Pacific Northwest with early shipments of *Crassostrea gigas* seed from Japan and is now endemic along the west coast of the United States (Sindermann and Lightner, 1988). This parasite has been implicated in sporadic mortalities of *Crassostrea gigas*, but the evidence has never been very strong. A recent, thorough, ten-year study (Davey, 1989) on a related species in mussels found no evidence of host mortality and the author argues forcefully that *Mytilicola* has been wrongly indicted in previous mortalities.

Potential implications: This parasite infects adult oysters and can be easily controlled by quarantine of broodstock in the hatchery.

In summary, quarantine of broodstock in a hatchery and the use of first generation offspring for any field studies, that is, compliance with ICES guidelines for introduction of non-native organisms, will prevent introduction of all disease agents listed above except viruses and the ovarian parasite *Marteilioides chungmuensis*, which is not known to cause mortality. If broodstock were limited to one source, the state of Washington, such problems could be avoided

because no pathogenic viruses are known in adult *Crassostrea gigas* from Washington, and *M. chungmuensis* is absent from that area. There are no published reports of a serious disease outbreak in *Crassostrea gigas* from Washington.

Risk Analysis for Introduction of Diseases with a non-endemic species: Crassostrea rivularis.

No parasite or disease analyses of *C. rivularis* have been reported in the scientific literature.

V. Methods

The **Protocol** is offered as a series of **Objectives**. For some of the individual **Objectives** it is appropriate to offer a specific set of methods to address the **Scenario** as described. This section provides appropriate detail to the relevant **Scenarios**.

Objectives 1 through 4 build upon experience already in hand. The following methods apply to *C. virginica, C. rivularis* and *C. gigas*. ICES protocols are applied to *C. gigas* and *C. rivularis* with all culture medium and effluent being appropriately treated prior to disposal. *C. virginica* broodstock will be obtained from Mobjack Bay, VA. We also have available selected broodstocks originating from Horse Head Reef in the James River, VA (a low salinity region where disease impact is low), and from several generations of selection against continuous MSX challenge in the Delaware Bay. Broodstock of *C. gigas* and *C. rivularis* are addressed elsewhere in the document.

Spawning procedures:

Oysters are conditioned at 20-22°C at the Virginia Institute of Marine Science oyster hatchery at Gloucester Point, VA. During conditioning the oysters are subjected to supplemental feeding with cultured phytoplankton (predominantly *Isochrysis galbana* Parke, clone T-ISO).

Thermal induction (28-30°C) of natural spawning is the preferred approach for all species. Spawning can be facilitated by the addition of sperm to the holding tank. Should thermal induction fail, gonadal products can be stripped from the ripe animals. Stripping can result in markedly decreased fertilization efficiencies due to immaturity of eggs or asynchrony in development of the eggs (Downing and Allen, 1987).

Larval culture through metamorphosis:

Larvae are cultured in the VIMS oyster hatchery using a modification of Loosanoff and Davis (1963) as described by Castagna et al., (in review). Water used for culture is passed through a sand filter, a charcoal bed and, finally, a 10 um GAF polypropylene bag filter. Cultured phytoplankton (predominantly *Isochrysis galbana* and *Thalassiosira nana*) are added as food. Larval concentration is maintained in the range 15/ml decreasing to 1/ml with increasing larval size. Tanks with volumes of 75 to 5000 l are available for larval culture as required. Water is changed three times per week at which time larvae are retained on appropriate size nylon sieves,

subsampled to provide a growth record from length measurement, rinsed in filtered sea water to remove adherent debris, and returned to the newly refilled tanks.

The cycle of water changes, feeding, etc. continues until larvae develop the characteristic, pigmented "eyespot" and protruding foot of the "pediveliger" stage that is competent to metamorphose to the attached benthic form. At this time larvae are induced to settle in the following manner. Larvae are introduced into a tank containing filtered seawater at 28-30°C, about 100,000 cultured phytoplankton cells/ml and a chosen substrate. For single oysters a clean oyster shell grit in shallow, mesh trays provides optimum substrate. For small experiments where handling and counting are critical a sheet mylar substrate can be used in a shallow tray. These trays are gently aerated for 72 hours to allow distribution of larvae. The post-set oysters are divided as required for further studies.

Objective 5, Culture of Larval Forms, proposes to examine temperature and salinity effects in a matrix design as follows:

Challenges will be made to three early life history stages of each species: veliger larvae, competent-to-metamorphose pediveliger larvae, and post settlement juveniles (spat), and to ripe, adult oysters. Data should also be collected as opportunity presents on spawning and egg viability. All experiments will be effected with diploid organisms (even though VIMS has capability to produce triploid organisms in quantity) because these are the reproductively active forms which would engage in a "interspecific interaction and/or competition" if actively reproducing populations of both were present in the Chesapeake Bay. The three sections focusing on early life history stages pose the scenario of fertilized eggs developing into larvae, and these planktonic stages being dispersed into a range of salinity and temperature environments. The accompanying questions that are posed relate to survival of those early life stages, with emphasis on comparison of *C. virginica* with non-native species.

Veliger larva studies:

A **minimum** of 2 spawns for each species (preferably 3) will be required for this component of the study, preferably at least 3 weeks apart to allow completion of one larval assay before the next one starts. With such intervals a chosen larval assay will run concurrently with the metamorphosis and spat assays from the prior spawning. Each spawn will be used to set up a large culture (500L) and a small culture (75L) using general culture methods given earlier. Two individual females or maternal groups will be isolated and used to produce these cultures. The large culture will be subsampled on day 1 for one set of replicate larvae to start growth/survival series at the chosen temperature and salinity values (given below). The remainder of this large culture will be maintained through metamorphosis to produce pediveligers and spat for the subsequent layer of T-S challenges. The small culture provides the true statistical replication and will be used to start replicate T-S series. The small culture is, therefore, only an overnight requirement. If enough spawning females cannot be obtained to effect replication within one experimental trial then the two temporally separate runs serve as replicates. Small scale closed systems will be used for T-S challenges and all larvae will be sacrificed at the end of the study.

All larvae will be examined as follows. Assume a maximum of 6 salinities (5, 10, 15, 20, 25, 30 ppt, essentially the entire range of oyster habitat in the Bay) X 3 temperatures (20, 25, 30°C, increasing from the minimum to induce spawning in both species). A 2L beaker will be the standard experimental container for these T-S challenges. This experimental design requires 18 beakers per experimental trial. If separate maternal spawns (replicates within one spawning event rather then temporally separated spawnings) are obtained then this increases to 36 beakers. Several temperature controlled rooms are available for this purpose. Salinity control is effected by dilution of filtered sea water of >30 ppt (see Mann, Campos and Luckenbach, 1991) or controlled addition of sea water salts. The latter approach has been used successfully in VIMS' laboratory for sensitive larval behavior work and found comparable to dilution of full strength sea water (unpublished data). All sea water is made up on the day of use and stored in Nalgene carboys prior to use.

Water change for the experimental challenges will be effected 3X per week, a frequency found optimal to growth in the VIMS oyster hatchery. Embryos will remain at the temperature and salinity of spawning (12-22 ppt depending upon season and operation of the VIMS oyster hatchery) for the first 24 hours after fertilization and will be transferred to the aforementioned 2L beakers at day 1 with the first water change. Options exist for temperature and salinity acclimation as part of the experimental protocol. A no acclimation protocol involves direct transfer to "experimental temperature and salinity" on day 1 from hatchery ambient. An acclimation protocol requires serial changes of sea water. Given circulation patterns in the Chesapeake Bay subestuaries, such as the James River, a rate of 2ppt/day might be a reasonable approach (see comments in Mann, 1988 and references therein on circulation). Initial stocking density of larvae will not exceed 20/ml. The choice of a 2L beaker as a replicate size is a compromise to provide sufficient statistical power and environmental range with practicality in handling that will require care in sampling and handling to minimize loss of larvae during the experimental trial. Water changes will be made on days 1, 3, 5, 8, 10, 12, 15 and so on. Prior to water changes larval numbers will be estimated by subsampling and counting in a Sedgewick Rafter cell. Algae food consumption will be estimated by counting residual food concentrations using a hemacytometer on a compound microscope. Unpublished data collected by Dr. B.J. Barber in 1990 strongly suggest that many of the outlying temperature-salinity combinations will be eliminated in the first week of the experiment, and that the optimal T-S combinations will be very obvious. If this is so only a limited number of T-S combinations may survive through to pediveliger stage. Larvae will be fed with Tahitian strain *Isochrysis galbana* as used in the VIMS hatchery operation. When fed at 100 cells/ul (=10^5 cells/ml = 10^8 cells/l) 36 beakers at 2l requires approximately 7.2 x 10^9 cells, or about 1.5l of food at standard hatchery culture concentrations of 5 x 10^6 cells/ml.

At each water change two subsamples (1ml each) of larvae will be taken from the known volume of water in the beaker (after thorough mixing) and counted as described earlier to estimate survival. These larvae will be fixed and stored in buffered formalin and subsequently measured for length (defined here as longest dimension parallel to the hinge line) to assess growth. When animals develop pigmented eyespots a mylar substrate (optimally preconditioned for 3-5 days in running seawater in the presence of live oysters, Dr. P.K. Baker, unpublished data from studies at VIMS, 1992-1993) can be provided for settlement. If pre-settlement numbers are known then

percentage settlement versus time after eyespot appearance can be estimated. If, however, this is precluded by low surviving numbers of larvae such data can be collected as described in the following section.

Pediveliger settlement studies:

This protocol is based on unpublished methods developed by Dr. S.M. Baker as part of a Ph.D. dissertation study at VIMS (Ph.D. 1994). Although developed for *C. virginica* it will be used here for all species.

Larvae will be grown to pediveliger stage in the hatchery as described. Known numbers of apparently competent to settle larvae (defined as having a well developed eyespot), subsampled from a single population, will be placed in petri dishes with salinity adjusted sea water and mylar substrate (preconditioned as described earlier). No settlement inducer will be used. Larval settlement will be assayed over a fixed time period (24 hours for completion of the attachment process) before removing the mylar, with attached larvae, and transferring it to a second petri dish containing salinity adjusted sea water plus neutral red. Uptake of neutral red facilitates subsequent counting of larvae. By this approach percentage of larvae settling and attaching can be estimated. It is important to note that larvae are sampled from a single population and do not provide valid statistical replication. Trials from sequential cultures provide replication.

Objective 6, Culture of early post settlement forms: physical requirements, is addressed by the following methods:

All species will be examined. Again, assume a design of 6 salinities (5, 10, 15, 20, 25, 30 ppt) X 3 temperatures (20, 25, 30°C). Spat for this study will be set on mylar sheet to facilitate handling and counting. The option exists to provide a chemical inducer to settlement. This provides a synchronized settlement. Unpublished data (S.M. Baker and R. Mann) obtained using DMSO as an inducer has shown this approach to be valuable when examining the time course of post settlement events; however, this is not critical in this design. Both "synchronized" and untreated options will be examined if time permits. T-S challenges will commence shortly after settlement and be sustained for a period of up to three weeks. Note that the focus here is post settlement; the attachment and settlement events **per se** have been covered in the previous section. It is probably better to use animals that are several weeks old to facilitate handling, counting and measurement. The standard container size will be a 10L white Nalgene bucket. There will be no simultaneous true replication of the treatments in this protocol because that would require simultaneous culture of two groups of larvae (duplicate substrates in the same or adjacent containers provide replication of handling stress but not biological replication because spat originate from one larval culture—these are pseudoreplicates). The experiment can, however, be repeated with temporal separation of spawns to provide replication. Water will be changed 3x/week with complete water changes and tank cleaning. Feeding concentration and algae species are as for larval challenges. Rather than periodic sacrificial sampling, as described for larval studies, estimates of survival (total counts after recording initial number of spat present) and length (from a subsample,

avoiding areas where growth may be inhibited by individual interactions) will be made on living material at every other water change. If this proves problematic in real time (improbable, but a worst case scenario) a photographic recording and image analysis technique developed in the VIMS laboratory (Roegner, 1990) will be employed to facilitate data collection. At the end of the experiment isolated individual animals will be removed from the substrate for biomass estimate.

Objective 7, Culture of early post settlement forms: macro organism interactions, will be effected as follows:

The scope of this **Objective** must clearly be limited because the possible interactions with predator and "pest" species native to the Chesapeake Bay are potentially enormous; however, we propose to build upon previous experience with *C. gigas* in quarantine systems (see section III.F.6 of this document) in maintaining target species or strains of species in a flume system supplied with unfiltered sea water. The influent water will contain larval forms of local invertebrates which have the opportunity to settle and grow with the experimental populations. This approach has proven successful in "infecting" *C. gigas* populations with *Polydora ligni* as described earlier (III.F.6). In this manner the experiment is observational and allows selection of the most important interactions to develop rather than enforce a chosen species interaction. This observational experiment can be effected on a continuing basis as soon as post settlement specimens become available.

Objective 8, Culture of early post settlement forms: disease organism interactions, will be effected by methods briefly described earlier (III.F.6) and in detail in Meyers et al (1991) for *P. marinus* challenges. MSX challenge requires in water challenge in a location of known activity. Details of a prior experiment are given in III.F.6.

Objective 9, Triploidy induction in remaining candidate species, is self explanatory.

Objective 10, In water challenge for MSX, will follow the design used in a previous study described in section III.F.6.

Objective 11, Spawning and Egg viability of Adult Oysters, addresses an issue that relates to range extension in an introduced population. The study will utilize the following procedures:

Unlike the previous sections which focus on early life history, this section proceeds in time and poses a scenario of settling stages having survived settlement and juvenile growth to a size that is capable of spawning. The question that is posed in this scenario is "what is the influence of salinity environment on the ability of the spawning oysters to provide viable eggs, and hence viable larvae?" There is surprisingly little quantitative published literature on the influence of salinity on either egg production or egg viability—yet in a situation where interspecific interactions exists or may exist such data is crucial to prediction of long term consequences. Apart from the obvious value of such data in predicting impact of non-indigenous introductions, the management implications of such data for native species in

isolation suggest grave consequences for the present fishery and management practices. Recent data collected in VIMS' long term studies of the James River seed oyster beds (Cox and Mann, 1992; Mann et al 1994) which examined both temporal changes in fecundity and egg viability (that percentage of the eggs which can produce viable embryos) through weekly sampling over a two year period strongly suggest that low salinities (<12 ppt), critical as refuge areas from the deleterious impact of both *H. nelsoni* and *P. marinus* and potential regions of interspecific competition between native and non-native oysters, may drastically reduce both fecundity and egg viability in a non-linear manner compared with higher salinities. For example, a decrease in salinity from 12 ppt to 10 ppt corresponds to a one order of magnitude decrease in mean fecundity for oysters of comparable size! Further, fecundity estimates have to be modified by consideration of egg viability, in that it is egg viability rather than fecundity that is strongly correlated with subsequent settlement data for oysters in the James during the study period. The implications of such data are several and serious. They suggest that...

(1) Present oyster populations are maintained in a spatially disproportionate manner with most oysters surviving in a narrow salinity "band" at salinities where disease and commercial exploitation has not yet eliminated them.

(2) Such "band" populations are vulnerable to interspecific competition by a competing species with a wide tolerance of estuarine salinities but apparently lacking the ability to survive salinities of <5ppt.

(3) Such "band" populations are probably the most intensely exploited because they are usually larger and considered more acceptable at market than oysters from lower salinities.

(4) Fecundity values alone do not represent potential reproductive contribution in low salinity populations.

(5) Standing stock measures in low salinity disproportionately overestimate potential reproductive contribution and therefore underestimate the gravity of the problem of broodstock preservation in fishery management.

The recruitment of an oyster into a particular location and salinity environment reflects only supply of larvae to that location and environmental conditions during that period. It does not necessarily reflect the range or mean salinities that characterize that location, yet it is the latter that will determine spawning ability of the resident oysters. In this section of the proposed comparative study quantitative estimates of fecundity and egg viability will be made for all three oyster species of comparable age and size maintained under comparable salinity environments.

Oysters will be approximately two years old at time of investigation. This is large enough to provide eggs but small enough to facilitate management of sufficient animals to provide sound statistical design to the experiment. Previous field studies (referenced earlier) have used a minimum of 10 female oysters per sampling event to estimate fecundity and egg viability with

all estimates being made on individual animals. Asynchrony in gametogenesis in field populations can provide considerable variability in assays within a sample; however, considerably less variability in laboratory manipulated stocks is expected because prior history of temperature and salinity exposure can be controlled.

C. virginica will be progeny of animals from local broodstocks. *C. gigas and C. rivularis* will be progeny from ICES treated protocol broodstocks. Following larval culture in the VIMS hatchery these animals will be maintained in the flowing sea water quarantine facility at VIMS, Gloucester Point. Both species will be maintained at ambient salinity during the summer through winter period. By early the following spring all animals should be devoid of any gametes if any were produced in year of birth, and therefore available for experimentation from an initially inactive state. Previous studies have demonstrated the importance of broodstock condition and conditioning on egg and larval viability (see Helm, Holland and Stephenson, 1973; Gallager and Mann, 1986; Gallager, Mann and Sasaki, 1986), although none have focused on salinity effects. The time course of conditioning both *C. virginica* and *C. gigas* are well documented (Price and Maurer, 1970 and Mann, 1979b, respectively) and have been successfully applied in the VIMS hatchery; data from Langdon and Robinson (unpublished personal communication) provides guidelines for studies with *C. rivularis*. Populations of all species will be conditioned under the same basic protocol. Ambient salinity varies between 12 and 20 ppt at site in spring. Successful spawnings have been obtained in this range by both *C. virginica* and *C. gigas* at the VIMS facility in past years; however, experience at <15 ppt with *C. gigas* is limited to one spawn. VIMS has yet to attempt lower than full sea water salinity conditioning or spawning with *C. rivularis*. The focus will include <15ppt, essentially the range occupied by the oysters remaining in the Chesapeake Bay at this time. Oysters will be conditioned at 15ppt, 12ppt and 9ppt in closed tank systems at controlled temperature with controlled feeding and periodic water changes (3x/week). Feeding and salinity adjustment procedures will be as for veliger, pediveliger and spat studies. The attainment of ripeness will be periodically assayed by sacrifice of individuals for egg examination from gonadal smears and thermal stimulation of such individuals to induce spawning (details as given earlier in section on general culture methods). A minimum of 50 animals of each species (and geographical type for *C. gigas*) will be conditioned at each salinity. This will allow for periodic removal for gonadal smear examination, and for three groups of ten animals each to be examined to assess fecundity and egg viability at weekly intervals after ripeness is attained. This design provides for examination of temporal change in fecundity and viability. Note that spawning is unlikely at the conditioning temperature, so spent individuals are expected to be a very small proportion of the total examined. Assay methods will be as used in previous studies. Briefly, these are as follows:

To estimate individual fecundity, whole (wet) oyster tissue is transferred to a commercial blender. After blending, the homogenate is washed through a 500 um sieve to remove large debris, and eggs retained on further washing through a 25 um sieve. Eggs are washed into a calibrated glass cylinder and the cylinder contents made up to a known volume with 1 um filtered sea water. The cylinder contents are thoroughly mixed and subsamples removed for counting using a Sedgewick Rafter cell under low power magnification on a compound

microscope. All counts are replicated. Fecundity estimates are obtained from proportional volumes of cylinder contents and subsample volumes.

Egg viability is determined as follows: Three aliquots, each of 50,000 eggs, are removed from eggs isolated in the fecundity estimation procedure. Each aliquot is transferred to a 100 ml beaker and made up to 50 ml volume. The resultant egg concentration is 100 eggs/ml. To each beaker an aliquot of sperm, isolated in the same manner after homogenization of male oysters from the same source and repeated passage of homogenate through a 20 um sieve, is added to give a final sperm concentration of 3×10^5 sperm/ml. After 45 minutes the sperm and egg mixture, now containing fertilized and developing eggs, is gently sieved through a 20 um sieve to remove excess sperm and the retained material returned to 100 ml beakers containing 50 ml of filtered sea water. The eggs are left to develop overnight and subsamples subsequently removed to count numbers of first shelled veliger larvae present. Percentage viability is estimated as that percentage of eggs transferred to the beaker prior to sperm addition resulting in active veliger larvae.

REFERENCES, PART 2

Ahmed, M. 1971. Oyster species of West Pakistan. Pakistan J. Zoology 3(2): 229-236.

Ajana, A.M. 1980. Fishery of the mangrove oyster, *Crassostrea gasar* Adanson (1757) in the Lagos area, Nigeria. Aquaculture 21 (2): 129-137.

Allen, M.J., R.J. Wolotira, Jr., T.M. Sample, S.F. Noel and C.R. Iten. 1988. Life history and harvest information for the Pacific oyster, *Crassostrea gigas* (Thunberg, 1793). NWAFC Tech. Mem. Ser.

Amemiya, I. 1926. Notes on experiments on the early developmental stages of the Portuguese, American, and English native oysters, with special reference to the effect of varying salinity. J. Mar. Biol. Assoc. U.K. 31 (1): 161-175.

Andrews, J.D. 1980. A review of introductions of exotic oysters and biological planning for new importations. Mar. Fish. Rev. 42(12): 1-11.

Azevedo, C. and A. Villalba. 1991. Extracellular giant rickettsiae associated with bacteria in the gill of *Crassostrea gigas* (Mollusca, Bivalvia). J. Invert. Pathol. 58: 75-81.

Barber, B.J., S.E. Ford and H.H. Haskin. 1988a. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. 1. Condition index and relative fecundity. J. Shellfish Res. 7: 25-31.

Barber, B.J., S.E. Ford and H.H. Haskin. 1988b. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. 11. Tissue biochemical composition. Comp. Biochem. Physiol. 91A: 603-608.

Bardach, J.E., J.H. Ryther and W.O. McLarney. 1972. Aquaculture: The Farming and Husbandry of Freshwater and Marine Organisms. John Wiley & Sons, Inc., New York, NY; 868 pp.

Beaumont, A.R. and J.E. Fairbrother. 1991. Ploidy manipulation in molluscan shellfish: A review. J. Shellfish Res. 10(1): 1-18.

Becker, C.D. and G.B. Pauley. 1968. An ovarian parasite (*Protista incertae sedis*) from the Pacific oyster, *Crassostrea gigas*. J. Invertebr. Pathol. 12: 425-437.

Boffi, A.V. 1979. Mollusco Brasileiros de Interesse Medico e Economico. Simbolo S.A. Industrias Graficas, Sao Paulo, Brasil; 182 pp.

Boveda, J.V.P. and R.J. Rodriguez. 1987. Supervivencia de la ostra de mangle *Crassostrea rhizophorae* (Gilding, 1828) a las variaciones de temperatura, salinidad y pH. Sociedad de Ciencias Naturales la Salle Memoria 47 (127-128): 217-231.

Bower, S.M. 1988. Circumvention of mortalities caused by Denman Island oyster disease during mariculture of Pacific oysters. Amer. Fish. Soc. Spec. Publ. 18: 246-248.

Breen, P.A. 1992. A review of models used for stock assessment in abalone fisheries. pp 253-275 in: Abalone of the World: Biology, Fisheries and Culture. S. A. Shepherd, M. J. Tegner, S. A. Guzman del Proo (Eds.). Fishing News Books, Blackwell Scientific, 608p.

Breese, W.P. and R.E. Malouf. 1977. Hatchery rearing techniques for the oyster *Crassostrea rivularis* Gould. Aquaculture 12: 123-126.

Bros, W.E. and B.C. Cowell. 1987. A technique for optimizing sample size (replication). J. Exp. Mar. Biol. Ecol. 114: 63-71.

Burreson, E. M., R. Mann and S.K. Allen. 1994. Field exposure of triploid *Crassostrea gigas* to *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) in the lower Chesapeake Bay. J. Shelf. Res. 13: 293.

Butler, P.A. 1949. Gametogenesis of the oyster under conditions of depressed salinity. Biol. Bull. 96(3): 263-269.

Cahour, A. 1979. *Marteilia refringens* and *Crassostrea gigas*. Mar. Fish. Rev. 41(1-2): 19-20.

Carreon, J.A. 1969. The malacology of Philippine oysters of the genus *Crassostrea* and a review of their shell characters. Proc. Nat. Shellfish. Assoc. 59: 104-115.

Castagna, M., M.C. Gibbons and K. Kurkowski. (In Review). Culture: Applications. Chapter 19 In: Eble, A., A. Rosenfield and V.S. Kennedy (Eds.). The Oyster *Crassostrea virginica*.

Chanley, P.E. 1958. Survival of some juvenile bivalves in water of low salinity. Proc. Nat. Shellfish. Assoc. 48: 52-65.

Chen, T.P. 1972. Status and problems of coastal aquaculture in Thailand. 74-83. In Pillay, T.V.R. (ed.). Coastal Aquaculture in the Indo-Pacific Region. Whitefriars Press, Ltd., London, U.K.

Comps, M. 1988. Epizootic diseases of oysters associated with viral infections. Amer. Fish. Soc. Spec. Publ. 18:23-27.

Comps, M. and J.R. Bonami. 1977. Infection virale associée à des mortalités ches l'huitre *Crassostrea gigas* th. C. R. Acad. Sci. Paris, Ser. D 285: 1139-1140.

Comps, M., M.S. Park and I. Desportes. 1986. Étude ultrastructurale de *Marteilioides chungmuensis* n. g., n. sp. parasite des ovocytes de l'huitre *Crassostrea gigas* Th. Protistologica 22(3): 279-285.

Comps, M. and Y. Pichot. 1991. Fine spore structure of a haplosporidan parasitizing *Crassostrea gigas*: taxonomic implications. Dis. Aquat. Org. 11: 73-77.

Cox, C. and R. Mann. 1992. Fecundity of oysters, *Crassostrea virginica* (Gmelin), in the James River, Virginia, U.S.A. J. Shellfish Res. 11(1): 47-52.

Davey, J.T. 1989. *Mytilicola intestinalis* (Copepoda: Cyclopoida): a ten year survey of infested mussels in a Cornish estuary, 1978-1988. J. Mar. Biol. Assoc. U. K. 69: 823-826.

Davis, H.C. 1958. Survival and growth of clam and oyster larvae at different salinities. Biol. Bull. 114 (1): 57-70.

Davis, H.C. and A. Calabrese. 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. U.S. Fish Wildl. Ser. Fish. Bull. 63(3): 643-655.

Desai, K.M., B. Patel and H. Dave. 1982. Laboratory rearing of eggs and larvae of edible oysters of the Gulf of Kutch. Proceedings of the Symposium on Coastal Aquaculture, Cochin, India, 1980 6: 704.

Dos Santos, A.E. and I.A. Nascimento. 1985. Influence of gamete density, salinity, and temperature on the normal development of the mangrove oyster, *Crassostrea rhizophorae* Guilding, 1828. Aquaculture 47 (4); 335-352.

Douglass, W.R. 1977. *Minchinia nelsoni* disease development, host defense reactions, and hemolymph enzyme alterations in stocks of oysters (*Crassostrea virginica*) resistant and susceptible to *Minchinia nelsoni* caused mortality. Ph.D. Dissertation. Rutgers University, New Brunswick, NJ. 232p.

Downing, S.L. and S.K. Allen, Jr. 1987. Induced triploidy in the Pacific oyster. *Crassostrea gigas*: Optimal treatments with cytochalasin B depend on temperature. Aquaculture 61: 1-15.

Durve, V.S. 1965. On the seasonal gonadal change and spawning in the adult oyster *Crassostrea gryphoides* (Schlotheim). J. Mar. Biol. Assoc. India. 7(2): 328-344.

Durve, V.S. 1967. On the nomenclature of two Indian backwater oysters. J. Mar. Biol. Assoc. India. 9(1): 173-178.

Elton, C.S. 1958. The ecology of invasions by animals and plants. Methuen and Co. Ltd., London. 181p.

Elston, R.A. 1984. Prevention and management of infectious diseases in intensive mollusc husbandry. J. World Maricult. Soc. 15: 284-300.

Elston, R.A. 1993. Infectious diseases of the Pacific oyster, *Crassostrea gigas*. Ann. Rev. Fish Dis. 3: 259-276.

Elston, R.A. , J.H. Beattie, C. Friedman, R. Hedrick and M.L. Kent. 1987. Pathology and significance of fatal inflammatory bacteraemia in the Pacific oyster, *Crassostrea gigas* Thünberg. J. Fish Dis. 10: 121-132.

Elston, R.A. and M.T. Wilkinson. 1985. Pathology, management and diagnosis of oyster velar virus disease (OVVD). Aquaculture 48: 189-210.

Farley, C.A., P.H. Wolf and R.A. Elston. 1988. A long-term study of "microcell" disease in oysters with a description of a new genus, *Mikrocytos* (g. n.), and two new species, *Mikrocytos mackini* (sp. n.) and *Mikrocytos roughleyi* (sp. n.). Fish. Bull. 86(3): 581-593.

Ford. S.E. 1986. Comparison of hemolymph proteins between resistant and susceptible oysters. *Crassostrea virginica*. exposed to the parasite *Haplosporidium nelsoni* (MSX). J. Invert. Pathol. 47: 283-294.

Ford. S.E. and H.H. Haskin. 1987. Infection and mortality patterns in strains of oysters *Crassostrea virginica* selected for resistance to the parasite *Haplosporidium nelsoni* (MSX). J. Parasitol. 73: 368-376.

Friedman, C.S. 1996. Haplosporidian infections of the Pacific oyster, *Crassostrea gigas* (Thunberg), in California, U. S. A. and Japan. J. Invert. Pathol. (In press).

Friedman. C.S., J.H. Beattie, R.A. Elston and R.P. Hedrick. 1990. Investigation of the relationship between the presence of a Gram-positive bacterial infection and summer mortality of the Pacific oyster, *Crassostrea gigas*, Thunberg. Aquaculture. 94(1): 1-16.

Friedman, C.S., D.F. Cloney, D. Manzer and R.P. Hedrick. 1991. Haplosporidiosis of the Pacific oyster, *Crassostrea gigas*. J. Invert. Pathol.58: 367-372.

Gallager, S.M. and R. Mann. 1986. Growth and survival of larvae of *Mercenaria mercenaria* and *Crassostrea virginica* relative to broodstock conditioning and lipid content of eggs. Aquaculture 56(2): 105-122.

Gallager, S.M., R. Mann and G.C. Sasaki. 1986. Lipids as an index of growth and viability in three species of bivalve larvae. Aquaculture 56(2): 81-104.

Glude, J.B. 1975. A summary report of Pacific coast oyster mortality investigations 1965-1972. Proc. 3rd U.S.-Japan Meeting on Aquaculture, 1974: 1-28.

Heral, M. and J.M. Deslous-Paoli. (1990). Oyster Culture in European Countries. In: Estuarine and Marine Bivalve Mollusc Culture. R. W. Menzel (Ed). CRC Press, Boca Raton, FL. pp 153-190.

Hedgecock, D. and N.B. Okazaki. 1984. Genetic diversity within and between populations of American oysters (*Crassostrea*). Malacologia 25 (2): 535-549.

Helm, M.M., D.L. Holland and R.R. Stephenson. 1973. The effect of supplementary algal feeding of a hatchery broodstock of *Ostrea edulis* L. on larval vigour. J. Mar. Biol. Assoc. U.K. 53:673-684.

Hine, P.M. , B. Wesney, and B.E. Hay. 1992. Herpes viruses associated with mortalities among hatchery reared larval Pacific oysters *Crassostrea gigas*. Dis. Aquat. Org. 12: 135-142.

His, E., R. Robert and A. Dinet. 1989. Combined effects of temperature and salinity on fed and starved larvae of the Mediterranean mussel *Mytilus galloprovincialis* and the Japanese oyster *Crassostrea gigas*. Mar. Biol. 100 (4): 455-463.

Hughes Games, W.L. (1977) Growing the Japanese oyster (*Crassostrea gigas*) in subtropical seawater fish ponds: 1. Growth rate, survival and quality index. Aquaculture, 11(3): 217-230.

Jhingran, V.G. and V. Gopalakrishnan. 1974. Catalogue of cultivated aquatic organisms. FAO Fisheries Technical Paper 130; 83 pp.

Johnson, P.T. 1984. Viral diseases of marine invertebrates. Helgolander Meeresunters. 37: 65-98.

Jones, S. 1970. The molluscan resources of India. Proceedings of the Symposium on Mollusca, Cochin, India, Part III: 906-918.

Joseph, M.M. and M.N. Madhyastha. 1984. Annual reproductive cycle and sexuality of the oyster *Crassostrea madrasensis* (Preston). Aquaculture 40 (3): 223-231.

Kamara, A.B., K.B. McNeil and D.B. Quayle. 1976. Tropical oyster culture: problems and prospects. 344-348. In Pillay, T. V. R. and Dill, W. A. (eds.). Advances in Aquaculture: FAO Technical Conference on Aquaculture, Kyoto, Japan, 1976. Fishing News Books, Ltd., Surrey, England.

Katkansky, S.C. and R.W. Warner. 1970. Sporulation of a haplosporidan in a Pacific oyster (*Crassostrea gigas*) in Humboldt Bay, California. J. Fish. Res. Bd. Canada 27(7): 1320-1321.

Kern, F.G. 1976. Sporulation of *Minchinia* sp. (Haplosporida, Haplosporidiidae) in the Pacific oyster *Crassostrea gigas* (Thunberg) from the Republic of Korea. J. Protozool 23(4): 498-500.

King, M.G. 1977. Cultivation of the Pacific oyster (*Crassostrea gigas*) in a non-tidal hypersaline pond. Aquaculture 11 (2): 123-136.

Kinne, O. (Ed.) 1983. Diseases of Marine Animals. Vol II, Introduction, Bivalvia to Scaphopoda. Biologische Anstalt Helgoland, Hamburg, 571p.

Koganezawa, A. 1975. Present status of studies on the mass mortality of cultured oysters in Japan and its prevention. Proc. 3rd U.S.-Japan Meeting on Aquaculture, 1974: 29-34.

Kong, C.P. and L.A. Luh. 1977. Notes on the efficiency of various materials tested as oyster spat collectors in Cowie Bay, Sabah. Malaysian Agricult. Jour. 50 (4): 462-479.

Kusuki, Y. (1990) Oyster culture in Japan and adjacent countries: *Crassostrea gigas* (Thunberg). In: Estuarine and Marine Bivalve Mollusc Culture. R. W. Menzel (Ed). CRC Press, Boca Raton, FL. pp 227-244.

Le Deuff, R.M., J.L. Nicolas, T. Renault and N. Cochennec. 1994. Experimental transmission of a herpes-like virus to axenic larvae of Pacific oyster, *Crassostrea gigas*. Bull. Eur. Assoc. Fish Pathol. 14: 69-72.

Le Gall, J.L. and O. Raillard. 1988. Influence de la temperature sur la physiologie de l'huitre *Crassostrea gigas*. Oceanis 14 (5): 603-608.

Loosanoff, V.L. 1958. Some aspects of behavior of oysters at different temperatures. Biol. Bull. 114 (1): 57-70.

Loosanoff, V.L. 1969. Maturation of gonads of oysters, *Crassostrea virginica*, of different geographical areas subjected to relatively low temperatures. Veliger 11 (3): 153-163.

Loosanoff, V.L. and H.C. Davis. 1952. Temperature requirements for maturation of gonads of northern oysters. Biol. Bull. 103 (1): 80-96.

Mane, U.H. 1978. Survival and behavior of oysters in water of low salinities at Ratnagiri on the west coast of India. J. Molluscan Studies 44 (2): 243-249.

Mann, R. (Ed.). 1979. Exotic species in mariculture. The MIT Press. Cambridge, MA. 363p.

Mann, R. 1979. Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* (Thunberg) and *Ostrea edulis* L. grown at sustained elevated temperatures. J. Mar. Biol. Ass. U.K. 59: 95-110.

Mann, R. 1981. The role of introduced bivalve mollusc species in mariculture. J. World Maricult. Soc. 14: 546-559.

Mann, R., B.M. Campos, and M.W. Luckenbach. (1991). Swimming rate and responses of larvae of three mactrid bivalves to salinity discontinuities. Mar. Ecol. Prog. Ser. 68:257-269.

Mann, R., R. Morales-Alamo, and J.S. Rainer. (1994). Reproductive activity of oysters, *Crassostrea virginica* Gmelin, in the James River, Virginia, during 1987-1988. J. Shellfish Res. 13(1): 157-164.

Menzel, R.W. 1974. Portuguese and Japanese oysters are the same species. Journal of the Fisheries Research Board of Canada 31 (4): 453-456.

Menzel, R.W. (Ed). 1990. Estuarine and Marine Bivalve Mollusc Culture. CRC Press, Boca Raton, FL. 361 pp.

Meyers, T.R. and S. Short. 1990. Summer mortalities and incidental parasitisms of cultured Pacific oysters in Alaska. J. Aquat. Anim. Health 2: 172-176.

Muranaka, M.S. and J.E. Lannan. 1984. Broodstock management of *Crassostrea gigas*: environmental influences on broodstock conditioning, Aquaculture 39 (1-4): 217-228.

Myhre, J.L. 1973. Levels of infection in spat of *Crassostrea virginica* and mechanisms of resistance to the haplosporidan parasite *Minchinia nelsoni*. M. S. Thesis. Rutgers University, New Brunswick. NJ. 102p.

Nell, J.A. and J.E. Holliday. 1988. Effects of salinity on the growth and survival of Sydney rock oyster (*Saccostrea commercialis*) and Pacific oyster (*Crassostrea gigas*) larvae and spat. Aquaculture 68 (1): 39-44.

Newball, S. and M.R. Carriker. 1983. Systematic relationship of the oysters *Crassostrea rhizophorae* and *C. virginica*: a comparative ultrastructural study of the valves. American Malacological Bull. 1: 35-42.

Newell, R.I.E. 1985. Physiological effects of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber and Mackin) on the American oyster *Crassostrea virginica* (Gmelin). J. Shellfish Res. 5: 91-95.

Nicolas, J.L., M. Comps and N. Cochennec. 1992. Herpes-like virus infecting Pacific oyster larvae, *Crassostrea gigas*. Bull. Eur. Assoc. Fish Pathol. 12: 11-13.

Pereya, W.T. 1964. Mortality of Pacific oysters, *Crassostrea gigas* (Thunberg), in various exposure situations in Washington. Proc. Nat. Shellfish. Assoc. 53: 51-63.

Pollard, D.A. and P.A. Hutchings. 1990. A Review of Exotic Marine Organisms Introduced to the Australian Region. II. Invertebrates and Algae. Asian Fisheries Science. 3: 223-250.

Price, K.S. and D. Maurer 1970. Holding and spawning Delaware Bay oysters out of season. Temperature requirements for maturation of gonads. Proc. Nat. Shellfish. Assoc. 61: 29-34.

Quayle, D.B. 1989. Pacific Oyster Culture in British Columbia. Can. Bull. Fish. Aqua. Sci. 218.

Rao, K.V. 1951. Observations on the probable effects of salinity on the spawning, development, and setting of the Indian backwater oyster, *Ostrea madrasensis* Preston. Proc. Indian Acad. Sci. 33: 231-256.

Rao, K.V. and K.N. Nayor. 1956. Rate of growth in spat and yearlings of the Indian backwater oyster *Ostrea madrasensis* Preston. Indian J. Fisheries 3 (2): 231-260.

Reeb, C.A. and J.C. Avise. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster. Genetics 124: 397-406.

Rheinhardt, R.D. and R. Mann. 1990. Development of epibenthic fouling communities on a natural oyster bed in the James River, Virginia. Biofouling: 2: 13-25.

Sandison, E.E. 1966. The effect of salinity fluctuations on the life cycle of *Gryphaea gasar* ((Adanson) Dautzenberg) in Lagos Harbor, Nigeria. J. Animal Ecol. 35 (2): 379-389.

Sandison, E.E. and M.B. Hill. 1966. The distribution of *Balanus pallidus stutsburi* Darwin, *Gryphaea gasar* ((Adanson)Dautzenberg), *Mercierella enigmatica* Fauvel and *Hydroides uncinata* (Philippi) in relation to salinity in Lagos Harbor and adjacent creeks. J. Animal Ecol. 35 (1): 235-250.

Shafee, M.S. and M.R. Sabatie. 1986. Croissance et Mortalite des Huitres dans la Lagune de Oualidia (Maroc). Aquaculture. 53:201-214.

Sindermann, C.J. and D.V. Lightner. 1988. Disease diagnosis and control in North American marine aquaculture. Elsevier, New York. 431p.

Stephen, D. 1980. The reproductive biology of the Indian oyster *Crassostrea madrasensis* (Preston): I. Gametogenic patterns and salinity. Aquaculture 21 (2): 139-146.

Tebble, N. 1966. British Bivalve Seashells. British Museum of Natural History, London, England; 212 pp.

Torigoe, K. 1981. Oysters in Japan. J. Sci. Hiroshima University, 29B(2): 291-419.

Wells, H.W. 1961. The fauna of oyster beds, with special reference to the salinity factor. Ecological Monographs 31 (3): 239-266.

Zenkevitch, L. 1963. Biology of the seas of the U.S.S.R. John Wiley & Sons, Inc., New York, NY; 955 pp.

APPENDIX A: House Joint Resolution No. 450

GENERAL ASSEMBLY OF VIRGINIA -- 1995 SESSION

HOUSE JOINT RESOLUTION NO. 450

Requesting the Virginia Institute of Marine Science to develop a strategic plan for molluscan shellfish research and begin the process of seeking necessary approvals for in-water testing of non-native oyster species.

Agreed to by the House of Delegates, February 4, 1995
Agreed to by the Senate, February 21, 1995

WHEREAS, the management and productivity of shellfish populations in Virginia's waters depend on a vigorous program of scientific investigation and research; and

WHEREAS, a range of important issues facing the native oyster supply demands further research including studies on oyster diseases, immunity, genetics and breeding; and

WHEREAS, further research is necessary to determine the potential for cultivating species not currently being cultured in Virginia's waters; and

WHEREAS, all shellfish research conducted by the agencies of the Commonwealth should be done in a coordinated and strategic fashion; now, therefore, be it

RESOLVED by the House of Delegates, the Senate concurring, That the Virginia Institute of Marine Science be requested to undertake the development of a strategic ten-year plan for molluscan shellfish research and to begin the process of seeking approvals in conformance with state, federal, and international laws and protocols for the in-water testing of oyster species not native to Virginia waters. The plan shall take into account the views of members of the shellfish industry and the interested public, related Chesapeake Bay regional, national and international initiatives, and shall, at a minimum, include: (i) an assessment of recent research on shellfish stocks, diseases, habitat and other facets germane to shellfish culture; (ii) research on oyster diseases, including studies of immunity, genetics and selective breeding for disease resistance; (iii) research necessary to identify suitable species for aquaculture and the development of methods for culture of those species; (iv) studies of the economic viability of candidate aquaculture species; (v) research on non-native species with respect to disease resistance and survivability in local waters; and (vi) an assessment of available funding vehicles and unmet needs to conduct the activities called for in the plan.

All agencies of the Commonwealth shall assist in the conduct of this study as requested by the Director of the Institute.

The Institute shall complete its work in time to submit its findings, including a report on the progress in seeking approvals for in-water testing of non-native oyster species, to the Governor and the 1996 Session of the General Assembly as provided in the procedures of the Division of Legislative Automated Systems for the processing of legislative documents.