APPENDIX B

ECOLOGICAL RISK ASSESSMENT FOR OYSTER RESTORATION ALTERNATIVES

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Note to Readers of Appendix B

Appendix B presents the results of an ecological risk assessment (ERA) conducted to assess the potential ecological consequences of the proposed action and alternatives for this PEIS. The decision to use an ERA as an assessment tool for this PEIS was based on the NRC's recommendation that the potential ecological outcomes of the introduction of the Suminoe oyster should be evaluated before deciding to introduce the species (NRC 2004). It was performed in consultation with an Ecological Risk Assessment Advisory Group (ERAAG), which consisted of risk assessment experts from NOAA, FWS, and EPA and served as the peer review panel for the ERA. This document is the final version of the ERA after completion of ERAAG's review, and reflects some additional revisions in response to the OAP's comments on the pre-draft PEIS. The ERAAG's comments and the ERA writing teams's responses are available at http://www.nao.usace.army.mil/OysterEIS /PeerReviews/homepage.asp.

Using an ERA as the basis for assessing the environmental consequences of a proposed action and alternatives is not a typical element of the NEPA process, and using an ERA to compare the *benefits* of a series of potential actions in addition to their risks also is atypical. These atypical uses of an ERA to support a PEIS have resulted in some significant inconsistencies between the ERA report and the Draft PEIS. The presentation of findings of the ERA in Appendix B is organized around eight risk questions derived from the NRC's report and defined further in response to the ERAAG's recommendations. Five of those questions pertain only to the proposed action to introduce the Suminoe oyster. In contrast, the evaluation of environmental consequences in Section 4.2 of the Draft PEIS is organized to describe the potential ecological effects of the proposed action and six alternatives on groups representing all components of the ecosystem of Chesapeake Bay, and subsequent parts of Section 4 evaluate the environmental consequences for other elements of the potentially affected environment as described in Section 3 of the Draft EIS. The ecosystem elements do not correspond directly to the risk questions, and the risk questions do not address many of the other elements of the potentially affected environment, such as endangered species and essential fish habitat. Given those disparities, the results of the ERA presented in this appendix had to be reorganized and, in some cases, extrapolated to contribute to evaluations presented throughout Section 4 of the Draft PEIS, but particularly in Section 4.2 (Other Ecosystem Components) and Section 4.3 (Water Quality).

Although the OAP was not the designated peer-review group for the ERA, it was responsible for the overall peer review of the PEIS prior to its publication. In that capacity, the OAP reviewed all appendices to the pre-draft PEIS during its review of that document in the summer of 2008. That review was the first opportunity that members of the OAP had to see how the ERA was conducted and how ODM results were used in ERA analyses. The OAP identified concerns about the use of ODM results in the ERA:

- use of ODM outputs to represent oyster population outcomes with insufficient accounting for model uncertainties
- how ODM results were combined with results of another model package, the Chesapeake Bay Environmental Management Package (CBEMP) to assess effects on some ecosystem components

In response to those concerns, analyses relating to ecological consequences of the proposed action and the alternatives presented in the pre-draft PEIS were substantially revised before the Draft PEIS was released to the public. Many of the findings of the ERA presented in this appendix, specifically those presenting ODM projections of the size of the oyster population 10 years after implementing an action, are no longer presented in Section 4 (Environmental Consequences) of the Draft PEIS. In addition, conclusions drawn from linear regressions relating ODM projections to CBEMP projections are no longer presented in Section 4. Effects of changes in oyster abundance on water quality and SAV are assessed using only CBEMP projections in Section 4.3 of the Draft PEIS, and two CBEMP publications from which results now reported in Section 4.3 were drawn have been added to the Draft PEIS as Appendix H. In some instances, in accordance with the OAP's concurrence that ODM results could be used for guidance, ODM outcomes were retained in Section 4 of the Draft PEIS for comparing the potential outcomes of some alternatives. Specifically, Section 4.2 presents the results of the Relative Risk Model (RRM), a tool used in the ERA that relies, in part, on ODM projections. The ODM results were used to estimate the extent to which the size of the population of oysters might change over time in specific state/salinity zones as a result of implementing each of the alternatives, and the interpretation of the RRM results emphasizes the differences in the magnitude and spatial distributions of those changes among alternatives. Although the time between the OAP's review of the pre-draft and the scheduled publication of the Draft PEIS allowed for revision of the analyses presented in Section 4 of the Draft PEIS, it was not sufficient to revise the ERA completely.

Ecological Risk Assessment for Oyster Restoration Alternatives

September 12, 2008

PREFACE

Preparation of this ecological risk assessment (ERA) was a collaborative effort between E^{x}ponent and Versar, Inc., and it was conducted in consultation with the Ecological Risk Assessment Advisory Group, consisting of Dr. Todd Bridges (USACE), Mr. Chris Guy (FWS), Simeon Hahn (NOAA), and Ms. Barbara Okorn (EPA). Versar worked with the ERAAG in developing the conceptual approach for the ERA, and E^{x}ponent implemented that approach. Lead author of the ERA was Dr. Charles Menzie, of E^{x}ponent, supported by Ms. Johanna Salatas and Mr. Ward Ted Wickwire. Dr. Lisa Methratta of Versar was lead author for Section 4.3, investigating the probability of a diploid Suminoe oyster introduction resulting from triploid aquaculture. Dr. William Richkus of Versar contributed to editing and writing of the document.

EXECUTIVE SUMMARY

The purpose of this Ecological Risk Assessment (ERA) is to evaluate the potential ecological risks and benefits associated with the proposed action and the alternative actions specified in the Programmatic Environmental Impact Statement (PEIS) for Oyster Restoration in Chesapeake Bay. The goal of the actions being evaluated, as stated in the PEIS, is to establish an oyster population that reaches a level of abundance in Chesapeake Bay comparable to that present during the period from 1920 to1970. The States of Maryland and Virginia see a need to restore the important ecological role of oysters in the Bay and the economic benefits of a commercial oyster industry.

The proposed action is to establish a naturalized, reproducing, and self-sustaining population of the Suminoe oyster, *Crassostrea ariakensis,* in the tidal waters of Maryland and Virginia through introductions beginning after the PEIS is completed while continuing efforts to increase the numbers of the native oyster, *Crassostrea virginica,* using best available restoration strategies and stock assessment techniques. Alternatives to the proposed action include:

> **Alternative 1 – No Action--Not taking the proposed action:** Continue Maryland's present Oyster Restoration and Repletion Programs, and Virginia's Oyster Restoration Program under current program and resource management policies and available funding using the best available restoration strategies and stock assessment techniques.
>
> **Alternative 2 – Expand Native Oyster Restoration Program:** Expand, improve, and accelerate Maryland's Oyster Restoration and Repletion Programs, and Virginia's Oyster Restoration Program in collaboration with Federal and private partners. This work would include but would not be limited to an assessment of cultch limitations and long-term solutions for this problem, and the development, production, and deployment of large quantities of disease resistant strain(s) of *C. virginica* (Eastern oyster) for brood stock enhancement.
>
> **Alternative 3 – Harvest Moratorium:** Implement a temporary harvest moratorium on native oysters and an oyster industry compensation (buy-out) program in Maryland and Virginia or a program that offers displaced oystermen on-water work in a restoration program.
>
> **Alternative 4 – Aquaculture:** Establish and/or expand state-assisted, managed, or regulated aquaculture operations in Maryland and Virginia using the native oyster species.
>
> **Alternative 5 – Aquaculture:** Establish state-assisted, managed, or regulated aquaculture operations in Maryland and Virginia using suitable triploid, nonnative oyster species.
>
> **Alternative 6 – Introduce and Propagate an Alternative Oyster Species (Other than C. *ariakensis*) or an Alternative Strain of C. *ariakensis*:** Introduce and propagate in the state-sponsored, managed, or regulated oyster restoration programs in Maryland and

Virginia, a disease resistant oyster species other than C. *ariakensis*, or an alternative strain of C. *ariakensis* from waters outside the U.S. in accordance with the ICES Code of Practices on the Introductions and Transfers of Marine Organisms 1994.

Alternative 7 – Establish a naturalized, reproducing, and self-sustaining population of *C. ariakensis* in the tidal waters of Maryland and Virginia through introductions beginning in 2005 (or when the EIS is completed) but discontinue efforts to restore *C. virginica*.

Alternative 8 – Combination of Alternatives

Alternative 6 could not be evaluated because of the lack of any other oyster species that is suitable for introduction into Chesapeake Bay and the lack of research into other strains of *C. ariakensis*. Alternative 8 was not evaluated because the lead agencies decided to wait until stakeholders have had an opportunity to comment on the alternatives evaluated in the draft PEIS before defining a combination of alternatives to be considered.

"Risk" is defined as "exposure to the chance of injury or loss; a hazard or dangerous chance." The term clearly refers specifically to the potential for an adverse or undesirable outcome. One goal of the proposed action and alternatives is to restore the desirable functions of oysters in the Bay; therefore, one risk being considered is the risk of failing to restore such functions. This ERA is an atypical risk assessment because it evaluates not only outcomes that may be viewed as adverse, but also those that may be considered beneficial, such as the degree to which each alternative might contribute to restoring the ecological functions of oysters. To the extent possible, the expected ecological outcomes of implementing the proposed action and alternatives are characterized as beneficial or adverse with regard to the goal of restoring the functions of oysters. Although multiple actions are evaluated, this ERA is not intended to be a comparative assessment. The lead agencies intend this ERA to describe the ecological outcomes of the proposed action and each alternative without comparing the alternatives in a comprehensive ranking. The findings of the ERA will be incorporated into the draft PEIS together with assessment findings for all other components of the affected environment (e.g., economics, archaeological resources). The draft PEIS will be a means of soliciting input from stakeholders. The lead agencies will use stakeholders' input to identify the most appropriate course of action to be defined and supported in the final PEIS.

The proposed action and alternatives represent diverse approaches to achieving oyster restoration that vary in methods, requirements, and species involved. The range of alternatives being considered reflects the variety of interests of stakeholders. Eight risk questions were developed as a framework for comparing these diverse alternatives based on the stated need for action, the goal of action, and the characteristics of the actions being evaluated. Many stakeholders consider the proposed action (i.e., introducing the nonnative oyster *C. ariakensis* to Cheaspeake Bay) to be highly controversial; consequently, it was the subject of an earlier, comprehensive review of available information about *C. ariakensis* and risks associated with introducing it in the Bay (NRC 2004). That review is considered to be a Tier 1 Risk Assessment for this ERA that provides background information about the kinds of risks that could arise as a result of the proposed action. During that preliminary assessment, the NRC identified five questions that could not be addressed at the time due to insufficient information. Although the NRC's review provided extensive information about current and past efforts to restore the population of *C. virginica* in the Bay, it did not evaluate the specific alternatives to the proposed action considered in this ERA and in the PEIS. The NRC's five questions are noted with asterisks in the following list of risk questions for this ERA:

1. Will the proposed action or alternatives achieve the stated goal for the size of the Bay-wide oyster population?
2. Will *C. ariakensis* provide ecosystem services similar to those afforded by *C. virginica*?*
3. Will *C. ariakensis* compete with *C. virginica* and other resident species, possibly leading to reduced populations or local extinction of these species?*
4. What is the potential for introduction and spread of diseases to other species in the Bay?*
5. Will *C. ariakensis* become an invasive or nuisance species?*
6. Will *C. ariakensis* disperse to areas outside of Chesapeake Bay and pose the kinds of risks identified above?*
7. Will the action result in an unintended introduction of *C. ariakensis* into Chesapeake Bay?
8. To what extent will the action influence ecosystem services in Chesapeake Bay?

Several methods of assessment were needed to account for the diversity of the proposed action and alternatives and for limitations of the tools and information available for this ERA. The likelihood that implementing the proposed action or the alternatives would result in achieving the stated restoration goal for oysters (Risk Question 1) was evaluated using exploratory demographic modeling with the Oyster Demographic Model (, which projects the growth of the Bay-wide population of oysters over a 10-year period. This exploratory modeling was applied only to the native oyster *C. virginica* because data were not considered adequate to support reliable estimates for *C. ariakensis.* Some exploratory modeling was performed to examine how populations might respond if mortality associated with diseases was reduced. The trajectory of population growth over 10 years following the implementation of a restoration strategy was the basis for speculating about changes in population size more than 10 years after implementing any of the actions. exploratory modeling General conclusions drawn from a synthesis of literature and the findings of recent research concerning the risks associated with introducing *C. ariakensis* were the primary means of addressing Risk Questions 2 through 6, which apply only to alternatives involving that species (i.e., the proposed action and Alternatives 5 and 7). Those conclusions are based on the assumption that *C. ariakensis* is successfully introduced, becomes well-established, and spreads widely throughout the Bay. The likelihood that cultivating triploid *C. ariakensis* (Alternative 5) would result in an unintended introduction into Chesapeake Bay (Risk Question 7) was evaluated using a combination of qualitative assessment (for accidental releases) and an estimate of the probability of development of a reproducing pair of diploids from triploids in aquaculture systems via several pathways. The chain of events and associated probabilities for the triploid-to-diploid risk assessment were developed using the *Generic*

Nonindigenous Aquatic Organisms Risk Analysis Review Process developed by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service. Finally, the consequences of the proposed action and alternatives for ecosystem services as reflected in water quality and other ecological receptors (Risk Question 8) were evaluated using the Relative Risk Model (RRM). Input for the RRM included the results of exploratory modeling of oyster populations and projections for water quality and submerged aquatic vegetation (SAV) from the Chesapeake Bay Environmental Modeling Package (CBEMP). The RRM describes the influences that changes in oyster biomass could have on water quality and other ecological receptors considered to represent the various components of the Chesapeake Bay ecosystem. A positive influence is any consequence of a change in oyster biomass that might support or encourage an increase in the abundance, health, or distribution of the receptor population. A negative influence is any consequence of a change in oyster biomass that might cause or contribute to a decrease in the abundance, health, or distribution of the receptor population. Where appropriate and possible, we discuss whether stakeholders are likely to consider such influences to be beneficial or adverse (i.e., a negative influence on one receptor, such as phytoplankton, might be viewed as a benefit if it increases water clarity and dissolved oxygen, while a negative influence on a different receptor, such as striped bass, might be considered adverse).

All ERAs have inherent uncertainty that relates to lack of knowledge about aspects of the analysis or limitations of relevant data and information. For example, a risk analyst might infer a particular outcome from laboratory studies without the support of reliable field observations to corroborate the inference. Acquiring more knowledge can reduce uncertainty, and research on the biology of *C. ariakensis* conducted over the last several years was designed to address important sources of uncertainty associated with the proposed action. Nevertheless, uncertainty remains, and decisions based on this ERA must recognize that uncertainty. For the decision-maker, this involves weighing the potential beneficial and adverse consequences of the decision along with the associated uncertainty. For the purpose of this assessment we describe the likelihood and/or magnitude of an outcome as negligible, low, moderate, or high. The criteria for assigning these qualitative terms are as follows:

- Negligible - If assumptions are met, there is no chance or virtually no chance that this outcome will occur.
- Low - The chance that the outcome will occur is very small.
- Moderate - An outcome is possible.
- High - The chance that an outcome will occur is large.

We also use relative terms to characterize the degree of uncertainty associated with the estimates of likelihood and magnitude:

- Low - The mechanisms of action are well understood and available information is sufficient to support a conclusion
- Moderate - We are reasonably sure of the conclusion, but some aspects of the mechanism of action or consequences are not well understood, and its magnitude may be uncertain.

- High - Available information (e.g. data from laboratory studies) provides some insight into mechanisms of action, but we are not sure about how interactions would be manifested in the environment (i.e., regarding either likelihood or magnitude).

The potential risks and benefits of the proposed action and alternatives and their associated uncertainties are addressed for each of the risk questions:

1. Will the proposed action or alternatives achieve the stated goal for the Bay-wide population of oysters?

The stated goal of oyster restoration in the Bay is to achieve an oyster population of the size present during the period 1920-1970. A rough estimate of that population size is 1.16×10^{10} market-size oysters. For the proposed action and Alternative 7, achievement of the restoration goal was assumed in order to evaluate the risks of introducing *C. ariakensis* as defined in the NRC's five questions. For Alternatives 1, 2 and 3, the results of exploratory modeling were used to project the size of a *C. virginica* population 10 years after implementation. Possible trends in population size beyond that period also were considered. For Alternatives 4 and 5, the number of oysters that might be produced annually in the maximum economically viable aquaculture industry in the Bay was estimated based on the projections of a market-demand model, and that number was compared to the restoration goal.

Proposed Action and Alternative 7- The future abundance of introduced *C. ariakensis* cannot be predicted at this time because supporting data about the vital statistics of the species in Chesapeake Bay are not considered reliable. At a qualitative level, the species grows faster, reproduces earlier, and is generally more disease resitant than *C. virginica.* If these factors are the sole or most important determinants for successful population growth, *C. ariakensis* could be expected to be more successful than *C. virginica* in the Bay. Several factors, however, could limit its ability to be established throughout the Bay. These include the continuing loss of hard-bottom habitat; competition with *C. virginica*; vulnerability to predators such as blue crabs; and vulnerability to diseases that are new to the species, such as *Bonamia*.

Alternative 1 – Continuing current restoration efforts was projected to result in a small increase (20%-30% at the 50th percentile) in the Bay-wide population of market-size oysters after 10 years. Most of the projected increase would occur in low salinity waters in Maryland. At the lower-bound estimate (i.e., 5th percentile) oyster biomass would decline in all areas of the Bay; at the higher bound estimate (95th percentile) biomass would increase in all areas, but the population would still be smaller than the restoration goal 10 years after implementation. One element of the assumed restoration activities (i.e., shell replenishment in Maryland) was terminated in 2007; therefore, the actual population level after 10 years would be even less than projected. The oyster population under this alternative is likely to decline indefinitely into the future because of the continuing decline in hard-bottom habitat.

The risk that implementing Alternative 1 would fail to achieve the restoration goal is considered high; that is, it is virtually certain that the goal would not be attained. The level of uncertainty associated with this risk is considered low to moderate due, in part, to recognized limitations of the exploratory modeling. The only potential mechanism by which the population of *C. virginica* might grow would be development of resistance to MSX and Dermo. Harvest is assumed to continue under Alternative 1, which would impede development of disease resistance in the population. The length of time required for a Bay-wide population of disease-resistant oysters to develop naturally is unknown, but it is likely to be substantial, and the availability of suitable habitat would continue to decrease during that time.

Alternative 2 – An enhanced restoration program was projected to result in an increase of 250% to 450% (50th percentile) in market-size oysters by year 10. Most of the increase would occur in lower salinity waters in Maryland. The projected increase is from a very small starting population; consequently, even at the 95th percentile projection, the population at year 10 would be substantially smaller than the restoration target. In addition, the rate of increase in the population was projected to level off in years 9 and 10 and would be likely to remain level or decline into the future. According to the hypothetical assessment scenario for this alternative, seed planting would increase through year 7 and remain constant in later years, suggesting that the projected increase through year 10 was driven primarily by seeding. At the lower-bound estimate (5th percentile) oyster biomass would decline throughout most of the Bay.

The risk that implementing Alternative 2 would fail to achieve the restoration goal is considered high. The level of uncertainty associated with this risk is considered moderate. Variation in outputs of exploratory modeling for this alternative was much greater than for Alternative 1. Differences between the 10th and 90th percentiles ranged up to more than 500% in some cases. Limitations of the exploratory modeling contribute to this level of uncertainty. Potential development of disease resistance, effect of harvest on the rate of development of disease resistance, and continuing loss of habitat are three factors that are not accounted for in exploratory modeling projections.

Alternative 3 – Continuing existing restoration programs and imposing a moratorium on harvest (assumed to be in place for at least 10 years) was projected to result in a 56% increase in the median (50th percentile) abundance of market-size oysters after 10 years. No substantial increasing trend is expected beyond the 10-year period. At the lower-bound estimate (5th percentile) oyster biomass would decline in much of the Bay.

The risk that implementing Alternative 3 would fail to achieve the restoration goal is considered moderate to high. The level of uncertainty associated with this risk is considered moderate. Limitations of the exploratory modeling contribute to this level of uncertainty. For instance, the exploratory modeling does not account for any development of disease resistance in the population of native oysters over time, but the elimination of harvest could contribute to more rapid development of disease resistance in the population than would occur under Alternatives 1 and 2, in which harvest would continue. Further loss of hard-bottom habitat over time would constrain the growth of the oyster population and also is not accounted for in the exploratory modeling.

Alternatives 4 and 5 – Although the two aquaculture alternatives employ different species (Alt. 4 = *C. virginica,* Alt. 5 = triploid *C. ariakensis*), the estimated maximum economically viable industry was assumed to be the same size for both alternatives for comparison with the restoration goal. The market-demand model projected that the maximum economically viable oyster industry in the Chesapeake Bay would involve an annual production of 2.6 million bushels of oysters, which equals roughly 683.7 million oysters. That number is well below the restoration goal.

The risk that implementing Alternatives 4 or 5 would fail to achieve the restoration goal is considered high. The level of uncertainty associated with this risk is considered low. A major factor contributing to confidence in this assessment of risk is that the likelihood of the maximum industry becoming established in the near future (i.e., 10 years) is considered to be low. Uncertainty is great about whether an industry of that size could ever be established or, once established, could be maintained. Although implementing either of these alternatives would be unlikely to achieve the Bay-wide oyster restoration goal, concentrations of aquaculture might develop in selected tributaries and result in localized oyster abundance equivalent to former abundance in those locations.

2. Will *C. ariakensis* provide ecosystem services similar to those afforded by *C. virginica*?

The NRC posed this question in its preliminary evaluation of risks associated with introducing *C. ariakensis* to Chesapeake Bay (NRC 2004). It is applicable only to the proposed action and Alternatives 5 and 7. Regarding Alternative 5, this question would apply only if an unintended introduction of diploids from triploid aquaculture results in a large, self-sustaining population of *C. ariakensis* (see Risk Question 7). In considering this risk question, the proposed introduction of *C. ariakensis* is assumed to be successful, and the species to be established throughout the range of *C. virginica* in the Bay. This question does not address the feasibility of an introduction, only the ecological consequences if the species were to become widespread.

The risk that *C. ariakensis* would not provide ecosystem services similar to those afforded by *C. virginica* is low. Ecological services considered in this ERA are those associated with provision of reef habitat for other Bay species, provision of food for other Bay species, and filtration capacity. *C. ariakensis*, if successful, is expected to populate historical oyster habitat and other hard substrates in the subtidal zone. Because *C. ariakensis* can tolerate high loads of suspended sediment and exist in muddy systems (albeit on shell), reefs of the species could provide localized benefits for SAV by buffering the action of waves and currents and by filtering suspended solids from the water. Both oyster species are expected to filter the same kinds of algae and suspended matter from the water. Reefs of *C. ariakensis* would provide habitat for other species; however, no studies have investigated if the small-scale structure of reefs of *C. ariakensis* or mixed-species reefs would attract and support the same biological community that reefs of *C. virginica* do.

The level of uncertainty associated with this conclusion is moderate. The uncertainty is related to inadequate understanding of all of the many and varied ways in which oysters interact with other components of the Bay ecosystem, as well as lack of knowledge about the characteristics of *C. ariakensis* reefs or mixed-species reefs in open waters of Chesapeake Bay. Although species interactions are considered the most important mechanisms by which changes in the abundance or kind of oysters in the Bay could influence other receptors, many of the specific details of these interactions are not well known or quantified. Uncertainty increases with the number of linkages between ecological receptors and oysters. We believe that the most important influences of oysters on ecological receptors in the Bay have been captured in using the Relative Risk Model to address Risk Question 8 and that existing information suggests that the magnitude of those influences would be small.

3. Will *C. ariakensis* compete with *C. virginica* and other resident species, possibly leading to reduced populations or local extinction of these species?

The NRC posed this question in its preliminary evaluation of risks associated with introducing *C. ariakensis* to Chesapeake Bay (NRC 2004). It is applicable only to the proposed action and Alternatives 5 and 7. Alternatives involving only *C. virginica* would not alter the existing kinds of competition between species within the Bay. Regarding Alternative 5, this question would apply only if an unintended introduction of diploids from triploid aquaculture results in a large, self-sustaining population of *C. ariakensis* (see Risk Question 7).

The risk that *C. ariakensis* would interact and compete with *C. virginica* is moderate to high. *C. ariakensis* grows faster, matures earlier, and is resistant to the diseases that affect *C. virginica* in Cheaspeake Bay; therefore, *C. ariakensis* could outcompete *C. virginica*. Because the species differ in their tolerances for stressors within the Bay, *C. virginica* could have an adaptive advantage in environments that are not favorable for *C. ariakensis* (e.g., intertidal areas and areas of low dissolved oxygen). A successful introduction of *C. ariakensis* could result in production of large quantities of shell that would benefit both oyster species; *C. virginica* larvae are known to settle on *C. ariakensis* shell. The suggestion that *C. ariakensis* could increase the shell budget at cultch areas is a speculation. The likelihood of that outcome would depend on the population growth of *C. ariakensis* and the longevity of its shell in the environment. In its native environment, *C. ariakensis* forms the base of reefs that support smaller species of oysters. The natural ranges *C. virginica* and *C. ariakensis* do not overlap; therefore, the possibility that they would form mixed-species reefs cannot be confirmed. The range of potential outcomes of competition between these species is broad: local extinctions of *C. virginica* are possible in environments that are favorable for *C. ariakensis*; either species might become dominant in localized areas; and mixed reefs may develop with varying proportions of each species. Gamete competition resulting from both species coexisting and spawning in the same location would favor the survival of the species present in greater abundance. The potential interactions between the two oyster species suggest the possibility of outcomes that are both positive and negative for *C. virginica*; therefore, the two species probably could coexist. Thus, although the risk of local extinction of *C. virginica* appears to be moderate, risk of Bay-wide extinction of *C. virginica* as an outcome of the proposed introduction appears low.

Several lines of evidence indicate that *C. ariakensis* would behave similarly to *C. virginica* with respect to choice of substrate for settlement. Larvae of *C. virginica* and *C. ariakensis* have the same requirements for clean, firm substrate to promote metamorphosis and attachment. Both species settle and form reefs on hard substrate (i.e., shell and rock). If *C. ariakensis* is successful in Chesapeake Bay, the species is expected to populate historical oyster habitat and other hard substrate in the subtidal zone. It is not expected to form large reefs outside of historical hard-bottom areas that would overtake other soft-bottom or SAV habitats. Horizontal expansion from existing hard-bottom areas and colonization of patches of shell could occur through shell accretion.

The uncertainty associated with these conclusions is moderate to high. This uncertainty stems from the limited knowledge and understanding of ecological interactions between the oyster species. Current knowledge is based largely on laboratory studies, limited field trials, and observations of *C. ariakensis* in its native range.

4. What is the potential for introduction and spread of diseases to other species in the Bay? (This does not include human health considerations, which are addressed in the PEIS)

The NRC posed this question in its preliminary evaluation of risks associated with introducing *C. ariakensis* to Chesapeake Bay (NRC 2004). It is applicable only to the proposed action and Alternatives 5 and 7. Alternatives involving only *C. virginica* would pose no risk of introducing new diseases, assuming that none of those alternatives would involve transporting *C.virginica* from other waters into Chesapeake Bay.

The risk of introducing diseases associated with alternatives involving the Oregon strain of *C. ariakensis* cultured at existing hatcheries in the Chesapeake Bay region would be negligible, assuming that ICES protocols are followed. More than three generations of *C. ariakensis* have been grown at hatcheries in the Chesapeake Bay region, and the stock is certified as disease free. If the Chesapeake Bay stock is supplemented with specimens from other facilities, a pathogenic virus could be introduced because they are difficult to detect. Once introduced into the Bay, *C. ariakensis* could be affected by endemic pathogens such as *Bonamia*. The risk that introducing pathogen-free *C. ariakensis* would result in an increase in subsequent invasions of newly introduced pathogens (e.g., in ballast water) that are capable of infecting bivalves is small; however, the risk would be proportional to the size of the population of *C. ariakensis* in Chesapeake Bay. *C. ariakensis* could serve as a reservoir for pathogens to which it is relatively resistant but that might affect *C. virginica.* The potential for *C. ariakensis* to transfer MSX and Dermo from affected *C. virginica* to unaffected native oysters and the magnitude of the effects would be proportional to the abundance *C. ariakensis* and proximity to affected *C. virginica*. Spatially variable environmental conditions such as salinity and water temperature also would influence this interaction. The risk of introduction and spread of new diseases associated with the proposed action and Alternatives 5 and 7 is considered negligible.

Uncertainty associated with this conclusion is low to moderate. This uncertainty results from limited knowledge and understanding regarding the basic biology and epidemiology of disease in *C. ariakensis*. The prevalence, infection rates, and transmission rates for some diseases are known from limited studies.

5. Will *C. ariakensis* become an invasive or nuisance species?

The NRC posed this question in its preliminary evaluation of risks associated with introducing *C. ariakensis* to Chesapeake Bay (NRC 2004). It is applicable only to the proposed action and Alternatives 5 and 7. Regarding Alternative 5, this question would apply only if an unintended introduction of diploids from triploid aquaculture results in a large, self-sustaining population of *C. ariakensis* (see Risk Question 7).

The risk that *C. ariakensis* would become an ecological nuisance within the Bay is low. *C. ariakensis* can settle on a variety of hard surfaces, including artificial materials; therefore, like other animals that settle on hard substrates, *C.ariakensis* poses some potential for fouling. *C. ariakensis* poses no risk of fouling structures or pipes in fresh water. Any risk of fouling associated with the species would be limited to subtidal locations in saline waters. The potential for *C. ariakensis* to become a nuisance species was evaluated by comparison with *C. gigas*, an oyster species that is considered a nuisance in the Wadden Sea in the Netherlands. The two species share characteristics such as rapid growth but differ in two important respects: *C. gigas* has a tough shell and is more resistant than *C. ariakensis* to natural predators such as birds and crabs; and *C. ariakensis* would be subject to harvesting in the Chesapeake Bay, whereas *C. gigas* in the Wadden Sea is not yet subject to harvesting. The case of *C. gigas* indicates that oysters can become an invasive nuisance; however, *C. gigas* appears to have a particular combination of characteristics that enhances that ability that *C. ariakensis* does not share.

The level of uncertainty associated with this conclusion is moderate to high because it is based on laboratory observations of settlement and limited field observations of *C. ariakensis* and because there is always uncertainty about how an introduced species will behave in a new environment. One important aspect of the proposed action is that it would be irreversible; if a diploid population of *C. ariakensis* were to become established in the Bay as a result of a purposeful introduction and become a nuisance species, it would not be possible to eradicate the species.

6. Will *C. ariakensis* disperse to areas outside of Chesapeake Bay and pose the kinds of risks identified above?

The NRC posed this question in its preliminary evaluation of risks associated with introducing *C. ariakensis* to Chesapeake Bay (NRC 2004). It is applicable only to the proposed action and Alternatives 5 and 7. Regarding Alternative 5, this question would apply only if an unintended introduction of diploids from triploid aquaculture results in a large, self-sustaining population of *C. ariakensis* (see Risk Question 7).

If *C. ariakensis* becomes established within Chesapeake Bay, the risk is high that it eventually would disperse to areas outside the Bay. Such dispersal would be more likely to the north of the Bay than to the south due to greater availability of suitable substrate. The species eventually could occur throughout a wide latitude parallel to its known native distribution, approximately from Connecticut to the Yucatan Peninsula, Mexico. The likelihood that *C. ariakensis* would compete with *C. virginica* in areas outside of the Bay or otherwise become an ecological nuisance depends on the species' ability to become established and develop reefs. The potential for competition was discussed in response to Risk Question 3. The presence of Bonamia in more saline ocean waters may limit natural dispersal of *C. ariakensis* along the Atlantic coast. In addition, at low numbers of adults within an area (relative to *C. virginica*), *C. ariakensis* would be at a competitive disadvantage due to the phenomenon of gamete sink. Aside from the moderate to high potential for competition with *C. virginica*, the risk that *C. ariakensis* could become an ecological nuisance by displacing species in soft-bottom or sandy habitats is low to moderate.

Uncertainty concerning the likelihood that *C. ariakensis* would disperse beyond the Bay is low to moderate because numerous dispersal mechanisms are possible (e.g., unauthorized physical transplants, attachment to boats or hard debris that is transported out of the Bay, larval dispersal). Uncertainty regarding the likelihood that *C. ariakensis* would compete effectively with *C. virginica* or otherwise become a nuisance is moderate to high. This uncertainty is due to limited knowledge and understanding of the ecological relationships between *C. ariakensis* and other estuarine receptors, which is based primarily on laboratory studies and limited field trials.

7. What is the likelihood that the action will result in an unintended introduction of *C. ariakensis* into Chesapeake Bay?

This question applies only to Alternative 5, aquaculture using triploid *C. ariakensis*. An unintended introduction is considered a risk for this alternative because the specification to use triploid *C. ariakensis*, which generally are believed to be unable to reproduce, reflects a compromise that addresses stakeholders' conflicting views about the desirability of introducing a nonnative species while attempting to attain the potential economic benefit of using the species. An unintentional introduction might result from aquaculture through any of several pathways, including accidental releases at hatcheries or field sites, undetected fertility of triploids, imperfect fidelity during the genetic crosses intended to produce triploids, and reversion from triploid to diploid among cultivated oysters in the field. The response to this risk question encompasses two general evaluations, a qualitative assessment of the risk of accidental release from hatcheries, and an estimate of the risk of introduction from field operations.

The risk of accidental release from a hatchery is considered moderate and would be proportional to the number of hatcheries producing triploid *C. ariakensis*. An accidental release from a hatchery could be the result of human error or of catastrophic destruction (e.g., hurricane). The risk would be greatest if multiple hatcheries are constructed throughout the Bay region to support large-scale, private aquaculture operations. Centralizing the production of larvae and spat to one or two locations that are certified specifically for those operations would reduce risk and allow for greater supervision of adherence to quarantine protocols. Uncertainty associated with this risk estimate is considered moderate to high because there is little basis for estimating the likelihood of catastrophic destruction of a hatchery or the rate of human error at facilities that do not yet exist. The lack of evidence of human error at university facilities that currently produce triploid *C. ariakensis* suggests that the rate of human error and the risk associated with it may be low under some circumstances.

The probability that in-field aquaculture operations would result in an unintentional introduction was estimated based on several assumptions. A maximum economically viable aquaculture industry was projected based on market-demand modeling, and a hypothetical scenario was developed to allocate that maximum number of cultivated oysters among locations that would be likely sites for large-scale aquaculture operations. The estimate of the probability of an unintended introduction was based on the underlying assumption that the maximum industry producing approximately 2.6 million bushels of oysters annually would be in place over a 10-year period. The number of diploids that could arise through all of the quantifiable pathways combined over one spawning season was estimated for a representative hypothetical site. After 10 years, the number of diploid oysters that could arise through all quantifiable pathways combined at a hypothetical operation representing 5% of the production of the maximum aquaculture industry was calculated to be 271 oysters. Across all aquaculture operations, the cumulative probability that cultivating triploid *C. ariakensis* could yield a reproductive population (defined as 2 individuals located in the same 1 m^2) over a 10-year time span was 8%. If escaped diploids survived for an extended period of time, and the level of triploid aquaculture activity remained high in fixed locations, the number of diploids at large in the Bay would continue to accumulate, and the risk of an unintentional introduction would increase proportionately with time.

Many factors could influence the risk of inadvertently introducing a reproductive population of *C. ariakensis* via aquaculture operations. The following factors would tend to lower the risk: (1) An aquaculture industry of the projected size is unlikely to be established within a decade or perhaps ever; a smaller industry would pose less risk. (2) The assessment assumes that the projected diploids would experience no mortality over 10 years, which is unlikely. (3) Two adjacent, reproductive oysters could be of the same sex or could fail to reproduce, failing to establish a reproductive population in the Bay. (4) The success of reproduction among very limited numbers of diploid adults could be so low that no sustainable population is ever established. Other factors would tend to increase the risk: (1) Continued aquaculture with triploids in the same location over many years or decades would increase the likelihood of achieving a founding population of escaped diploids in natural habitats adjacent to aquaculture operations. (2) Continued aquaculture with triploids in the same location over an extended period of time would increase the cumulative accidental losses of triploids into local waters; if escaped triploids survive, and the probability of reversion of triploids to diploids increases with time, ultimately, a large number of escaped triploids could revert to produce a founding population of diploids. (3) Especially favorable conditions for reproduction might occasionally occur in a location in which escaped diploids are present, resulting in a large production of larvae that would accelerate dispersal throughout the Bay.

Given the many unknowns in the two component evaluations for this question and the variety of possible pathways of introduction, no specific level of risk can be determined for the overall likelihood that implementing Alternative 5 would result in an unintended introduction. The level of uncertainty associated with evaluating this risk is high due to lack of information about many contributing factors.

The possibility of unintentionally introducing a reproductive population of *C. ariakensis* via cultivating triploids can be combined with the discussion of the ecological outcomes of an intentional introduction of the species to evaluate overall outcomes. If an unintentional introduction eventually leads to the abundance projected for an intentional introduction, the ecological outcomes would be equivalent to those of the proposed action. Barring a catastrophic release from a hatchery, however, significantly more than 10 years probably would be required to achieve the 10-year abundance projected for an intentional introduction because of the small number of oysters (diploid or triploid) expected to be released from aquaculture operations in any given year.

8. To what extent will the action influence ecosystem services in Chesapeake Bay?

The RRM was used to characterize the relative degree and direction of the influences of changes in oyster abundance in Chesapeake Bay on water quality and various ecological receptors such as phytoplankton, SAV, fish, and wildlife. The increases and decreases in oyster biomass projected for each alternative are described in response to Risk Question 1. The RRM results summarized here describe expected ecological outcomes for the 50th percentile projections of oyster biomass. The 50th percentile, or median, is a measure of central tendency. For most alternatives to the proposed action, exploratory modeling projections at the 5th percentile suggest that the biomass of oysters in the Bay would continue to decline despite implementation of the alternative; therefore, the positive influences on fish and wildlife receptors described below for the 50th percentile projections might not occur.

Projected increases in oyster abundance and biomass generally would result in negligible to small improvements in water quality (dissolved oxygen, suspended solids, water transparency) at the scale of the six broad state/salinity zones evaluated in this ERA because projected changes in biomass are small for most alternatives. Moreover, changes in water quality evaluated on the Bay-wide scale are small. Larger influences on suspended solids and transparency may occur within individual segments of the Bay or individual tributaries depending on local increases in oyster biomass and associated filtration capacity of the local oyster population.

Increases in oyster abundance and biomass generally would have positive influences on most fish and wildlife receptors. For species that depend on oyster reefs for habitat or food, influences would be proportional to the growth and sustainability of oyster hard-bottom. These proportional increases are described in the response to the first risk question. The alternatives vary in their relative success in sustaining oyster hard-bottom throughout the Bay. Alternative 2 could produce an increase in hard-bottom in lower salinity waters, primarily in Maryland, but not in higher salinity waters. These gains would be short-lived if the enhanced restoration activities were terminated because the gains appear to be dependent on seed planting. Alternative 3 was predicted to result in an increase in oyster hard-bottom in most areas; the greatest increases would occur in low salinity areas. The predictions do not account for the potential benefit of the development of disease resistance.

Most species of fish and wildlife that do not depend directly on oysters for food or habitat would experience small positive influences proportional to increases in oyster biomass at the scale of the broad state/salinity zones. Influences could be greater at smaller spatial scales (at the segment or tributary level). Evidence from the literature indicates that the largest positive influences of oyster restoration would occur in shallow, semi-enclosed, low-salinity segments of Chesapeake Bay.

Increases in oyster biomass projected for the proposed action and alternatives would have small negative influences on phytoplankton because oysters filter phytoplankton from the water. A "negative" influence means simply that the amount of phytoplankton would decrease; the term is not intended to convey a value judgment regarding whether that decrease should be considered an adverse or beneficial outcome for the Bay. Ecological receptors that depend on phytoplankton for food could experience associated small negative influences. All these influences would be very small within the 10-year assessment period. Based on these results, changes in the abundance and biomass of oysters projected to be at or below the stated restoration goal for oysters in the Bay would be unlikely to result in ecologically significant shifts in food webs of the Bay.

Overall, results based on exploratory modeling projections of oyster biomass suggest that increases in oysters would provide the greatest benefit for ecosystem components that use oyster reefs as habitat or sources of food or that are localized to areas where oysters are abundant. This conclusion is consistent with the premise that oysters exert influences at local scales. Chesapeake Bay segments that would benefit most from oyster restoration have historical oyster bars, are comparatively shallow, and flush slowly. These physical characteristics would allow oysters to exert an influence on the water column. Other influences would be small; therefore, influences for other ecosystem components that relate directly to habitat, food, or water quality are considered small.

This evaluation of the influences of the proposed action and alternatives on ecological conditions in the Bay incorporates uncertainty attributable to a variety of sources. Uncertainty arises as a result of the complexity of the Bay's ecosystem and our limited ability to identify and incorporate the full range of interactions among its various components. The analysis described in this ERA was simplified to provide a consistent means of evaluating the ecological consequences of the proposed action and alternatives. Nevertheless, we feel that the general structure of the analysis is reliable and that it captures the significant ecological changes and influences. Uncertainty increases with the number of linkages between ecological receptors and oysters; however, given that the most significant influences have been captured and that we are confident in our assessment that they would be small, we do not anticipate large ecological influences associated with any more subtle relationships that this analysis does not represent explicitly. Uncertainty arises from using biomass projections limited to a 10-year period. Although important changes could begin to occur within that period, later changes in abundance could result in increases or decreases in the magnitude of influences captured in the RRM.

The characterizations of positive or negative ecological influences summarized here were based on one or more models: the exploratory modeling, which projects the relative change in abundance of oysters; the CBEMP, which translates changes in oyster biomass into changes in phytoplankton, SAV, zooplankton, benthic invertebrates, and water quality; and the degree of association between those ecological receptors and selected species of fish and wildlife. Each of these models incorporates some level of uncertainty; therefore, the projections should be viewed as relative indicators, not absolute values. Generally, the level of uncertainty associated with RRM outputs is considered moderate. These outputs are largely qualitative in nature and are intended to indicate the relative influences of the proposed action and alternatives. Uncertainty should be considered high if model outputs are expected to provide a quantitative assessment of how ecological receptors might actually respond in the Bay.

Reducing Uncertainty

The uncertainties identified above could be reduced by conducting focused studies. Investigating a few specific questions would be especially valuable for reducing key uncertainties:

- **Examine the longevity of *C. ariakensis* shell in the waters and sediments of Chesapeake Bay.** If this species is to be successful at developing and sustaining populations, the integrity of its shell in the environment will be important for maintaining and perhaps increasing the shell inventory at cultch sites in the Bay.
- **Develop a monitoring program for detecting the presence of diploid *C. ariakensis* in areas adjacent to aquaculture operations and hatcheries.** Monitoring would provide data on the rate of production of diploids, the spatial distribution of escaped diploids, and the need for implementing an adaptive management strategy to prevent collocation of adult diploid *C. ariakensis.*
- **Develop metrics for judging the potential for *C. ariakensis* to become a nuisance for ecological receptors and human uses within the Bay and in areas outside of the Bay.** This would begin with reaching agreements concerning mode by which the species might pose a nuisance.
- **Use triploid *C. ariakensis* to examine the kinds of communities that develop on a *C. ariakensis* reef in Chesapeake Bay and measure the influences of *C. ariakensis* on local ecology and water quality.** This would involve constructing reefs resembling those that occur in the species' native habitat.
- **Conduct additional research on rate and probability of reversion of triploid *C. ariakensis* to a diploid state.** More reliable information on this phenomenon would contribute to a more precise assessment of risk that large scale triploid aquaculture could result in unintended diploid introduction of this species.
- **Conduct research to estimate the rate at which *C. virginica* develops natural resistance to Dermo and MSX, the probability that disease resistance could be established throughout the Chesapeake Bay oyster stock, and the time required to develop that resistance.** This would require laboratory studies, field studies and various types of population modeling to assess the manner in which disease resistance may develop and expand geographically throughout the Bay.
- **Design and implement monitoring programs that would provide data needed to accurately characterize population dynamics of the Chesapeake Bay native oyster stock.** Good data on the current size of the oyster population and such vital parameters as natural mortality rates, fishing mortality rates and growth rates are required to develop reliable and reasonably accurate population models that can be used to investigate the efficacy of alternative management strategies for the Bay's oyster population.

Table ES-1. Summary of the Ecological Risk Assessment for proposed action and alternatives

Summary of Responses to Risk Questions

	Proposed Action and Alternative 7	**Alternative 1**	**Alternative 2**	**Alternative 3**	**Alternative 4**	**Alternatives 5**
1. Risk of failing to achieve oyster restoration goal.	Cannot be determined at this time Uncertainty: High	High Uncertainy: Low to moderate	High Uncertainty: Moderate	High Uncertainty: Moderate	High Uncertainty: Low	High Uncertainty: Low
2. Risk that *C. ariakensis* would not provide ecological services similar to *C. virginica.*	Low Uncertainy: Moderate	Negligible[1]	Negligible	Negligible	Negligible	Low[2] Uncertainty: Moderate
3. Risk of competition between *C. ariakensis* and *C. virginica* and local extinction of *C. virginica.*	Moderate to high for competition; moderate for local extinction; low for Bay-wide extinction Uncertainty: Moderate to high	Negligible	Negligible	Negligible	Negligible	Moderate to high for competition; moderate for local extinction; low for Bay-wide extinction Uncertainty: Moderate to high
4. Risk of introducing new diseases	Negligible Uncertainty: Low to moderate	Negligible	Negligible	Negligible	Negligible	Negligible Uncertainty: Low to moderate
5. Risk that *C. ariakensi* would become an invasive or ecological nuisance species in the Bay.	Low Uncertainty: Moderate to high	Negligible	Negligible	Negligible	Negligible	Low Uncertainty: Moderate to high
6. Risk of dispersal of *C. ariakensis* outside of Chesapeake Bay.	High Uncertainty: Low to moderate	Negligible	Negligible	Negligible	Negligible	High, if an unintended introduction of diploids results in a large population Uncertainty: Moderate to high
7. Risk of an unintentional introduction of *C. ariakensis* into the Bay.	Negligible	Negligible	Negligible	Negligible	Negligible	Undetermined (see text). Uncertainty: High
8a. Potential for improvement in water quality (i.e., increase in dissolved oxygen and reduction is suspended solids).	If successfully introduced, low at large geographic scales; moderate at local level Uncertainty: High	Negligible Uncertainty: Low	Low in low-salinity waters in MD; low to moderate at local levels Uncertainty: Low	Low, but lower than for Alternative 2; low to moderate at local levels. Uncertainty: Low	Negligible, except at local level Uncertainty: Low	Negligible, except at local level Uncertainty: Low

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[1] Alternatives that include only *C. virginica* pose no risk of producing outcomes associated with *C. ariakensis*; therefore, based on the risk categories defined for this ERA, those risks are designated as negligible.

[2] For this and all subsequent risk questions, the risk that Alternative 5 would produce an outcome is evaluated based on the assumption that cultivating triploids results in establishing a diploid population in the Bay at large. The risk of that outcome is evaluated in response to Risk Question 7.

Table ES-1. Summary of the Ecological Risk Assessment for proposed action and alternatives

Summary of Responses to Risk Questions

	Proposed Action and Alternative 7	**Alternative 1**	**Alternative 2**	**Alternative 3**	**Alternative 4**	**Alternatives 5**
8b. Potential for improvement in extent and viability of living oyster hard-bottom.	If successfully introduced, moderate to high Uncertainty: High	Negligible Uncertainty: Low	Low to moderate in MD waters; negligible in VA waters Uncertainty: Moderate	Low in most areas Uncertainty: Moderate	Negligible to low if on-bottom culture employed Uncertainty: Low	Negligible Uncertainty: Low
8c. Potential for benefits to fish and wildlife that depend directly on oysters for habitat or food.	If successfully introduced, high Uncertainty: High	Negligible Uncertainty: Low	Proportional to increase in living oyster hard-bottom Uncertainty: Moderate	Less than Alternative 2; greatest potential in low-salinity waters of MD Uncertainty: Moderate	Negligible to low Uncertainty: Low	Negligible Uncertainty: Low
8d. Potential for benefits to fish and wildlife that depend indirectly on oysters for habitat or food.	Expected to be low and proportional to increase in living oyster hard-bottom Uncertainty: High	Negligible Uncertainty: Low	Negligible to low over large geographic scales; moderate at local scale in MD low salinity waters Uncertainty: Moderate	Lower than for Alternative 2, but expected in both MD and VA. Uncertainty: Moderate	Negligible over large scales, but low at local level Uncertainty: Low	Negligible over large scales, but low at local level Uncertainty: Low
8e. Risk of adverse ecological effects associated with increases in oyster biomass.	Expected to be low for phytoplankton and plankton-based food web Uncertainty: Moderate	Negligible Uncertainty: Low	Low for phytoplankton and plankton-based food web in MD low salinity waters Uncertainty: Low	Low for phytoplankton and plankton-based food web, lower than for Alternative 2 Uncertainty: Low	Negligible at large geographic scales; negligible to low at local level Uncertainty: Low	Negligible at large geographic scales; negligible to low at local level Uncertainty: Low

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1.0 INTRODUCTION

The purpose of this Ecological Risk Assessment (ERA) is to evaluate potential ecological risks and benefits associated with implementing the proposed action or any of the alternative actions specified in the Programmatic Environmental Impact Statement (PEIS) for Oyster Restoration in Chesapeake Bay. This ERA, along with other information, will be used in the NEPA process to assist the decision makers to make an informed, balanced decision. The ERA does not evaluate potential options for managing the oyster fishery (e.g., restrictions on gear types, harvest limits) and does not define the sustainable level of oyster harvest in Chesapeake Bay. Nevertheless, the oyster population models developed for this risk assessment may prove to be useful tools for evaluating strategies for managing the oyster fishery. Similarly, this ERA does not address social and economic risks and other concerns that are not ecological; the PEIS addresses those issues. This ERA differs somewhat from typical ERAs because it captures both positive and negative outcomes of the alternatives. Managers asked for this more complete ecological picture to fully inform the PEIS. The context for the ERA includes the potential that alternatives will meet certain goals. The primary goal is to restore the abundance of oysters to a specified level. Additional ecological goals or expectations associated with restoring oysters include an improvement in overall water quality (e.g., increased dissolved oxygen) and the enhancement of overall ecological services.

The **proposed action** is to establish a naturalized, reproducing, and self-sustaining population of an Asian species, *Crassostrea ariakensis,* in the tidal waters of Maryland and Virginia through introductions beginning after the PEIS is completed while continuing efforts to increase the numbers of the native oyster, *Crassostrea virginica,* using best available restoration strategies and stock assessment techniques. Reproductive (i.e., diploid) *C. ariakensis* would be propagated from existing third or later generations of the Oregon stock of this species, in accordance with the International Council for the Exploration of the Sea's Code of Practices on the Introductions and Transfers of Marine Organisms 1994 (ICES 1995).

Alternatives to the proposed actions include:

> **Alternative 1 – No Action--Not taking the proposed action:** Continue Maryland's present Oyster Restoration and Repletion Programs, and Virginia's Oyster Restoration Program under current program and resource management policies and available funding using the best available restoration strategies and stock assessment techniques.
>
> **Alternative 2 – Expand Native Oyster Restoration Program:** Expand, improve, and accelerate Maryland's Oyster Restoration and Repletion Programs, and Virginia's Oyster Restoration Program in collaboration with Federal and private partners. This work would include but would not be limited to an assessment of cultch limitations and long-term solutions for this problem, and the development, production, and deployment of large quantities of disease resistant strain(s) of *C. virginica* (Eastern oyster) for brood stock enhancement.

Alternative 3 – Harvest Moratorium: Implement a temporary harvest moratorium on native oysters and an oyster industry compensation (buy-out) program in Maryland and Virginia or a program that offers displaced oystermen on-water work in a restoration program.

Alternative 4 – Aquaculture: Establish and/or expand state-assisted, managed, or regulated aquaculture operations in Maryland and Virginia using the native oyster species.

Alternative 5 – Aquaculture: Establish state-assisted, managed, or regulated aquaculture operations in Maryland and Virginia using suitable triploid, nonnative oyster species.

Alternative 6 – Introduce and Propagate an Alternative Oyster Species (Other than C. *ariakensis*) or an Alternative Strain of *C. ariakensis*: Introduce and propagate in the state-sponsored, managed, or regulated oyster restoration programs in Maryland and Virginia, a disease resistant oyster species other than C. *ariakensis*, or an alternative strain of C. *ariakensis* from waters outside the U.S. in accordance with the ICES 2005 Code of Practices on the Introductions and Transfers of Marine Organisms.

Alternative 7 – Establish a naturalized, reproducing, and self-sustaining population of *C. ariakensis* in the tidal waters of Maryland and Virginia through introductions beginning in 2005 (or when the EIS is completed) but discontinue efforts to restore *C. virginica*.

Alternative 8 – Combination of Alternatives

The intended purpose of the proposed action or one of the alternatives, as stated in the PEIS, is to restore oysters to the Chesapeake Bay. The states of Maryland and Virginia see a need to restore the ecological role of oysters in the Bay and the economic benefits of a commercial fishery by increasing the population of the native oyster, establishing a population of an ecologically compatible nonnative species that would restore these lost functions, enhancing aquaculture in the Bay, or a combination of these actions.

The NRC synthesized considerations about intentional introductions of nonnative oysters into Chesapeake Bay in a literature review and summary (NRC 2004). That review is considered to be an initial or Tier 1 Risk Assessment for this ERA. The NRC presented a detailed analysis of the available literature and research concerning the potential introduction of *C. ariakensis* into Chesapeake Bay and provided extensive information about current and past efforts to restore the population of *C. virginica* in the Bay. The NRC review did not address the specific alternatives to the proposed action addressed in this ERA and in the PEIS. This ERA builds on the information presented in the NRC's report and addresses the outstanding ecological risk questions it described as a Tier 2 Risk Assessment for the proposed action and selected alternatives. This ERA also considers the broader ecological implications of the alternatives for representative components of the Bay's ecosystem.

No analysis of the ecological consequences of Alternative 6 was possible because no Bay-specific research findings are available for any other species or strain, and the potential increase in oyster stock could not be projected for any other species or strain. Alternative 8 was not evaluated because the lead agencies decided to wait until stakeholders have had an opportunity to comment on the alternatives evaluated in the draft PEIS before defining a combination of alternatives to be considered.

"Risk" is defined as "exposure to the chance of injury or loss; a hazard or dangerous chance." The term clearly refers specifically to the potential for an adverse or undesirable outcome. This ERA is an atypical risk assessment because it evaluates not only outcomes that may be viewed as adverse, but also those that may be considered beneficial. One goal of the proposed action and alternatives is to restore desirable functions of oysters in the Bay; therefore, one risk being considered is the risk of failing to restore such functions. To the extent possible, the expected ecological outcomes of implementing the proposed action and alternatives are characterized as beneficial or adverse with regard to the goal of restoring the functions of oysters. Although multiple actions are evaluated, this ERA is not intended to be a comparative assessment. The lead agencies intend this ERA to describe the ecological outcomes of the proposed action and each alternative without comparing the alternatives in a comprehensive ranking. The findings of the ERA will be incorporated into the draft PEIS together with assessment findings for all other components of the affected environment (e.g., economics, archaeological resources). The draft PEIS will be a means of soliciting input from stakeholders. The lead agencies will use stakeholders' input to identify the most appropriate course of action to be defined and supported in the final PEIS.

The proposed action and alternatives represent diverse approaches to achieving oyster restoration that vary in methods, requirements, and species involved. The range of alternatives being considered reflects the variety of interests of stakeholders. Eight risk questions were developed as a framework for comparing the alternatives based on the stated need for action, the goal of action, and the characteristics of the actions being evaluated. In its preliminary assessment of the status of knowledge about *C. ariakensis* and the possible risks of introducing it in Chesapeake Bay, the NRC identified five questions that could not be addressed at the time due to insufficient information. The NRC's five questions are noted with asterisks in the following list of risk questions evaluated in this ERA.

1. Will the proposed action or alternatives achieve the stated goal for the size of the Bay-wide oyster population?
2. Will *C. ariakensis* provide ecosystem services similar to those afforded by *C. virginica*?*
3. Will *C. ariakensis* compete with *C. virginica* and other resident species, possibly leading to reduced populations or local extinction of these species?*
4. What is the potential for introduction and spread of diseases to other species in the Bay?*
5. Will *C. ariakensis* become an invasive or nuisance species?*
6. Will *C. ariakensis* disperse to areas outside of Chesapeake Bay and pose the kinds of risks identified above?*

7. Will the action result in an unintended introduction of *C. ariakensis* into Chesapeake Bay?
8. To what extent will the proposed action and alternatives influence ecosystem services in Chesapeake Bay?

Several methods of assessment were needed to account for the diversity of the proposed action and alternatives and for limitations of the tools and information available for this ERA. The likelihood that implementing the proposed action or the alternatives would result in achieving the stated restoration goal for oysters (Risk Question 1) was evaluated by conducting exploratory modeling using the Oyster Demographic Model (ODM), which projects the growth of the Bay-wide population of oysters over a 10-year period. The trajectory of population growth over that time period was the basis for speculating about changes in population size more than 10 years after implementing any of the actions. Available information about *C. ariakensis* biology was insufficient to project the growth of a population in Chesapeake Bay with any certainty;therefore, exploratory modeling results were not available for this species. We examined the potential growth of a population of *C. virginica* with lower mortality and greater resistance to disease; this provided a benchmark for possible growth of *C. ariakensis*. General conclusions drawn from a synthesis of literature and the findings of recent research concerning the risks associated with introducing *C. ariakensis* were the primary means of addressing Risk Questions 2 through 6, which apply only to alternatives involving that species (i.e., the proposed action and Alternatives 5 and 7). Those conclusions are based on the assumption that *C. ariakensis* is successfully introduced, becomes well-established, and spreads widely throughout the Bay. The likelihood that cultivating triploid *C. ariakensis* (Alternative 5) would result in an unintended introduction into Chesapeake Bay (Risk Question 7) was evaluated using a combination of qualitative assessment (for accidental releases) and an estimate of the probability of development of a reproducing pair of diploids from triploids in aquaculture systems via several pathways. The chain of events and associated probabilities for the triploid-to-diploid risk assessment were developed using the *Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process* developed by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service. Finally, the consequences of the proposed action and alternatives for ecosystem services as reflected in water quality and other ecological receptors (Risk Question 8) were evaluated either qualitatively (in the case of a successful introduction of *C. ariakensis*) or by using the Relative Risk Model (RRM). Input for the RRM included exploratory modeling projections of oyster populations and projections for water quality and submerged aquatic vegetation (SAV) from the Chesapeake Bay Environmental Modeling Package (CBEMP). The RRM describes the influences that changes in oyster biomass could have on water quality and other ecological receptors considered to represent the various components of the Chesapeake Bay ecosystem. A positive influence is any consequence of a change in oyster biomass that might support or encourage an increase in the abundance, health, or distribution of the receptor population. A negative influence is any consequence of a change in oyster biomass that might cause or contribute to a decrease in the abundance, health, or distribution of the receptor population. Where appropriate and possible, we discuss whether stakeholders are likely to consider such influences to be beneficial or adverse (i.e., a negative influence on one receptor, such as phytoplankton, might be viewed as a benefit if it increases water clarity and dissolved oxygen, while a negative influence on a different receptor, such as striped bass, might be considered adverse).

The ERA follows USEPA's ecological risk assessment process (Figure 1-1). The process begins with Problem Formulation (USEPA 1992, 1998) and proceeds to evaluate the degree to which stressors pose risks with reference to selected assessment endpoints. Stressors can be chemical, biological, or physical in nature. The alternatives for oyster restoration have the potential to introduce a broad range of stressors, as is discussed in Section 2 of this ERA. Assessment endpoints refer to the ecological components that are the focus of the evaluation. For this ERA, the assessment endpoints include the sustainability of oyster species and other species that represent various ecological components of the Bay. Problem Formulation is presented in Section 2, which describes the proposed action and alternatives, the Chesapeake Bay ecosystem, and the ecological resources that may be affected by changes in oyster populations. Problem Formulation also includes the assessment endpoints selected for evaluation in this ERA. These assessment endpoints focus the analysis and were developed through consultation with various parties involved in decision-making.

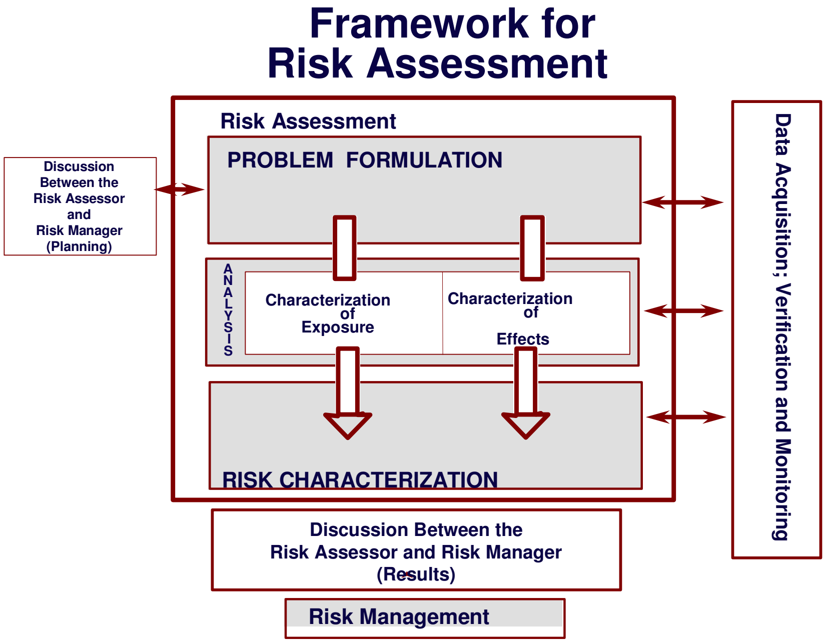

Figure 1-1. Framework for ecological risk assessment (USEPA 1992)

Section 3 describes the approach and methods used to assess the risks and potential benefits of each alternative.

Section 4 presents the results of the risk analyses for the proposed action and each of the alternatives and describes the uncertainties associated with the analyses. The risks and potential benefits of the proposed action and alternatives and their associated uncertainties are addressed for each of the risk questions.

All ERAs have inherent uncertainty that relates to lack of knowledge about aspects of the analysis or limitations of relevant data and information. For example, a risk analyst might infer a particular outcome from laboratory studies without the support of reliable field observations to corroborate the inference. Acquiring more knowledge can reduce uncertainty, and research on the biology of *C. ariakensis* conducted over the last several years was designed to address important sources of uncertainty associated with the proposed action. Nevertheless, uncertainty remains, and decisions based on this ERA must recognize that uncertainty. For the decision-maker, this involves weighing the potential beneficial and adverse consequences of the decision along with the associated uncertainty. For the purpose of this assessment we describe the likelihood and/or magnitude of an outcome as negligible, low, moderate, or high. The criteria for assigning these qualitative terms are as follows:

- Negligible - If assumptions are met, there is no chance or virtually no chance that this outcome will occur.
- Low - The chance that the outcome will occur is very small.
- Moderate - An outcome is possible.
- High - The chance that an outcome will occur is large.

We also use relative terms to characterize the degree of uncertainty associated with the estimates of likelihood and magnitude:

- Low - The mechanisms of action are well understood and available information is sufficient to support a conclusion.
- Moderate - We are reasonably sure of the conclusion, but some aspects of the mechanism of action or consequences are not well understood, and its magnitude may be uncertain.
- High - Available information (e.g., data from laboratory studies) provides some insight into mechanisms of action, but we are not sure about how interactions would be manifested in the environment (i.e., regarding either likelihood or magnitude).

2.0 PROBLEM FORMULATION

Problem formulation is the process of generating and evaluating preliminary hypotheses about why human actions may cause (or have caused) ecological effects. This process provides the foundation for an ERA. An ERA can be based on what is known as well as on what can be extrapolated from what is known (USEPA 1998). The function of an ERA is to synthesize information to contribute to management decisions. An ERA is not, in and of itself, a scientific research effort; therefore, it is not possible or necessary to incorporate every aspect of every ecosystem component or every potential interaction into an ERA. This ERA for oyster restoration in Chesapeake Bay is complex because it must encompass the potential consequences throughout the entire Bay ecosystem of diverse alternatives being considered for restoring the ecological and economic functions of a declining oyster population. The challenge was to formulate the problem so as to cover the wide range of potential ecological consequences associated with the various alternatives. We have attempted to assess likely or foreseeable effects succinctly. This ERA is not intended to duplicate prior or continuing efforts, but rather to synthesize available information about all potential outcomes of the proposed action and alternatives. The level of analysis for each of the eight risk questions identified in Section 1 and for each ecosystem element considered with regard to consequences for ecological services is commensurate with the amount and quality of data and information available.

Risk Question 1 (Will the proposed action or alternatives achieve the stated goal for the size of the Bay-wide oyster population?) addresses the potential for growth of the oyster population over time expected to result from implementing the proposed action and each of the alternatives. Management agencies consider the restoration goal, an oyster population of the average size that existed between 1920 and 1970 (estimated at 1.16×10^{10} market-size oysters; see PEIS Appendix A), to be reasonably representative of the size of a self-sustaining oyster population that could provide significant ecological services and support a reasonable oyster fishery. The magnitude of ecological services provided by oysters in Chesapeake Bay is considered to be proportional to the biomass of oysters present. The projected outcomes used to address this risk question, therefore, also serve as input for addressing Risk Question 8 about the consequences of the proposed action and alternatives for ecological services in the Bay. The risk being assessed is that implementing the proposed action or one of the alternatives would **fail** to achieve the restoration goal.

Risk Questions 2 through 6 apply only to the proposed action and Alternatives 5 and 7, all of which involve *C. ariakensis*. These NRC (2004) identified these questions as being unresolvable at the time due to the lack of data and information. Research conducted since then provided the basis for addressing those questions through a review and synthesis of the most relevant findings. The evaluations of risk presented in this ERA assume that *C. ariakensis* will be successfully naturalized and become abundant and widespread throughout the Bay. The premise for that assumption is that risks would be proportional to the size of the population of *C. riakensis* within the Bay. The evaluations use qualitative terms to describe the likelihood of the outcomes suggested in the five risk questions and the degree of uncertainty associated with answering each question. Those conclusions are supported by a brief review and synthesis of the findings of relevant research.

Risk Question 7 (Will the action result in an unintended introduction of *C. ariakensis* into Chesapeake Bay?) applies only to Alternative 5, aquaculture with triploid *C. ariakensis.* An unintended introduction is considered a risk for this alternative because the specification to use triploid *C. ariakensis*, which generally are believed to be unable to reproduce, reflects a compromise that addresses stakeholders' conflicting views about the desirability of introducing a non-native species while attempting to attain the potential economic benefit of using the species. The quantitative difference between Alternative 5 and the proposed action and Alternative 7 is that an unintentional introduction would occur as a result of much smaller and more localized introductions; consequently, the rates of increase and dispersal would be much slower than expected following a purposeful introduction. The *Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process* was used in developing the approaches for estimating the probability that cultivating triploid *C. ariakensis* in large-scale aquaculture operations would result in a reproductive population in the Bay.

Risk Question 8 (To what extent will the proposed action and alternatives influence ecosystem services in Chesapeake Bay?) is applicable to the proposed action and all of the alternatives. To formulate the problem for addressing this risk question, the major components of the Chesapeake Bay ecosystem that may be affected by the proposed action or alternatives were defined, and species or variables that represent those components were identified based on the ecological requirements of the species (e.g., habitat and food). The representativeness of species or variables selected for use in the ERA was taken into account in assessing the level of uncertainty associated with estimates of risk, as is discussed in Section 4.

This section presents a brief overview of the habitat and ecology of Chesapeake Bay and the role of oysters within it (Section 2.1), the actions considered in the risk assessment (Section 2.2), the mechanisms by which the selected ecological receptors could be affected by the alternative actions (Section 2.3), and the assessment and measurement endpoints evaluated in the ERA (Section 2.4).

2.1 OVERVIEW OF THE HABITAT OF CHESAPEAKE BAY[3]

Chesapeake Bay is the largest estuary in the U.S. An estuary is a partially enclosed body of water formed where fresh water from rivers and streams flows into the ocean (http://www.epa.gov/owow/estuaries/about1.htm). Estuaries are influenced by the tides but protected from the full force of ocean waves, winds, and storms by reefs, barrier islands, or peninsulas that define an estuary's seaward boundary. Within an estuary, fresh water mixes with salt water, and each contributes its own chemical and physical characteristics. This creates a range of environments that support a wide variety of plants and animals. The Chesapeake Bay is a complex ecosystem that includes several different kinds of habitat and ecological communities. Geologically, Chesapeake Bay is less than 10,000 years old. The Bay proper is approximately 200 miles long, stretching from Havre de Grace, Maryland, to Norfolk, Virginia. Troughs from the ancient Susquehanna River basin form a deep channel along much of the length of the Bay. This channel allows passage of large commercial vessels. The Bay varies in width from about

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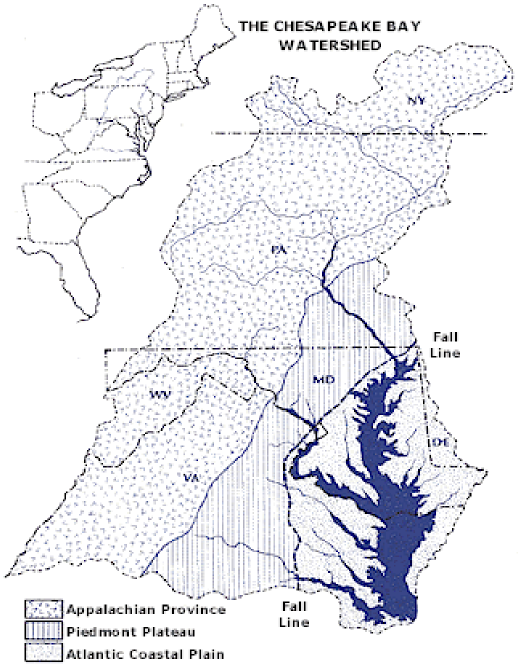
[3] This general description of the Chesapeake Bay is adapted from Reshetiloff 1995 and the Chesapeake Bay Program web page (http://www.chesapeakebay.net), with some general editing.

3.4 miles near Aberdeen, Maryland, to 35 miles at its widest point near the mouth of the Potomac River. Although Chesapeake Bay is long and wide, its average depth is only about 21 feet. Because the Bay is so shallow, it is more sensitive to temperature fluctuations and wind than the open ocean. Including its tidal tributaries, the Bay has approximately 11,684 miles of shoreline. Fifty major tributaries flow into the Bay. Most of the fresh water entering the Bay (80% to 90%) comes from tributaries on the northern and western sides, and these inputs influence the spatial extent and distribution of salinity zones. Chesapeake Bay is commonly evaluated in terms of segments defined by state, geomorphology, and salinity characteristics. Bay segments established for many of the USEPA's Chesapeake Bay Program projects were employed in this analysis. This ERA focuses on the six broad zones defined by state and salinity and groups the segments within those zones (Section 3-4). This ERA does not address the tidal freshwater tributaries of the Chesapeake.



From: EPA, Chesapeake Bay Program

Chesapeake Bay lies totally within the Atlantic Coastal Plain, but the watershed includes parts of the Piedmont Province and the Appalachian Province. The tributaries that feed the Bay from these three different geological provinces contribute waters carrying minerals, nutrients, and sediments that reflect a broad geochemical range. The Atlantic Coastal Plain is flat and low. Its maximum elevation is about 300 feet above sea level. It is supported by a bed of crystalline rock covered with southeasterly dipping layers of unconsolidated sand, clay, and gravel. Water draining through this material dissolves many minerals, including iron, calcium, and magnesium. The fall line, which ranges from 15 to 90 miles west of the Bay, forms the boundary between the Piedmont Plateau and the Coastal Plain. Waterfalls and rapids clearly mark this line, which is close to Interstate 95. Here, the elevation rises to 1,100 feet. The Piedmont Plateau extends to the Appalachian Mountains in the west. Several types of dense crystalline rock, including slates, schists, marble, and granite, compose the eastern side of the Piedmont. Rocks of the Piedmont tend to be impermeable, and water from the eastern side is low in the calcium and magnesium salts. The western side of the Piedmont consists of sandstones, shales, and siltstones, underlain by limestone. This limestone bedrock contributes calcium and magnesium to its water, making it hard. The Appalachian Province lies in the western and northern parts of the watershed. Sandstone, siltstone, shale, and limestone form the bedrock. These areas, characterized by mountains and valleys, are rich in coal and natural gas deposits. Water from this province flows to the Bay mainly via the Susquehanna River.

Salinity is a key factor affecting the physical make-up of the Bay. Salinity increases with depth, and fresh water tends to remain at the surface. Salinity is greatest at the mouth of the Bay, where seawater from the Atlantic Ocean enters. Salinity levels within the Bay vary seasonally and from year to year, depending on the volume of fresh water flowing into the Bay. Because the greatest volume of fresh water enters the Bay from northern and western tributaries, isohalines tend to show a southwest to northeast tilt. The rotation of the earth, or the Coriolis force, deflects flowing water to the right in the Northern Hemisphere so that saltier water moving up the Bay is deflected toward the Eastern Shore.

Temperature changes the rate of chemical and biological reactions within the water. Because the Bay is shallow, it has a very limited capacity to store heat. As a result, water temperature fluctuates throughout the year, ranging from 0 °C to 29 °C (32-84 °F). These changes in water temperature influence when plants and animals feed, reproduce, move locally, or migrate. The temperature profile of the Bay is fairly predictable. During spring and summer, surface and shallow waters are warmer than deeper waters, and the coldest water is found at the bottom. Turbulence of the water helps to break down this layering during the winter.

Water circulation transports plankton, fish eggs, shellfish larvae, sediments, dissolved oxygen, minerals, and nutrients throughout the Bay. Circulation is driven primarily by the movement of fresh water from the north and salt water from the south. Circulation causes nutrients and sediments to be mixed and resuspended. This mixing creates a zone of maximum turbidity that fish and other organisms often use as a nursery area because of the large amount of nutrients available there. Weather contributes to circulation when wind promotes mixing and when variations in the volume of rainfall influence the vertical and horizontal distributions of salinity within the Bay.

The waters of the Bay also transport sediments. As suspended sediments settle to the bottom, they can smother benthic plants and animals. Sediments suspended in the water column cause the water to become turbid, decreasing the light available to support the growth of submerged aquatic vegetation (SAV). The characteristics of sediments in the Bay vary according to their place of origin. In the upper Bay and tributaries, sediments are fine-grained silts and clays that are light and can be carried long distances. As these sediments move through the Bay, the particles slowly descend into the denser saline layer. There, the particles may reverse direction and flow back up toward tidal tributaries with the lower layer of water. As the upstream flow decreases, the sediments settle to the bottom. Sediments in the middle Bay are mostly made of silts and clays. These sediments are mainly derived from shoreline erosion. In the lower Bay, the sediments are sandier and heavier. These particles result from shoreline erosion and inputs from the ocean. Such heavy sediments drop to the bottom fairly rapidly, remain near their original sources, and are less likely to be resuspended than finer silts. Some nutrients and contaminants readily adsorb to sediments; consequently, areas of high sediment deposition sometimes have high concentrations of nutrients and persistent contaminants.

Like temperature and salinity, the chemical composition of the water also helps determine the distribution and abundance of plant and animal life within the Bay. The waters of the Chesapeake contain organic and inorganic materials, including dissolved gases, nutrients, inorganic salts, trace elements, heavy metals, and potentially toxic chemicals. Major constituents of saline water include chlorides, sodium, magnesium, calcium, and potassium. Dissolved salts are important to the life cycles of many organisms. Some fish spawn in fresh or slightly brackish water and must move to more saline waters as they mature. These species have internal mechanisms that enable them to cope with the changes in salinity. Metals such as mercury, lead, chromium, and cadmium also occur in low concentrations. Unlike seawater, which has a relatively constant composition, the chemical composition of fresh water varies depending upon the soil and rocks that the water has come in contact with. Both fresh and salt water contain natural dissolved materials, such as the by-products of decomposition. Organisms living in the Bay also release compounds directly into the water.

Dissolved oxygen and carbon dioxide are essential for most animals inhabiting the Bay. The amount of available oxygen is affected by salinity and temperature. Cold water can hold more dissolved oxygen than warmer water, and fresh water holds more than saline water. Oxygen is transferred from the atmosphere into the surface waters by diffusion and the aerating action of the wind. It is also a by-product of photosynthesis by floating and rooted aquatic plants and phytoplankton. Because photosynthesis requires light, production of oxygen by aquatic plants is limited to areas of shallow water, usually less than six feet deep. Surface water is nearly saturated with oxygen most of the year, whereas bottom waters range from saturated to anoxic. During the winter, respiration levels of organisms are relatively low; vertical mixing is good; and stratification of the water column by salinity or temperature is minimal. As a result, dissolved oxygen is plentiful throughout the water column. During the spring and summer, increased levels of respiration by animals and microbes and greater stratification may reduce vertical mixing, resulting in low levels of dissolved oxygen in deep water. In the autumn, when surface waters cool, vertical mixing occurs, and deep waters are re-oxygenated. Carbon dioxide is a by-product of animal respiration and provides the carbon that plants use to produce new tissue during photosynthesis. Carbon dioxide is more soluble in water than oxygen. Its availability is affected by temperature and salinity in much the same way as the availability of oxygen.

Nutrients like nitrogen and phosphorus occur naturally in water, soil, and air. Just as the nitrogen and phosphorus in fertilizer aid the growth of agricultural crops, both nutrients are vital to the growth of plants and animals in the Bay. Nitrogen is essential to the production of plant and animal tissue. Plants and animals use it to synthesize protein. Nitrogen enters the ecosystem in several chemical forms and also occurs in other dissolved or particulate forms, such as in the tissues of living and dead organisms. Some bacteria and blue-green algae can extract nitrogen gas from the atmosphere and transform it into organic nitrogen compounds. This process, called nitrogen fixation, cycles nitrogen between organic and inorganic components. Other bacteria release nitrogen gas back into the atmosphere as part of their normal metabolism in a process called denitrification. Denitrification removes about 25 percent of the nitrogen entering the Bay each year. Phosphorus is another key nutrient in the Bay's ecosystem. In the water, phosphorus occurs in dissolved organic and inorganic forms, often attached to particles of sediment. This nutrient is essential to cellular growth and reproduction. Phytoplankton and bacteria assimilate and use phosphorus in their growth cycles. Phosphates, the organic form, are preferred, but organisms will use other forms of phosphorus when phosphates are unavailable. In the presence of oxygen, high concentrations of phosphates in the water will combine with suspended particles. These particles eventually settle to the Bay bottom and are temporarily removed from the cycling process. Phosphates often become long-term constituents of bottom sediments. Phosphorus compounds in the Bay generally occur in greater concentrations in less saline areas, such as the upper part of the Bay and tributaries. Overall, phosphorus concentrations vary more during summer than during winter.

When present in excess, nutrients are pollutants. Sewage treatment plants, industries, vehicle exhaust, acid rain, and runoff from agricultural, residential, and urban areas are additional sources of nutrients entering the Bay. Excess amounts of phosphorus and nitrogen cause rapid growth of phytoplankton, creating dense populations, or blooms. These blooms become so dense that they reduce the amount of sunlight available to SAV. Without sufficient light, plants cannot carry out photosynthesis to produce the food they need to survive. Algae may also grow directly on the surface of SAV, blocking light. Another hazard of nutrient-enriched algal blooms that are not consumed by zooplankton comes after the algae die. As the blooms decay, oxygen is used up in decomposition. This can lead to inadequate levels of oxygen in the water, which can harm or kill aquatic organisms. Besides nutrients, other toxics and pollutants create problems in the Bay. Heavy metals, insecticides, herbicides, and a variety of synthetic products and by-products can be toxic. These contaminants reach the Bay through municipal and industrial wastewater; runoff from agricultural, urban, and industrialized areas; and atmospheric deposition.

The Bay watershed provides habitat for more than 3,000 migratory and resident species of fish and wildlife. Kinds of habitat range from hardwood forests to saltwater marshes. Upland Bay habitats support insects, amphibians, reptiles, birds, and mammals. Forests and Bay islands are particularly critical habitat for waterfowl. Most Bay fish are pelagic, living in the open waters of the Bay. Rivers and streams provide habitat for anadromous species, including striped bass, alewife, American and hickory shad, and shortnose and Atlantic sturgeon.

Shallow water habitat is critical for many life stages of invertebrates, fish, and waterfowl. Shallow water structures, such as submerged grasses and marshes, are essential nursery habitats for invertebrates and fish. Other bottom-dwelling species include invertebrates, some fish, and the blue crab. Many benthic invertebrates are filter feeders that help maintain water quality by filtering suspended organic particles out of the water. Oysters are also reef-building invertebrates, providing additional structure that supports other shellfish, finfish, and crabs.

Oysters fill an ecological niche in the Chesapeake Bay ecosystem that intertwines with nearly all other elements of the community. The native oyster tolerates wide variations in the Bay's environment, including substantial fluctuations in temperature, salinity, and suspended sediments; therefore, it is widespread throughout the Bay. Oysters historically played an important role in the Bay ecosystem by providing habitat for numerous other species and by removing suspended material, such as sediments and plankton, from the water column. Because other species of wildlife in the Bay may use different habitats at different times during their life cycles, their interactions with oysters may be diverse and variable over time. Changes in oyster populations, therefore, can affect a wide range of species and biological communities in many different ways. Large-scale commercial harvesting and diseases of oysters have led to dramatic declines in the population in Chesapeake Bay, and consequent effects on the ecosystem are apparent. The various components of the ecosystem of Chesapeake Bay being addressed in this ERA are described in Section 2.4.

2.2 IDENTIFICATION OF STRESSORS

Stressors refer to chemical, biological, or physical factors that influence ecological conditions at the level of a single species, a biological community, or an entire ecosystem. Most ERAs focus on a particular kind of stressor, such as the presence of chemical contaminants in the environment. In such ERAs, receptors are identified based on the pathways by which the contaminants may move through the food chain and ecosystem. Other ERAs may focus on a particular biological or physical stressor, and the USEPA and other agencies have applied the ecological risk assessment framework to these situations. USEPA also developed a framework for considering the cumulative risk associated with multiple stressors (USEPA 2003). This ERA evaluating alternatives for oyster restoration is more complex than typical ERAs because it considers a suite of chemical, biological, and physical stressors associated with a variety of alternatives that differ in the amounts, spatial distributions, and species of oysters. The stressors also will vary over these spatial scales. Stressors – chemical, biological, and physical – could increase or decrease in magnitude as a result of a change in the population of oysters in the Bay; consequently, they could have positive influences, negative influences, or both on the ecological receptors of the Bay.

Exploratory modeling using the Oyster Demographic Model (see PEIS Appendix A) was the primary tool for addressing Risk Question 1 (achievement of the oyster restoration goal). The exploratory modeling projected changes in populations by simulating the effects of management actions on three rates that determine the size of an oyster population, which biologists call vital rates. The three vital rates are (1) the rate at which larval oysters are produced and successfully reach the spat life-stage (i.e., recruitment), (2) the rate of growth of individual oysters, and (3) the rate at which oysters die (i.e., mortality). These rates vary with environmental conditions and the characteristics of the particular species of oyster and interact in ways that are difficult to envision without conducting complex calculations. The exploratory modeling was a tool for integrating empirical data about the vital rates of oysters with additional information about their interactions with management actions (e.g., harvest and stocking) and the environment to project the size of the oyster populations. From a risk assessment perspective, the exploratory modeling accounts for most of the factors that might be considered stressors on oyster population growth (e.g., salinity conditions, disease mortality, harvest mortality); however, some important stressors (e.g., decline in oyster habitat over time) are not reflected in the model. The details and limitations of the exploratory modeling are summarized in Section 3 and described in detail in PEIS Appendix A.

In the evaluation of Risk Questions 2 through 6, which pertain to the proposed action and Alternatives 5 and 7, *C. ariakensis* is considered to be the stressor. The risk questions address outcomes that many stakeholders consider to be undesirable. Those risks are evaluated by considering recent research related to the biology of *C. ariakensis* and the ecology of Chesapeake Bay. Those evaluations assume that *C. ariakensis* will be naturalized successfully and will become abundant and widespread throughout the Bay.

Risk Question 7, regarding the potential for an unintended introduction of *C. ariakensis*, pertains to Alternative 5 only. To the extent that an unintended introduction might occur, the introduced population would constitute the stressor, as described for Risk Questions 2 through 6.

In addressing Risk Question 8, we considered the following categories of stressors, all of which are influenced by differences in the abudance and distribution of oysters: changes in habitat, food, predators, and water quality. The positive and negative influences of stressors associated with the proposed action and each alternative are identified, and we evaluate how those influences might affect representative ecological receptors without expressing value judgments regarding whether those influences are "good" or "bad" for the Bay as a whole. The term "influence" describes the range of potential positive and negative effects that changes in oyster abundance (measured as biomass) might cause among oysters and other ecological components of the Bay. A positive influence is any consequence of a change in oyster biomass that might support or encourage an increase in the abundance, health, or distribution of the receptor population. A negative influence is any consequence of a change in oyster biomass that might cause or contribute to a decrease in the abundance, health, or distribution of the receptor population. For example, a negative influence on the amount of algae means that the increased filtration expected to be associated with an increase in the abundance of oysters could reduce the abundance of algae. This "negative" influence is different than a value judgment concerning whether reducing the abundance of algae in the Bay should be considered a beneficial outcome or an adverse one. A positive influence means that an increase in the abundance of oysters could increase the availability of food or habitat for other species, without comment regarding the value of that change. The term influence is broader than the term "risk," which typically refers only to the potential for adverse outcomes of some action or agent. The expected ecological influences of restoring oysters in Chesapeake Bay (e.g., provision of habitat and increase in water clarity) are widely perceived to be beneficial potential outcomes of the proposed action and alternatives. The consequences of introducing a nonnative species to achieve restoration goals may include those beneficial outcomes as well as others that many stakeholders perceive to be adverse (e.g., introduction of new diseases, extinction of the native species, alterations in the interactions of other species in the Bay).

As noted earlier, this atypical ERA addresses both the the potential beneficial outcomes of increasing the oyster population in Chesapeake Bay and the risks associated with actions being considered to achieve that objective. Considering both positive and negative influences provides a more complete picture for managers of the overall consequences of alternatives for oysters and other ecological components of the Bay. A comprehensive evaluation of the ecological consequences of the proposed action and alternatives requires this broader perspective. This ERA captures the major kinds of potential influences of the proposed action and alternatives on the major ecological receptors in the Bay; however, oysters may interact with these and other ecological receptors in subtle ways that we do not understand. The potential significance of such unknown relationships is discussed in Section 4.7.

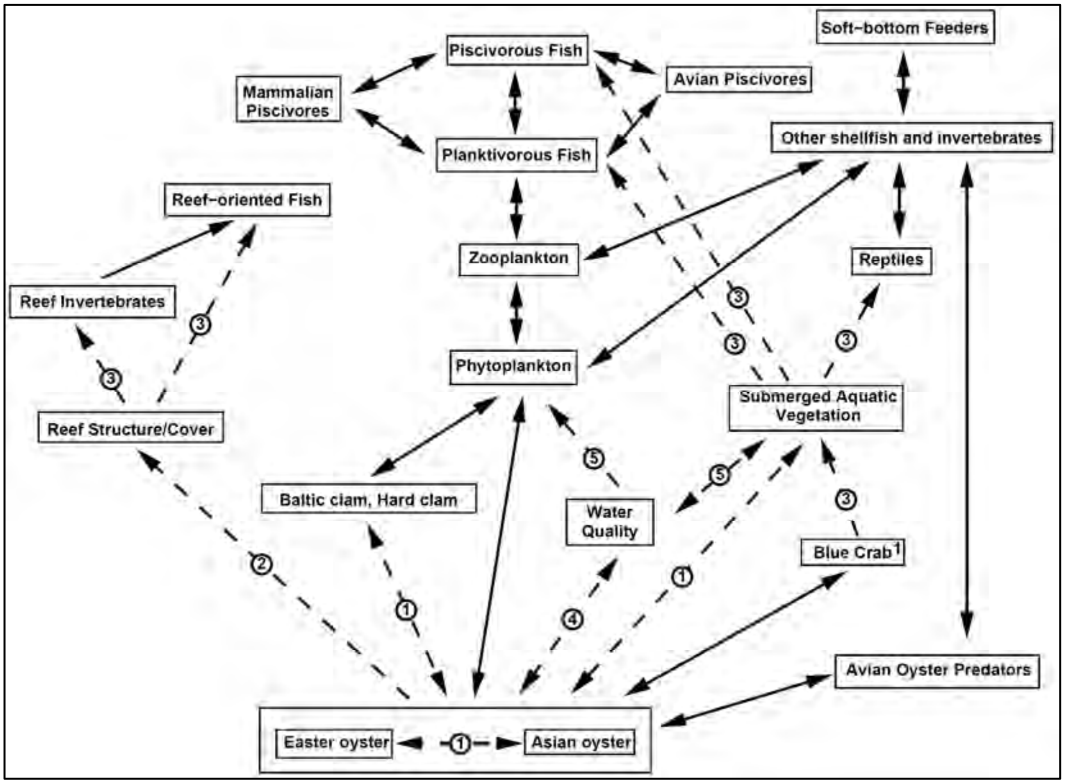
2.3 IDENTIFICATION OF MECHANISMS OF EFFECT AND RECEPTORS

The exploratory modeling was the primary means of addressing Risk Question 1. That model was constructed to include as many of the factors that influence oyster population growth as possible, and to account for all mechanisms through which that influence is exerted. The size of the oyster population can be considered to be the receptor for that risk question.

Mechanisms of effect and receptors differ widely for Risk Questions 2 through 6 because of the broad range of potential outcomes encompassed by those questions. Findings of the most relevant recent research are summarized and integrated in descriptive evaluations of risk in response to those questions. For example, regarding Risk Question 3 (competition between the two species), *C. virginica* is considered the receptor, and all potential mechanisms by which the two species might interact are described and evaluated using current research findings. Because the NRC's five questions address outcomes that many stakeholders consider undesirable, the evaluations of the risks suggested in those questions assume successful introduction of *C. ariakensis* resulting in widespread establishment of the species throughout the Bay. That assumption represents a conservative view of the possible outcomes (i.e., a worst case scenario; failure or limited success of an introduction would reduce or eliminate risks associated with most of those questions). Regarding Risk Question 7, if an unintended introduction of *C. ariakensis* were to occur, the mechanisms of effect and receptors would be the same as for Risk Questions 2 through 6

Addressing Risk Question 8 requires identifying the mechanisms by which changes in oyster abundance might affect receptor species. All biota inhabiting the Chesapeake Bay are potential receptors of the "stress" caused by changes in oyster populations. We designed an assessment approach to simplify the enormous task of evaluating risk across so many potential receptors while encompassing the major mechanisms by which changes in oyster populations could affect the ecosystem of Chesapeake Bay. This ERA does not address all possible mechanisms of effect; it considers only those mechanisms that have a reasonable likelihood of influencing a receptor. The major mechanisms of potential effect do not apply for all alternatives; moreover, many of the potential effects cannot be quantified. Despite the fact that Chesapeake Bay is one of the most intensively studied estuarine ecosystems in the world, many of the quantitative parameters of the structure and function of the Bay's complex ecosystem have not been described fully. Given these levels of complexity and uncertainty, much of this assessment is qualitative and descriptive. We have attempted to identify all potentially affected ecological resources to the extent that risk assessors, advisors, and managers require for an adequate and appropriate assessment of the relative risks of the proposed action and alternatives being considered for restoring oysters in Chesapeake Bay.

Mechanisms of effect can be direct (e.g., a change in oysters as habitat) or indirect (e.g., changes in algal abundance and subsequent changes in water clarity). We quantitatively evaluated three major mechanisms of direct and indirect effects: changes in habitat, food, and water quality. Other effects were considered qualitatively. We identified direct and indirect mechanisms of effect for selected receptors by considering the role of oysters within the Chesapeake Bay ecosystem and estimating the relative likelihood that a potential receptor would be affected by changes in the abundance or kind of oysters present in the Bay. Figure 2-1 is a conceptual model of the interactions between oysters and other components of the Bay's ecosystem. The figure illustrates that changes in the oyster population could affect many receptors in many ecological niches throughout the system.



[1] Blue crab predation on soft-bottom clams may also be affected by oyster abundance. Arrow not shown.

Figure 2-1. Conceptual model of mechanisms of effect through which oysters and receptors interact. Solid lines indicate predator-prey relationships. Dashed, numbered lines indicate the following other types of relationships: (1) competition for space, (2) change in the amount of oyster-reef habitat, (3) change in habitat/cover, (4) change in nutrients or other water chemistry because of changes in oyster abundance, (5) change in production of phytoplankton or submerged aquatic vegetation because of changes total dissolved solids, light attenuation, or dissolved oxygen.

Oysters together with other ecological components of the Bay provide a range of ecological services. Some of these services are easily recognized, such as the presence of species of recreational, commercial, or aesthetic value to humans. Others contribute to improved water quality. Others are important for providing habitat or food. Based on these considerations, we identified the major components of the ecosystem that might be affected by changes in oyster populations and selected representative species from these components for which to evaluate the positive and negative influences associated with changes in oyster abundance. The representative species were selected based on well-known characteristics of the Bay ecosystem, particularly as summarized in "Habitat Requirements for Chesapeake Bay Living Resources" (Funderburk et al. 1991). When representative species could not be identified logically for a particular ecosystem component, or when the assessment approach was not applicable at the species level, the entire community of organisms that perform a similar ecosystem function was defined as a receptor. Receptors include animals and plants near the base of food webs as well as selected species of fish and wildlife. Decision makers and managers should recognize that influences on these representative species could be reflected to varying degrees among all species that are part of the same ecosystem component or community.

To further clarify and simplify the assessment, we classified the ecosystem components and key representative species into two groups: those likely to be affected through direct interactions with oysters and those likely to be affected indirectly through their interactions with species that interact directly with oysters. The interactions are shown in Table 2-1.

Table 2-1. Summary of the assessment and measurement endpoints for the oyster ERA

	Receptor	**Mechanism(s) and Types of Interactions**	**Representative Species**	**Assessment Endpoint**	**Section Describing Interaction**
1.	Benthic Hard Bottom	-Competition between *C. virginica* and *C. ariakensis* for food or substrate (direct)	*C. virginica* *C. ariakensis*	1	2.4.2.1.1
2.	Benthic Soft Bottom	-Competition with oysters for food or substrate (indirect)	Baltic clam Hard clam	1	2.4.2.1.2
3.	Submerged Aquatic Vegetation	-Change in water total suspended solids and dissolved nutrient concentrations caused by oysters (indirect)	Community	1, 2	2.4.2.2
4.	Blue Crab	-Change in abundance of oysters to prey upon (direct) -Change in habitat available to larval blue crabs via changes in submerged aquatic vegetation caused by oysters (indirect)	Blue crab	1	2.4.2.3
5.	Phytoplankton	-Change in amount or selectivity of predation by oysters (direct)	Community	1, 2	2.4.2.4
6.	Zooplankton	-Competition with oysters for planktonic food (indirect)	Copepod *A. tonsa*	1	2.4.2.5
7.	Planktivorous Fish	-Competition with oysters for planktonic food (indirect)	Menhaden Bay anchovy	1	2.4.2.6.1
8.	Reef-oriented Fish	-Change in the amount of food available on oyster reefs (direct) -Change in the amount cover provided by oyster reefs (direct)	Black sea bass Croaker Naked goby	1	2.4.2.6.2
9.	Piscivorous Fish	-Change in the abundance of prey fish caused by oysters (direct)	Blue fish Striped bass	1	2.4.2.6.3
10.	Reptiles	-Change in abundance or composition of invertebrate prey items caused by oysters (direct and indirect) -Change in distribution of vegetative cover caused by oysters (indirect)	Loggerhead turtle Diamondback Terrapin	1,3	2.4.2.6.4
11.	Avian Oyster Predators	-Change in abundance of shellfish prey items caused by oysters (indirect)	American oyster Catcher	1	2.4.2.7
12.	Avian Piscivores	-Change in abundance of prey fish caused by oysters (direct and indirect)	Bald eagle American osprey	1,3	2.4.2.8
13.	Avian Soft-bottom Feeders	-Change in abundance or composition of invertebrate prey items caused by oysters (indirect)	Black duck Canvasback duck	1,3	2.4.2.9
14.	Mammalian Piscivores	-Change in abundance of prey fish caused by oysters (direct and indirect)	Raccoon River otter	1,3	2.4.2.10
15.	Water Quality	-Changes in the extent of filtration by oysters (direct)	NA	4	2.4.2.11

[1] Assessment endpoint is the abundance of the receptor species, which may be affected through changes in rates of growth, survival, or reproduction.

[2] Assessment endpoint is the species composition the receptor community, which may be affected as above.

[3] Assessment endpoint is the distribution of the receptor species, which may be affected by migrations or changes in abundance.

[4] Assessment endpoints are water light attenuation, chlorophyll levels, nutrient levels, algal biomass, primary production, and bottom dissolved oxygen levels

Direct mechanisms of effect are those in which a receptor is directly affected by the size (abundance or biomass), spatial distribution, or characteristics of the oyster population. Direct receptor species interact directly with oysters (e.g., compete for the same substrate space) or depend on oysters or oyster reefs (e.g., require oyster reef habitat for successful completion of their life cycle). Direct receptors can be categorized by their ecological roles. Clearly, oysters themselves are a critical receptor for this ERA. Populations of *C. virginica*, *C. ariakensis,* or both could be affected directly by the proposed action or alternatives (if relevant according to alternative, see Section 4.4). Oyster-dependent species, such as the naked goby, would be affected directly by changes in the amount and distribution of their oyster-reef habitat. In Figure 2-1, those key species connected to oysters by only a single link are considered to be affected by direct mechanisms. Certainty about the influence of oysters on species that use oysters for food and habitat is likely to be greater than certainty about influences on species that are indirectly associated with oysters.

Indirect receptors are species that do not compete directly with oysters for food, but might be affected if one or more of their forage species was influenced by changes in oyster populations. Species connected by more than one link in Figure 2-1 are considered to be affected through indirect mechanisms. A good example of such a species is the blue crab. This species could be affected through habitat changes related to changes in the amounts of oyster reef (a direct effect) and SAV (an indirect effect of oyster-related changes in water clarity) as well as through direct and indirect linkages to food sources. Menhaden is an example of a species that is completely indirectly related to oysters. This species would not be greatly influenced by habitat changes but could be influenced indirectly through changes in algal biomass and water quality. The level of uncertainty in the risk assessment is likely to increase with increasing numbers of linkages (distance from) between receptors and oysters because particular uncertainties are associated with each link.

2.4 IDENTIFICATION OF ASSESSMENT AND MEASUREMENT ENDPOINTS

Assessment endpoints are explicit expressions of the actual environmental value that is to be protected, operationally defined by an ecological entity and its attributes (USEPA 1998). For example, the abundance of the receptor species may be considered to be an assessment endpoint. Measurement endpoints are the outcomes of the methods or means by which the assessment endpoints are approximated or represented. Measurement endpoints are generally surrogates for assessment endpoints and are necessary because, in most cases, assessment endpoints cannot be observed or measured directly. For example, it may not be possible to estimate the abundance of a species, but estimating the density of that species may be feasible through standard sampling techniques. Typically, measurement endpoints are the result or outcome of field surveys or laboratory studies that may be useful for evaluating the assessment endpoints in relation to relevant scientific literature. Identification of quantitative assessment and measurement endpoints for all of the risk questions is not possible for this atypical ERA, given the diverse nature of the questions and the limitations of available information. The endpoints identified here apply only to Risk Questions 1 and 8.

2.4.1 Chesapeake Bay Oyster Population Endpoint

Risk Question 1 focuses on the population of oysters in Chesapeake Bay because the stated goal of the proposed action is to restore oysters to the Bay by increasing their abundance (Section 1). The target population size (1.16×10^{10} market-size oysters) was estimated using harvest levels and fishing mortality rates for 1920 through 1970 (see PEIS Appendix A). Achieving the restoration goal may result in economic benefits, but this ERA evaluates only ecological outcomes. The primary assessment endpoint used to evaluate potential effects on the oyster population in Chesapeake Bay is the degree to which an alternative achieves the target population size after 10 years. Model results are provided only for *C. virginica.* The exploratory modeling projected oyster population growth over a period of only 10 years because the uncertainty associated with model projections increases with each time step. Extending projections beyond 10 years would have produced estimates that the model developers considered to be unacceptably unreliable. In addition, although the amount of habitat used as input to the exploratory modeling was kept constant over the assessment period, studies have shown a continuing decline in habitat over time (approximately 35% per decade), but the rate of habitat decline could not be projected and incorporated into the exploratory modeling. As a result, reliability of population projections beyond 10 years was considered by model developers to be further compromised. The exploratory projections of the size of the oyster population 10 years after implementing an action are useful for comparing outcomes among the alternatives; futhermore, the trajectory of change in the population over time indicated in model projections as well as additional information provided a basis for informed speculation about changes in population size further into the future.

2.4.2 Chesapeake Bay Ecosystem Endpoints

Assessment endpoints for representative ecological receptors all involve considering how changes in the abundance, spatial distribution, and kinds of oysters would influence ecological components of the Bay. These influences could be either positive or negative and could be manifested through changes in habitat, food, water quality, and other factors. The following sections describe how we evaluated these assessment endpoints. The assessment and measurement endpoints considered in this ERA are summarized in Figure 2-1 and Table 2-1.

2.4.2.1 Benthic Community

Benthic, or bottom-dwelling, organisms in Chesapeake Bay live in a variety of environments, ranging from intertidal flats to deep channels. Distinct benthic communities are associated with different habitats, including mudflats, marshes, SAV beds, and oyster reefs. The benthos of habitats that remain submerged during low tide occupy mostly soft substrates. Benthic communities are structured by the physical and chemical environment as well as by complex interactions among species in the ecosystem. As a result, they can serve as an indicator of the environmental status of the location in which they reside. In 2006, 59% of the Bay's benthic habitat was considered degraded according to the Benthic Index of Biotic Integrity (CBP 2007). The percentage of degraded area in 2006 was substantially larger than the percentages in

2004 and 2005, probably as a result of the extended persistence of low dissolved oxygen during 2006. Research has suggested that reducing the amounts of nutrients, sediments, and chemical contaminants entering the Bay could improve the health of the benthic community (CBP 2007).

In the Chesapeake Bay, the distribution and kinds of benthic organisms (> 500 μm) are strongly correlated with salinity and are further influenced by sediment type, patterns of dissolved oxygen, and other physical factors (Diaz and Schaffner 1990; Llansó et al. 2002). The diversity and density of organisms generally increase with increasing salinity. Tidal freshwater habitats are numerically dominated by tubeworms and insect larvae, and the nonnative Asian clam (*Corbicula fluminea)* contributes to large biomass. Mildly to moderately salty regions exhibit greater diversity of organisms and feeding types than are observed in freshwater habitats. Shoals and channels within regions of medium salinity (i.e., mesohaline – 5 to 18 ppt) exhibit great densities of bivalves (e.g., clams, oysters), except where concentrations of dissolved oxygen are inadequate to support them. Segmented worms (i.e., polychaete annelids), small crustaceans, and suspension-feeding bivalves (*Rangia cuneata, Macoma* spp.) dominate these areas. The blue crab (*Callinectes sapidus*) is an important predator of bivalves, including young oysters, in these regions of the estuary. Areas of high salinity typically support a large variety of organisms. Suspension feeding polychaetes and tunicates are important contributors to biomass in high salinity environments, and their filtering capacity is comparable to that of bivalves in lower salinity environments. Oyster reefs and the polychaete annelid *Chaetopterus variopedatus* provide hard substrate for species-rich epifaunal (i.e., species that live on the surface of the bottom) communities (Dauer et al. 1982; Schaffner 1990).

Benthic communities play a central role in the transfer of materials from the water column to higher levels in the food web. Suspension-feeding organisms, such as oysters, transfer particles from the water column to the benthos by depositing nutrient-rich organic matter to the bottom. This process of biodeposition supports benthic primary production and stimulates the exchange of various forms of nitrogen and nitrogen compounds from one part of the system to another (Newell et al. 2002). Benthic organisms contribute to energy flow by focusing and transforming biological energy and transferring that energy to organisms higher in the food web. Benthic productivity constitutes the base that supports most fishery species. Much of the productivity of fisheries in the Chesapeake Bay is linked directly to the benthos through feeding (Virnstein 1977; Holland et al. 1988; Diaz and Schaffner 1990).

Oysters clearly are very important within the Chesapeake Bay ecosystem and are beneficial to the ecosystem in many ways. Oyster reefs provide substrate and shelter for invertebrates and finfish. They increase substrate for epibenthic organisms, enhancing filter-feeding populations of barnacles, mussels, tunicates, and sponges. Oysters control turbidity, the biomass of phytoplankton, and the composition of the phytoplankton community through selective feeding (Newell 1988). Oyster populations stimulate nitrification and denitrification processes by mediating changes in the water column, sediment, and nutrients (Newell et al. 2004). They release dissolved inorganic nutrients year around, which stimulates phytoplankton productivity rather than biomass.

We determined that the most concise means of incorporating the benthic ecosystem component into the assessment for this ERA was to consider two distinct communities, hard bottom and soft bottom. These two communities adequately encompass the major mechanisms of effect by which oysters could influence the benthic component of the Bay's ecosystem.

2.4.2.1.1 Hard Bottom

Oyster is the dominant species in hard-bottom habitats in Chesapeake Bay and the appropriate representative species for that habitat. Functioning oyster reefs provide and maintain habitat for numerous other epifaunal species, including barnacles, mussels, encrusting bryozoans, and sponges. In the absence of functioning oyster reefs, these organisms compete with oysters for limited space on hard surfaces such as pilings, rip-rap, shells, and boat bottoms. Successive generations of oysters growing on the shells of previous generations gradually accrete large, three-dimensional structures that can compensate for sedimentation, if the rate of growth of the oyster reef exceeds the rate of sedimentation. Thus, the role of oysters as "ecosystem engineers" is of special importance to the wide variety of organisms that depend on hard surfaces during some portion of their life histories. We considered oysters to be both a stressor and receptor in this assessment, depending on the alternative being evaluated and the risk question being addressed. For example, a reproductive population of *C. ariakensis* could impose stress on the native oyster species as evaluated under risk question 3, and large shifts in the abundance of oysters could influence the structure of the ecosystem as evaluated under risk question 8.

2.4.2.1.2 Soft Bottom

The soft-bottom benthic community, which predominates in Chesapeake Bay, interacts with oysters in a variety of ways. Some of the soft-bottom species can be found on substrate created by oysters, which can serve as a refuge from predation. Oysters augment the organic content of sediments in adjacent soft-bottom habitats through biodeposition, which increases the nutritive potential of the substrate for organisms occupying those habitats (Newell 1988; Dame 1993). Biodeposits contain a large proportion of organic matter (Newell and Jordan 1983) and provide a medium for the growth of bacteria, which deposit-feeding benthic organisms depend upon for energy (Levinton et al. 2001). An increase in biodeposits generally produces increased benthic productivity. Biodeposits also change the physical and chemical characteristics of sediments, including sediment texture, grain size, and chemical gradients (Pryor 1975; Risk and Moffat 1977; Dame 1993). These changes tend to increase the diversity of benthic fauna.

Suspension-feeding bivalves, such as clams, dominate the soft-bottom benthic community in mesohaline regions of the Chesapeake Bay (Holland et al. 1987). Two key species of bivalves considered to be representative of the soft-bottom benthic community are the hard clam (*Mercenaria mercenaria*) and the Baltic clam (*Macoma balthica*). These two species occupy different salinity regimes, covering the range of salinities in which oysters occur (*M. mercenaria* is found predominantly in higher salinities and *M. balthica* in lower salinities), and both are filter-feeding infauna (i.e., species that live completely or mostly buried within the bottom sediment). The major potential mechanism for these species to interact with oysters is through competition for food and space. Competition for space could occur on a local scale if an increase in oyster population causes an expansion of hard-bottom habitat over existing soft-bottom habitat. Increased competition between clams and oysters for food could result in a reduction in the abundance of infaunal bivalves. Such a reduction could trigger a shift in crab predation pressure from clams to oysters, since clams are important food items for blue crabs and epibenthic fish (Hines et al. 1990). Such interactions are considered in the ecological risk assessment.

Populations of deposit-feeding and filter-feeding benthic invertebrates are expected to be negatively influenced if oysters prevail over these species in a competition for food, space, or both. The Chesapeake Bay Environmental Model Package (CBEMP) results provide quantitative projections of the effects of changes in the oyster population on the populations of other bivalve filter feeders. In addition to using these findings to evaluate the risks and benefits of the oyster restoration alternatives for soft-bottom benthos, we also examined the existing data and literature about the species' habitat and food requirements compared with those of the oyster to estimate the level of competition that might occur and predict the responses of these two species to changes in the availability of food and habitat. The indirect consequence of a potential shift in crab predation was considered qualitatively. The magnitude of response expected would be proportional to the size of the oyster population projected to occur in response to each alternative using the oyster demographic model.

2.4.2.2 Submerged Aquatic Vegetation (SAV)

The term submerged aquatic vegetation (SAV) refers to both marine angiosperms (the so-called true seagrasses) and freshwater macrophytes that occupy Chesapeake Bay and its tributaries (http://www.vims.edu/bio/sav/). SAV encompasses 19 taxa from 10 families of vascular macrophytes and 3 taxa from one family of freshwater macrophytic algae, the Characeae, but excludes all other algae. The SAV community as a whole is being evaluated as an important ecological component of the Bay. The reason for considering SAV collectively is that monitoring data for SAV is available as acreage in the Bay (regardless of species), and modeling used to assess responses of SAV to changes in oyster abundance (Section 3) does not distinguish among species. The SAV community of Chesapeake Bay and its tidal tributaries includes 15 species (exclusive of the algae). Eel grass (*Zostera marina*) is found only in the lower reaches of the bay. Nonnative Eurasian watermilfoil (*Myriophyllum spicatum)*, sago pondweed (*Stuckenia pectinata*), redhead grass (*Potamogeton perfoliatus*), wild celery (*Vallisneria americana*), water starwort (*Callitriche* sp.), curly pondweed (*Potamogeton crispus*), common elodea (*Elodea canadensis*), water stargrass (*Heteranthera dubia*), hydrilla (*Hydrilla verticillata*), coontail (*Ceratophyllum demersum*), southern naiad (*Najas guadalupensis*), and spiny naiad (*Najas minor*) are less tolerant of high salinities and are found in the middle and upper reaches of the bay (SAV Restoration Workgroup 2005; Stevenson and Confer 1978; Orth et al. 1979; Orth and Moore 1981, 1984). Widgeon grass (*Ruppia maritima*) and horned pondweed (*Zannichellia palustris*) are tolerant of a wide range of salinities and are found throughout the Chesapeake Bay. SAV plays a critical role in the Chesapeake Bay ecosystem, serving as a sediment stabilizer, important habitat for juvenile fish and crabs, food for waterfowl, and a seasonal nutrient sink that can help offset the growth of algae. Due to degradation of water quality in Chesapeake Bay, SAV populations today are greatly reduced in both density and abundance compared with levels documented in the early 1960s (Kemp et al. 2005). In 2006, SAV experienced a 25 percent decrease throughout the Bay, reaching the lowest level since 1989 and representing only about 32 percent of the Chesapeake Bay Program's restoration goal for SAV (CBP 2007).

Oysters can affect the SAV community indirectly by inducing changes in water quality and providing physical protection for plants. Filtration by oysters can increase the penetration of light through the water due to the removal of suspended sediment and phytoplankton, thereby potentially improving growing conditions for SAV. SAV are known to benefit from the presence of oyster reefs, which dampen wave energy (Turner et al. 1999; Heiss and Bortone 1999). Historically, the presence of tall, three-dimensional oyster bars in fairly deep water may have reduced shoreline wave energy, thereby helping to prevent SAV from being dislodged or damaged.

We evaluated risks and benefits for this component of the Bay's ecosystem at the community level, but we accounted for potential changes in the diversity of species of SAV in the Bay. The primary mechanism of effect for the SAV community would be indirect as a result of changes in water quality induced by oyster populations (Figure 2-1). Oyster filtration results in increased light penetration through the water as a result of direct removal of suspended solids and indirect reduction in phytoplankton through removal of nutrients. We used modeled projections of the oyster populations expected to result from each of the oyster restoration alternatives as inputs to a model to predict the effects on SAV (Section 3). The measurement endpoints for SAV, therefore, are the estimates of abundance and distribution of SAV provided by that model. As noted in Section 3 and Section 4.7, uncertainties arising from the application of the oyster demographic model and the CBEMP contribute to uncertainties in estimating influences on SAV and other receptors. We also considered the findings reported in the scientific literature concerning the responses of SAV to changes in water quality. Such literature provided the basis for qualitatively assessing potential changes in species diversity in the SAV community in response to water quality changes.

2.4.2.3 The Blue Crab

The blue crab is difficult to associate with any single component of the Bay ecosystem because it occupies a variety of aquatic habitats ranging from the mouth of the Bay to fresher rivers and creeks and occurs at different trophic levels during various stages of its life cycle. Throughout the year, crabs may burrow into the bottom, shed and mate in shallow waters and beds of SAV, or swim freely in open water. The first life stage of a blue crab, called the zoea, lives a planktonic, free-floating existence. After several molts, the zoea reaches its second larval stage: the megalops. Following recruitment to the estuary, blue crab megalopae metamorphose into the first crab stage. These small crabs (2-3 mm) prefer habitats that can provide refuge from predation. In the Chesapeake Bay, SAV is preferred habitat for juvenile blue crabs from the first crab stage to approximately 20 mm (Orth and van Montfran 1982, 1987). Although the presence of SAV can enhance survival of juvenile blue crabs, it is not essential for strong year classes; large harvests have occurred during periods of low SAV coverage. In the absence of SAV, macro-algae and oyster reefs may provide the necessary structural refuges (Brumbaugh 1996). Both juvenile and adult blue crabs forage on the bottom and hibernate there through the winter. During spring, blue crabs migrate from the southern part of the Chesapeake to tidal rivers and northern portions of the Bay. During the rest of the year, adult blue crabs are dispersed throughout the Bay.

Blue crabs are opportunistic predators; they exploit prey species at sizes that are most common in each of the habitats they visit (Micheli 1997). Although adult oysters are too large for blue crabs to open and prey upon (reviewed in White and Wilson-Ormond 1996), crabs feed readily and opportunistically on juvenile oysters (Eggleston 1990). Oysters attain a partial refuge from predation at low densities (Eggleston 1990), but predation by blue crabs might increase with increasing oyster abundance. Mobile predators such as the blue crab produce strong direct effects of predation and disturbance on the benthic communities in Chesapeake Bay (Hines et al. 1990). Changes in the community structure and population density of predators and of prey species resulting from complex interactions with introduced species usually have cascading trophic effects that alter the entire structure of an ecosystem, as documented for the Hudson River estuary (Strayer et al. 1999) and San Francisco Bay (Carlton et al. 1990). An increase in the oyster population could increase the food supply for blue crabs and, indirectly, the availability of refuge habitat for juvenile crabs. An increase in the abundance of SAV resulting from increased filtration of Bay waters by oysters could enhance the blue crab population by providing more refuge for juvenile crabs.

The population of blue crab could be affected via both direct and indirect mechanisms. An increase in the oyster population could result in an increase in food supply for adult blue crabs and in an increase in refuge habitat for juvenile crabs (Figure 2-1). Changes in SAV expected to result from improvements in water quality due to increased filtration by a larger population of oysters could contribute indirectly to the enhancement of habitat for juvenile crabs. The potential magnitude of these effects was assessed using modeled projections of oyster population size expected in response to each of the alternatives and modeled projections of changes in SAV expected in response to the predicted populations of oysters (see Section 3). Information in the published scientific literature and the preliminary findings of continuing research funded by NOAA and Maryland DNR were used to evaluate rates of oyster predation by blue crabs, their food preferences, and relationships between SAV and changes in the blue crab population.

2.4.2.4 Phytoplankton

Phytoplankton are minute, free-floating aquatic plants. Phytoplankton communities in Chesapeake Bay are structured by salinity, temperature, light, and the availability of nutrients (Harding 1994). Although an abundant supply of phytoplankton provides more food for organisms at higher trophic levels, too much phytoplankton can harm the overall health of the Chesapeake Bay. An excess of nutrients in the estuary can result in large algal blooms. If left ungrazed, excess phytoplankton from such large blooms die and sink to the bottom. The process of decay of this excess organic matter consumes oxygen and worsens the seasonal oxygen depletion in the bottom waters of the Bay (Section 2.1). This seasonal oxygen depletion is detrimental to organisms such as fish and shellfish and can result in high rates of mortality of fish during summer. Scientists use the Phytoplankton Index of Biotic Integrity to establish the environmental status of the habitats in which the communities reside. In 2006, 69 percent of the

Bay areas sampled were occupied by phytoplankton communities that were considered degraded (CBP 2007).

Major groups of phytoplankton in the Chesapeake Bay include diatoms (Bacillariophyta), golden-brown algae (Chrysophyta), green algae (Clorophyta), blue-green algae or cyanobacteria (Cyanophyta), dinoflagellates (Pyrrophycophyta), cryptomonads (Cryptophyta), and micro-flagellates (Prasinophyta, Euglenophycota, Protozoa). Diatoms dominate the spring bloom, which constitutes the greatest algal biomass of the year. The timing, position, and magnitude of the spring bloom vary greatly between years and depend largely on flow (Harding 1994). Dinoflagellates replace diatoms during the summer, but at much reduced concentrations. Large blooms of dinoflagellates and cyanobacteria, which sometimes occur during spring and summer, produce red tides that are toxic to fish, shellfish, and sometimes humans. Red tides are prevalent on the western side of the Bay and at the mouths of certain tributaries and can result in significant economic losses due to closures of shellfish beds. *Pfiesteria piscicida*, a harmful species of phytoplankton that has appeared recently in the Bay, has been implicated in massive fish kills and some human illness.

Phytoplankton is the principle food of oysters. The native Eastern oyster is an active suspension feeder that exhibits complex feeding responses when exposed to seasonal variations in temperature and suspended organic particulate matter (reviewed in Langdon and Newell 1996). Larval oysters feed on a wide range of suspended particulate matter, including bacteria (Baldwin and Newell 1991). Oyster larvae that are offered plankton ranging from 0.2 µm to 30 µm feed preferentially on the 20-µm to 30-µm size-fraction, which is dominated by heterotrophic protozoans and dinoflagellates (Baldwin and Newell 1991). Oyster larvae typically ingest particles between 0.5 and 12 µm but will consume larger particles (16 to 30 µm) when blooms of dinoflagellates of that size are present (Baldwin and Newell 1995). A mixed algal diet has been shown to be superior to single-species diets for the growth of juvenile oysters (Enright et al. 1986). Although detrital complexes (i.e., non-living organic matter and attached bacteria) contribute to the nutritional requirements of the native oyster (Langdon and Newell 1990; Crosby et al. 1990), most of the carbon incorporated into oysters' tissues is derived from phytoplankton (Haines 1977). In one study, adult Eastern oysters in a salt-marsh estuary fed preferentially on phototrophic nanoflagellates (Wetz et al. 2002).

Oysters affect the phytoplankton community both directly and indirectly. The primary effect is direct: selective feeding reduces phytoplankton biomass and alters the species composition of the community. Many studies have demonstrated that benthic suspension feeders exert top-down control on phytoplankton production in freshwater, estuarine, and coastal waters (Cohen et al. 1984; Riemann et al. 1988; Cloern and Alpine 1991). Phytoplankton densities were 40% to 60 % lower in a 6-km to 8-km segment of the Potomac River with the highest densities of an Asian clam (*Corbicula fluminea*) than in upstream or downstream areas with fewer clams (Cohen et al. 1984). Daily rates of primary productivity in northern San Francisco Bay were much lower after the introduction of another Asian clam, *Potamocorbula amurensis*, when compared with pre-invasion levels, which led to a dramatic decline in annual phytoplankton production (Cloern and Alpine 1991). Based on feeding rates and densities in the field, researchers concluded that this decline in primary production was a result of the consumption of phytoplankton by *P. amurensis*. Results of a study by Newell et al. (2002) suggest that an ecosystem dominated by benthic primary production may develop in shallow waters when reduced turbidity associated with bivalve feeding increases light penetration to a level that can sustain benthic microalgal production. Turbidity is reduced when bivalves filter phytoplankton and inorganic particles larger than 3 µm from the water column and transfer undigested material to the sediment surface in their feces and pseudofeces (collectively called biodeposits).

The primary mechanism for oysters to affect the phytoplankton community is direct, by altering phytoplankton biomass and community composition as a result of selective feeding. The release of dissolved inorganic nutrients into the water column by oysters and the resuspension of oyster-generated biodeposits would be expected to contribute to phytoplankton productivity year round. We consider those effects to be of lesser significance because an increase in the oyster population is expected to reduce or eliminate the large blooms of phytoplankton that result from excess nutrient inputs to the Bay. Measurement endpoints for evaluating the risks and benefits of the oyster restoration actions for the phytoplankton community were chlorophyll level and primary production. Scientific literature and the results of continuing studies funded to support the EIS were used to characterize and qualitatively evaluate potential changes in the composition of the phytoplankton community in response to changes in the level of predation by oysters, based on the food preferences of the subject species of oyster.

The Suminoe oyster is generally believed to use the same food resources as the Eastern oyster (NRC 2004). Although the diet and particle-selection behavior of *C. virginica* have been studied in detailed laboratory studies (Newell and Jordan 1983; Shumway et al. 1985), no similar field or laboratory studies have been conducted specifically to evaluate the diet composition of *C. ariakensis* in terms of preferred species, size, or biovolume of phytoplankton or to examine the nonnative's ability to take up other suspended solids such as bacterioplankton. Laboratory studies conducted for aquaculture purposes have found that *C. virginica* and *C. ariakensis* both consumed similar algal diets (diatoms and flagellates) provided in culture (Langdon and Robinson 1996). A comparative study of the responses of *C. virginica* and *C. ariakensis* to two species of algae that produce large seasonal blooms in Chesapeake Bay showed that clearance rates were similar for both oysters for the two bloom-producing species of algae tested and the standard algal formula provided as food in hatcheries (Brownlee 2006).

2.4.2.5 Zooplankton

Zooplankton are minute, aquatic invertebrate animals, including the free-floating larval stages of oysters, clams, and crabs. Zooplankton communities in Chesapeake Bay act as the middle step between the very productive phytoplankton and bacteria at the bottom of the food chain and the many economically important species at higher levels in the food chain (i.e., trophic levels), such as fish and their larvae. Zooplankton consume phytoplankton and bacteria and can be a regulating force over these communities. In turn, excretion by zooplankton is one of the most significant recycling mechanisms that supply phytoplankton with nitrogen and phosphorus for growth. Brownlee and Jacobs (1987) reviewed the composition and distribution of zooplankton in Chesapeake Bay. Protozoans, rotifers, and copepod nauplii dominate the microzooplankton (< 200 µm). Dominant mesozooplankton (> 200 µm) species are the copepods *Acartia tonsa* and *Eurytemora affinis* in Maryland and *Acartia hudsonica* in Virginia.

Copepods account for greater than 65% of all species collected in zooplankton monitoring programs in Chesapeake Bay. Cladocerans, barnacle nauplii, and polychaete larvae are important at certain times of the year and in particular salinity regimes. In summer, gelatinous species of zooplankton (especially ctenophores) are important predators of copepods and oyster larvae. Zooplankton communities in the freshwater and oligohaline regions of the Chesapeake Bay are diverse, and their abundance and biomass are usually high. Abundance, biomass, and diversity are generally lower in the mesohaline and polyhaline zones, although high densities of larval polychaetes, mollusks, and decapods occur in specific areas.

The major consumers of zooplankton are larval fish, adult fish of certain species, ctenophores (*Mnemiopsis leidyi*), and jellyfish (e.g., the sea nettle *Chrysaora quinquecirrha*). Fish such as the bay anchovy (*Anchoa mitchili*) feed primarily on zooplankton, and particularly on *A. tonsa* (Peebles et al. 1996). Bivalve larvae in the free-floating stage, known as veligers, can be considered part of the zooplankton and are subject to the same predators. Purcell et al. (1991) found that while in its medusa stage, the sea nettle captures bivalve larvae but does not ingest them; they estimated predation of bivalve larvae by ctenophores to be 0.2% to 1.7% per day in the Chesapeake Bay. They concluded that the sea nettle is not an important predator of bivalve larvae but may reduce their mortality by consuming ctenophores, which do prey upon the veligers. Breitburg and Fulford (2006) found a significant decrease in the abundance of sea nettles in Chesapeake Bay since the mid 1980s and a simultaneous increase in the biovolume of ctenophores (i.e., milliliters of ctenophores per cubic meter of water). They estimated that ctenophores currently consume an average of 10 to 25 percent of oyster larvae throughout the summer and may consume 40 to 100 percent of oyster larvae locally in areas of peak density of ctenophores.

Because of its ubiquity and importance in the trophic structure of the Bay, we selected *A. tonsa* to represent the zooplankton community. *A. tonsa* is the dominant copepod species in the mesohaline portion of the Chesapeake Bay from April to October (Brownlee and Jacobs 1987). The ability of *A. tonsa* to thrive on various kinds of food, including phytoplankton, microzooplankton, and detritus, may enable it to maintain a high production rate under widely different conditions (White and Roman 1992a).

The primary mechanism of effect of the proposed action and alternatives on the zooplankton community would be indirect, through competition between oysters and the representative species for planktonic food (i.e., the mechanism of effect is as a result of changing availability of food resources on which all the species may depend). A change in food resources could cause changes in the rates of growth and reproduction of zooplankton, ultimately resulting in changes in abundance. Modeled projections of the biomass of mesozooplankton and microzooplankton at oyster populations attained as a result of each oyster management alternative were the measurement endpoints. Scientific literature and results of on-going studies were used to characterize prey preferences and predation rates of oysters, *A. tonsa*, and Cnidarians.

2.4.2.6 Pelagic Community

The nektonic, or swimming, community of the Chesapeake Bay is quite broad. This group includes fish, some crustaceans (e.g., shrimp and crabs), and some other invertebrates (e.g., worms). Approximately 267 species of fish can be found in the Chesapeake Bay (Murdy et al. 1997; White 1989). Other species such as sea turtles and, occasionally, marine dolphins and whales also enter the Bay.

The fishes of Chesapeake Bay are either resident or migratory. Resident species, like the killifish, tend to be smaller than migratory species and generally do not travel great distances. They normally occur in shallow water, where they feed on a variety of invertebrates. Other resident species, such as yellow and white perch, travel short distances from brackish to freshwater areas of the upper Bay or tributaries during late winter and spring to spawn. Migratory fish fall into two categories: (1) anadromous fish, which spawn in the Bay or its tributaries, and (2) catadromous fish, which spawn in the ocean. Anadromous fish migrate varying distances to spawn in fresh water. Striped bass spawn in the tidal freshwater areas of the Bay and major tributaries; younger fish remain in the Chesapeake to feed while many adults migrate to ocean waters after spawning. Shad and herring are truly anadromous, traveling from the ocean to fresh water to spawn and returning to the ocean to feed. Eels are the only catadromous species in Chesapeake Bay. Although they live in the Bay for long periods, eels eventually migrate to ocean waters in the Sargasso Sea to spawn. Other migratory fish use the Bay strictly for feeding. Some species, like croaker, drum, menhaden, weakfish, and spot, journey into the Bay while still in their larval stage to take advantage of the rich supply of food. Bluefish generally enter the Bay as juveniles or adults.

2.4.2.6.1 Planktivorous Fish

Planktivorous fish are a key part of the food web in the Chesapeake Bay. They consume small organisms that drift or swim in the water column, collectively called plankton, and are preyed upon by larger fishes such as striped bass and bluefish, known as piscivores. The larval and early juvenile stages of all fish species in the Bay feed on plankton; however, the bay anchovy and menhaden are the only two major species in the Bay that feed primarily on plankton throughout their life cycles. Because oysters also feed on some types of plankton, planktivorous fish might interact with oysters through competition for food. If overlapping food resources become scarce, growth, reproduction, and survival of planktivorous fishes could be reduced if oyster abundance increases. The appearance of zebra mussels in the Great Lakes is an extreme example of how a significant increase in the abundance of shellfish can affect other components of the ecosystem as a result of high filtration rates; this example is discussed in more detail in Section 4.5 of the EIS.

The small bay anchovy occurs in coastal waters from Maine to Yucatan. It is the most abundant fish in the Bay and is a major source of food for nearly all predatory fish. Humans do not exploit the species because of its small size. The population of bay anchovy fluctuates greatly from year to year but has exhibited a declining trend since about 1994. Recruitment of juveniles into the population depends partly on the concentration of planktonic food available

(Jung and Houde 2004). The bay anchovy is particularly sensitive to pollution (Bechtel and Copeland 1970; Livingston 1975), which could affect its abundance in the Bay. Although bay anchovies and oysters both consume plankton, they prefer different types. Bay anchovies feed primarily on zooplankton, particularly the copepod Acartia tonsa (Peebles et al. 1996). Oysters consume some zooplankton, but most of their diet consists of phytoplankton (Haines 1977); therefore, the potential for direct competition between oysters and anchovies is limited. A variety of indirect interactions are possible, however. For example, A. tonsa consumes phytoplankton, which may be reduced by oysters, thereby affecting the food supply for bay anchovy.

Menhaden occur in coastal and estuarine waters from Nova Scotia to northern Florida. They are abundant in the Bay during the spring, summer, and fall, but generally migrate south to the Carolinas during the winter. In addition to being a major source of food for striped bass and other piscivorous fishes, menhaden support one of the largest fisheries in the U.S. Menhaden are used for fishmeal, fish oil, and bait for other fisheries. Although the stock is considered healthy, recruitment of juveniles into the population has declined recently, and harvest limits are in effect for Chesapeake Bay (Cosby et al. 2007; ASMFC 2006). Menhaden are planktivorous throughout their life cycle but undergo a series of changes in feeding behavior as they grow and develop (Friedland et al. 1989). Larvae feed on small plankton of all kinds, but juveniles are obligate filter feeders and consume mostly phytoplankton (June and Carlson 1971; Govoni et al. 1983). As juveniles grow into adults, their diets gradually shift to include more zooplankton (Durbin and Durbin 1975). Amorphous organic matter composed of dissolved detritus and decaying plants also constitutes a substantial proportion of their diet in some environments (Lewis and Peters 1994). Oysters consume mainly phytoplankton (Haines 1977) but also detritus and other material (Langdon and Newell 1990; Crosby et al. 1990); therefore, the potential for competition for food between oysters and menhaden would be most intense during menhaden's juvenile and early adult stages.

The primary mechanism of effect of the proposed action and alternatives on this sector of the fish community would be through competition with oysters for planktonic food (i.e., a direct mechanism). A change in food resources could result in changes in rates of growth and reproduction of planktivorous fish, ultimately resulting in a change in their abundance. Representative species of the planktivorous fish community include populations of the Bay anchovy and menhaden. The primary information for evaluating the influence of the alternatives on these species was the scientific literature and continuing studies investigating the food preferences of the subject oyster species and the prey requirements of planktivorous fish. We characterized influences on these species by considering influences on their food (zooplankton and phytoplankton).

2.4.2.6.2 Reef-Oriented Fish

Oyster bars, which are remnants of the oyster reefs that were present historically in the Bay (Hargis 1999), provide habitat for several species of fish, many of which are important in commercial and recreational fisheries. Although some tropical fish reside on reefs throughout their life cycles, few Bay species exhibit this pattern. The naked goby *(Gobiosoma bosci)*, a small forage species, resides on oyster bars throughout its juvenile and adult lifestages (Breitburg

1991) and is considered an exclusively reef-dwelling species. Black sea bass *(Centropristis striata)*, which is considered to be a temperate reef fish, is found seasonally on oyster bars and other hard substrates and structures in the mid- and lower-Bay during warm months. Although black sea bass generally migrate to ocean waters during the winter, they are reef-dependent for a significant portion of each year. A third category of reef-oriented fish includes species that use a variety of habitats but frequent hard-bottom habitat, such as oyster bars; the Atlantic croaker *(Micropogonias undulates)* is an example of such reef-aggregating species. These three species, naked goby, black sea bass, and Atlantic croaker, represent the suite of species that orient to and may be affected by changes in the availability of oyster-reef habitat.

Breitburg et al. (2000) discussed the role that restored oyster reefs may play in enhancing the production of finfish and decapod crustaceans, such as crabs; however, the role of oyster bars in the population dynamics of reef-oriented fish species has not been documented. Several studies have investigated differences in the abundance of reef-oriented species among sites with and without oyster bars and reefs. Harding and Mann (2001) documented patterns of species richness, abundance, and size-specific use of habitat by transient fish along a gradient from complex reef habitat through simple sand bottom in the Piankatank River, Virginia. They found that as habitat complexity increased, the size and abundance of transient fish increased. They concluded that oyster reefs may be important habitat, although not essential, for the species investigated, which included Atlantic croaker, Atlantic menhaden, striped bass, and weakfish. Peterson et al. (2003) used results from six different field studies to estimate the enhancement of production for several species of fish that could be attributed to restoration of oyster-reef habitat. They classified the species evaluated in the study into two groups: (1) species that recruit exclusively to reefs, such as naked goby and oyster toadfish, and (2) species that aggregate around reefs, such as black sea bass and bay anchovy. For the second group, the investigators noted that the absence of reef habitat did not limit their production, but the presence of reef habitat augmented it. Rodney and Paynter (2006) compared macrofaunal assemblages on restored and non-restored oyster reefs in mesohaline regions of the Bay. They found that densities of demersal fish, primarily naked goby, were four times greater on the restored reefs than on the unrestored reefs. They also found that densities of fish prey species were much greater on restored reefs, 20 times greater than on unrestored reefs for amphipods, for example.

An increase in the amount (area and volume) of oyster reef in Chesapeake Bay could directly affect the populations of some species of reef-oriented fish and indirectly affect others through increases in the availability of prey items and valuable habitat associated with reefs. For the exclusively reef species, represented by the naked goby, an increase in the amount of available habitat could directly affect the size of the population. For the reef-dependent species, represented by black sea bass, an increase in the amount of available habitat and the resultant increase in food resources could affect the population size. For reef-aggregating species, represented by Atlantic croaker, a change in reef habitat could change the food resources associated with the habitat and, thus, the size of the croaker population. The scientific literature describes the relationship between population size and reef habitat and prey requirements for species that feed on reef-dependent prey items. Literature that detailed the relationship between reefs and populations of these relevant prey species was also evaluated. The measurement endpoints for all species in this group were comparisons of modeled projections of the quantity of oyster reef (area, volume, and distribution) expected in response to the each oyster restoration alternative with the habitat and feeding requirements of the representative species. Although the three subgroups within this ecosystem component could be affected via both direct and indirect mechanisms, literature providing quantitative characterizations of these relationships was limited; therefore, the assessment of this ecosystem component is both quantitative and qualitative. We characterized the magnitudes of influence as proportional to the magnitudes of change for oysters.

2.4.2.6.3 Piscivorous Fish

The piscivorous segment of the Bay's fish community includes some of the most sought-after species in recreational and commercial fisheries. Species such as striped bass, bluefish, weakfish, and Spanish mackerel can be found seasonally and are sought by anglers throughout the Bay. Striped bass, an anadromous species, and bluefish, a marine species that uses the Bay as a nursery area, can be considered to be representative of the piscivorous segment of the fish community. In Chesapeake Bay, the population of striped bass has increased considerably over the past decade due to responsible fishery management, but susceptibility to disease and availability of prey significantly influence the abundance of the species (CBP 2007).

Changes in oyster populations in the Bay could affect these fish species indirectly through the food chain. The complete mechanism is that a change in the oyster population (abundance and distribution) could affect planktivorous fish through competition for prey, and piscivorous fish would then be affected by the associated change in the availability of their fish and non-fish prey. The scientific literature describes how the abundance of the representative species, striped bass and bluefish, could change in response to changes in the availability of food resources. The measurement endpoint is a comparison of the modeled projection of abundance and distribution of oysters expected to result from each of the oyster restoration alternative and the associated competitive effects on planktivorous fish with the feeding requirements of piscivores in Chesapeake Bay. Only indirect pathways exist for piscivorous species, with multiple links from oysters to the receptor species. We characterized the influences on piscivorous fish species by evaluating influences on their prey.

2.4.2.6.4 Reptiles

Of the seven sea turtles found throughout the world, four appear seasonally in the Chesapeake Bay. The loggerhead turtle (*Caretta caretta*) accounts for close to 90% of the summer population of sea turtles in the Bay and, therefore, is a representative species for this assessment. The loggerhead turtle is on the Federal list of threatened species and on Maryland's and Virginia's lists of threatened species. Juvenile loggerheads enter the Chesapeake Bay during the late spring and early summer (Lutcavage and Musick 1985) and migrate out of the Bay from late September to early November, as water temperatures drop (Klinger and Musick 1995). They have been documented throughout the main stem of the Bay as far north as the Magothy River and in several of the tributaries, including the Potomac, Patuxent, Choptank, and Severn rivers. The Bay provides ideal foraging habitat for the development of juvenile sea turtles. Loggerheads eat a variety of foods including horseshoe crabs, crustaceans, jellyfish, and mollusks. They concentrate their feeding along channels near the mouths of rivers and areas of the Bay deeper than 13 feet.

The diamondback terrapin (*Malaclemys terrapin*) is the Maryland state reptile and another representative species. It is the only North American turtle that lives exclusively in brackish water. Diamondbacks feed mostly on mollusks, especially snails, clams, and mussels. Diamondbacks are non-migratory and spend their entire lives in local creeks, salt marshes, and coves. Whitelaw and Zajac (2002) demonstrated that resource availability may not be the primary driver of terrapin distribution. Distribution may be driven more by the physical structure, plant density, and tidal amplitude of the creeks in which they reside. Diamondbacks and, particularly, their nests are susceptible to predation by raccoons, crabs, crows, gulls, rats, muskrats, foxes, skunks, and mink. Because of the appeal of terrapin as a gourmet delicacy, harvest pressure decimated terrapin populations throughout the Bay by the early 1900s. To aid in conserving the population, the state of Maryland passed legislation in 2007 banning the commercial harvest of terrapins in state waters.

The mechanism of effect for loggerheads and terrapins is indirect; changes in the oyster population could change the availability of prey items for these species, specifically clams, crabs, mussels, cnidarians (for loggerheads), and SAV, in the Chesapeake Bay. The scientific literature describes prey requirements for these species. The measurement endpoints for this community are comparisons of the modeled projections of the abundance and distribution of oysters expected to result from each oyster restoration alternative and the resultant effects, if any, on the distribution and abundance of clams and mussels with the available information about food requirements for sea turtles and terrapins, as well how predators of these species might be affected.

2.4.2.7 Avian Oyster Predators

Numerous avian species in the Chesapeake Bay watershed feed on SAV and benthos, including species commonly found on oyster bars. An important representative species is the American oystercatcher (*Haematopus palliates*). Oystercatchers are large shorebirds with strong white or black-and-white markings. They consume oysters and other shellfish and have powerful, brightly colored bills that they use to open the shells of bivalves. Oystercatchers were once hunted almost to the point of extinction but are now conspicuous shorebirds found throughout the Chesapeake Bay region.

Several studies have shown that a decrease in shellfish stocks negatively affects the oystercatcher population (Goss-Custard et al. 2003; Atkinson et al. 2003; Tuckwell and Nol 1997). When the abundance of shellfish is low, the birds can survive on alternative prey species, but these species often do not enable the birds to maintain good body condition (Smit et al. 1998). Tuckwell and Nol (1997) showed that kleptoparasitism of oystercatcher eggs by other species (e.g. gulls) increases when oystercatchers are feeding on non-oyster shellfish.

The primary mechanism of effect for oystercatchers is through a change in the availability of oysters for prey. A secondary mechanism of effect could be through competition between oysters and other shellfish that could shift the prey-suite for oystercatchers. We characterized influences on these birds by evaluating the influences on their prey base.

2.4.2.8 Avian Piscivores

Many avian species use the abundant fish populations of the Chesapeake Bay as their primary food sources. Two of the species documented best in the literature are the bald eagle (*Haliaeetus leucocephalus*) and the North American osprey (*Pandion haliaetus*).

The bald eagle is a large raptor that is on the Federal list of threatened species and on state lists of threatened species in Maryland and Virginia. Bald eagles require large areas of undisturbed mature forest close to aquatic foraging areas. Bald eagles eat fish when they are available but will shift to a variety of other birds, mammals, and turtles – both live and as carrion – when fish are scarce. The Chesapeake Bay may once have provided habitat for as many as 3,000 breeding pairs of bald eagles. The population declined dramatically due to habitat destruction, poaching, and contamination by DDT. In 1973, the bald eagle was listed as endangered in 43 of the lower 48 states. After a ban on the use of DDT, the population slowly began to increase, and the bald eagle was reclassified as threatened in 1995 and delisted in 2007.

The osprey is the only diurnal bird of prey that feeds exclusively on live fish. The species is situated at the top of the aquatic food chain and is a good indicator of habitat destruction, dwindling fish populations, and environmental contamination. Ospreys build conspicuous nests on tall, offshore structures such as channel markers and duck blinds to protect their young and to be located near their food supply. Ospreys eat a host of fish species and are vulnerable to predation by animals, such as raccoons.

The mechanism of effect for both of these avian species is indirect: a change in the oyster population could cause changes in the populations of planktivorous fish (particularly menhaden) through competition for food, which could affect avian piscivores. The scientific literature describes the prey requirements of avian piscivores, as represented by the osprey and bald eagle. We characterized the influences on these bird species by considering the influences on their prey.

2.4.2.9 Avian Bottom Feeders

The Chesapeake Bay is located along the Atlantic flyway, which channels the annual seasonal flights of millions of migratory waterfowl to the Bay. The shallow waters and wetlands of the Bay and its temperate climate offer a fertile and diverse environment for waterfowl. There are four categories of Chesapeake Bay waterfowl: dabbling ducks, diving ducks, geese, and swans. All four kinds depend on agricultural areas, bay bottom, and wetlands for food and nesting habitat.

The black duck (*Anas rubripes*) is a good representative of a benthic-feeding avian species. The black duck is a medium to large dabbling duck that is most similar to the mallard (*Anas platyrhynchos*), but it lacks the male mallard's characteristic green head and white collar around the neck. Black ducks feed on a combination of plants and animals. They forage underwater by dabbling and upending. Their diet consists mainly of the seeds of grasses, sedges, pondweeds, and other aquatic vegetation. They will also readily eat snails, Baltic clams, ribbed mussels, and fish (Krementz 1991). Black ducks depend upon the condition of the bottom of the bays and wetlands in which they feed. Diving ducks, such as canvasbacks (*Aythya valisineria*), depend on aquatic habitats throughout their life cycles. They feed on plants and animals in wetlands and shallow benthic habitats. Canvasbacks in Chesapeake Bay once consumed wild celery almost exclusively, but the decline in wild celery caused the species to shift its diet to small clams. As a bottom feeder, canvasbacks are likely to be able to forage on and around oyster bars.

Neither black duck nor canvasback duck, nor any of the other waterfowl known to inhabit Chesapeake Bay, feed directly on oysters to any significant extent; however, canvasbacks and other diving ducks feed on or around oyster bars. Hooked mussels, a major prey item of diving ducks, are a major component of epibenthic communities on oyster bars, and oyster bars are believed to be a major source of this prey for the ducks (Blankenship 2007). The primary mechanism of interaction with oysters for these benthic-feeding birds is indirect, through changes in the kinds and distribution of benthic invertebrates that could result from competition with oysters for food and habitat. The literature describes the food requirements of black ducks and canvasbacks. The measurement endpoints for this community are comparisons of modeled projections of the abundance and distribution of the oyster population expected to result from each oyster restoration alternative and the associated change, if any, in the distribution of SAV and small benthic invertebrates in shallow-water habitats with available information about the food supply for black ducks and canvasbacks. We characterized influences on these species by evaluating influences on their food.

2.4.2.10 Mammalian Piscivores

Many piscivorous mammals inhabit the shores and waters of Chesapeake Bay. Although these mammals do not feed directly on oysters to any significant extent, a change in oyster populations could affect them indirectly through competition between oysters and planktivorous fish, which are food for piscivorous mammals. Although indirect effects such as these are extremely difficult to quantify and may be undetectable, this ecosystem component is included as part of a standard approach to ecological risk assessment analyses. Two representative species are the raccoon and the river otter.

The raccoon (*Procyon lotor*) is an omnivorous nocturnal mammal that prefers to inhabit trees, preferably near streams, springs, or rivers. Raccoons feed on mice, insects, fish, and frogs (Dewey and Fox 2001).

The river otter (*Lontra canadensis*) spends most of its life in the rivers, marshy ponds, and wooded riparian areas of the Chesapeake and its tributaries. River otter populations are increasing on Maryland's Eastern Shore, and some otters have been captured and released in other parts of the state where they had become scarce. River otters feed on fish, crayfish, crabs, frogs, and small mammals (Dewey and Ellis 2003).

The literature describes the prey requirements of mammalian piscivores, as represented by river otters and raccoons. The measurement endpoint for this community is a comparison of modeled projections of the abundance and distribution of oysters expected to result from each oyster restoration alternative and associated effects on fish communities with available information regarding the dietary requirements of the representative piscivorous mammals. We characterized the influences on these species by considering influences on their food.

2.4.2.11 Water Quality

The water quality characteristics of Chesapeake Bay and their role in controlling the biotic components of the Chesapeake Bay ecosystem were described in Section 2.1. Parameters of water quality have both direct and indirect relationships with the Bay's oyster population. For this ERA, we considered only the relationships in which changes in the oyster population may cause changes in water quality. Water quality parameters that may directly affect oysters (e.g., salinity, circulation patterns) are considered in the models that were used in this assessment (Section 3).

Oysters can directly affect the concentration of total suspended solids (TSS, which includes suspended sediments, phytoplankton, and other particulate matter). Oysters remove material from the water column through filtration and deposit it in the sediments. This results in and increase in the amount of light that reaches SAV. Biodeposition also results in sequestration of nutrients in the sediment; consequently, oysters may contribute indirectly to reducing primary production. A reduction in primary production would contribute to a decrease in the amount of organic matter that decomposes in the deeper portions of the Bay, a reduction in biological oxygen demand, and an improvement in oxygen levels in the Bay. The assessment endpoints for water quality are dissolved oxygen at the bottom, TSS, and algal biomass.

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3.0 APPROACH AND METHODS

The approach used to evaluate the eight risk questions (RQs) concerning ecological outcomes of the proposed action and alternatives consists of four parts:

1. An evaluation of the risk of failing to achieve the oyster restoration goal (i.e., target Bay-wide abundance; RQ 1)
2. An evaluation of ecological risks of the proposed action as defined in five questions derived from the NRC's preliminary assessment of the risks of introducing *C. ariakensis* into Chesapeake Bay (NRC 2004; RQs 2 through 6).
3. An evaluation of the likelihood that cultivating triploid *C. ariakensis* (Alternative 5) would give rise to a reproductive population that would pose the same risks identified for the proposed action (RQ 7).
4. An evaluation of the relative influences of the alternatives to the proposed action on ecological conditions in Chesapeake Bay as a result of changes in oyster biomass using the Relative Risk Model (RQ 8). The potential ecological influences of the proposed action were evaluated qualitatively based on the assumption that *C. ariakensis* is successfully established within the Bay.

This approach differs from the original plan for the ERA. The original approach for the ERA that was described to stakeholders in numerous presentations over the past five years has been modified substantially; therefore, a brief review of a few of the changes that led to the present four-part approach may be useful. The ERA approach evolved as a result of interactions among stakeholders participating in preparing the EIS, in response to peer review of tools being used in the ERA, through interactions with the Ecological Risk Assessment Advisory Group (ERAAG), and through interactions with the Oyster Advisory Panel (OAP). The original concept for conducting the ERA was to develop an oyster demographic model (ODM) to project the size of the oyster population at the end of a 10-year assessment period. Projections were to be calculated for only 10 years because uncertainty increases with each time step, and extending the model beyond 10 years would have produced projections that the developers considered to be increasingly unreliable. In addition, the amount of habitat used as input to the ODM was kept constant over the 10-year assessment period; however, studies have shown continuing decline in habitat over time. The inability to project the rate of habitat decline and incorporate it into the ODM would have further compromised the reliability of population projections beyond 10 years. Although ODM projections end at year 10, the results show a trajectory of population change over time that provides a basis for informed speculation about changes in population sizes further into the future. The ODM outputs at year 10 were then to be used as input to the Chesapeake Bay Environmental Model Package (CBEMP) to predict changes in water quality and SAV (Section 3.4). A weight-of-evidence approach based on available literature and research findings was to be used to address the potential ecological effects of the projected changes in oyster abundance on the other receptor species described earlier.

Several limitations of the ODM were identified during its development and in peer review by the OAP. Uncertainties about the structure of the model and inputs to it were recognized and had to be acknowledged in interpreting the model results for *C. virginica* populations; nevetheless, it was concluded that ODM outputs for *C. virginica* could prove useful for comparing outcomes, given that all model limitations and uncertainties were acknowledged. Most importantly, the OAP concluded that adapting the ODM to quantitatively project the growth of a population of *C. ariakensis* in the Bay would involve unacceptably large and unknown uncertainties. The ODM, therefore, was not adapted to make species-specific population projections for *C. ariakensis*. A few exploratory model runs of the ODM were used, however, to provide insight about how key factors, specifically greater resistance to disease and faster growth rates, might affect the growth of a representative population of oysters with those characteristics. These are not used to represent *C. ariakensis* but are used in a limited way to compare the ecological influences of changes in oyster biomass. Section 3.1 describes how the ODM was used to address RQ 1. Exploratory modeling output also served as input in the approach used to address RQ 8.

Because of the uncertainties associated with using the exploratory modeling to project the potential abundance and biomass of *C. ariakensis* in Chesapeake Bay, the decision was made to assess the potential ecological outcomes of implementing the proposed action through a descriptive evaluations of risks identified by the NRC (2004; RQ 2 through RQ 6) based on the most current research findings. Those evaluations are based on the assumption that *C. ariakensis* would be successfully naturalized and become abundant and widespread throughout the Bay. The premise for this assumption is that risks would be proportional to the size of the population of *C. ariakensis* within the Bay. This assumption does not constrain the evaluation to any particular time frame, and the evaluation is qualitative in that it is not based on a specific estimate of abundance or spatial distribution of the species. The evaluation is presented as narrative responses to RQs 2 through 6. Section 3.2 describes the approach.

The *Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process* (ANSFT 1996) was used to develop an approach for estimating the risk that cultivating triploid *C. ariakensis* in large-scale aquaculture operations (Alternative 5) would result in establishing a diploid population in the Bay. Section 3.3 describes the approach used to address RQ 7.

The results of exploratory modeling combined with projections from the CBEMP were used to provide insights about the relative ecological influences of changes in oyster biomass among the alternatives. These insights suggest how changes in water quality and the availability or quality of habitat and food resulting from changes in oyster biomass might influence ecological receptors. The Relative Risk Model (RRM) was used to present these influences on a relative scale so that alternatives can be compared. Section 3.4 describes this approach for addressing RQ 8.

3.1 EVALUATING THE RISK OF FAILING TO ACHIEVE THE OYSTER RESTORATION GOAL (RQ 1)

Although failure to achieve the oyster restoration goal is not an "ecological risk," per se, the size of the population of oysters that might result from implementing the proposed action or an alterative is the primary factor that determines how those management strategies might affect the Chespeake Bay ecosystem at some time in the future. Managers consider the restoration goal established for the PEIS to represent an oyster population that would provide needed ecological services and support an oyster fishery. Understanding the relative capability of the proposed action and alternatives to meet that goal is useful for evaluating their potential to afford the desired level of ecological services.

The exploratory modeling projected changes in oyster populations over time by simulating the effects of management actions on three rates that determine the size of an oyster population, which biologists call vital rates. The three vital rates considered in the exploratory modeling are (1) the rate at which larval oysters are produced and successfully reach the spat life-stage (i.e., recruitment), (2) the rate of growth of individual oysters, and (3) the rate at which oysters die (i.e., mortality). These rates vary with environmental conditions and the characteristics of particular species of oyster and interact in ways that are difficult to envision without conducting complex calculations. The exploratory modeling is a tool for integrating empirical data about the vital rates of oysters with additional information about their interactions with management actions (e.g., harvest and stocking) and the environment to project the size of the oyster populations. Output from a larval transport model served as input to the exploratory modeling to account for sources and dispersal of oyster larvae throughout existing habitat. The exploratory modeling also provides an estimate of the uncertainty in outcomes that is caused by variability in vital rates. This source of uncertainty is estimated by conducting many simulations (i.e., 1,000) in which vital rates are selected randomly from the range of values measured in previous studies. That is, the exploratory modeling is a Monte Carlo simulation. Although the goal established for the PEIS clearly refers to a Bay-wide oyster population, Mann and Powell (2007) emphasized that oysters in Chesapeake Bay cannot be considered a single population. The Bay's metapopulation actually comprises numerous exporting source populations and importing sink populations that interact in very complex ways that vary from year to year. The exploratory modeling is the only assessment tool currently available that attempts to account for that complexity.

Several limitations of the model were identified during development and subsequent peer review by OAP: (1) The existing population of Eastern oysters in Chesapeake Bay could not be estimated reliably because no Bay-wide survey designed specifically to estimate abundance has been conducted. (2) Insufficient data exist from which to model the rate at which habitat for oysters is reduced by sedimentation, removal, or biological and physical decay. (3) Parameterizations of vital rates for *C. virginica* are based on data sets that are spatially and temporally limited and may not fully reflect the range of values that occurs in the Bay. (4) Vital population rates for *C. ariakensis* have been measured in contained research studies that are geographically limited; Bay-wide values cannot be estimated unless the species is introduced. Despite its limitations and deficiencies, the demographic model is the only tool currently available that integrates information about the dynamics of the Eastern oyster population in the Bay to address the goals established for the PEIS. For this reason, it has been used to compare the relative effects of alternatives on Eastern oyster populations.

Appendix A of the PEIS provides details about the structure, inputs, and outputs of the exploratory modeling. Harvestable biomass (expressed as either biomass or numbers of market-size oysters, depending on application) is the output metric used in this ERA because it provides a common link between the exploratory modeling and the output of models that relate changes in oyster biomass to changes in water quality and other ecological conditions (i.e., oyster parameters in the CBEMP). The exploratory modeling was used to project growth of the Bay-wide oyster population over a 10-year period for the alternatives involving only *C. virginica*. The trajectory of population growth over that time period together with relevant research findings were considered in speculating about changes in population size more than 10 years after implementing a management strategy. Available information about the biology of *C. ariakensis* was insufficient to project the growth of a population in Chesapeake Bay with any certainty and therefore no estimates for this species are provided in this ERA. The exploratory modeling incorporates variability and yields probabilistic results through Monte Carlo simulations based on 1,000 individual "runs" of the model. The 50th percentile projections of oyster biomass are discussed in the ERA to provide insight about ecological implications at the central tendency of possible changes in oyster biomass. The 5th and 95th percentile projections are described to illustrate the range of variability in results. The projected total Bay-wide population of market-size oysters for each of the alternatives was segmented into six state/salinity zones to provide input for the Relative Risk Model used to address RQ 8. These zones were established based on some geographical limitations of exploratory modeling projections and because they reflect the major salinity zones within which certain aquatic communities of Chesapeake Bay are commonly characterized (Figure 3-1).

Using a model to predict changes in oyster biomass involves uncertainty. The sources of uncertainty and their potential significance for the outputs of exploratory modeling are summarized in Section 4.7 and discussed in greater detail in Section 5 and Appendix A of the PEIS. The exploratory modeling did not account for several important influences on oyster populations, such as the changes in the amount of shell substrate (i.e., the "shell budget") that strongly influence size and rate of growth of the oyster population and the development of disease resistance in *C. virginica*.

3.2 EVALUATING RISK QUESTIONS 2 THROUGH 6

The NRC (2004) posed the following questions concerning actions that involve introducing *C. ariakensis* into Chesapeake Bay:

- RQ 2 - Will *C. ariakensis* provide ecosystem services similar to those afforded by *C. virginica?*
- RQ 3 - Will *C. ariakensis* compete with *C. virginica* and other resident species possibly leading to reduced populations or local extinction of these species?
- RQ 4 - What is the potential for introduction and spread of diseases to other species in the Bay? (This does not include human health considerations, which are addressed in the PEIS.)
- RQ 5 - Will *C. ariakensis* become an invasive or nuisance species?
- RQ 6 - Will *C. ariakensis* disperse to areas outside of Chesapeake Bay and pose the kinds of risks identified in above?

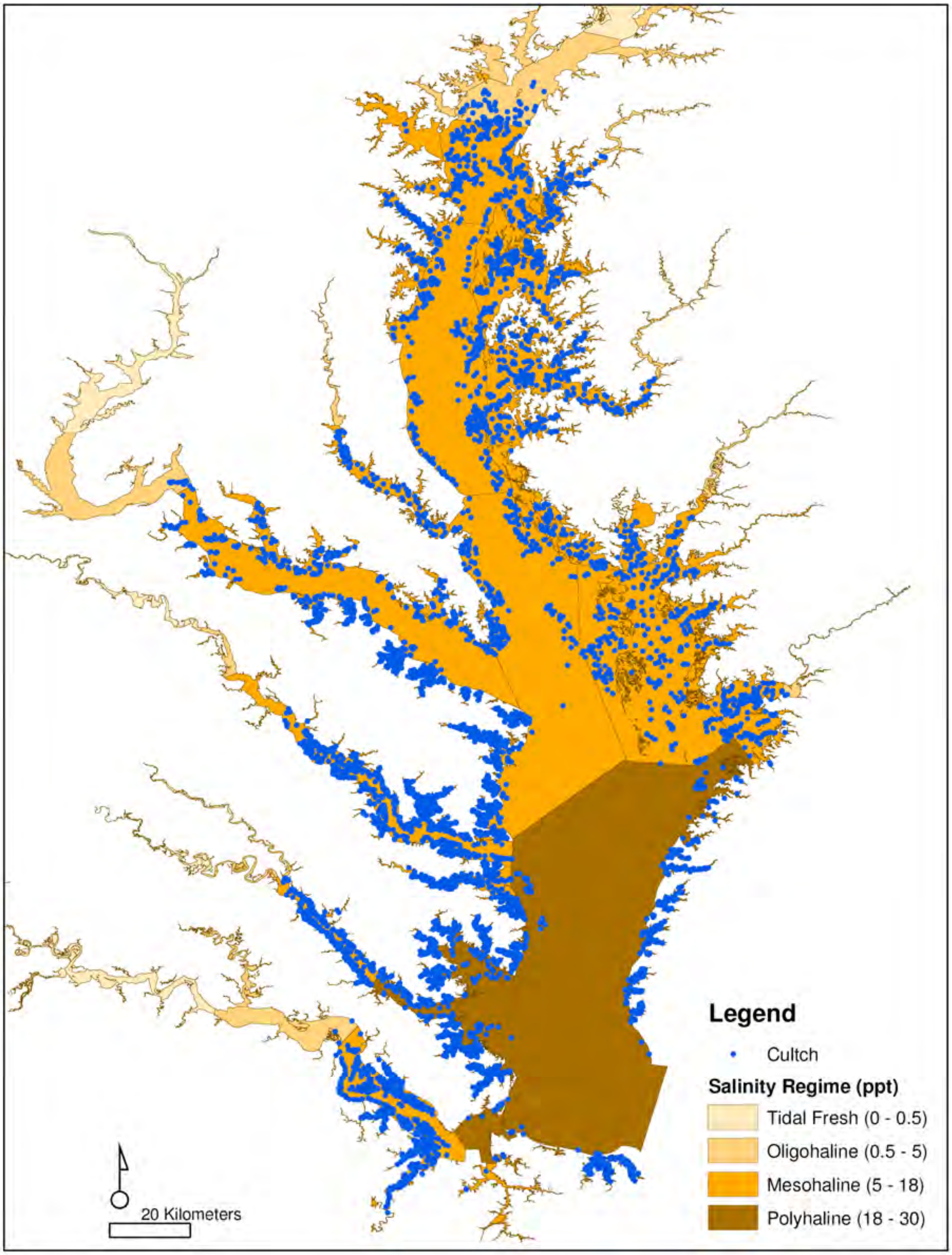


Figure 3-1. Distribution of existing oyster cultch in Chesapeake Bay, with salinity zones; the data presented represent the habitat layer used as input to the oyster demographic model described in PEIS Appendix A.

The NRC (2004) presented extensive and comprehensive information about *C. ariakensis* and the outcomes of introductions of nonnative oyster species into environments throughout the world. That information served as the basis for descriptive assessments of ecological risks of the proposed action and Alternatives 5 and 7. Beginning in 2003, numerous research studies were initiated to investigate various aspects of the biology of *C. ariakensis* and its interactions with *C. virginica* and other aquatic species of Chesapeake Bay. Many of the studies were designed specifically to address aspects of the NRC's questions. Many of those research projects have been completed, but several are still in progress. The descriptive assessments of risk presented as responses to RQs 2 through 6 drew upon findings of completed and continuing studies, using whatever information was available at the time to answer each of the questions to the extent possible. Attachment A summarizes the status of the extensive *C. ariakensis* research projects funded by NOAA. The research has answered many questions, helped to better define and begin to answer other questions, and highlighted data gaps and continuing research challenges. This ERA identifies remaining uncertainties and their implications in addressing each of the risk questions.

Several kinds of reviews helped to enhance the presentation and analyses of information in this element of the ERA. Individual researchers who have studied *C. ariakensis* were asked to review the presentation and interpretation of their research in this ERA. These individuals were sent Section 4.2 of this report. Many of the researchers provided comments, updates on research, and suggested edits to help align the interpretation with their results. Researchers also responded to specific questions about various aspects of the biology of *C. ariakensis.* Finally, the report was peer reviewed by the Ecological Risk Assessment Advisory Group (ERAAG). This group consists of four individuals with expertise in the field of ecological risk assessment who represent various federal agencies. These members of the ERAAG were involved in formulating the problem to be addressed in this ERA, designing the overall approach, and reviewing early drafts of the report. The ERAAG's peer-review comments were the primary basis for clarifying the document and addressing technical questions.

3.3 EVALUATING THE RISK THAT CULTIVATING TRIPLOID *C. ARIAKENSIS* WOULD GIVE RISE TO A REPRODUCTIVE POPULATION IN THE BAY (RQ 7)

One risk associated with a large-scale, long-term aquaculture industry using triploid *C. ariakensis* (Alternative 5) is that it could result in an unintended introduction of diploid *C. ariakensis* into the Bay. An introduction is considered a risk of this alternative because the specification to use triploid *C. ariakensis*, which generally are believed to be unable to reproduce, reflects a compromise that attempts to address stakeholders' conflicting views about the desirability of introducing a nonnative species while attaining the potential economic benefit of using the species. An unintended introduction of diploids, therefore, would be an undesirable outcome. The number of diploid individuals that might be introduced unintentionally via Alternative 5 would be considerably fewer than the number to be deliberately introduced by implementing the proposed action or Alternative 7; consequently, the time required to establish a Bay-wide, self-sustaining population would be much greater. The ultimate ecological consequences of the presence of an established population of *C. ariakensis* in Chesapeake Bay would be the same, and these are addressed in assessments of the proposed action and Alternative 7

(Sections 4.2 and 4.4). A comprehensive evaluation of the risk of developing a self-sustaining population of diploids as a result of cultivating triploids requires identifying all pathways by which a population might arise, and attempting to estimate the probabilities of each step in the sequence of necessary events within each of those pathways. An approach based on the *Generic Nonindigenous Aquatic Organisms Risk Analysis Review Process* (ANSFT 1996) was used to estimate the overall likelihood that aquaculture of triploid *C. ariakensis* would result in releasing diploids into the Bay at large (i.e., outside of aquaculture enclosures) and establishing a potentially reproductive population.

The ANSFT's methodology is a modified version of the risk assessment process developed by the USDA's Animal and Plant Health Inspection Service for evaluating the introduction of plant pests (Orr et al. 1993). The original objective of the ANSFT's *Review Process* was to evaluate the risk of introducing non-indigenous organisms and to determine the risk-management steps required to mitigate the calculated risk. This method applies a standardized process to identify how the evaluation was initiated and then outlines a method for assessing risk and managing that risk. Multiple pathways may be evaluated if several organisms or pathways of introduction are involved. We modified the *Review Process* to evaluate the pathways of potential introduction resulting from large-scale aquaculture of triploid *C. ariakensis*, focusing on the portion of the process that addresses risk assessment.

The first step in assessing risk as outlined in the *Review Process* is to identify interested parties and gather their input. Several experts were involved in identifying the pathway steps and associated probabilities, including Stan Allen (Virginia Institute of Marine Science), Roger Newell (University of Maryland Center for Environmental Science-Horn Point), Mark Luckenbach (VIMS), Ken Paynter (University of Maryland), A.J. Erskin (VSC), Doug Lipton (University of Maryland), and Chris Guy (U.S. Fish and Wildlife Service).

Collecting pathway data is the next step in the *Review Process*. This step requires knowledge of the origin of organisms in the pathway, the number of individuals moving through the pathway, the intended use of the pathway, past experiences with the pathway, any previous risk assessments on this or related pathways, and previous mitigation activities for this or related pathways.

Organism risk assessment, the next component of the *Review Process*, is the most important and complex step. Although the risk assessment model that underlies the organism assessment step may consider both the "probability of establishment" and the "consequence of establishment" (e.g., economic, environmental, social or political), for the purposes of the ERA, we were interested in exploring only the probabilities associated with establishment; the consequences of establishment are evaluated in the PEIS. We generated a flow diagram depicting a sequence of events (e.g., reversion, fertilization, survival, maturation, escape) required to yield diploid *C. ariakensis* under a hypothetical scenario of triploid aquaculture. The aquaculture scenario was defined with input from stakeholders specifically to provide a context for this risk assessment (see PEIS Appendix C). Each event is specific to relevant life stages, and the independent probability (or range of probabilities) associated with each event is reported. The product of these probabilities represents the number of diploid *C. ariakensis* produced at an aquaculture location in the Chesapeake Bay under the circumstances of the hypothetical scenario. This scenario was designed to reflect the maximum level of aquaculture that is economically feasible and sustainable given the estimated market demand for oysters; therefore, the magnitude of risk calculated for this scenario represents the greatest level of risk anticipated from Alternative 5. Using GIS layers showing the distribution of oyster bar habitat from the Maryland Bay Bottom Survey, this section further explores the probability that diploid *C. ariakensis* produced through the chain of events could settle in densities sufficient for successful reproduction. When data were not available for *C. ariakensis*, the most relevant existing information for congeners was included in the model. If no quantitative data were available, we included qualitative information.

Important topics to address in the risk assessment were the number of individuals per site in the founding aquaculture populations, the probability that triploids could be fertile, the probability that diploids could arise from triploids through reversion, the probability that diploids could be deployed with the triploids, and the probability that diploids could be released due to an accident at the hatchery or in the field. Probabilities for fertility, the encounter rate of gametes, fertilization, larval survivorship, substrate availability, metamorphosis, and post-settlement mortality also were considered. In all instances, data sources are documented. Although the *Review Process* specifically addresses the potential for a newly colonized population to spread beyond its place of origin at this step, this ERA does not address the likelihood of spread. This approach treats the establishment of any reproductive population of *C. ariakensis* as a negative consequence of the aquaculture alternative.

The advantage of the *Review Process* methodology is that once the relevant events are established, and probabilities are estimated for each event, we can explore the predictions of the model given a known set of controllable conditions, such as the initial size and spatial arrangement of the aquaculture population. One disadvantage for this evaluation is that relevant data are extremely limited, and levels of uncertainty are difficult to estimate based on the information available. Limitations in the data for the chain of events included the following:

- Some probabilities were based on a small number of individuals of *C. ariakensis*,
- In some cases, no data on *C. ariakensis* were available, and data for *C. virginica* were substituted.
- Data available for *C. ariakensis* were for individuals grown in different environmental conditions than those expected at the aquaculture sites considered for this evaluation.
- Data were taken from experiments that were not explicitly designed for the purposes of the *Review Process*.

Another disadvantage is that we have assumed that individual events in the chain leading to establishment of a diploid population are independent, but they may not be. For example, the probability of reversion may be affected by the probability of escape because older escapees that are not harvested are expected to have a higher rate of reversion. No independent experts have reviewed the approach developed to estimate the probability of diploids arising from triploid aquaculture; however, the approach was modified in response to the ERAAG's questions and comments.

3.4 EVALUATING THE RELATIVE INFLUENCES OF ALTERNATIVES ON ECOLOGICAL CONDITIONS IN THE BAY (RQ 8)

Increases in oyster biomass within the Bay generally are presumed to result in increases in ecological services. However, there are uncertainties about the relative magnitudes of these increases as a function of amount of oyster biomass and whether changes in services might be considered negative or positive for the Bay ecosystem. For example, under some alternatives oyster biomass might decline in some areas while increasing in others, resulting in both diminishment and increase of oyster-related ecological services, depending on geographical location. Further, the NRC (2004) suggested that "if the nonnative oyster became invasive and the population was not kept in check by harvesting or by native predators, it is conceivable that *C. ariakensis* could reach sufficient densities to shift the bay ecosystem back toward benthic dominance rather than pelagic dominance." This kind of shift would result from a large reduction in phytoplankton biomass in the Bay due to filtration by oysters. Although such a shift might restore conditions that existed historically when the native oyster was very abundant, it could significantly reduce the supply of food available to animals associated with the plankton-based food-webs, including zooplankton, larval fish, fish that feed on phytoplankton or zooplankton, and fish that feed on the planktivorous fish. This ERA uses the RRM to identify the kinds of changes in ecological services that might result from implementing the alternatives and to characterize them in a standardized manner, including those with both positive and negative influences on receptors.

The RRM was selected to compare alternatives with respect to influences on ecological services because (1) it provides relative rather than absolute metrics of risks and benefits, which the lead agencies for this project preferred; (2) it is straightforward and has been used in a variety of applications; and (3) regional Federal and State agencies have become familiar with the methodology via using it for an on-going evaluation of the Delaware River Estuary. Outside of the mid-Atlantic region, the RRM has been used to evaluate factors related to declines of Pacific herring (Landis et al. 2004), environmental conditions in the Willamette and McKenzie rivers in Oregon (Luxon and Landis 2005), rain forest preserves in Brazil (Moraes et al. 2002), and other regional assessments (Landis 2005). It has been suggested as a means for addressing regional issues with multiple stressors (Menzie et al. 2007). For this ERA, the RRM has been modified somewhat to provide a consistent numerical framework for comparing relative influences of alternatives on ecological receptors of the Bay. The relative influences on ecological services are examined over a 10-year time horizon as that is the chosen period for modeling changes in oyster biomass. As discussed elsewhere, while this time period provides insight into the relative changes in oyster biomass among alternatives, increases and/or decreases in oysters may continue beyond this horizon. The likelihood that *C. ariakensis* would compete with the native oyster or become a nuisance species is considered elsewhere in this report as it is not possible to make reliable forecasts for changes in the biomass of this species over ten-year or longer time frames.

The RRM uses ordinal numerical scores to classify the relative importance or magnitude of stressors, effects, and estimates of ecological consequences. These are derived for a set of characteristics or attributes. In this case, the characteristics being evaluated are the ecological receptors and conditions of the Bay identified in Section 2, Problem Formulation. The numerical scores reflect the positive or negative influences of an alternative on each ecological receptor expected as result of the changes in oyster biomass associated with that alternative. Positive and/or negative influences on these receptors and conditions reflect the relative influences of the alternatives on ecological services as a whole. Differences among the alternatives can be examined by comparing influences on a particular receptor/condition or on the aggregate of receptors and conditions.

Relating positive and negative influences on ecological receptors to ecological services requires further explanation and qualification. Increases in oyster biomass are likely to influence many of the ecological receptors positively because of associated increases in habitat, food, or water quality. The phytoplankton-based food web could be negatively influenced by increases in oyster biomass. Decreases in phytoplankton abundance could result in both increases and decreases in ecological services with respect to individual ecological receptors or conditions. Ecological services that would be enhanced by a decrease in phytoplankton include increased growth of SAV due to increased water clarity and increases in dissolved oxygen. Yet, such negative influences on phytoplankton could negatively influence the phytoplankton-based food web and the ecological services associated with species that depend on that food web. When considering overall influences on ecological services, the scales of such influences must be borne in mind. Improvements in water quality associated with decreases in phytoplankton may have ecological benefits for most species, including those that depend on phytoplankton for food. Nevertheless, at some level of reduction, food could become limiting for species that depend on phytoplankton, which would result in the situation suggested by NRC (2004) involving a shift in the Bay ecosystem from pelagic dominance back toward benthic dominance. Whether such a shift would or could occur or whether it would result in a net loss or gain of ecological services is beyond the ability of this ERA to predict. It is possible to describe the relative influences of alternatives on phytoplankton and the associated food web over a 10-year period and gain some insight into relative magnitudes and associated potential consequences.

The relative influences of the alternatives on ecological conditions (habitat, food, and water quality) in the Bay are examined for six state/salinity zones: Maryland oligohaline (MD OH), Maryland mesohaline (MD MH), Maryland polyhaline (MD PH), Virginia oligohaline (VA OH), Virginia mesohaline (VA MH), and Virginia polyhaline (VA PH). These zones were established based on some geographical limitations of exploratory modeling projections and because they reflect the major salinity zones within which certain aquatic communites of Cheapeake Bay are commonly characterized. Each of these zones is made up of several Chesapeake Bay segments, as shown in Table 3-1. The number of segments used in the analysis described below varied among these four state/salinity zones and ranged from three for the MD OH zone to 25 for the MD MH zone. Table 3-1 also describes the physical characteristics of the segments.

Table 3-1. State/salinity zones for analyses of relative ecological influences

Segment	Water Body	Perim-eter (m)	Area (m^2)	Area (acres)[(a)]	Volume (m^3)	# of Cultch Areas[(b)]	Total Acres of Cultch	Cultch Area/ Total Area
Maryland Oligohaline (> 0.5-5 ppt)								
CHSOH	Chester	124641	14790537	36541	28875000			
CHOOH	Choptank	145797	15037649	3716	45000000			
ELKOH	Elk	138710	37270004	9210	1.01E+08			
GUNOH	Gunpowder	163323	41998392	10378	64250000			
CB2OH	Mainbay	246410	275239520	68013	1.24E+09	68	598.19	0.0088
MIDOH	Middle	93914	16214070	4007	25000000			
NANOH	Nanticoke	238038	16455330	4066	45000000			
PAXOH	Patuxent	76397	14243456	3520	27180000			
POCOH	Pocomoke	116755	13821501	3415	18000000	2	22.04	0.0064
POTOH	Potomac	312495	214963696	53119	8.52E+08	11	535.32	0.010
SASOH	Sassafras	161366	33085712	8176	84187500			
Maryland Mesohaline (> 5-18 ppt)								
BIGMH	Big Annemessex	162996	29067984	7183	43625000	9	39.08	0.0054
CHSMH	Chester	363647	119290907	29477	4.55E+08	111	1065.85	0.036
CHOMH2	Choptank	206492	74200120	18335	2.67E+08	58	435.67	0.024
CHOMH1	Choptank	537319	242057248	59814	9.45E+08	188	1791.04	0.030
EASMH	Eastern Bay	619132	234558868	57961	9.97E+08	183	2074.28	0.036
FSBMH	Fishing Bay	295582	83505552	20634	1.43E+08	42	382.09	0.019
HNGMH	Honga River	246072	97719184	24147	1.86E+08	67	1341.3	0.055
LCHMH	Little Choptank	407700	89578958	22135	2.08E+08	52	4001.92	0.18
LYNPH	Lynnhaven	289316	19607176	4845	16730000	214	528.93	0.11
MAGMH	Magothy	121642	26541486	6559	76500000	8	3174.03	0.48
CB3MH	Mainbay	145496	361585728	89350	2.39E+09	205	3980.44	0.044
CB4MH	Mainbay	326788	908849967	224582	9.24E+09	253	3394.23	0.015
CB5MH	Mainbay	842496	1474652418	364394	1.54E+10	832	3566.28	0.0098
MANMH	Manokin	275258	60788916	15021	89500000	28	323.57	0.021
NANMH	Nanticoke	219270	48357788	11949	97250000	17	74.52	0.0062
PATMH	Patapsco	339736	93604632	23130	4.52E+08	3	7.05	0.00030
PAXMH	Patuxent	348050	107580204	26584	5.61E+08	84	1430.32	0.054
POCMH	Pocomoke	483373	195923574	48414	3.55E+08	142	1800.19	0.037
POTMH	Potomac	1277757	887864640	219396	5.79E+09	871	8560.08	0.039
RHDMH	Rhode	43830	9110563	2251	20312500	2	9.79	0.0043
SEVMH	Severn	153435	29387340	7262	1.13E+08	39	415.88	0.057
SOUMH	South	129040	23982120	5926	67000000	18	175.24	0.030
TANMH	Tangier Sound	1046674	897937604	221885	4.02E+09	234	3074.98	0.014
WSTMH	West	58041	11303989	2793	20375000			
WICMH	Wicomico	285770	35116516	8677	56420000	6	146.59	0.017
Maryland Polyhaline (>18 ppt)								
Some portions of the MD Mesohaline segments fall into polyhaline waters. This is taken into account within the Oyster Demographic Model. These areas can vary depending on freshwater inflows. As a result, there can be some mismatch between salinity designations for Chesapeake Bay segments and salinity at specific oyster bars over the modeling period.								

Table 3-1. (Continued)

Segment	Water Body	Perim- eter (m)	Area (m^2)	Area (acres)(a)	Volume (m^3)	# of Cultch Areas(b)	Total Acres of Cultch	Cultch Area/ Total Area
Virginia Oligohaline (> 0.5-5 ppt)								
JMSOH	James	271459	127749032	31567	4.32E+08	3	23.55	0.00075
CHKOH	James-Chickahominy	355816	27969270	6911	48562500			
RPPOH	Rappahannock	112097	19536530	4828	53580000			
MPNOH	York-Mattaponi	100741	7952139	1965	35000000			
PMKOH	York-Pamunkey	119417	14093807	3483	66680000			
Virginia Mesohaline (> 5-18 ppt)								
EBEMH	Eliz- East branch	99682	5774440	1427	6460000			
LAFMH	Eliz-Lafayette	87952	5754146	1422	3390000	5	43.66	0.031
SBEMH	Eliz- South branch	171896	8393598	2074	27730000			
WBEMH	Eliz- West branch	56237	6006832	1484	6310000	4	2.31	0.0016
JMSMH	James	552699	304241056	75180	9.77E+08	614	7746.88	0.10
PIAMH	Piankatank	286397	69774176	17242	2.01E+08	456	1083.93	0.063
RPPMH	Rappahannock	724298	323830688	80020	1.48E+09	602	5242.18	0.066
CRRMH	Rapp-Corrotoman	146952	23483608	5803	65687500	267	431.13	0.074
YRKMH	York	321194	94595793	23375	2.76E+08	233	2832.91	0.12
Virginia Polyhaline (> 18 ppt)								
ELIPH	Elizabeth River	64695	21152682	5227	1.15E+08	1	75.88	0.014
JMSPH	James	120958	76561904	18919	4.34E+08	26	107.94	0.0057
CB8PH	Mainbay	146606	412427744	101913	3.17E+09	11	81.14	0.00080
CB6PH	Mainbay	278815	743353039	183686	6.5E+09	171	486.4	0.0026
CB7PH	Mainbay	956822	1520821583	375803	1.35E+10	366	1952.32	0.0052
MOBPH	Mobjack Bay	987544	342714372	84687	1.34E+09	857	3907.75	0.046
YRKPH	York	209230	68414728	16906	4.01E+08	146	1064.84	0.063

Source:
(a) CBP 2004
(b) Greenhawk and Barker 2007. pers. comm.

We considered four ecological factors associated with changes in oyster biomass that could influence other ecological receptors in the Bay: habitat, food, predation, and water quality. We used a standardized scoring system to relate changes in oyster biomass to the overall influence on each ecological receptor. These scores range between –5 and +5, and 0 indicates no influence. This scale provides the basis for comparing the *relative* positive and negative influences of the various alternatives. Some of the scores are derived from predicated changes associated with changes in oyster biomass; others are derived based on the degree of influence these changes may have on fish and wildlife species. The predicted changes are derived from a combination of two models: the exploratory modeling, which predicts changes in oyster biomass, and the CBEMP, which was used to translate these changes into changes in water quality and certain ecological receptors. The CBEMP predicts influences for oyster hard-bottom, dissolved oxygen, total suspended solids, phytoplankton, zooplankton, benthic biomass, and SAV. Influences on the remaining fish and wildlife receptors were evaluated by considering how the predicted influences might subsequently influence habitat, food, or other environmental conditions relevant to specific groups of fish and wildlife.

To standardize the scores, we associated a value of "5" with a change of approximately an order-of-magnitude in oyster biomass (800% to 1,200%). We selected this range based on (1) a review of exploratory modeling outputs that indicated that the largest modeled values were about an order-of-magnitude, and (2) the stated goal of oyster restoration to achieve levels comparable to the 1920 to 1970 period. The estimated restoration goal (1.16×10^{10} oysters) is about an order-of-magnitude greater than current levels of oyster biomass. These two considerations indicated that relative influences would fall within about an order-of-magnitude and served as a bound on assigning scores with a maximum value of "5". A value of "5" for oysters also indicates that the population within a zone is large. The following scores represent intermediate degrees of change for oyster biomass: 0.1 or –0.1 (1% to 10%), 1 or –1 (10% to 100%), 2 or –2 (100% to 400%), 3 or –3 (400% to 600%), 4 or –4 (600% to 800%). The increments of this scale obviously are not even throughout its range. The two small "bins" near the center of the scale (–0.1 to 0 and 0 to 0.1) capture very small influences, allowing us to show small differences among alternatives while preserving the ability to show the larger changes that might result from implementing some of the alternatives. Influences expected to result in changes of less than 1% were assigned a 0.

We used the same numerical scoring system applied to oysters for representing the degree of influence that changes in oyster biomass might have on all other ecological receptors. This standardizes scores across receptors. In some cases, these relative influences are derived from the model predictions described above. Thus, for example, a predicted 30% increase in SAV over a 10-year period would be assigned a value of "1" because it falls within the 10% to 100% bin; a decrease in phytoplankton of 8% would be assigned a value of –0.1 because it falls within the –1% to –10% bin.

For fish and wildlife species, the degree of influence associated with changes in oysters was derived by applying association factors to scores derived from the predictions described above. An association factor reflects the degree to which a species of fish or wildlife uses or depends directly on a particular kind of habitat or food. Indirect relationships between changes in oyster biomass and influences on an ecological receptor are reflected through the combination of the underlying direct relationships. An example of an indirect relationship is the influence that oysters have on ospreys. Ospreys do not eat oysters or use oyster reefs for habitat, but they do prey on fish that are directly or indirectly influenced by changes in oyster biomass. Association factors range between "0" (almost no direct dependency) and "1" (almost complete direct dependency). For example, menhaden feed almost exclusively on phytoplankton; therefore, an association factor of "1" is assigned to the relationship between phytoplankton (as a food source) and menhaden. In contrast, avian soft-bottom feeders (ducks) have a mixed diet of submerged aquatic vegetation and benthic invertebrates. Based on the literature, the proportions appear to be about 40% plant matter and 60% invertebrates; therefore, association factors of 0.4 (for plants) and 0.6 (for benthic invertebrates) were assigned as the food-related association factors for this category of receptors. The uncertainties associated with assigning association factors are discussed later in this section and in Section 4.7.

Influences of changes in oyster biomass on dissolved oxygen (DO) were evaluated by categorizing changes in concentrations of DO relative to ecologically important threshold values such as 3 mg/l or 5 mg/l.

RRM scores were derived in one of three ways, as described in the following sections.

3.4.1 Scores for Oysters

RRM scores for oysters were derived from the exploratory modeling results by determining the degree of change in oyster biomass for each of the state/salinity zones by dividing the increase or decrease in oyster biomass over the modeled 10-year period by the average starting biomass for all six state/salinity zones. This normalized the increases and decreases so that small changes in a state/ salinity zone with a low starting biomass did not appear to be large relative to other state/salinity zones. The resultant degrees of change were sorted into the bins described above (e.g., 1% to 10%, 11% to 100%) and assigned a corresponding RRM score.

We used two projections from the probabilistic output provided by the exploratory modeling: the 50th percentile (an estimate of central tendency), and the 95th percentile (an upper bound estimate on what might occur). We did not calculate the influences that would occur at a lower bound projection, such as the 5th percentile. Changes in oyster biomass at the 5th percentile were considerably smaller than those at the 50th. The directions of ecological influences at that level of change in oyster biomass would be the same, but the magnitudes would be too small to contribute meaningfully to the analysis.

The maximum RRM score for oysters was “5”. That value reflects the modeled range of change obtained from the exploratory modeling and indicates oyster biomass that reaches the stated restoration goal. Some areas of the Bay received negative RRM scores for some alternatives. These scores reflect cases in which the biomass of oysters is expected to decrease in response to implementing the alternative.

3.4.2 Scores for Ecological Receptors Modeled in the CBEMP

We used the CBEMP, a comprehensive mathematical model of physical and eutrophication processes in the Bay and its tidal tributaries (Cerco and Noel 2004) to relate changes in oyster biomass to changes in water quality and other ecological responses. Attachment B of this ERA presents a summary description of the CBEMP. As a result primarily of time limitations, CBEMP model runs using exploratory modeling projected oyster biomass as inputs were not made for each of the alternatives. Instead, effects on water quality and SAV were extrapolated from the outputs of six CBEMP model runs that were initiated with different starting oyster scenarios (Attachment B). Cerco and Noel generated output for each of the six scenarios for a 10-year period. This resulted in 60 modeled values for each combination of oyster and response variable (6 scenarios x 10 years) for each Chesapeake Bay segment. We analyzed that CBEMP oyster/response variable output to identify relationships at the state/salinity scale. That involved combining Chesapeake Bay segments into state/ salinity zones. Attachment B describes how the segments were combined. The CBEMP outputs were used in a regression analysis to quantify relationships between oyster biomass and water quality and ecological conditions. We recognized that combining Chesapeake Bay segments at the state/salinity scale would dampen strong relationships within a specific segment by combining it with segments where relationships were weak; however, no segment-specific CBEMP modeling was conducted as part of this ERA.

Regression equations were derived to identify significant relationships between oyster biomass and the response variables (Attachment B). In cases where data were limited (because of the limited number of segments available), we applied the regression equation for one state/ salinity zone (i.e., mesohaline) to an adjacent state/salinity zone (i.e., oligohaline). This adjustment was required because of differences in how salinity zones were defined in the CBEMP and how they were applied in aggregating exploratory modeling results. Chesapeake Bay segments used in the CBEMP are designated as oligohaline, mesohaline and polyhaline based on long-term salinity records. exploratory modeling results were aggregated according to salinity zones by calculating the average salinity at each oyster bar over the 1,000 model runs for each alternative, and then aggregating oyster biomass for all bars where average salinities were within the ranges established for each salinity zone. The modeled salinity values at many bars differed from year to year, and many bars changed back and forth between oligohaline and mesohaline over all model runs. The result was that many oyster bars that geographically would fall into a mesohaline Bay segment were categorized based on averaged modeled salinity values as being oligohaline. Oligohaline zones were especially underrepresented in the CBEMP outputs but were very important with respect to projected changes in oyster biomass. For this reason, we assigned the relationships derived for MD MH to MD OH (these zones extend into each other) and VA MH to VA OH (again, these zones extend into each other).

For the purpose of examining relationships at the scale of a state/salinity zone, the individual model outputs for the segments were aggregated to provide values corresponding to the geographical extent of each of the four state/salinity zones. As appropriate for the measure, this was either as total biomass (as in the case of oysters and SAV) or on a unit-area or unit-volume basis. These 60 derived aggregated values took into account the relative areas and/or volumes of the Chesapeake Bay segments relative to that of the state/salinity zone. The regression equations were incorporated into the RRM to translate relative changes in oyster biomass to relative changes in ecological conditions. These relative changes were estimated as percent changes and translated to ordinal values as described in Section 3-4. RRM scores for water quality (i.e., dissolved oxygen and total suspended solids), phytoplankton, zooplankton, benthic invertebrates, and SAV were derived by applying the regression equations to translate exploratory modeling oyster biomass outcomes to outcomes for the individual ecological receptors.

The following example describes how this was accomplished for SAV using data from analysis of Alternative 2a. Because oysters filter water, increases or decreases in oyster biomass influence the concentrations of algae and suspended solids in the water. Increased filtration associated with an increase in oyster biomass is predicted to reduce the concentrations of algae and suspended particulate matter. One result is increased water clarity, which contributes to enhancing the growth of SAV. Figure 3-2 illustrates the relationship between SAV biomass and oyster biomass for the MD MH zone. The 60 values displayed in the graph were derived as the aggregate of the segments in this zone as described above. The figure shows the relationships between the total biomass of SAV in the zone in metric tons and the biomass of harvestable oysters in terms of kilograms of carbon (dry weight).

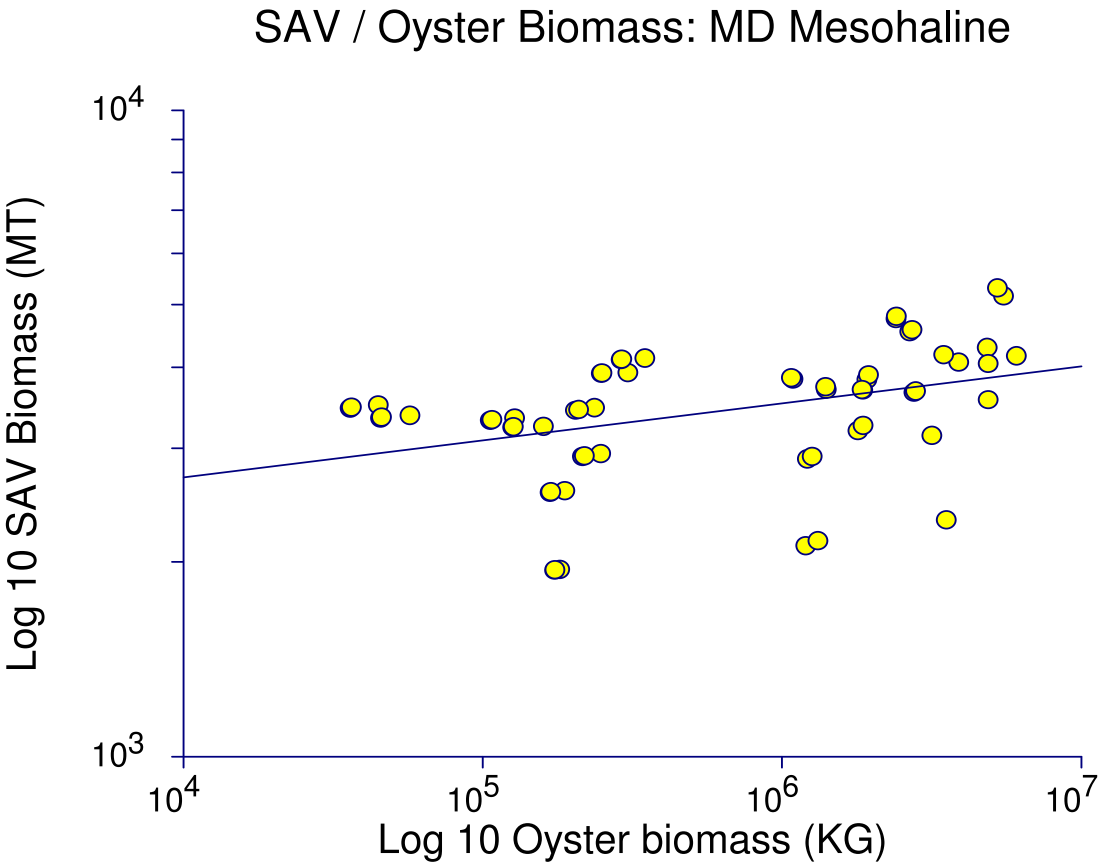


Figure 3-2. SAV biomass as a function of oyster biomass predicted from 60 CBEMP model runs carried out by Cerco and Noel (6 oyster biomass scenarios X 10 years). Each point represents a composite response of SAV in the Bay segments considered to be in the Maryland mesohaline zone.

The relationship is positive and statistically significant. The associated regression equation is:

SAV (MT) = 3161 + 2.3 E-04 * Oyster Biomass (KG)

In this example, the 95th percentile oyster biomass of market-size oysters is predicted to change from a starting value of 13,750 kg to 200,400 kg over the 10-year model period. Using the regression equation, SAV is predicted to change from 3,165 MT to 3,228 MT. This is an increase of about 2% and was assigned an RRM score of 0.1 because it falls within the range of 1% to 10%.

After reviewing all regression model results, we noticed that the statistical relationships between oyster biomass and various ecological components for the MD OH zone often did not appear to be consistent with known biological relationships or were insignificant. An example is shown in Figure 3-3 for SAV in MD OH.

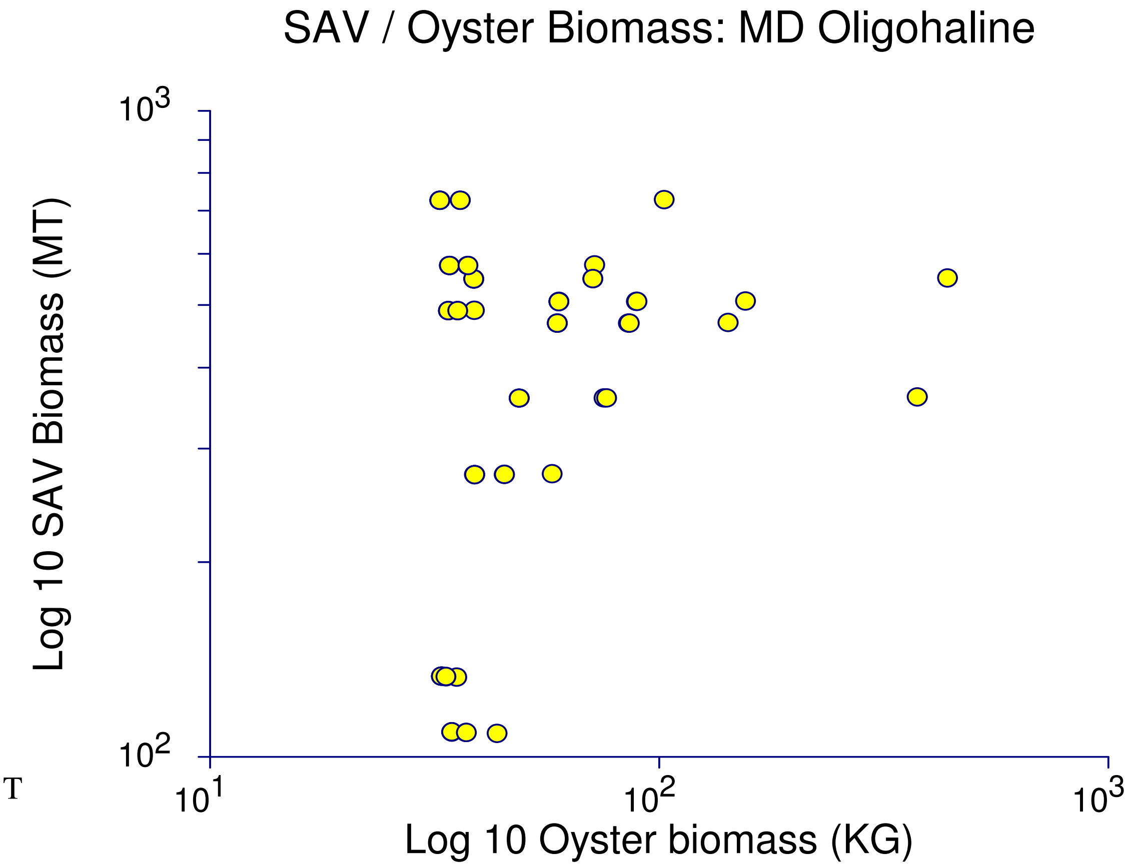


Figure 3-3. SAV biomass as a function of oyster biomass as predicted from 60 CBEMP model runs carried out by Cerco and Noel (6 oyster biomass scenarios X 10 years). Each point represents a composite response of SAV in the Bay segments considered to be in the Maryland oligohaline zone.

Upon inspection of the results we found only three Chesapeake Bay segments in Maryland that were classified in the CBEMP as oligohaline (Table 3-1). In contrast, 25 segments in Maryland were classified as oligohaline; consequently, the data set for MD MH was larger than for MD OH. This is reflected in the larger range of oyster biomass depicted for MD MH than fro MD OH in the figures shown above. We also knew that waters described as oligohaline in the exploratory modeling results included segments that were classified as mesohaline in the CBEMP. For these reasons we did not use the counterintuitive or insignificant results derived for the MD OH segments. Instead, we applied the regressions derived for MD MH from the CBEMP to the exploratory modeling model outputs for the MD OH zone.

We reviewed other relationships and noted situations where the CBEMP either did not include or underrepresented segments for particular areas. These were handled as follows: algae and zooplankton (MD MH was used for MD OH and VA MH was used for VA OH), benthic deposit feeders (MD MH was used for MD OH and VA PH was used for other Virginia waters).

The resultant degrees of predicted change in each of ecological receptors for which predictions were made with the CBEMP were sorted into the bins described above and assigned a corresponding RRM score.

3.4.3 Scores for Other Ecological Receptors

The CBEMP does not provide predictions for fish and wildlife species; therefore, RRM scores for those receptors were derived by characterizing the relative influences of changes in oyster biomass and changes in the receptors considered in the CBEMP on the representative fish, reptiles, birds, and mammals. The characterization was accomplished by combining information about the degree of change in oysters and other ecological components as represented in their individual RRM scores with the degrees of association between the fish and wildlife receptors and these other ecological components. The following algorithm represents this procedure:

$$RRM_{receptor} = \sum (RRM_{habitats} \text{ x } AF_{habitats}) + \sum (RRM_{foods} \text{ x } AF_{foods})$$

where:

$RRM_{receptor}$ = The calculated RRM score for a fish or wildlife ecological receptor

$RRM_{habitats}$ = The RRM scores for oysters, SAV or "other" as these reflect changes in the habitat afforded by these ecological components.

$AF_{habitats}$ = An assigned association factor that reflects the relative degree of association (reliance) between the ecological receptor and each type of habitat

RRM_{foods} = The RRM scores for ecological components (e.g., oysters, phytoplankton, and various fish) that are used as food by other fish and wildlife species.

AF_{foods} = An association factor that reflects the relative degree of association (reliance) between the ecological receptor and each type of food

The maximum score a receptor can receive is equivalent to the score assigned to the change in oyster biomass for a particular alternative and geographic area. An example of such a receptor is the naked goby, a fish that depends directly on oyster reefs for habitat and food. If the change in oyster over a ten-year period yields a score of "3", then a reef-dependent species such as the naked goby also receives a score of "3". All other fish and wildlife species are only partially dependent either directly on indirectly on oysters for habitat and food and, thus, will have RRM scores less than those assigned to oysters and the animals that depend directly upon them.

The scores can be an aggregate of values such as 0.1 and 1 that represent small influences as well as values such as 2 through 5 that represent large influences. This was done so that the occurrence of small influences could be captured. Such a mix of values can lead to aggregate values that may confound direct interpretation of the specific value derived for a receptor. However, upon inspection of the results, the influence of this potential confounding factor is negligible on a relative basis for the following reasons: if there ar a few influences, usually one dominates. If there are many influences (there can be up to 7), the individual influences are diminished because of lower association factors. As a result, the RRM results do indicate the relative magnitudes of influences among alternatives on the various ecological receptors and the aggregate of these receptors.

3.4.3.1 Assignment of Association Factors

The association factors are subjective values between 0 and 1 assigned to reflect the relative importance of a particular kind of habitat or food to an ecological receptor. Dr. Charles Menzie of Exponent assigned the association factors based on a review of the literature and the goal of achieving an internally consistent set of association factors. An association factor of 0 represents no association, and a factor of 1 represents complete association (preference or dependency). These cases are relatively easy to identify based on the ecology of species. A species either does or doesn't rely primarily on oysters for habitat or for food. Assigning association factors for direct relationships in which and animal uses a variety of habitats or eats a variety of foods is more difficult. In those cases, it is necessary to apportion the partial relationships among the various habitats and foods and to use information about the ecology of the receptors to guide that apportionment. In all these cases, the sum of association values for habitats or foods is equal to or less than 1. This preserves relationships across receptors.

Most scientists will recognize the relative importance of particular kinds of habitat and food to the various ecological receptors. Although opinions may vary among scientists about the specific values of some intermediate association factors, most scientists familiar with the ecology of the various receptors probably would identify the same kinds of habitat and food as important and would agree on the extreme values (i.e., 0 and 1). Because the RRM scores are all tied to relative changes in oyster biomass, an internally consistent set of association factors should reasonably reflect the predictable influences of changes in oyster biomass on the representative species of fish and wildlife. As noted, to insure consistency among alternatives and across receptors, the sum of the assigned association factors within a category (e.g., habitat or food) was set equal to or less than 1, reflecting the relative degree of dependency of the ecological receptors on resources in that category. Confidence in the relative magnitudes of the association factors is greatest for species such as reef fish (e.g., naked goby), whose abundance is directly and positively related to the availability of oyster reefs as sources of habitat and food.

Confidence in the degrees of association for other species is less certain, and this source of uncertainty is discussed further in Section 4.7. The approach employed here is designed only to provide an indication of degrees of influence of each of the alternatives relative to each other, not to evaluate limiting factors for recptors.

Two sets of association factors were developed. The first set represents a base analysis and reflects only known preferences for various types of habitat and food (Table 3-2). Factors between 0.4 and 0.6 were assigned for stronger associations or where literature indicated that a receptor used a food or habitat resource in this particular proportion, and factors between 0.1 and 0.25 were assigned for weaker associations or when an ecological receptor relies on several categories of food or habitat. An example of the former is the avian soft-bottom feeder which consists of duck species that feed on aquatic plants and on benthic invertebrates. Because there are two predominant sources of food for this group, association was apportioned across two sources of food, SAV and benthic invertebrates. Because reports of dietary habits indicated that greater use of invertebrates as food than plants, we chose to divide the total association for food (i.e., 1) as follows: 0.4 for SAV and 0.6 for benthic invertebrates. An example of the latter is piscivorous fish represented by the striped bass. This receptor group eats a variety of fish as well as crabs; therefore, based on dietary information for this receptor, the the association for food (i.e., 1) was apportioned as 0.2 blue crabs, 0.4 forage fish (e.g., silversides and mummichogs), and 0.4 planktivorous fish (e.g., menhaden). This reflects the larger proportion of fish in the diet of the receptor, and the fact that this receptor also eats crustaceans.

It is possible to create finer divisions across food or habitat categories but all of these divisions would total to "1" or less than "1" and would reflect similar underlying oyster-related influences. In cases where food preferences include oyster-dependent (as food), benthic-dependent, and plankton-dependent elements, oysters can positively influence some categories and negatively influence others. For example, blue crabs and forage fish are treated as part of the striped bass diet and are expected to be positively influenced by increases in oyster biomass. This positive influence translates to a positive influence on the striped bass. Striped bass also eat planktivorous fish such as the menhaden. Increases in oyster biomass are expected to have a negative influence on the plankton-based food-web; therefore, the proportions of food in the diet can shape the degree of positive or negative influence on a particular receptor. Association factors reflect what is known about the receptors. The uncertainties associated with the use of the association factors is discussed further in Section 4.7.

The second set of association factors has been adjusted to account for the spatial coverage of oyster cultch in the Bay (Table 3-3). This set was assigned by setting the factor for all associations that involved receptors using oysters for food or habitat equal to the median value of the fractions of bottom area that contain cultch in the Chesapeake Bay segments (Table 3-1). Using the relative area of oyster cultch provided a lower measure of association that recognizes that ecological receptors can use this habitat or food source only in proportion to its presence. The median fraction across all segments is 0.03 (a central value of 3%). This median value is within a factor of three of the median values for the various state/salinity zones and provides a reasonable central estimate for he purpose of this analysis. This value is approximately an order of magnitude less than the association factors unadjusted for the proportion of cultch in a Bay segment.

Table 3-2. Association factors unadjusted for proportion of oyster cultch

Ecological Receptor	**Habitat Associations**			**Food Associations**									
	Hard Bottom	**SAV**	**Other**	**Phyto-plank-ton**	**SAV and/or Associated Inverte-brates**	**Zoo-Plank-Ton**	**Oysters or Inverte-brates on Oyster Reefs**	**Other Benthic Inverte-brates**	**Blue Crab**	**Forage Fish**	**Plank-tivorous Fish**	**Pisci-vorous Fish**	**Avian Soft-bottom Feeders**
Blue crab	0.1	0.5	0.4			0.2	0.2	0.4		0.2			
Forage Fish	0.2	0.4	0.4		0.2	0.2	0.2	0.4					
Plantivorous fish - phytoplankton			1	1									
Plantivorous fish – zooplankton			1			1							
Reef oriented fish	1												
Piscivorous fish			1						0.2	0.4	0.4		
Avian oyster predators			1				0.4	0.4	0.2				
Avian piscivores			1						0.2	0.2	0.2	0.2	0.2
Avian soft-bottom feeders			1		0.4			0.6					
Mammals			1				0.2	0.2	0.2	0.4			
Reptiles			1		0.25		0.25	0.25	0.25				

Table 3-3. Association factors based on area of oyster coverage in Chesapeake Bay

Ecological Receptor	Habitat Associations			Food Associations									
	Hard Bottom	SAV	Other	Phyto-plank-ton	SAV and/or Associated Inverte-brates	Zoo-plank-ton	Oysters or Inverte-brates on Oyster Reefs	Other Benthic Inverte-brates	Blue Crab	Forage Fish	Plank-tivorous Fish	Pisci-vorous Fish	Avian Soft-bottom Feeders
Blue crab	0.03	0.5	0.4			0.2	0.03	0.4		0.2			
Forage Fish	0.03	0.4	0.4		0.2	0.2	0.03	0.4					
Planktivorous fish – phytoplankton			1	1									
Planktivorous fish – zooplankton			1			1							
Reef oriented fish	1												
Piscivorous fish			1						0.2	0.4	0.4		
Avian oyster predators			1				0.03	0.4	0.2				
Avian piscivores			1						0.2	0.2	0.2	0.2	0.2
Avian soft-bottom feeders			1		0.4			0.6					
Mammals			1				0.03	0.2	0.2	0.4			
Reptiles			1		0.25		0.03	0.25	0.25				

Association factors that reflect preferences for habitat or food are based, in part, on the review of ecological characteristics provided in Section 2.4.2. Water quality is not included as an association factor but is discussed in the results. We defined three kinds of habitat: oyster hard bottom, SAV, and other (sand and soft bottom or open-water areas away from reefs). The sum of the habitat-related association factors is always 1, and we apportion this among the three types of habitat categories. Oyster abundance was modeled with respect to a defined set of oyster bars. The number and sizes of these bars are kept constant in the exploratory modeling. Increases and decreases in oyster biomass on these fixed bars were assumed to translate into proportional increases and decreases of habitat or food for species that rely on oyster reefs for those resources, based on life history characteristics of those species summarized in Section 2.4. The degree of precision of such proportionality need not be established, since it is kept constant in the evaluation of the alternatives. Increased oyster biomass could mean an increase in the horizontal area of coverage for a bar, a vertical increase, or both. Either type of change would provide more habitat or food. Decreases in biomass would translate to a loss of habitat or food value. Reef fish are considered to have a strong positive habitat association with oyster hard bottom. Although these species may also use other structures, we relied on a strong association to emphasize the potential contribution of oysters to this category of ecological receptors. This is why we set the association factor for this ecological receptor as equal to the RRM score for the oysters.

SAV provides important habitat and food for a variety of ecological receptors. As indicated in Table 3-2 and 3-3, we assigned relatively high habitat association factors for blue crabs (0.5) and forage fish (0.4). The 0.5 value assigned for blue crabs in relationship to SAV reflects our understanding of the importance of SAV in the early lifecycle of blue crabs (Section 2.4.2.3). Hines (2007) recently reviewed habitat characteristics for blue crabs and underscored the apparent importance of SAV for juvenile stages. He also noted that SAV is not a requirement for this stage and that juvenile crabs can use other structures in the absence of SAV. This is why a value of 0.5 is used rather than a higher value. In addition, Hines (2007) noted that blue crabs use a variety of habitats throughout their life cycle; therefore, we apportioned the remainder of the association to these other habitat types. We emphasized the importance of SAV because it is preferred by early life stages.

The forage fish category includes small species that inhabit near shore areas, such as mummichogs and Atlantic silversides. Although these species are not specifically called out as assessment endpoints, this group is important in the diet of piscivorous fish, and we included this category to evaluate influences on fish and wildlife at higher trophic levels. Both species can use a variety of near shore habitats; however, mummichogs and silversides often are more abundant in SAV. The species may choose SAV environments as a refuge from predators or the effects of storms (http://era.noaa.gov/Regional Analyses of Restoration Planning; http://www.fisheries.vims.edu/multispecies/femap/Fish%20Pages/Atlantic%20Silverside.htm; Rozas and Odum 1987a, 1987b, 2004; Jacques van Montfrans 2004; Bain and Eckerlin 2006). These species – especially mummichogs – also use other structure, such as that provided by oyster hard bottom. These species – especially silversides – also use near-shore areas with sand or mud bottoms. Mummichogs and silversides feed on a broad variety of invertebrates with an emphasis on benthic organisms (Gilmurray and Daborn 1981; Weisberg et al. 1981; Weisberg and Lotrich 1982; Fay et al. 1983; Kneib 1997; USACE 1998; James-Pirri et al. 2001). We assigned feeding-related association factors that reflect the preference for benthic invertebrates as well as a preference for specific habitats where invertebrates may be abundant and for which forage fish show some preference (SAV and oyster hard bottom). Table 3.2 reflects our understanding of the apportionment of association across habitats as follows: 0.2 for reefs (to reflect the association with structure), 0.4 for SAV (to reflect the known association of these species with SAV beds), and the remainder, 0.4, for other bottom types as indicated in the literature cited above. The apportionment of food items as reflected in the selected association factors also is based on our understanding from the literature cited above: 0.2 for invertebrates associated with SAV, 0.2 for zooplankton, 0.2 for invertebrates living on oyster reefs, and 0.4 for benthic invertebrates in sand or soft bottom areas.

SAV and oyster hard bottom were not considered to provide important habitat for other fish and wildlife. However, these species may use these habitats as foraging areas, and this is represented in association factors for the food categories. SAV may be one of a number of spawning areas for two planktivores that are not specifically called out as representative species: alewife and blue-back herring. Alewife spawn in a variety of habitats, including large rivers, small streams, ponds, and large lakes over a wide range of substrates such as gravel, sand, detritus, and submerged vegetation (http://www.dnr.state.md.us/fisheries/fishfacts/herring.asp). In areas where alewife and blueback herring co-exist, blueback herring use a greater variety of spawning sites, including shallow areas covered with vegetation, rice fields, swampy areas, and small tributaries upstream from the tidal zone. The two representative planktivores that are called out as representative species – Bay anchovy and menhaden – tend to spawn and live in open water areas without specific structure or SAV (Castellanos and Rozas 2001; Bain and Eckerlin 2006). Food-related association factors include menhaden's preference for phytoplankton and the bay anchovy's preference for zooplankton (Section 2.4.2.6.1).

Two species represent piscivorous fish, bluefish and striped bass. Bluefish spawn offshore (Bigelowe and Schroeder1953). Habitat requirements cannot be determined accurately, but bluefish appear to use a variety of estuarine and offshore environments (Shepherd and Packer 2005). Harding and Mann (2001) observed a preference among bluefish for foraging over areas of oyster hard bottom in a tributary of Chesapeake Bay. We consider this to be reflected in the association between piscivorous fish and forage fish, which, in turn, have a positive association with oyster hard bottom. Striped bass spawn in fresh water or nearly fresh tributaries (Bain and Bain 1982). The eggs and larvae are carried with the currents. Juvenile and young striped bass use the tributaries and near-shore areas of the Bay for foraging. As they grow, their diet shifts to predominantly fish. This includes the category of forage fish. Adult bluefish and striped bass feed on a range of fish species, but they display a preference for planktivores such as the bay anchovy, menhaden, and other species. In addition, adult striped bass will prey on select invertebrates such as blue crabs (Booth and Gary 1993; Walter and Austin 2003). Juvenile bluefish will feed on the megalopae stage of blue crabs (Parthree et al. 2006). We considered both bluefish and striped bass as open-water, migratory species that use oyster hard bottom and SAV as sources of food but not as critical habitat. We assigned association factors for food categories that reflect the diversity of the diets of bluefish and striped bass. These factors are weighted heavily to fish (0.8 for the combination of forage and planktivorous fish); we included an association factor of 0.2 for predation on blue crabs, primarily by striped bass.

We assumed that wildlife species (birds, mammals, and reptiles) do not use oyster hard bottom and SAV as habitat; however, many species of wildlife could use these and other areas as sources of food. These associations with oysters are reflected in the food categories. The American oystercatcher feeds on oysters and other shellfish, benthic invertebrates, and crabs. We reflected this by assigning association factors for these various food groups that correspond with the preferences of this bird species. Avian soft-bottom feeders include the black duck and the canvasback duck. The black duck feeds primarily on invertebrates but also on plant material. The canvasback duck feeds primarily on plants but also eats invertebrates. (Reinecke and Owen 1980; Lewis and Garrison 1984; http://www.chesapeakebay.net/waterfowl.htm; http://www.epa.gov/med/grosseile_site/indicators/canvasback.html). To represent this mixed diet we assigned association factors for SAV and for benthic invertebrates.

Both ospreys and eagles feed on fish. In addition, eagles often forage on small mammals and birds, including ducks (http://www.nps.gov/choh/naturescience/baldeagles.htm). Because ducks are one of the ecological receptors, we included them in the diet of eagles.

Otters and raccoons represent the piscivorous mammals. The bulk of the river otter's diet is fish; however, otters are opportunistic and will feed on a variety of prey depending on availability and ease of capture (USEPA 1993). Depending on availability, otters also may consume many other kinds of vertebrates and invertebrates. Crustaceans and shellfish are among the more important aquatic biota. Although otters exhibit a preference for aquatic biota, raccoons feed on food sources in both terrestrial and aquatic environments. We assigned association factors that reflect the mixed diet of otters. Raccoons share some of the otter’s diet, but the balance is made up of foods from terrestrial areas.

Turtles have a varied diet of invertebrates (Section 2.4.2.6.4). These may be obtained from a wide variety of areas and habitats. We reflected this with a distribution of association factors across categories of invertebrate prey. As with other distributions of association factors across food categories, these total to “1”.

3.4.3.2 Example Calculation of an RRM Score

The method for deriving an RRM score for an ecological receptor that is not modeled with the CBEMP is illustrated for the blue crab. The following example is for Alternative 3 (harvest moratorium) in the Maryland oligohaline zone. For this alternative, the normalized increase in oyster biomass over the 10-year period was 543%. This change falls into the 400% to 600% bin of the RRM scale, giving it a score of 3. Blue crabs feed on a variety of kinds of food that might be influenced by changes in oyster biomass, including several that are modeled in the CBEMP. The projected change in oyster biomass within this zone was estimated to result in the following changes in ecological receptors considered in the CBEMP:

- SAV: 1.13% with a corresponding RRM score of 0.1
- phytoplankton: -0.28% with a corresponding RRM score of 0
- mesozooplankton: -0.7% with a corresponding RRM score of 0
- deposit feeding benthic invertebrates: -0.32% with a corresponding RRM score of 0

In addition, crabs can feed on forage fish. An RRM score of 1.26 was calculated for forage fish for Alternative 3 in the Maryland oligohaline zone. Using those RRM scores as background information and the factors assigned for the degrees of association between the blue crab and its various sources of food, the RRM score for blue crabs was calculated as follows:

0.1 (AF for oyster reef as habitat) x 3 (RRM score for oysters) = 0.3

0.5 (AF for SAV as habitat) x 0.1 (RRM score for SAV) = 0.05

0.2 (AF for oysters as food) x 3 (RRM score for oysters) = 0.6

0.2 (AF for zooplankton as food for larval crabs) x 0 (RRM score for zooplankton) = 0

0.2 (AF for benthic invertebrates as food) x 0 (RRM score for benthic invertebrates) = 0

0.2 (AF for forage fish as food) x 1.26 (RRM score for forage fish) = 0.25

The sum of the above yields an RRM score for blue crabs of 1.2.

4.0 RISK ANALYSES

In light of the inherent uncertainties in the absolute values of exploratory modeling outputs, the focus of this assessment is primarily on the relative magnitude of outcomes and comparing possible changes in abundance or biomass of market-size oysters among alternatives and areas. Relative magnitude is of particular importance because exploratory modeling outputs serve as input into the RRM, which is used to address RQ 8. Estimates of abundance derived from the exploratory modeling, however, are the only means available for addressing RQ 1. Biomass and numbers of market-size oysters are the exploratory modeling output metrics used because this size-class of oysters reflects the reproductive population. Moreover, Cerco and Noel (2005) used this size category to model changes in ecological conditions and water quality, and their results were used in the RRM to addres RQ 8. We focused on differences in the magnitude of change in the biomass of oysters between years 1 and 10 among the alternatives and on the spatial characteristics of those differences across the 6 state/salinity zones. We used the starting biomass and the ending biomass after 10 years as predicted by the exploratory modeling to estimate the magnitude of change for each alternative. We examined changes between starting harvestable biomass and the 50th and 95th percentile projections of biomass after 10 years but also describe 5th percentile outcomes. The 10-year horizon is the benchmark for comparing all alternatives; however, the exploratory modeling output depicts a trajectory of population change over time (PEIS Appendix A) that provides a basis for informed speculation about changes in population size further into the future. The uncertainties in exploratory modeling projections and evaluations are described in narrative form in other parts of this section, and a discussion of the implications of each uncertainty for estimates of risk is presented.in Section 4.7.

4.1 RQ 1 - WILL THE PROPOSED ACTION OR ALTERNATIVES ACHIEVE THE STATED GOAL FOR THE SIZE OF THE BAY-WIDE OYSTER POPULATION?

4.1.1 Projections for Introducing a Nonnative Oyster (Proposed Action and Alt. 7)

Predicting the success and resultant biomass of a nonnative oyster introduced into Chesapeake Bay encompasses considerable uncertainty. The OAP concluded that adapting the exploratory modeling to quantitatively project the growth of a population of *C. ariakensis* in the Bay would involve large and unknown uncertainties because of the lack of data about many important life-history characteristics of the species and how those characteristics might be displayed or altered in the Bay environment. In concurrence with OAP's comments regarding the exploratory modeling, the modeling team did not adapt the model to predict the growth of a diploid population of *C. ariakensis* in Chesapeake Bay; therefore, the question of whether the proposed action would achieve the stated goal cannot be answered at this time.

4.1.2 Projections for *C. virginica* Alternatives (Alternatives 1, 2a, 2b and 3)

The alternatives addressed here include: Alternative 1 (no action, current management actions), Alternative 2a (current programs but increased restoration efforts), Alternative 2b (current programs but maximum restoration efforts), and Alternative 3 (current programs but harvest moratorium). The variations in oyster biomass across the state/salinity zones for each of the alternatives involving only *C. virginica* are shown in Figures 4-1 through 4-4.

Under Alternative 1, at the lower-bound estimate (i.e., 5th percentile) oyster biomass would decline in all areas of the Bay; at the upper-bound estimate (95th percentile) biomass would increase in all areas. In numerical terms, the Bay-wide number of market-size oysters after 10 years in the 50th percentile projection was 330.2 million; the 5th and 95th percentile values were 116 million and 593 million, respectively. These values are all substantially less than the PEIS goal of 11.6 billion. One element of the assumed restoration activities (i.e., shell replenishment in Maryland) was terminated in 2007; therefore, the actual population level after 10 years would be even less than projected. The oyster population under this alternative is likely to decline indefinitely into the future because of the continuing decline in hard-bottom habitat.

The risk that implementing Alternative 1 would fail to achieve the restoration goal is considered high; that is, it is virtually certain that the goal would not be attained. The level of uncertainty associated with this risk is considered low to moderate due, in part, to recognized limitations of the exploratory modeling. The only potential mechanism by which the population of *C. virginica* might grow would be development of resistance to MSX and Dermo. Harvest is assumed to continue under Alternative 1, which would impede development of disease resistance in the population. The length of time required for a Bay-wide population of disease-resistant oysters to develop naturally is unknown, but it is likely to be substantial, and the availability of suitable habitat would continue to decrease during that time.

The enhanced restoration programs under Alternative 2 were projected to result in increases of 250% to 450% (50th percentile) in market-size oysters by year 10. Although these increases are substantial, they are from a very small starting population. Most of the increase would occur in lower salinity waters in Maryland. Numerically, the Bay-wide number of market-size oysters after 10 years under Alternative 2a in the 50th percentile projection was 1.4 billion; the 5th and 95th percentile values were 576 million and 2.0 billion, respectively. The 95th percentile figure is closer to the PEIS goal of 11.6 billion than 95th percentile projection for Alternative 1, but most of the enhanced population would occur in Maryland waters. In addition, the rate of increase in the population was projected to level off in years 9 and 10 and would be likely to remain level or decline into the future. According to the hypothetical assessment scenario for this alternative, seed planting would increase through year 7 and remain constant in later years, suggesting that the projected increase through year 9 was driven primarily by seeding and would not extend further into the future without continued seeding.

The risk that implementing Alternative 2 would fail to achieve the restoration goal is considered high. The level of uncertainty associated with this risk is considered moderate. Variation in exploratory modeling outputs for this alternative was much greater than for Alternative 1. Differences between the 10th and 90th percentiles ranged up to more than 500% in

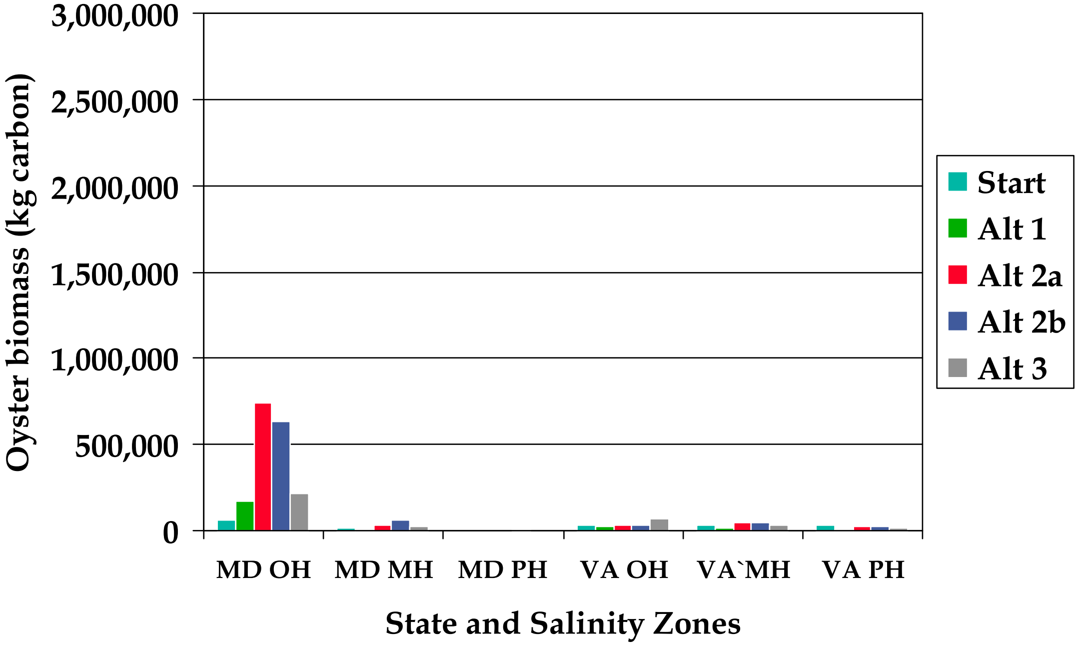


Figure 4-1. Relative changes in biomass (linear scale) for *C. virginica* alternatives (50th percentile)

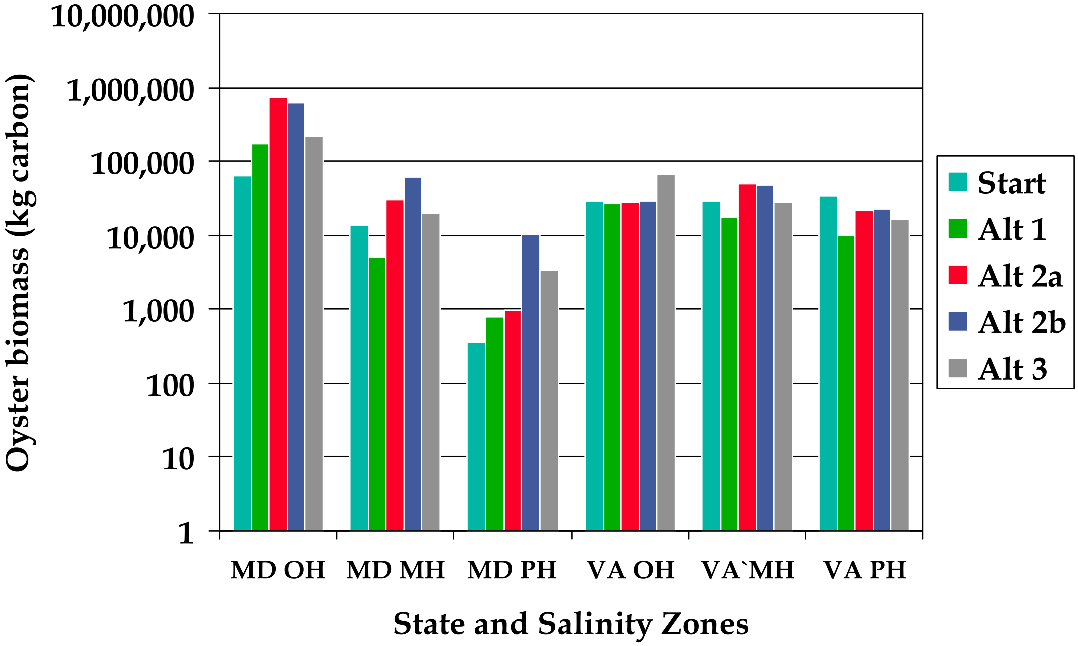


Figure 4-2. Relative changes in biomass (log scale) for *C. virginica* alternatives (50th percentile)

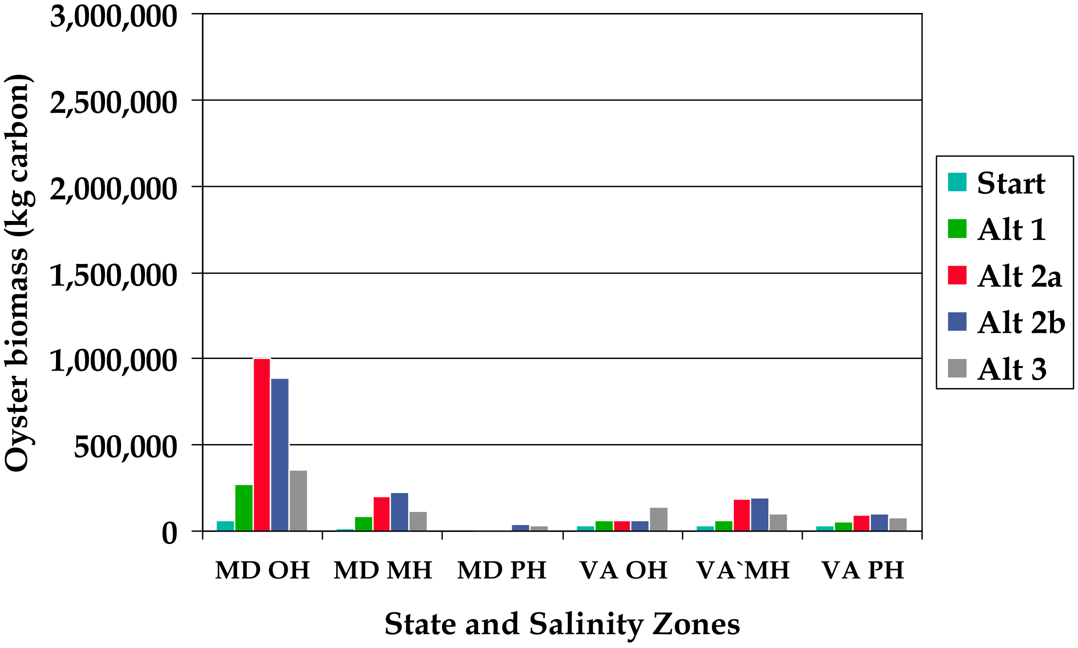


Figure 4-3. Relative changes in biomass (linear scale) for *C. virginica* alternatives (95th percentile)

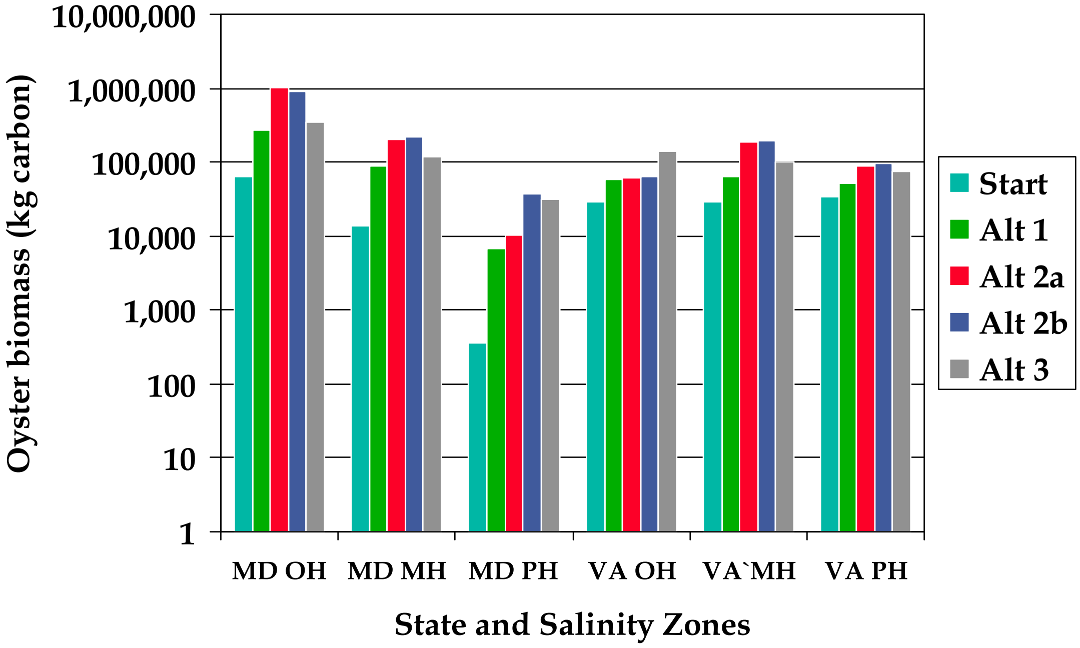


Figure 4-4. Relative changes in biomass (log sale) for *C. virginica* alternatives (95th percentile)

some cases. Limitations of the exploratory modeling contribute to this level of uncertainty. Potential development of disease resistance, effect of harvest on the rate of development of disease resistance, and continuing loss of habitat are three factors that are not accounted for in exploratory modeling projections.

Under Alternative 3, a havest moratorium was projected to result in a 56% increase in the median (50th percentile) abundance of market-size oysters after 10 years. No substantial increasing trend would be expected beyond the 10-year period based on the trajectory of population change over time. At the lower-bound estimate (5th percentile) oyster biomass would decline in much of the Bay. The Bay-wide number of market size oysters after 10 years under in the 50th percentile model run was 469.3 million; the 5th and 95th percentile values were 166.1 million and 948.3 million, respectively. These values are all substantially less than the PEIS goal of 11.6 billion.

The risk that implementing Alternative 3 would fail to achieve the restoration goal is considered moderate to high. The level of uncertainty associated with this risk is considered moderate. Limitations of the exploratory modeling contribute to this level of uncertainty. For instance, the exploratory modeling does not account for any development of disease resistance in the population of native oysters over time, but the elimination of harvest could contribute to more rapid development of disease resistance in the population than would occur under Alternatives 1 and 2, in which harvest would continue. Further loss of hard-bottom habitat over time would constrain the growth of the oyster population and also is not accounted for in the exploratory modeling.

4.1.3 Projections for Alternatives to Promote Aquaculture (Alternatives 4 and 5)

The distribution of biomass for the aquaculture alternatives is shown in Figures 4-5 and 4-6. The biomass and location of cultivated oysters was estimated based on the aquaculture scenario documented in Appendix C of the PEIS. In that appendix, scenarios are established that are considered to be reasonable representations, both in size and location, of a large-scale aquaculture industry in the Bay. For the purpose of this evaluation we assumed that cultivated market-size oysters of either species (i.e., *C. virginica* for Alternative 4, or *C. ariakensis* for Alternative 5) would be maintained each year at the same locations and in the same concentrations as developed for the scenarios. Biomass was estimated from the number of oysters using the conversion

$$\text{Biomass (g carbon)} = 0.0002115 \times H^{1.74575}$$

where H is shell height. This relationship was obtained by dividing the conversion from shell height to dry weight reported by Mann & Evans (1998) by two to estimate grams of carbon (C. Cerco, USACE, pers. comm.). We assumed the shell height of an oyster of median market size to be 77 mm. Biomass at a given shell height differs between diploid *C. virginica*, triploid *C. virginica*, and *C. ariakensis.* Triploid Suminoe oysters produce greater biomass per unit shell length (i.e., are heavier at a given shell size) than Eastern oysters. In studies conducted in 2005 and 2006, a biomass index (gm wet weight/mm shell length) for triploid Suminoe oysters for the

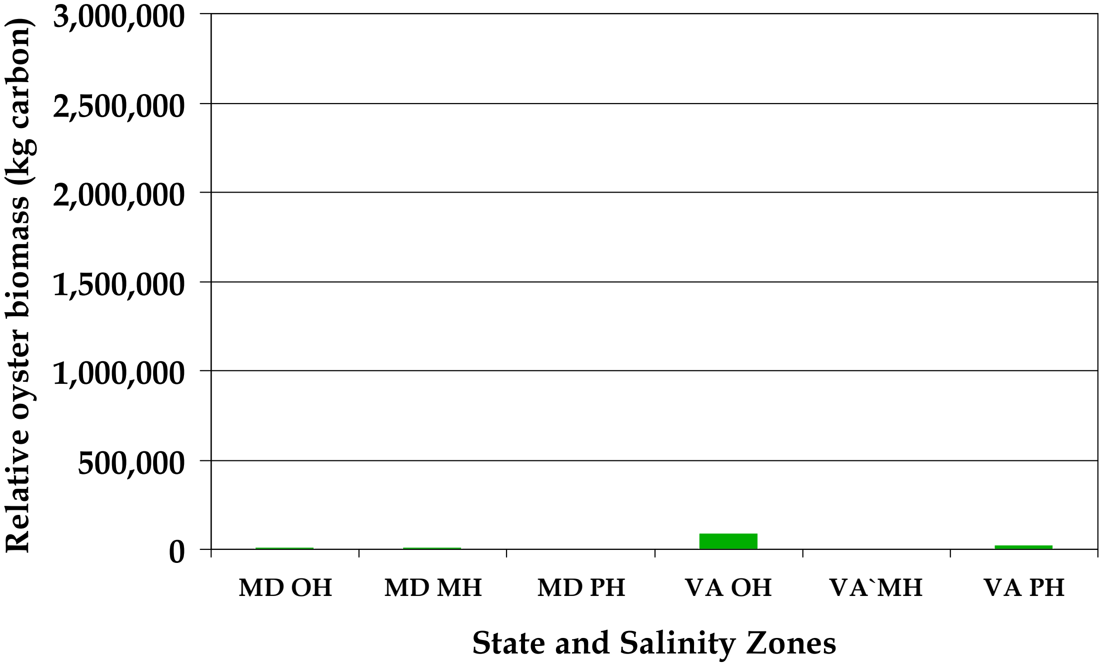


Figure 4-5. Estimated biomass of cultivated oysters (linear scale)

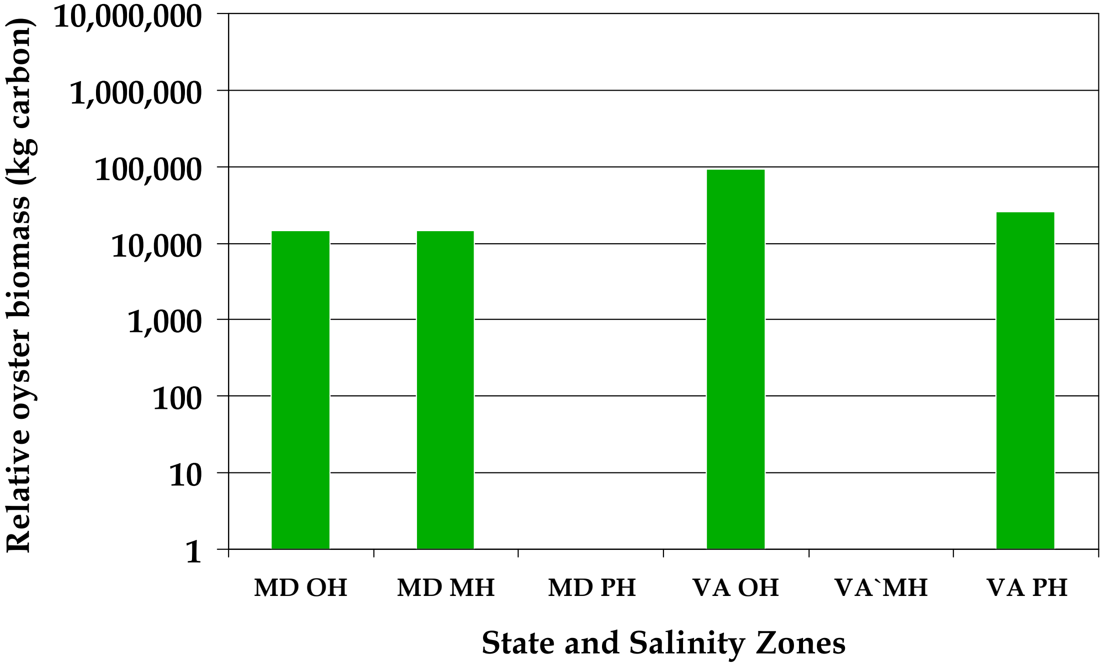


Figure 4-6. Estimated biomass of cultivated oysters (log scale)

period January to October was 80% greater than the index for diploid Eastern oysters, and 30% to 60% greater than the index for triploid Eastern oysters (data provided by Dr. S. Allen, VIMS). Although Alternatives 4 and 5 are considered together here for simplicity, Alternative 5 would result in greater biomass, on the order of 50% greater on average; therefore, the ecological effects of the alternative would be greater.

Numerically, the maximum economically viable oyster aquaculture industry in the Bay was estimated to have an annual production of 2.6 million bushels, or about 683.7 million oysters. That figure is much less than the restoration goal of 11.6 billion oysters. The risk that implementing Alternatives 4 or 5 would fail to achieve the restoration goal is considered high. The level of uncertainty associated with this risk is considered low. A major factor contributing to confidence in this assessment of risk is that the likelihood of the maximum industry becoming established in the near future (i.e., 10 years) is considered to be low (see PEIS Section 5.1). Uncertainty is great about whether an industry of that size could ever be established or, once established, could be maintained. Although implementing either of these alternatives would be unlikely to achieve the Bay-wide oyster restoration goal, concentrations of aquaculture might develop in selected tributaries and result in localized oyster abundance equivalent to former abundance in those locations. The estimates of oyster biomass projected for the aquaculture alternatives were used to predict changes in water quality and algal biomass using the CBEMP for assessing RQ 8. Algal biomass, in turn, influences benthic and planktonic food webs. The resultant changes in oysters and other ecological conditions were translated to potential changes in ecological conditions and evaluated using the RRM.

4.2 ASSESSMENT OF RISK QUESTIONS 2 THROUGH 6 ADDRESSING THE INTRODUCTION OF *C. ARIAKENSIS* INTO THE CHESAPEAKE BAY

Each of the following subsections begins with a conclusion that states the degree of risk and the attendant uncertainty followed by a summary of the information that supports the assessment.

4.2.1 RQ 2 – Will *C. ariakensis* provide ecosystem services similar to those afforded by *C. virginica*?

Conclusion: The risk that *C. ariakensis* would not provide ecosystem services similar to those afforded by *C. virginica* is low. Ecological services considered in this ERA are those associated with provision of reef habitat for other Bay species, provision of food for other Bay species, and filtration capacity. *C. ariakensis*, if successful, is expected to populate historical oyster habitat and other hard substrates in the subtidal zone. The level of uncertainty associated with this conclusion is moderate. The uncertainty is related to inadequate understanding of all of the many and varied ways in which oysters interact with other components of the Bay ecosystem, as well as lack of knowledge about the characteristics of *C. ariakensis* reefs or mixed-species reefs in open waters of Chesapeake Bay.

The NRC posed this question in its preliminary evaluation of risks associated with introducing *C. ariakensis* to Chesapeake Bay (NRC 2004). It is applicable only to the proposed action and Alternatives 5 and 7. Regarding Alternative 5, this question would apply only if an unintended introduction of diploids from triploid aquaculture results in a large, self-sustaining population of *C. ariakensis* (Section 4.3). In considering this risk question, the proposed introduction of *C. ariakensis* is assumed to be successful, and the species to be established throughout the range of *C. virginica* in the Bay. This question does not address the feasibility of an introduction, only the ecological consequences if the species were to become widespread.

The risk that *C. ariakensis* would not provide ecosystem services similar to those afforded by *C. virginica* is low. Ecological services considered in this ERA are those associated with provision of reef habitat for other Bay species, provision of food for other Bay species, and filtration capacity. *C. ariakensis*, if successful, is expected to populate historical oyster habitat and other hard substrates in the subtidal zone. Because *C. ariakensis* can tolerate high loads of suspended sediment and exist in muddy systems (albeit on shell), reefs of the species could provide localized benefits for SAV by buffering the action of waves and currents and by filtering suspended solids from the water. Both oyster species are expected to filter the same kinds of algae and suspended matter from the water.

One important aspect of ecosystem services provided by oysters relates to reef-building capability. Despite some potential differences in reef formation between the two species, *C. ariakensis* is a reef former and would support other communities. Luckenbach (2007) observed that *C. ariakensis* is less adept at growing in high densities than the native oyster and that it does not produce as much vertical relief above the substrate; nevertheless, it does exhibit some capability to do both. Kingsley-Smith et al. (2007) observed that triploid *C. ariakensis* exhibits some characteristics of reef formation when grown in cages in Chesapeake Bay. Guo (pers. comm. 2008) observed that *C. ariakensis* forms reefs in its native habitat. Oyster reefs in Nantong China are 0.2 to 0.8 m tall and consist primarily of *C. ariakensis* with some small *C. sikamea* on top. Guo also noted that *C. ariakensis* reefs are found in or near large river systems that have high sediment loads and soft bottoms. *C. ariakensis* is usually the basal species in reef assemblages and provide substrate for other oyster species. Guo noted that *C. ariakensis* reefs in China usually do not have many other species of invertebrates attached (although polychaetes are common); he speculated that this be related to the muddy water in which *C. ariakensis* usually lives. Luckenbach (pers. comm. 2008) noted that reef-oriented species did become associated with experimental *C. ariakensis* reefs described in Kingsley-Smith et al. (2007). Organisms also became associated with shell-only controls, and Luckenbach indicated that such species probably would become associated with any structure placed on the bottom. He noted that we do not know if the composition of communities associated with oyster reefs would differ between the two oyster species. Differences might be small and ecologically insignificant, or subtle differences could lead to larger, system-level changes. The Luckenbach et al. "tile study" (Section 4.2.2.2) also raises interesting questions about the potential for co-existence of these two species. Interspecific competition for space clearly occurred, but it had the surprising effect of changing growth form—increasing shell growth in the vertical direction—and reducing the biomass of both species. This seems to suggest that mixed-species populations could result in more reef-like development than monospecific populations of *C. ariakensis.* As Guo noted, *C. ariakensis* typically occurs with other oyster species.

Another ecosystem service that relates to the maintenance of habitat type involves the production of shell. New shell and increased area of shell covering the bottom are necessary for the sustainability of oysters and other species that require hard substrate. To the extent that *C. ariakensis* could succeed in existing hard-bottom areas, the species would contribute to the production and sustainability of shell and could cause an increase in the area of hard bottom. If *C. ariakensis* were successful in this regard, the production and sustainability of shell could benefit the population of *C. virginica* because the shell would provide the substrate necessary for settlement and development of young *C. virginica.* This suggestion is speculative; no data are available to support the possibility of significant accretion of *C. ariakensis* shell. Carnegie (pers. comm. 2008) speculated that neither oyster species is likely to reclaim vast areas of soft bottom in the absence of some sort of engineering, like shell planting. He further noted that the thin shells of *C. ariakensis* would not only be more susceptible to crushing by crabs, but also to worm burrowing, and probably to the natural decay processes that negatively affect shell budgets over time.

The level of uncertainty associated with this conclusion is moderate. The uncertainty is related to inadequate understanding of all of the many and varied ways in which oysters interact with other components of the Bay ecosystem, as well as lack of knowledge about the characteristics of *C. ariakensis* reefs or mixed-species reefs in open waters of Chesapeake Bay. Although species interactions are considered the most important mechanisms by which changes in the abundance or kind of oysters in the Bay could influence other receptors, many of the specific details of these interactions are not well known or quantified. Uncertainty increases with the number of linkages between ecological receptors and oysters. We believe that the most important influences of oysters on ecological receptors in the Bay have been captured in using the RRM to address RQ 8 and that existing information suggests that the magnitude of those influences would be small. Details of the findings of additional studies that we believe support this assessment are presented in the discussion of RQ 3, Section 4.2.2, below

4.2.2 RQ 3 - Will *C. ariakensis* compete with *C. virginica* and other resident species, possibly leading to reduced populations or local extinction of these species?

Conclusion: Risk is moderate to high that *C. ariakensis* would interact with and compete with *C. virginica.* The two species might be able to co-exist within the Bay because some environments would be more favorable to one than the other, and they can co-exist within reefs. Although most of the interactions described below are negative in nature, one positive interaction is possible. A successful population of *C. ariakensis* might produce shell for colonization by oyster spat. If that occurred, a naturalized population of *C. ariakensis* could contribute to the sustainability of both oyster species in Chesapeake Bay in the absence of a shell replenishment program. Uncertainty about the nature and extent of competitive interactions between the oyster species is moderate to high because nearly all of the available information comes from laboratory studies and limited field trials. No studies to date support the potential for a positive interaction; it is presented only as a possibility.

This risk question is applicable only to the proposed action and Alternatives 5 and 7. Alternatives involving only *C. virginica* would not alter the existing kinds of competition between species within the Bay. Regarding Alternative 5, this question would apply only if an unintended introduction of diploids from triploid aquaculture results in a large, self-sustaining population of *C. ariakensis* (see Risk Question 7).

The NRC (2004) reached the following conclusion regarding competition:

> *Based on the limited data available, it appears that* C. ariakensis *has environmental tolerances that make it well suited for growth and reproduction in the Chesapeake Bay and in other similar estuarine habitats on the Atlantic and Gulf coasts. It is likely to compete with the native oyster, although differences in environmental tolerances might result in these two species occupying different habitats if* C. ariakensis *becomes established in the bay.*

The NRC (2004) recommended further study to investigate the potential competition between the two species: (1) Develop a better understanding of *C. ariakensis* biology in the Chesapeake Bay under various temperature and salinity regimes, particularly its growth rate, reproductive cycle, larval behavior, and settlement patterns in different hydrodynamic regimes; size-specific, post-settlement mortality rates; and susceptibility to native parasites, pathogens, and predators. (2) Determine the ecological interactions of *C. ariakensis* and *C. virginica* at all life stages, including interspecific competition and reef-building capacity.

A conceptual model of potential interactions is provided in Figure 4-7. We focused on three potential ways that *C. ariakensis* and *C. virginica* could compete with respect to sustainability of their populations within the Bay:

1. Overlap in ecological requirements and susceptibility to chemical, biological and physical stressors; the greater the overlap the greater the potential for interspecies competition.
2. Relative differences in settlement success and growth and the ability to co-exist on reefs.
3. Interference with reproductive success.

The oyster species also may compete with one another for food; however, the availability of food does not seem to be a limiting factor at present. A reduced food supply, to the extent it would happen, would affect both species.

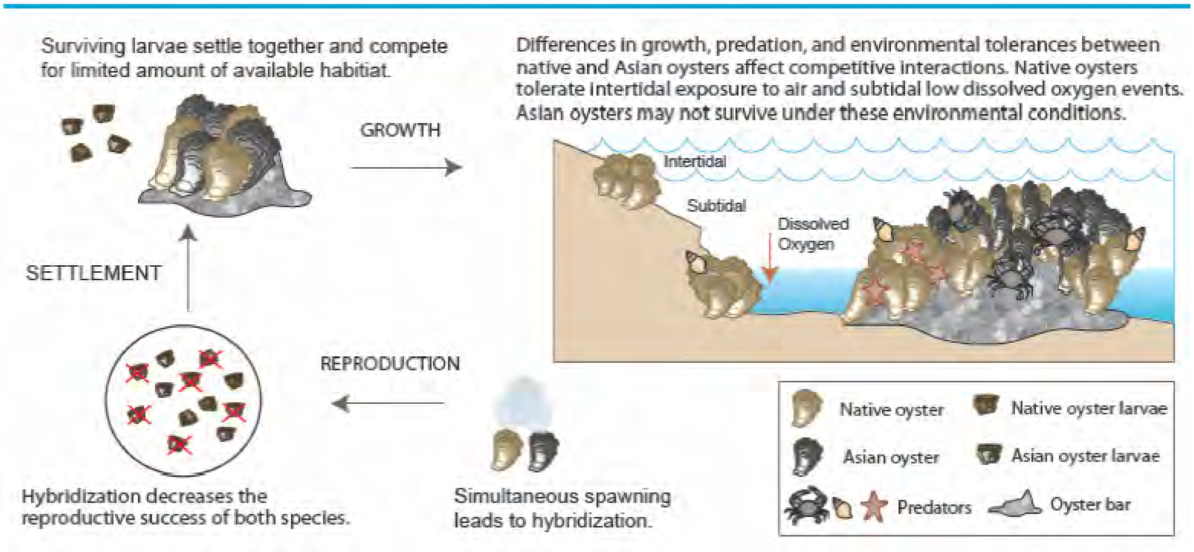


Figure 4-7. Conceptual model of potential competitive interactions between *C. ariakensis* and *C. virginica* (from Bushek et al. 2007)

4.2.2.1 Overlap in Ecological Requirements and Susceptibility to Stressors

The NRC (2004) indicated that initial studies suggested that *C. ariakensis* grows in waters with a range of salinities, grows rapidly compared to native oysters, and is resistant to MSX and Dermo diseases. This environmental flexibility would allow *C. ariakensis* to occupy and reproduce in the same habitats as *C. virginica*. In addition to 'sharing' space, they would also share food resources; consequently, competition is likely. Although there is broad overlap in the ecological requirements of the two species, differences in the abilities of the species to occupy different environments may favor one species over another. These differences are highlighted below

Tolerance to Atmospheric Exposure in Intertidal Habitats

Kingsley-Smith et al. (2007) conducted cage experiments with triploid *C. ariakensis* and observed almost complete mortality in intertidal habitats. In simulated tidal exposures comparing *C. ariakensis* and *C. virginica*, Luckenbach and Kingsley-Smith (2006) found that *C. virginica* exhibited greater survival than *C. ariakensis* in intertidal treatments. In particular, *C. virginica* was able to persist in the mid intertidal and low intertidal treatments, whereas *C. ariakensis* experienced almost complete mortality in all three intertidal treatments. They also found that *C. ariakensis* grew faster than *C. virginica* in the subtidal treatment. The authors noted that the low survival of *C. ariakensis* in the intertidal treatments suggests that, in the wild, *C. virginica* might be able to escape competition with the nonnative species and that the faster growth of *C. ariakensis* is subtidal habitat supports the possibility of a niche separation between the species if they were to co-exist in Chesapeake Bay. They noted that *C. ariakensis* is not reported to be an intertidal species in the Ariake Sea, Japan.

In North Carolina, triploid *C. ariakensis* deployed in the field demonstrated similarly low mortality rates in intertidal and subtidal placements (Bishop and Peterson 2006). During the winter, *C. ariakensis* in subtidal habitat outgrew those in intertidal habitat; however, during the spring, when fouling severely affected subtidal oysters, intertidal oysters displayed faster growth rates (Bishop and Peterson 2006).

Intertidal habitat is not extensive in the Bay; however, the limited habitat that is available could offer *C. virginica* some refuge from competition for space with *C. ariakensis*. *C. virginica* appears to be able to tolerate intertidal conditions better than *C. ariakensis*, which would give it a competitive advantage in such environments.

Tolerance to Exposure to Low Dissolved Oxygen

Matche and Barker (2007) examined mortality among juvenile oysters following experimental exposure to anoxia/hypoxia. All trials were conducted at a temperature of 30 °C; therefore, the study represents a worst-case scenario. They found that *C. ariakensis* was significantly less tolerant of anoxia than *C. virginica* through 144 hours of exposure, after which all *C. ariakensis* were dead, but only 51% of *C. virginica* were dead. *C. ariakensis* was also significantly less tolerant than *C. virginica* through 192 hours of exposure to a declining hypoxic regime with 10% and 20% initial DO saturation. In both sets of experiments, *C. ariakensis* was less tolerant than *C. virginica*. The authors concluded that juvenile *C. ariakensis* are significantly less able to tolerate anoxia and hypoxia than juvenile *C. virginica*.

Harlan and Paynter (2006) obtained similar results in experiments conducted at temperatures of 10 °C and 20 °C. The lower tolerance of *C. ariakensis* was observed at DO saturations of 0%, decreases of 20% to 13%, and decreases of 10% to 6%. The authors noted that these differences cannot be explained by differences in metabolic rates. Instead, they appear to reflect behavioral differences between the oysters. In particular, *C. ariakensis* continued to gape (keep its shell open) in anoxic/hypoxic conditions, whereas *C. virginica* shut its valves. The authors suggested that this behavioral difference is likely to be due to differences in metabolic pathways. Metabolic differences (or some other unidentified factor) afford a competitive advantage to *C. virginica* in waters that periodically experience anoxic or hypoxic conditions. Paynter et al. (in press) noted that the greater mortality of triploid *C. ariakensis* in cage experiments in the Patuxent River could be due to their sensitivity to hypoxia.

C. virginica appears to be able to tolerate low dissolved oxygen conditions better than *C. ariakensis,* which gives it a competitive advantage in environments that periodically experience low dissolved oxygen (DO).

Tolerance to Diseases

C. ariakensis (triploid) appears to be more tolerant of Dermo than *C. virginica* (diploid) based on the results of a study performed by Calvo et al. (2000). MSX was absent in *C. ariakensis* but present in *C. virginica.* The field studies performed to date (i.e., over the last 5 to 6 years) show no evidence that *C. ariakensis* is killed by MSX or Dermo (NOAA 2005). This finding is limited to field tests of triploids in aquaculture growing conditions (typically not on-bottom). This potentially greater resistance to disease is a key reason for expecting *C. ariakensis* to have a greater rate of survival than *C. virginica.*

C. ariakensis can be infected by Dermo (Moss et al. 2006; Vasta et al.) despite the observed survival of triploid *C. ariakensis* in aquaculture. Experimental field exposures indicated that *C. ariakensis* acquired *P. marinus* infections at a similar rate to *C. virginica* but that infection intensities remained lower, at least during the first year of exposure (Breitburg et al. 2007). In cage experiments with triploids of both species, Paynter et al. (in press) found that prevalences and weighted prevalences of *P. marinus* in both *C. virginica* and *C. ariakensis* remained relatively low throughout the experiment, but *C. virginica* consistently acquired higher prevalences and weighted prevalences than *C. ariakensis.* Moss et al. (2006) found that *C. ariakensis* hemocytes can kill *P. marinus*, the cause of Dermo, but only when the number of parasites present is small. Even when few parasites are present, some portion of the parasite survives and eventually proliferates when transferred to culture medium. If the parasite is present in greater numbers, it appears to survive exposure to hemocytes and plasma and proliferates. The research indicates that the hemocytes of neither *C. virginica* nor *C. ariakensis* are able to kill all infecting *P. marinus* trophozites, and these retain their proliferating capacity. Preliminary evidence suggests that infected *C. ariakensis* can transmit *P. marinus* to *C. virginica* in an aquaculture setting. No field experiments to test *C. ariakensis*-to-*C. virginica* transmission of *P. marinus* have been conducted to date. No *Haplosporidium* spp infections (the cause of MSX) have been detected in *C. ariakensis* so far.

C. ariakensis can be infected and killed by the oyster pathogen *Bonamia*. Mass mortalities of triploid *C. ariakensis* were observed in experimental deployments in North Carolina (Burreson et al. 2004). The possibility that this pathogen could infect *C. ariakensis* introduced to the Bay has received considerable attention. Infection of *C. ariakensis* by the virulent type of *Bonamia* appears to be influenced by salinity (Audemard et al. in press). Infection is greatest at salinities of 30 ppt and declines with decreasing salinities; therefore, there may be a geographic limit of infection because salinities in the Bay are lower than in the Atlantic Ocean. Carnegie (pers. comm. to Menzie, 2008) noted that the effects of *Bonamia* on *C. ariakensis* may be high at salinities of 25 ppt or more, and moderate to high at 22 to 25 ppt; only at 18 ppt and lower is there confidence that the effects of *Bonamia* on *C. ariakensis* would be low. *C. virginica* is resistant to this species of *Bonamia*, which affords *C. virginica* a competitive advantage at higher salinities.

Resistance to Predators

Oysters are vulnerable to several predators, and differential predation intensity probably would depend on oyster life stage, location within the Bay (state/salinity zones), type of predator, and oyster species. Juvenile *C. virginica* appear to be somewhat less susceptible to predators than juvenile *C. ariakensis*. Results are equivocal for larvae of the species. Breitburg et al. (2007) examined predation of larvae of the two species by the ctenophore *Mnemiopsis leidyi*. They found that ctenophores had a 50% higher preference for 10- to 13-day old *C. ariakensis* larvae than for *C. virginica* larvae of similar ages but observed no difference in predation of younger oyster larvae.

Newell et al. (2007a) examined the potential for differences in predation pressure on juvenile *C. ariakensis* and *C. virginica* growing attached to large pieces of cultch in a series of choice experiments. Predators included four species of mud crabs (*Rhithropanopeus harrisii, Eurypanopeus depressus, Panopeus herbstii,* and *Dyspanopeus sayi*), the blue crab (*Callinectes sapidus)*, and flatworms (*Stylochus* spp.). All four species of mud crab and the blue crab significantly ($P < 0.05$) selected *C. ariakensis* compared to *C. virginica,* but predation by flatworms of both sizes did not differ significantly between oyster species. The authors concluded that because *C. ariakensis* exposed to predators continued to have a weaker shell (i.e., compression strength is 64% less than that of *C. virginica)* relative to *C. virginica*, the natural suite of crab and flatworm predators in Chesapeake Bay could serve to control the abundance of feral *C. ariakensis*. They noted that the situation in the natural environment may be sufficiently different in some locations to enable *C. ariakensis* to compensate for its greater vulnerability to predation by crabs. Bishop and Peterson (2006) also found that cultchless, triploid *C. ariakensis* were more susceptible to predation than cultchless, triploid *C. virginica*. They noted that predators that crush shells or mechanically open prey items could limit the abundance of *C. ariakensis.*

Kennedy and Newell et al. (in press) examined predation by starfish and oyster drills in addition to the crabs and flatworms discussed above. They concluded that starfish species favored *C. virginica* over *C. ariakensis* when given a choice. Oyster drills seemed to be attracted to *C. virginica.* The authors noted that continuing preference for *C. virginica* indicates that drills are using some chemical effluent to track oysters as a food source. They suggested that drills would continue to be a more important predator of *C. virginica* than of *C. ariakensis* in a field situation where both species of oysters were living sympatrically.

4.2.2.2 Relative Differences in Settlement Success and Growth

Oysters settle and form reefs on discrete hard-bottom locations within Chesapeake Bay. Use of these subtidal locations by the two species (*C. virginica* uses intertidal areas as well) and the relative degree of success of each species will depend on competition for space and the ability to co-exist in mixed-species populations. Some insights into the relative success of populations and reefs of mixed oysters including *C. ariakensis* can be gleaned from observations of reefs in its native waters off the coast of China. Guo et al. (2007) sampled extensively along the coast of China. They noted that *C. ariakensis* is the dominant species in at least 5 of the 12 sites where it is found and is clearly a reef-builder. *C. ariakensis* is not found in monospecific reefs; it always co-exists with other *Crassostrea* species in its native range. In most reefs, *C. ariakensis* is the dominant and basal species (i.e., the large oysters at basal positions are *C. ariakensis*, and the small oysters on top are *C. gigas* or *C. sikamea*). Guo (pers. comm. 2008) noted that he finds no difference in substrate preference between *C. ariakensis* and *C. virginica*. Both would settle on shells regardless of whether the shells are on soft or hard bottoms. *C. ariakensis* can survive systems with high sediment load by growing upward.

One primary consideration for successful oyster restoration using an introduced species is the likelihood that one or both species would be attracted to and settle on appropriate reef materials. Tamburri et al. (in press) examined this for various settlement materials that might be used in Chesapeake Bay. They found that both species prefer to colonize natural substrates (e.g., shell) covered with biofilms. Waterborne chemical cues emitted by adult oysters enhance substrate attachment for all larval forms, but cues do not appear to be species specific; consequently, both species probably would choose the same types of natural materials on which to settle and would be attracted by similar chemical cues. In cage experiments, Paynter et al. (in press) observed that spat of *C. virginica* readily settled on *C. ariakensis.*

Newell et al. (2007b) examined competitive interactions between diploid *C. virginica* and diploid *C. ariakensis* with respect to larval settlement through to development of reefs. The results are preliminary. They observed several factors that favored the success of *C. ariakensis*:

1. Post-settlement mortality in mesocosms was lower for *C. ariakensis* (~55%) than for *C. virginica* (~80%) even though predator pressure (in the absence of crabs) was about the same.
2. *C. ariakensis* had a significantly faster rate of shell growth than *C. virginica.*
3. *C. ariakensis* can continue to grow at lower temperatures than can *C. virginica.*
4. *C. ariakensis* matures to reproductive status faster than does *C. virginica*.

Newell et al. (2007b) concluded that a more rapid accumulation of biomass may give *C. ariakensis* a long-term competitive advantage over *C. virginica*, especially in combination with the possibly earlier and more intense reproductive development of *C. ariakensis.*

As noted earlier, salinity influences the growth rates of the two species. *C. ariakensis* grows faster than *C. virginica* at the higher salinities present in Virginia waters, and the difference decreases with decreasing salinity. The salinity gradient within Chesapeake Bay probably would influence the degree to which differential growth rates affect competition between the species. The effect of differential growth rates would be expected to be greater in Virginia than in Maryland. The work of Paynter et al. (in press) suggested that the differences in growth rate may be related to the ability of *C. ariakensis* to grow over a larger portion of the year than *C. virginica.*

Luckenbach (2006) examined the success of larval settlement for mixed species assemblages. The data are limited but indicate that post-settlement survival of recruits was high (> 90%) regardless of whether *C. virginica* settled on substrates (tiles) occupied by adult *C. ariakensis* or whether *C. ariakensis* settled on substrate occupied by adult *C. virginica.* Lukenbach (2006) also observed differences in the growth forms of the species (Figure 4-8). Although the “horizontal” form of *C. ariakensis* was more common, the authors also observed growth forms similar to that of *C. virginica.*

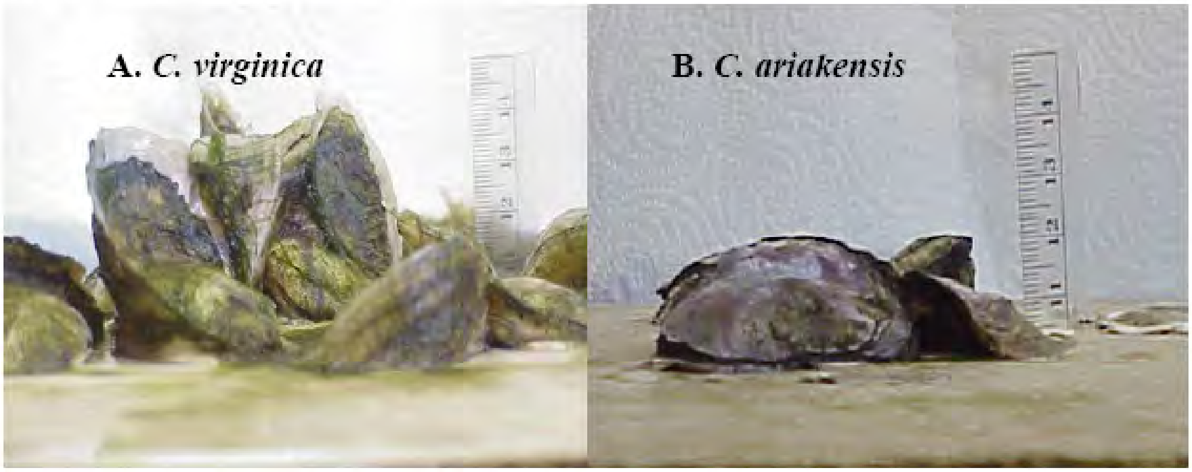


Figure 4-8. Common growth forms of *C. virginica* (A) and *C. ariakensis* (B). From Luckenbach (2006).

C. ariakensis has been observed to build reefs in its native range (Guo et al. 2007). Figure 4-9 shows pictures of natural reefs off the coast of China. Kingsley-Smith et al. (2007) observed reef-forming characteristics in their cage experiments with triploid *C. ariakensis* in Chesapeake Bay. Within the context of their experiments, *C. ariakensis* clearly exhibited the ability to form complex "reef" structures. Their experiments constrained both the number of age classes of experimental oysters (1) and the number of oysters per shell (5 to 13); consequently, neither species was able to form a true natural reef. In contrast to results from previous quarantine experiments, *C. ariakensis* grew well in dense clumps and formed complex habitat similar to (but not exactly the same as) *C. virginica*.

Luckenbach (2006) explored interactions between *C. ariakensis* and *C. virginica* when the species are growing together on a common substrate. The research provides insights into how the species would respond to one another when growing together within the same reef system. The study revealed significant interspecific competition for space between *C. ariakensis* and *C. virginica.* Reduced growth rates for both species were observed in mixed-species treatments compared to single-species treatments. An example of this is given in Figure 4-10 for soft-tissue biomass. They found that the presence of even a small number of one species on a tile generally caused a reduction in the average growth rate of the other species on that tile.

Figure 4-9. Examples of *C. ariakensis* reefs in the species' native range (from Guo et al., 2007).

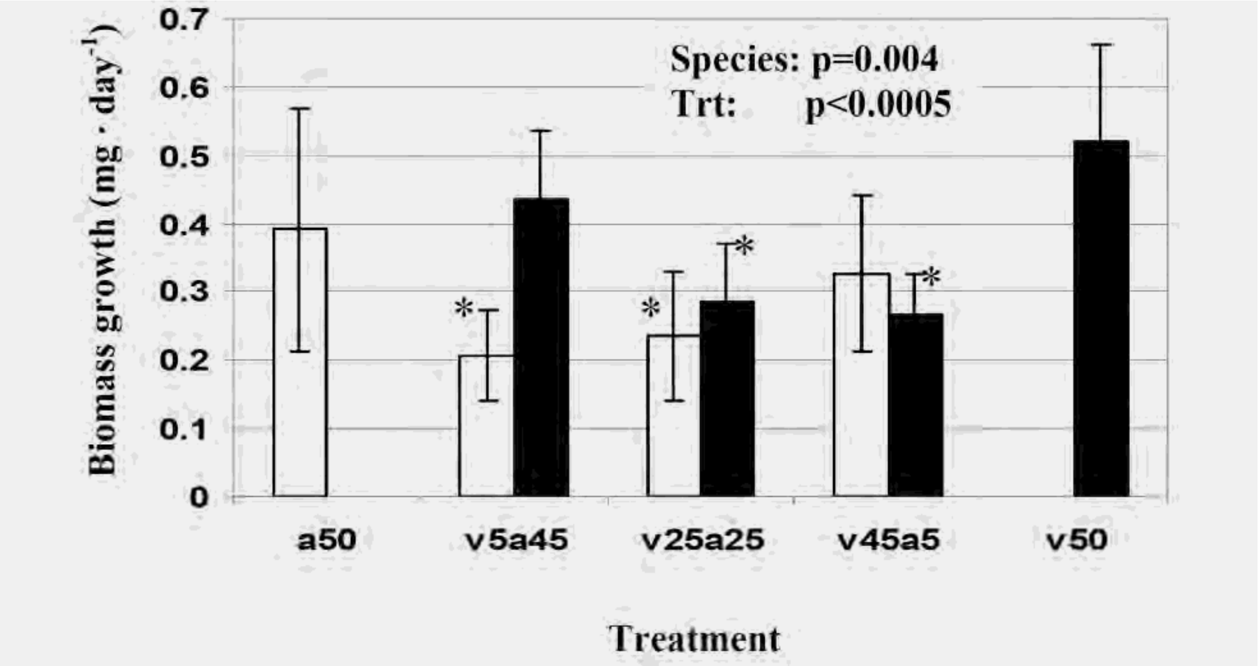
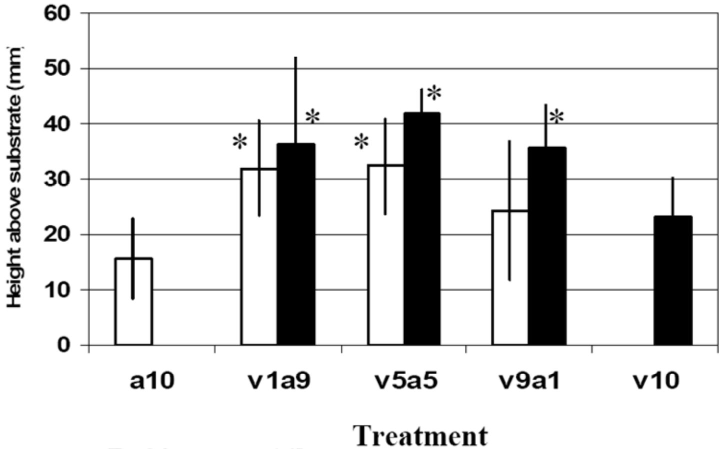


Figure 4-10. Growth rates under intra- and inter-specific competition for *C. ariakensis* (open bars) and *C. virginica* (filled bars) with a total density of 50 oysters per 100 cm^2. Significant differences between intra and inter-specific growth are indicated with a "*". From Luckenbach (2006).

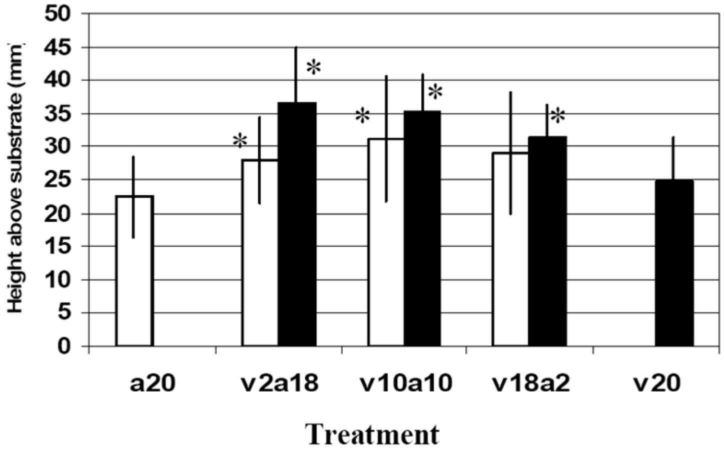
4.2.2.3 Interference with Reproductive Success

We use the term "reproductive success" to mean successful spawning combined with the successful production of viable oyster larvae. The primary issues concerning the potential for interference include the degree to which one species prevents or stimulates spawning of the other species, the degree to which cross-fertilization occurs between *C. ariakensis* and *C. virginica*, and the viability of hybrids. The dampened reproductive success of one or both species has been referred to as a "gamete sink."

No evidence collected to date has indicated that either species of oyster prevents the other from spawning. The time of spawning of the two oyster species could overlap, and the research of Meritt et al. (2005a, 2005b) suggests that one species can induce the other to spawn. If hybrids are formed, all development ceases after 8 to 10 days, and the hybrid larvae die (Allen et al. 1993).



B. 20 oysters/tile



C. 50 oysters/tile

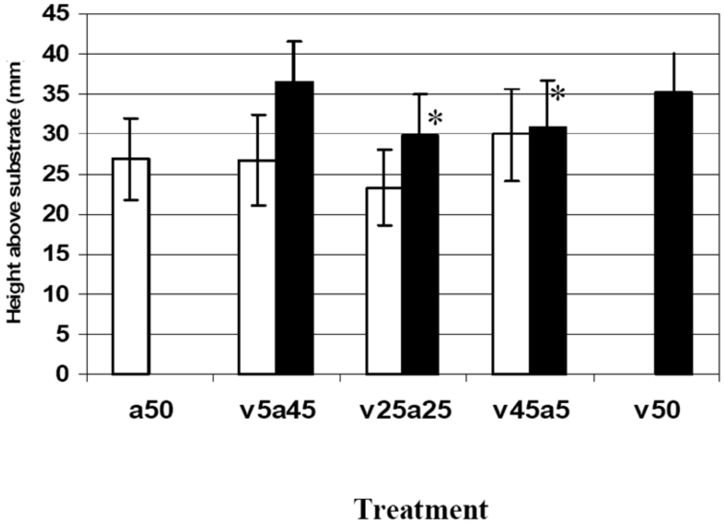


Figure 4-11. Vertical shell height above the substrate for each species, with intra- and inter-specific competition. From Luckenbach (2006).

Bushek et al. (2006) examined the relative vulnerability of the two species to hybridization. They observed reductions in fertilization rates and a gamete sink under conditions of synchronous spawning and cohabitation. The magnitude of the interaction depended on gamete concentrations and mixing, indicating that increasing the distance between the species would reduce the interaction. They also found that *C. virginica* sperm appeared to be more likely to sequester *C. ariakensis* eggs than the converse interaction, indicating a potential competitive advantage for *C. virginica*. Bushek (pers. comm. 2008) proposed the following four possible interspecific interactions, assuming that spawning seasons would overlap in Chesapeake Bay, that similar environmental cues trigger both species (increased temp and/or salinity), and that gametes from one species would stimulate the other to spawn:

1. Hybridization is likely to occur at some frequency, and the resultant hybrids would not be viable.
2. *C. virginica* may have a small competitive advantage over *C. ariakensis* in this interaction because *C. virginica* sperm are more likely to fertilize *C. ariakensis* eggs than the other way around.
3. The likelihood of cross-fertilization will decrease as gametes are diluted because about 10 times as much sperm is needed to successfully fertilize an egg from another oyster species as to fertilize eggs from the same species. Gregarious setting on oyster shell could increase the likelihood of subsequent interactions between adults of the two species during spawning. Young oysters, which are likely to be male, would be attached to older adults, which are likely to be female.

Given the various factors that will influence the success of spawning, Bushek believes that it is nearly impossible to predict how these interactions will influence the success of either species through time (pers. comm. 2008).

This overview of available information on both species suggests that the risk that *C. ariakensis* would interact and compete with *C. virginica* is moderate to high. *C. ariakensis* grows faster, matures earlier, and is resistant to the diseases that affect *C. virginica* in Cheaspeake Bay; therefore, *C. ariakensis* could outcompete *C. virginica*. Because the species differ in their tolerances for stressors within the Bay, *C. virginica* could have an adaptive advantage in environments that are not favorable for *C. ariakensis* (e.g., intertidal areas and areas of low dissolved oxygen). A successful introduction of *C. ariakensis* could result in production of large quantities of shell that would benefit both oyster species; *C. virginica* larvae are known to settle on *C. ariakensis* shell. The suggestion that *C. ariakensis* could increase the shell budget at cultch areas is a speculation. The likelihood of that outcome would depend on the population growth of *C. ariakensis* and the longevity of its shell in the environment. While in its native environment *C. ariakensis* forms the base of reefs that support smaller species of oysters, the natural ranges *C. virginica* and *C. ariakensis* do not overlap; therefore, the possibility that they would form mixed-species reefs cannot be confirmed. The range of potential outcomes of competition between these species is broad: local extinctions of *C. virginica* are possible in environments that are favorable for *C. ariakensis*; either species might become dominant in localized areas; and mixed reefs may develop with varying proportions of each species. Gamete competition resulting from both species coexisting and spawning in the same location would favor the survival of the species present in greater abundance. The potential interactions between the two oyster species suggest the possibility of outcomes that are both positive and negative for *C. virginica*; therefore, the two species probably could coexist. Thus, although the risk of local extinction of *C. virginica* appears to be moderate, the risk of Bay-wide extinction of C. virginica as an outcome of the proposed introduction appears low.

Several lines of evidence indicate that *C. ariakensis* would behave similarly to *C. virginica* with respect to choice of substrate for settlement. Larvae of *C. virginica* and *C. ariakensis* have the same requirements for clean, firm substrate to promote metamorphosis and attachment. Both species settle and form reefs on hard substrate (i.e., shell and rock). If *C. ariakensis* is successful in Chesapeake Bay, the species is expected to populate historical oyster habitat and other hard substrate in the subtidal zone. It is not expected to form large reefs outside of historical hard-bottom areas that would overtake other soft-bottom or SAV habitats. Horizontal expansion from existing hard-bottom areas and colonization of patches of shell could occur through shell accretion.

The uncertainty associated with these conclusions is moderate to high. This uncertainty stems from the limited knowledge and understanding of ecological interactions between the oyster species. Current knowledge is based largely on laboratory studies, limited field trials, and observations of *C. ariakensis* in its native range.

4.2.3 RQ 4 - What is the potential for introduction and spread of diseases to other species in the Bay? (This does not include human health considerations, which are addressed in the PEIS)

Conclusion: If ICES protocols are followed, introducing *C. ariakensis* would pose a negligible risk of introducing diseases. Some risk exists for *C. ariakensis* to be affected by endemic pathogens such as *Bonamia.* The risk that the introduction of pathogen-free *C. ariakensis* would result in an increase in subsequent infections by introduced pathogens (e.g., in ballast water) that are capable of infecting bivalves is small, although it would be proportional to the population size of *C. ariakensis*. Uncertainty related to this conclusion is moderate to low.

This question is applicable only to the proposed action and Alternatives 5 and 7. Alternatives involving only *C. virginica* would pose no risk of introducing new diseases, assuming that none of those alternatives would involve transporting *C.virginica* from other waters into Chesapeake Bay.

Introduction and spread of disease as a result of a planned introduction of *C. ariakensis* could occur at two points: (1) as part of the initial introduction and (2) subsequently, due to the susceptibility of *C. ariakensis* to diseases introduced to the Bay after a reproductive population of *C. ariakensis* has become established. The first of these risks is judged to be low. The NRC (2004) report reached the following conclusion with respect to the potential for introduction and spread of diseases if *C. ariakensis* is introduced as planned:

> *Strict application of the ICES protocols reduces the risk of co-introduction of undesirable organisms, including most pathogens and parasites. Oversight of the importation and deployment of the new species and prevention of a rogue introduction (an unsanctioned, illegal, direct release of reproductive nonnative oysters) will be required to prevent release of "hitchhiking" species.*

Research has indicated a potential for transmission of diseases from stocks located off the coast of China if a strain of *C. ariakensis* obtained from outside U.S. waters is introduced (i.e., Alternative 6) without following ICES protocols. Moss et al. (2007) screened 1,358 oyster samples from Asia and the VIMS hatchery and identified locations in waters off the coast of China where the prevalence of a herpes-like virus (OsHV), *Perkinsus* spp, or both was relatively heavy in a variety of bivalve species. Importantly, none of the samples from the VIMS hatchery were infected with OsHV, but *Perkinsus marinus* was detected in two of three hatchery groups examined.

Reece et al. (2007) examined the potential for transmission of diseases from introduced oysters taken from the wild (China) to other species bivalve species. All of the Chinese oysters imported to use as the parasite source that had tissue and DNA available to analyze were determined to be *Crassostrea hongkongensis*, a species with which *C. ariakensis* can be easily confused based on visual identification. Still, this species reflects the potential for a wild oyster from China to serve as a vector for disease to other bivalves within Chesapeake Bay. The research showed that the pathogen *Perkinsus beihaiensis* could be transmitted from this oyster to other bivalve species including *C. virginica, C. ariakensis,* and the clam *Mercenaria mercenaria.*

The NRC (2004) report does not address the possibility that an established population of *C. ariakensis* could provide a reservoir for future diseases that may be introduced to the Bay and subsequently pose a risk to other shellfish species. This additional, <u>incremental</u> ecological risk to other bivalve species (clams, mussels, oysters) in the Bay as a result of future shellfish diseases associated with the presence of *C. ariakensis* is judged to be low. The logic for this conclusion is as follows: *C. ariakensis* will be one of many bivalve species in the Bay. If a pathogen introduced in the future is able to infect a variety of bivalve species, those species are already present, and the addition of *C. ariakensis* provides only one other possible conduit. The absence of *C. ariakensis* would not eliminate the future ecological risk; thus, the presence of *C. ariakensis* would represent a small incremental additional risk. This incremental risk would be proportional to the size of the *C. ariakensis* population. The NRC report also does not address the issue of whether a *C. ariakensis* populaiton could lose it's disease resistance over time. Such a phenomenon has never been reported in the literature and would appear unlikely. Uncertainty related to this conclusion is moderate to low. This uncertainty results from limited knowledge and understanding regarding the basic biology and epidemiology of disease in *C. ariakensis*. The prevalence, infection rates, and transmission rates for some diseases are known from limited studies.

4.2.4 RQ 5 - Will *C. ariakensis* become an invasive or nuisance species?

> **Conclusion: Aside from the potential for competition with *C. virginica,* the risk that *C. ariakensis* would become an ecological nuisance in Chesapeake Bay is low. It is not expected to form large reef systems outside of historical hard-bottom areas that would overtake other soft-bottom and SAV habitats. It can withstand high sediment loads and muddy substrates when attached to shell. The species, therefore, could be effective at reducing total suspended solids, which would be a benefit to SAV. The level of uncertainty associated with this conclusion is moderate to high because it is based on laboratory observations of settlement and limited field observations of triploid *C. ariakensis*.**

This question is applicable only to the proposed action and Alternatives 5 and 7. Regarding Alternative 5, this question would apply only if an unintended introduction of diploids from triploid aquaculture results in a large, self-sustaining population of *C. ariakensis* (Section 4.3).

The NRC's (2004) report indicated that the major concern and greatest uncertainty relates to the likelihood that *C. ariakensis* would become an invasive or nuisance species and threaten the ecological integrity of the Chesapeake Bay and adjacent waters along the Atlantic coast or in the Gulf of Mexico. The NRC noted that it is exceedingly difficult to predict whether a marine species has the potential to become an "invasive" or a "nuisance" species; however, certain attributes correspond with an increased potential: fast growth, high reproductive rates, and tolerance of a wide range of environmental conditions.

The terms "invasive" and "nuisance" require some definition. Executive Order 13112 defines an "invasive species" as an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health. The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 defines an ''aquatic nuisance species'' as a nonindigenous species that threatens the diversity or abundance of native species or the ecological stability of infested waters, or commercial, agricultural, aquacultural, or recreational activities that depend on such waters. The National Invasive Species Council (ISAC 2006) provided further clarification about how to consider "invasiveness:"

> *Invasive species are those that are not native to the ecosystem under consideration and that cause or are likely to cause economic or environmental harm or harm to human, animal, or plant health. Plant and animal species under domestication or cultivation and under human control are not invasive species. Furthermore for policy purposes, to be considered invasive, the negative impacts caused by a nonnative species will be deemed to outweigh the beneficial effects it provides. Finally, a nonnative species might be considered invasive in one region but not in another. Whether or not a species is considered an invasive species depends largely on human values. By attempting to manage invasive species, we are affirming our economic and environmental values. Those nonnative species judged to cause overall economic or environmental harm or harm to human*

> *health may be considered invasive, even if they yield some beneficial effects. Society struggles to determine the appropriate course of action in such cases, but in a democratic society that struggle is essential.*

This ERA considers the potential for ecological harm. The NRC (2004) and the ERAAG provided perspectives about what might constitute ecological harm in the case of introducing *C. ariakensis* in Chesapeake Bay. The NRC speculated that *C. ariakensis* could reach sufficient densities to shift the Bay's ecosystem from pelagic dominance back toward benthic dominance with resultant shifts in species composition and abundance at higher trophic levels. Of course, the same thing could happen if the native oyster rebounded. Both species, however, might be limited by diseases and harvesting. The NRC noted benefits associated with such an ecological shift: (1) reducing standing stocks of phytoplankton might facilitate improvement of water quality and reduce populations of gelatinous zooplankton, and (2) an increase in SAV could have beneficial secondary effects on associated invertebrates and waterfowl. Although this ecological shift could restore the system to an earlier state, some individuals who use the Bay for various purposes may find these changes unwelcome. Certainly perceptions will differ concerning what represents an unwelcome ecological change. For example, the NRC noted that populations of pelagic finfish (e.g., menhaden, striped bass) might be reduced, whereas species that rely directly or indirectly on benthic productivity (e.g., sheepshead, bluefish) might be positively affected. The NRC also expressed concern that rapid population expansion of a nonnative oyster could displace native oysters and other fouling species. Furthermore, the NRC noted that the nonnative oyster could become a major fouling species, thereby increasing the costs of maintaining water intake pipes, boat hulls, and so forth. The ERAAG expressed concern about the possibility that an introduced nonnative species could occupy habitats outside of existing hard-bottom areas and displace SAV or soft-bottom benthic communities. Available research is used to address the likelihood of such ecological changes.

Certain constraints on *C. ariakensis* should be noted with regard to its potential to invade other habitats. The most important is salinity. *C. ariakensis* can grow in waters ranging from oligohaline to polyhaline. It would not move into the tidal freshwater habitats of the Bay and its tributaries. Its movement into polyhaline waters might be limited because of its susceptibility to the disease *Bonamia,* which could be present in such environments.

We discussed competition with the native oyster in the previous section and concluded that the two species are likely to compete but that the outcomes of competitive interactions are uncertain. If *C. ariakensis* were to be successful at sustaining populations in the Bay, competition would occur in subtidal zones, on hard-bottom substrates, in waters that do not experience low dissolved oxygen, and at salinities of less than 18 to 22 ppt (i.e., where *Bonamia* would be a cause of mortality). In these environs, *C. ariakensis* would have advantages over *C. virginica* based on laboratory studies and field observations with the species in aquaculture. The principal advantages are that *C. ariakensis* grows faster, continues to grow over a longer period of the year, and matures faster than *C. virginica*. *C. virginica* has some advantages over *C. ariakensis*, including greater tolerance of predators, greater tolerance of low dissolved oxygen, and the ability to withstand exposure in intertidal areas; therefore, we concluded that the two species could co-exist. In its native range, *C. ariakensis* reefs typically support other oyster species (Guo, pers. comm.2008). Within these reef systems, *C. ariakensis* is the larger basal species, and other smaller species can live upon these animals. Because *C. virginica* will settle on *C. ariakensis* shell (e.g., Paynter et al, in press), both species might be able to co-exist within the same reef systems, as *C. ariakensis* does in multispecies reefs off the coast of China.

The potential for ecosystem changes was examined is Section 4.4. For the population sizes evaluated, the influences on other ecological components would be small and generally positive. Negative influences would be related to reduction in phytoplankton and subsequent effects on species that depend on phytoplankton for food. These negative effects would be very small. (Many would judge influences that tend to reduce phytoplankton to provide a beneficial effect on overall water quality.) This is due, in part, to the fact that oysters are restricted to particular areas and water depths. If *C. ariakensis* were to repopulate the existing hard-bottom cultch areas, it would still cover a relatively small fraction of bottom area within the Bay. This evaluation considers a particular population size and time window. If *C. ariakensis* were to naturalize successfully, populations could exceed those examined in this ERA; however, this might occur long after the 10-year evaluation period.

We examined the potential that *C. ariakensis* could alter habitats beyond the hard-bottom areas in the Bay on which oysters have been located historically (i.e., areas currently or historically occupied by SAV or that are part of the soft-bottom community) by examining the following evidence:

- **Preferences for substrates on which to settle**. If *C. ariakensis* settles on a broad range of substrates, the likelihood that it would occupy habitats outside the traditional hard-bottom areas would increase.
- **Sustainability of shell that would support horizontal extension of traditional hard-bottom areas**. If the shell of *C. ariakensis* is able to withstand decay and can accumulate such that it increases the footprint of hard bottom, the likelihood that it could overgrow other types of habitat would increase. (This potential also would result in a potential positive effect on the ecosystem because it would restore reef habitat.)
- **Evidence from natural or introduced populations of *C. ariakensis* in other locations**. If *C. ariakensis* has exhibited the potential to overgrow habitats or form large, continuous reef systems in other areas, the likelihood that this would occur in Chesapeake Bay and surrounding estuaries would increase.
- **Evidence from introduced populations of other oysters in other locations**. If other introduced oyster species have exhibited the ability to dominate areas outside of hard-bottom environments, a species whose biology is not completely understood could exhibit these same characteristics.

Tamburri et al. (in press) examined the preferences and stimuli for settlement of *C. virginica* and *C. ariakensis* for various potential settlement materials in the Bay. They found that both species strongly prefer to colonize natural substrates (e.g., shell) covered with biofilms. Waterborne chemical cues emitted by adult oysters enhanced substrate attachment for all larval forms, but cues did not appear to be species specific; consequently, both species probably would choose the same kinds of natural materials on which to settle and would be attracted by similar chemical cues. Tamburri et al. (2008) also conducted settlement experiments with soft-bottom substrate (mud) from a mudflat. Neither species settled on this sediment. In settlement experiments with mixtures of shell and sediment, settlement did occur, but the oysters settled on exposed shell surfaces. The occurrence of singleton *C. ariakensis* in soft sediments in China reported in Tamburri et al. (2008) probably reflects settlement on shell present on the surface of the sediment (Newell, pers. comm. 2008; Guo, pers. comm. 2008). The ability of *C. ariakensis* to settle on scattered shell provides some evidence that the species could form a local colony in a region dominated by soft sediment. Guo (pers. comm. 2008) further noted that *C. ariakensis* can survive in systems with high sediment loads by growing upward, and that it is very common for *C. ariakensis* to be partially buried in mud. Although Guo's observations suggest that *C. ariakensis* can occupy soft sediments, the availability of at least some fragments of shell upon which to settle appears to be a prerequisite. Overall, research suggests that the probability that *C. ariakensis* would colonize substrates other than hard bottom is low.

The ability of oysters to naturally extend the area of hard bottom beyond current footprints depends on the long-term integrity of shell. Carnegie (pers. comm. 2008) noted that neither oyster species is likely to reclaim vast areas of soft bottom in the absence of some sort of engineering, like shell planting. He noted further that the thin shells of *C. ariakensis* would not only be more susceptible to crushing by crabs, but also to burrowing by worms, and probably to the natural decay processes that negatively affect shell budgets over time. Therefore, although a growing population of *C. ariakensis* might be able to contribute shell and expand the area of hard bottom in the Bay, the process probably would be slow.

The NRC (2004) described the introduction of *C. ariakensis* to the Pacific coast. The species was inadvertently introduced to Oregon with shipments of *C. gigas* and *C. sikamea* spat from Japan in the 1970s. Although *C. ariakensis* seed has been outplanted on intertidal mudflats or suspended from floating rafts repeatedly at several sites from Washington to central California, no established wild populations have been reported on the west coast of the U.S. The NRC (2004) noted that this may be due to the cold water. Some insights concerning the species' ability to form extensive reefs that would overtake other habitats can be gained from observations of *C. ariakensis* in its native waters. *C. ariakensis* typically occurs in mixed reefs with other oyster species. Although *C. ariakensis* species can tolerate soft sediments, it prefers to settle on shell. Guo (pers. comm. to Menzie 2008) noted that although *C. ariakensis* can form extensive reefs, such reefs occur only in estuaries close to large rivers; *C. virginica* reefs are more wide spread. The distribution of *C. ariakensis* is wide, but clearly river-dependent. Kingsley-Smith et al. (2007) observed characteristics of reef formation among *C. ariakensis* in caged experiments in Chesapeake Bay.

Insights can be drawn from studies of other introductions of oysters. Ruesink et al. (2005) reviewed many such cases and offered a few observations concerning the potential for an introduced species to grow into other habitats. They noted that introduced oysters can develop reef systems in otherwise large expanses of soft-sediment estuarine and lagoonal seascapes. With respect to the issue of whether an introduced oyster would convert habitats, Ruesink et al. (2005) noted that few data exist on the rate of conversion of native habitats, such as unvegetated tidal flats or eelgrass, into introduced oyster reefs. They noted, however, that some evidence suggests that oyster reefs can reduce eelgrass cover directly. In western Canada, eelgrass (*Zostera* *marina*) was relatively rare down slope from dense populations of *C. gigas*, and transplanted shoots survived poorly relative to transplants within natural eelgrass beds located away from reefs.

Nehring (2006) reported on the invasive nature of the Pacific oyster, *C. gigas*, in Europe. This species is very adept at attaching to available hard substrates, including other shellfish, and subsequently forming clumps of oysters that eventually develop into reefs. The species has become established in the Wadden Sea, where many beds of *C. gigas* are now rapidly developing into solid reefs at several sites in the region. Nehring noted that the Pacific oyster is expected to take over in mudflats on the German and Danish coasts of the North Sea, both as an ecosystem engineer generating solid reefs and as a competitive suspension feeder. *C. gigas* tends to settle in the same locations and on the same tidal level as the native blue mussel (*Mytilus edulis*). The overgrowth of mussels and preemption of space, and possibly also competition for phytoplankton and filtering of larvae, probably would diminish the native blue mussel. *C. ariakensis* is similar to *C. gigas* in some respects: it has a fast growth rate and can attach to a variety of substrates. However, based on cage experiments with triploids (Kingsley-Smith et al., 2007; Paynter et al., in press) salinity might be a factor affecting growth rates of *C. ariakensis,* which tends to grow faster at higher salinities. *C. gigas* has tough shells and is more resistant than *C. ariakensis* to natural predators such as birds and crabs. Another situation-specific difference is that *C. ariakensis* would be subject to harvesting in the Chesapeake Bay, whereas *C. gigas* in the Wadden Sea is not yet subject to harvesting. The case of *C. gigas* indicates that oysters can become an invasive nuisance species; however, *C. gigas* appears to have a particular combination of characteristics that enhances that its ability. *C. ariakensis* shares some but not all of these.

C. ariakensis can settle on a variety of surfaces including artificial ones (Luckenbach et al.2006); therefore, as with other animals that settle on hard substrates, there is some potential for fouling. This is discussed in greater detail in the EIS. The species would not present a fouling problem for structures or pipes in freshwater. To the extent that *C. ariakensis* fouls hard surfaces, these would be in subtidal locations (Luckenbach and Kingsley-Smith 2006).

The level of uncertainty associated with this conclusion is moderate to high because it is based on laboratory observations of settlement and limited field observations of *C. ariakensis* and because there is always uncertainty about how an introduced species will behave in a new environment. One important aspect of introduction of a reproducing population is that it would be irreversible; if a population of *C. ariakensis* were to become established in the Bay as a result of a purposeful introduction and were to become a nuisance species, eradication would not be possible.

4.2.5 RQ 6 - Will *C. ariakensis* disperse to areas outside of Chesapeake Bay and pose the kinds of risks identified above?

Conclusion: The risk is high that *C. ariakensis* eventually would disperse outside Chesapeake Bay, if the species successfully established a reproductive population there. Such dispersal would be more likely to the north of the Bay

than to the south. The likelihood that *C. ariakensis* would compete with *C. virginica* in areas outside of the Bay or otherwise become a nuisance depends on the species' ability to become established and develop reefs. *C. ariakensis* could be somewhat limited in its ability to disperse along the Atlantic coast due to infection with *Bonamia.* In addition, at low numbers of adults within an area (relative to *C. virginica*), *C. ariakensis* would be at a competitive disadvantage due to the phenomenon of gamete sink; this could change over time as *C. ariakensis* becomes more abundant. Aside from competition with *C. virginica,* the risk that *C. ariakensis* could become a nuisance is small to moderate. The level of uncertainty concerning the likelihood that *C. ariakensis* would eventually disperse beyond Chesapeake Bay is low to moderate. The level of uncertainty regarding the likelihood that *C. ariakensis* would effectively compete with *C. virginica* or otherwise become a nuisance is moderate to high.

This question is applicable only to the proposed action and Alternatives 5 and 7. Regarding Alternative 5, this question would apply only if an unintended introduction of diploids from triploid aquaculture results in a large, self-sustaining population of *C. ariakensis* (see Risk Question 7).

The NRC (2004) concluded that if reproductively viable populations of *C. ariakensis* are established in the Bay, the species is highly likely to spread outside the Bay. The NRC noted a variety of unintentional and intentional transport mechanisms (e.g., larval transport by water currents, transport of larvae and adults by ship traffic, human movement of adults) that could hasten the spread of *C. ariakensis* to regions outside the Bay.

Available research and discussions with the researchers suggest that this dispersal is more likely to occur to the north than to the south of the Bay. This results, in part, from the availability of suitable environments for oyster settlement and growth. Intertidal environments constitute most of the oyster habitat south of the Bay. This type of environment does not appear to be favorable for *C. ariakensis.* Subtidal oyster habitat is more prevalent north of the Bay; consequently, that area is more likely to support populations of *C. ariakensis.* Researchers also indicated that expansion of *C. ariakensis* into polyhaline waters and along the coasts may be limited by disease because of the species' greater vulnerability to *Bonamia* at high salinities. The rate of dispersal would depend on many factors. Natural dispersal would be determined by larval behavior, salinity, and water circulation patterns. Oysters also could be transported intentionally or as fouling organisms on the hulls of boats. The native range of *C. ariakensis* spans a broad range from Korea to Vietnam (41 N to 20 N); the latitude of its native range corresponds to the area between Connecticut and the Yucatan Peninsula, Mexico. Guo (pers. comm. 2008) noted that, given this broad range, diseases might not be a permanent obstacle to long-distance dispersal. He further noted that observations of the species in its native range suggest that it tends to inhabit estuaries associated with large river systems.

Scarpa et al. (2007) examined the potential for growth and reproduction of *C. ariakensis* in sub-tropical environments. They observed that *C. ariakensis* did not grow as well as *C. virginica* during autumn in Florida. They did not suggest an explanation for this difference.

They also observed increased mortality of *C. ariakensis*, perhaps due to *Bonamia.* The data are limited but, to the extent that they can provide some insight, they suggest that *C. ariakensis* would not compete strongly with *C. virginica* in subtropical areas of the U.S.

Uncertainty concerning the likelihood that *C. ariakensis* would disperse beyond the Bay is low to moderate because numerous dispersal mechanisms are possible (e.g., unauthorized physical transplants, attachment to boats or hard debris that is transported out of the Bay, larval dispersal). Uncertainty regarding the likelihood that *C. ariakensis* would compete effectively with *C. virginica* or otherwise become a nuisance is moderate to high. This uncertainty is due to limited knowledge and understanding of the ecological relationships between *C. ariakensis* and other estuarine receptors, which is based primarily on laboratory studies and limited field trials.

4.3 RQ 7 - WHAT IS THE LIKELIHOOD THAT THE ACTION WILL RESULT IN AN UNINTENDED INTRODUCTION OF C. ARIAKENSIS INTO CHESAPEAKE BAY?

This question applies only to Alternative 5, aquaculture using triploid *C. ariakensis*. An introduction is considered a risk for this alternative because the specification to use triploid *C. ariakensis*, which generally are believed to be unable to reproduce, reflects a compromise that addresses stakeholders' conflicting views about the desirability of introducing a nonnative species while attempting to attain the potential economic benefit of using the species. An unintentional introduction might result from aquaculture through any of several pathways, including accidental releases at hatcheries or field sites, undetected fertility of triploids, imperfect fidelity during the genetic crosses intended to produce triploids, and reversion from triploid to diploid among cultivated oysters in the field. The response to this risk question encompasses two general evaluations, a qualitative assessment of the risk of accidental release from hatcheries, and an estimate of the risk of introduction from field operations.

The objective of this section is to evaluate the likelihood that the implementation of a full-scale aquaculture industry using <u>triploid</u> *C. ariakensis* could lead to an unintended or accidental introduction of <u>diploid</u> *C. ariakensis* into the Bay. This involved identifying, and to the extent possible, quantifying a sequence of events that could lead to the presence of reproductive, diploid *C. ariakensis* at large in the Bay in sufficient densities to result in the establishment of a reproductive population. A potential reproductive population is considered to begin with two collocated, reproductive, diploid *C. ariakensis.* Collocation is defined as individuals sharing a space of 1 m^2. In other words, oysters within a meter of each other have a chance of spawning simultaneously, resulting in fertilization. Although field operations for cultivating triploid *C. ariakensis* could give rise to a reproductive diploid population, the probability of that outcome is low based on the calculations presented below. The analysis is based on a large number of assumptions; consequently, much uncertainty accompanies the estimates. All of the assumptions were made conservatively, such that the likelihood of producing a reproductive population would be overestimated rather than underestimated.

The evaluation is organized in two parts. The first part presents the pathways by which individual, diploid *C. ariakensis* could result from various aspects of the aquaculture operations.

Each pathway in the chain of events has multiple steps, and each step has an associated probability. The outcome of the chain of events is expressed as the number of diploid individuals that might result from each pathway. We estimated this for a representative aquaculture operation (PEIS Appendix C). The second part involves estimating the likelihood that the resultant diploids would be collocated and, therefore, would represent an initiating spawning pair. We estimated this for the combined influences of all aquaculture operations within a tributary where aquaculture operations might occur. A third requirement for a reproductive population to be established involves the success of the progeny from the first pair. We discuss this third step qualitatively but did not evaluate it quantitatively.

The confidence with which these probabilities can be estimated is a function of the amount and quality of the relevant information available. Given the paucity of such information, outcomes from this model must be viewed with caution. Given the uncertainties, estimates used for each step in each pathway were selected to ensure that the defined risk would be conservative (i.e., values that would tend to overestimate probabilities of events).

4.3.1 Part 1. Probability of Producing Reproductive Individuals of *C. ariakensis*

This part of the analysis presents the chain of events by which individual, diploid *C. ariakensis* could result from various aspects of aquaculture operations using triploid *C. ariakensis*. The results of this analysis provide insight into the size of the resultant population of diploid individuals and, thus, the potential for a starting spawning population. This information is used in the second part of the analysis to estimate the probability of co-occurrence of reproductive individuals. The estimates were developed for a representative aquaculture operation. The number of oysters for a representative operation was set at 714,285 total oysters based on the output of Dr. Doug Lipton's economic demand model (PEIS Appendix D). The estimates can then be scaled for aggregated operations within individual Chesapeake Bay segments.

The biology of *C. ariakensis* is complex, from its pattern of genetic inheritance to its effects on ecosystem function. The establishment of this sequence of events and the estimation of probabilities required the input and advice of a number of scientists and professionals from across the region. These contributors represent academic institutions, government laboratories, commercial oystermen, and private companies (Table 4-1). This exercise benefited greatly from their contributions.

Table 4-1. Contributors to the triploid-to-diploid risk analysis

Contributor	**Affiliation**
Stan Allen	Virginia Institute of Marine Science
A. J. Erskin	Virginia Seafood Research Council
Chris Guy	U.S. Fish and Wildlife Services
Doug Lipton	University of Maryland
Mark Luckenbach	Virginia Institute of Marine Science
Charlie Menzie	Exponent

All available sources of information for *C. ariakensis* were collected and evaluated for potential contribution to the chain of events. Individuals listed in Table 4-1 were pivotal in pointing us toward many valuable resources, which included peer-reviewed journal articles, conference proceedings, and annual reports written for funding agencies by principal investigators. Additionally, the Virginia Seafood Council trials represent some of the largest studies of *C. ariakensis,* and the information from these studies also was valuable. When information for *C. ariakensis* was not available, information for an ecologically similar congener such as *C. virginica* or *C. gigas* was substituted. The amount of ecological information for *C. ariakensis* relevant to this exercise was extremely limited.

The purpose of this exercise is to estimate the probability of accidentally introducing diploid *C. ariakensis* into the wild as a result of cultivating triploid *C. ariakensis*. The model developed here is a sequence of events that would have to occur in order for this introduction to occur. Several steps in the pathways represent major life-history stages for *C. ariakensis*. Numerical probabilities that describe the likelihood of successfully completing some of these life-history stages are available. Some events could not be described with a single probability due to lack of information. In these instances, we used algebraic relationships to relate that probability to some set of measurable ecological factors upon which that probability is thought to depend. Unknown probabilities were estimated using these equations.

Six major pathways were identified that might result in a diploid introduction (Figure 4-12). The first two deal with the possibility that triploid fertility could ultimately give rise to diploids in the field. This could occur when fertile triploids mate with other fertile triploids (Pathway A) or with diploids that might arise from one of the other pathway steps (Pathway B). The next two pathways explore the series of steps that would lead directly to the occurrence of a rare diploid amongst the oysters deployed to the field either through lack of fidelity of the 4n X 2n cross (Pathway C) or through reversion of triploids to diploids (Pathway D). The latter two pathways consider the likelihood of accidental release of triploid adults from the field site (Pathway E) or of adults or larvae from a hatchery (Pathway F).

4.3.1.1 Pathway A: Triploid Fertility (Mating Triploids with Diploids)

<u>A1/B1. Probability of Triploids among Deployed Oysters</u> *(P_{trip})*

Triploid *C. ariakensis* will be produced through genetic crosses with tetraploid (4n) and diploid (2n) oysters. Currently, the production of genetic triploids is thought to be nearly 100% efficient. Triploids can be distinguished from diploids using flow cytometry. At the present time, fourteen 4n X 2n spawns are known to have produced 49 diploid progeny out of the 60,061 putative triploids examined (Attachment E). This is approximately 1 in 1000 (or 0.001). A newer ploidy certification method involves using mesh sieves to sort putative triploids by size. This process has been used twice and in both instances, 0 diploids were found out of 3,042 larvae (in 2006) and 10,560 larvae (in 2007), respectively. This suggests that the production of genetic triploids produced no diploids in these subsamples. The model takes the conservative approach in assuming that diploids do arise from the 4n X 2n cross at a rate of 1 in 1000 and that they are not eliminated prior to deployment.

Production of Diploids from Initial Starting Population

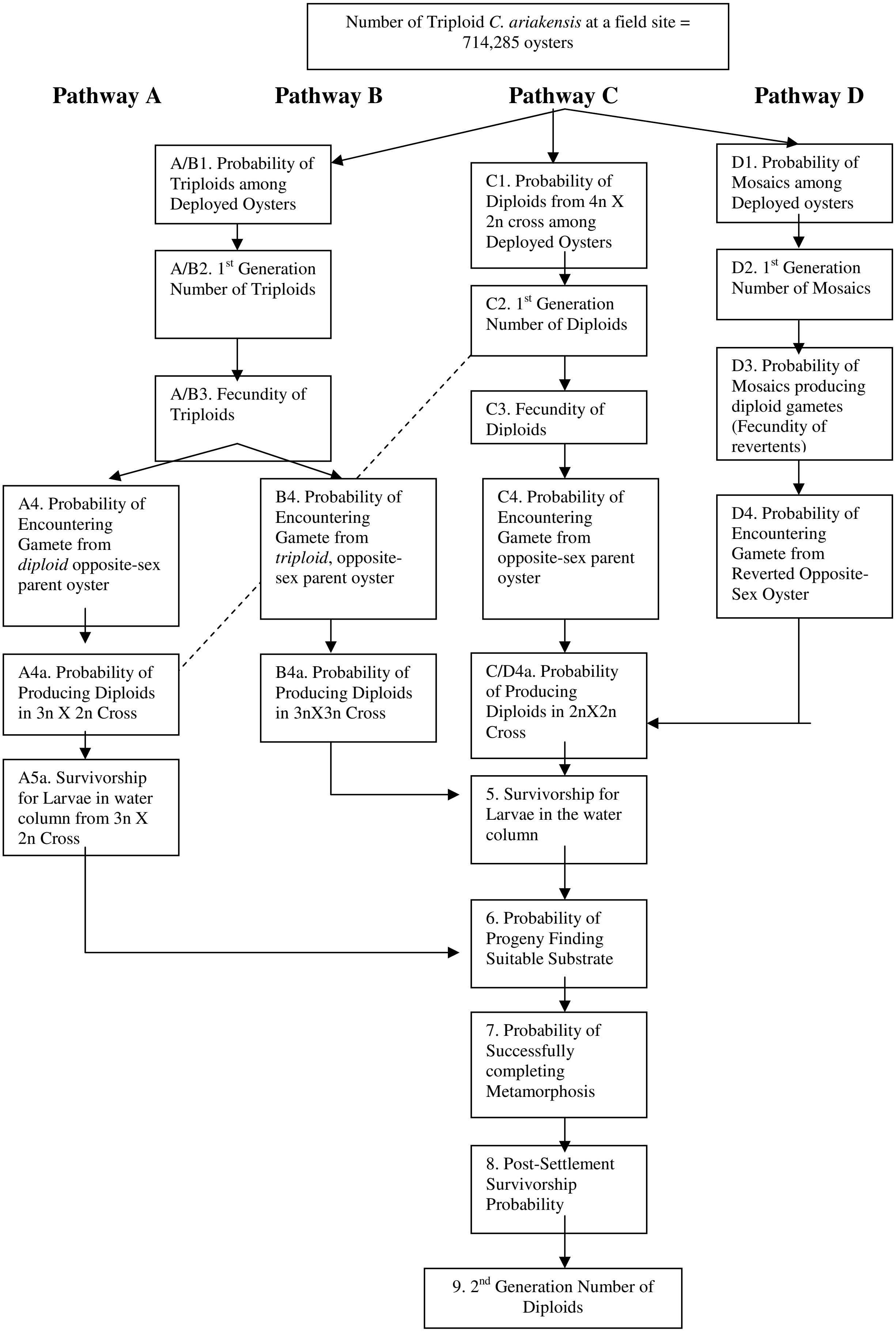
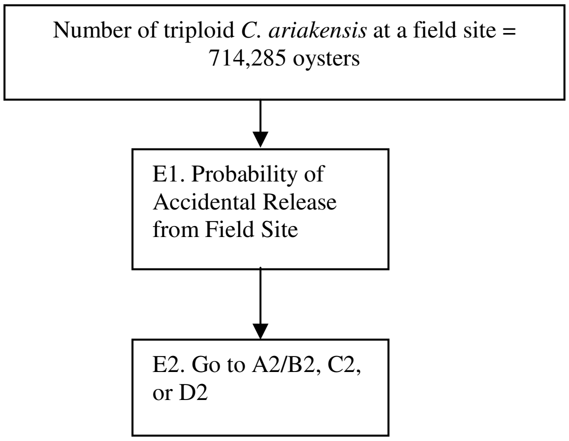


Figure 4-12 Model of pathways for triploid-to-diploid risk assessment

Accidental Release of Post-Recruitment Oysters from Field Site

Pathway E



Accidental Release from Hatchery

Pathway F

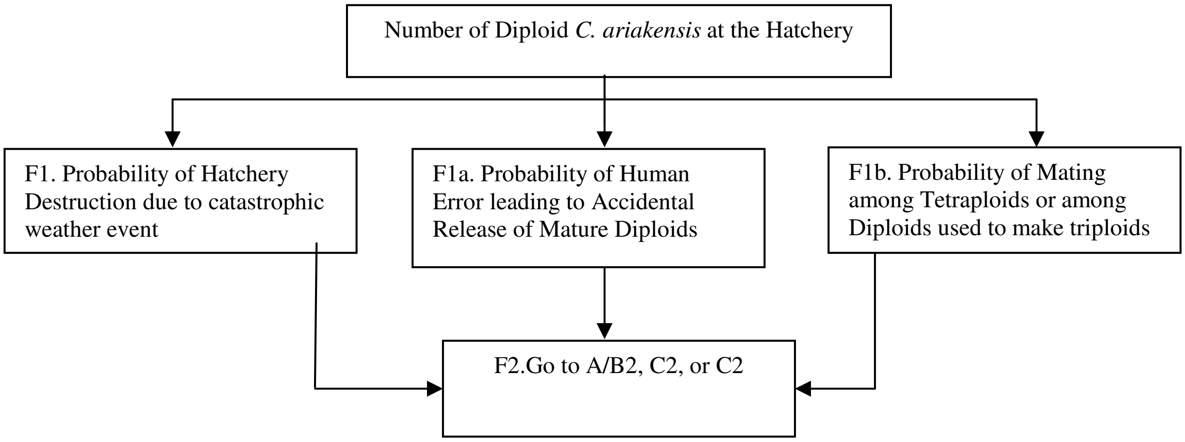


Figure 4-12. (Continued)

P_{trip} = 99.9%
= 0.999

A/B2. First Generation Number of Triploids (N_{trip})

If the production of genetic triploids is less than 100% efficient, then it is possible for this number to be minimally smaller than the total number of oysters deployed. This number may be calculated by multiplying the total number of *C. ariakensis* deployed to the field site times the probability that those oysters are indeed triploids. For the model, 00.0% of the 714,285 *C. ariakensis* deployed per site are presumed to be triploids.

N_{trip} = 714,285 oysters * P_{trip}
= 714,285 *0.999
= 713,570 triploid oysters

A/B3 Total Fecundity of Triploids (# of viable gametes) (Triploid F_{tot})

Although their presumed sterility is the basis for using triploids in aquaculture, triploid *C. ariakensis* do produce viable gametes (Attachment E). This step in the chain determines fecundity (F_{tot}), or the number of gametes produced by the oysters. For populations that have size stratification, F_{tot} can be determined by summing together size-specific individual fecundities (Mann and Evans 1998). Lacking *apriori* knowledge of the population size distribution, we will use a conservative estimate of the standard-sized oyster at the time of harvest in aquaculture (Length = 77mm). This number represents the mid-point of the 76-80 mm harvest size class used in the demographic model (PEIS Appendix A). Note that because aquaculture triploids are removed from the water when they reach market size, this would be a conservative, maximum estimate for fecundity in this event chain.

Weight = $0.000423 * L^{1.7475}$
Weight = $0.000423 * 77^{1.7475}$
= 0.837 mg

$F_{ind} = 39.06 * \text{Weight}^{2.36}$ (r^2=0.887, d.f.=24)
= $39.06 * 0.837^{2.36}$
= 25.70

$F_{tot} = N_{trip} * F_{ind}$
= 713,570 * 25.7
= 18,338,749 eggs m^{-2}

Where L = standard size oyster (mid-point of 75-80 mm size class; size at harvest)
N_{trip} = number of triploids

This set of equations has been used to determine the fecundity of regular, diploid *C. virginica*. However, triploid *C. ariakensis* are expected to have reduced fertility rates compared to diploids. Although reproductive potential has not been studied in *C. ariakensis*, much work has examined fertility in *C. gigas,* and we used that information. The *relative fecundity* of *C. gigas* triploids compared to diploids has been reported to be between 2% (Guo and Allen 1994) and 13.4% (Gong et al. 1994).

Triploid $F_{tot} = F_{tot} * 0.134$
$= 18{,}338{,}749$ eggs $m^{-2} * 0.134$
$= 2{,}457{,}392$ eggs m^{-2}

Salinity can influence the rate of fecundity. We can multiply F_{tot} by a modifying term (F_S) to account for the effect of salinity on fecundity. F_S is a proportional term that ranges between 0 and 1.0 with zero referring to no effect and 1.0 referring to a total effect. The estimate of this effect is based on limited data for *C. virginica.* In samples collected from the James River in Virginia, the lowest salinity at which eggs were found was 8.5‰ (Mann et al. 1994). Thus below salinity of 8.5‰, $F_S=0$ (no viable eggs). The mean salinity in this location was 13.5‰. Based on this information, Mann and Evans (1998) developed the following set of relationships to describe how salinity, S, influences the modifying term, F_S.

If Salinity > 13.5, then $F_S = 1.0$
If Salinity < 13.5, then $F_S = [(S-8.0)/13.5-8.0)] \times 1.0$
$= (S-8.0)/5.5$

Disease can also influence the rate of fecundity. We can multiply F_{tot} by a modifying term (F_D) to account for the effect of disease on fecundity. F_D is a proportional term that would range between 0 and 1.0 with zero referring to no effect and 1.0 referring to a total effect. Data on the effects of disease on fecundity are scarce for *C. ariakensis* and its congeners. In accordance with Mann and Evans (1998), we will leave F_D fixed at 1.0

Triploid $F_{overall}$ = Triploid $F_{tot} * F_S * F_D$

For the purpose of this exercise, we will assume that $F_S=1.0$ and $F_D=1.0$, and thus that Triploid $F_{overall}$ = Triploid F_{tot}.

A4. Probability of Encountering a Gamete from a Diploid, Opposite-sex Parent Oyster (F_f)

After gametes are spawned, the next step is for an egg cell to encounter a sperm cell in the water column and become fertilized. The fertilization efficiency F_f is a density dependent coefficient with values ranging between 1.0 (100% of eggs are fertilized) and 0 (no eggs are fertilized).

Triploid populations often have a skewed sex ratio with a greater proportion of males in the population compared to females (Zhou 2002; Erskine 2003). As a conservative estimate, the model assumes a 50:50 sex ratio. Eggs are more energetically expensive to produce; consequently, compared to sperm cells, egg cells are produced in fewer numbers and generally limit reproduction. This, therefore, is a conservative estimate because it overestimates the number of eggs likely to be present. The model assumes that triploids and diploids spawn synchronously and completely.

The model uses a relationship published by Levitan (1991) for the fertilization efficiency for echinoderms. These equations developed for echinoderms have been applied to *C. virginica* and *C. ariakensis* in previous modeling studies (Mann and Evans 1998; Luckenbach pers. comm.). Fertilization rate is defined as a decreasing logarithmic function of oyster density. That is, fertilization is great at high densities but decreases rapidly as density decreases. The model assumes that the 714,285 oysters at the field site are grown in spatial aggregations with densities of 500 per 1 m^{-2} unit, where a unit is defined as an oyster float (e.g., Taylor float) or cage. Luckenbach also made this assumption (Luckenbach pers. comm.). This is likely to be a conservative overestimate of density because most of the units in use actually have a somewhat greater area (Luckenbach pers. comm.).

Log %Fertilization = 0.72 (log Oyster Density) + 0.49

Rearranging this equation gives:

$\% \text{ Fertilization} = 0.49 * (\text{Oyster Density})^{0.72}$

$\% \text{ Fert}_{TT} = 0.49 * (500 \text{ oysters per m}^{-2})^{0.72}$
$= 43\%$
$= 0.43$

This number is applied as a correction factor (F_f) in the model ranging between 0 and 1. To convert the percent fertilization calculated above to a correction factor, it is divided by 100.

$F_f = \% \text{ Fert}_{DT} / 100$
$F_f = 0.43 / 100$
$= 0.0043$

<u>A4a. Probability of Producing viable Diploids in 3n X 2n Cross</u> (P_{DTdip})

Only a portion of the progeny produced in a 3n X 2n mating will be diploids. Experimental matings of *C. gigas* indicate that the proportion of progeny surviving after 9 months that are diploid can range between 98% (Gong et al. 1994) and 100% (Guo and Allen 1994). The larger, more conservative estimate of 100% will be used here.

$P_{DTdip} = 1.0$

<u>A5a. Survivorship for Larvae from 3n X 2n Cross</u> (Triploid S_{larvae})

Larvae resulting from at least one triploid parent have lower rates of survivorship relative to pure diploid crosses. Several values have been reported for *C. gigas* survival to two months (spat stage; Guo and Allen 1994; Gong et al. 1994). The survivorship rate for genetic triploids is approximately 0.3140% (S. Allen, pers. comm.) and is the most relevant value for our purposes. This was also the largest and, therefore, most conservative estimate of survivorship.

Triploid S_{larvae}=0.003140

6. Probability of Finding Suitable Substrate, P_{sub}

If a larva survives transport in the water column, it must next locate suitable substrate on which to settle. Successful settlement of oyster larvae requires hard substrate that is free of debris. This probability will depend on the proportion of available habitat in the vicinity of the field site. The probability of finding suitable substrate (P_{sub}) ranges between 0 and 1.00, where a 0 probability indicates that no larvae find suitable substrate and a 1.00 probability indicates that all larvae locate suitable habitat. For oysters, this probability is primarily a function of how much shell habitat is available. Let us assume that an area of shell that occupies one square meter of the bottom has a height of 1 cm and a volume of 10l. We can then use the following relationships to estimate this probability (Mann and Evans 1998):

If shell volume > 10 l per m^2, then P_{sub} = 1.0
If shell volume < 10 l per m^2, then P_{sub} = 0.1 * shell volume (unitless)

A conservative estimate then is that all settling oyster larvae are able to find suitable substrate.

P_{sub}=1.0

7. Probability of Successfully Completing Metamorphosis (P_{meta})

After a larva settles on appropriate habitat, it undergoes metamorphosis. The probability of this event (P_{meta}) can vary between 0 and 1.00. A 0 probability indicates that no settled larvae successfully complete metamorphosis, and a 1.00 probability indicates that all settled larvae successfully complete this process. Estimates for this probability come from hatchery operations. At the VIMS Oyster Hatchery, this probability is typically around 0.25, and we used this estimate for the chain of events.

P_{meta} = 0.25

8. Probability of Post-Settlement Survivorship (S_{juv})

Individuals that complete metamorphosis successfully may experience subsequent mortality due to numerous biotic and abiotic factors. The probability of surviving the post-settlement period (S_{juv}) is a function of mortality calculated on a daily time step (M_{juv}) and the number of days (d) until reaching the midpoint of the juvenile size class (Mann and Evans 1998). The number of days, d, depends upon the rate of individual growth. Cumulative mortality to 8mm is expected to be approximately 93%, giving a cumulative survival rate of 7% (Roegner and Mann 1995). This assumes linear growth at a rate of 0.29 mm per day, taking about 29 days to reach 8mm. These data are based on information from hatcheries for *C. virginica*.

$$S_{juv} = (1-M_{juv})^{d}$$
$$= 0.07$$

9. 2nd Generation Number of Diploids: Final Step in all of the Pathways

The product of the probabilities in each pathway yields the cumulative risk of introducing diploid *C. ariakensis* in terms of number of oysters for one spawning event. This model is iterated per spawning event such that the number of second-generation, diploid *C. ariakensis* would then contribute to step C2 in the model. Reproduction between two newly produced diploid *C. ariakensis* from the final step will only occur when these individuals are within sufficient proximity to one another and spawn synchronously. Thus, when the model is iterated, the spatial proximity of reproducing diploid *C. ariakensis* to each other becomes important. The model assumes that the collocation of two opposite-sex, synchronously spawning diploids within the same 1 m^2 is required for successful mating. Where diploid larvae settle will depend upon many factors, including hydrodynamic regimes and larval settling behavior (North et al. 2006).

4.3.1.2 Pathway B: Triploid Fertility (Mating Triploids with Triploids)

Pathway B begins with steps A/B1 through A/B3 (see above) and continues here with step B4.

B4. Probability of Encountering a Gamete from a Diploid, Opposite-sex Parent Oyster (F_f)

After gametes are spawned, the next step is for an egg cell to encounter a sperm cell in the water column and become fertilized. The fertilization efficiency F_f is a density dependent coefficient with values ranging between 1.0 (100% of eggs are fertilized) and 0 (no eggs are fertilized).

Triploid populations often have a skewed sex ratio with a greater proportion of males in the population compared to females (Zhou 2002; Erskine 2003). As a conservative estimate, the model assumes a 50:50 sex ratio. Eggs are more energetically expensive to produce; consequently, compared to sperm cells, egg cells are produced in fewer numbers and generally limit reproduction. This, therefore, is a conservative estimate because it overestimates the number of eggs likely to be present. The model assumes that triploids and diploids spawn synchronously and completely.

The model uses a relationship published by Levitan (1991) for the fertilization efficiency for echinoderms. These equations developed for echinoderms have been applied to *C. virginica* and *C. ariakensis* in previous modeling studies (Mann and Evans 1998; Luckenbach pers. comm.). Fertilization rate is defined as a decreasing logarithmic function of oyster density. That is, fertilization is great at high densities but decreases rapidly as density decreases. The model assumes that the 714,285 oysters at the field site are grown in spatial aggregations with densities of 500 per 1 m^{-2} unit, where a unit is defined as an oyster float (e.g., Taylor float) or cage. Luckenbach also made this assumption (Luckenbach pers. comm.). This is likely to be a conservative overestimate of density because most of the units in use actually have a somewhat greater area (Luckenbach pers. comm.).

Log %Fertilization = 0.72 (log Oyster Density) + 0.49

Rearranging this equation gives:

% Fertilization = 0.49 * $(\text{Oyster Density})^{0.72}$

% Fert_{TT} = 0.49 * $(500 \text{ oysters per } m^{-2})^{0.72}$
= 43%
= 0.43

This number is applied as a correction factor (F_f) in the model ranging between 0 and 1. To convert the percent fertilization calculated above to a correction factor, it is divided by 100.

F_f = % Fert_{DT} /100
F_f = 0.43 / 100
= 0.0043

B4a. Probability of Producing Diploids in a 3n X 3n Cross

Only a portion of the progeny produced in a 3n X 3n mating will be diploids. Experimental matings among triploid *C. gigas* were carried out, and the ploidy level of juveniles was analyzed using flow cytometry after one year. This analysis found that at that time, 4% of the progeny were diploid (Guo and Allen 1994). The model uses this as an estimate of the percentage of offspring from the mating that are diploid.

P_{TTdip} = 0.04

B5. Survivorship of larvae produced 3n X 3n Cross (Triploid S_{larvae})

Larvae resulting from at least one triploid parent have lower rates of survivorship relative to pure diploid crosses. Several values have been reported for *C. gigas* survival to two months (spat stage; Guo and Allen 1994; Gong et al. 1994). The survivorship rate for genetic triploids is approximately 0.3140% (S. Allen, pers. comm.) and is the most relevant value for our purposes. This was also the largest and, therefore, most conservative estimate of survivorship.

Triploid S_{larvae}=0.003140

From here, Pathway B continues on at Step 6 (see above).

4.3.1.3 Pathway C: Imperfect Fidelity of 4n X 2n Crosses

C1. Probability of Producing in the 4n X 2n Cross and Deploying Diploids P_{dip}

Several genetic processes could lead to the production of diploids from crossing tetraploids with diploids (Attachment E). Triploids can be distinguished from diploids using flow cytometry. At the present time, 14 spawns are known to have produced 49 diploid progeny out of the 60,061 putative triploids examined (Attachment E). This is approximately 1 in 1000 (or 0.001). A newer ploidy certification method involves using mesh sieves to sort putative triploids by size. This process has been used twice, and in both instances, 0 diploids were found out of 3,042 larvae (in 2006) and 10,560 larvae (in 2007), respectively. This suggests that the production of genetic triploids produced no diploids in these subsamples. The model takes the conservative approach in assuming that diploids do arise from the 4n X 2n cross at a rate of 1 in 1000 and that they are not eliminated prior to deployment.

$P_{dip} = 0.001$

C2. First Generation Number of Diploids (N_{dip})

This represents the number of diploid oysters that are deployed that arose from the 4n X 2n crosses. This number also considers the efficiency with which diploids can be detected and removed before deployment. This number is calculated by multiplying the total number of *C. ariakensis* deployed to the field site by the probability that diploids arose in that population during the 4n X 2n cross.

$N_{dip} = N_{tot} * P_{dip}$

$= 714{,}285 * 0.001$
$= 714$

C3. Average Fecundity of Diploids, Diploid F_{tot}

This step in the chain determines fecundity (F_{tot}), or the number of gametes produced by diploid oysters. For populations that have size stratification, F_{tot} can be determined by summing together size-specific individual fecundities (Mann and Evans 1998). Lacking a prior knowledge of the population size distribution, we will use a conservative estimate of the standard-sized oyster at the time of harvest in aquaculture (Length = 77mm). This number represents the mid-point of the 76 to 80 mm harvest size-class used in the demographic model (PEIS Appendix A). Diploids arising from a 4n X 2n cross may have reduced fertility compared to “normal” diploids. The model conservatively estimates that the fecundities of these two types of diploids are equivalent.

$\text{Weight} = 0.000423 * L^{1.7475}$
$\text{Weight} = 0.000423 * 77^{1.7475}$
$= 0.837 \text{ mg}$

F_{ind} = 39.06 * Weight $^{2.36}$ (r^2=0.887, d.f.=24)
= 39.06 * 0.837 $^{2.36}$
= 25.70

Diploid F_{tot}= N_{dip} * F_{ind}
= 714 * 25.7
= 18,350 eggs m^{-2}
Where L = standard size oyster
N_{dip} = number of diploids

Salinity can influence the rate of fecundity. The model multiplies F_{tot} by a modifying term (F_S) to account for the effect of salinity on fecundity. F_S is a proportional term that ranges between 0 and 1.0 with zero referring to no effect and 1.0 referring to a total effect. The estimate of this effect is based on limited data for *C. virginica*. In samples collected from the James River in Virginia, the lowest salinity at which eggs were found was 8.5‰ (Mann et al. 1994). Thus below salinity of 8.5‰, F_S=0 (no viable eggs). The mean salinity in this location was 13.5‰. Based on this information, Mann and Evans (1998) developed the following set of relationships to describe how salinity, S, influences the modifying term, F_S.

If Salinity > 13.5, then F_S=1.0
If Salinity < 13.5, then F_S = [(S-8.0)/13.5-8.0)] X 1.0
=(S-8.0)/5.5

Disease can also influence the rate of fecundity. The model multiplies F_{tot} by a modifying term (F_D) to account for the effect of salinity on fecundity. F_D is a proportional term that would range between 0 and 1.0 with zero referring to no effect and 1.0 referring to a total effect. Data on the effects of disease on fecundity are scarce for *C. ariakensis* and its congeners. In accordance with Mann and Evans (1998), we will leave F_D fixed at 1.0.

Diploid $F_{overall}$ = Diploid F_{tot} * F_S * F_D

For the purpose of this exercise, the model assumes that F_S=1.0 and F_D=1.0, and thus that Diploid $F_{overall}$ = Diploid F_{tot}.

<u>*C4. Probability of Encountering Gamete from Opposite-sex Parent Oyster*</u> *(F_f)*

As in step A4, this probability depends upon the density of oysters (i.e. the proximity to mates and the sex ratio of the population). We assumed that the population would have a 50:50 male to female sex ratio and that spawning would be synchronous across the population. Using Levitan's (1991) equation, this rate may be estimated as:

% Fertilization = 0.49 * (Oyster Density)$^{0.72}$

Approximately 714,285 oysters would be deployed over a 1-acre area (4047 square meters) at the field site. Oysters probably would be grouped together in spatial aggregations. This model makes the same assumption as the Luckenbach model (Luckenbach pers. comm.)

that oysters are grown in densities of 500 per 1 m^2 unit, where a unit is defined as an oyster float (e.g., Taylor float). The basic form of the model assumes that zero diploids are produced from the 4n X 2n cross. (If N_{dip} is >0, then the %Fertilization will also be >0).

$$\begin{aligned}\% \text{ Fertilization } &= 0.49 * (500 \text{ oysters } m^{-2} * 0.001)^{0.72} \\ &= 0.30\% \\ &= 0.0030\end{aligned}$$

This number is applied as a correction factor (F_f) in the model ranging between 0 and 1. To convert the percent fertilization calculated above to a correction factor, it is divided by 100.

$F_f = \%$ Fertilization / 100
$F_f = 0.0030 / 100$
$= 0.000030$

C/D4a. Probability of Producing Diploid Offspring from a 2n X 2n Cross (P_{DDdip})

Offspring from a 2n X 2n cross are expected to be diploid in nearly all cases. The model conservatively estimates this probability to be 100%.

$P_{DDdip} = 100\%$
$= 1.0$

5. Survivorship for Larvae in the water column (S_{larvae})

Larvae in the water column may experience mortality due to biotic and abiotic stressors. We can estimate the daily rate of larval mortality, M_{larvae}, as a proportion of the initial larval population dying on a daily time step. The rate of survivorship, S_{larvae}, may be expressed as the proportion surviving, or 1.00 - the mortality rate. Assuming that the rate of mortality increases exponentially with time, the following set of relationships can be used to estimate survivorship.
$S_{larvae} = (1-M_{larvae})$

The proportion of larvae surviving after x days = $(1-M_{larvae})^x$

The development period from competency to metamorphosis for oysters lasts approximately 21 days. Using data from the Oyster Hatchery at Gloucester Point, VA, M_{larvae} can range from 0.1-0.07. Computing the range of S_{larvae} based on these data, survivorship rates range from 0.109 to 0.217. We used the higher and, thus, more conservative estimate of this rate.

$S_{larvae}=0.217$

From here, Pathway C continues at Step 6 (see above).

4.3.1.4 Pathway D: Reversion from Triploid to Diploid

D1. Probability of Mosaics among Deployed Oysters (P_{mos})

Cells within genetic triploids may revert to the diploid state. If an individual oyster contains both triploid and diploid cells, it is said to be a mosaic. Reversion may be examined using the terms "incidence" and "intensity," which are typically associated with disease spread. The *incidence* of reversion refers to the number of animals in the population that exhibit mosacism. The *intensity* of reversion then refers to the proportion of cells within an individual oyster that have reverted.

The incidence of mosaics in *C. ariakensis* populations is low. Two estimates of this rate are 1.20% (Erskine 2003) and 0.78 (VSC 2003) for genetic *C. ariakensis* triploids up to market size. Reversion is rarely observed in the first year of life (Zhou 2002; Erskine 2003; S. Allen pers. comm.); therefore, oysters will be likely to be harvested before reversion occurs. Reversion increases over time and is more common during rapid periods of growth. This may explain why the incidence of reversion would be expected to be greater in rapidly growing species such as *C. ariakensis* and *C. virginica*. The model uses the larger, more conservative of the two estimates stated above.

$P_{mos} = 1.2\%$
$\quad = 0.012$

D2. First Generation Number of Mosaics (N_{mos})

This represents the number of diploid/triploid mosaics that arise in the initial starting population. The rate of mosacism increases with oyster age, so this number will depend on the amount of time the oyster is left in the water.

$N_{mos} = P_{mos} * N_{tot}$
$\quad = 0.012 * 714{,}285$
$\quad = 8{,}571$ oysters at the field site

D3. Probability that Mosaics Produce Gametes (Revertent F_{tot})

This probability estimates how many offspring that reverted individuals could be expected to produce. The fecundity of these species relates to the intensity of reversion. Reversion *intensity* is the proportion of cells in a mosaic that revert to the diploid state. This value can be detected analytically in the lab using flow cytometry. Following a set of 13 oysters during the VSC trial in 2000-2002, researchers found that reversion intensity increased gradually over time and that the intensity of reversion varied among individuals (Attachment E).

For the purpose of this exercise, the model is especially concerned with the reversion rate of gonadal tissue. If gonadal tissue reverts to the diploid state (2n), meiosis could yield haploid (1n) gametes that could mate with another haploid gamete from *C. ariakensis*. The resulting offspring would be diploid. Because reversion is rare to begin with, finding individuals to assay for reproductive potential is difficult. Using flow cytometry to study 53 gonads of reverted *C. ariakensis* males, researchers found 0 samples that produced 1n sperm (Attachment E).

Mosaic $F_{tot} = 0$

D4. Probability of Encountering a Gamete from a Reverted, Opposite-Sex Oyster (F_f)

Although the preceding zero probability in this pathway indicates that this pathway ultimately yields zero diploids, we will carry through the pathway for the sake of completeness. If gametes are formed by a revertent, then the next step is for the gametes to encounter gametes from another revertent of the opposite sex. As in A3, this probability is a function of oyster density and the sex ratio of the population. We assumed a conservative sex ratio of 50 males to 50 females.

The formula used to calculate this probability in general is (Mann and Evans 1998):

$$\% \text{ Fertilization} = 0.49 * (\text{Oyster Density})^{0.72}$$

As discussed in D1, the incidence of reversion is conservatively estimated to be 1.2% of the oyster population. We will make the same assumption as Luckenbach (Luckenbach model, pers. comm.), that oysters are grown in densities of 500 per 1 m^2 unit, where a unit is defined as an oyster float (e.g., Taylor float). The model further assumes that the 8,571 diploids are evenly distributed across 1924 oyster units (714,285 total oysters at the field site divided by 500 oysters per 1 m^2 oyster units). Multiplying the density of oysters times the incidence of reversion yields:

$$\begin{aligned} \% \text{ Fert}_{Mos} &= 0.49 * [(\text{Oyster Density}) * 0.012)]^{0.72} \\ &= 0.49 * (500 * 0.012)^{0.72} \\ &= 1.78\% \\ &= 0.0178 \end{aligned}$$

This number is applied as a correction factor (F_f) ranging between 0 and 1. To convert the percent fertilization calculated above to a correction factor, it is divided by 100.

$$\begin{aligned} F_f &= \% \text{ Fert}_{Mos} / 100 \\ F_f &= 0.0178 / 100 \\ &= 0.000178 \end{aligned}$$

From here, Pathway D continues on at step C/D4a (see above).

4.3.1.5 Pathway E: Accidental Release of Adults from Field Site

E1. Probability of Accidental Release from Field Site (P_{rel})

After deployment at the field site, some post-recruitment oysters could be accidentally lost from their containment due to factors such as storms, boat encounters, etc., and would never be harvested. To develop some rough estimate of this risk, the model uses information available from studies that have involved deploying triploid *C. ariakensis* in the Bay over the past several years. The table included here as Attachment C, which was prepared by Maryland Environmental Service, lists the details of all of the field trials and research experiments involving *C. ariakensis* deployments. The release events occurred as a result of several factors. In one instance, a Taylor float broke free from the PVC floats because the plastic ties used to secure it failed. In another study, an anchor struck and dragged one of the cages six feet. This caused the cage to break open and the oysters to fall out. Although in many instances the escaped oysters were recovered, the model considers the total number of oysters accidentally lost to be unrecoverable as a conservative estimate for this risk. This may be an underestimate of this risk because escapement could occur without detection.

P_{rel} = (Total number of release incidents) / (Total number of studies) * (Number of oysters lost during study / total number of oysters in those studies)

= (4 incidents / 134 studies) * (2204 lost oysters / 135,400 oysters in studies where oysters were lost)
= 0.03 * 0.016
= 0.0005

E2. Go to A2/B2, C2, or D2

An accidentally released triploid *C. ariakensis* may go on to contribute to the local triploid, diploid, or mosaic populations as described in Pathways A, B, C, and D. The likelihood that any of these escaped oysters would revert to the diploid state and, therefore, contribute to Pathway D would increase with the amount of time spent in the water.

4.3.1.6 Pathway F: Accidental Release from the Hatchery

F1. Probability of Hatchery Destruction due to Catastrophic Weather Event (P_{hatch})

A natural catastrophe such as a hurricane could destroy the facility and cause the release of the entire broodstock. If we assume that the facility is constructed to withstand such an event then this probability is expected to be minimal. The model does not attempt to estimate this probability, but rather simply recognizes that it exists.

F1a. Probability of Accidental Release of Mature Diploids (P_{hatch})

Human error at the hatchery could lead to a release of mature diploids. If we assume that hatchery operation protocols are properly followed, then we can also expect this event to have a small likelihood of occurrence. The model does not attempt to estimate this probability, but rather simply recognizes that it exists.

F1b. Probability of Matings Between Initial Tetraploids or Diploids Used to Produce Triploids (P_{hatch})

Another potential for accidental release of diploids from a hatchery could involve the initial diploid by tetraploid matings which are intended to produce triploids. Triploids are produced by mating tetraploid males to diploid females. If either the diploids or tetraploids used in those crosses proceeds to change sex, then either the diploids or tetraploids could potentially mate with each other. These matings could give rise to diploid offspring which could subsequently escape from the hatchery (M. Luckenbach, pers. comm.). The model does not attempt to estimate this probability, but rather simply recognizes that it exists.

The risk of accidental release via any one of the events described in F1, F1a, or F1b would be particularly high if large-scale private aquaculture were to occur with hatchery locations distributed throughout the Bay. Private aquaculture on such an expansive scale could increase the likelihood of accidental violations of the stringent biosecurity protocols developed by the ICES. One way to minimize this likelihood would be to centralize the production of larvae and spat in its most sensitive quarantine phase to one or two locations that are certified specifically for these operations (S. Allen, pers. comm.). Centralization of operations would allow for greater oversight and adherence to quarantine protocols.

F2. Go to A/B2, C2, or D2

Escaped oysters could contribute to the number of diploid, triploid, or mosaic oysters in the Bay; therefore, Pathway F may continue on in Steps A/B2, C2, or D2. These oysters, however, are not expected to be harvested on a regular schedule. The likelihood that any of these escaped oysters would revert to the diploid state and, therefore, contribute to Pathway D would increase with the amount of time spent in the water.

4.3.1.7 Estimates of Reproductive Individuals

The product of the probabilities in each chain described above yields the number of diploid *C. ariakensis* expected to arise from each pathway after one spawning season for a representative aquaculture operation with triploid *C. ariakensis* (Table 4-2). The greatest cumulative risk for any one pathway was 0.78 diploid oysters/year yielded by Pathway A (Table 4-2). Pathways B, C, and D yielded 0.03, 0.002, and 0 diploids, respectively, and make a negligible contribution to the total estimate over one generation. Pathways D and E feed into one of the first four pathways. For Pathway F, the model refrains from assigning specific probabilities, and no cumulative risk was calculated. To date, there have been no accidents involving releases at the hatcheries, and no data for estimating such probabilities are available. Table 4-2 also shows the number of diploid oysters that might be produced over a 10-year period for a tributary system within which there are 44 operations. The total number of diploid oysters at an example aquaculture location from all pathways is 271. Many assumptions are made in estimating the probabilities at each step in the chain of events because of the paucity of information available about the basic biological processes of *C. ariakensis*. Because of this, the level of uncertainty associated with these estimates is high.

4.3.2 Part 2. Probability of a Collocated Reproductive Pair of *C. ariakensis*

The above calculations assumed that all viable larvae would find suitable settlement substrate. The model does not address how individuals would be dispersed in space and whether individuals would be collocated and, thus, represent a spawning pair. We estimated the probability of a spawning pair at the scale of an aggregate of aquaculture operations in the Nanticoke River. This system was selected because it constituted a definable subbasin within the Bay and was designated as a candidate for highly localized aquaculture in the development of aquaculture scenario to be evaluated in the EIS (PEIS Appendix C). Our estimate is based on three simplifying assumptions: (1) oyster larvae would not be dispersed out of the system, and all diploid individuals would settle in the system; (2) these individuals would experience no mortality and would accumulate over time, increasing their density and potential for co-occurrence; and (3) the individuals would settle on appropriate hard-bottom area within the system. This estimate considers the potential for co-occurrence after a decade of operations within the system. The first two assumptions are conservative because some of the individuals could settle in areas outside the Nanticoke and, thus, reduce density and the potential for co-occurrence, and because some individuals would die as a result of predation and other factors. Young *C. ariakensis* are known to be particularly vulnerable to predation by blue crabs (Newell et al. 2007a). The third assumption presumes that settlement occurs randomly within acceptable hard-bottom substrate. This assumption may not be conservative because larvae may be drawn to other settled oysters. This attraction could involve attraction to natural populations of *C. virginica* that may be in the river. Some of the individual diploid oysters, however, probably would settle on oysters in aquaculture and subsequently be removed from the system during harvest. To the extent that this occurs, this would represent a source of mortality or removal of individual diploid animals from the system.

The assumption that larvae would be retained within the system is supported by modeling studies. The spatial and temporal variation in larval dispersal is influenced by hydrodynamic regimes (advection and turbulence) and species-specific differences in vertical swimming behaviors (North et al. 2006). Laboratory studies have demonstrated that *C. ariakensis* veligers tend to swim toward the bottom and remain there, where, in the Bay, they would be subjected to more landward water flows (Newell et al. 2005). A coupled hydrodynamic and larval transport model predicted that *C. ariakensis* larvae were more likely to be retained within the basin where they were produced than to be transported to a different basin due to their vertical orientation in the water column (North et al. 2006). Data for oyster-bar habitat (GIS layers from the Maryland Bay Bottom Survey, MBBS) in each Chesapeake Bay segment suggest that the potential settling area is large (e.g., Nanticoke Creek, 8,900,000 m^2). We used the area of the oyster-bar habitat

Table 4-2. Summary of calculations for each pathway.

Pathway	**P_{trip}, P_{dip} P_{mos} P_{rel} or P_{hatch}**	**N_{trip} or N_{dip} (oysters at the field site)**	**Triploid or Diploid F_{tot} (eggs m^{-2})**	**F_f**	**P_{DTdip} P_{TTdip} or P_{Dddip}**	**S_{larvae}**	**P_{sub}**	**P_{meta}**	**S_{juv}**	**No. of Oysters**	**No. of Oysters after 10 years example location (44 hatcheries)**
A Triploid Fertility (Triploid X Diploid)	0.999	714,285	2,459,855 eggs m^{-2}	0.0043	1.0	0.00314	1.0	0.25	0.07	0.58	256
B Triploid Fertility (Triploid X Triploid)	0.999	714,285	2,459,855 eggs m^{-2}	0.0043	0.04	0.00314	1.0	0.25	0.07	0.03	14
C Diploids from Imperfect Fidelity	0.001	715	18,376 eggs m^{-2}	0.00003	1.0	0.217	1.0	0.25	0.07	0.002	1
D Diploids from Reversion	0.012	8,580	0	0.000178	1.0	0.217	1.0	0.25	0.07	0	0
E Escape from the Field Site	0.0005	481									
F Escape from the Hatchery											
TOTAL for example location											271

within the Nanticoke River to consider the potential for collocation of two oysters that may result from the aggregate of all aquaculture operations within that system

Within the spatial extent of settlement, the spatial proximity between newly settled diploids will directly affect the likelihood that they will mate and produce a subsequent generation. Reproduction between two newly produced, diploid *C. ariakensis* would occur only if the individuals were close enough to each other in space, and if they spawned in synchrony. The model assumes that two opposite-sex, diploid individuals must be within 1 m^2 of each other for successful reproduction to take place (C. Guy, pers. comm.).

To explore the roles of dispersal density and mate proximity, a series of calculations was carried out to estimate the expected density of diploids after 10 years. The Nanticoke River location was chosen to illustrate this exercise because it is a spatially discrete location for which an aquaculture scenario has been described. This location would have 5% of the total aquaculture operations (875 operations total*5%=44 operations at Nanticoke River). As shown in Table 4-2, after 10 years of continued aquaculture, and assuming that all oysters survived, 271 diploid oysters would be produced from the Nanticoke River operations. To determine this number, first the number of operations was multiplied by the number of oysters yielded per spawning cycle at an example location, and then multiplied by 10 spawning cycles. This calculation was made for each pathway, and the sum of the products equals the cumulative number of diploid oysters after 10 years across all pathways at an example location (271). The amount of available oyster bar habitat on which larvae could settle at the Nanticoke River location is approximately 8,900,000 m^2 (MBBS GIS Layers). The following statements of probability were used to derive an estimate of the likelihood that two or more diploids could occur in the same 1m^2 after 10 years.

Because of the low density of oysters (271 over 10 years) and the large area of appropriate habitat for settlement, we assumed that settlement would occur randomly within the appropriate habitat. We recognize that the presence of *C. virginica* adults might attract individual *C. ariakensis,* and that distribution could be patchy depending on the spatial distribution of *C. virginica* adults. Each species could affect spawning in the other; however, the progeny of such cross-fertilization are non-viable, an effect referred to as the gamete sink (Bushek et al. 2006). Presuming random settlement, the spatial distribution of settled diploid *C. ariakensis* can be envisioned as assigning oysters randomly to a grid with N, 1-m^2 squares, where each square represents a potential site for co-occurrence and, thus, the potential for a spawning pair. The probability of 2 or more oysters on a square is P=1-P(0). For the first oyster, the probability of landing on an unoccupied square is 1. For the second oyster it is (N-1)/N, for the third oyster (N-2)/N, for the m*th* oyster (N-m+1)/N. Thus, multiplying probabilities together through the m*th* oyster:

$$P(0) = \frac{(N-1)!}{(N-m)!}\frac{1}{N^{m-1}} \tag{1}$$

This gives an exact result. For N-m>>1 Sterling's formula can be used for the factorials:

$$x! \cong \sqrt{2\pi} \quad x^{x+1/2} e^{-x} \tag{2}$$

to give a form more convenient for computation:

$$P = 1 - \frac{(N-1)^{N-1/2}}{(N-m)^{N-m+1/2}} \frac{e^{-(m-1)}}{N^{m-1}} \tag{3}$$

To avoid very large numbers it may be easier to calculate ln P(0), exponentiate it, and subtract it from 1.

$$\ln P(0) = (N-1/2)\ln(N-1) - (N-m+1/2)\ln(N-m) - (m-1)\ln N - m + 1$$

For N=8,900,000 and m=271 (at the end of year 10), the probability of co-occurrence is:

lnP(0)=-0041, P(0)=0.9959, and P=0.004.

Thus, the likelihood that two or more diploid *C. ariakensis* would occur in the same 1 m^2 after 10 years is 0.004, or a 0.4% chance that the aquaculture of triploid *C. ariakensis* could result in densities of *C. ariakensis* thought to be necessary for subsequent reproduction within this particular river. We have not estimated the probability for all areas where aquaculture might occur. The Nanticoke operations are thought to represent 5% of the total. If the assumptions identified above hold on average for other areas, this could translate to a probability of about 8% over a 10-year period. This, of course, assumes that none of the diploid oysters die during this period, and that they are retained within a system with no extensive dispersion to other areas, which would reduce densities and decrease the probability of a co-occurrence. The probability of co-occurrence of two diploid *C. ariakensis* is related to both the number of triploid oysters in aquaculture and the availability of substrate. Each of these factors - an increase in abundance and a decrease in available substrate - would affect the density of individuals and the potential for two individuals to be collocated. Over a period greater than 10 years, this risk would be expected to cumulatively increase, but whether the probability eventually becomes 100% would depend on the longevity and fate of the gradually increasing numbers of diploids that are being projected.

The NRC (2004) indicated that, given what is known about the biology of *C. ariakensis*, "it is very likely that *C. ariakensis* is capable of establishing wherever *C. virginica* was established in the Chesapeake Bay, with the exception of areas where sedimentation now prevents or inhibits larval settlement" (p. 202). This report concludes that triploid aquaculture in combination with strict accountability and best management practices "would probably have little impact on total oyster abundance" (p. 235). However, the NRC's (2004) report repeatedly emphasizes that the basic understanding of *C. ariakensis* is too limited in scope to make firm conclusions with low levels of uncertainty.

This series of computations makes several assumptions. First, it assumes that diploids are distributed randomly in space. This is unlikely to be the case because larvae exhibit settling behaviors and are subject to fluctuations in hydrodynamic forcing factors, such as advection and turbulence (North et al. 2006). Second, all oyster-bar habitat is assumed to be equivalent. This also is unlikely to be true. The coupled hydrodynamic-larval transport model predicted that some oyster bars would be larval "sinks" (i.e., receiving more larvae than they produce), whereas many more bars would be strong producers of oyster larvae (i.e. producing more larvae than they receive; North et al. 2006). Producer bars were particularly frequent on the Eastern Shore of Maryland. If *C. ariakensis* were to populate a strong producer bar, then this population could be a source of oysters both within the basin where that bar is located and beyond. Third, this model assumes that the biological (larval behavior) and physical (advection and turbulence) characteristics of the region, which are generally thought to affect the distribution of settling larvae, are unimportant. Given these assumptions, this estimate must be viewed with caution.

The likelihood of having collocated diploid individuals could be small to large. This likelihood would be small if: (1) an aquaculture industry of the projected size was unable to become established within a decade or ever in the future, (2) ICES quarantine protocols are followed properly, (3) aquaculture facilities are concentrated into a central location, (4) if diploids are deployed at low densities to reduce the concern that two diploids could reproduce, (5) if the period between deployment and harvest does not overlap with the reproductive season, (6) diploid eggs, larvae, or juveniles suffer high mortality (e.g. predation from blue crabs), (7) suitable habitat is not available for settling diploid larvae, (8) larvae settle on "sink" bars (sensu North et al. 2006), (9) competition for space is strong for settling larvae, (10) collocated oyster recruits are of the same sex or fail to successfully reproduce, (11) diploid Suminoe oysters become susceptible to diseases in the Bay**,** (12) the rate of reproduction of diploid adults is so limited that no sustainable population is ever established.

Conversely, this likelihood would be large if: (1) ICES quarantine protocols are not followed properly, (2) aquaculture facilities are widely distributed throughout the Bay magnifying the potential for human error, (3) continuous aquaculture occurs in the same location over many years, (4) if oysters are deployed at high densities, (5) if the period between deployment and harvest overlaps with a reproductive season, (6) the number of escaped revertants accumulates over time, (7) larvae are readily able to find suitable habitat on which to settle, (8) larvae settle on a "producer" bar (sensu North et al. 2006), (9) competition for space is weak for settling larvae, (10) diploid eggs, larvae, or juveniles suffer low mortality (e.g., predation from blue crabs) and survive indefinitely, (11) diploid Suminoe oysters continue to resist diseases occurring in the Bay, (12) a rare storm event strong enough to damage or destroy hatchery or aquaculture facilities occurs.

Given the many unknowns in the two component evaluations for this question and the variety of possible pathways of introduction, no specific level of risk can be determined for the overall likelihood that implementing Alternative 5 would result in an unintended introduction. Some stakeholders believe that an unintended introduction is a certainty if large scale triploid aquaculture is implemented; however, no probablity analyses have been published to support this view. The level of uncertainty associated with evaluating this risk is high due to lack of information about many contributing factors.

The possibility of unintentionally introducing a reproductive population of *C. ariakensis* via cultivating triploids can be combined with the discussion of the ecological outcomes of an intentional introduction of the species to evaluate overall outcomes. If an unintentional introduction eventually leads to the abundance projected for an intentional introduction, the ecological outcomes would be equivalent to those of the proposed action. Barring a catastrophic release from a hatchery, however, significantly more than 10 years probably would be required to achieve the 10-year abundance projected for an intentional introduction because of the small number of oysters (diploid or triploid) expected to be released from aquaculture operations in any given year.

4.4 RQ 8 - TO WHAT EXTENT WILL THE ACTION INFLUENCE ECOSYSTEM SERVICES IN CHESAPEAKE BAY?

The Relative Risk Model (RRM) used for this evaluation portrays the degree of influence (positive or negative) that changes in oyster biomass might have on ecological receptors and water quality in each state/salinity zone. The details of the approach are provided in Section 3-4 and Attachment B. The following summary will remind the reader of the steps in the procedure:

1. With the exception of the proposed action, exploratory demographic modeling was used to project changes in oyster biomass over a 10-year period for each of the alternatives.
2. The Chesapeake Bay Environmental Model Package (CBEMP) was used to relate changes in oyster biomass to percent changes in phytoplankton, zooplankton, benthic biomass, submerged aquatic vegetation (SAV), dissolved oxygen, and total suspended solids. We extrapolated from the results of existing output of the CBEMP to account for changes in oyster biomass projected by demographic modeling, as described in Attachment B
3. Percent changes in oyster biomass and in the modeled ecological conditions derived in Step 2 were translated into RRM scores that range between –5 and +5. A value of "5" represents approximately an order-of-magnitude (10 x) change.

RRM scores for fish and wildlife species were derived by multiplying the degree(s) of association with food sources, habitats, or both with the change in those food sources and habitats as reflected in their individual RRM scores. Two sets of association values were used for fish and wildlife; the first set reflects values based on the literature and interviews with experts (Table 3-2). The second set is based on the median value of bottom area that is covered by cultch (~ 0.03; Table 3-3). The first set of association factors for fish and wildlife is about an order of magnitude greater than the second set.

RRM scores are assigned to degrees of change or influence as follows: 0.1 or –0.1 (1% to 10%), 1 or –1 (10% to 100%), 2 or –2 (100% to 400%), 3 or –3 (400% to 600%), 4 or –4 (600% to 800%), 5 or –5 (>800%). Annual and long-term variability in ecological receptors varies among receptor groups. Phytoplankton, zooplankton, and benthic invertebrates undergo substantial seasonal and annual variation. Other receptors that can vary substantially from year to year include SAV and blue crabs. For such receptors, annual variability can be greater than a factor of 2 (100%), and often is much greater; therefore, influences that correspond with changes in abundance of these receptors of less than a factor of 2 were designated as "small" relative to other factors that influence ecological receptors in the Bay. For relatively long-lived receptors such as oysters, piscivorous fish, and various species of birds, changes of a factor of 2 are not uncommon over longer time scales (e.g., 10-years); therefore, RRM scores of 0.1 or –0.1 and 1 or –1 (changes of a factor of 2 or less over a 10-year period) were considered "small" from an ecological standpoint.

Results are presented first by alternative and then by state/salinity zones. RRM scores are presented as stacked histograms. These are not intended as cumulative total scores of positive or negative but do indicate the extent to which groups of receptors are either positively or negatively influenced under the various alternatives. The stacks comprise the individual RRM scores for each of the ecological receptor groups. These histograms enable readers to compare the potential ecological effects of the proposed action and alternatives across state/salinity zones or across alternatives. They display the combined, relative, positive and negative influences associated with changes in oyster hard bottom and associated changes in other ecological conditions. The maximum relative risk score that any ecological receptor can receive is "5." When scores were combined (stacked in the figures), the scales for the stacked histograms had to be adjusted to accommodate the combinations. Three scales are used to present the stacked histogram results for the RRM scores: ± 30 for the base case, which reflects the association values in Table 3-2, ± 5 for the results using the association values in Table 3-3 and excluding results for oyster hard bottom and associated reef-dependent fish, and ± 10 for comparing alternatives for each state/salinity zone. These three scales facilitate visual comparisons and are kept constant for each of the kinds of results. Individual RRM scores for each receptor are always between -5 and +5, as described in Section 3. Matrices showing the actual numerical scores for each ecological receptor group are provided in Section 4.6. These matrices were developed to support management decisions.

A few key kinds of relationships are helpful to bear in mind when evaluating the RRM results. Increased oyster biomass could have a positive influence on certain receptors by providing increased food or habitat. Examples of these positive influences are reef-oriented fish and bird species that feed on oysters. Increased oyster biomass could have a negative influence (an inverse relationship) on phytoplankton biomass because oysters feed on phytoplankton and can reduce the standing crop. The reduction in phytoplankton subsequently could have a negative influence on animals that feed on phytoplankton, such as zooplankton and menhaden. Because filtration by oysters can reduce suspended solids in the water, increases in oyster biomass could improve water clarity, which might result in greater growth of SAV. Greater growth of SAV could have a positive influence on species that rely on these plants for food or habitat. Because reductions in phytoplankton and suspended solids could result in a smaller input of organic matter to sediments, increases in oyster biomass could have a negative influence on soft-bottom benthos, which feed on material in and on the sediments. Subsequent influences of changes in oyster biomass on various fish and wildlife species depend primarily on the degree to which these species rely on habitats or food items that are positively or negatively influenced by changes in oyster biomass.

Several sources of uncertainty in the analysis are described in Section 4.7. Two sources of uncertainty are considered to some degree within the RRM results. The first relates to the demographic modeling of oyster biomass. These results are provided as distributions (PEIS Appendix A); we chose a central value of these distributions (the 50th percentile) and an upper-bound value (the 95th percentile) to examine the differences among alternatives. A second source of uncertainty relates to how well we understand the ecological associations between changes in oyster biomass and influences on other ecological conditions and receptors. For fish and wildlife species, we used two sets of association factors to depict possible relationships. The first set tends to reflect strong dependencies, and the second set reflects dependencies that are tied to the relative coverage of bottom area by oysters.

4.4.1 Influences on Dissolved Oxygen and Total Suspended Solids

Newell (1988) estimated that, at one time, the oyster population of Chesapeake Bay would have been able to clear a volume of water equal to that of the Bay in two to four days, suggesting that a fully restored oyster population might be capable of controlling spring phytoplankton blooms that contribute to low dissolved oxygen conditions during the summer. Other researchers also have discussed this potential beneficial role of oysters in controlling water quality in the Bay (Jackson et al. 2001; Ruesink et al. 2005; Kemp et al. 2005); however, the hypothesis has been the subject of debate. Pomeroy et al. (2006) and Fulford et al. (2007) argued that the potential role of oysters in controlling algae in the Bay has been overstated and that the various populations of suspension-feeding benthos now present in the Bay should have a filtration capacity approaching that of the pre-Colonial population of oysters. Yet, they do not appear to be controlling algal blooms. Those authors concluded that achieving the restoration goal for oysters in the Bay (i.e., average population level over the period 1920-1970) would be unlikely to result in a significant, Bay-wide reduction in phytoplankton biomass. In a reply publication, Newell et al. (2007b) critiqued those conclusions and maintained that increasing the population of oysters by orders of magnitude could have important effects on water quality and ecological conditions in the Bay.

These competing scientific arguments rely on specific sets of assumptions about the timing, spatial distribution, and magnitude of filtration by oysters that are beyond the level of detail that could be addressed in this ERA. Clearly, the greater the number of oysters, the greater the amount of water they would filter. Based on this underlying assumption, projections of oyster biomass generated through exploratory oyster population modeling were used in the ERA, in conjunction with outputs from another model, the Chesapeake Bay Environmental Modeling Package (CBEMP; Cerco and Noel, 2005a, 2005b, 2006). CBEMP outputs provided insights into possible water quality effects at the scale of Bay segments and regions. This segment-level evaluation complements the six broad areas (two states and three salinity zones) considered in the ERA. The CBEMP is described in detail in Attachment B. Further consideration of the limitations of modeled oyster abundance estimates since completion of the ERA led to the conclusion that potential water quality consequences of the oyster restoration alternatives would best be addressed by using only CBEMP outputs, framed within a context of the likelihood of changes in oyster abundance that might occur under the various alternatives.

One major limitation of all assessments conducted in this ERA is that they consider water quality responses on large spatial scales (state/salinity zones). The analyses are not capable of characterizing potential localized changes in water quality within tributaries or smaller areas. CBEMP results do allow potential small-scale effects to be investigated. The only water quality parameters considered here are those for which outputs from the CBEMP were available: dissolved oxygen (DO) and total suspended solids (TSS). Submerged aquatic vegetation (SAV; underwater grasses), while not a water quality parameter, is also addressed here because it is the ecosystem component that is most closely linked to and responsive to changes in TSS. The CBEMP model outputs used in the ERA analysis and discussed here are static and do not account for any changes in inputs to that model package that might occur over the 10-year assessment period (e.g., increases or decreases in nutrient loading to the Bay); therefore, the evaluations of the potential effects on water quality described here consider only the relationship between water quality and changes in oyster abundance and assume that all other factors would remain constant.

4.4.1.1 Proposed Action: Introduce the Suminoe Oyster and Continue Efforts to Restore the Eastern Oyster

As discussed earlier, projections of abundance and biomass of a Suminoe oyster population in the Bay, were it to be introduced, were not possible. In addressing ecological risk issues, it was assumed that the introduced species would be successful and become widely distributed throughout the Bay. This assumption was considered to be a "worst case" scenario from the perspective of those stakeholders who believe that introducing a nonnative oyster is undesirable. This same assumption was made to consider the effects of the proposed action on what water quality and other components of the Bay ecosystem.. Clearly, if the proposedr introduction were unsuccessful, oyster abundance would not increase significantly and water quality would remain unchanged.

Insights into the effects on water quality and ecological benefits of achieving particular levels of oyster abundance and biomass can be gained from the modeling work performed by Cerco and Noel (2005a, 2005b, 2006). The starting population for their modeling was the average over the period 1991 to 2000 from Jordan et al (2002), which was somewhat higher than the estimated levels for more recent years. The modeled 10-fold increase in oyster biomass was not distributed evenly throughout the Bay. Oyster abundance in the Maryland increased by a factor of 50, whereas abundance in Virginia exhibited only a 4-fold increase; this result ws consistent with patterns shown in exploratory modeling. For the Bay as a whole, Cerco and Noel (2005a) projected that a 10-fold increase in oyster biomass from that starting level would result in a 0.25 mg/l increase in summer-average, bottom dissolved oxygen in the deep waters of the bay (depth > 12.9 m). Oxygen levels below 5 mg/l of water affect the behavior and survival of fish. Concentrations below 2 mg/l are considered to be severely hypoxic and affect the structure, distribution, and productivity of benthic organisms, including oysters. In recent decades, an average of 5.25% of the Bay mainstem volume was hypoxic. An increase of 0.25 mg/l would not alter that condition to any significant degree. The explanation for the small increase in DO was that filtration of phytoplankton from the water column was estimated to result in a net removal of 30,000 kg per day of nitrogen through sediment denitrification and sediment retention. Because oysters remove suspended matter (total suspended solids, TSS) in shallow areas, enhancement of SAV in response to improved water clarity was projected. Calculated summer-average SAV biomass improved by 25% with a 10-fold increase in oyster biomass.

As discussed earlier, oysters are most likely to affect water quality locally rather than Bay-wide. Cerco and Noel (2005a) investigated such potential localized effects by selecting 3 of the 35 Bay segments used in their modeling for detailed examination of effects on the regional scale (Figure 4-13). These segments provided a range of geometry and environmental conditions and included a deeper, mainstem segment of the Bay (CB4), an eastern embayment that encompasses the mouth of the Choptank River (EE2), and the Big Annemessex River (ET9). Oysters can live in only a portion of CB4 but can inhabit most of the other two segments. A summary of influences of oyster abundances/biomass on conditions in these segments is provided in Figures 4-14 to 4-16.

The effects on dissolved oxygen of a 10-fold increase over historical oyster levels are shown in Figure 4-14 a through c. The figures indicate changes on the order of 0.5 mg/l in summer bottom water for a 10-fold increase in oysters at the scale of a Bay segment, which is a larger effect than projected on a Bay-wide basis. Larger changes would occur if oysters were restored to historic levels. Increases in dissolved oxygen were projected for segments CB-4 and EE-2; however, a counter-intuitive decrease was predicted for segment ET-9. This decrease occured because of the large reduction in phytoplankton through filtering by oysters reduces the oxygen production of the phytoplankton, which is lower than respiration in this location.

A similar presentation of changes inwater transparency (i.e., a reduction in TSS) associated with different levels of oyster abundance is shown in Figure 4-15 a through c.

Figure 4-16 illustrates that filtration by oysters removes suspended material that decreases attenuation and increases light penetration. These influences have a strong effect on water clarity and, consequently, on the growth of SAV. The response of SAV to the increase in light is illustrated in Figure 4-16 a through c. SAV biomass nearly triples for a 10-fold increase in oyster density and increases by greater than a factor of 4 for restoration to historic oyster densities.

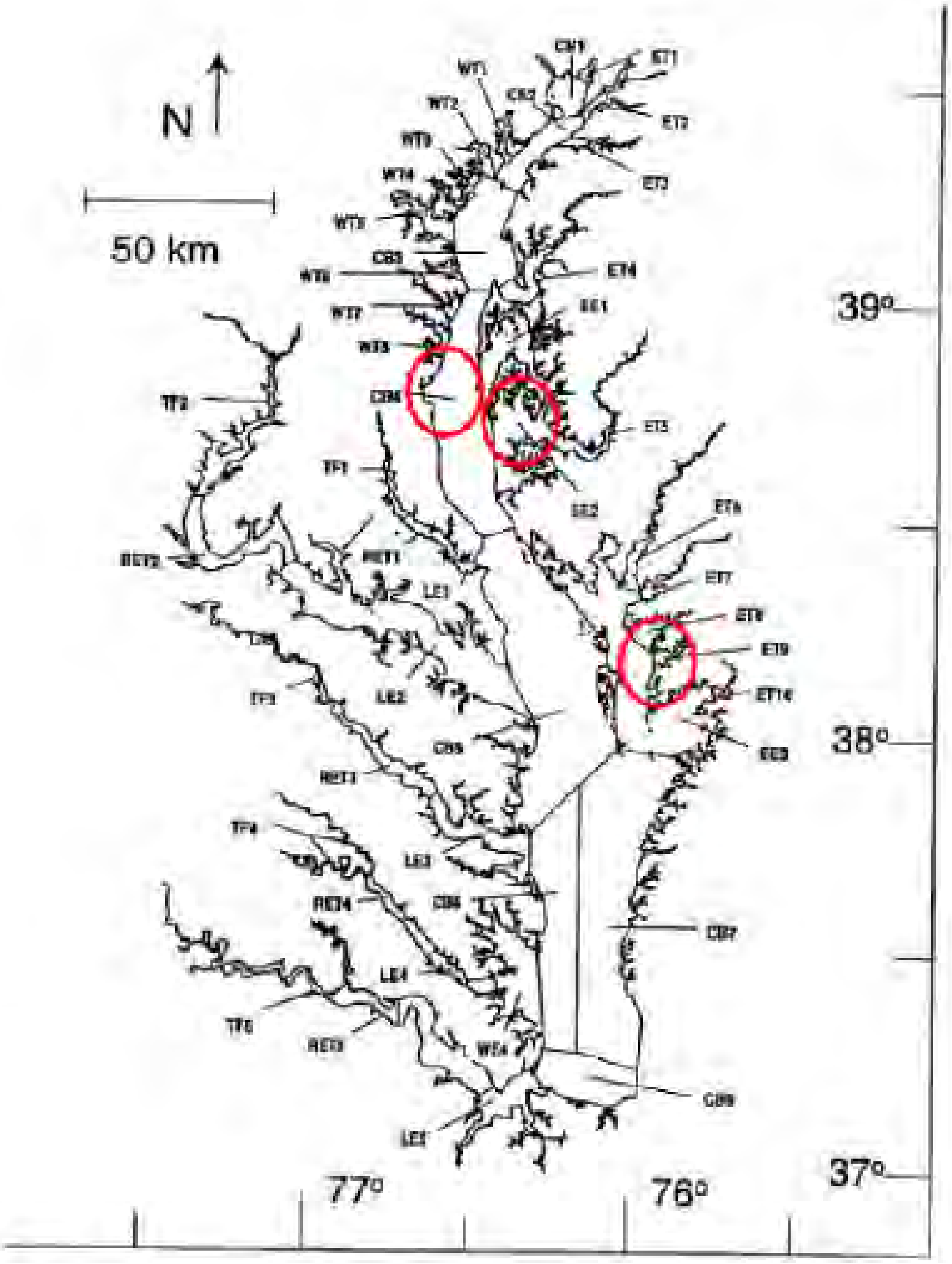


Figure 4-13. Selected segments for detailed evaluation of the effects of oysters.

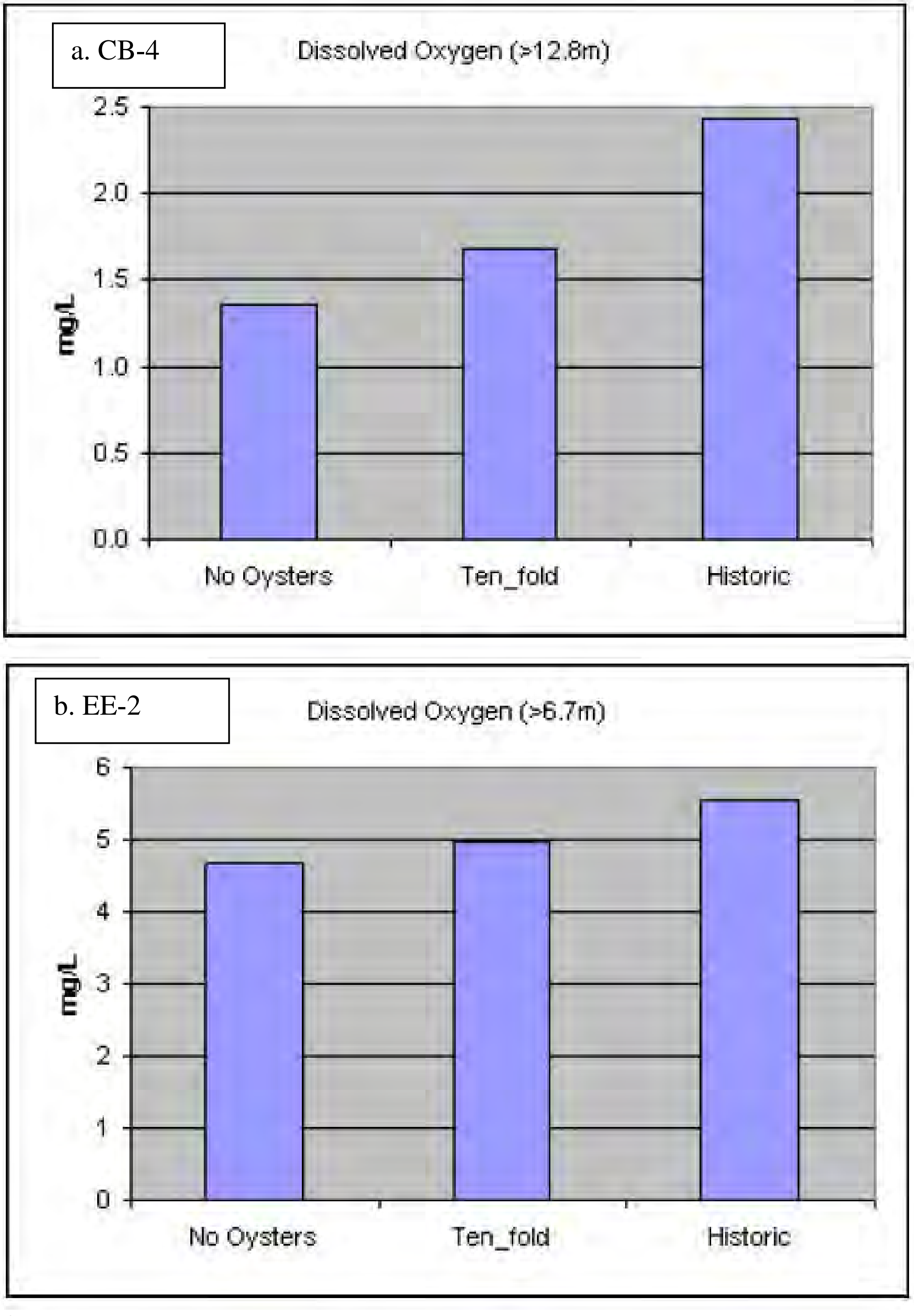


Figure 4-14. Summer bottom dissolved oxygen in Bay Segments CB-4(a), EE-2(b) and ET-9(c) with no oysters, an increase in oyster biomass to 10 times present levels, and under historic levels of oyster abundance.

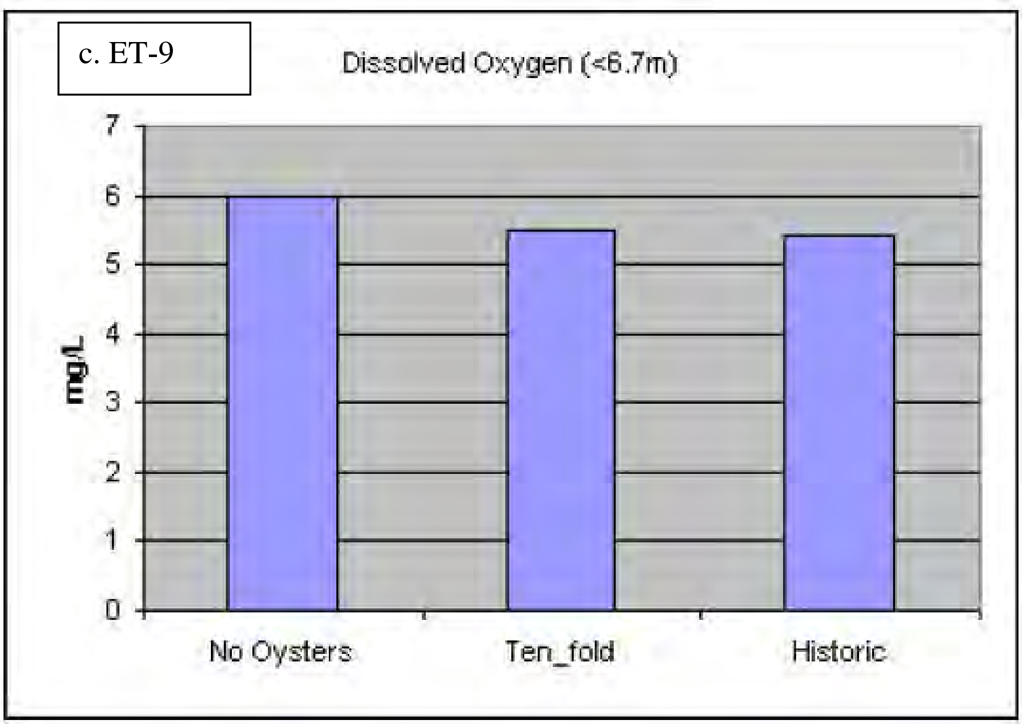


Figure 4-14. (Continued)

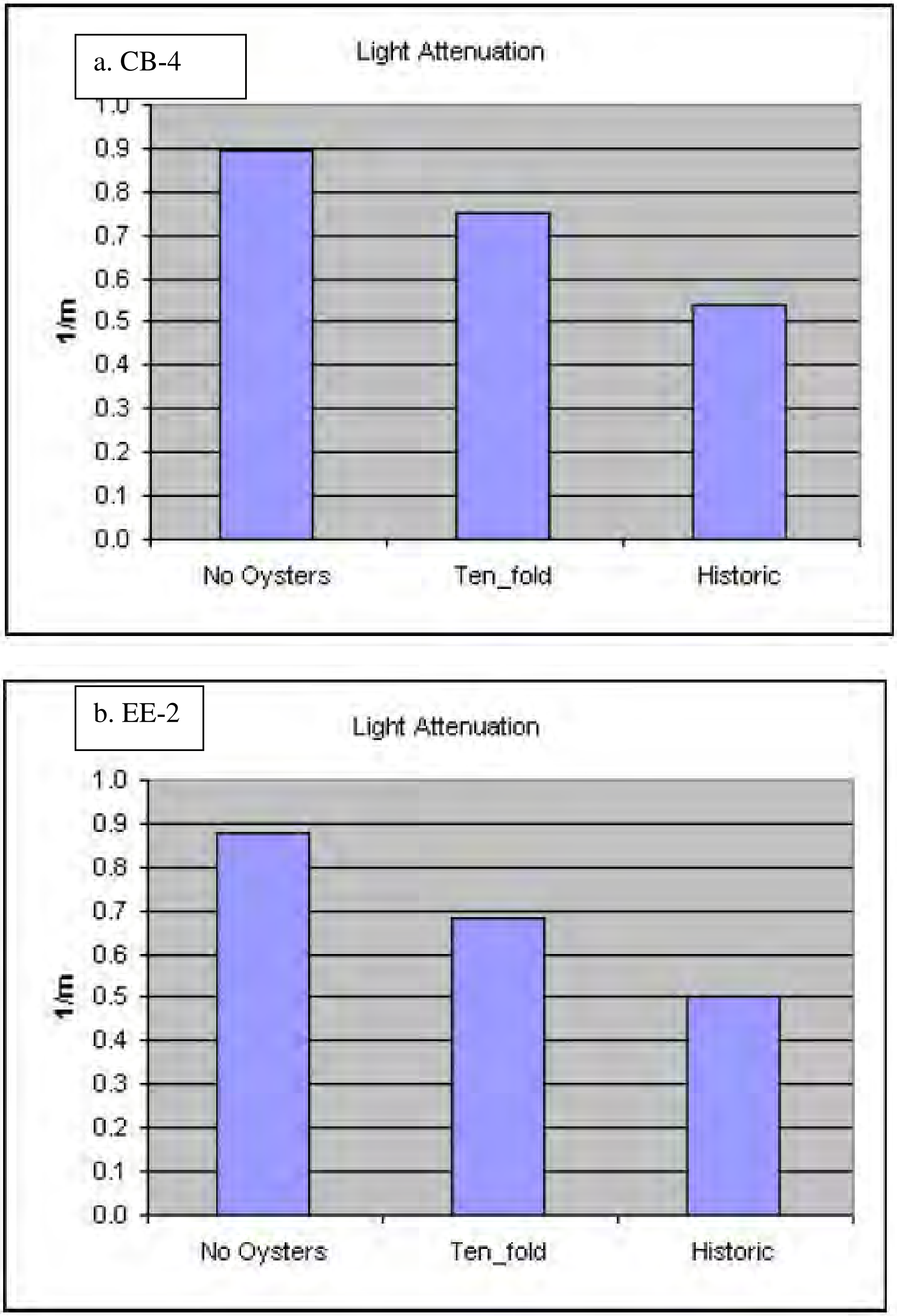


Figure 4-15. Light attenuation (highest with lowest TSS) in Bay Segments CB-4(a), EE-2(b) and ET-9(c) with no oysters, an increase in oyster biomass to 10 times present levels, and under historic levels of oyster abundance. Light attenuation is defined as the rate of decrease of light per unit of vertical distance in the water column (1/m).

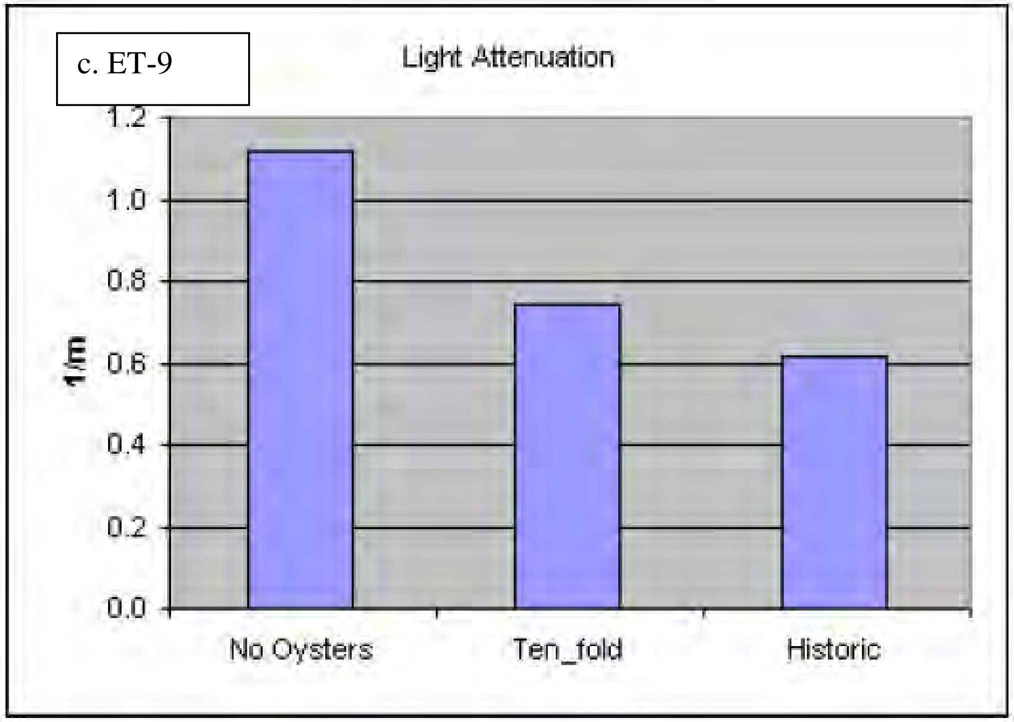


Figure 4-15. (Continued)

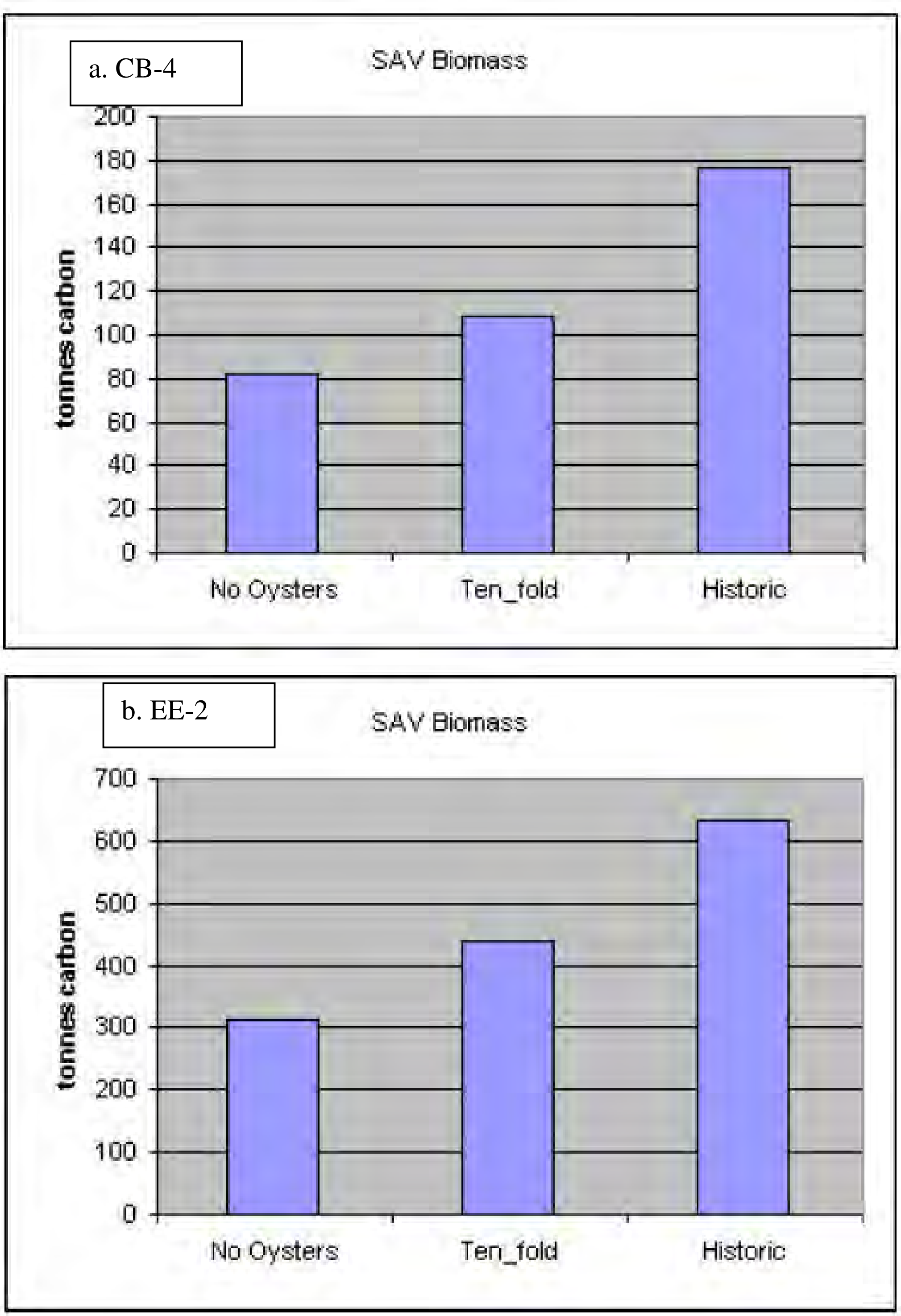


Figure 4-16 Biomass of SAV in Bay Segments CB-4(a), EE-2(b) and ET-9(c) with no oysters, an increase in oyster biomass to 10 times present levels, and under historic levels of oyster abundance.

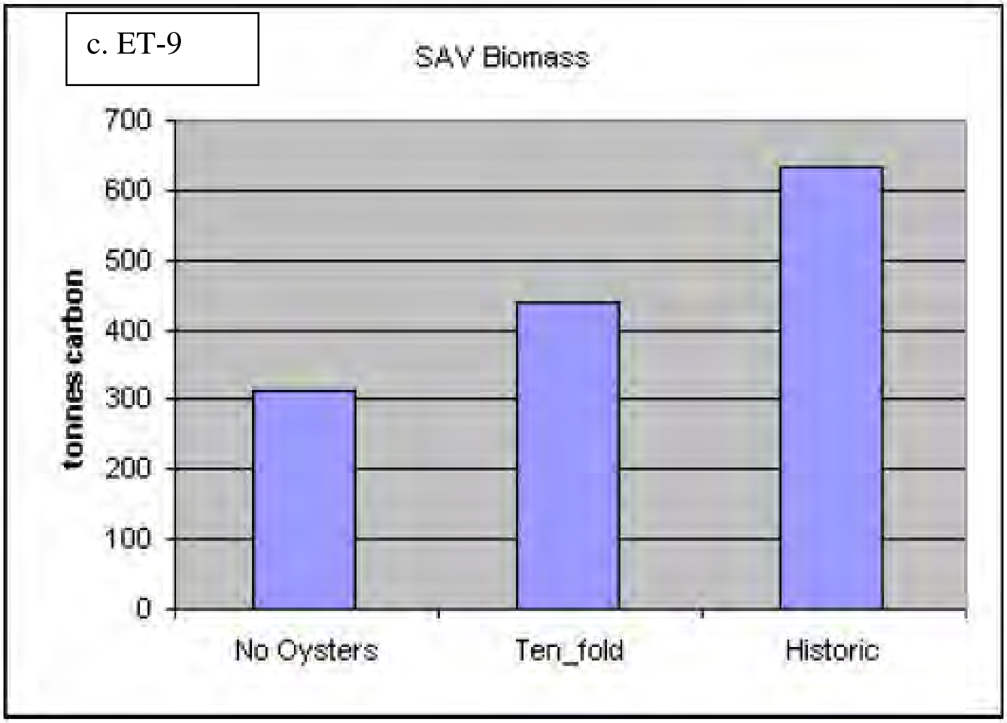


Figure 4-16. (Continued)

Cerco and Noel (2005a) noted that oxygen levels in their modeling results did not respond to increases in oysters as much as might be expected. They suggested that the smaller response occurred because oysters are found in the shoals rather than in the deeper portions of the Bay mainstem. Phytoplankton production over the mainstem settles to the bottom waters and contributes to anoxia. In the shoals, oysters would remove the phytoplankton biomass before it settled to the bottom. A more subtle explanation lies in the origins of mainstem anoxia. Oxygen depletion in the upper Bay does not originate solely with excess production in the overlying waters. Rather, oxygen depletion is accumulated as net circulation moves bottom water up the channel from the mouth of the Bay (Kuo 1991, Cerco 1995). Improving dissolved oxygen in the upper Bay requires reducing oxygen demand in the lower Bay. The oyster restoration strategies considered in this EIS would do nothing to diminish oxygen demand in the lower Bay and, consequently, would have a limited effect on the upper Bay. Cerco and Noel (2005a) noted that, despite the uncertainties in their approach for relating oyster biomass to ecological changes using the CBEMP, they believe their basic findings regarding the nature and magnitude of restoration benefits are valid. They found their results to be consistent with the earlier findings of Officer et al. (1992) and Gerritsen et al. (1994) and with the recent findings of Newell and Koch (2004). Benthic controls of algal production are most effective in shallow, spatially limited regions, as in the example of the dark false mussel in the Magothy River (Bergstrom 2004, pers. comm..). The ability to influence deep regions of large spatial extent is limited by the location of oysters in the shoals and by exchange processes between the shoals and deeper regions.

Because it is not possible to project the size and distribution of an introduced population of Suminoe oysters, CBEMP modeling results cannot be applied directly to deterimine how Suminoe oysters might affect dissolved oxygen and SAV in the Bay. If the proposed introduction is assumed to be successful, a 10-fold increase in oyster biomass might be a reasonable expectation, given the small starting population of the species, and water quality could be expected to improve by at least the magnitude projected by the CBEMP. Because the Suminoe oyster is resistant to MSX and Dermo, a successful introduction could result in oyster abundance in the higher salinity waters in the Bay that would exceed the values assumed by Cerco and Noel (2005a) and result in greater improvement in water quality in that portion of the Bay.

4.4.1.2 Alternative 1: No-Action

Given that a Bay-wide increase in oyster abundance is unlikely under Alternative 1, no changes in water quality would be expected. Although some increase might be expected in lower salinity waters in Maryland, the magnitude of the projected change in oyster biomass probably would be insufficient to affect water quality at the geographic scale of the ERA analysis (six state/salinity zones).

4.4.1.3 Alternative 2: Enhance Restoration

The ERA, concluded that no significant changes in water quality would result from changes in the size of the oyster population projected for Alternative 2. Some very small decreases in TSS were projected for low salinity areas in Maryland, which is consistent with the larger increase in oyster biomass that might be expected in that state/salinity zone. If all of the projected increase in oyster biomass were to be concentrated in a limited location, the potential for changes in water quality would be significant. This is consistent with the findings of Cerco and Noel (2005a), which showed that influences on water quality will be greatest at the scale of individual segments and especially for shallow semi-enclosed areas. In particular, there may be a noticeable positive influence on water clarity in select, low-salinity segments in the upper Bay. This would reduce TSS and enhance the growth of SAV in those locations.

4.4.1.4 Alternative 3: Harvest Moratorium

Although changes oyster abundance under a harvest moratorium could not be predicted, at a minimum the changes would be similar in location and magnitude to those expected to occur under Alternative 1. Oyster abundance could be expected to increase in high salinity areas, but those increases are not likely to be sufficient to affect water quality,even on a local scale. The magnitude of change in oyster biomass probably would be insufficient to affect water quality at the geographic scale of the analysis. Small changes might occur at the scale of individual segments in the lower salinity areas in Maryland, consistent with the findings of Cerco and Noel (2005a).

4.4.1.5 Alternative 4: Cultivate Eastern Oysters

The annual production of the predicted maximum aquaculture industry in the Bay (i.e., 2.6 million bushels annually distributed over 9 possible locations for concentrated aquaculture operations) was converted to biomass and grouped according to the 6 state/salinity zones to project potential water quality effects based on CBEMP output. No discernable changes in DO or TSS were projected; however, the scale of the analysis conducted in the ERA is too large to detect local effects, such as in an individual tributary. Changes in water quality that might result from implementing Alternative 4 would be a function of the cumulative size of aquaculture operations (i.e., the number of oysters being farmed), the distribution of operations within bodies of water, and the hydrodynamics of the host waters. Although concentrating oyster production in a limited area would offer the greatest potential to affect water quality locally, aquaculture operators also would have to account for the availability of a sufficient supply of food (e.g., phytoplankton) in order to achieve economically viable growth rates. For example, an area with a high rate of phytoplankton production would be desirable to ensure good oyster growth rates, but the rate of phytoplankton consumption by densely farmed oysters could exceed the rate of phytoplankton production. In that circumstance, the growth rate of the oysters could be constrained or reduced, and the efficiency of the aquaculture operation would be reduced. Optimal positioning of aquaculture operations, so that rate of phytoplankton consumption by oysters (and, thus, the rate of oyster growth) is kept in balance with the rate of phytoplankton production, might not produce the maximum potential improvement in local water quality.

4.4.1.6 Alternative 5: Cultivate a Nonnative Oyster

Alternatives 4 and 5 were treated similarly in the evaluation of water quality effects, while acknowledging that growth rates and the ratio of biomass to shell height of triploid Suminoe oysters are greater than those of both diploid and triploid Eastern oysters. Negligible effects on water quality were projected on the scale of the state/salinity zones for the predicted maximum aquaculture industry considered for Alternative 5. Some differences between Alternative 4 and Alternative 5 are possible. The faster growth rate and shorter time to grow to market size for triploid Suminoe oysters might result in fewer Suminoe oysters (one cohort) than Eastern oysters (two to three cohorts) being present in the Bay at any one time and less area within the Bay being occupied by aquaculture operations. The Suminoe oyster's faster growth may be a function of greater filtering and food consumption; therefore, cultivating the species may have a greater effect on water quality locally than cultivating the same number of Eastern oysters. The same factors discussed under Alternative 4 (i.e., size of the water body, hydrodynamics, and oyster densities) would control the extent to which Alternative 5 would affect water quality locally.

4.4.1.7 Alternative 6: Introduce an Alternative Nonnative Oyster or an Alternative Strain of the Suminoe Oyster

No nonnative oyster other than the Suminoe oyster has been demonstrated to be a good candidate for cultivation in Chesapeake Bay. Although several other strains of the Suminoe oyster are maintained within hatcheries in the Bay region, comprehensive studies have investigated only the Oregon strain. No information is available to support an evaluation of the potential fate and ecological consequences of using another nonnative species or another strain of the Suminoe oyster. The effects of Alternative 6 on water quality could not be evaluated because no Bay-specific research findings are available on any other species or strain and because population sizes could not be predicted for any other species.

4.4.1.8 Alternative 7: Introduce the Suminoe Oyster and Discontinue Efforts to Restore the Eastern Oyster

This alternative is similar to the proposed action, except that it calls for ceasing efforts to restore the Eastern oyster. The modest increase in the number of Eastern oysters projected to occur under Alternative 1, would represent only a small fraction of the total potential population for Alternative 7; therefore, the consequences of Alternative 7 for water quality would be the same as those for the proposed action.

4.4.1.9 Alternative 8: Combination of Alternatives

This alternative would consist of a combination of several of the alternatives, possibly including the proposed action. The combination that would best meet oyster restoration objectives will be identified after stakeholders have had an opportunity to review and comment on evaluations of the individual alternatives. No specific combination of alternatives is evaluated in this ERA.

4.4.2 Ecosystem Services Assoicated with the Proposed Action

Risk Question 8 (To what extent will the action influence ecosystem services in Chesapeake Bay?) is only applicable in the same manner to the proposed action and all of the alternatives if the answer to Risk Question 2 (Will Suminoe oysters provide ecosystem services similar to those afforded by Eastern oysters?) is positive. As a result, it is necessary to first address Question 2 in the evaluation of the proposed action. If the answer is shown to be yes, then any differences among the proposed action and the alternatives would be a result solely of the potential differences in resultant oyster abundance among them. As noted earlier, Risk Question 2 applies to the proposed action and Alternative 7, directly, and would apply to Alternative 5 only if an unintended introduction of diploids from triploid aquaculture were to result in a large, self-sustaining population of Suminoe oysters.

In considering this Risk Question 2, the proposed introduction of the Suminoe oyster is assumed to be successful, and the species to be established throughout the range of the Eastern oyster in the Bay. This assumption was considered to be a “worst case” scenario from the perspective of those stakeholders who believe the introduction of a nonnative oyster is undesirable. That is, the assessments in this section did not investigate the feasibility of an introduction; they simply examined the potential consequences if the species were to become abundant and widespread.

If Suminoe oysters establish populations throughout the Bay, the risk that they would not provide ecosystem services similar to those afforded by Eastern oysters is considered to be low. Ecological services considered in this ERA were those associated with provision of reef habitat for other Bay species, provision of food for other Bay species, and filtration capacities of both oyster species. If the proposed introduction were successful, Suminoe oysters would be expected to populate historical oyster habitat and other hard substrates in the subtidal zone. Because the Suminoe oyster can tolerate high loads of suspended sediment and exist in muddy systems (albeit on shell), reefs of the species could provide localized benefits for SAV by buffering the action of waves and currents and by filtering suspended solids from the water. Both oyster species are expected to filter the same kinds of algae and suspended matter from the water. Reefs of the Suminoe oyster would provide habitat for other species; however, no studies have investigated if the small-scale structure of reefs of Suminoe oyster or mixed-species reefs would attract and support the same biological community that reefs of the Eastern oyster do. As already discussed, the Suminoe oyster does not appear likely to overgrow soft bottom areas. If the Suminoe oyster were to expand into soft-bottom areas, however, that expansion could begin to compensate for the significant loss of hard-bottom habitat that has occurred in recent decades (approximately 70% loss over the past 20 years; Attachment A of PEIS Appendix A). Based on the results of the research conducted to date and summarized earlier in this ERA, the presence of a self-sustaining population of Suminoe oysters in Chesapeake Bay would pose a negligible to low risk of diminishing the level of ecosystem services provided by oysters. The proposed introduction would be likely to increase services in proportion to the magnitude of increase in the biomass of oysters in the Bay.

The level of uncertainty associated with this conclusion is considered moderate. The uncertainty is related to inadequate understanding of all of the many and varied ways in which oysters interact with other components of the Bay ecosystem, as well as lack of knowledge about the characteristics of Suminoe oyster reefs or mixed-species reefs in open waters of Chesapeake Bay. Although species interactions are considered the most important mechanisms by which changes in the abundance or kind of oysters in the Bay could influence other receptors, many of the specific details of these interactions are not well known or quantified. Uncertainty increases with the number of linkages between ecological receptors and oysters.

Based on the conclusion that the Suminoe and Eastern oysters are likely to provide similar ecological services in Chesapeake Bay, the extent to which the proposed action would influence ecosystem services in Chesapeake Bay would be a function of the extent to which it resulted in an increase in oyster abundance. As discussed earlier, available data and information and analysis tools are not sufficient to allow prediction of such a result; consequently, the RRM was not employed to evaluate the potential ecological effects of the proposed action. The Sumino oyster's resistance to MSX and Dermo suggests that it may be able to survive and prosper in higher salinity waters in the Bay, where the Eastern oyster is currently most significantly affected by disease. But it appears to be at an adaptive disadvantage to the Eastern oyster in intertidal areas and areas of low dissolved oxygen. The Suminoe oyster also appears to be susceptible to *Bonamia* in the highest salinities, which could limit significant growth of the Suminoe oyster population to mesohaline and oligohaline waters. The likelihood that an introduction would result in expansion and growth of a population of Suminoe oysters throughout the Bay would depend on the relative importance of the positive factors (primarily disease resistance and higher growth rate) and negative factors (primarily susceptibility to predation, competition with the Eastern oyster, and continuing loss of hard-bottom habitat) that could influence the species in Chesapeake Bay. If the effects of the positive factors were to be much greater than the effects of the negative factors, the proposed action would likely result in a substantial increase in ecological services in the Chesapeake Bay. These would include the services related to providing food and habitat, the buffering of SAV and shorelines against waves and currents, and water quality benefits primarily related to an increase in water clarity. Influences would be greatest at the scale of individual segments. Oysters would have the greatest effects within shallow semi-enclosed bays, as was discussed with respect to water quality. The habitat provided by oysters and their influences on algae, SAV, and water quality will affect the other ecological receptors, including the fish and wildlife of the Bay. The relative degree of influence that the Suminoe oyster has on other ecological receptors is expected to be proportional to changes in oyster biomass in a manner similar to the influences portrayed for the native oyster. These influences are mainly positive. There can be some negative influences associated with reducing the biomass of algae. These include negative influences on species that rely on planktonic algae for food. For the scale of anticipated reductions, these negative influences on algae biomass will have positive influences on other ecological receptors that use SAV.

4.4.3 Ecosystem Services for Alternative 1 Based on RRM Evaluation

This alternative involves continuing Maryland's oyster restoration and repletion programs, and Virginia's oyster restoration program using current resource management policies, available funding, and the best available restoration strategies and stock assessment techniques. The positive and negative influences on various ecological receptors are illustrated in Figure 4-17 for the base analysis (50th percentile of biomass estimates) and Figure 4-18 (95th percentile of biomass estimates). The largest positive influence is in the MD OH zone. The negative values for MD MH, VA OH, and VA PH are associated with declines of oysters under Alternative 1 for the 50th percentile biomass model estimates. Even greater declines would occur if the 5th percentile value were used for estimating future oyster abundance and biomass. This alternative resulted in small influences (RRM values of 0.1 or -0.1) for benthic soft-bottom, SAV, phytoplankton, zooplankton, and fish and wildlife species that depend on these for food or habitat. Larger influences were observed for species that depend on oysters for food or habitat according to the association values given in Table 3-2.

Figure 4-18, which reflects the 95th percentile of oyster biomass 10 years after implementation of the alternative, predicts positive influences related to increases in oysters for all state/salinity zones. These positive influences would be greatest in the MD OH and MD MH zones. Figures 4-17 and 4-18 reflect influences that are directly related to increases or decreases in oysters.

In addition to the association values provided in Table 3-2, influences on fish and wildlife species that depend on oysters directly or indirectly for food and habitat were evaluated using a second set of association values (Table 3-3) that were derived using a median value of the bottom area (0.03) that oysters occupy relative to total areas within the Chesapeake Bay segments. The results of using this second set of association values are provided in Figures 4-19 and 4-20 for the modeled estimates of the 50th and 95th percentiles of oyster biomass. Oysters and reef-oriented fish are excluded from these figures because their values are the same as those displayed in Figures 4-17 and 4-18, and excluding them from the figures improves the visibility of the smaller influences on other ecological receptors. Scales for display of the stacked histograms were changed (from 30 to 5) to help the reader see the RRM scores for the other receptors.

Figures 4-19 and 4-20 reflect small positive and negative influences of increasing and decreasing oyster biomass on ecological receptors. These all have individual RRM scores of less than 1.

4.4.4 Ecosystem Services for Alternatives 2a and 2b Based on RRM Evaluation

Alternatives 2a and 2b involve expanding, improving, and accelerating Maryland's oyster restoration and repletion programs and Virginia's oyster restoration program in collaboration with Federal and private partners. This work would include an assessment of cultch limitations and long-term solutions for this problem and the development, production, and deployment of large quantities of disease resistant strain(s) of *C. virginica* to enhance the brood stock. Alternatives 2a and 2b differ in magnitude of effort. The former places more restoration effort in areas of low salinity, and the latter places more effort in areas of medium to high salinity.

4.4.4.1 Alternative 2a

Figures 4-21 and 4-22 present RRM results for Alternative 2a for the 50th and 95th percentile biomass estimates. These figures illustrate positive and negative influences that are directly related to increases and decreases in oyster biomass. The species with visibly evident RRM scores in these figures all depend directly or indirectly on oysters for food or habitat. The largest responses are evident in MD OH, MD MH, and VA MH. Ecological receptors that could be negatively influenced by increases in oyster biomass include species in benthic soft-bottom, phytoplankton, and zooplankton communities, and fish and wildlife that depend on these for food. As indicated in the figures, the negative or positive influences on these ecological receptors are small (i.e., they are not visible in the histograms). This is most obvious in Figure 4-22, which reflects the 95th percentile oyster biomass. Responses for the 5th percentile are simply smaller than those for the 50th percentile.

Results using the second set of association values that reflect the area of oyster coverage (from Table 3-3) are shown in Figures 4-23 and 4-24. The figures show results for ecological receptors other than oysters and reef-oriented fish to highlight the small changes in these other groups. The scores for oysters and reef-oriented fish are the same as in Figures 4-21 and 4-22. All the influences on the groups of ecological receptors are small (less than 1). Although very small, the negative influence that increasing oyster biomass has on phytoplankton and animals that depend on phytoplankton can be seen within the MD OH zone. This type of negative influence is also evident for VA MH for the 95th percentile of biomass estimates for oysters. The RRM scores for these individual groups of receptors are -0.1, which reflect influences of 1% to 10%.

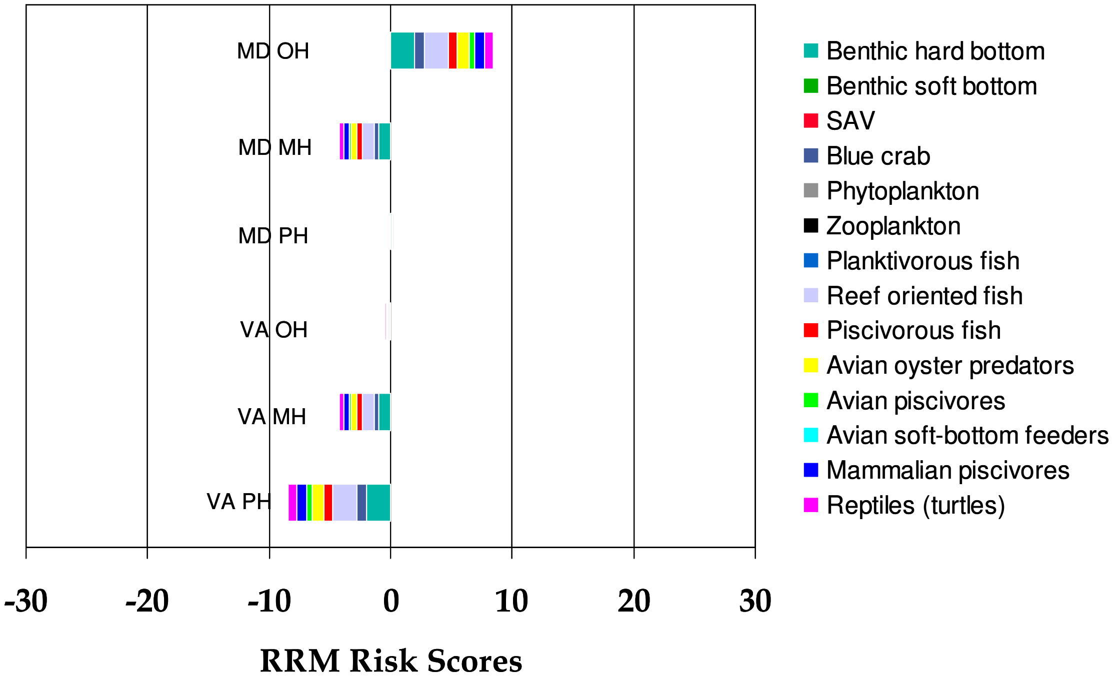


Figure 4-17. RRM risk scores for Alternative 1, 50th percentile oyster biomass

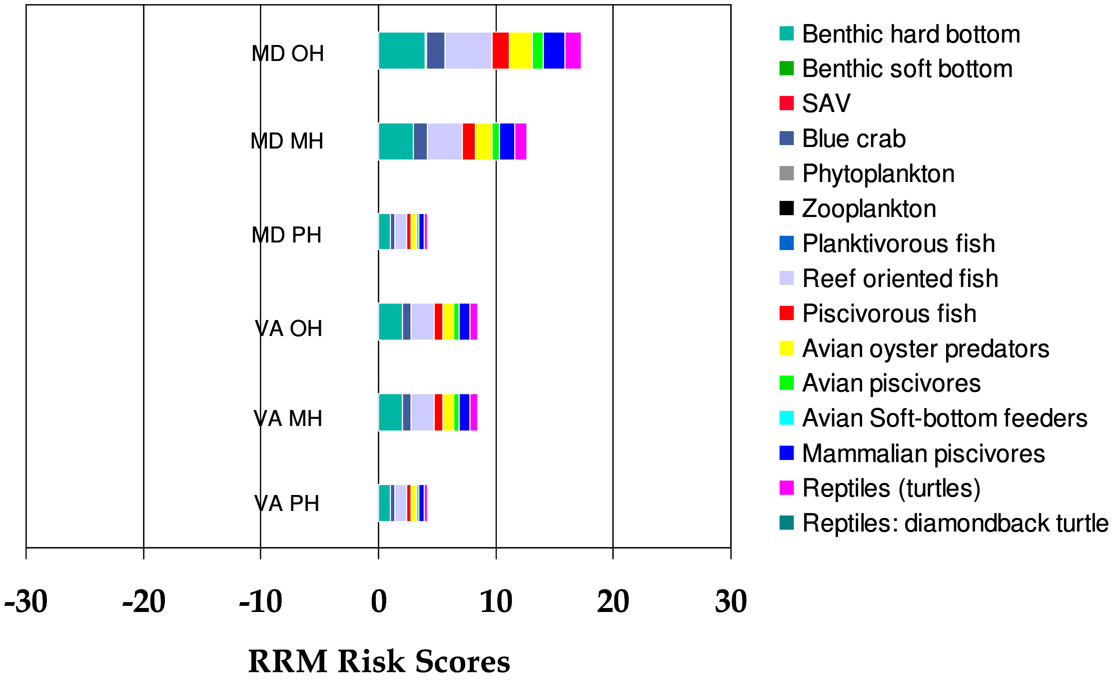


Figure 4-18. RRM risk scores for Alternative 1, 95th percentile oyster biomass

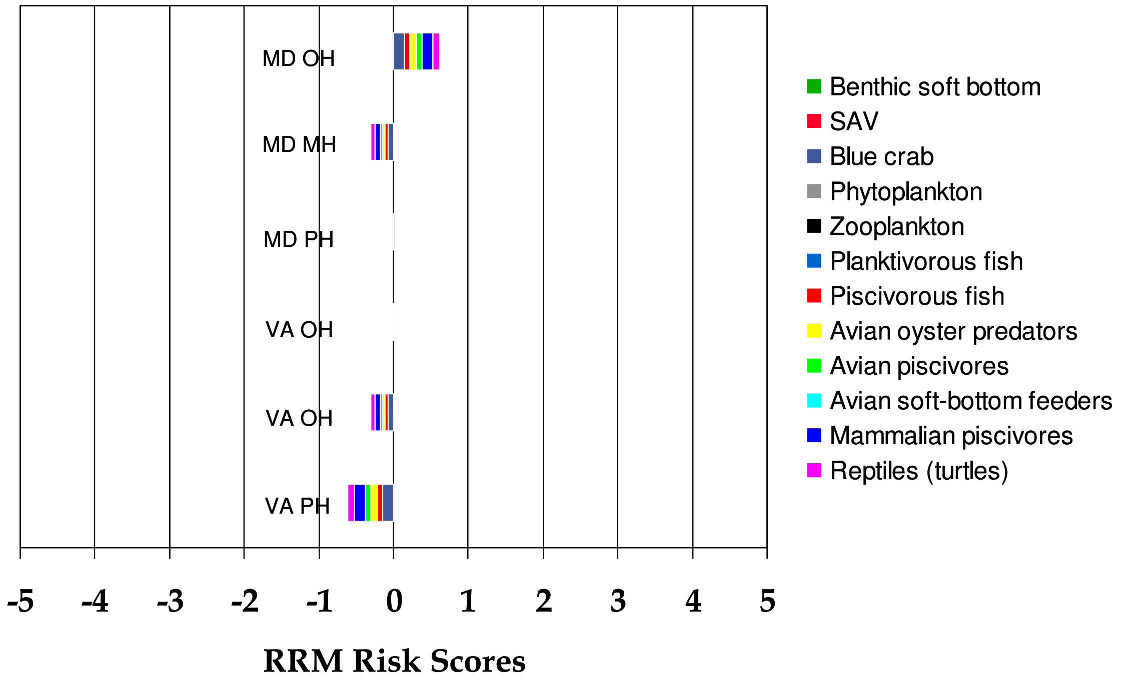


Figure 4-19. RRM risk scores for Alternative 1, 50th percentile oyster biomass and association factor 0.03

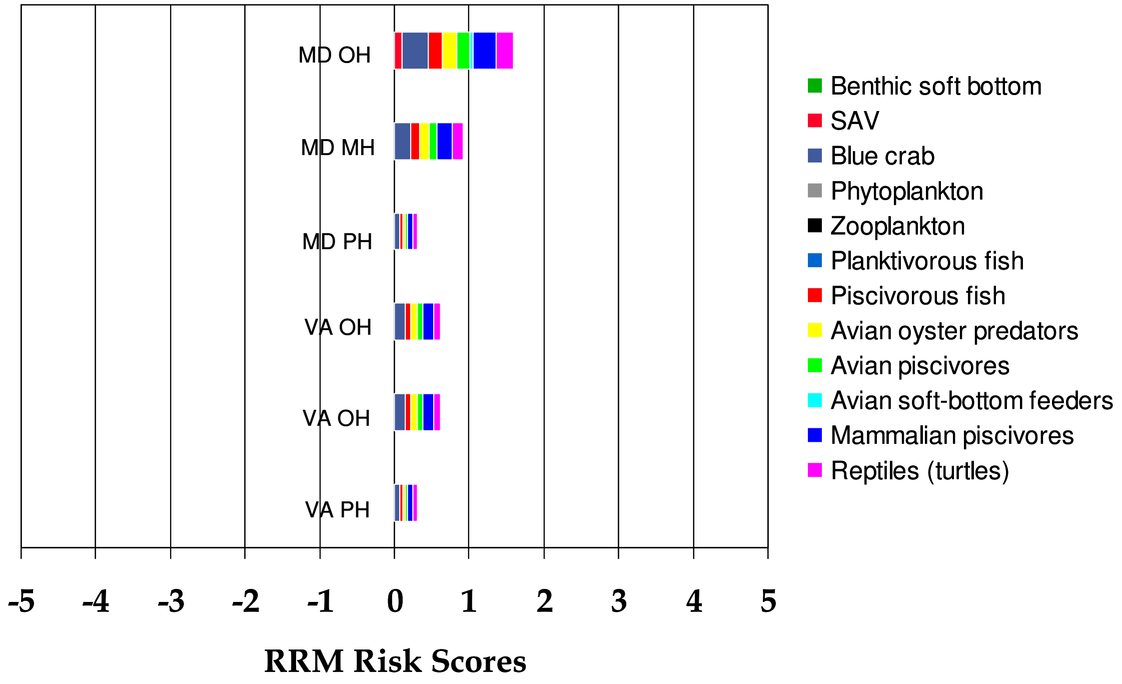


Figure 4-20. RRM risk scores for Alternative 1, 95th percentile oyster biomass and association factor 0.03

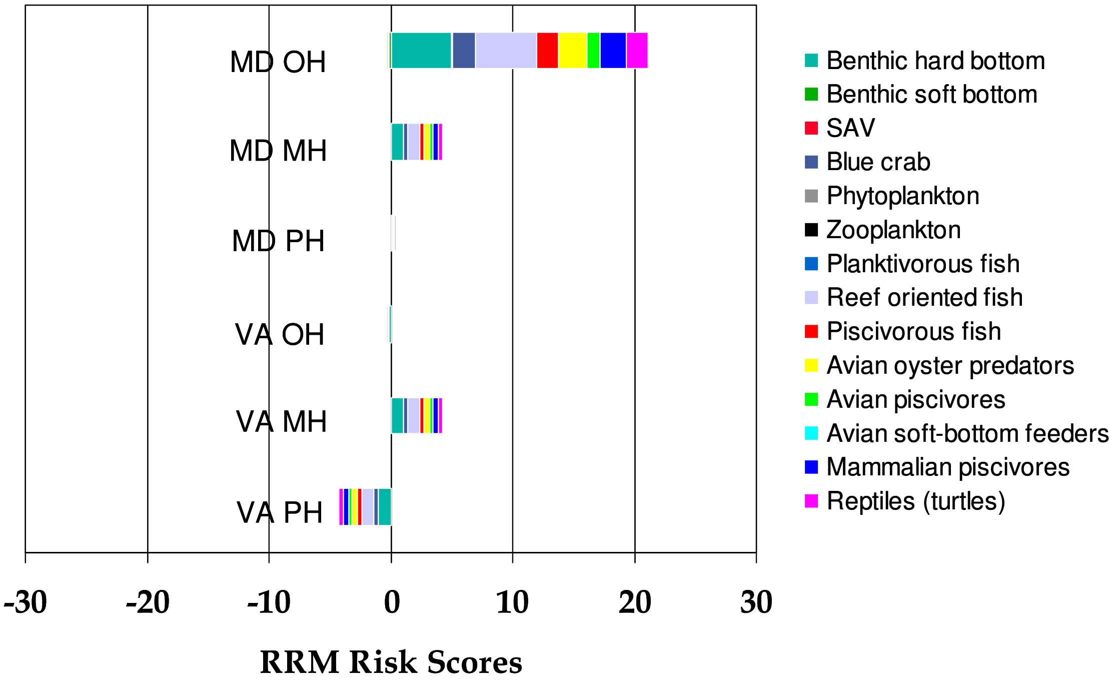


Figure 4-21. RRM risk scores for Alternative 2a, 50th percentile oyster biomass

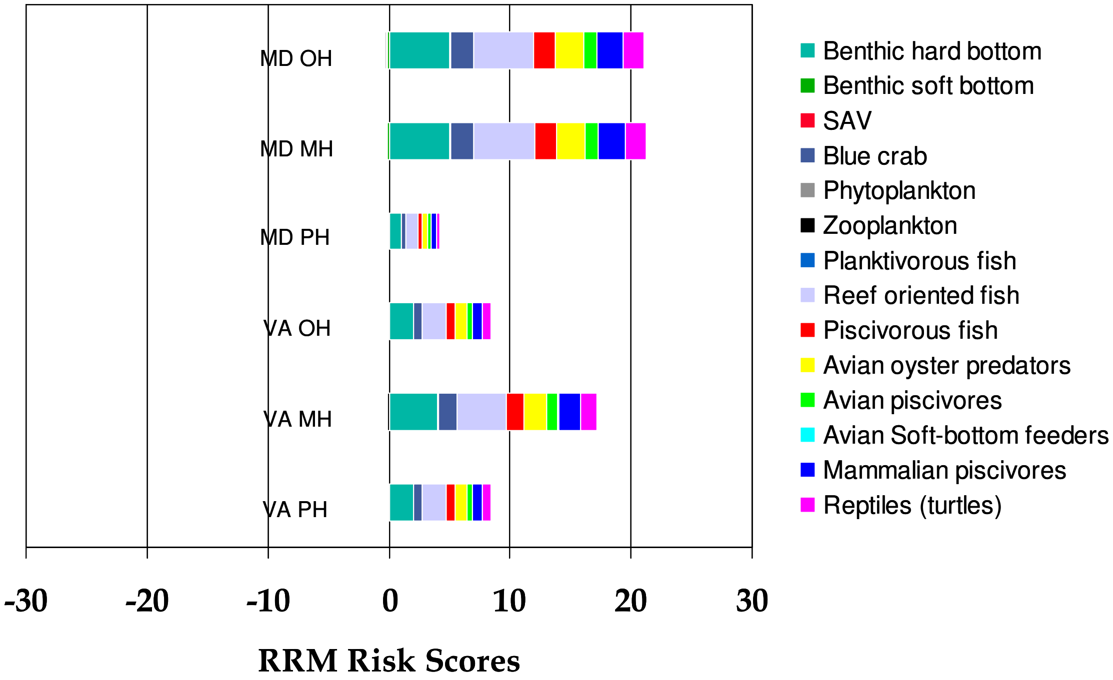


Figure 4-22. RRM risk scores for Alternative 2a, 95th percentile oyster biomass

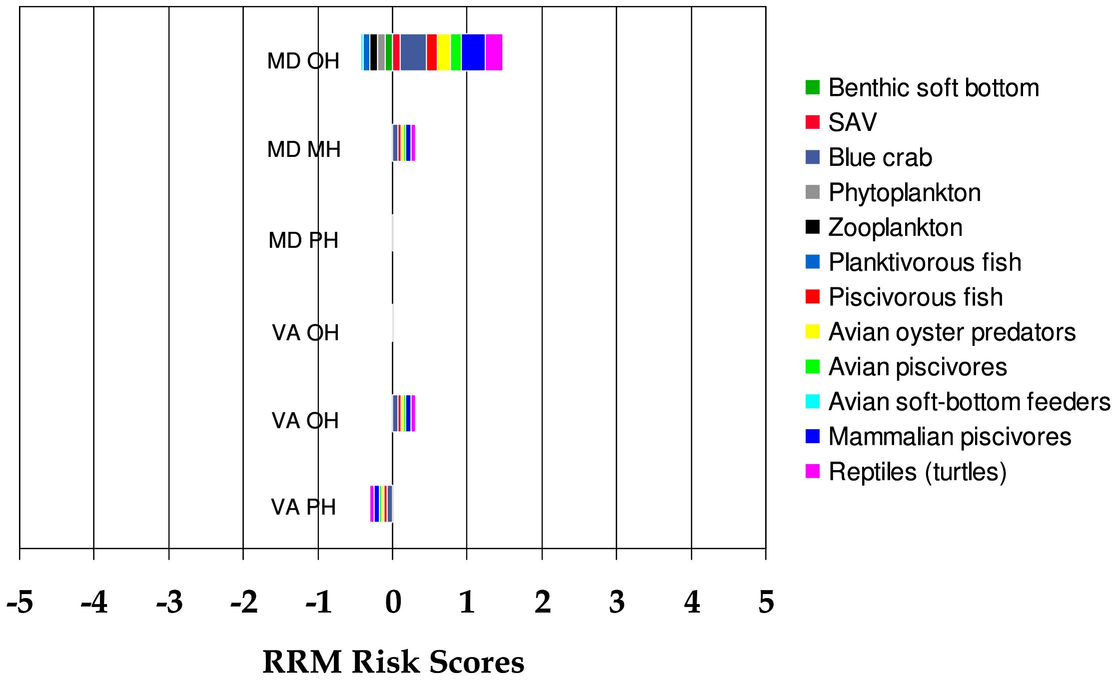


Figure 4-23. RRM risk scores for Alternative 2a, 50th percentile oyster biomass and association factor 0.03

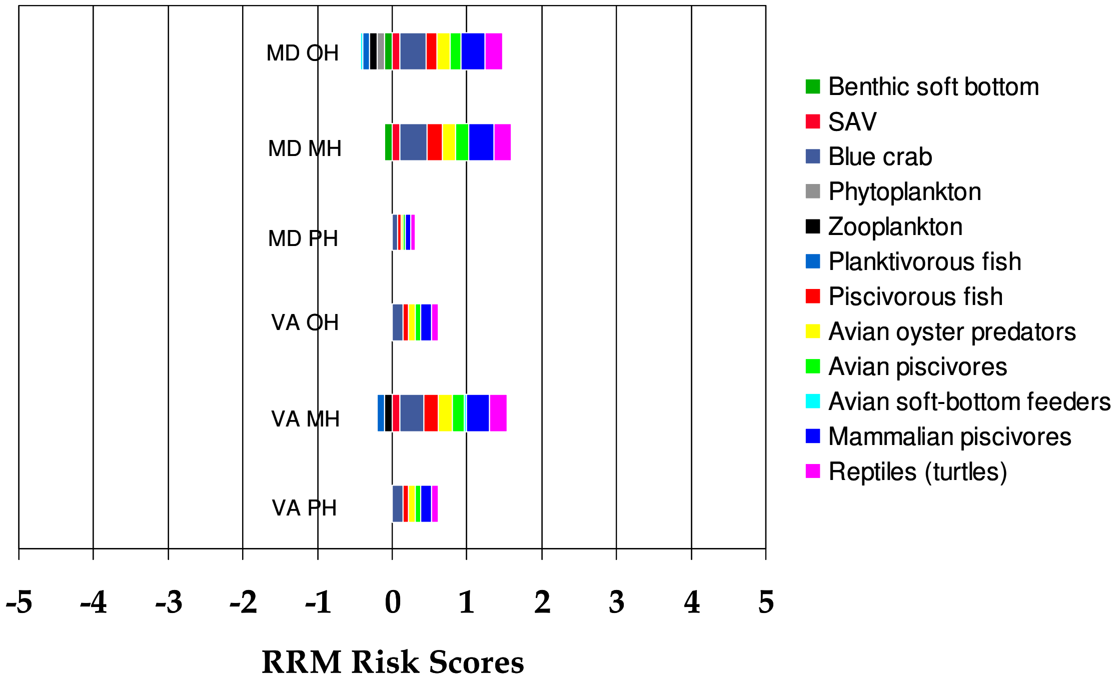


Figure 4-24. RRM risk scores for Alternative 2a, 95th percentile oyster biomass and association factor 0.03

4.4.4.2 Alternative 2b

Figures 4-25 and 4-26 present RRM results for Alternative 2b for the 50th and 95th percentile biomass estimates using the association factors in Table 3-2. These illustrate positive and negative influences that are directly related to increases and decreases in oyster biomass. The largest responses are in MD OH, MD MH, and VA MH. Results using the second set of association factors (Table 3-3) are shown in Figures 4-27 and 4-28. These figures reveal the small negative influence that increasing oyster biomass could have on phytoplankton and the animals – other than oysters - that rely on phytoplankton for food.

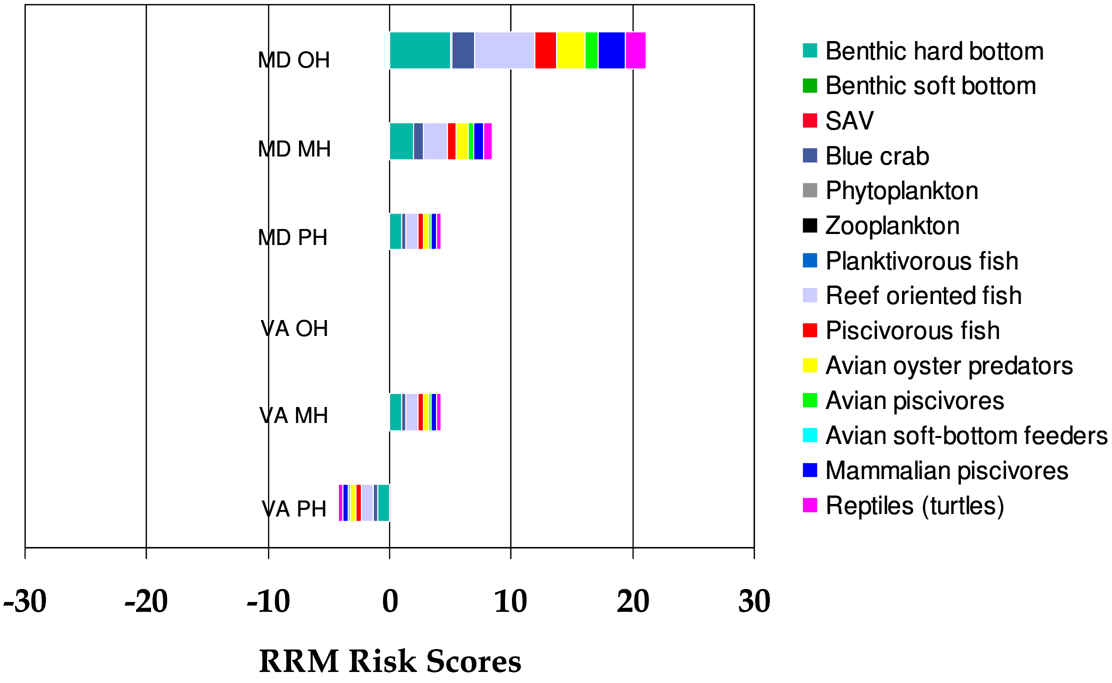


Figure 4-25. RRM risk scores for Alternative 2b, 50th percentile oyster biomass

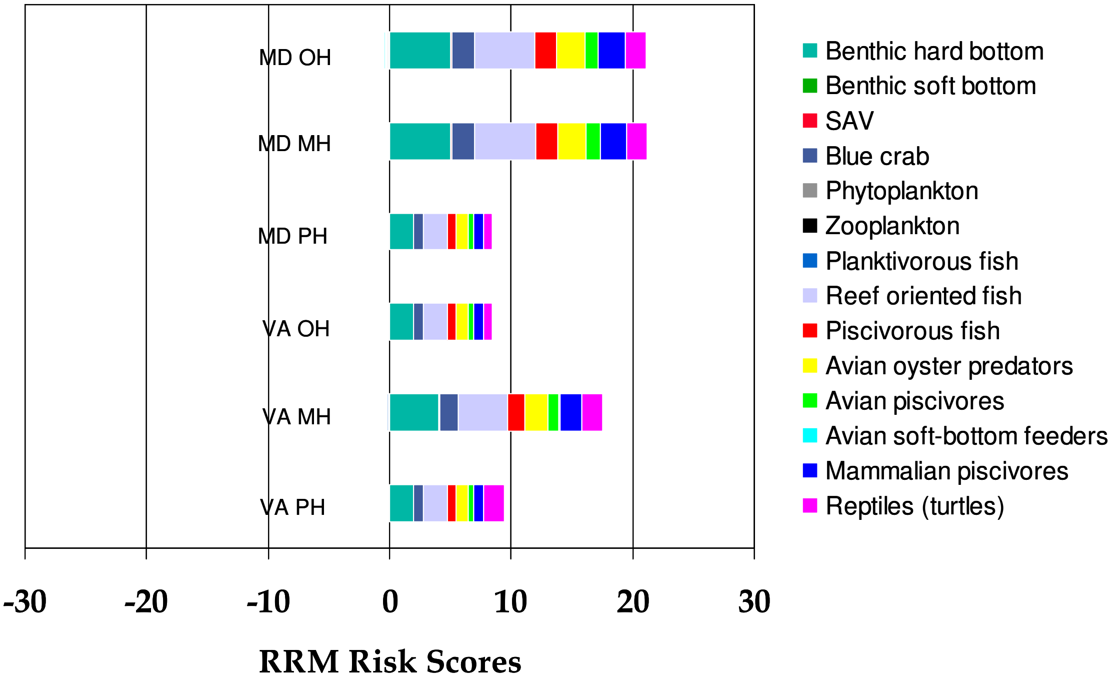


Figure 4-26. RRM risk scores for Alternative 2b, 95th percentile oyster biomass

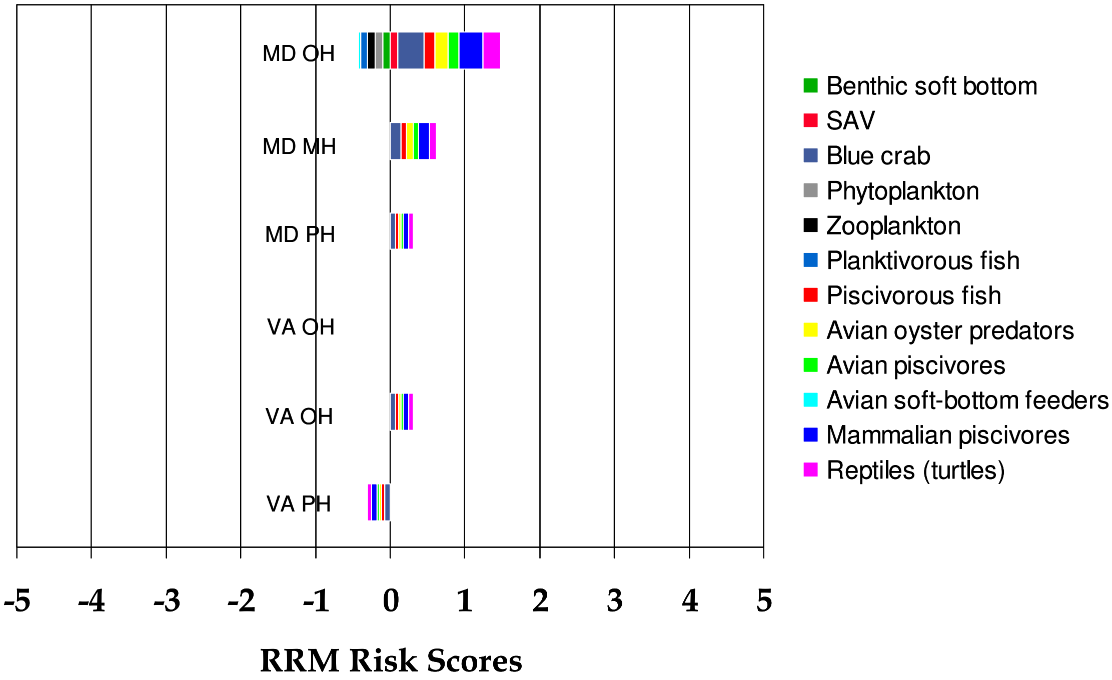


Figure 4-27. RRM risk scores for Alternative 2b, 50th percentile oyster biomass and association factor 0.03

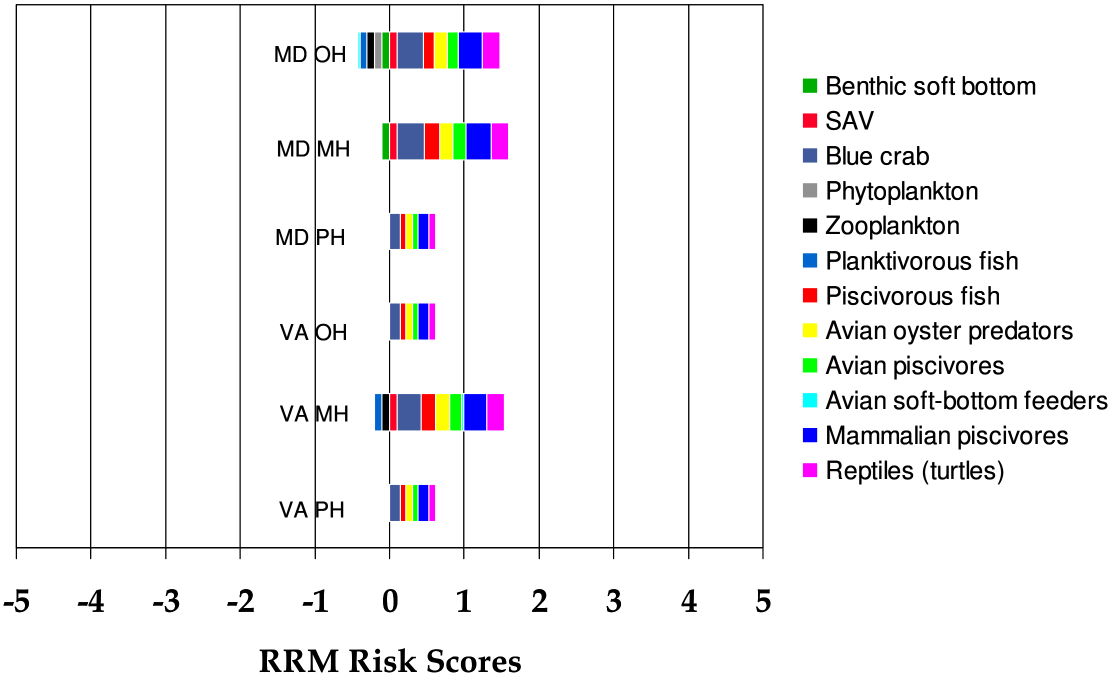


Figure 4-28. RRM risk scores for Alternative 2b, 95th percentile oyster biomass and association factor 0.03

4.4.5 Ecosystem Services for Alternative 3 Based on RRM Evaluation

Alternative 3 involves implementing a temporary harvest moratorium on native oysters and a compensation (buy-out) program for participants in the oyster industry in Maryland and Virginia, or a program under which displaced oystermen are offered on-water work in a restoration program. Most influences track directly and positively with changes in oyster biomass (Figures 4-29 through 4-32). Small (fractional) negative influences on the planktonic food web are indicated for MD OH and VA OH for estimated 95th percentile biomass with the smaller set of association factors (Figure 4-32).

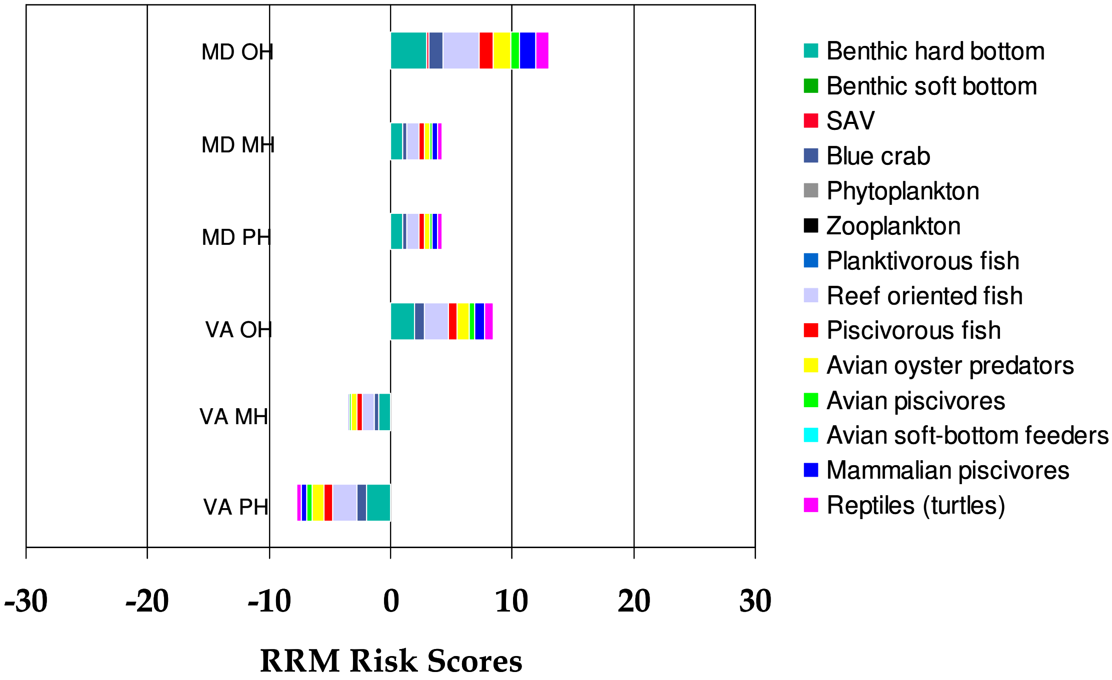


Figure 4-29. RRM risk scores for Alternative 3, 50th percentile oyster biomass

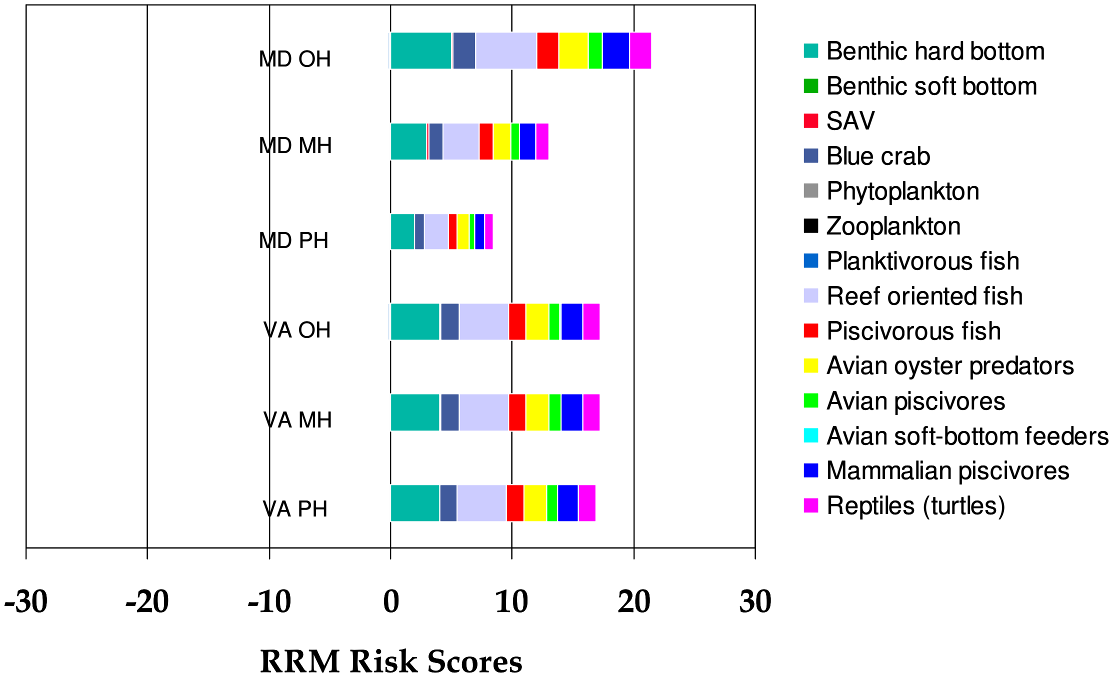


Figure 4-30. RRM risk scores for Alternative 3, 95th percentile oyster biomass

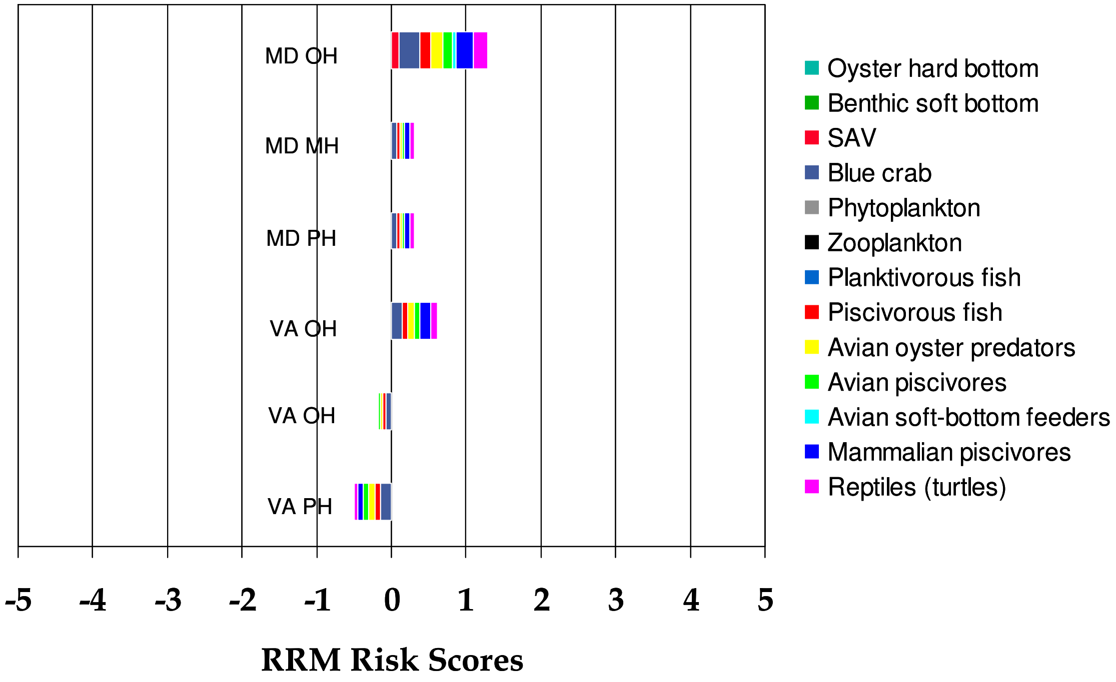


Figure 4-31. RRM risk scores for Alternative 3, 50th percentile oyster biomass and association factor 0.03

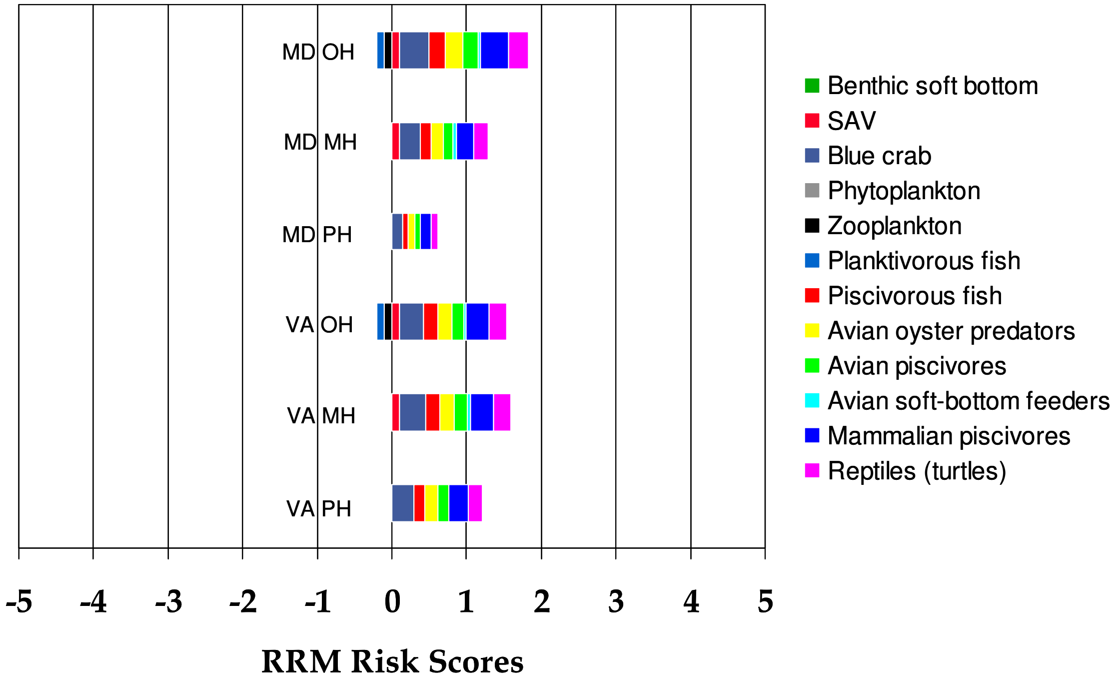


Figure 4-32. RRM risk scores for Alternative 3, 95th percentile oyster biomass and association factor 0.03

4.4.6 Ecosystem Services for Alternatives 4 and 5 Based on RRM Results

The RRM was used to examine how the aquaculture alternatives might influence other ecological receptors in the Bay. Because both alternatives involve estimates of cultivated biomass, they are combined here. Aquaculture of triploid *C. ariakensis* (Alternative 5) is evaluated further in Section 4.3 with respect to the potential for releasing diploids into the Bay at large. Additional information about the ecological effects of intensive aquaculture operations is presented in Attachment D.

The effects of aquaculture on ecological receptors are expected to differ depending on how the aquaculture is implemented. On-bottom, unconfined operations would enhance hard-bottom habitat and the receptors that depend on it. Confined aquaculture in off-bottom cages might contribute some additional habitat, whereas confined aquaculture in floats would provide less. We assumed that all aquaculture operations for *C. ariakensis* (Alternative 5) would be confined and that most operations would be in cages on or near the bottom. We assumed that aquaculture operations for *C. virginica* (Alternative 4) would involve unconfined placement on the bottom. This would make the oysters available for temporary habitat and/or food; however, the cultivated area would be disturbed periodically to harvest. For the purpose of this assessment we considered the maximum influences that oysters might have on other ecological receptors. To this end, we treated oysters in aquaculture in two ways. First, we treated cultivated oysters in the same way as natural populations and used the association factors from Table 3-2 (Figure 4-33). We expect that the ecological influences of these oysters would be less than those associated with natural populations. Second, we assumed that these oysters would have a negligible direct influence as habitat or food but that they would offer filtration capacity and the indirect effects associated with this biological function. The influences associated with this second set of assumptions are illustrated in Figure 4-34.

The results show that if cultivated oysters provide habitat and food, the largest influences would be in the Virginia oligohaline and polyhaline zones (Figure 4-33). These influences reflect the greater expected aquaculture in those zones. If the direct effect of the habitat and food provided by cultivated oysters is assumed to be negligible, the filtration capacity of the oysters would have small influences on other ecological receptors in the Virginia oligohaline and polyhaline zones (Figure 4-34).

4.4.7 Ecosystem Services for Alternatives 6 and 7 Based on RRM Results

Alternative 6 involves introducing and propagating a nonnative species other than C. *ariakensis* in the tidal waters of Maryland and Virginia. Alternative 7 involves introducing *C. ariakensis* and discontinuing efforts to increase the abundance of *C. virginica*. These introductions would be conducted in accordance with the Code of Practices on the Introductions and Transfers of Marine Organisms 1994 (ICES 1995).

No demographic modeling was conducted for these alternatives; however, insights can be gained by reviewing Section 4.2.

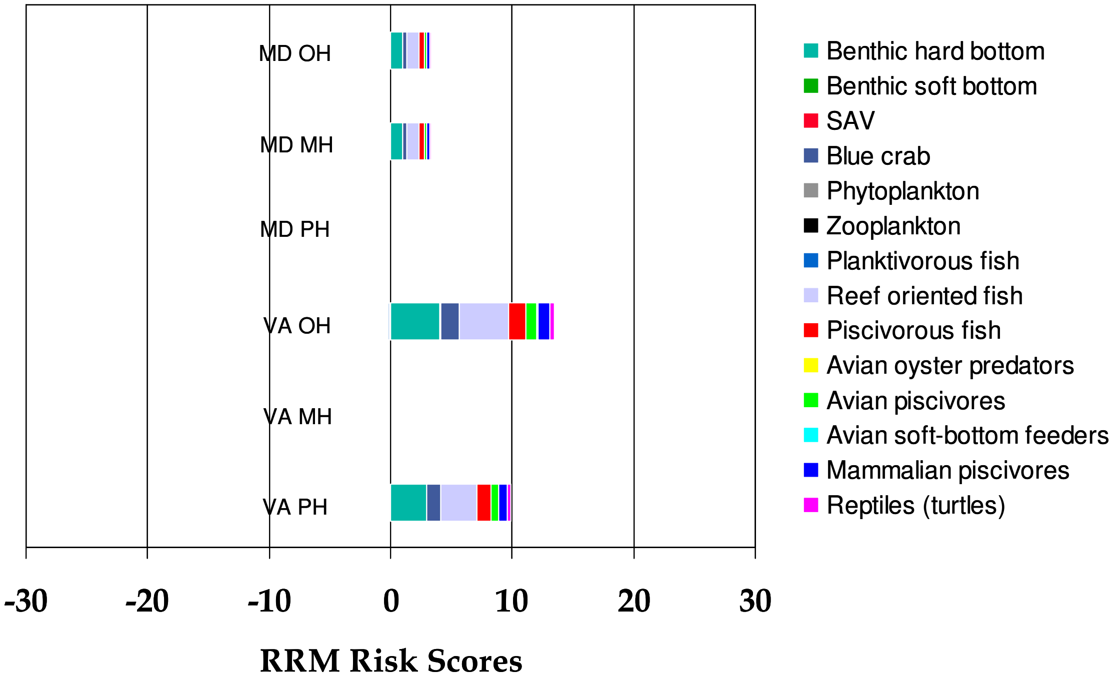


Figure 4-33. RRM scores for Alternatives 4 and 5 assuming that cultivated oysters provide habitat and food (except for bird and mammal predators of oysters) comparable to natural oysters and using the association factors from Table 3-2.

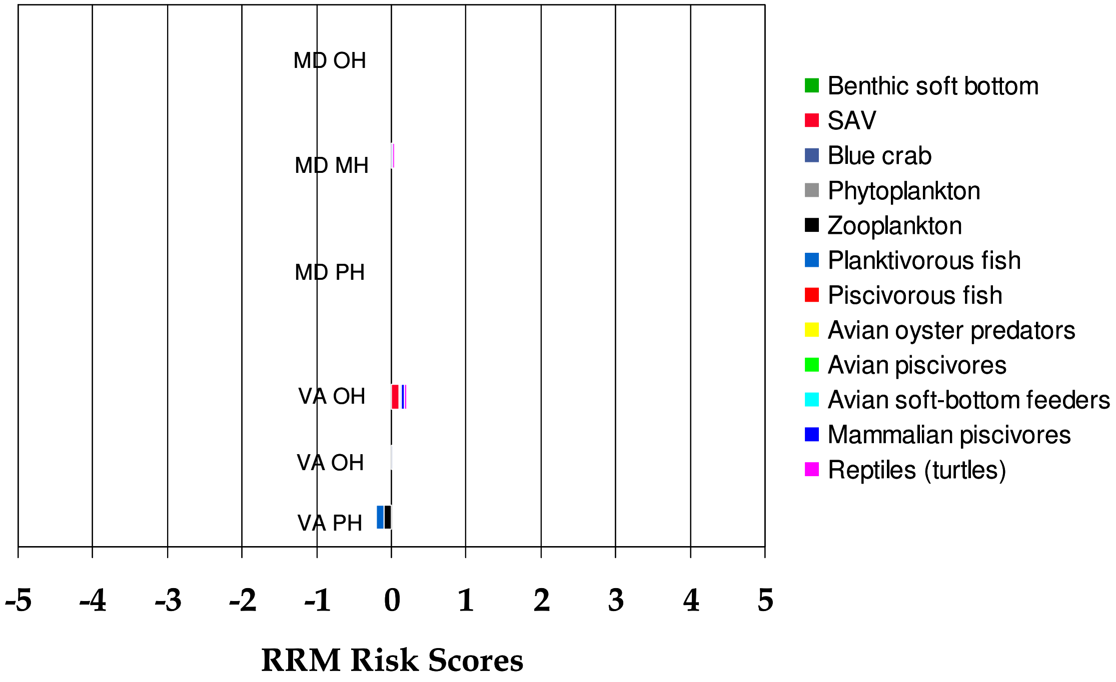


Figure 4-34. RRM scores for Alternatives 4 and 5, assuming that cultivated oysters provide negligible direct habitat or food for other ecological receptors.

4.4.8 Comparison of Alternatives for State/Salinity Zones

Figures 4-35 to 4-40 compare the ecological influences of the alternatives for each state/salinity zone based on the RRM results. These figures are based on 50th percentile estimates of oyster biomass. The figures also include ecological influences associated with hypothetical introductions of a species that can grow throughout the Bay unencumbered by diseases (shown as Scenarios 1 and 2). These comparative scenarios are not the expected outcomes for *C. ariakensis* because the demographics of that species within Chesapeake Baycannot be modeled reliably at this time. Reef-dependent fish were excluded from these figures to enable greater visualization of influences on other ecological receptors. Influences on reef-dependent fish are proportional to changes in oyster biomass. The second set of fish and wildlife association values (Table 3-3) was used to derive RRM scores for these figures to enable the reader to visualize these influences along with the small influences on benthic soft-bottom, SAV, phytoplankton, and zooplankton that are derived from the CBEMP model runs.

The largest increases or decreases are related to the change in the benthic hard-bottom community, for which oyster is the representative species. Introducing an oyster that has the vital characteristics reflected in Scenarios 1 and 2 (less mortality and a faster growth rate) was the only strategy that provided positive influences in all state/salinity zones. All other positive and negative influences are small. For the MD OH zone, the restoration alternatives (2a and 2b) yielded outcomes similar to those that might be achieved by introducing a different oyster species. Positive responses for the benthic hard-bottom community were less evident in the MD MH and MD PH zones. The harvest moratorium (Alternative 3) had small positive influences in VA OH and VA MH waters, and restoration efforts had a small positive influence in VA MH waters. None of the alternatives other than introduction of an oyster with the vital characteristics reflected in Scenarios 1 and 2 resulted in positive influences in polyhaline waters of Virginia.

Alternatives that resulted in order-of-magnitude increases in the benthic hard-bottom community also resulted in small positive influences on SAV, blue crabs, and piscivorous fish and wildlife. Small negative influences are all associated with reductions in phytoplankton and the influence this would have on zooplankton, planktivorous fish, and benthic soft-bottom invertebrates. All these potential negative influences are small (i.e., RRM scores of less than 1).

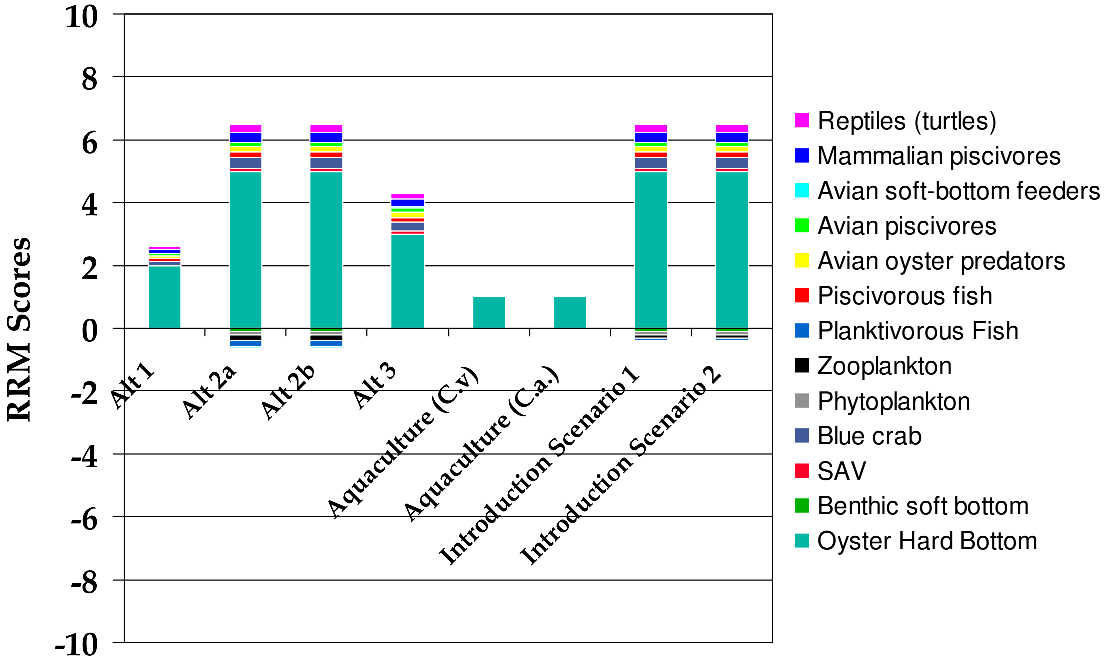


Figure 4-35. Comparison of alternatives for the MD OH zone

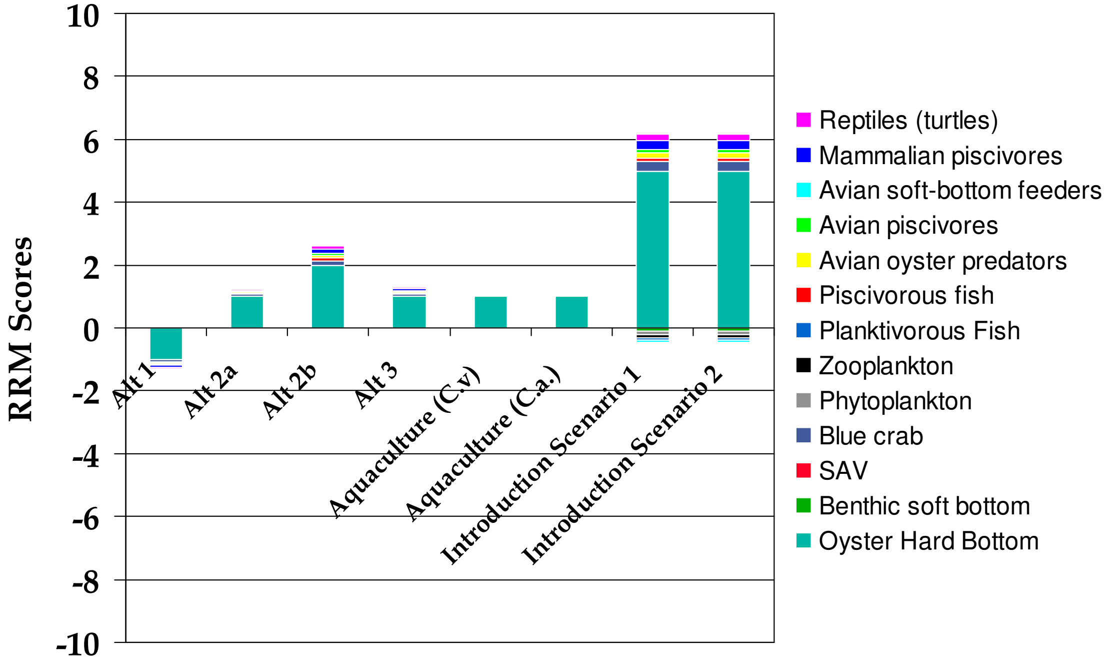


Figure 4-36. Comparison of alternatives for the MD MH zone

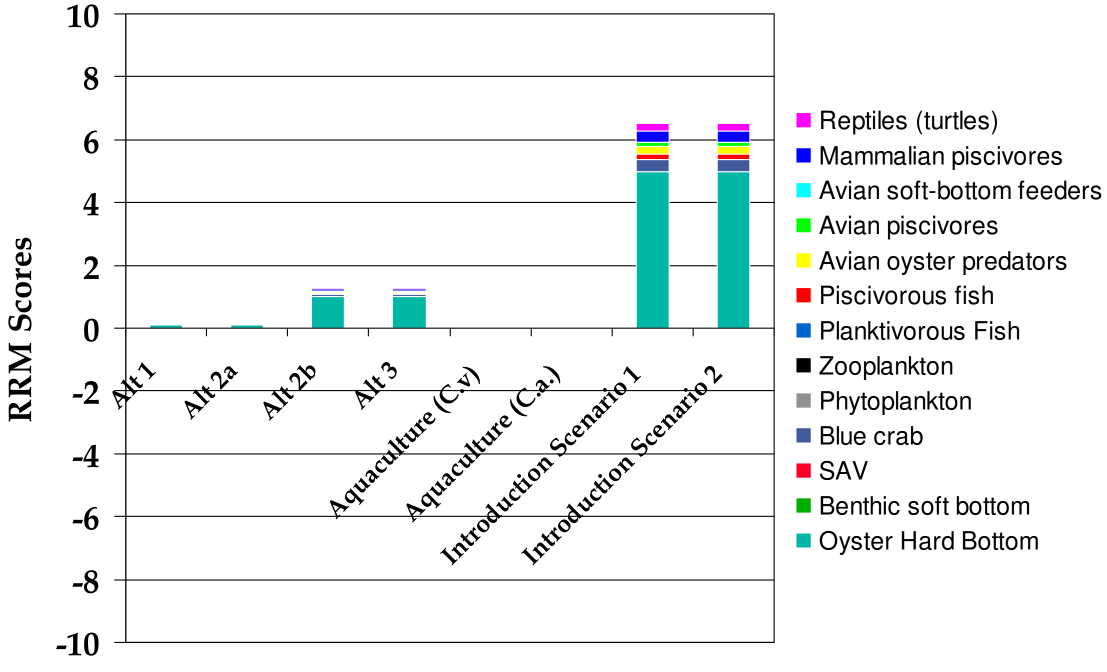


Figure 4-37. Comparison of alternatives for the MD PH zone

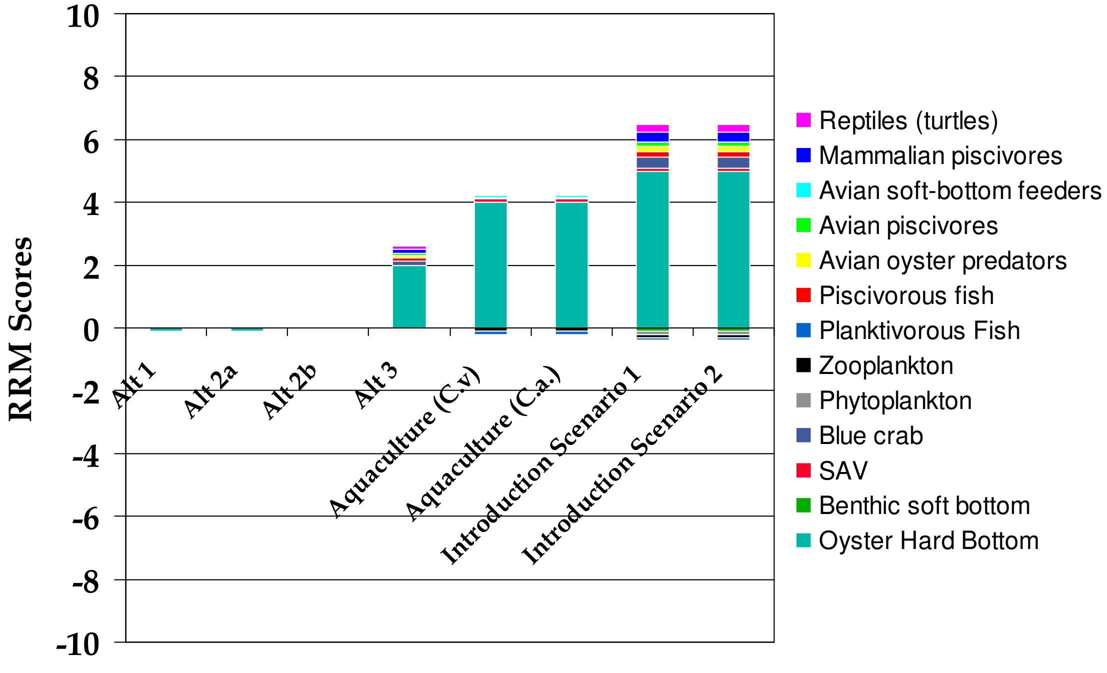


Figure 4-38. Comparison of alternatives for the VA OH zone

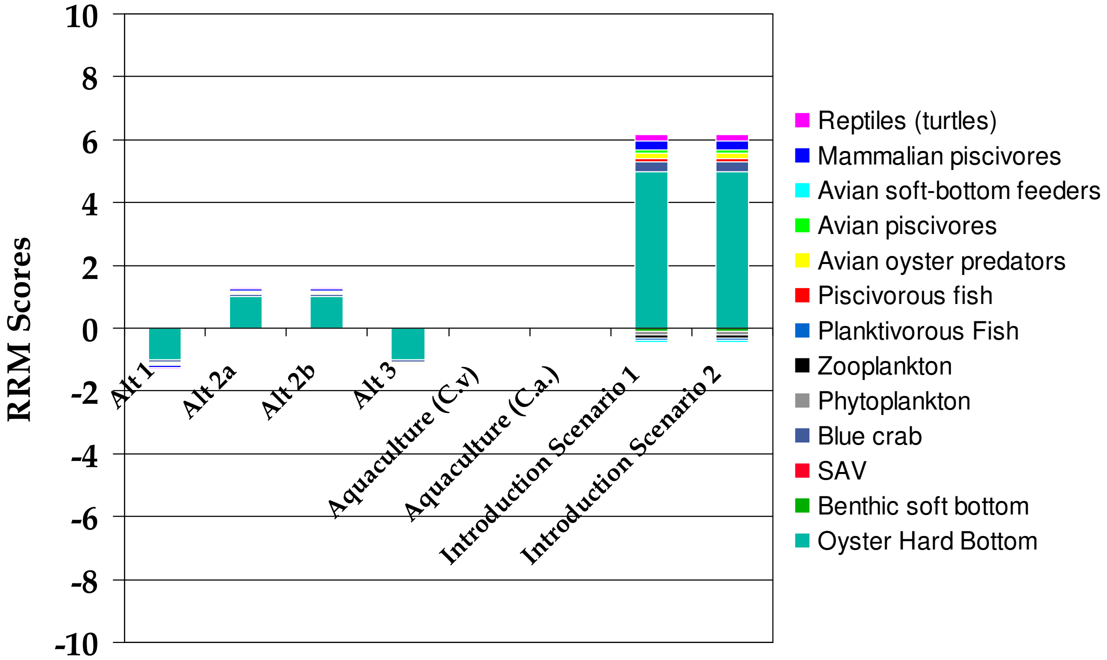


Figure 4-39. Comparison of alternatives for the VA MH zone

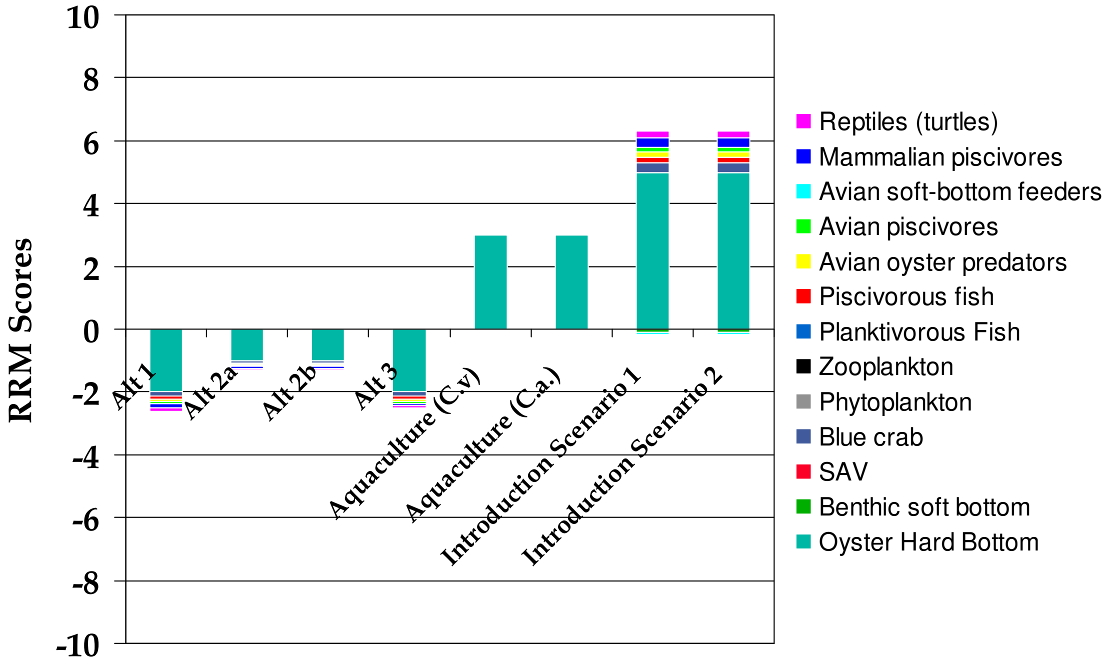


Figure 4-40. Comparison of alternatives for the VA PH zone

4.4.9 Summary of RRM Analyses

The RRM was used to examine the relative positive and negative influences that the alternatives could have on ecological receptors and water quality in Chesapeake Bay. Tables 4-3 and 4-4 (based on 50th percentile modeled biomass) and 4-5 and 4-6 (based on 95th percentile modeled biomass) provide a simplified overview of the RRM results in the decision matrices that were developed to support the overall EIS. Separate tables were developed for Maryland and Virginia because it is important to display the differences between the states in the expected outcomes of the alternatives. The tables display values derived for different degrees of association between oysters and selected species of fish and wildlife. When RRM values ranged over more than one significant digit among the salinity zones within a state's waters, the lower and upper values of that range are displayed for the alternative. When the RRM scores did not vary much across salinity zones, a single value is shown. Cells are color-coded to facilitate comparisons across alternatives. The colors indicate small, moderate, and large positive or negative influences.

Restoration alternatives generally resulted in more success in Maryland than in Virginia. As a result, positive influences on fish and wildlife were greater in Maryland waters. Under some alternatives, oysters declined in Virginia waters resulting in small negative influences on ecological receptors that benefit from oysters.

RRM scores differ between the sets of calculations based on the 50th and 95th percentiles of modeled oyster biomass in some ways. For Maryland waters, the 95th percentile biomass values produced more positive RRM scores (Table 4-5) than 50th percentile biomass values (Table 4-3). This difference reflects the generally positive effect of increasing oyster biomass on most ecological receptors. This is also true for Virginia waters (Tables 4-4 and 4-6).

The matrices can be used to describe risks with respect to the various assessment end-points:

- **Phytoplankton** - Increases in oyster abundance and biomass are expected to have, at most, a small influence on phytoplankton biomass. Increases in oyster abundance and biomass over the ranges considered in this ERA could result in a small negative influence on phytoplankton.
- **Zooplankton** - Increases in oyster abundance and biomass are expected to have, at most, a small influence on zooplankton populations. Increases in oyster abundance and biomass over the ranges considered in this ERA could result in a small negative influence on zooplankton.
- **Planktivorous fish** - Increases in oyster abundance and biomass are expected to have, at most, a small influence on planktivorous fish as a result of influences on either phytoplankton or zooplankton. Increases in oyster abundance and biomass over the ranges considered in this ERA could result in a small negative influence on planktivorous fish.

Table 4-3. RRM results for ecological receptors in Maryland at 50th percentile biomass

Maryland Waters

Decision Matrix Related to Ecological Risks (Positive and Negative Influences)

Ecological Receptors	**Low mortality Bay-wide**		**Alternative 1**		**Alternative 2(a)**		**Alternative 3**		**Alternative 4**		**Alternative 5**		**Alternative 8 (2 or 3)**	
Benthic hard bottom (*C. virginica*)	**See Text**		-1	2	0.1	5	1	3	**not natural**				0.1	5
Benthic hard bottom	5										**not natural**			
Benthic soft bottom	-0.1	0	0		-0.1	0	0		0		0		-0.1	0
SAV	0	0.1	0		0.	0.1	0	0.1	0		0		0.	0.1
Blue crab	1.9		-0.4	0.8	0.04	1.9	0.4	1.2	0	0.4	0	0.4	0.04	1.9
Blue crab assuming lower association with oysters	0.3		-0.1	0.1	0.1	0.4	0.1	0.3	0		0		0.1	0.4
Phytoplankton	-0.1	0	0		-0.1	0	0		0		0		-0.1	0
Zooplankton	-0.1	0	0		-0.1	0	0		0		0		-0.1	0
Planktivorous fish – phytoplankton	-0.1	0	0		-0.1	0	0		0		0		-0.1	0
Planktivorous fish – zooplankton	-0.1	0	0		-0.1	0	0		0		0		-0.1	0
Reef oriented fish	5		-1	2	0.1	5	1	3	0	1	0	1	0.1	5
Piscivorous fish	1.8		-0.4	0.7	0.04	1.8	0.4	1.1	0	0.1	0	0.1	0.04	1.8
Piscivorous fish assuming lower association with oysters	0.1	0.2	-0.04	0.08	0	0.15	0.04	0.2	0		0		0	0.2
Avian oyster predators	2.3	2.4	-0.5	1	0.02	1.1	0.5	1.4	0		0		0.02	1.4
Avian oyster predators assuming lower association with oysters	0.2		-0.04	0.1	0	0.2	0.03	0.15	0		0		0	0.2
Avian piscivores	1.1		-0.2	0.5	0.02	1.1	0.2	0.7	0	0.1	0	0.1	0.02	1.1
Avian piscivores assuming lower association with oysters	0.1	0.2	-0.03	0.1	0	0.1	0.03	0.1	0		0		0	0.1
Avian soft-bottom feeders	-0.06	0	0		-0.02	0	0	0.04	0		0		-0.02	0.04
Mammalian piscivores	2.2		-0.4	0.9	0.04	2.2	0.4	1.3	0	0.24	0	0.24	0.04	2.2
Mammalian piscivores assuming lower association with oysters	0.3		-0.04	0.1	0.01	0.3	0.07	0.2	0				0.01	0.3
Reptiles (turtles)	1.7		-0.4	0.7	0.03	1.7	0.35	1	0	0.1	0	0.1	0.03	1.7
Reptiles (turtles) assuming a lower association with oysters	0.2		-0.05	0.1	0	0.2	0.05	0.2	0		0		0	0.2
Water Quality	0		0		0		0		0		0		0	

🟥	High negative influence (RRM scores <-3 to -5)
🟧	Moderate negative influence (RRM scores <-1 to -3)
🟫	Low negative influence (RR scores <0 to -1
⬜	No influence (RRM = 0}
🟦	Low positive influence (RRM scores >0 to 1)
🟦	Moderate positive influence (RRM scores >1 to 3)
🟦	High positive influence (RR scores >3 to 5)

Table 4-4. RRM results for ecological receptors in Virginia at 50th percentile biomass

Virginia Waters

Evaluation Criteria: Decision Matrix Related to Ecological Risks (Positive and Negative Influences)

Ecological Receptors	**Low mortality Bay-wide**		**Alternative 1**		**Alternative 2(a)**		**Alternative 3**		**Alternative 4**		**Alternative 5**		**Alternative 8 (4 or 5)**	
Benthic hard bottom (*C. virginica*)	**See Text**		-0.1	-2	-1	1	-2	2	**not natural**				**not natural**	
Benthic hard bottom	5										**not natural**			
Benthic soft bottom	-0.1		0		0		0		0		0		0	
SAV	0	1	0		0		0		0	0.1	0	0.1	0	0.1
Blue crab	1.85	2.45	-0.04	-0.8	-0.4	0.4	-0.8	0.8	0	1.56	0	1.56	0	1.56
Blue crab assuming lower association with oysters	0.3	0.35	-0.01	-0.1	-0.07	0.07	-0.14	0.14	0	0.02	0	0.02	0	0.02
Phytoplankton	-0.1	0	0		0		0		0		0		0	
Zooplankton	-0.1	0	0		0		0		0	-0.1	0	-0.1	0	-0.1
Planktivorous fish – phytoplankton	-0.1	0	0		0		0		0		0		0	
Planktivorous fish – zooplankton	-0.1	0	0		0		0		0	-0.1	0	-0.1	0	-0.1
Reef oriented fish	5		-0.1	-2	-1	1	-2	2	0	4	0	4	0	4
Piscivorous fish	1.8	2	-0.04	-0.72	-0.4	0.4	-0.7	0.7	0	1.5	0	1.5	0	1.5
Piscivorous fish assuming lower association with oysters	0.1	0.2	-0.08	0	-0.04	0.04	-0.08	0.08	0	0.02	0	0.02	0	0.02
Avian oyster predators	2.3	2.45	-0.05	-0.95	-0.5	0.5	-0.95	0.95	0		0		0	
Avian oyster predators assuming lower association with oysters	0.2		-0.09	0	-0.04	0.04	-0.09	0.09	0		0		0	
Avian piscivores	1.1	1.4	-0.02	-.5	-0.2	0.2	-0.5	0.5	0	1	0	1	0	1
Avian piscivores assuming lower association with oysters	0.1		-0.07	0	-0.03	0.03	-0.1	0.1	0	0.02	0	0.02	0	0.02
Avian soft-bottom feeders	-0.06	.34	0		0		0		0	0.04	0	0.04	0	0.04
Mammalian piscivores	2.1	2.5	-0.04	-0.9	-0.4	0.4	-0.9	0.9	0	1	0	1	0	1
Mammalian piscivores assuming lower association with oysters	0.3		-0.01	-0.1	-0.07	0.07	-0.1	0.1	0	0.02	0	0.02	0	0.02
Reptiles (turtles)	1.7	2	-0.03	-0.7	-0.35	0.35	-0.7	0.7	0	0.4	0	0.4	0	0.4
Reptiles (turtles) assuming a lower association with oysters	0.2		-0.1	0	-0.05	0.05	-0.1	0.1	0	0.03	0	0.03	0	0.03
Water Quality	0		0		0		0		0		0		0	

🟥	High negative influence (RRM scores <-3 to -5)
🟧	Moderate negative influence (RRM scores <-1 to -3)
🟧	Low negative influence (RR scores <0 to -1
⬜	No influence (RRM = 0}
🟦	Low positive influence (RRM scores >0 to 1)
🟦	Moderate positive influence (RRM scores >1 to 3)
🟦	High positive influence (RR scores >3 to 5)

Table 4-5. RRM results for ecological receptors in Maryland at 95th percentile biomass

Maryland Waters

Evaluation Criteria: Decision Matrix Related to Ecological Risks (Positive and Negative Influences)

Ecological Receptors	**Low mortality Bay-wide**		**Alternative 1**		**Alternative 2(a)**		**Alternative 3**		**Alternative 4**		**Alternative 5**		**Alternative 8 (2 or 3)**	
Benthic hard bottom (*C. virginica*)	**See Text**		1	4	1	5	2	5	**not natural**				2	5
Benthic hard bottom)	5										**not natural**			
Benthic soft bottom	-0.1	0	0		-0.1	0	0		0		0		0	
SAV	0	1	0		0	0.1	0	0.1	0		0		0	0.1
Blue crab	1.9	2.2	0.4	1.6	0.4	1.9	0.8	1.9	0	0.4	0	0.4	0.8	1.9
Blue crab assuming lower association with oysters	0.4	0.7	0.07	0.35	0.07	0.35	0.14	0.4	0		0		0.14	0.4
Phytoplankton	-0.1	0	0		-0.1	0	0		0		0		0	
Zooplankton	-1	0	0		-0.1	0	-0.1	0	0		0		-0.1	0
Planktivorous fish – phytoplankton	-0.1	0	0		-0.1	0	0		0		0		0	
Planktivorous fish – zooplankton	-1	0	0		-0.1	0	-0.1	0	0		0		-0.1	0
Reef oriented fish	5		1	4	1	5	2	5	0	1	0	1	2	5
Piscivorous fish	1.8	1.9	0.4	1.4	0.4	1.8	0.7	1.8	0	0.1	0	0.1	0.7	1.8
Piscivorous fish assuming lower association with oysters	0.19	0.36	0.04	0.19	0.04	0.2	0.08	0.2	0		0		0.08	0.2
Avian oyster predators	2.4		0.5	1.9	0.5	2.3	1	2.4	0		0		1	2.4
Avian oyster predators assuming lower association with oysters	0.2	0.25	0.04	0.19	0.04	0.18	0.03	0.18	0		0		0.03	0.18
Avian piscivores	1.1	1.3	0.2	0.9	0.2	1.1	0.5	1.2	0	0.1	0	0.1	0.5	1.2
Avian piscivores assuming lower association with oysters	0.17	0.31	0.03	0.18	0.03	0.17	0.07	0.2	0		0		0.07	0.2
Avian soft-bottom feeders	0	0.3	0.04	0	-0	0	0	0.04	0		0		0	0.04
Mammalian piscivores	2		0.4	1.8	0.4	2.1	1	2.2	0	0.24	0	0.24	1	2.2
Mammalian piscivores assuming lower association with oysters	0.3	0.5	0.07	0.3	0.07	0.3	0.14	0.37	0				0.14	0.37
Reptiles (turtles)	1.7	2	0.3	1.4	0.35	1.7	0.7	1.7	0	0.1	0	0.1	0.7	1.7
Reptiles (turtles) assuming a lower association with oysters	0.2	0.5	0.05	0.2	0.05	0.2	0.1	0.3	0		0		0.1	0.3
Water Quality	0		0		0		0		0		0		0	

High negative influence (RRM scores <-3 to -5)
Moderate negative influence (RRM scores <-1 to -3)
Low negative influence (RR scores <0 to -1
No influence (RRM = 0}
Low positive influence (RRM scores >0 to 1)
Moderate positive influence (RRM scores >1 to 3)
High positive influence (RR scores >3 to 5)

Table 4-6. RRM results for ecological receptors in Virginia at 95th percentile biomass

Virginia Waters

Evaluation Criteria: Decision Matrix Related to Ecological Risks (Positive and Negative Influences)

Ecological Receptors	**Low mortality Bay-wide**		**Alternative 1**		**Alternative 2(a)**		**Alternative 3**		**Alternative 4**		**Alternative 5**		**Alternative 8 (3, 4, 5)**	
Benthic hard bottom (*C. virginica)*	**See Text**		1	2	2	4	4		**not natural**				4	
Benthic hard bottom	5										**not natural**		**not natural**	
Benthic soft bottom	-0.1	-1	0		0		0		0		0		0	
SAV	0	1	0		0	0.1	0	0.1	0	0.1	0	0.1	0	0.1
Blue crab	1.4	2.4	0.4	0.8	0.8	1.6	1.5	1.6	0	1.56	0	1.56	1.5	1.6
Blue crab assuming lower association with oysters	-0.1	0.9	0.07	0.14	0.14	0.33	0.3	0.35	0	0.02	0	0.02	0.3	0.35
Phytoplankton	-0.1	0	0		0		0		0		0		0	
Zooplankton	-1	0	0		-0.1	0	-0.1	0	0	-0.1	0	-0.1	-0.1	0
Planktivorous fish – phytoplankton	-0.1	0	0		0		0		0		0		0	
Planktivorous fish – zooplankton	-1	0	0		-0.1	0	-0.1	0	0	-0.1	0	-0.1	-0.1	0
Reef oriented fish	5		1	2	2	4	4		0	4	0	4	4	
Piscivorous fish	1.6	2	0.4	0.7	0.7	1.5	1.4	1.5	0	1.5	0	1.5	1.4	1.5
Piscivorous fish assuming lower association with oysters	-0.1	0.48	0.04	0.08	0.08	0.18	0.15	0.19	0	0.02	0	0.02	0.15	0.19
Avian oyster predators	1.9	2.4	0.5	1	1	1.9	1.9		0		0		1.9	
Avian oyster predators assuming lower association with oysters	-0.3	0.29	0.04	0.09	0.09	0.19	0.18	0.19	0		0		0.18	0.19
Avian piscivores	0.8	1.4	0.2	0.4	0.5	0.9	0.9		0	1	0	1	0.9	
Avian piscivores assuming lower association with oysters	-0.2	0.49	0.03	0.07	0.07	0.16	0.14	0.18	0	0.02	0	0.02	0.14	0.18
Avian soft-bottom feeders	-0.6	0.3	0		0	0.04	0	0.04	0	0.04	0	0.04	0	0.04
Mammalian piscivores	1.7	2.5	0.4	0.9	0.9	1.8	1.7	1.8	0	1	0	1	1.7	1.8
Mammalian piscivores assuming lower association with oysters	-0.1	0.65	0.07	0.14	0.14	0.3	0.27	0.31	0	0.02	0	0.02	0.27	0.31
Reptiles (turtles)	1.4	2.1	0.3	0.7	0.7	1.4	1.4		0	0.4	0	0.4	1.4	
Reptiles (turtles) assuming a lower association with oysters	-0.1	0.6	0.05	0.1	0.1	0.2	0.2	0.23	0	0.03	0	0.03	0.2	0.23
Water Quality	0		0		0		0		0		0		0	

🟥	High negative influence (RRM scores <-3 to -5)
🟧	Moderate negative influence (RRM scores <-1 to -3)
🟧	Low negative influence (RR scores <0 to -1
	No influence (RRM = 0}
🟦	Low positive influence (RRM scores >0 to 1)
🟦	Moderate positive influence (RRM scores >1 to 3)
🟦	High positive influence (RR scores >3 to 5)

- **Submerged Aquatic Vegetation (SAV)** - Increases in oyster abundance and biomass are expected to have a small positive influences on SAV because additional oysters could reduce suspended matter in the water column and increase water clarity. Increased oyster reefs could provide a buffer against waves that would benefit SAV. Positive influences would be greater at local levels where oyster biomass is higher per unit area.
- **Soft-bottom benthic communities** - Increases in oyster biomass could have a negative influence on soft-bottom benthic communities if additional oysters reduce the amount of organic matter reaching the sediments.
- **Blue crabs** - This species would benefit from alternatives that increase oyster abundance and biomass. Crabs can use oysters as a food source. They also benefit from the increased SAV that could result from increased oyster biomass.
- **Reef-oriented fish** - These fish would benefit from alternatives that increase oyster biomass. These fish rely on oyster reefs to varying degrees for food and habitat.
- **Piscivorous fish** - These fish would benefit from alternatives that result in an increase in oyster biomass because oysters contribute to habitats (oyster reefs and SAV) that support forage fish. In addition, some piscivorous fish feed on blue crabs.
- **Birds that feed on oysters** - These birds would benefit from alternatives that increase the abundance and biomass of oysters.
- **Piscivorous birds** - These birds would benefit from alternatives that result in an increase in oysters because they would prey on a wide variety of fish, including forage fish that would benefit from increased oyster abundance.
- **Birds that feed on soft-bottoms** - These birds would be both positively and negatively influenced because they feed on benthic invertebrates in soft-bottom environments, as well as on SAV. The former may be negatively influenced by increased oyster abundance, and the latter may be positively influenced.
- **Piscivorous mammals** - These mammals would benefit from alternatives that result in an increase in oysters because they would prey on a wide variety of fish, including forage fish that would benefit from increased oyster abundance. They could also prey on oysters.
- **Turtles** - These animals would be positively influenced by increases in oysters because they feed, in part, in environments that are positively influenced by oysters.

Alternatives 4 and 5, which promote aquaculture, would provide a source of oysters that might have some localized positive and negative effects. The presence of cultivated oysters would increase filtration capacity at the local scale, and this would remove algae and other particulates from the water column. The presence of aquaculture operations might also have localized negative effects. The operations would occupy a certain amount of space and volume, which could reduce habitat for other benthic organisms. The operations would involve repeated removal and replacement of units, which would cause periodic physical disruption of the local environment. The waste of concentrated numbers of oysters accumulating within the sediments beneath an aquaculture operation could affect the quality of those sediments.

4.5 PERSPECTIVES ON THE ECOLOGICAL ROLE OF OYSTERS FROM THE LITERATURE

Modeling and the projections of the RRM suggested that changes in oyster biomass would result in only small influences on the benthic soft-bottom community, SAV, phytoplankton, zooplankton, and the fish and wildlife that rely upon these as food or habitat. The RRM also indicated the possibility of small positive influences on a variety of species that depend directly or indirectly on oysters for food and habitat. This section examines other investigators' perspectives concerning the degree to which changes in oyster abundance might be reflected in changes in other ecological receptors.

A key assumption about the potential ecological role of oysters in the Bay involves their ability to filter water, thereby influencing the abundance of algae. Following from that assumption is the potential that an increase in the abundance of oysters in the Bay could influence ecological components and water quality conditions that are related to algal abundance. Newell (1988) estimated that, at one time, the oyster population would have been able to clear a volume of water equal to that of the Bay in two to four days. Based on this estimate, Newell suggested that restoring the oyster population could control spring phytoplankton blooms. Jackson et al. (2001), Ruesink et al. (2005), and Kemp et al. (2005) also discussed the potential role of oysters in controlling phytoplankton. To the contrary, Pomeroy et al. (2006) and Fulford et al. (2007) argued that the potential role of oysters in controlling algae in the Bay has been overstated. Pomeroy et al. (2006) concluded that lack of access to all Bay water and low spring-time filtration rates would prevent oysters from controlling the spring bloom and the resulting summer hypoxia. They suggested that a multispecies guild of suspension feeders now present in the Bay should have a filtration capacity approaching that of the pre-Colonial population of oysters, but it still does not control the bloom. Fulford et al. (2007) used a bivalve filtration model to examine the ability of oysters to reduce phytoplankton abundance. They concluded that achieving the restoration goals for oysters in the Bay would be unlikely to result in significant, Bay-wide reductions in phytoplankton biomass. Like Pomeroy et al. (2006), Fulford et al. (2007) noted several spatial and temporal mismatches between oysters and phytoplankton that may limit the ability of oysters to influence amounts of algae Bay-wide. Newell et al. (2007) maintained that increases in oysters by orders of magnitude could have important effects on water quality and ecological conditions in the Bay.

These arguments depend on specific sets of assumptions about the timing, spatial distribution, and magnitude of filtration by oysters. Clearly, the greater the number of oysters, the greater the potential amount of water that they would filter. Some of the differences reflected in the literature relate to different assumptions about oyster abundance. The ERA is based on particular increases or decreases in particular regions of the Bay; therefore, its outcomes are more specific. The ERA relies upon the results of the CBEMP to convert changes in oyster biomass into possible effects on water quality, phytoplankton, submerged aquatic vegetation, zooplankton, and soft-bottom benthic organisms. Newell et al. (2007) cited the CBEMP as a refinement over previous approaches. For the degrees of change in oyster biomass considered in the ERA, which may be limited relative to the magnitudes of change considered by Newell et al. (2007), the CBEMP results suggest that there would be no large-scale effects on water quality and ecological conditions. In comments provided on Attachment B of the ERA, Carl Cerco (pers.

comm. 2008) noted that restoring oysters is unlikely to have a large effect on the extensive, deep, open waters that, by volume, comprise major proportions of the state/salinity zones used in the ERA to characterize the influence of oysters on water quality. The objective of the PEIS for Oyster Restoration specifying restoration of a Bay-wide oyster population constrained the ERA to a large-scale assessment that would be relatively insensitive to small-scale, local effects.

The conclusions of Pomeroy et al (2006), Fulford et al. (2007), and Cerco (pers. comm. 2008) support the results presented in Section 4.4 indicating that the projected changes in oyster biomass associated with the proposed action and alternatives would have only small influences on conditions at the scale of the state/salinity zones considered within this ERA. Cerco and Noel (2005a) noted that, despite the uncertainties in their approach for relating oyster biomass to ecological changes using the CBEMP, they believe their basic findings regarding the nature and magnitude of restoration benefits are valid. They found their results to be consistent with the earlier findings of Officer et al. (1992) and Gerritsen et al. (1994) and with the recent findings of Newell and Koch (2004). Benthic controls of algal production are most effective in shallow, spatially limited regions. In these shallow regions, removal of solids from the water column by oysters enhances adjacent SAV beds. The ability to influence deep regions of large spatial extent is limited by the location of oysters in the shoals and by exchange processes between the shoals and deeper regions.

The available literature is consistent with our findings regarding the ecological implications of oysters for providing food and habitat and for affecting water quality in the Bay. The ecological benefits of oyster restoration activities are likely to be greatest in shallow spatially limited areas. Ecological effects at the scales of the larger state/salinity zones could occur but would be small.

As noted, the RRM results are presented at the scale of a state/salinity zone. These large geographic areas dampen stronger relationships that may exist at the scale of an individual segment of the Chesapeake Bay. An example is given here based on CBEMP model estimates. Attachment B shows relationships between oyster biomass and ecological responses at the state/salinity scale. One of these relationships - SAV/Oyster - is shown below for the MD MH zone. It shows a significant but small change over an increase in oyster biomass of two and a half orders of magnitude. Figure 4-41 reflects area-weighted relationships at a geographic scale of a state/salinity zone. These can be contrasted to the stronger relationship that can occur for an individual Chesapeake Bay segment within this zone (Figure 4-42). The important influence of local conditions at the level of a tributary or embayment was also described earlier in connection with the effects that oysters can have on water quality.

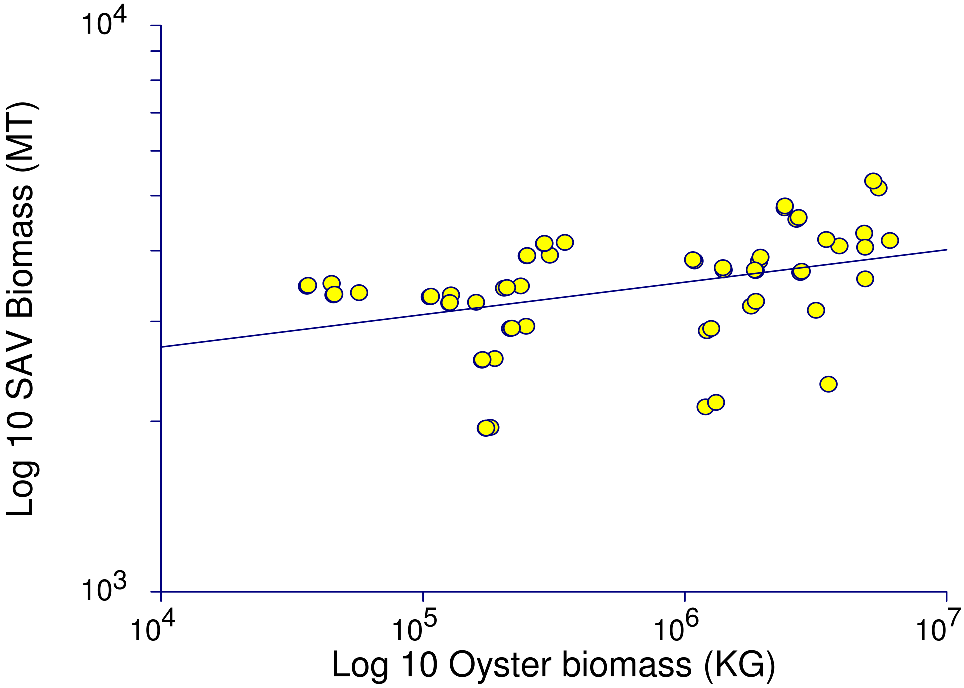


Figure 4-41. Relationship between SAV and oyster biomass for the MD MH zone based on CBEMP outputs.

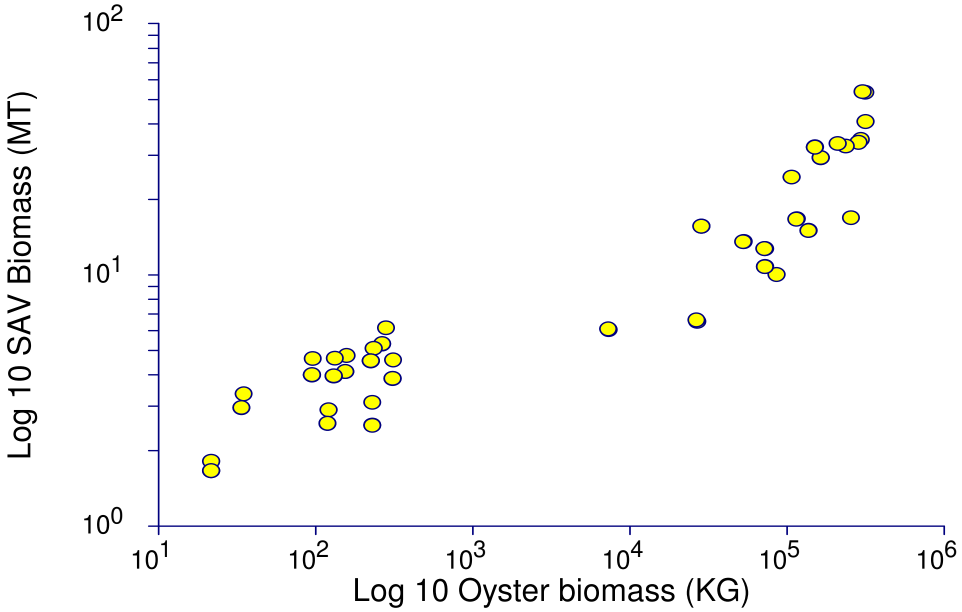


Figure 4-42. Relationship between SAV and oyster biomass for CB Segment CHOMH2 based on CBEMP outputs.

4.6 UNCERTAINTIES AND THEIR IMPLICATIONS FOR DECISIONS

Uncertainty arises from lack of knowledge concerning various aspects of risk analyses. An example of an area of uncertainty concerns the degree to which *C. ariakensis* could generate shell that would accumulate in the environment of the Bay over time. Uncertainty is distinguished from variability, which is a natural property of environmental systems. An example of variability includes variations in environmental factors (water flows and temperature) that affect the population dynamics of oysters. Some components of natural variability have been incorporated into the exploratory modeling and CBEMP. This is why the exploratory modeling results are provided as probabilities. The CBEMP incorporates variability in water flows, which is a major source of natural variability within the Bay.

All ERAs have inherent uncertainties and must contend with natural variability inherent in environmental systems. Nevertheless, decisions generally must be made that take this uncertainty into account. For the decision-maker, this involves weighing the potential positive and negative consequences of the decision and the attendant uncertainties associated with each. The degree of uncertainty associated with risk assessments is variable because our understanding of some aspects of a complex system may be better than it is for others; therefore, identifying sources of uncertainty and recognizing their implications is important for making a sound decision. Many of the uncertainties associated with introducing a nonnative species of oyster into Chesapeake Bay have been highlighted in previous sections. Here we summarize uncertainties with respect to understanding the ecological system of Chesapeake Bay, the magnitudes of influences among ecological components, the biology of *C. ariakensis,* the nature of interactions between *C. ariakensis* and *C. virginica,* the potential for *C. ariakensis* to migrate out of the Bay and become a nuisance species, and the potential for large-scale cultivation of triploid *C. ariakensis* to generate a reproductive diploid population in the Bay. We begin each of the following sections with a brief statement concerning our perspectives on the implications of the uncertainties for decision-makers. Ultimately, decision-makers will need to judge the information for themselves in light of other factors that weigh on those decisions.

4.6.1 The Ecological System of Chesapeake Bay

The analysis described in this ERA was simplified to develop and implement a comparative framework; nevertheless, we feel that the general structure of the analysis is reliable and that it captures the significant ecological changes and influences. Uncertainty increases with the increasing numbers of linkages between ecological receptors and oysters; however, given that the most significant influences have been captured and that we are confident in our assessment that they would be small, we do not anticipate large influences associated with any more subtle relationships that this analysis does not represent explicitly.

The positive or negative influences of changes in oyster abundance on other ecological components of the Bay depend, in part, on the nature of the interactions (i.e., direct or indirect) between oysters and other components of the Bay ecosystem. The analyses in the ERA that are based on the Relative Risk Model emphasize interactions involving food, habitat, and water quality. Although these interactions are considered the most important mechanisms by which changes in the abundance or kind of oysters in the Bay could influence other receptors, many of the specific details of these interactions are not well known and quantified; furthermore, other interactions that are not considered within the ERA may be important for determining the outcomes of implementing the proposed action or alternatives

We have confidence in the broad general ecological relationships identified as part of Problem Formulation (Section 2); however, these relationships and the delineation of habitat and food groups were simplified for the analyses presented in the ERA. The ERA considers three broad categories of habitat: oyster reefs, SAV, and other environments (predominantly soft-bottom or sand) because oyster reef and SAV habitat are the two kinds of habitat expected to change most as a result of changes in oyster abundance. Little change is expected in the physical characteristics of other areas. Microhabitats that are important for some species, however, could be affected. For example, the physical structure of a *C. ariakensis* reef could differ from that of a *C. virginica* reef, and those differences might create new microhabitats that favor different species than those typically associated with native oyster reefs in Chesapeake Bay. "Healthy" populations of *C. virginica* produce continuous horizontal and vertical reefs. These types of continuous reefs with elevated relief afford habitat, influence water flow, and buffer waves; consequently, their presence has been considered beneficial for protecting SAV and near-shore environments. Studies indicate that *C. ariakensis* also forms reefs, but the extent to which reefs will develop in the Bay remains uncertain. In addition, *C. ariakensis* might populate subtidal structures to a greater extent than does *C. virginica*.

Food groups are also simplified and include the following categories: phytoplankton, zooplankton, SAV and/or associated invertebrates, oysters or invertebrates on oyster reefs, other benthic invertebrates, blue crabs, forage fish (small near-shore species), planktivorous fish (primarily herring species), piscivorous fish, and avian soft-bottom feeders (ducks). These reflect major ecological groups for the ERA, but each group comprises many species that could respond to differing degrees to changes in oyster abundance and may vary in suitability as food. For example, phytoplankton includes diatoms, green algae, dinoflagellates, and other groups. These vary in size and nutritive value, and some species could have adverse effects on animals that ingest them; therefore, combining all algal species into a single group introduces uncertainty. This also applies to other large groupings. In general, however, an increase in a category of food (more algae, more benthic invertebrates, more forage fish) will tend to have a positive influence on the animals that feed on these groups. The major uncertainty concerns the potential for a shift from desirable to undesirable species within a group. The potential for such changes is small, perhaps negligible, given the magnitudes of change in the abundance of oysters projected in the ERA because the influence of the small changes in abundance of oysters on phytoplankton and other groups of ecological receptors appears to be small.

The influence of habitat and food on a group of ecological receptors is represented by sets of association factors. These were assigned based on the literature that describes general food and habitat preferences. The groups and species within each group could exhibit different preferences from those shown in the tables. With few exceptions, changes in the relative influence of habitats and food do not change the direction of influences (positive vs. negative), and although the degree of influence might vary, such differences are expected be small.

As was described in Section 3, the approach used in this ERA was to identify the major Bay ecosystem components and select receptor species considered to be representative of each component. This simplified approach does not account for the various kinds of interactions that occur among all of the species that comprise the Chesapeake Bay ecosystem. However, because the main objective of this ERA is to compare outcomes of the proposed action and alternatives, and because existing information suggests that both oyster species appear capable of providing the same types of ecological services within the Bay ecosystem, the outcomes of a simplified approach are considered to provide a reasonable basis for comparison of the actions being considered. One major uncertainty not accounted for in any of the analyses is how Chesapeake Bay water quality and habitat conditions might change over time. A decline in hard bottom habitat appears likely to continue into the foreseeable future, decreasing the potential for success of any oyster restoration program. Changes in water quality in the future that might adversely or beneficially affect both species of oysters would affect outcomes but cannot be predicted.

4.6.2 Magnitudes of Influences among Ecological Components

The two largest uncertainties decision-makers should consider are associated with making assessments based on expected conditions at the end of a 10-year period following implementation and current limitations on our ability to predict the extent to which *C. ariakensis* would become established in the Bay. Important changes could begin to occur within the 10-year period, and influences could continue long after the 10-year period. The most important of these possible changes involve (1) declines in the abundance of *C. virginica* following initial restoration efforts, and (2) the possibility that major changes associated with introducing *C. ariakensis* could occur after the 10-year period. Projections of the abundance of an introduced species are highly uncertain and do not consider all of the factors that may influence the success of *C. ariakensis* in Chesapeake Bay. This ERA analysis suggests that a *C. ariakensis* population would have the potential to grow rapidly over a 10-year period. If *C. ariakensis* could accomplish that, its populations could be expected to continue to grow until reaching some unpredicatable maximum carrying capacity of the environment; however, uncertainty about the performance of *C. ariakensis* in Chesapeake Bay is high.

The projected magnitudes of positive or negative influences reported in this ERA were based on three components of the analyses: the demographic model that projects the relative change in abundance of oysters; the CBEMP model that translates these changes into changes in phytoplankton, SAV, zooplankton, benthic invertebrates, and water quality; and the degree of association between these ecological receptors and fish and wildlife. The analysis relies on one or more of these three models, each of which has inherent uncertainties.

Because each of the alternatives involving natural populations includes estimates derived from the demographic model, uncertainties associated with that model are reflected in all the alternatives. The sources of uncertainty in the demographic model and their potential significance for model outputs are discussed in PEIS Appendix A. The demographic model does not account for several important influences on oyster populations, such as the amount of shell substrate required to achieve a target oyster population (i.e., the “shell budget”) and changes in the shell budget. The outputs of the demographic model are considered more reliable for projecting changes in *C. virginica* than for *C. ariakensis.* Although modeling the growth of a population of a hypothetical species with vital rates that approximate some characteristics of *C. ariakensis* provides insights that can be used to evaluate risks, these outputs are not reliable for estimating how well *C. ariakensis* would do within the Bay. The model projections are best viewed as illustrating a potential for population growth.

The demographic model was run for a 10-year period for each of the alternatives involving natural populations. Projections were only calculated for ten years because the uncertainty associated with model projections increases with each time step, and extending projections beyond ten years would have produced projections that the model developers considered to be increasingly unreliable. In addition, the amount of habitat used as input to the exploratory modeling was kept constant over the 10-year assessment period, although studies have shown continuing decline in habitat over time. Because the rate of habitat decline could not be projected and incorporated into the exploratory modeling, model developers considered the reliability of population projections beyond 10 years to be further compromised. Although exploratory modeling projections end at year10, the trajectory of population change over time is shown in model output, which allowed for informed speculation about changes in population sizes further into the future.

Although the 10-year period provides a baseline for comparisons, it does not reflect some potentially important trends. The first involves the enhanced restoration alternatives (2a and 2b). Enhanced restoration can lead to increases in oyster abundance and biomass, especially in Maryland waters; however, the exploratory modeling outputs suggest that populations would level off and could decline beginning in year 9, when spat seeding is no longer increasing. In the absence of a continually increasing enhancement program, the biomass of oysters may decline after the 10-year assessment period. Conversely, for the harvest moratorium alternative, if the elimination of harvest contributed to an increase in rate of disease resistance development, populations of *C. virginica* might increase at some time in the future. We noted the inherent uncertainty associated with using the demographic model to project changes in the abundance and biomass of a hypothetical oyster that approximates some of the vital rates of *C. ariakensis.* That uncertainty reflects lack of knowledge about the biology of *C. ariakensis* and its population dynamics within the Bay. Assuming a select set of life-history characteristics, the demographic model showed that a nonnative species would increase in abundance and biomass over the 10-year modeling period. The key assumption that supports the projected growth is low mortality; however, studies of the susceptibility of *C. ariakensis* to predation (especially by crabs) and disease (i.e., *Bonamia* in more saline waters) suggest that the projections of the demographic model might overestimate survival rates of naturalized *C. ariakensis*. The abundance and biomass of *C. ariakensis*, therefore, probably would be less than those projected for the hypothetical nonnative species. If *C. ariakensis* could sustain a population, the growth of that population could exceed the model projections over a longer time frame (e.g., 20 to 100 years out). Whether or not this would occur is unknown. If *C. ariakensis* were harvested, as is expected, that pressure combined with other sources of mortality (crabs and diseases) could constrain population numbers.

Variability is reflected to some degree in the demographic model by conducting numerous runs under variable environmental conditions (PEIS Appendix A). The RRM model relied on the 50th and the 95th percentiles of the projections of the demographic model. This covers a range of possibilities for resultant oyster populations; however, the demographic modeling does not reflect the full range of uncertainties associated with estimating oyster populations. Still, only small influences on other ecological receptors were detected over the range of alternatives and associated changes in oyster abundance.

Two areas of uncertainty are associated with use of the CBEMP. The first relates to the uncertainties associated with projecting changes in ecological response factors and water quality. The CBEMP is based on mechanistic relationships and is considered a reasonable basis for estimating changes at each step. Because the model links relationships together, however, uncertainties arise at each step in translation. The ability to check modeled projections of biological responses against actual changes of biota within the Bay is limited. We examined the available literature to help explain the magnitudes of changes or influences projected by the models.

The second source of uncertainty stems from the need to extrapolate from a set of existing model runs (Attachment B) instead of running the CBEMP specifically for the abundances of *C. virginica* projected for Alternatives 1, 2, and 3 using the demographic model. This extrapolation was accomplished by examining the relationships between model outputs (e.g., for phytoplankton) for various modeled projections of oyster abundance. Significant direct and inverse relationships were identified. Uncertainties arise in the analysis because it involved a translation from existing model estimates instead of estimates projected uniquely for the alternatives. In addition, the CBEMP model was run for individual CB segments. The ERA is conducted at a larger spatial scale (six state/salinity zones); therefore, the existing model runs from the CBEMP were aggregated. This can have the effect of dampening relationships that may exist at smaller spatial scales. Stronger relationships between oysters and other ecological receptors than those revealed in this ERA would be expected in select tributaries and segments.

The RRM results indicate that changes in oyster populations would have small positive or negative influences on other ecological receptors. Confidence in the direction of these influences is adequate, but confidence in the magnitudes of the influences is less so. For this reason, we also examined the available literature on the potential influence of oysters on other ecological receptors. From this we concluded that, to the extent that oysters exert an influence on ecological receptors other than those that rely directly on oyster reefs for food or habitat, this influence is small over the expected ranges of oyster abundance and at the scales of state/salinity zones. This is consistent with the results of the RRM and lends a degree of confidence to those results. As noted above, some tributaries and segments probably would experience a greater degree of influence than is revealed for the larger state/salinity zones. We conclude, as others have, that the main ecological benefits of oyster restoration would be local. Bay-wide changes in water quality (e.g., oxygen levels in the main Bay) and significant reductions in nutrients are unlikely to result from the restoration efforts.

4.6.3 Biology of *C. ariakensis*

Many uncertainties surround the biological characteristics of *C. ariakensis.* This ERA relies on information from laboratory studies and limited field observations off the coast of China. Although the available data are insufficient to support reliable predictions about how *C. ariakensis* would fare in the Bay, they do provide useful insights into the potential risks and benefits that could be associated with a successful introduction. If a population of *C. ariakensis* were to become established in the Bay, it would not be possible reverse that action and irradicate the species.

Section 4.4 describes much of the research that has been carried out to investigate how *C. ariakensis* might behave in Chesapeake Bay. All research consists of focused investigations of oysters' responses to particular sets of variables under controlled conditions. A study of *C. ariakensis* reefs in the coastal waters of China revealed that the species can develop reefs that include other oyster species. These reefs are most common in estuaries associated with rivers, and the species can establish reefs in systems with high sediment loads. We assumed that *C. ariakensis* would form reefs in Chesapeake Bay and would reach the abundance levels suggested by modeling oysters with the desired characteristics of growth and survival. Our reasoning was that risks from *C. ariakenis* would be related to the ability of this species to become abundant; therefore, the projections of increased abundance, regardless of whether they would actually occur, were appropriate for use in the ERA. The uncertainty that remains about the potential for both oyster species to provide the same ecological services has significant implications, since an introduction of a non-native species would be irreversible. If the introduced species were in fact to affect other ecosystem components in a manner different from the native species and the result was undesirable, there is no corrective action that could be taken.

4.6.4 Interactions between *C. ariakensis* and *C. virginica*

Despite the uncertainties about how *C. ariakensis* would fare in the Bay and the population sizes that might be achieved, available information is sufficient to conclude that if *C. ariakensis* were successfully introduced, it would compete with *C. virginica.*

Section 4.4 describes research investigating the potential for competitive interactions between *C. ariakensis* and *C. virginica.* All research consisted of controlled laboratory experiments. The research indicated differences between *C. ariakensis* and *C virginica* that could translate into the potential for competitive interactions between them within the Bay. The research also suggested that the ecological niches that these species are likely to occupy in Chesapeake Bay do not overlap completely. Because the insights come entirely from laboratory studies, and our level of knowledge about the probable behavior of *C. ariakensis* in the Bay was not substantially increased with field observations of populations of *C. ariakensis* in its native environment, uncertainty is high about how the potential competitive interactions observed in the laboratory would be manifested in the field. We concluded that such interactions could exist and could pose a risk; however, we also must acknowledge the possibility that the two species could coexist within the Bay because their ecological niches may not overlap completely. We also must acknowledge the potential for the presence of *C. ariakensis* to exert a positive influence on *C. virginica* by increasing the amount of shell habitat available for settlement of larvae.

4.6.5 Potential for *C. ariakensis* to Disperse Outside of Chesapeake Bay and Become a Nuisance Species in Other Coastal Estuaries

Confidence is high that if a reproductive population of *C. ariakensis* is established in Chesapeake Bay, the species would be dispersed to adjacent areas. The likelihood that it would become an ecological nuisance is less certain. The influence of *C. ariakensis* elsewhere is expected to be similar to its influence in the Bay, where it could compete with *C. virginica.*

It could also have a positive influence on this species. Given the various ways in which movement could occur (e.g., larval transport pathways, intentional or unintentional transport of adult oysters by humans) and the wide range of suitable environmental conditions, we believe it is more probable than not that such dispersal would occur eventually. The rates of dispersal and establishment are uncertain. We are uncertain about whether the species would become abundant in other areas or would become an ecological nuisance. We concluded that an abundant population of *C. ariakensis* in the Bay would be unlikely to become a nuisance (as perceived by Virginia and Maryland) with respect to the ecological response factors we evaluated in the ERA because *C. ariakensis* would be harvested and is expected to develop primarily on existing hard-bottom areas. A decision to introduce *C. ariakensis* into the Bay would be made with knowledge of the potential for an adverse interaction between the two oyster species.

Interactions between the two oyster species could occur in areas outside of Chesapeake Bay, which could be viewed as a nuisance. The degree to which *C. ariakensis* would cause ecological harm within or outside Chesapeake Bay depends on the biology of the species. *C. ariakensis* shares some characteristics with *C. gigas,* a species that has been invasive and is considered a nuisance in some European waters. Both species grow faster than native oyster species and mature earlier. Both species have the ability to settle on available hard substrates. *C. gigas* is more resistant to predators than *C. ariakensis* and is less susceptible to harvest pressure in the waters in which it has become established. *C. gigas* is also able to use a wider variety of habitats than *C. ariakensis.* Although *C. ariakensis* exhibits some characteristics that would enable it to spread, the species appears to have a lower potential to be invasive than *C. gigas.* No strong evidence is available to support the notion that *C. ariakensis* would overtake other habitats. The nuisance potential of *C. ariakensis* with respect to human uses of Chesapeake Bay is evaluated in the EIS.

If *C. ariakensis* is able to populate hard substrate in subtidal areas of the Bay, it could be dispersed to other areas where it might also grow and spread. The species' potential for success within Chesapeake Bay, therefore, might be viewed as a potential threat (i.e., a nuisance) to areas outside of the Bay. The existing information on the biology of *C. ariakensis* indicates that some biological characteristics would contribute to success, whereas other factors might limit the degree to which populations would grow. These factors appear to be well supported by available research, but information is lacking about how they would be manifested in the Bay. Factors that favor population growth include the fast growth rate and early maturation of the species. Factors that may limit population growth include vulnerability to predation (especially by crabs), susceptibility to disease at higher salinities, and potentially poor sustainability of shell. This last factor has not been tested; however, the shells of *C. ariakensis* appear to be thinner and might decay faster than those of *C. virginica* and *C. gigas.* On balance, *C. ariakensis* could be successful in portions of Chesapeake Bay; however, it seems unlikely that it would grow unchecked.

4.6.6 Potential for Cultivation of Triploids to Generate Reproductive *C. ariakensis*

Several uncertainties are associated with predicting whether cultivating triploid *C. ariakensis* would lead to the establishment of a reproductive population at large in the Bay. To the extent that this could occur, we believe that the process would be slow and could be monitored and managed.

Section 4.3 describes most of the uncertainties associated with evaluating the likelihood that cultivating triploid *C. ariakensis* would lead to the establishment of a reproductive diploid population in the Bay. Several complete pathways that could lead from triploid aquaculture to the establishment of a reproductive population are possible. Uncertainties arise, in part, from assumptions concerning the probabilities of occurrence at each step in these pathways. Where information for estimating these component probabilities was scarce, we made conservative estimates (i.e., we assumed higher probabilities). In some cases, we relied on information for other species (e.g., *C. gigas*) or other phyla (e.g., echinoderms). Although we believe that the estimates are conservative, uncertainty in the analysis is moderate to high because of the need to evaluate probabilities as a chain of events, each of which has attendant uncertainty. Centralization of hatchery operations at one or two facilities would allow for greater oversight and adherence to ICES quarantine protocols and perhaps minimize the likelihood of accidental breaches in biosecurity. However, what could not be accounted for in these calculations were the probabilities of human error and rare events (e.g., hurricanes) and also the cumulative probability of all factors over a very extended period of time. Because of the numerous factors that might reduce or increase the probability of an unintended introduction, the authors could not assign a risk level to this outcome. If an unintended introduction were to occur, it would be considered undesirable to the extent that the non-native species did not provide the same ecological services as the native species. But if the introduction were to occur, it would be irreversible, as in the case of an intended introduction.

We feel confident that, the process leading to establishment of a reproductive population as a result of an unintended introduction would be very slow. This would afford an opportunity to monitor environments around aquaculture operations for signs of reproductive individuals. We assumed that the probability of spawning would be increased if diploid larvae settle on appropriate habitat within the water body in which their cultivated parent generation was held. Individuals that settle at great distances from the aquaculture area would have a very low probability of contributing to a reproductive population. Implementing a monitoring program, therefore, would be feasible as part of an adaptive management strategy. Such a program could help to quantify the likelihood of establishing a reproductive population by looking for incidents of settlement of *C. ariakensis.* That information could be used to reduce the probability of occurrence through management actions.

4.6.7 Studies that Could Reduce Uncertainties

The uncertainties identified above could be reduced by conducting studies. Investigating a few specific questions would be especially valuable for reducing key uncertainties:

- **Examine the longevity of *C. ariakensis* shell in the waters and sediments of Chesapeake Bay.** If this species is to be successful at developing and sustaining populations, the integrity of its shell in the environment will be important for maintaining and perhaps increasing the shell inventory at cultch sites in the Bay.
- **Develop a monitoring program for detecting the presence of diploid *C. ariakensis* in areas adjacent to aquaculture operations and hatcheries.** Monitoring would provide data on the rate of production of diploids, the spatial distribution of escaped diploids, and the need for implementing an adaptive management strategy to prevent collocation of adult diploid *C. ariakensis.*
- **Develop metrics for judging the potential for *C. ariakensis* to become a nuisance for ecological receptors and human uses within the Bay and in areas outside of the Bay.** This would begin with agreements concerning mechanisms by which the species might pose a nuisance.
- **Use triploid *C. ariakensis* to examine the kinds of communities that develop on a *C. ariakensis* reef in Chesapeake Bay and measure the influences of *C. ariakensis* on local ecology and water quality.** This would involve constructing reefs resembling those that occur in the species' native habitat.
- **Conduct additional research on rate and probability of reversion of triploid *C. ariakensis* to a diploid state.** More reliable information on this phenomenon would contribute to a better assessment of risk that triploid aquaculture could result in unintended diploid introduction of this species.
- **Conduct research to estimate the rate at which *C. virginica* may develop resistance to both dermo and MSX, and the probability and time frame within which disease-resistance could be established throughout the Chesapeake Bay oyster stock.** This would require laboratory studies as well as field studies to assess the manner in which disease resistance may develop and expand geographically throughout the Bay.
- **Design and implement monitoring programs that would provide data needed to accurately characterize population dynamics of the Chesapeake Bay oyster stock**. Good data on such vital parameters as natural mortality rates, fishing mortality rates and growth rates are required to develop reliable and reasonably accurate population models that can be used to investigate the efficacy of alternative management strategies for the Bay's oyster fishery.

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ATTACHMENT A

SUMMARY OF NOAA-FUNDED *C. ARIAKENSIS* RESEARCH

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EIS Proj. #	Funding Agencies	Principal Investigator	NOAA Grant ID	Study Title	Last Progress Report Date	Scheduled Final Report Due/Done	Final Report Complete	Peer Review Complete ?	Journal Published ?	Comments
1	DNR / NOAA	Newell	NA04 NMF457 0423	Quantifying the response of different strains of Crassostrea ariakensis larvae to environmental change under spatially realistic conditions	Dec-07	Jun-08	Yes	Partial	None Identified	A peer review is planned for the final report. Some peer review on the initial progress reports was completed. The PI's are planning to eventually publish results from the'04-'05 larval studies.
2	DNR	Merritt, Allen	n/a	Evaluation of gametogenesis and spawning cues for diploid C. ariakensis -environmental risk & establishment of diploid brood stock populations	none	Feb-06	Yes	No	None Identified	A peer review is planned for the final report.
3	DNR / NOAA	Newell	NA04 NMF457 0414, 0418	Long-term mesocosm studies of competitive interactions between diploid C.v. and C.a.	Apr-08	May-09	No	No	None Identified	A summary report for peer review is expected in June (Breitburg). The research will continue through 2008.
4	DNR	Merritt, Allen	n/a	Spawning Interactions Between Crassostrea ariakensis and Crassostrea virginica, does the proposed introduction of a new species pose a recruitment threat to native oysters? ("Gamete Sink Study")	Apr-06	Jul-06	Yes	No	None Identified	A peer review is planned for the final report.
5	DNR / NOAA	Burreson, Carnegie, Audemard	NA04 NMF457 0421, 0422	Susceptibility of C.a. to the oyster pathogen Bonamia ostreae and to Bonamia sp. recently discovered in C.a. in NC / Potential Impact of Bonamia sp. parasitism on the Asian oyster Crassostrea ariakensis	Sep-07	Dec-07	Yes	Partial	Yes	A peer review is planned for the final report. One of the reports is posted on the NOAA website. A peer review of the final DNR report and initial progress reports (for work conducted under the NOAA grant) was completed. Dr. Burreson did not provide a response, however, four peer reviewed journal articles have been published and there are two manuscripts in preparation.
6	DNR / NOAA	Tamburri	NA04 NMF457 0420	Behavior, substrate selection and survival of C.a. pediveliger larvae and juveniles to variation in environmental conditions	Dec-06	Aug-07	Yes	Yes	Yes	A peer review of the final progress report for the DNR funding was completed. A manuscript was provided to the peer review group as a response to the peer review comments. The peer review group stated that the overall, the re-analysis of the results and the inferential statistics provided in the manuscript support the clear trends in the data. A final published peer reviewed paper has been received. The final report for this new research is available on the NOAA website. No additional peer review is planned.

EIS Proj. #	Funding Agencies	Principal Investigator	NOAA Grant ID	Study Title	Last Progress Report Date	Scheduled Final Report Due/Done	Final Report Complete	Peer Review Complete ?	Journal Published ?	Comments
7	Various Grants & DNR	Luckenbach, Paynter, Lin, Richardson	n/a	"Ecological Studies of C.a. in China and Japan" (The Keith Campbell Foundation for the Environment); "Investigation of a C.a. reef in Laizhou Bay, China" (MD DNR)	Oct-04	May-05	Yes	No	None Identified	This research did not result in a final report. No peer review is planned.
8	DNR	Zohar	n/a	Research and Development Studies on Crassostrea ariakensis	Nov-05	Feb-06	Yes	Yes	None Identified	A peer review was completed on the 6 page final summary report to DNR and on progress reports. Initial peer review comments indicated that additional work was necessary. This research was not completed. Dr. Vasta continued his work under two NOAA grants (EIS project #34 and #37).
9	DNR	Zohar	n/a	Environmental Tolerance Studies on Crassostrea ariakensis	Dec-04	Feb-06	Yes	Yes	None Identified	Peer review comments indicated that this project's main value has been to raise some fundamental questions about the feasibility of introducing this species into Chesapeake Bay at the larval stage. The recommended that these questions should be formalized as objectives for further study.
10	DNR	McLean	n/a	Power Plant Assessment		completed	Yes	No	Yes	A peer review is planned for the final report.
11	DNR / NOAA	Reece	NA04 NMF457 0248	Comparing microbiological characteristics of C. ariakensis and C. virginica with respect to uptake and elimination of bacterial and viral pathogens in situ	Apr-08	Dec-08	No	No	None Identified	This research will continue through 2008. No peer review is planned.
12	DNR	Matche, Driscoll	n/a	Sensitivity to Hypoxia – Comparison of Crassostrea ariakensis and Crassostrea virginica	none	Jun-06	Yes	No	None Identified	A peer review is planned for the final report.
13	DNR / NOAA	Paynter	NA05 NMF457 1237	Metabolic Differences between Crassostrea ariakensis and Crassostrea virginica at Varying Temperature and Salinity – Is C. ariakensis sensitive to low oxygen?	Mar-06	Jun-07	Yes	No	None Identified	A peer review is planned for the final report. A report is available on the NOAA website.
14	DNR	Mann	n/a	Estimating oyster age and growth rate (A Population Model for the Oyster C. ariakensis)	none	2006	Yes	No	Yes	The final report was a published journal article. A peer review will be planned for the final report if necessary.

EIS Proj. #	Funding Agencies	Principal Investigator	NOAA Grant ID	Study Title	Last Progress Report Date	Scheduled Final Report Due/Done	Final Report Complete	Peer Review Complete ?	Journal Published ?	Comments
15	DNR	North	n/a	Larval Transport Model	Feb-05	Jul-06	Yes	Yes	Yes	Peer review is complete. The four reviewers accepted the results of the research. The peer review report is available on the Norfolk District Corps website.
16	EPA / DNR	Cerco	n/a	Evaluating Ecosystem Effects of Oyster Restoration in Chesapeake Bay	Sep-05	Feb-07	Yes	Yes	Yes	The documentation for this modeling was peer reviewed by the Chesapeake Bay Program. The use of the model output data is being reviewed by the peer reviewers for the Ecological Risk assessment and oyster demographic model. The peer review report is available on the Norfolk District Corps website.
17	DNR	UMD, Versar (Volstad)	n/a	Ecological Risk Assessment (ERA) Note: funding for this project includes the Demographic Modeling (peer review by OAP)		Jun-08	Yes	Partial	None Identified	For the Oyster Demographic Model, a peer review was completed by 7 reviewers. This peer reviewers accepted the model with some conditions. The peer review is available on the Norfolk District Corps website. For the Ecological Risk Assessment, a peer review is underway and will be available on the Norfolk District Corps website.
18	DNR	Lipton	n/a	Background Economic Analysis for the EIS (November 2005) & Background Economic Analysis for the aquaculture alternatives for the EIS		Apr-08	Yes	Partial	Yes	A peer review is underway and will be available on the Norfolk District Corps website.
19	DNR	Paolisso	n/a	Cultural Assessment	May-07	Feb-08	Yes	Yes	Yes	Peer review comments were favorable. Report and peer review are available on the Norfolk District Corps website.
20	DNR / NOAA	Luckenbach	NA05 NMF457 1240	Comparative performance of triploid Crassostrea ariakensis and C. virginica in bottom habitats in Virginia and Maryland	Apr-08	Jul-08	No	No	Yes	A final report is expected in August 2008. A peer review will be completed at this time if necessary.
21	VA Graduate Marine Science Consortium	Reece, Allen	n/a	Analysis of genetic variation in Crassostrea ariakensis: Evaluation of germplasm resources for broodstock development (Project number: R/A-33)	none	2004	Yes	No	Yes	If necessary, a peer review will be planned for the final report.

EIS Proj. #	Funding Agencies	Principal Investigator	NOAA Grant ID	Study Title	Last Progress Report Date	Scheduled Final Report Due/Done	Final Report Complete	Peer Review Complete ?	Journal Published ?	Comments
22	NOAA (Sea Grant)	Burreson, Allen, Reece	n/a	Potential pathogens of Crassostrea ariakensis in its native range in China and in established populations in Washington, USA (Sea Grant R/MG-030-01)	none	2005	Yes	Yes	None Identified	A peer review of the final DNR report is complete. Dr. Burreson was unwilling to address the peer review comments, but the comments were not "bad" comments and the report was just an 8 page sea grant project record report. It has Kimberly Reece's name on it although Eugene Burreson was listed by NOAA as the lead PI. The peer review group was the Disease PRG
23	NOAA	Bushek, Ford, Guo	NA04 NMF457 0427	A histological investigation of oyster parasites and pathology in three Chinese estuaries containing varying mixtures of coexisting oyster species including Crassostrea ariakensis	Nov-07	Feb-08	Yes	Partial	None Identified	A peer review of the initial progress reports was completed. Dr. Bushek provided a brief response. The Final report will be sent for peer review ASAP. A report is available on the NOAA website The peer review group was the Disease PRG
24	NOAA	Cordes, Reece	NA04 NMF457 0432	Assessing levels of genetic variation within and among native populations and hatchery stocks of the Suminoe oyster Crassostrea ariakensis using a suite of molecular markers	Mar-07	Aug-07	Yes	No	Yes	A number of papers have been published in peer reviewed journals. If necessary, a peer review will be planned for the final report. The final report is available on the NOAA website.
25	NOAA	Guo	NA04 NMF457 0424	Genetic and ecological structure of oyster estuaries in China and factors affecting success of Crassostrea ariakensis	Apr-08	Feb-09	No	No	Yes	A number of papers have been published in peer reviewed journals. A final report is expected in July 2008. A peer review will be completed at this time.
26	NOAA (Sea Grant)	Paynter		Caged Crassostrea ariakensis deployment in Chesapeake Bay: growth, disease, Polydora infestation, and mortality in 3 and 4 year old non-native oysters	none	Jul-07	Yes	n/a	None Identified	Note: No report. Research continued as project #33
27	NOAA	Luckenbach		Competitive interactions between Crassostrea virginica and C. ariakensis	none	Oct-06	Yes	No	None Identified	No peer review planned. This project was continued as project 3
28	NOAA	Newell		Assessing the potential for natural predators to control the spread of the Suminoe oyster, Crassostrea ariakensis	none	2005	Yes	No	None Identified	No peer review planned. This project was continued as project 30
29	NOAA	Breitburg	NA05 NMF457 1232	Will predation mortality differ for larvae of native and non-native oysters?	Apr-08	May-09	No	No	None Identified	Research will continue through 2008. Two peer reviewed journal articles have been published. A summary report has been submitted for peer review.

EIS Proj. #	Funding Agencies	Principal Investigator	NOAA Grant ID	Study Title	Last Progress Report Date	Scheduled Final Report Due/Done	Final Report Complete	Peer Review Complete ?	Journal Published ?	Comments
30	NOAA	Kennedy, Newell	NA04 NMF457 0425	Predation by polyhaline invertebrate predators on young non-native oysters, Crassostrea ariakensis, in Chesapeake Bay	Apr-08	Jun-08	Yes	Yes	Yes	Two journal published papers, Marine Biology 152: 449-460, and "Discriminatory predation on oysters (In Press)," were accepted by NOAA as peer reviewed final reports.
31	NOAA	Bushek	NA04 NMF457 0428	Fertilization interference between Crassostrea ariakensis and C. virginica	Dec-06	2007	Yes	No	Yes	If necessary, a peer review will be planned for the final report.
32	NOAA	Luckenbach	NA04 NMF457 0429	Comparative post-settlement growth and survival in the Suminoe oyster Crassostrea ariakensis exposed to intertidal emersion	Mar-06	Jun-06	Yes	No	Yes	If necessary, a peer review will be planned for the final report.
33	NOAA	Paynter	NA05 NMF457 1239	Caged Crassostrea ariakensis deployment in Chesapeake Bay: growth, disease, Polydora infestation, and mortality in 3 and 4 year old non-native oysters	Jul-06	Jun-07	Yes	No	Yes	If necessary, a peer review will be planned for the final report.
34	NOAA	Vasta	NA05 NMF457 1242	Susceptibility of Crassostrea ariakensis to Bonamia species: potential for increased disease transmission between oyster species	Dec-06	Jul-07	Yes	Partial	Yes	A peer review on initial research was completed. No completed peer review to date. The final report will be sent for peer review ASAP.
35	NOAA	Reece	NA04 NMF457 0430, 0431	Potential for Crassostrea ariakensis to serve as a vector for exotic pathogens in Chesapeake Bay	Apr-08	Jun-08	No	Partial	Yes	A peer review on initial research was completed. A final report is expected in July 2008. A peer review will be completed at this time. Three peer reviewed journal articles have been published and there is one manuscript in preparation. A progress report for 5/1/08 with mostly new information was received.
36	NOAA	Gilbert	NA05NMF457 1234	The use of non-native oysters in the restoration of Chesapeake Bay oyster populations and the potential threats posed by harmful algae	Apr-08	Dec-08	No	No	Yes	A progress summary report for peer review dated 31-Dec-08 will be peer reviewed. Research will continue through 2008.

EIS Proj. #	Funding Agencies	Principal Investigator	NOAA Grant ID	Study Title	Last Progress Report Date	Scheduled Final Report Due/Done	Final Report Complete	Peer Review Complete ?	Journal Published ?	Comments
37	NOAA	Vasta	NA05 NMF457 1243	Evaluation of Crassostrea ariakensis as a potential sink or reservoir for pathogens of humans and shellfish	Dec-07	Oct-08	No	No	Yes	No completed peer review to date. There are two manuscripts in preparation. The summary report has been completed for peer review. A final report is anticipated soon.
38	NOAA	Mitchelmore	NA05 NMF457 1230	Does C. ariakensis accumulate more microbial pathogens than C. virginica increasing the pathogenic risk for human consumption?	Dec-06	Sep-07	Yes	No	None Identified	A final report is available on the NOAA website. A manuscript is in preparation. A peer review will be completed if necessary.
39	NOAA (Sea Grant)	Govoni		Comparison of bacteria uptake and depuration rates between the Suminoe oyster Crassostrea ariakensis and the American oyster Crassostrea virginica	none	2006	Yes	No	None Identified	A NOAA Technical Memorandum was published. If necessary, a peer review will be planned for the final report.
40	NOAA	Graczyk	NA04 NMF457 0426	Environmental tolerance-dependent competition between adult Crassostrea ariakensis and C. virginica in recovering and retaining waterborne disease agents in relation to water salinity	Dec-05	Mar-06	Yes	No	Yes	A final report is available on the NOAA website. A peer review will be completed if necessary.
41	NOAA (Sea Grant)	Marshall		Non-native oyster trials for aquaculture (NC)	none	May-05	Yes	No	Yes	Economic data from this research used in the economic assessment for the EIS has been reviewed as part of the economic assessment (see #18). A number of research reports have been published in Peer reviewed Journals. A final report is available on the NOAA website. A peer review of the aquaculture results will be completed if it is determined to be necessary.
42	NOAA	Allen	NA05 NMF457 1241	Comparative economic evaluation of triploid C. ariakensis and triploid disease-resistant C. virginica: Companion trial to 2005 VSC deployment	Sep-07	2007	Yes	No	None Identified	Economic data from this research used in the economic assessment for the EIS has been reviewed as part of the economic assessment (see #18). A final report is available on the NOAA website. A peer review of the aquaculture results will be completed if it is determined to be necessary. (Manuscripts are in preparation.)
43	NOAA	Southworth	NA05 NMF457 1235	The potential for using triploid Crassostrea virginica for on bottom culture in Chesapeake Bay	Dec-07	Mar-08	Yes	No	None Identified	A final report is available on the NOAA website. A peer review will be completed if necessary.

EIS Proj. #	Funding Agencies	Principal Investigator	NOAA Grant ID	Study Title	Last Progress Report Date	Scheduled Final Report Due/Done	Final Report Complete	Peer Review Complete ?	Journal Published ?	Comments
44	NOAA	Wieland	NA05 NMF457 1231	Supply and Management of Oyster Harvests in the Chesapeake Bay: An examination of historical factors and their implications for introduction of non-native oysters and targeted alternatives	Jan-08	May-08	Yes	Partial	None Identified	A number of reports are currently in peer review. Two reports are available on the NOAA website. One final report is pending. (Manuscripts are in preparation)
45	NOAA	Allen	NA04 NMF457 0433	Biological material support for studies on Crassostrea ariakensis	Apr-08	Dec-08	No	No	None Identified	Research is scheduled through 2008. No peer review is planned. (Manuscript in preparation)
46	NOAA	Allen	NA03NMF457 0379	Biosecurity and comparative field trials of triploid Crassostrea ariakensis with C. virginica	n/a	n/a	n/a	n/a	None Identified	Note: this is biosecurity reporting - there is no analysis planned and no conclusions. A report is available on the NOAA website.
46	NOAA	Allen		VSC Trials - Ongoing Biosecurity Reporting	n/a	n/a	n/a	n/a	None Identified	Note: this is biosecurity reporting - there is no analysis planned and no conclusions (A manuscript is in preparation)
47	NOAA	Luckenbach, Mann, North	NA06 NMF457 0246	Developing a relationship between Gamete Concentration, Turbulent Mixing and Fertilization Efficiency in C.a.	Apr-08	Sep-08	No	No	None Identified	Research is scheduled through 2008. No peer review is planned.
48	NOAA	Scarpa	NA06 NMF457 0245	Growth and Reproduction of C.a. in a U.S. Sub-Tropical Environment: EIS Ramifications	Apr-08	Dec-08	No	No	None Identified	Research is scheduled through 2008. No peer review is planned.
	NOAA	Wang, Shen	NA04 NMF457 0361	Assessing Asian Oyster Dispersion and Potential Risk of Reproduction Using a Chesapeake Bay Hydrodynamic Model with Age-Distribution Function	none	Jan-07	Yes	No	None Identified	If necessary, a peer review will be planned for the final report. A report is available on the NOAA website.
	NOAA	O'Connell	NA07 NMF457 0305	Wrap-up tasks for the Programmatic Environmental Impact Statement (EIS) (funds MES and Versar)	Apr-08	Dec-08	No	No	None Identified	No peer review is planned
	NOAA	Barnes (ERF)	NA07 NMF457 0306	ERF 2007 support	n/a	Apr-08	Yes	No	None Identified	No peer review is planned
	NOAA	Beal (ASMFC)	NA06 NMF457 0281	EIS PDT support for ASMFC	Apr-08	n/a	No	No	None Identified	No peer review is planned
	NOAA	Allen, Frank, Lawale	NA07 NMF457 0311	Breeding and domestication of C. virginica lines and varieties for oyster culture in Chesapeake Bay	Apr-08	Dec-08	No	No	None Identified	No peer review is planned

EIS Proj. #	Funding Agencies	Principal Investigator	NOAA Grant ID	Study Title	Last Progress Report Date	Scheduled Final Report Due/Done	Final Report Complete	Peer Review Complete ?	Journal Published ?	Comments
	NOAA	Harding, Mann, Southworth	NA07 NMF457 0313	Impacts of Oyster Aquaculture on Shallow Water Ecosystems in a Chesapeake Bay	Apr-08	Nov-08	No	No	None Identified	No peer review is planned
	NOAA	Wesson	NA07 NMF457 0314	An Evolution of Extensive Oyster Aquaculture on Private Leased Ground in Virginia Using Triploid Native Oysters	Jan-08	Mar-09	No	No	None Identified	No peer review is planned
	NOAA	Luckenbach	NA06 NMF457 0293	Sponsorship for special issue of the Journal of Shellfish Research to disseminate findings on C. ariakensis research	n/a	Feb-08	Yes	No	None Identified	No peer review is planned
	NOAA	Congrove	NA06NMF4570004	Feasibility study on one-year grow-out of market size triploid Crassostrea ariakensis	n/a	Apr-08	Yes	No	None Identified	No peer review is planned

ATTACHMENT B

MODELED RELATIONSHIPS BETWEEN OYSTER BIOMASS, WATER QUALITY, AND ECOLOGICAL RESPONSES

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1.0 Introduction

This appendix describes the analysis undertaken to explore the modeling results of Carl Cerco and Mark Noel using the Chesapeake Bay Environmental Modeling Package (CBEMP), which relates changes in water quality and ecological responses to changes in oyster biomass. Several documents describe the methods of Cerco and Noel:

> Assessing a Ten-Fold Increase in the Chesapeake Bay Native Oyster Population:A Report to the EPA Chesapeake Bay Program. July 2005. Prepared by Carl F. Cerco and Mark R. Noel of the US Army Engineer Research and Development Center, Vicksburg, MS
>
> Evaluating Ecosystem Effects of Oyster Restoration in Chesapeake Bay: A Report to the Maryland Department of Natural Resources. September 2005. Prepared by Carl F. Cerco and Mark R. Noel of the US Army Engineer Research and Development Center, Vicksburg, MS
>
> Ecosystem Effects of Oyster Restoration in Virginia Habitat and Lease Areas: A Report to the Maryland Department of Natural Resources. Draft. April 2006. Prepared by Carl F. Cerco and Mark R. Noel of the US Army Engineer Research and Development Center, Vicksburg, MS

Three models are at the heart of the CBEMP. Distributed flows and loads from the watershed are computed with a highly modified version of the HSPF model (Bicknell et al. 1996). These flows are input to the CH3D-WES hydrodynamic model (Johnson et al. 1993), which computes three-dimensional intratidal transport. Computed loads and transport are input to the CE-QUALICM eutrophication model (Cerco and Cole 1993), which computes algal biomass, nutrient cycling, dissolved oxygen, and numerous other constituents and processes. The eutrophication model incorporates a predictive sediment-diagenesis component (DiToro and Fitzpatrick 1993) and living resources components, including benthos (Meyers et al. 2000), zooplankton (Cerco and Meyers 2000), and submerged aquatic vegetation (Cerco and Moore 2001). Documentation is available at the Chesapeake Bay Program web site http://www.chesapeakebay.net/modsc.htm. Nutrient and solids loads are computed on a daily basis for 94 subwatersheds of the 166,000-km^2 Chesapeake Bay watershed and are routed to individual cells of the models based on local watershed characteristics and drainage area contributing to the cell. The hydrodynamic and eutrophication models operate on a grid of 13,000 cells. The grid contains 2,900 surface cells (0.4 km2) and employs nonorthogonal, curvilinear coordinates in the horizontal plane. Z coordinates are used in the vertical direction, which is up to 19 layers deep. Depth of the surface cells is 2.1 m at mean tide and varies as a function of tide, wind, and other forcing functions. Depth of subsurface cells is fixed at 1.5 m. A band of littoral cells that is 2.1 m deep at mean tide adjoins the shoreline throughout most of the system. Ten years, 1985 through 1994, are simulated continuously using time steps of 0.5 minutes (hydrodynamic model) and 0.15 minutes (eutrophication model).

The CBEMP was peer reviewed extensively during and after its development (C. Cerco, pers. comm., May 2008). An expert panel, consisting of Dr. Roger Newell and Dr. W. Michael Kemp, both of the University of Maryland, Horn Point, provided advice and reviewed products during development. Throughout the development of the model, Cerco and Noel made quarterly presentations about the CBEMP to the Chesapeake Bay Program Modeling and Research Subcommittee (MARS) and the Living Resources Subcommittee; question-and-answer sessions and critiques followed each presentation. MARS approved the model in October 2005. The CBEMP provided the basis for the peer-reviewed publication: Cerco, C., and Noel, M. (2007). Can oyster restoration reverse cultural eutrophication? *Estuaries and Coasts*, 30(2), 331-343.

Cerco and Noel modeled changes in oyster biomass over a 10-year period, but the scenarios they modeled did not correspond precisely with the alternatives being considered in the PEIS. Because the CBEMP was not run specifically to address the issues being considered in the Ecological Risk Assessment (ERA), a simplified approach using the available CBEMP outputs to project environmental consequences of the EIS alternatives was developed in consultation with Carl Cerco. Cerco and Noel developed model runs for the following six scenarios used in our evaluations of relationships:

- Oyster biomass for the 1994 Base Case (OYS30)
- Oyster biomass for the 1994 Base Case with treatment (Maryland) (OYS30_LOOP_wtreatment)
- Oyster biomass for the Base Case with restoration (OYS36)
- Oyster biomass for a 10-fold increase (OYS31)
- Oyster biomass for a 10-fold increase with restoration (OYS37)
- Oyster biomass for the 1920-1970 period (OYS28 1920-1970)

Cerco and Noel provided the results of these model runs to the ERA and EIS writing teams for each segment of Chesapeake Bay and for each modeled year (1 through 10). These model results cover a range of oyster biomass values for each Chesapeake Bay segment. We examined how water quality and ecological responses changed across this range of biomass for each segment. That analysis revealed strong relationships in some segments but not in others. For purpose of the ERA, we grouped the segments used by Cerco and Noel according to zones defined by state and salinity: Maryland oligohaline (MD OH), Maryland mesohaline (MD MH), Maryland polyhaline (MD PH), Virginia oligohaline (VA OH), Virginia mesohaline (VA MH), and Virginia polyhaline (VA PH). These zones were established based on some geographical limitations of ODM population projections but also because they reflect the major salinity zones within which biologists commonly characterize certain aquatic communities of Chesapeake Bay. In addition, the ODM projections are highly variable at the level of a segment, and aggregating segments into larger geographic areas dampened some of this variability.

The model results from Cerco and Noel included several segments for which oyster biomass was assigned only a nominal value that did not change during the model runs. All of those segments had the same temporal fluctuations; therefore, we concluded that water quality and ecological responses in those nominal- (non-) oyster segments reflected annual fluctuations in water flows. We presume that Cerco and Noel included the segments with nominal values of oyster biomass to facilitate the modeling computations. Because these segments did not provide insight into relationships between oyster biomass and water quality or other ecological responses, we did not include them in analyses for the ERA.

Model projections were sufficient to examine relationships for four of the six state/salinity zones: Maryland oligohaline, Maryland mesohaline, Virginia mesohaline, and Virginia polyhaline. Table A-1 lists the Chesapeake Bay segments included in these four zones.

Table A-1. Chesapeake Bay segments used to evaluate the state/salinity zones for the ERA. The ERA evaluation considered only the segments in which oyster biomass changed among modeling scenarios and model years.

MD Oligohaline	MD Mesohaline	VA Mesohaline	VA Polyhaline
CB2OH	BIGMH	CRRMH	CB6PH
CHOOH	CB3MH	JMSMH	CB7PH
POCOH	CB4MH	PIAMH	CB8PH
POTOH (SAV)	CB5MH	RPPMH	ELIPH
	CHOMH1	YRKMH	JMSPH
	CHOMH2		MOBPH
	CHSMH		YRKPH
	EASMH		
	FSBMH		
	HNGMH		
	LCHMH		
	MAGMH		
	MANMH		
	NANMH		
	PATMH		
	PAXMH		
	POCMH		
	POTMH		
	RHDMH		
	SEVMH		
	SOUMH		

Table A-1. Chesapeake Bay segments used to evaluate the state/salinity zones for the ERA. The ERA evaluation considered only the segments in which oyster biomass changed among modeling scenarios and model years.

MD Oligohaline	MD Mesohaline	VA Mesohaline	VA Polyhaline
	TANMH		
	WICMH		

The number of segments used in the analysis varied among these four state/salinity zones. For each of the 60 model outputs (6 scenarios x 10 years), we calculated values for total oyster biomass for all segments within a zone and also an area-weighted or volume-weighted value for a water quality or ecological response factor. Dissolved oxygen was handled differently, as described in Section 2.

We used linear regression to determine if the relationship between oyster biomass and a response factor was statistically significant in the CBEMP projections. Because response factors are likely to reflect a host of other conditions within the segments, the variability in a response often was considerable within and among modeled scenarios. We were interested in ascertaining the nature and magnitude of relationships within each state/salinity zone to enable us to evaluate the implications of changes in oyster biomass expected to result from each of the alternatives; therefore, we used the regression calculations to identify the direction of the relationship (direct or inverse) and the relative magnitude of the relationship. Outputs of regression analyses that were statistically significant are provided at the end of this document.

After reviewing model results, we noticed that the statistical relationships between oyster biomass and various ecological components for the MD OH region often were inconsistent with known biological relationships or were insignificant. Inspection revealed that the MD OH zone included only four Chesapeake Bay segments, whereas the MD MH zone included 23 segments, despite the fact that ODM projections categorized a more substantial proportion of oyster bars as oligohaline. This discrepancy resulted from differences between how salinity zones were established in the CBEMP and how they were applied to aggregate ODM results. Chesapeake Bay segments used in the CBEMP are designated as oligohaline, mesohaline, or polyhaline based on long-term salinity records. ODM results were aggregated according to salinity by determining the average salinity at each oyster bar over the 1000 model runs for each alternative, and then aggregating oyster biomass for all bars where average salinities were within the ranges defined for each salinity zone. The modeled salinity values at many bars differed from year to year, and many bars changed back and forth between oligohaline and mesohaline over all model runs. The result was that many oyster bars that geographically would fall into a mesohaline Bay segment were categorized as oligohaline based on averaged modeled salinity values. The larger data set available for MD MH is evident in the larger range of oyster biomass depicted for mesohaline segments than for oligohaline segments in the figures presented in this appendix. We were aware that waters classified as oligohaline in the ODM included large portions of Chesapeake Bay segments that were classified as mesohaline in the Chesapeake Bay Program; consequently, we did not use the CBEMP results for oligohaline segments to evaluate ecological responses to changes in oyster biomass in the MD OH zone. Instead, we applied the regressions derived from the CBEMP for the MD MH zone to the ODM projections for the MD OH zone. We reviewed other relationships and noted situations where the CBEMP either did not include or underrepresented segments for particular areas when compared with the ODM. These were used as follows in the ERA: algae and zooplankton (MD MH was used for MD OH, and VA MH was used for VA OH), benthic deposit feeders (MD MH was used for MD OH and VA PH was used for other Virginia waters).

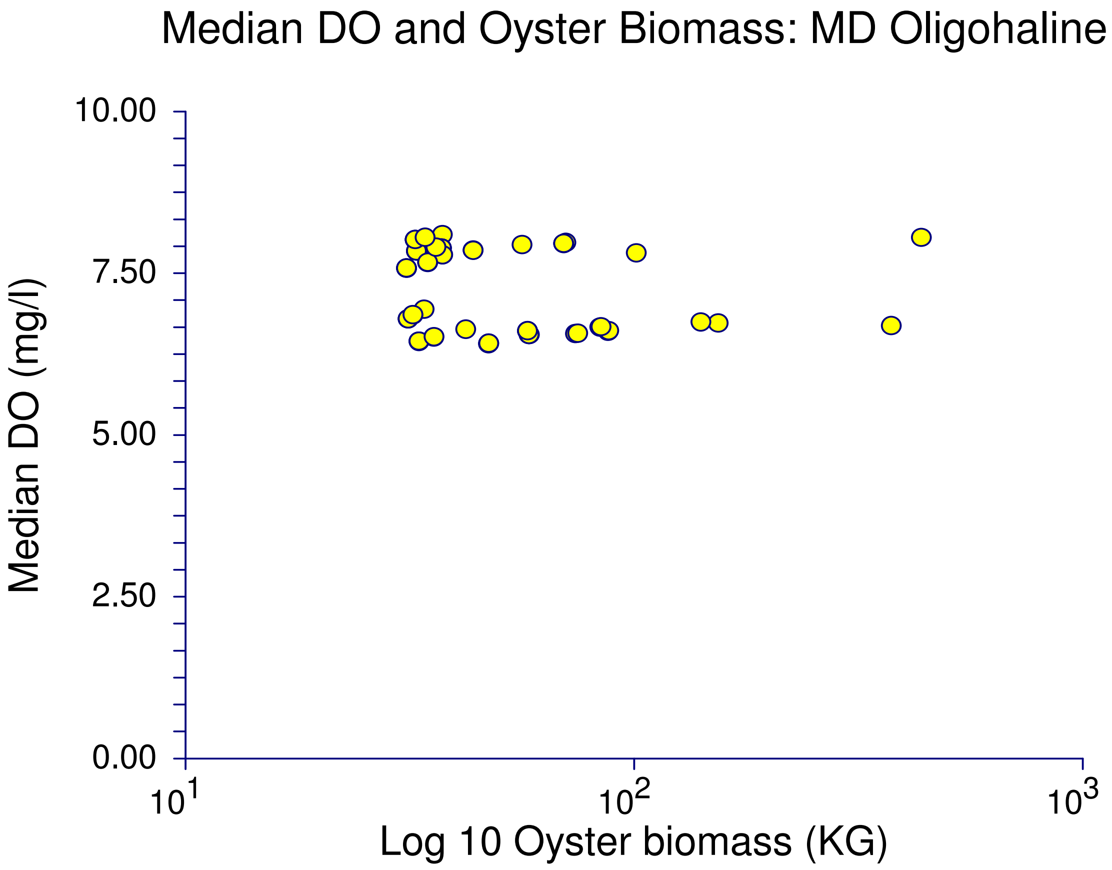
Each of the following sections begins with a brief overview and a description of how information was used in the ERA. Information is presented for the following response factors: dissolved oxygen and total suspended solids, submerged aquatic vegetation, algae, zooplankton, benthic deposit feeders, and other filter feeders. Information is presented in the form of scatter plots. Regression lines are shown only for statistically significant relationships. Details of the regressions, including the criteria for determining statistical significance, are provided at of the end of this document.

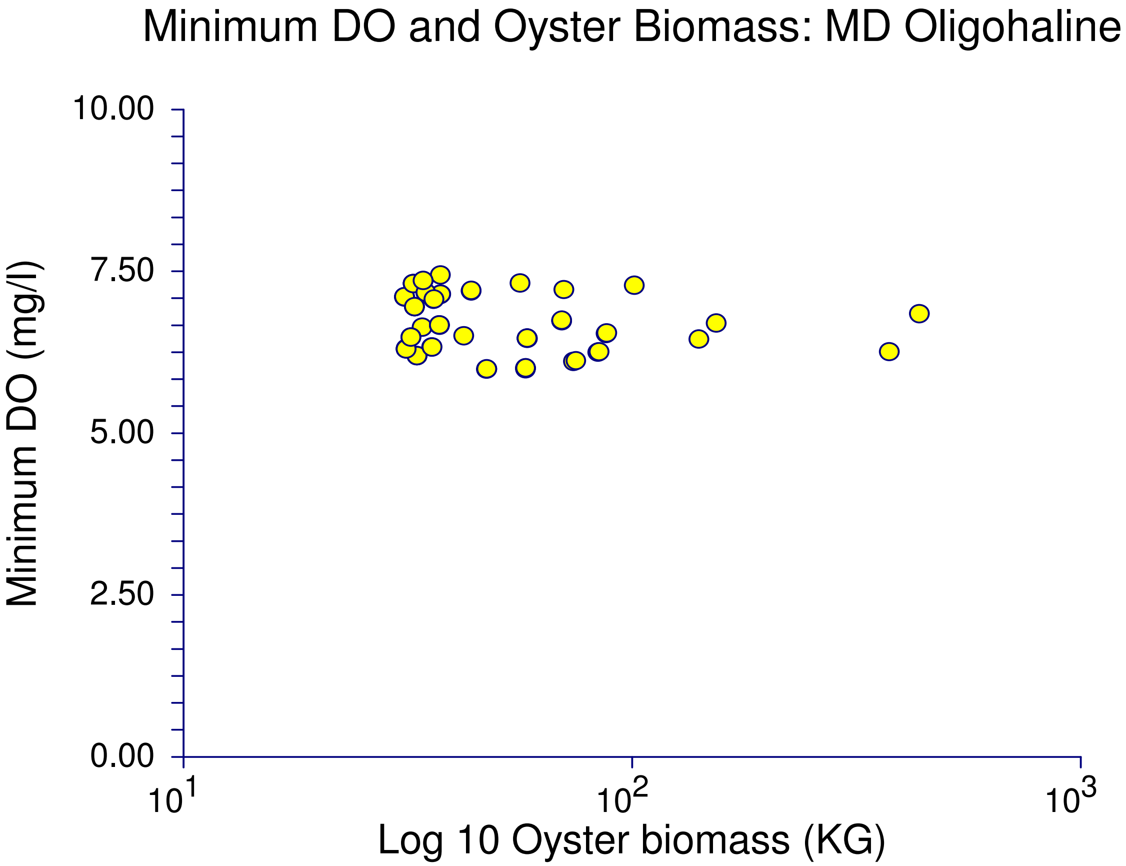
2.0 Relationship of Dissolved Oxygen to Oyster Biomass

Two relationships between oyster biomass and dissolved oxygen were examined, median oxygen for segments and the minimum oxygen for segments, because they are the most useful for characterizing central tendency and the likelihood of ecologically important changes, such as an increase in oxygen in a zone that frequently experiences low DO. For the RRM, we were interested in absolute changes in oxygen relative to various standards or guidelines.

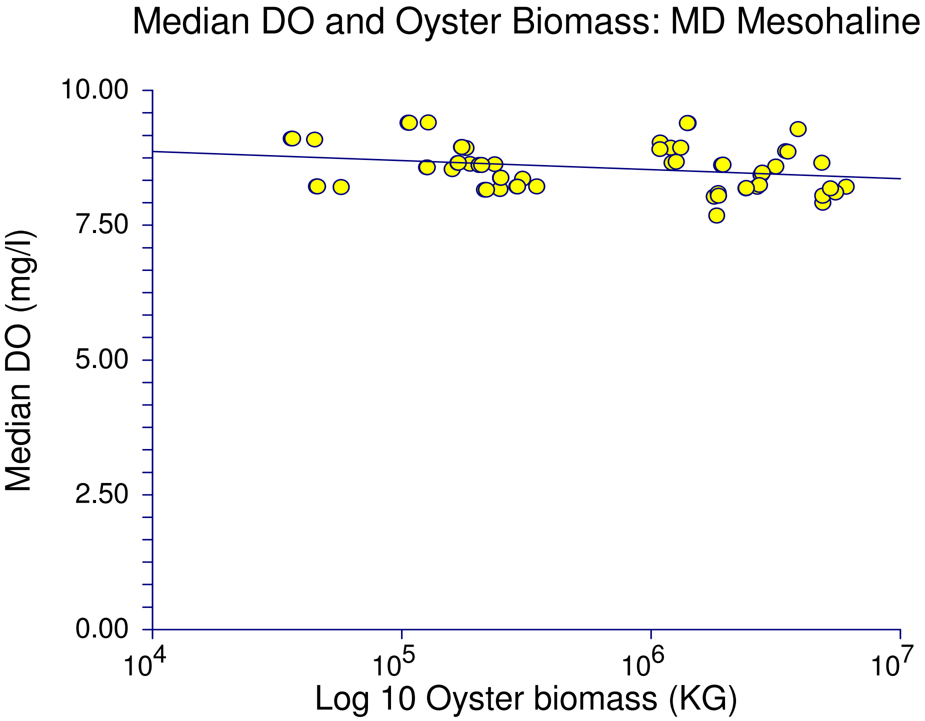
The values for the segments were taken directly from the output of the CBEMP, without weighting for the relative areas of the segments; area weighting was used to evaluate some other biological responses. Only one significant relationship was detected: an inverse relationship between biomass and median DO for the MD MH zone. This inverse relationship is the opposite of the expected response of DO to an increase in oyster biomass and was not observed in the Cerco and Noel evaluation. We believe that aggregating segments into state/salinity zones masked small positive relationships and that the one inverse relationship probably is an anomalous statistical result. Modeled oyster biomass in this zone spanned three orders of magnitude, which is greater than the range of biomass values in the MD OH and VA MH zones; therefore, the inability to detect significant relationships in those zones may be related to their smaller ranges of oyster biomass values. The VA PH zone had oyster biomass values that spanned several orders of magnitude; however, we found no significant relationships between oyster biomass and dissolved oxygen, or most other response factors of interest in this assessment, within that zone.

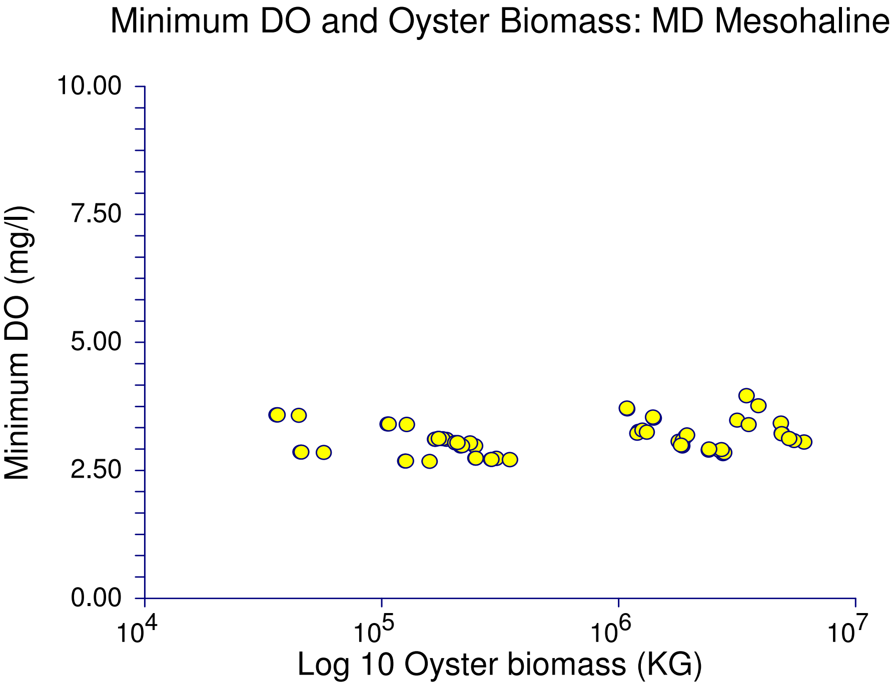
Maryland Oligohaline Zone




Minimum DO and Oyster Biomass: MD Oligohaline
10.00
7.50
5.00
2.50
0.00
Minimum DO (mg/l)
10^1
10^2
10^3
Log 10 Oyster biomass (KG)

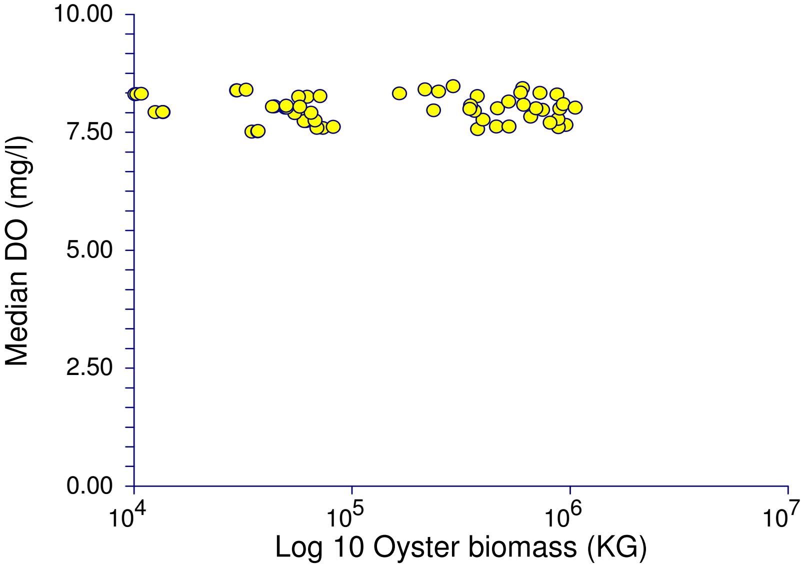
Maryland Mesohaline Zone



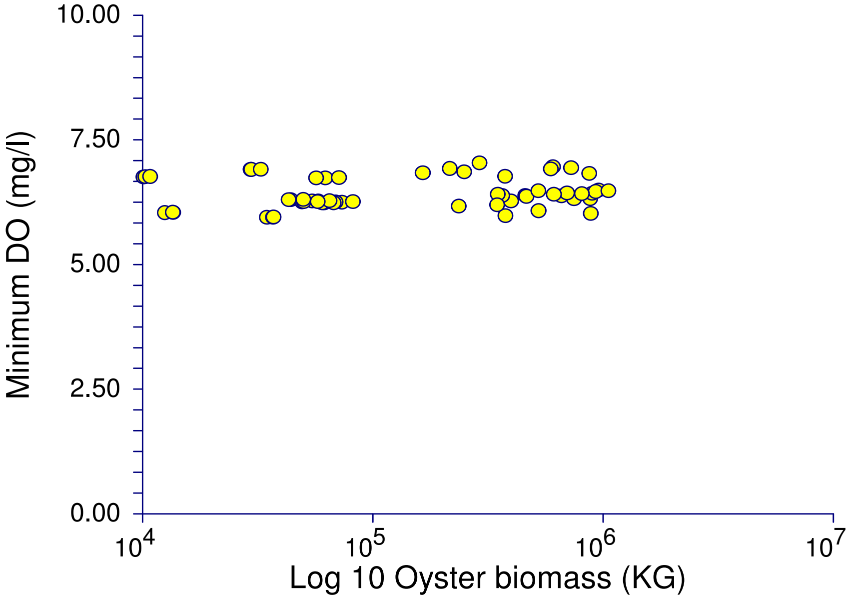


Virginia Mesohaline Zone

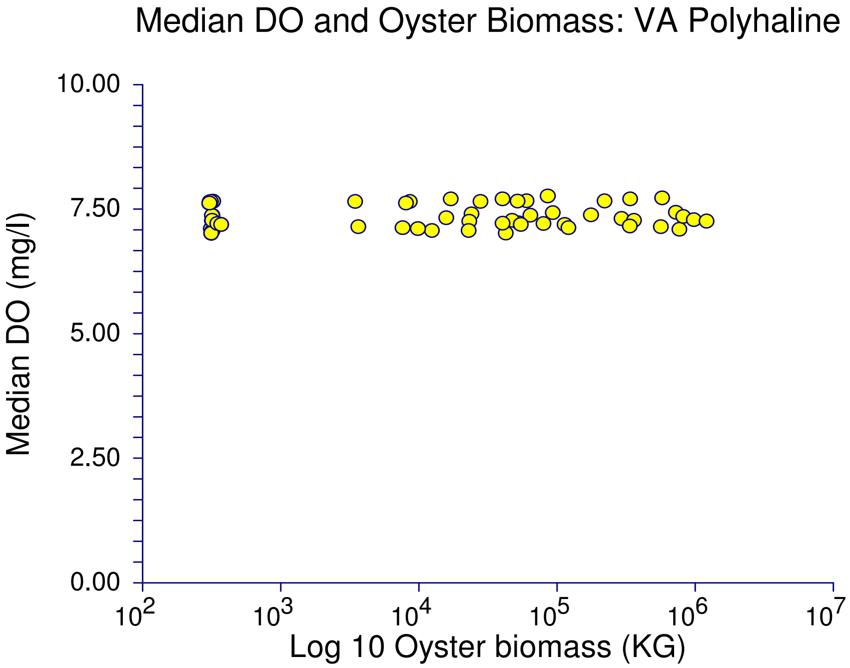
Median DO and Oyster Biomass: VA Mesohaline

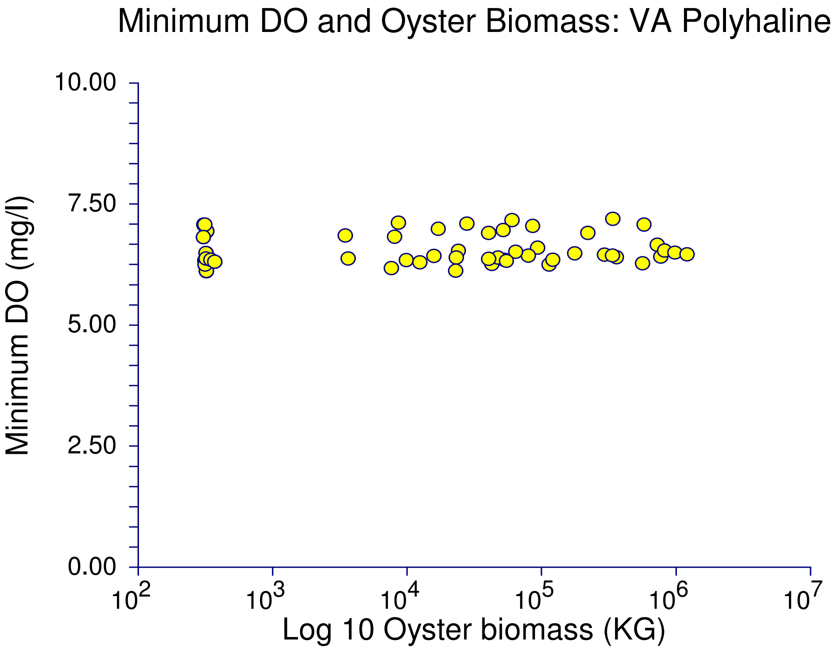


Minimum DO and Oyster Biomass: VA Mesohaline



Virginia Polyhaline

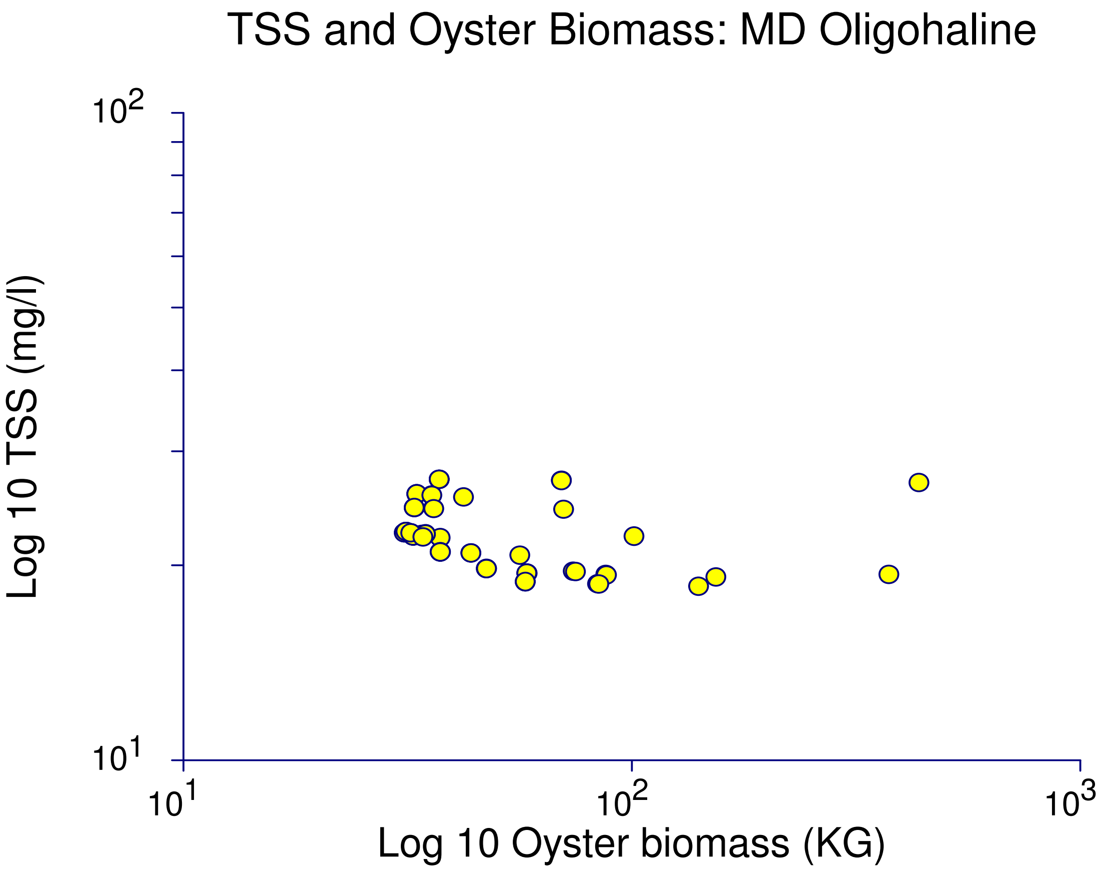




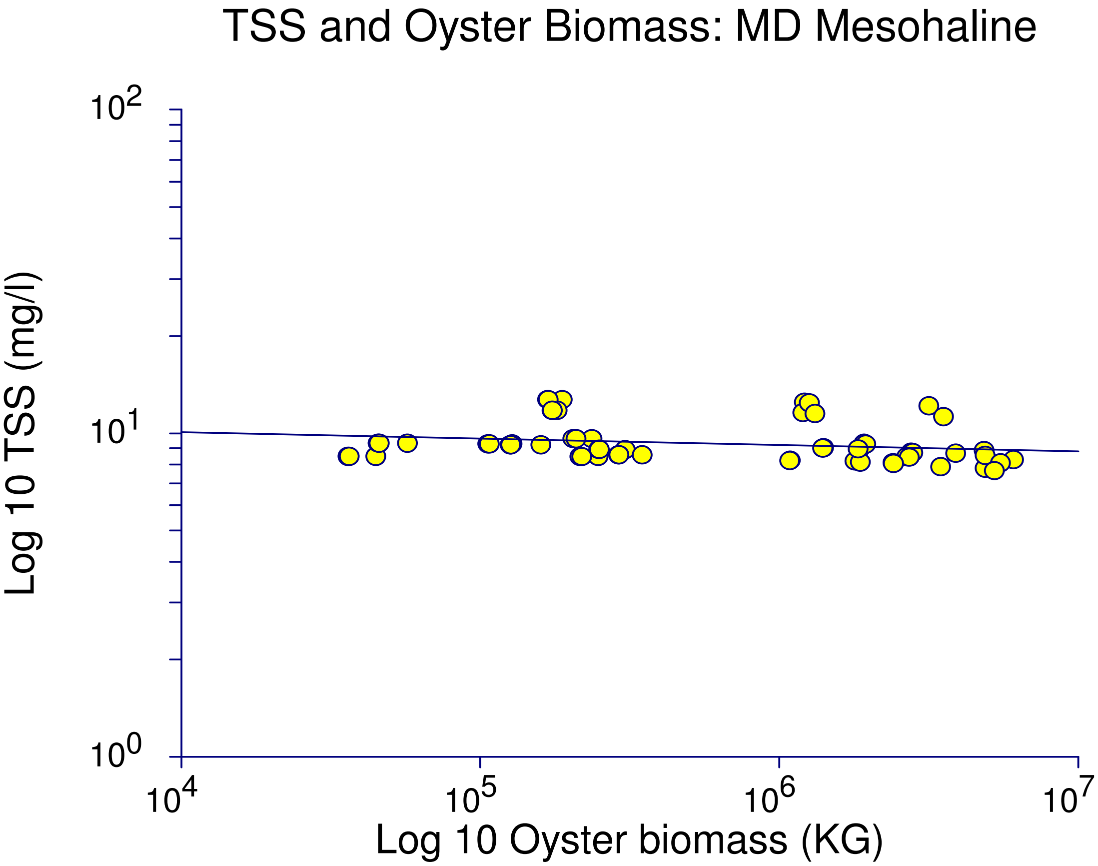
3.0 Relationship of TSS to Oyster Biomass

Estimates of total suspended solids (TSS) in each Chesapeake Bay segment were weighted by the volumes of the segments to obtain volume-weighted estimates of TSS for each segment for each model run (one run per year for 10 years for each of 6 scenarios, for a total of 60 runs per segment). The only significant relationship detected was an inverse one in the MD MH zone. This inverse relationship is expected because oysters filter particulate matter from the water. Modeled oyster biomass in the zone spanned three orders of magnitude, which is greater than the range of values in the MD OH and VA MH zones; therefore, the inability to detect relationships in those zones may be related to their smaller ranges of oyster biomass values. The VA PH zone had oyster biomass that spanned several orders of magnitude; however, we found no significant relationships between oyster biomass and TSS, or most other response factors of interest in this assessment, within that zone.

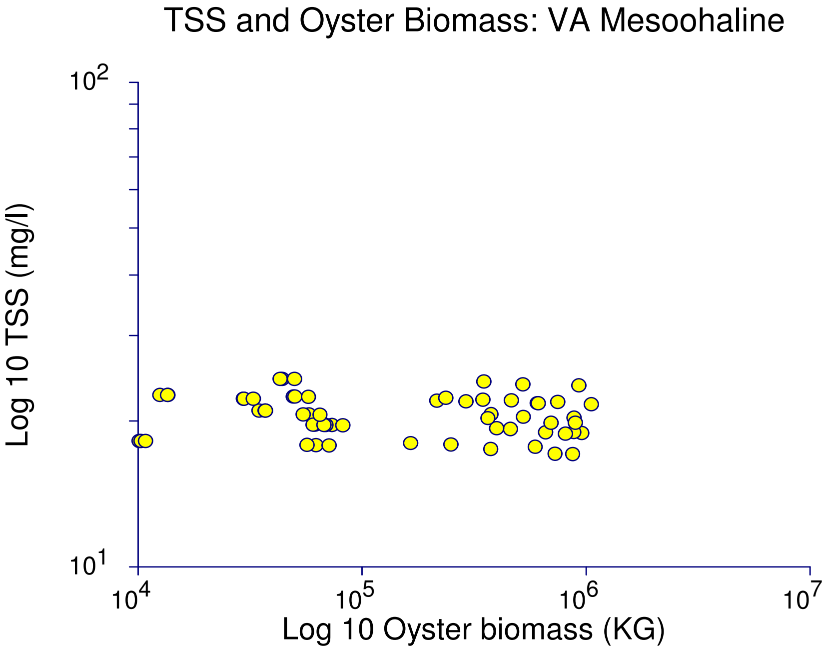
Maryland Oligohaline



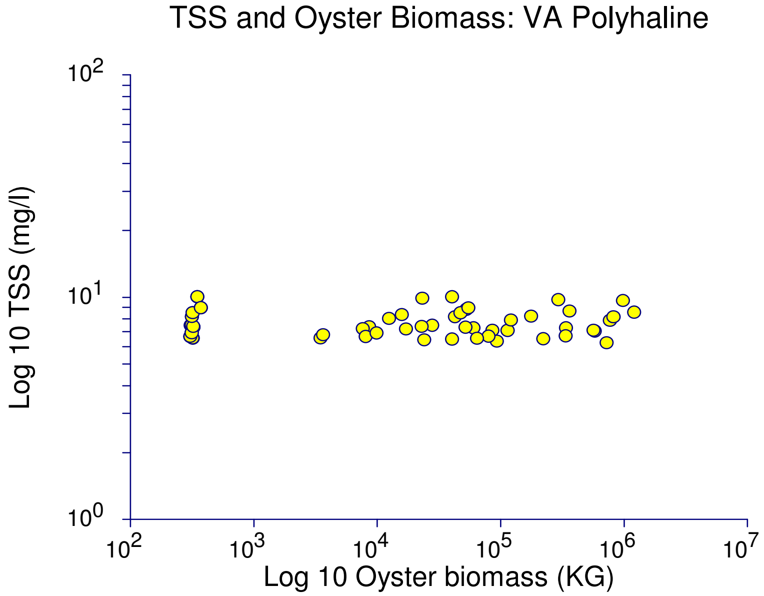
Maryland Mesohaline



Virginia Mesohaline



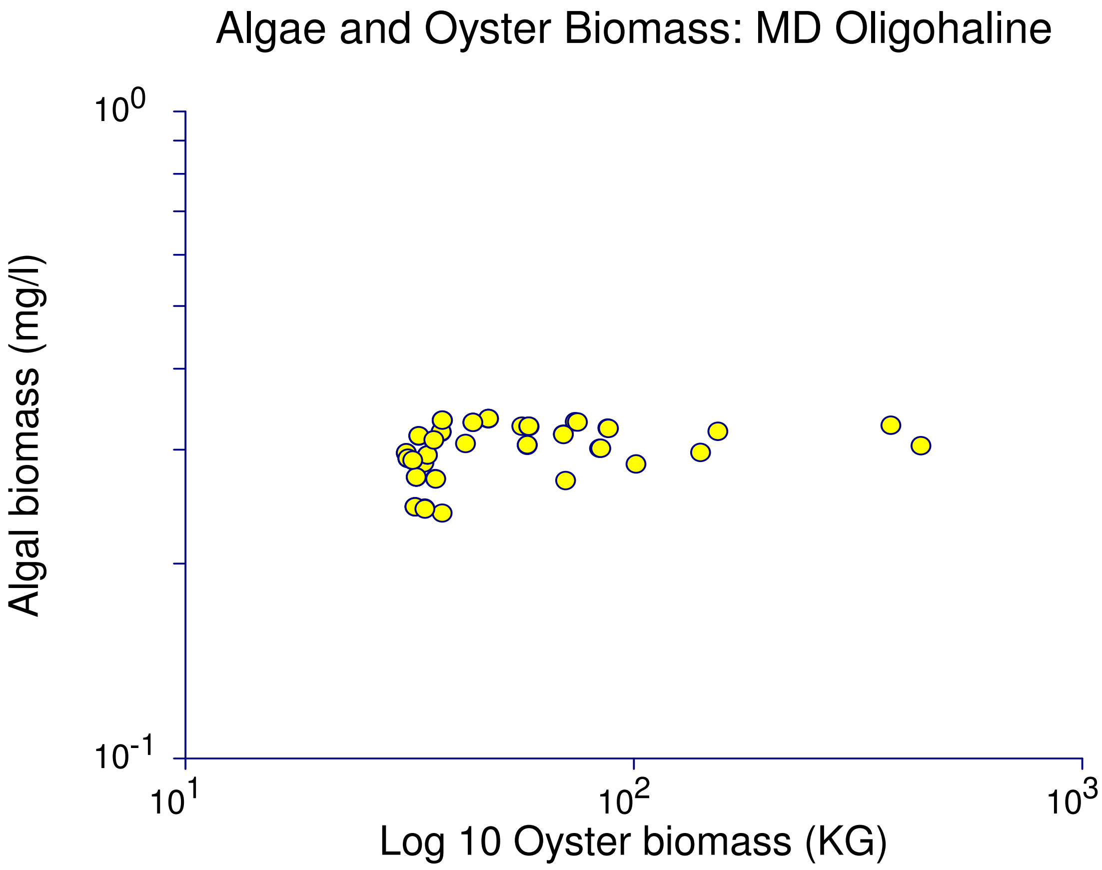
Virginia Polyhaline



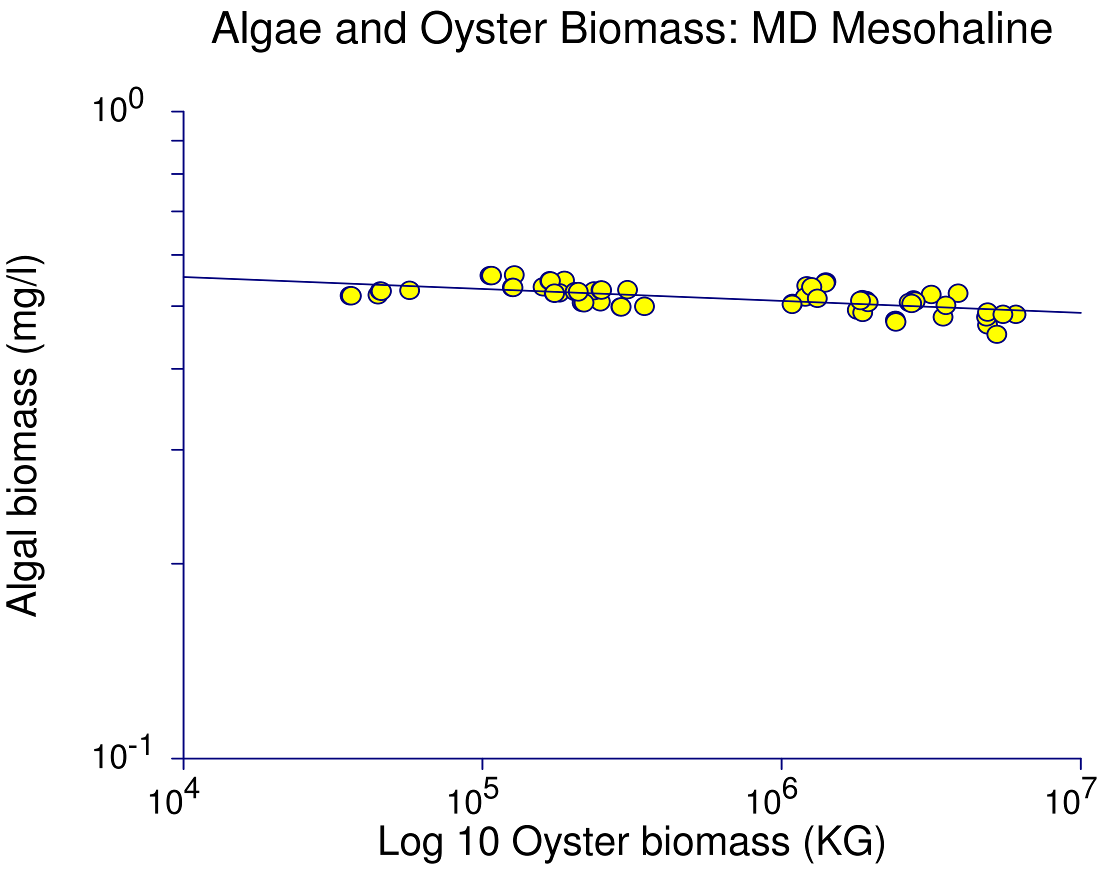
4.0 Relationship of Algal Biomass to Oyster Biomass

Estimates of algal biomass in each Chesapeake Bay segment were weighted by the volumes of the segments to obtain a volume-weighted estimate of algal biomass for each segment for each model run (one run per year for 10 years for each of 6 scenarios, for a total of 60 runs per segment). The only significant relationships detected were inverse ones in the MD MH and VA MH zones. These inverse relationships are expected because oysters filter algae from the water; therefore, an increase in the abundance of oysters translates to an increase in filtration and a potential decrease in the abundance of algae. Modeled oyster biomass for the MD OH zone spanned a little over one order of magnitude, and no significant relationship was detected over this small range. Oyster biomass in the VA PH zone spanned several orders of magnitude; however, we found no significant relationships between oyster biomass and algal biomass, or most other response factors of interest in this assessment, within that zone.

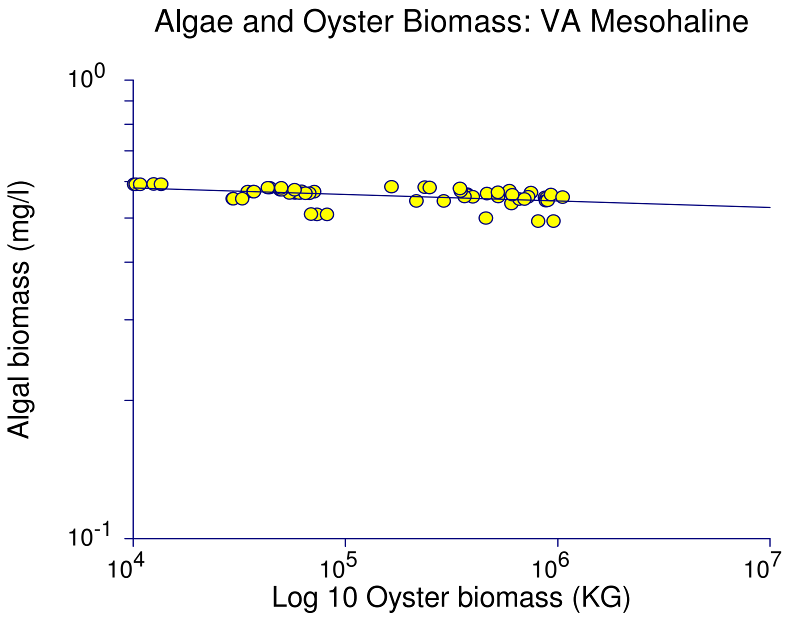
Maryland Oligohaline



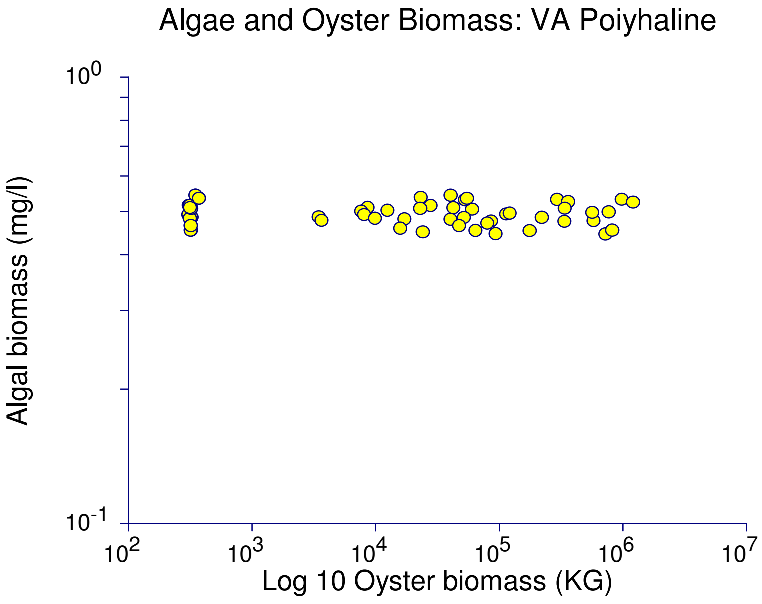
Maryland Mesohaline



Virginia Mesohaline



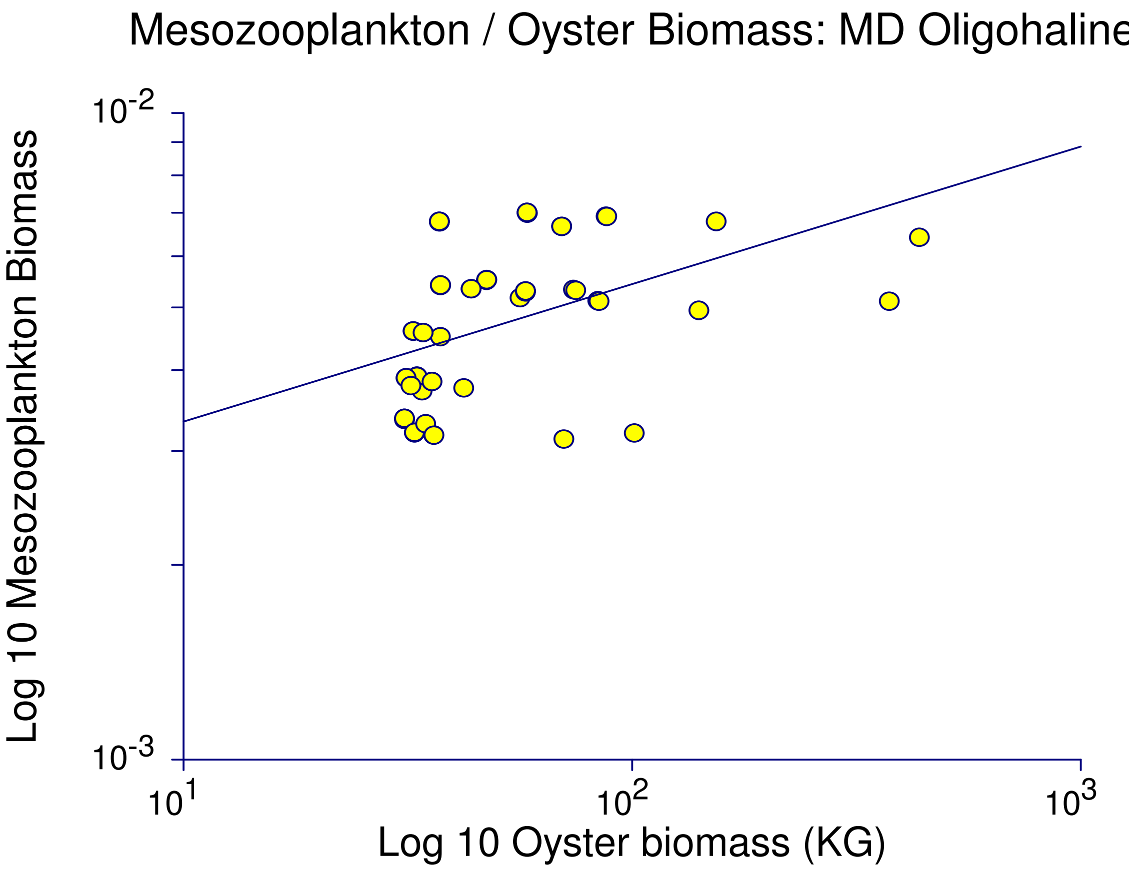
Virginia Polyhaline



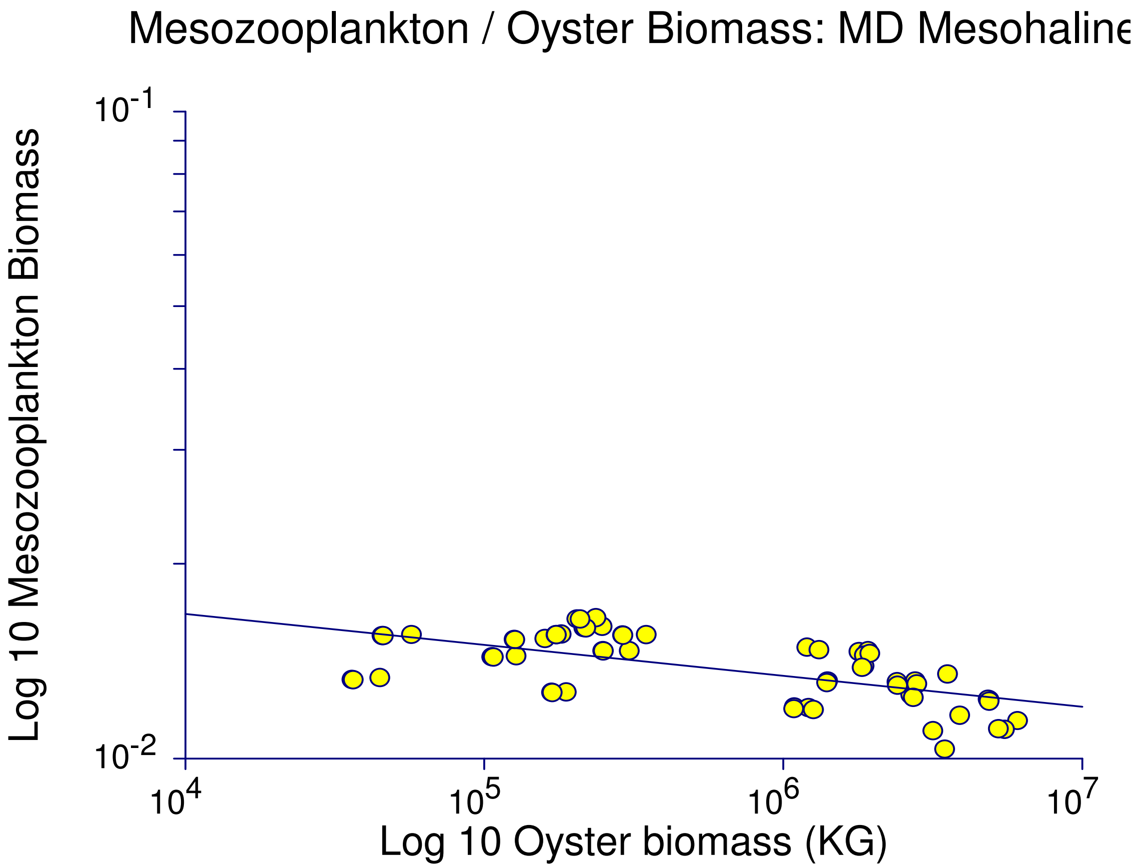
5.0 Relationship of Mesozooplankton to Oyster Biomass

Estimates of mesozooplankton biomass in each Chesapeake Bay segment were weighted by the volumes of the segments to obtain a volume-weighted estimate for each segment for each model run (one run per year for 10 years for each of 6 scenarios, for a total of 60 runs per segment). Significant inverse relationships were detected in the MD MH and VA MH zones. This is the expected nature of the relationship because oysters are expected to decrease algae, and decreases in algae are expected to result in a decrease in zooplankton. The regression equations were used in the RRM to relate changes in oyster biomass to influences on zooplankton. A significant direct relationship was detected in the MD OH zone, where modeled oyster biomass spanned a little over an order of magnitude. This direct relationship probably reflects the small set of values available for the analysis. Oyster biomass in the VA PH zone spanned several orders of magnitude; however, we found no significant relationship between oyster biomass and mesozooplankton biomass, or other response factors of interest in this assessment, within that zone.

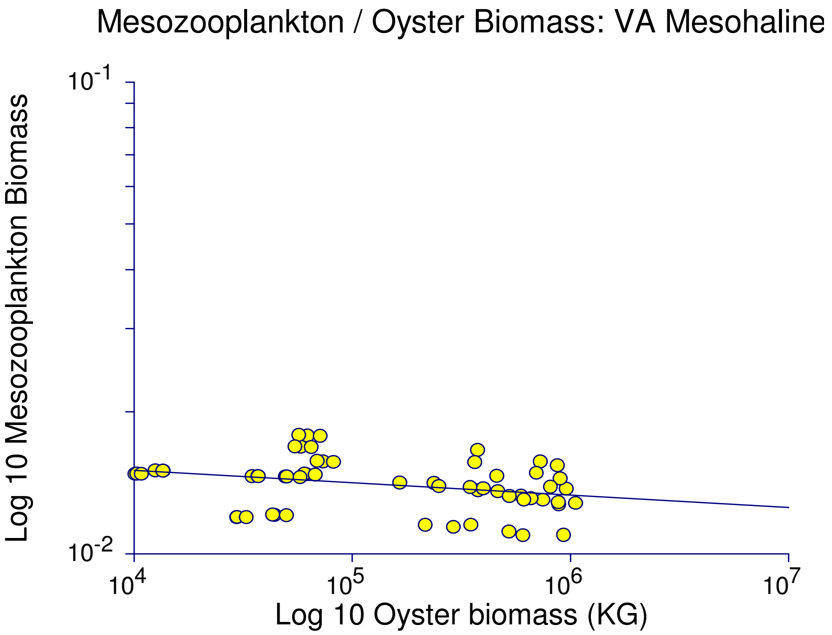
Maryland Oligohaline



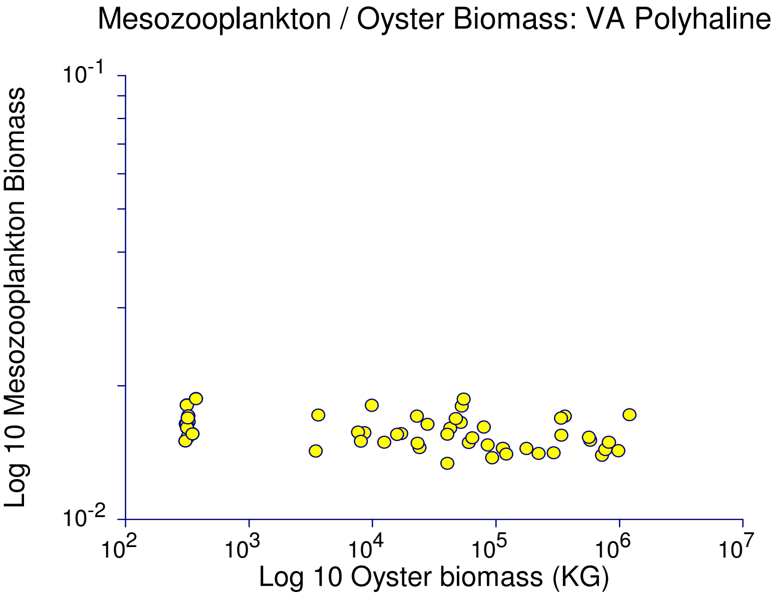
Maryland Mesohaline



Virginia Mesohaline



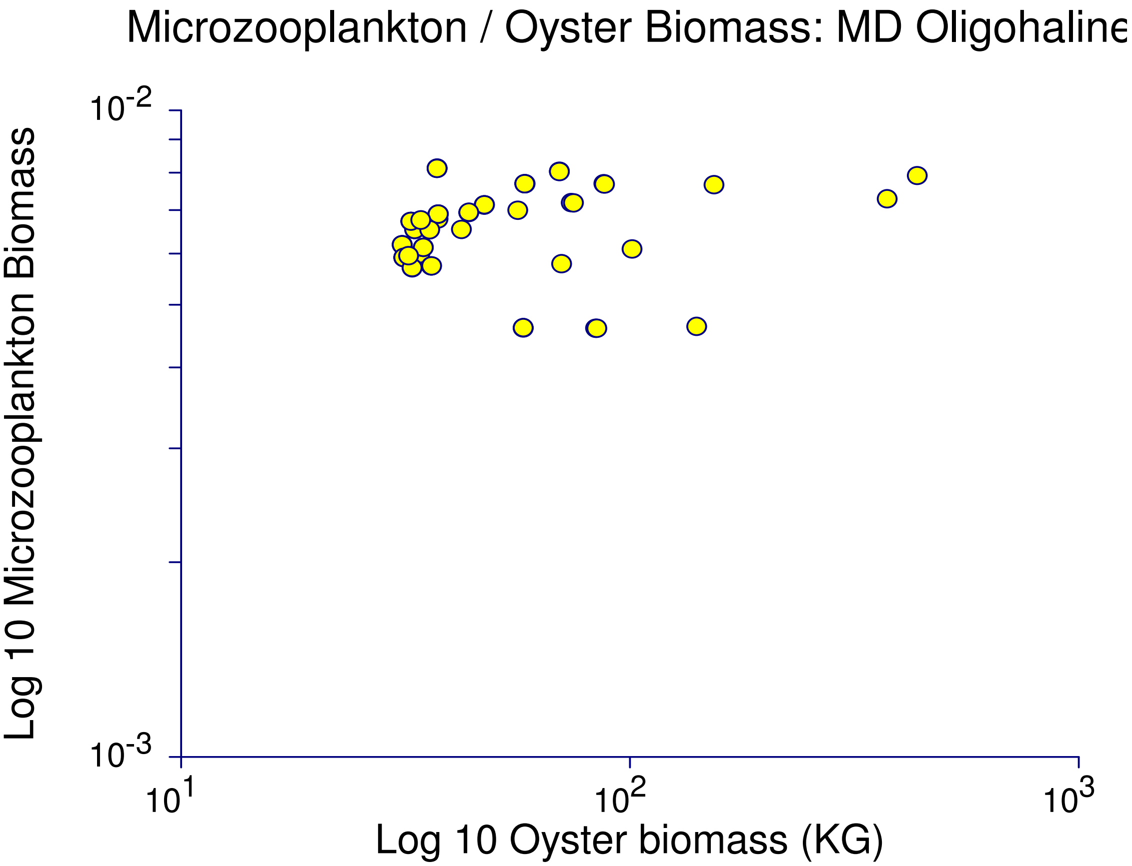
Virginia Polyhaline



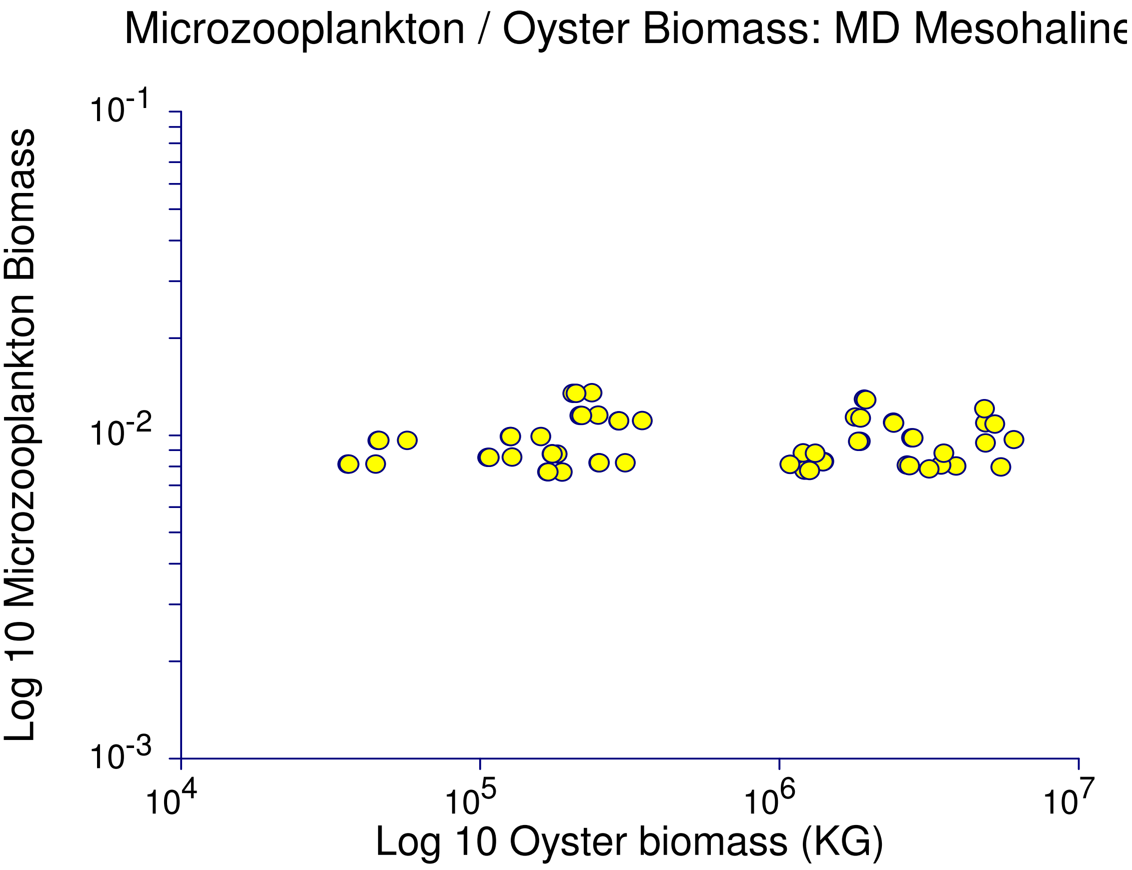
6.0 Relationship of Microzooplankton to Oyster Biomass

Modeled estimates of microzooplankton biomass in each Chesapeake Bay segments were weighted by the volumes of the segments for each model run to obtain a volume-weighted estimate for each of the 60 model runs. No statistically significant relationships were detected between modeled microzooplankton biomass and modeled oyster biomass.

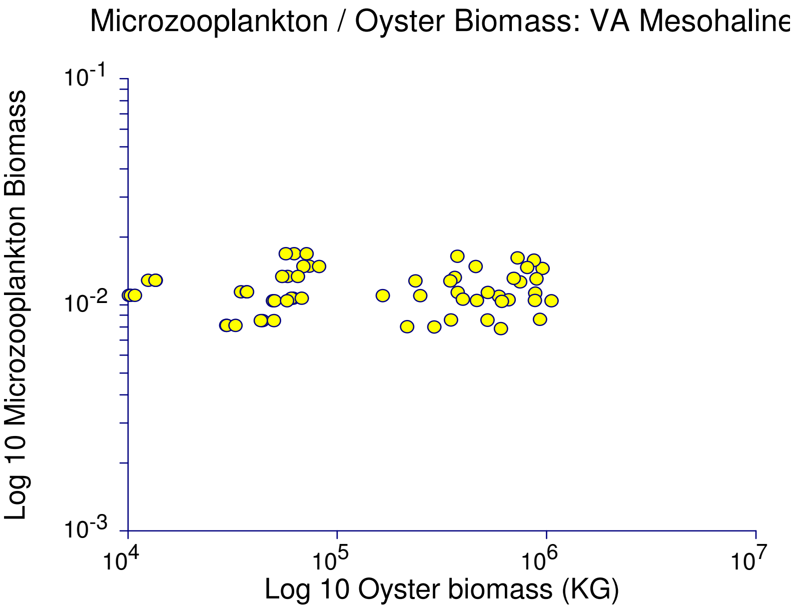
Maryland Oligohaline



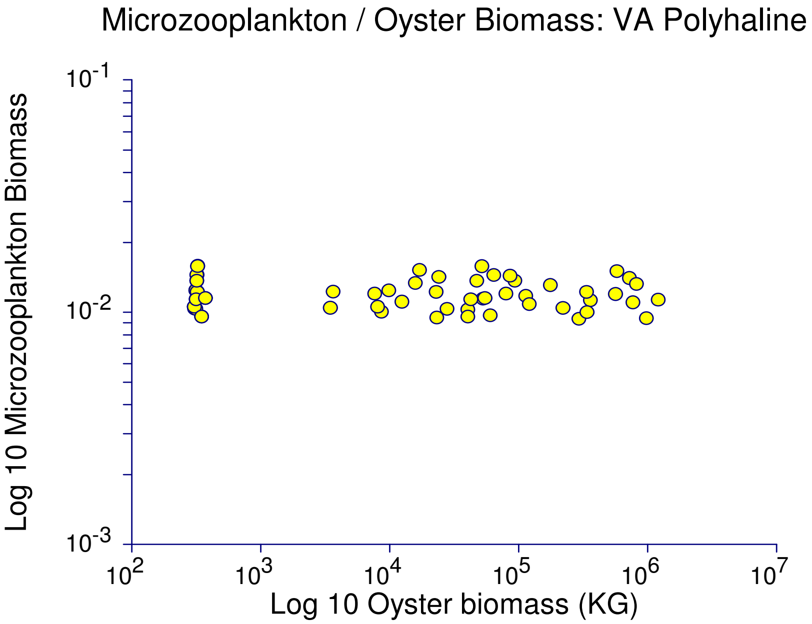
Maryland Mesohaline



Virginia Mesohaline



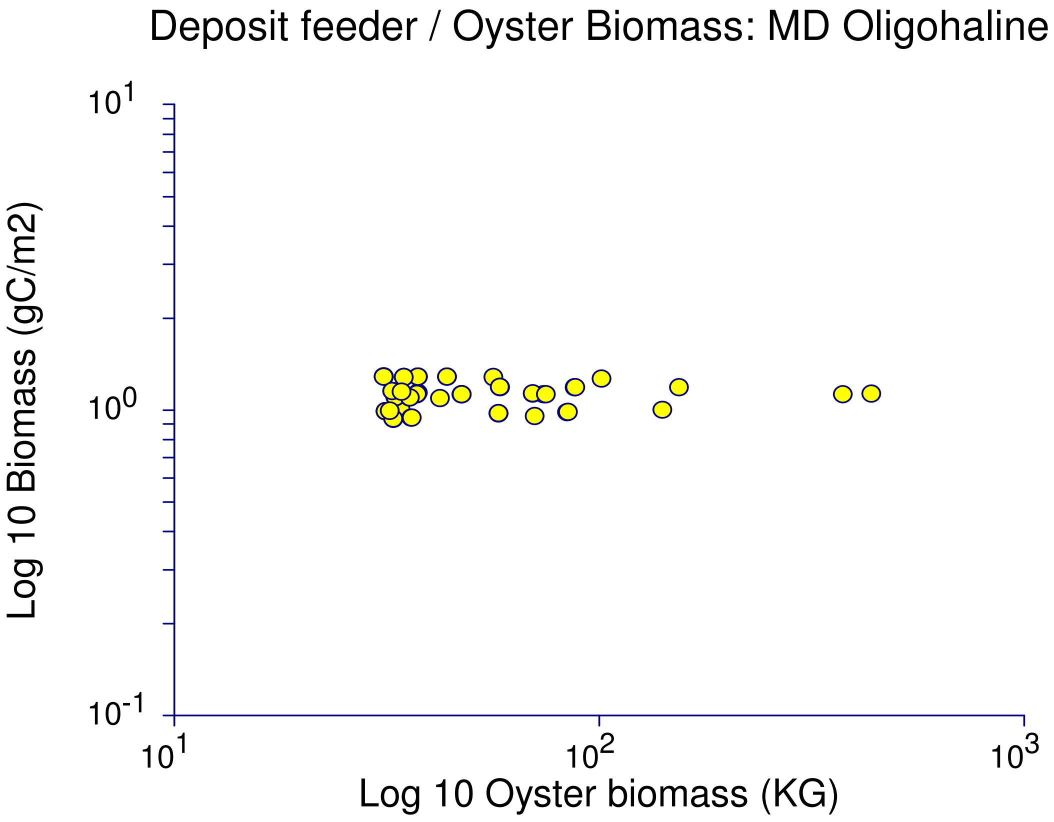
Virginia Polyhaline



7.0 Relationship of Deposit Feeders to Oyster Biomass

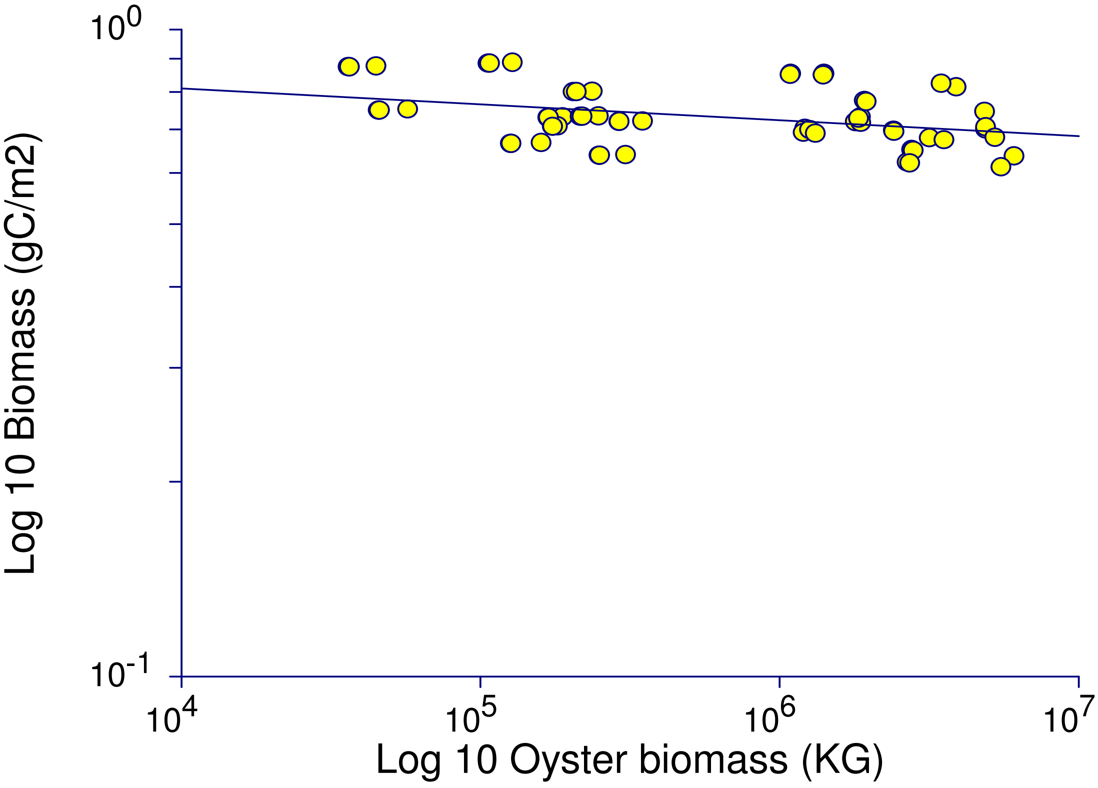
Modeled estimates of the biomass of benthic deposit feeders in each Chesapeake Bay segment were weighted by the areas of the segments. Area was used as a weighting factor because benthic invertebrates inhabit the sediments, and their abundance is usually expressed on a per-unit-area basis. Significant inverse relationships were found in the MD MH and VA MH zones. These inverse relationships were expected because filtration of suspended particulate matter and algae by oysters is expected to reduce the load of these sources of food that reaches the sediments, potentially having a negative influence on the invertebrates that live there. The regression equations for these relationships were used in the RRM to relate changes in oyster biomass to influences on benthic invertebrates. Modeled oyster biomass for the MD OH zone spanned a little over an order of magnitude, and the inability to detect relationships may reflect this small range of oyster biomass. Oyster biomass in the VA PH zone spanned several orders of magnitude; however, we found no significant relationships between oyster biomass and the biomass of benthic deposit feeders, or other response factors of interest in this assessment, within that zone.

Maryland Oligohaline

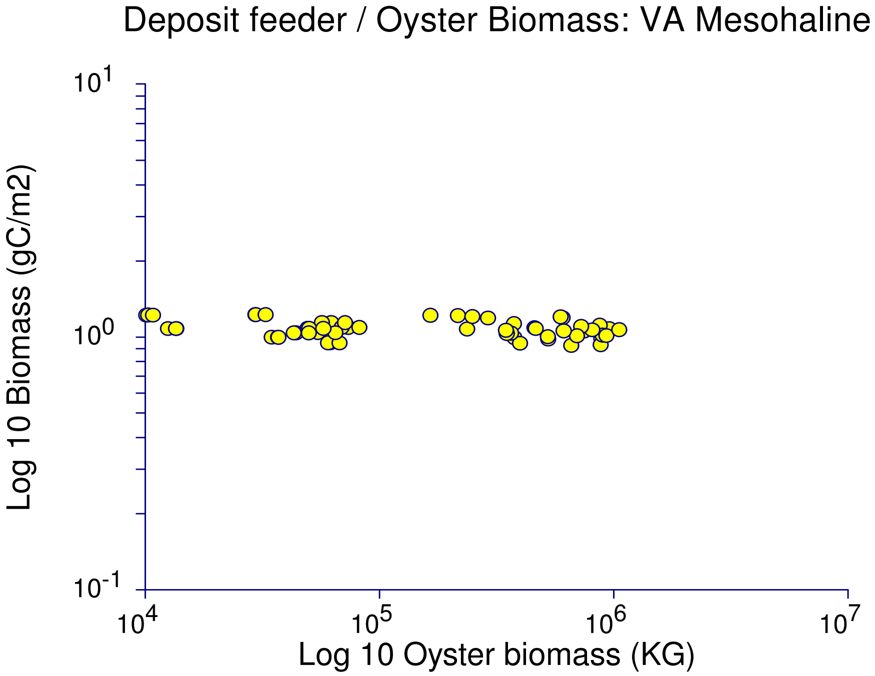


Maryland Mesohaline

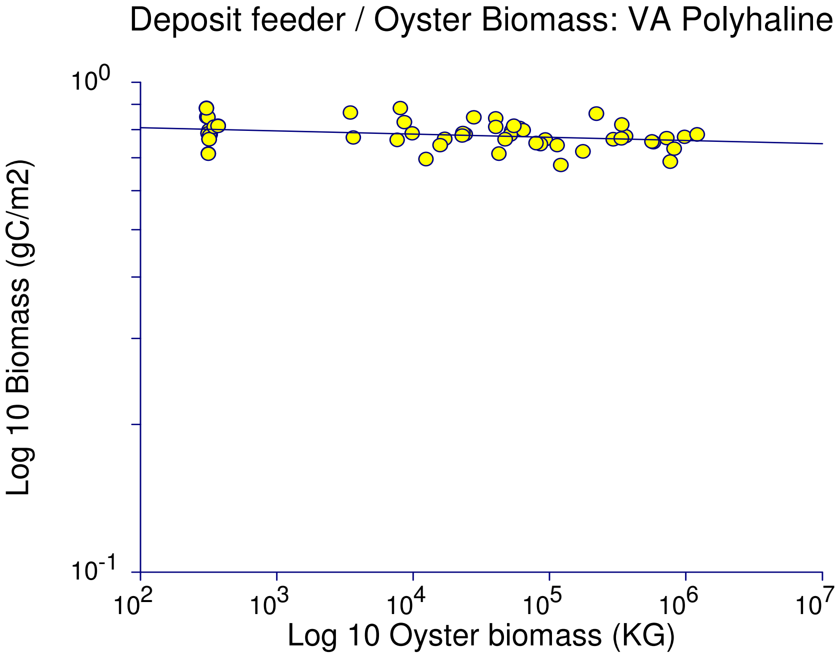
Deposit feeder / Oyster Biomass: MD Mesohaline



Virginia Mesohaline



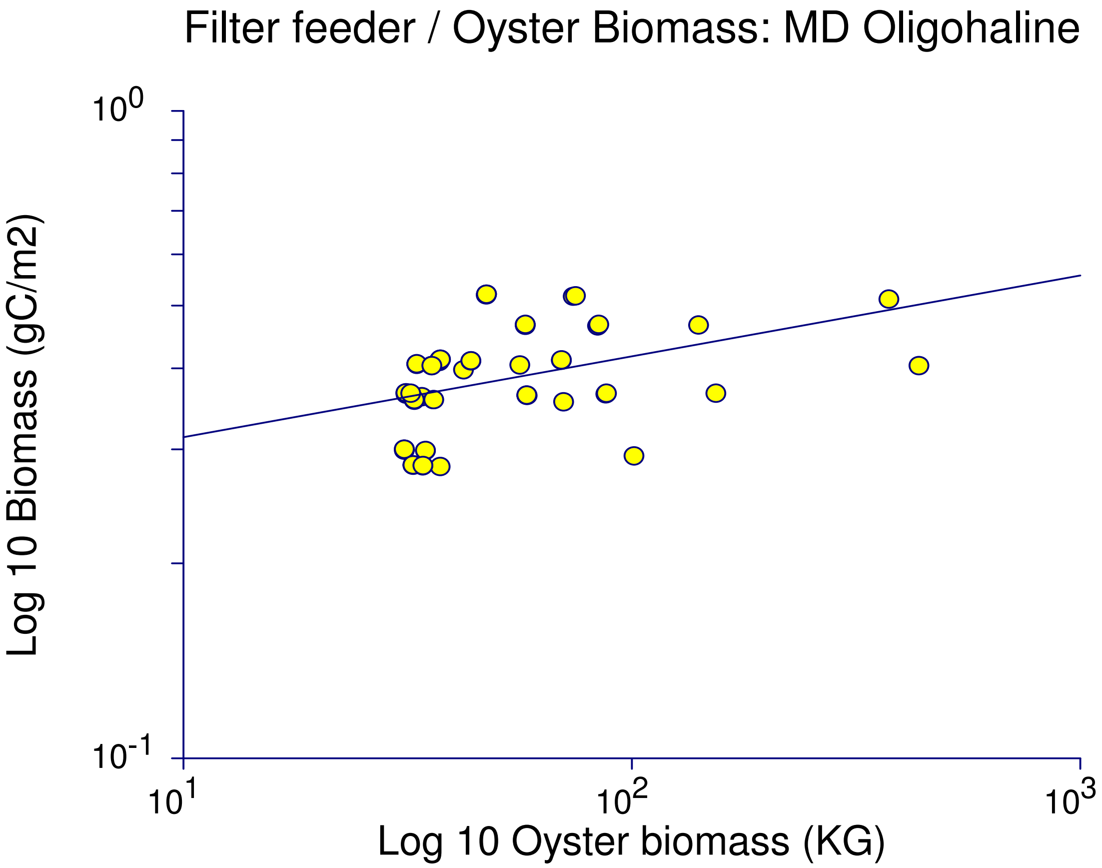
Virginia Polyhaline



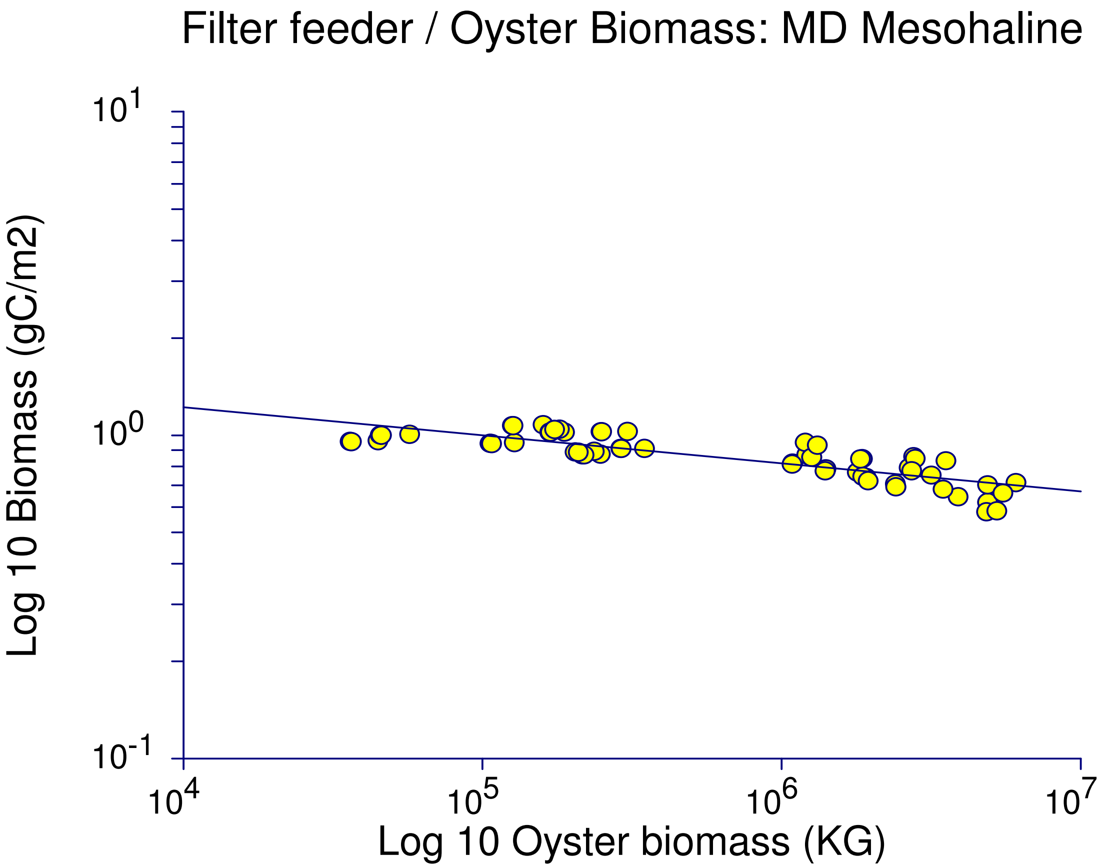
8.0 Relationship of Other Filter Feeders to Oyster Biomass

Modeled estimates of the biomass of other filter feeders (not benthos) in each Chesapeake Bay segment were weighted by the areas of the segments to obtain an area-weighted estimate of the biomass of other filter feeders for each of the 60 model runs. Significant inverse relationships were detected in the MD MH, VA MH, and VA PH zones. As discussed for deposit feeders, this is an expected relationship because increasingly abundant oysters would have a negative effect on potential food sources for other filter feeders. A significant direct relationship was detected in the MD OH zone, where modeled oyster biomass spanned a little over an order an order of magnitude. The positive relationship observed within this zone may reflect variability over a small range.

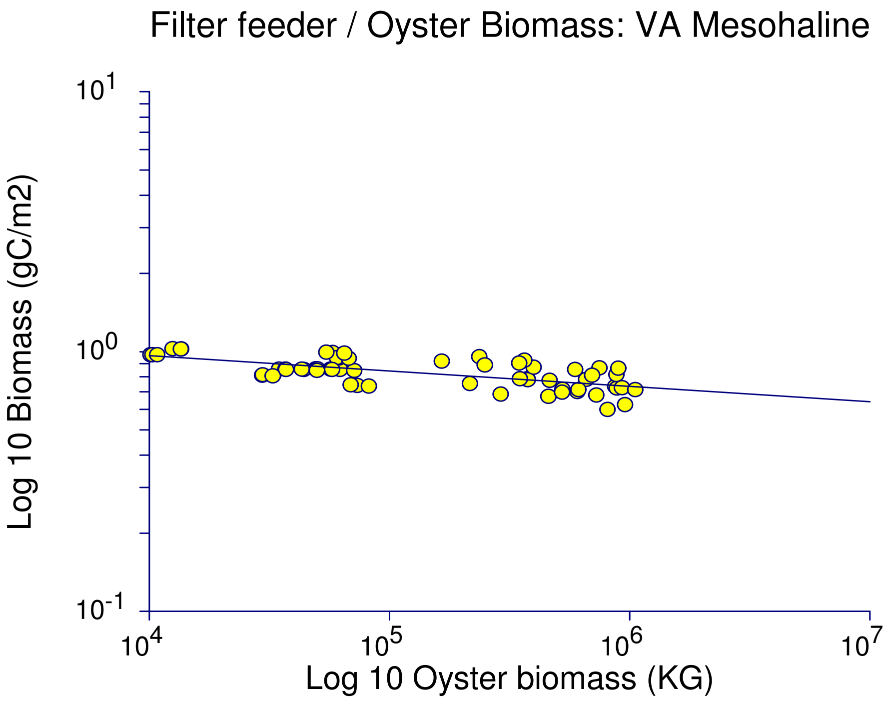
Maryland Oligohaline



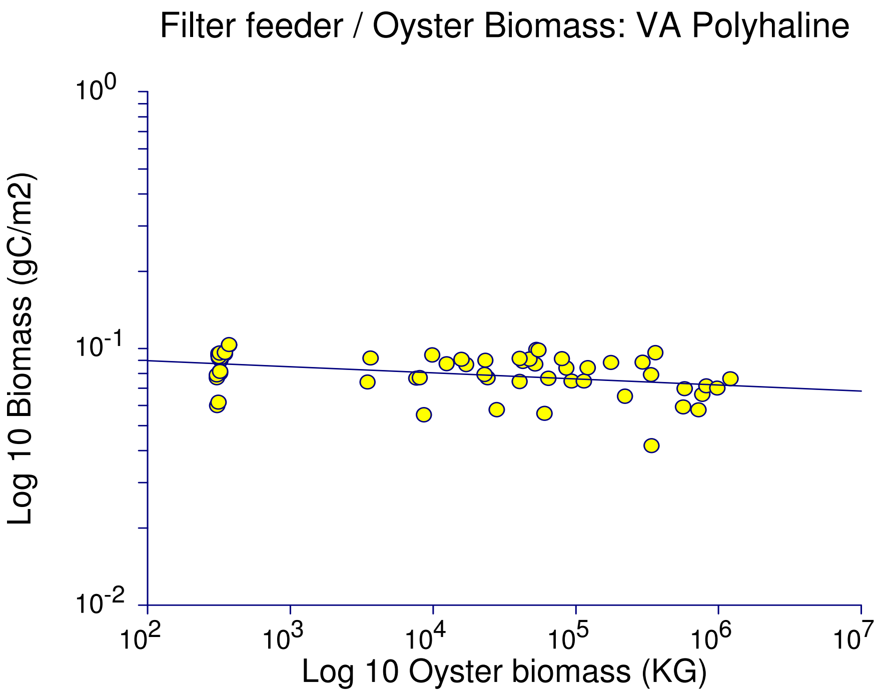
Maryland Mesohaline



Virginia Mesohaline



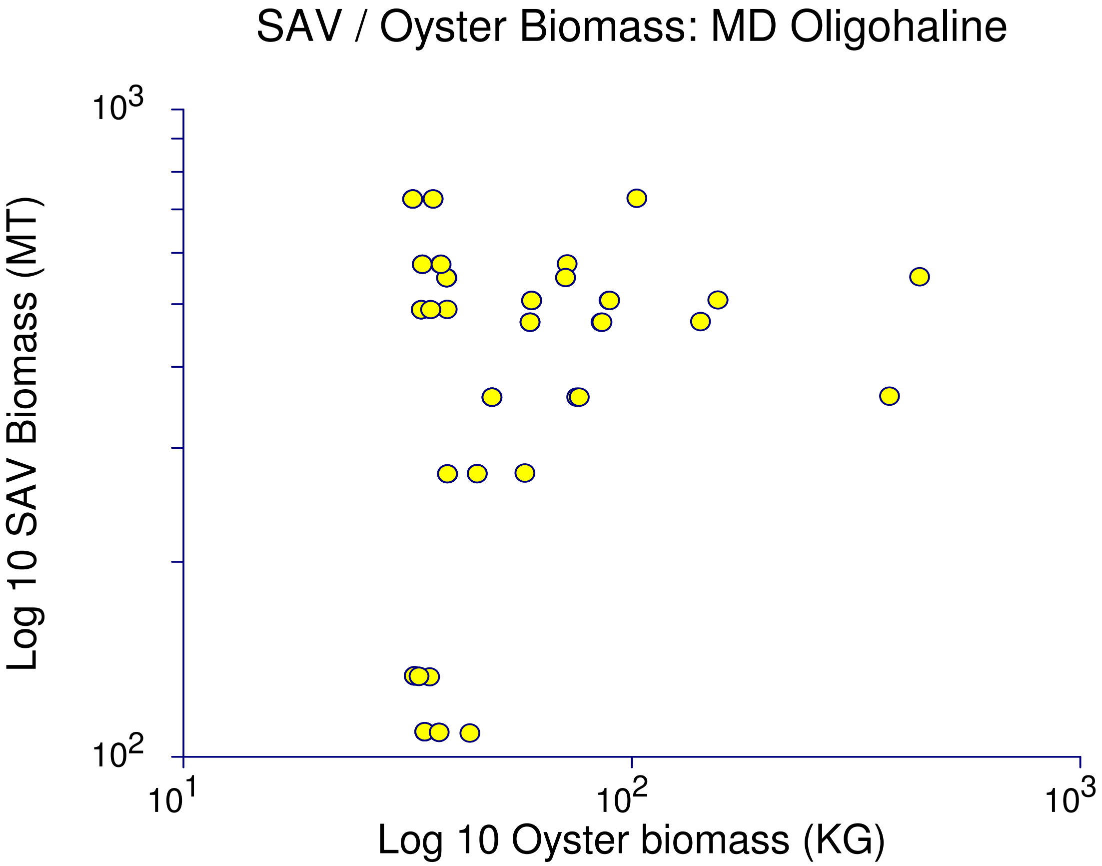
Virginia Polyhaline



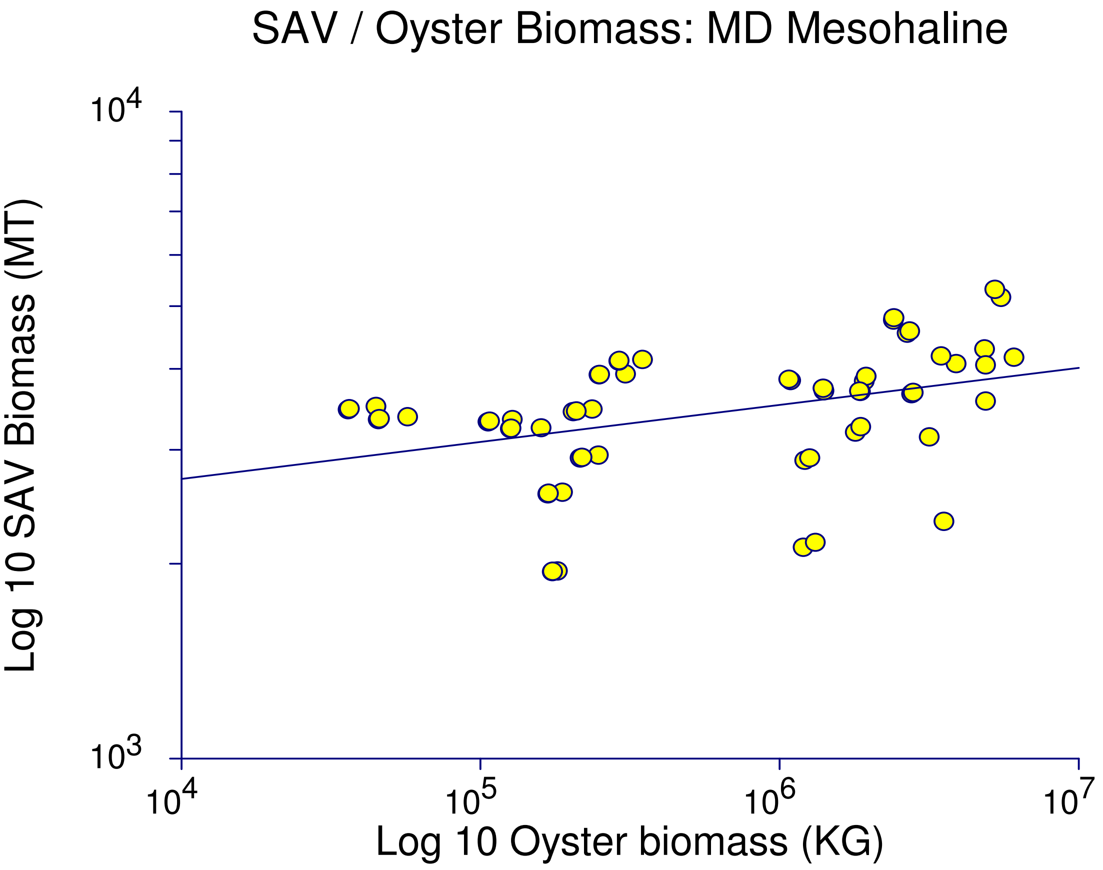
9.0 Relationship of SAV to Oyster Biomass

Modeled estimates of the biomass of SAV were summed for each group of Chesapeake Bay segments within a state/salinity zone for each of the 60 model runs. We used this statistic because SAV is expressed as total biomass per segment, and no area adjustments were needed. Oyster biomass also was summed for this analysis. Significant direct relationships were detected in the MD MH and VA MH zones. This was the expected nature of the relationship because increased filtration by oysters increases water clarity, which encourages greater growth of plants. The regression equations from these relationships were used in the RRM to calculate the influence of changes in oyster biomass on SAV. Modeled oyster biomass in the MD OH zone spanned a little over an order of magnitude, and the inability to detect a relationship may reflect the narrow range of modeled oyster biomass values. Oyster biomass in the VA PH zone spanned several orders of magnitude; however, we found no significant relationships between oyster biomass and the biomass of SAV, or most other response factors of interest in this assessment, within that zone.

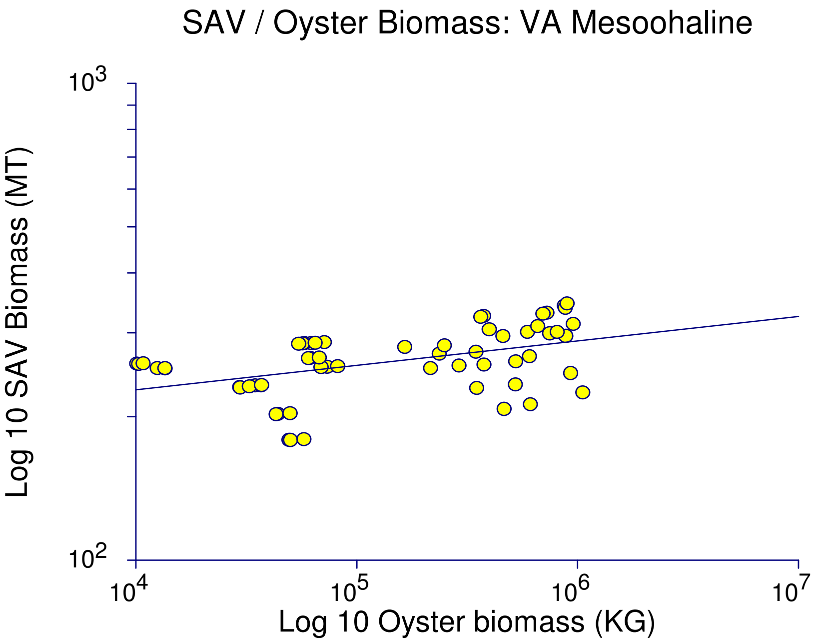
Maryland Oligohaline



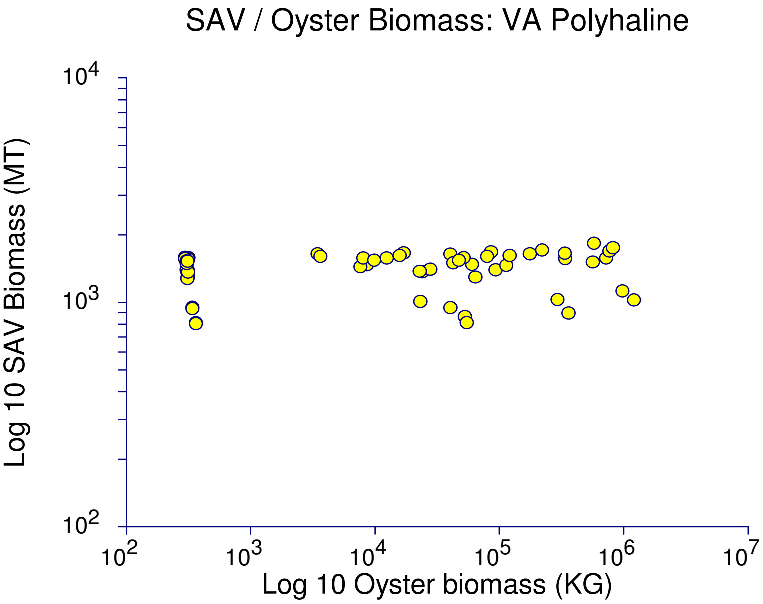
Maryland Mesohaline



Virginia Mesohaline

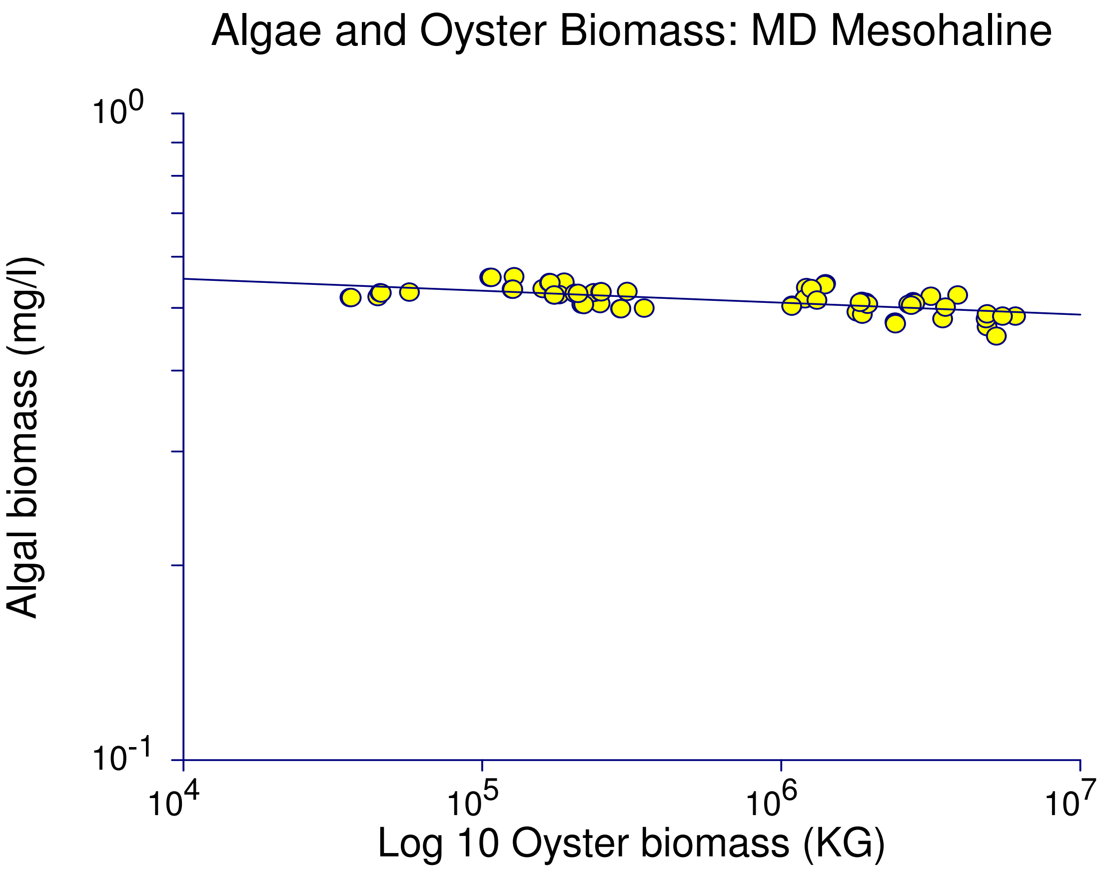


Virginia Polyhaline



10.0 Regression Summary

This section presents the results of the regressions. Some explanation is needed in order to properly understand how these results are used. First, only regressions that have slopes that are significantly different from “0” are used to relate changes in oyster biomass to changes in other ecological conditions. This is accomplished by using the regression model for the relationship. For example, as described earlier there is a negative relationship between algal biomass and oyster biomass for the Maryland Mesohaline zone. This was displayed earlier in this attachment as follows:



The regression model for this negative relationship is:

Algal biomass (mg/l) = 0.529 + (-9.53E-09 * oyster biomass (kg))

As the figure and regression model show the slope, though significant, is only slightly negative. The regression also shows that the slope of the line (-9.53E-09) is a relatively small value when algal biomass in mg/l is contrasted with oyster biomass in kg. As a result, because many of the summary statistics provided as standard output from the statistics package are truncated at four decimal places, the values for the slope appear in these tables as “0.0000”. The actual slope of the line can be found in the regression models at the end of each summary.

DO: MD Mesohaline

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Database C:\DOCUMENTS AND SETTINGS\CA ... STER\REGRESSION WORKSHEET.S0
Y = C2 X = C1

Run Summary Section

Parameter	**Value**	**Parameter**	**Value**
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	8.6742	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.0769	Sum of Weights	60.0000
Correlation	-0.2774	Coefficient of Variation	0.0482
Mean Square Error	0.1709768	Square Root of MSE	0.4134934

Summary Statement

The equation of the straight line relating C2 and C1 is estimated as: C2 = (8.6742) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 8.6742 with a standard error of 0.0712. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.0769. The correlation between C2 and C1 is -0.2774.

A significance test that the slope is zero resulted in a t-value of -2.1988. The significance level of this t-test is 0.0319. Since 0.0319 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 8.6742. The lower limit of the 95% confidence interval for the intercept is 8.5317 and the upper limit is 8.8168.

Y = C2   X = C1

Regression Estimation Section

Parameter	**Intercept B(0)**	**Slope B(1)**
Regression Coefficients	8.6742	0.0000
Lower 95% Confidence Limit	8.5317	0.0000
Upper 95% Confidence Limit	8.8168	0.0000
Standard Error	0.0712	0.0000
Standardized Coefficient	0.0000	-0.2774
T Value	121.7915	-2.1988
Prob Level (T Test)	0.0000	0.0319
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	0.5802

Regression Estimation Section (Continued)

Parameter	**Intercept B(0)**	**Slope B(1)**
Regression of Y on X	8.6742	0.0000
Inverse Regression from X on Y	9.9179	0.0000
Orthogonal Regression of Y and X	8.6734	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(8.67421949656446) + (-7.09386515663854E-08) * (C1)

TSS: MD Mesohaline

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Y = C2 X = C1

Run Summary Section

Parameter	**Value**	**Parameter**	**Value**
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	9.7535	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.0882	Sum of Weights	60.0000
Correlation	-0.2970	Coefficient of Variation	0.1470
Mean Square Error	1.901279	Square Root of MSE	1.378869

Summary Statement
The equation of the straight line relating C2 and C1 is estimated as: C2 = (9.7535) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 9.7535 with a standard error of 0.2375. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.0882. The correlation between C2 and C1 is -0.2970.

A significance test that the slope is zero resulted in a t-value of -2.3686. The significance level of this t-test is 0.0212. Since 0.0212 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 9.7535. The lower limit of the 95% confidence interval for the intercept is 9.2781 and the upper limit is 10.2289.

Regression Estimation Section

Parameter	Intercept B(0)	Slope B(1)
Regression Coefficients	9.7535	0.0000
Lower 95% Confidence Limit	9.2781	0.0000
Upper 95% Confidence Limit	10.2289	0.0000
Standard Error	0.2375	0.0000
Standardized Coefficient	0.0000	-0.2970
T Value	41.0670	-2.3686
Prob Level (T Test)	0.0000	0.0212
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	0.6441
Regression of Y on X	9.7535	0.0000
Inverse Regression from X on Y	13.6034	0.0000
Orthogonal Regression of Y and X	9.7533	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(9.75351165650047) + (-2.54826617342111E-07) * (C1)

Algae: MD Mesohaline

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Y = C2 X = C1

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.5291	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.4728	Sum of Weights	60.0000
Correlation	-0.6876	Coefficient of Variation	0.0329
Mean Square Error	2.869363E-04	Square Root of MSE	1.693919E-02

Summary Statement
The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.5291) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.5291 with a standard error of 0.0029. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.4728. The correlation between C2 and C1 is -0.6876.

A significance test that the slope is zero resulted in a t-value of -7.2116. The significance level of this t-test is 0.0000. Since 0.0000 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.5291. The lower limit of the 95% confidence interval for the intercept is 0.5232 and the upper limit is 0.5349.

Regression Estimation Section

Parameter	**Intercept B(0**	**Slope B(1)**
Regression Coefficients	0.5291	0.0000
Lower 95% Confidence Limit	0.5232	0.0000
Upper 95% Confidence Limit	0.5349	0.0000
Standard Error	0.0029	0.0000
Standardized Coefficient	0.0000	-0.6876
T Value	181.3334	-7.2116
Prob Level (T Test)	0.0000	0.0000
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	1.0000
Regression of Y on X	0.5291	0.0000
Inverse Regression from X on Y	0.5446	0.0000
Orthogonal Regression of Y and X	0.5299	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(.529072583718186) + (-9.53128123818518E-09) * (C1)

Algae: VA Mesohaline

Database C:\DOCUMENTS AND SETTINGS\CA ... STER\REGRESSION WORKSHEET.S0
Y = C2 X = C1

Run Summary Section

Parameter	**Value**	**Parameter**	**Value**
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.5706	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.1775	Sum of Weights	60.0000
Correlation	-0.4213	Coefficient of Variation	0.0396
Mean Square Error	4.926369E-04	Square Root of MSE	2.219543E-02

Summary Statement

The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.5706) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.5706 with a standard error of 0.0040. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.1775. The correlation between C2 and C1 is -0.4213.

A significance test that the slope is zero resulted in a t-value of -3.5381. The significance level of this t-test is 0.0008. Since 0.0008 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.5706. The lower limit of the 95% confidence interval for the intercept is 0.5626 and the upper limit is 0.5786.

Regression Estimation Section

Parameter	**Intercept B(0)**	**Slope B(1)**
Regression Coefficients	0.5706	0.0000
Lower 95% Confidence Limit	0.5626	0.0000
Upper 95% Confidence Limit	0.5786	0.0000
Standard Error	0.0040	0.0000
Standardized Coefficient	0.0000	-0.4213
T Value	142.9429	-3.5381
Prob Level (T Test)	0.0000	0.0008
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	0.9356
Regression of Y on X	0.5706	0.0000
Inverse Regression from X on Y	0.6161	0.0000
Orthogonal Regression of Y and X	0.5705	0.0000

Notes:

The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model

(.570587472143749) + (-3.16725192302769E-08) * (C1)

Mezozooplankton: MD Mesohaline

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Y = C2 X = C1

Run Summary Section

Parameter	**Value**	**Parameter**	**Value**
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.0148	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.4902	Sum of Weights	60.0000
Correlation	-0.7002	Coefficient of Variation	0.0833
Mean Square Error	1.331706E-06	Square Root of MSE	1.153996E-03

Summary Statement

The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.0148) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.0148 with a standard error of 0.0002. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.4902. The correlation between C2 and C1 is -0.7002.

A significance test that the slope is zero resulted in a t-value of -7.4681. The significance level of this t-test is 0.0000. Since 0.0000 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.0148. The lower limit of the 95% confidence interval for the intercept is 0.0144 and the upper limit is 0.0152.

Regression Estimation Section

Parameter	**Intercept B(0)**	**Slope B(1)**
Regression Coefficients	0.0148	0.0000
Lower 95% Confidence Limit	0.0144	0.0000
Upper 95% Confidence Limit	0.0152	0.0000
Standard Error	0.0002	0.0000
Standardized Coefficient	0.0000	-0.7002
T Value	74.6729	-7.4681
Prob Level (T Test)	0.0000	0.0000
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	1.0000
Regression of Y on X	0.0148	0.0000
Inverse Regression from X on Y	0.0159	0.0000
Orthogonal Regression of Y and X	0.0149	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(1.48426593874664E-02) + (-6.7242500079472E-10) * (C1)

Mesozooplankton: MD Oligohaline

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Y = C2 X = C1

Run Summary Section

Parameter	**Value**	**Parameter**	**Value**
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.0045	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.0848	Sum of Weights	60.0000
Correlation	0.2912	Coefficient of Variation	0.2526
Mean Square Error	1.488957E-06	Square Root of MSE	1.220228E-03

Summary Statement
The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.0045) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.0045 with a standard error of 0.0002. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.0848. The correlation between C2 and C1 is 0.2912.

A significance test that the slope is zero resulted in a t-value of 2.3185. The significance level of this t-test is 0.0240. Since 0.0240 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.0045. The lower limit of the 95% confidence interval for the intercept is 0.0041 and the upper limit is 0.0049.

Regression Estimation Section

Parameter	**Intercept B(0)**	**Slope B(1)**
Regression Coefficients	0.0045	0.0000
Lower 95% Confidence Limit	0.0041	0.0000
Upper 95% Confidence Limit	0.0049	0.0000
Standard Error	0.0002	0.0000
Standardized Coefficient	0.0000	0.2912
T Value	21.2744	2.3185
Prob Level (T Test)	0.0000	0.0240
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	0.6256

Regression of Y on X	0.0045	0.0000
Inverse Regression from X on Y	0.0010	0.0001
Orthogonal Regression of Y and X	0.0045	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(4.50332613881758E-03) + (5.29740549135315E-06) * (C1)

Mesozooplankton: VA Mesohaline

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Y = C2 X = C1

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.0147	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.1075	Sum of Weights	60.0000
Correlation	-0.3279	Coefficient of Variation	0.1171
Mean Square Error	2.736222E-06	Square Root of MSE	1.654153E-03

Summary Statement
The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.0147) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.0147 with a standard error of 0.0003. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.1075. The correlation between C2 and C1 is -0.3279.

A significance test that the slope is zero resulted in a t-value of -2.6432. The significance level of this t-test is 0.0105. Since 0.0105 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.0147. The lower limit of the 95% confidence interval for the intercept is 0.0141 and the upper limit is 0.0153.

Regression Estimation Section

Parameter	Intercept B(0)	Slope B(1)
Regression Coefficients	0.0147	0.0000
Lower 95% Confidence Limit	0.0141	0.0000
Upper 95% Confidence Limit	0.0153	0.0000
Standard Error	0.0003	0.0000
Standardized Coefficient	0.0000	-0.3279
T Value	49.3381	-2.6432
Prob Level (T Test)	0.0000	0.0105
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	0.7387
Regression of Y on X	0.0147	0.0000
Inverse Regression from X on Y	0.0192	0.0000
Orthogonal Regression of Y and X	0.0167	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(1.46775897224357E-02) + (-1.76339468728881E-09) * (C1)

Benthic Deposit Feeder: MD Mesohaline

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Y = C2 X = C1

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.7601	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.1140	Sum of Weights	60.0000
Correlation	-0.3376	Coefficient of Variation	0.0986
Mean Square Error	5.281913E-03	Square Root of MSE	7.267677E-02

Summary Statement
The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.7601) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.7601 with a standard error of 0.0125. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.1140. The correlation between C2 and C1 is -0.3376.

A significance test that the slope is zero resulted in a t-value of -2.7312. The significance level of this t-test is 0.0083. Since 0.0083 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.7601. The lower limit of the 95% confidence interval for the intercept is 0.7350 and the upper limit is 0.7851.

Regression Estimation Section

Parameter	**Intercept B(0)**	**Slope B(1)**
Regression Coefficients	0.7601	0.0000
Lower 95% Confidence Limit	0.7350	0.0000
Upper 95% Confidence Limit	0.7851	0.0000
Standard Error	0.0125	0.0000
Standardized Coefficient	0.0000	-0.3376
T Value	60.7165	-2.7312
Prob Level (T Test)	0.0000	0.0083
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	0.7660
Regression of Y on X	0.7601	0.0000
Inverse Regression from X on Y	0.9360	0.0000
Orthogonal Regression of Y and X	0.7623	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(.760059135978896) + (-1.54872405598578E-08) * (C1)

Benthic Deposit Feeder: VA Polyhaline

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Y = C2 X = C1

Run Summary Section

Parameter	**Value**	**Parameter**	**Value**
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.7905	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.0663	Sum of Weights	60.0000
Correlation	-0.2574	Coefficient of Variation	0.0582
Mean Square Error	2.080404E-03	Square Root of MSE	4.561144E-02

Summary Statement

The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.7905) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.7905 with a standard error of 0.0067. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.0663. The correlation between C2 and C1 is -0.2574.

A significance test that the slope is zero resulted in a t-value of -2.0290. The significance level of this t-test is 0.0471. Since 0.0471 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.7905. The lower limit of the 95% confidence interval for the intercept is 0.7771 and the upper limit is 0.8038.

Regression Estimation Section

Parameter	**Intercept B(0)**	**Slope B(1)**
Regression Coefficients	0.7905	0.0000
Lower 95% Confidence Limit	0.7771	0.0000
Upper 95% Confidence Limit	0.8038	0.0000
Standard Error	0.0067	0.0000
Standardized Coefficient	0.0000	-0.2574
T Value	118.4863	-2.0290
Prob Level (T Test)	0.0000	0.0471
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	0.5141
Regression of Y on X	0.7905	0.0000
Inverse Regression from X on Y	0.8801	0.0000
Orthogonal Regression of Y and X	0.7903	0.0000

Notes:

The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model

(.790473516619447) + (-4.48096930892558E-08) * (C1)

Other filter feeders: MD Oligohaline

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Y = C2   X = C1
```

Run Summary Section

```
Parameter                Value              Parameter                  Value
Dependent Variable       C2                 Rows Processed             60
Independent Variable     C1                 Rows Used in Estimation    60
Frequency Variable       None               Rows with X Missing        0
Weight Variable          None               Rows with Freq Missing     0
Intercept                0.3712             Rows Prediction Only       0
Slope                    0.0003             Sum of Frequencies         60
R-Squared                0.0748             Sum of Weights             60.0000
Correlation              0.2735             Coefficient of Variation   0.1700
Mean Square Error        4.347574E-03       Square Root of MSE         6.593614E-02
```

Summary Statement

The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.3712) + (0.0003) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.3712 with a standard error of 0.0114. The slope, the estimated change in C2 per unit change in C1, is 0.0003 with a standard error of 0.0001. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.0748. The correlation between C2 and C1 is 0.2735.

A significance test that the slope is zero resulted in a t-value of 2.1656. The significance level of this t-test is 0.0345. Since 0.0345 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0003. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0005. The estimated intercept is 0.3712. The lower limit of the 95% confidence interval for the intercept is 0.3484 and the upper limit is 0.3941.

Regression Estimation Section

```
Parameter                            Intercept B(0)      Slope B(1)
Regression Coefficients                      0.3712          0.0003
Lower 95% Confidence Limit                   0.3484          0.0000
Upper 95% Confidence Limit                   0.3941          0.0005
Standard Error                               0.0114          0.0001
Standardized Coefficient                     0.0000          0.2735

T Value                                     32.4566          2.1656
Prob Level (T Test)                          0.0000          0.0345
Reject H0 (Alpha = 0.0500)                      Yes             Yes
Power (Alpha = 0.0500)                       1.0000          0.5674

Regression of Y on X                         0.3712          0.0003
Inverse Regression from X on Y               0.1666          0.0036
Orthogonal Regression of Y and X             0.3712          0.0003
```

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(.371247059209371) + (2.67370706035864E-04) * (C1)

Other filter feeders: MD Mesohaline

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Y = C2 X = C1

Run Summary Section

Parameter	**Value**	**Parameter**	**Value**
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.9656	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.7168	Sum of Weights	60.0000
Correlation	-0.8466	Coefficient of Variation	0.0824
Mean Square Error	5.10401E-03	Square Root of MSE	7.144236E-02

Summary Statement
The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.9656) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.9656 with a standard error of 0.0123. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.7168. The correlation between C2 and C1 is -0.8466.

A significance test that the slope is zero resulted in a t-value of -12.1154. The significance level of this t-test is 0.0000. Since 0.0000 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.9656. The lower limit of the 95% confidence interval for the intercept is 0.9410 and the upper limit is 0.9902.

Regression Estimation Section

Parameter	**Intercept B(0)**	**Slope B(1)**
Regression Coefficients	0.9656	0.0000
Lower 95% Confidence Limit	0.9410	0.0000
Upper 95% Confidence Limit	0.9902	0.0000
Standard Error	0.0123	0.0000
Standardized Coefficient	0.0000	-0.8466

Regression Estimation Section (Continued)

Parameter	**Intercept B(0)**	**Slope B(1)**
T Value	78.4692	-12.1154
Prob Level (T Test)	0.0000	0.0000
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	1.0000
Regression of Y on X	0.9656	0.0000
Inverse Regression from X on Y	1.0046	0.0000
Orthogonal Regression of Y and X	0.9661	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(.965605035724311) + (-6.75337334396573E-08) * (C1)

Other filter feeders: VA Mesohaline

Run Summary Section

Parameter	**Value**	**Parameter**	**Value**
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.8947	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.3809	Sum of Weights	60.0000
Correlation	-0.6172	Coefficient of Variation	0.1003
Mean Square Error	6.966332E-03	Square Root of MSE	8.346456E-02

Summary Statement
The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.8947) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.8947 with a standard error of 0.0150. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.3809. The correlation between C2 and C1 is -0.6172.

A significance test that the slope is zero resulted in a t-value of -5.9742. The significance level of this t-test is 0.0000. Since 0.0000 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.8947. The lower limit of the 95% confidence interval for the intercept is 0.8647 and the upper limit is 0.9248.

Regression Estimation Section

Parameter	Intercept B(0)	Slope B(1)
Regression Coefficients	0.8947	0.0000
Lower 95% Confidence Limit	0.8647	0.0000
Upper 95% Confidence Limit	0.9248	0.0000
Standard Error	0.0150	0.0000
Standardized Coefficient	0.0000	-0.6172
T Value	59.6076	-5.9742
Prob Level (T Test)	0.0000	0.0000
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	1.0000
Regression of Y on X	0.8947	0.0000
Inverse Regression from X on Y	0.9962	0.0000
Orthogonal Regression of Y and X	0.8948	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(.894746543888807) + (-2.01106348760965E-07) * (C1)

Other filter feeders: VA Polyhaline

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Y = C2 X = C1

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	0.0840	Rows Prediction Only	0
Slope	0.0000	Sum of Frequencies	60
R-Squared	0.1442	Sum of Weights	60.0000
Correlation	-0.3798	Coefficient of Variation	0.1561
Mean Square Error	1.607449E-04	Square Root of MSE	1.267852E-02

Summary Statement
The equation of the straight line relating C2 and C1 is estimated as: C2 = (0.0840) + (0.0000) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 0.0840 with a standard error of 0.0019. The slope, the estimated change in C2 per unit change in C1, is 0.0000 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.1442. The correlation between C2 and C1 is -0.3798.

A significance test that the slope is zero resulted in a t-value of -3.1264. The significance level of this t-test is 0.0028. Since 0.0028 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0000. The lower limit of the 95% confidence interval for the slope is 0.0000 and the upper limit is 0.0000. The estimated intercept is 0.0840. The lower limit of the 95% confidence interval for the intercept is 0.0802 and the upper limit is 0.0877.

Regression Estimation Section

Parameter	**Intercept B(0)**	**Slope B(1)**
Regression Coefficients	0.0840	0.0000
Lower 95% Confidence Limit	0.0802	0.0000
Upper 95% Confidence Limit	0.0877	0.0000
Standard Error	0.0019	0.0000
Standardized Coefficient	0.0000	-0.3798
T Value	45.2730	-3.1264
Prob Level (T Test)	0.0000	0.0028
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	0.8674
Regression of Y on X	0.0840	0.0000
Inverse Regression from X on Y	0.1001	0.0000
Orthogonal Regression of Y and X	0.0837	0.0000

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(8.39563196582696E-02) + (-1.91926640738662E-08) * (C1)

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Y = C2 X = C1

Run Summary Section

Parameter	**Value**	**Parameter**	**Value**
Dependent Variable	C2	Rows Processed	60
Independent Variable	C1	Rows Used in Estimation	60
Frequency Variable	None	Rows with X Missing	0
Weight Variable	None	Rows with Freq Missing	0
Intercept	3160.8935	Rows Prediction Only	0
Slope	0.0002	Sum of Frequencies	60
R-Squared	0.2658	Sum of Weights	60.0000
Correlation	0.5155	Coefficient of Variation	0.1859
Mean Square Error	423776.7	Square Root of MSE	650.9814

Summary Statement

The equation of the straight line relating C2 and C1 is estimated as: C2 = (3160.8935) + (0.0002) C1 using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero, is 3160.8935 with a standard error of 112.1279. The slope, the estimated change in C2 per unit change in C1, is 0.0002 with a standard error of 0.0001. The value of R-Squared, the proportion of the variation in C2 that can be accounted for by variation in C1, is 0.2658. The correlation between C2 and C1 is 0.5155.

A significance test that the slope is zero resulted in a t-value of 4.5819. The significance level of this t-test is 0.0000. Since 0.0000 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0002. The lower limit of the 95% confidence interval for the slope is 0.0001 and the upper limit is 0.0003. The estimated intercept is 3160.8935. The lower limit of the 95% confidence interval for the intercept is 2936.4452 and the upper limit is 3385.3418.

Regression Estimation Section

Parameter	Intercept B(0)	Slope B(1)
Regression Coefficients	3160.8935	0.0002
Lower 95% Confidence Limit	2936.4452	0.0001
Upper 95% Confidence Limit	3385.3418	0.0003
Standard Error	112.1279	0.0001
Standardized Coefficient	0.0000	0.5155
T Value	28.1901	4.5819
Prob Level (T Test)	0.0000	0.0000
Reject H0 (Alpha = 0.0500)	Yes	Yes
Power (Alpha = 0.0500)	1.0000	0.9945
Regression of Y on X	3160.8935	0.0002
Inverse Regression from X on Y	2221.2943	0.0009
Orthogonal Regression of Y and X	3160.8935	0.0002

Notes:

The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model

(3160.89352217283) + (2.32712448107273E-04) * (C1)

SAV: VA Mesohaline

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Y = C2   X = C1

Run Summary Section
Parameter                  Value              Parameter                   Value
Dependent Variable         C2                 Rows Processed              60
Independent Variable       C1                 Rows Used in Estimation     60
Frequency Variable         None               Rows with X Missing         0
Weight Variable            None               Rows with Freq Missing      0
Intercept                  244.2540           Rows Prediction Only        0
Slope                      0.0001             Sum of Frequencies          60
R-Squared                  0.2543             Sum of Weights              60.0000
Correlation                0.5043             Coefficient of Variation    0.1355
Mean Square Error          1282.314           Square Root of MSE          35.80941


Summary Statement
The equation of the straight line relating C2 and C1 is estimated as: C2 = (244.2540) + (0.0001) C1
using the 60 observations in this dataset. The y-intercept, the estimated value of C2 when C1 is zero,
is 244.2540 with a standard error of 6.4400. The slope, the estimated change in C2 per unit change in
C1, is 0.0001 with a standard error of 0.0000. The value of R-Squared, the proportion of the variation
in C2 that can be accounted for by variation in C1, is 0.2543. The correlation between C2 and C1 is
0.5043.

A significance test that the slope is zero resulted in a t-value of 4.4478. The significance level of this t-
test is 0.0000. Since 0.0000 < 0.0500, the hypothesis that the slope is zero is rejected.

The estimated slope is 0.0001. The lower limit of the 95% confidence interval for the slope is 0.0000
and the upper limit is 0.0001. The estimated intercept is 244.2540. The lower limit of the 95%
confidence interval for the intercept is 231.3629 and the upper limit is 257.1451.


Regression Estimation Section
Parameter                          Intercept B(0)       Slope B(1)
Regression Coefficients                  244.2540           0.0001
Lower 95% Confidence Limit               231.3629           0.0000
Upper 95% Confidence Limit               257.1451           0.0001
Standard Error                             6.4400           0.0000
Standardized Coefficient                   0.0000           0.5043

T Value                                   37.9274           4.4478
Prob Level (T Test)                        0.0000           0.0000
Reject H0 (Alpha = 0.0500)                    Yes              Yes
Power (Alpha = 0.0500)                     1.0000           0.9921

Regression of Y on X                     244.2540           0.0001
Inverse Regression from X on Y           185.7873           0.0003
Orthogonal Regression of Y and X         244.2540           0.0001
```

Notes:
The above report shows the least-squares estimates of the intercept and slope followed by the corresponding standard errors, confidence intervals, and hypothesis tests. Note that these results are based on several assumptions that should be validated before they are used.

Estimated Model
(244.253996053114) + (6.42372059580864E-05) * (C1)

11.0 References

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2000 - 2007 Research and Field Trials - Table of Potential Escapement Events for *Crassostrea ariakensis*

Notes:

This table includes all documented research and aquaculture field trials that were performed on the East Coast of the United States using triploid Crassostrea ariakensis.
Information contained in this table was obtained from several sources, including documentation of industry field trials, summary reports for EIS-funded research projects and personal communication.
Accuracy and level of detail of information provided in this table, as well as the comprehensiveness of the table itself, is limited to the accuracy of information and detail provided to the preparer and/or contained in the data source.
Numbers of spat/animals deployed are estimates. Consult data source for exact number and age of animals or spat deployed as well as precise deployment location and method.
See attached "Data Source" spreadsheet for source of information provided in this table.
All of the triploid Crassostrea ariakensis oysters that were used in these field trials and research projects originated from the Virginia Institute of Marine Sciences (VIMS) laboratories.
Escapement refers to the loss of oysters from cages.
**Items in red denote documented escapement events.*

Order	**Source(s) of information**	**Data Sources**	**Principal Investigator or Grower (Company Name) / Landside Location**	**Season**	**Date of Cage Deployment**	**Cage Locations**	***C. ariakensis* # allowed at site (per permit) or actual # of oysters deployed at site**	**Probability Diploid**	**Estimated Total Number of Diploids**	**Estimated Rate of Triploid to Diploid Reversion**	**Accidental *C. ariakensis* Oyster Release Incidents**	**Evaluation of the likelihood that these oysters could establish a viable population**
1	Virginia Marine Resources Commission (VMRC)	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2000	York River	1,000	4 per 3,660	1.0	Less Than 1%	None	N/A
2	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2000	Chincoteague	1,000	4 per 3,660	1.0	Less Than 1%	None	N/A
3	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2000	Folly Creek	1,000	4 per 3,660	1.0	Less Than 1%	None	N/A
4	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2000	Piankatank River	1,000	4 per 3,660	1.0	Less Than 1%	None	N/A
5	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2000	Rappahannock River	2,000	4 per 3,660	2.1	Less Than 1%	None	N/A
6	VMRC	VSC / VMRC Field Trial	VA (Grower - Not Provided)	Spring	2001	Pocomoke Sound (Saxis)	5,376	4 per 3,660	5.8	Less Than 1%	None	N/A
7	VMRC / US Fish and Wildlife Service (FWS)	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2001	Rappahannock River	25,200	4 per 3,660	27.5	Less Than 1%	Four (4) oysters found were thought to have been from a group deployed near Kellum's oyster house during the 2000 - 2002 VSC field trials. The group in reference was deployed as spat on shell in net enclosure on the bottom of a tidal pool.	The oysters were picked up by hand in 2004. All oysters were tested and found to be triploid. (Jim Wesson, VMRC)
8	VMRC	VSC / VMRC Field Trial	VA (Grower Not Known)	Spring	2001	Rappahannock River	8,600	4 per 3,660	9.3	Less Than 1%	None	N/A
9	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2001	Folly Creek	12,600	4 per 3,660	13.7	Less Than 1%	None	N/A
10	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2001	York River	8,000	4 per 3,660	8.7	Less Than 1%	None	N/A
11	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2001	York River	8,600	4 per 3,660	9.3	Less Than 1%	None	N/A
12	VMRC	VSC / VMRC Field Trial	Milford Haven, VA (Grower Not Provided)	Spring	2001	Gwynn Island	8,600	4 per 3,660	9.3	Less Than 1%	None	N/A
13	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2001	Cherrystone Creek	5,376	4 per 3,660	5.8	Less Than 1%	None	N/A
14	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2001	Chincoteague	12,600	4 per 3,660	13.7	Less Than 1%	None	N/A
15	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Spring	2001	Coan River	7,600	4 per 3,660	8.3	Less Than 1%	None	N/A
16	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Fall	2001	James River	3,266	4 per 3,660	3.5	Less Than 1%	None	N/A
17	VMRC	VSC / VMRC Field Trial	VA (Grower Not Provided)	Fall	2001	Little Wicomico River	8,200	4 per 3,660	8.9	Less Than 1%	None	N/A
18	VMRC	VSC / VMRC Field Trial	VA (Grower Not Known)	Spring	2001	Yeocomico River	8,600	4 per 3,660	9.3	Less Than 1%	None	N/A
19	US Army Corp of Engineers - Norfolk District (Norfolk District) / FWS	VSC Field Trial	Shore Seafood, Saxis, VA	Fall	2003	Pocomoke Sound (Saxis)	100,000	0.000652	65.2	Less Than 1%	One large individual oyster (approx 200 mm in length) was found in April 2006 at the Shore seafood site, when the 2006 spill occurred (see below). It was assumed to be associated with the 2003 trial at this site.	Not Provided
20	Norfolk District / FWS	VSC Field Trial	Shores and Ruark, Urbanna, VA	Fall	2003	Rappahannock River	100,000	0.000652	65.2	Less Than 1%	None	N/A
21	Norfolk District / FWS	VSC Field Trial	Purcell's Seafood, Burgess, VA	Fall	2003	Little Wicomico River	100,000	0.000652	65.2	Less Than 1%	None	N/A

Order	Source(s) of information	Data Sources	Principal Investigator or Grower (Company Name) / Landside Location	Season	Date of Cage Deployment	Cage Locations	*C. ariakensis* # allowed at site (per permit) or actual # of oysters deployed at site	Probability Diploid	Estimated Total Number of Diploids	Estimated Rate of Triploid to Diploid Reversion	Accidental *C. ariakensis* Oyster Release Incidents	Evaluation of the likelihood that these oysters could establish a viable population
22	Norfolk District	VSC Field Trial	Accomac Aquafarms, Accomac, VA	Fall	2003	Folly Creek	100,000	2 per 3000	66.6	Less Than 1%	None	N/A
23	Norfolk District / FWS	VSC Field Trial	Mason Seafood, Chincoteague, VA	Fall	2003	Chincoteague	100,000	0.0003	30.0	Less Than 1%	None	N/A
24	Norfolk District / FWS	VSC Field Trial	Sea Farms Inc., Hudgins, VA	Fall	2003	Gwynn Island (Milford Haven)	100,000	0.000652	65.2	Less Than 1%	None	N/A
25	Norfolk District / FWS	VSC Field Trial	Bevans Oyster Company, Kinsale, VA	Fall	2003	Yeocomico River	100,000	0.000652	65.2	Less Than 1%	None	N/A
26	Norfolk District / FWS	VSC Field Trial	Leggett & Crew, Yorktown, VA	Fall	2003	York River	100,000	0.000652	65.2	Less Than 1%	None	N/A
27	FWS	VSC Field Trial	Mobjack Bay Seafood, Ware Neck, VA		2004 (?)	Ware River, Mobjack Bay	100,000	0.000652	65.2	Less Than 1%	None	N/A
28	Norfolk District / FWS	VSC Field Trial	Shores and Ruark, Urbanna, VA	Fall	2005	Rappahannock River	100,000	0.0013	130.0	Less Than 1%	None	N/A
29	Norfolk District / FWS	VSC Field Trial	Mobjack Bay Seafood, Ware Neck, VA	Fall	2005	Ware River, Mobjack Bay	100,000	0.0003	30.0	Less Than 1%	None	N/A
30	Norfolk District / FWS	VSC Field Trial	Sea Farms Inc., Hudgins, VA	Fall	2005	Gwynn Island (Milford Haven)	100,000	0.0013	130.0	Less Than 1%	None	N/A
31	Norfolk District / FWS	VSC Field Trial	Purcell's Seafood, Burgess, VA	Fall	2005	Little Wicomico River	100,000	0.0013	130.0	Less Than 1%	None	N/A
32	Norfolk District / FWS	VSC Field Trial	Atlantis Seafood, Norfolk, VA	Fall	2005	Broad Bay (Virginia Beach)	100,000	0.0003	30.0	Less Than 1%	None	N/A
33	Norfolk District / FWS	VSC Field Trial	Mason Seafood, Chincoteague, VA	Fall	2005	Chincoteague	100,000	0.0013	130.0	Less Than 1%	None	N/A
34	Norfolk District / FWS	VSC Field Trial	Cowart Seafood, Lottsburg, VA	Fall	2005	Coan River	100,000	0.0013	130.0	Less Than 1%	None	N/A
35	Norfolk District / VMRC / FWS	VSC Field Trial	Accomac Aquafarms, Accomac, VA	Fall	2005	Folly Creek	100,000	0.0013	130.0	Less Than 1%	One oyster was found. It was thought to have been from a group deployed in 2005. It was picked up by hand from an area that a cage had landed when it broke away in a storm in 2005. The area was surveyed in December at low tide, when the ground was not covered with water (intertidal), and there were no others.	The oyster was picked up by hand in December 2007 by VMRC. The oyster that was found was tested and found to be triploid. (Jim Wesson, VMRC)
36	Norfolk District / FWS	VSC Field Trial	Shore Seafood, Saxis, VA	Fall	2005	Pocomoke Sound (Saxis)	100,000	0.0013	130.0	Less Than 1%	A spill of approximately 1,600 oysters occurred in April 2006 when the basket in the Taylor float broke free from the PVC floats after the plastic ties that secured it broke.	A clam rake was used to clean the area directly under the float. An area of five (5) square meters around the float was also surveyed. The Project Manager at the time felt confident that 95-99% of the oysters were recovered. The bottom under the float is made up of a half meter of anoxic mud. Any oysters left there would have little chance of survival.
37	Norfolk District / FWS	VSC Field Trial	Bevans Oyster Company, Kinsale, VA	Fall	2005	Yeocomico River	100,000	0.0013	130.0	Less Than 1%	None	N/A
38	FWS	VSC Field Trial	Shore Seafood, Saxis, VA	Fall	2006	Pocomoke Sound (Saxis)	100,000	0.0003	30.0	Less Than 1%	None	N/A
39	Norfolk District / FWS	VSC Field Trial	Mobjack Bay Seafood, Ware Neck, VA	Fall	2006	Ware River, Mobjack Bay	100,000	0.0003	30.0	Less Than 1%	None	N/A
40	Norfolk District / FWS	VSC Field Trial	Sea Farms Inc., Hudgins, VA	Fall	2006	Gwynn Island (Milford Haven)	100,000	0.0003	30.0	Less Than 1%	None	N/A
41	Norfolk District / FWS	VSC Field Trial	Little River Seafood, Reedville, VA	Fall	2006	Little Wicomico	100,000	0.0003	30.0	Less Than 1%	None	N/A
42	Norfolk District / FWS	VSC Field Trial	Purcell's Seafood, Burgess, VA	Fall	2006	Little Wicomico River	100,000	0.0003	30.0	Less Than 1%	None	N/A
43	Norfolk District / FWS	VSC Field Trial	Accomac Aquafarms, Accomac, VA	Fall	2006	Folly Creek	100,000	0.0013	130.0	Less Than 1%	None	N/A
44	Norfolk District / FWS	VSC Field Trial	Cowart Seafood, Lottsburg, VA	Fall	2006	Coan River	100,000	0.0003	30.0	Less Than 1%	None	N/A
45	Norfolk District / FWS	VSC Field Trial	Lynnhaven Oyster Co., Virginia Beach, VA	Fall	2006	Broad Bay	100,000	0.0003	30.0	Less Than 1%	None	N/A
46	Norfolk District / FWS	VSC Field Trial	Atlantis Seafood, Norfolk, VA	Fall	2006	Broad Bay	100,000	0.0001	10.0	Less Than 1%	None	N/A
47	Norfolk District / FWS	VSC Field Trial	Mason Seafood, Chincoteague, VA	Fall	2006	Chincoteague	100,000	0.0003	30.0	Less Than 1%	None	N/A

Order	Source(s) of information	Data Sources	Principal Investigator or Grower (Company Name) / Landside Location	Season	Date of Cage Deployment	Cage Locations	*C. ariakensis* # allowed at site (per permit) or actual # of oysters deployed at site	Probability Diploid	Estimated Total Number of Diploids	Estimated Rate of Triploid to Diploid Reversion	Accidental *C. ariakensis* Oyster Release Incidents	Evaluation of the likelihood that these oysters could establish a viable population
48	Norfolk District / FWS	VSC Field Trial	Bouden Seafood, Chincoteague, VA	Fall	2006	Chincoteague	100,000	0.0003	30.0	Less Than 1%	None	N/A
49	Norfolk District / FWS	VSC Field Trial	Bell Seafood, Cape Charles, VA (Oyster, VA)	Fall	2006	Crow Bay (seaside)	100,000	0.0003	30.0	Less Than 1%	None	N/A
50	Norfolk District / FWS	VSC Field Trial	Bevans Oyster Company, Kinsale, VA	Fall	2006	Yeocomico River	100,000	0.0003	30.0	Less Than 1%	None	N/A
51	Norfolk District	VSC Field Trial	VA (Grower Not Provided)	Fall	2007	Broad Bay	70,000	0.0001	7.0	Less Than 1%	None	N/A
52	Norfolk District	VSC Field Trial	Mason Seafood, Chincoteague, VA	Fall	2007	Chincoteague	70,000	0.0001	7.0	Less Than 1%	None	N/A
53	Norfolk District	VSC Field Trial	Bouden Seafood, Chincoteague, VA	Fall	2007	Chincoteague	70,000	0.0001	7.0	Less Than 1%	None	N/A
54	Norfolk District	VSC Field Trial	Cowart Seafood, Lottsburg, VA	Fall	2007	Coan River	70,000	0.0001	7.0	Less Than 1%	None	N/A
55	Norfolk District	VSC Field Trial	Accomac Aquafarms, Accomac, VA	Fall	2007	Folly Creek	70,000	0.0001	7.0	Less Than 1%	None	N/A
56	Norfolk District	VSC Field Trial	Little River Seafood, Reedville, VA	Fall	2007	Little Wicomico River, VA	70,000	0.0001	7.0	Less Than 1%	None	N/A
57	Norfolk District	VSC Field Trial	Purcell's Seafood, Burgess, VA	Fall	2007	Little Wicomico River, VA	70,000	0.0001	7.0	Less Than 1%	None	N/A
58	Norfolk District	VSC Field Trial	Mobjack Bay Seafood, Ware Neck, VA	Fall	2007	Ware River, Mobjack Bay	70,000	0.0001	7.0	Less Than 1%	None	N/A
59	Norfolk District	VSC Field Trial	Shore Seafood, Saxis, VA	Fall	2007	Pocomoke Sound (Saxis)	70,000	0.0001	7.0	Less Than 1%	None	N/A
60	Norfolk District	VSC Field Trial	Bevans Oyster Company, Kinsale, VA	Fall	2007	Yeocomico River	70,000	0.0001	7.0	Less Than 1%	None	N/A
61	FWS	Research	Ken Paynter, University of Maryland Center for Environmental Science (UMCES) (MD Location)	Not Provided	2004	Choptank River	200	0.000652	0.1	Less Than 1%	None	N/A
62	FWS	Research	Ken Paynter, UMCES (Chesapeake Bay Lab, MD Location)	Not Provided	2004	Patuxent River	200	0.000652	0.1	Less Than 1%	Oysters from a 2004 Paynter research trial (162 oysters) escaped at Chesapeake Bay Lab.	More information is needed.
63	FWS	Research	Ken Paynter, UMCES (MD Location)	Not Provided	2004	Severn River	200	0.000652	0.1	Less Than 1%	None	N/A
64	FWS	Research	Ken Paynter, UMCES (VA Location)	Not Provided	2004	York River	200	0.000652	0.1	Less Than 1%	None	N/A
65	FWS	Research	Mark Luckenbach, VIMS (MD Location)	Not Provided	2004	Patuxent River	541	0.000652	0.4	Less Than 1%	None	N/A
66	FWS	Research	Mark Luckenbach, VIMS (MD Location)	Not Provided	2004	Severn River	614	0.000652	0.4	Less Than 1%	None	N/A
67	MD DNR	Research	Richard McLean, Calvert Cliffs Nuclear Power Plant on the Chesapeake Bay	Not Provided	2004	Chesapeake Bay	198	0.000652	0.1	Less Than 1%	None	N/A
68	Research Report	Research	Eugene Burreson, VIMS (NC Locations)	Summer	2004	Various (Neuse River Estuary to mouth of Newport River - Bogue Sound)	800	Not Provided	Not Provided	Not Provided	Not Provided	Not Provided
69	Research Report	Research	Eugene Burreson, VIMS (NC Locations)	Fall	2004	Various (Neuse River Estuary to mouth of Newport River - Bogue Sound)	1,600	Not Provided	Not Provided	Not Provided	Not Provided	Not Provided
70	Research Report	Research	Eugene Burreson, VIMS (NC Locations)	Fall	2004	Bogue Sound, NC	"several hundred returned"	Not Provided	Not Provided	Not Provided	Not Provided	Not Provided
71	FWS	Research	Karen Hudson, VIMS (VA Location)	Not Provided	2005	Chincoteague	721	0.0013	0.9	Less Than 1%	None	N/A
72	FWS	Research	Karen Hudson, VIMS (VA Location)	Not Provided	2005	Rappahannock River	673	0.0013	0.9	Less Than 1%	None	N/A
73	FWS	Research	Ken Paynter, UMCES (MD Location)	Not Provided	2005	Choptank River	200	0.000652	0.1	Less Than 1%	None	N/A
74	FWS	Research	Ken Paynter, UMCES (MD Location)	Fall	2005	Patuxent River	200	0.000652	0.1	Less Than 1%	None	N/A

Order	Source(s) of information	Data Sources	Principal Investigator or Grower (Company Name) / Landside Location	Season	Date of Cage Deployment	Cage Locations	*C. ariakensis* # allowed at site (per permit) or actual # of oysters deployed at site	Probability Diploid	Estimated Total Number of Diploids	Estimated Rate of Triploid to Diploid Reversion	Accidental *C. ariakensis* Oyster Release Incidents	Evaluation of the likelihood that these oysters could establish a viable population
75	FWS / VMRC	Research	Ken Paynter, UMCES (MD Location)	Fall	2005	Severn River, Carr Creek	200	0.000652	0.1	Less Than 1%	In July 2007 more than 600 *C. ariakensis* oysters were released into the Severn River after an anchor struck and dragged one of the cages six (6) feet, breaking the cage open. Cages were 10 ft x 10 ft x 2 ft (height), made of galvanized steel pipe, covered with chain link and screwed into ground. Each cage contained 25, 2 ft x 2 ft trays.	Over 557 oysters were recovered with 100 unaccounted for. Some of these could have died prior to the incident. Any oysters not recovered from within the cage or the immediate vicinity around the cage were likely killed by physical damage or burial when the cage was dragged. Testing revealed that 100% of oysters sampled were sterile triploids.
76	FWS	Research	Ken Paynter, UMCES (VA Location)	Fall	2005	York River	200	0.000652	0.1	Less Than 1%	None	N/A
77	FWS	Research	Mark Luckenbach, VIMS (MD Location)	Not Provided	2005	Patuxent River	541	0.000652	0.4	Less Than 1%	None	N/A
78	FWS	Research	Mark Luckenbach, VIMS (MD Location)	Not Provided	2005	Severn River	614	0.000652	0.4	Less Than 1%	None	N/A
79	Research Report	Research	Eugene Burreson, VIMS (NC Locations)	Spring, Summer, Winter, Fall	2005	Bogue Sound, NC	"several hundred"	Not Provided	Not Provided	Not Provided	Not Provided	Not Provided
80	FWS	Research	Karen Hudson, VIMS (VA Location)	Not Provided	2006	Chincoteague	260	0.00031	0.1	Less Than 1%	None	N/A
81	FWS	Research	Karen Hudson, VIMS (VA Location)	Not Provided	2006	Coan River	350	0.00031	0.1	Less Than 1%	None	N/A
82	FWS	Research	Karen Hudson, VIMS (VA Location)	Not Provided	2006	Yeocomico River	450	0.00031	0.1	Less Than 1%	None	N/A
83	FWS	Research	Howard Kator, VIMS (VA Location)	Fall	2006	James River	400	0.00031	0.1	Less Than 1%	None	N/A
84	FWS	Research	Ken Paynter, UMCES (MD Location)	Not Provided	2006	Choptank River	200	0.000652	0.1	Less Than 1%	None	N/A
85	FWS	Research	Ken Paynter, UMCES (MD Location)	Not Provided	2006	Patuxent River	200	0.000652	0.1	Less Than 1%	None	N/A
86	FWS	Research	Ken Paynter, UMCES (MD Location)	Not Provided	2006	Severn River	200	0.000652	0.1	Less Than 1%	None	N/A
87	FWS	Research	Ken Paynter, UMCES (VA Location)	Not Provided	2006	York River	200	0.000652	0.1	Less Than 1%	None	N/A
88	FWS	Research	Mark Luckenbach, VIMS (MD Location)	Not Provided	2006	Patuxent River	541	0.000652	0.4	Less Than 1%	None	N/A
89	FWS	Research	Mark Luckenbach, VIMS (MD Location)	Not Provided	2006	Severn River	614	0.000652	0.4	Less Than 1%	None	N/A
90	FWS	Research	Denise Breitburg, Smithsonian Environmental Research Center (SERC) (MD Location)	Not Provided	2007	Rhode River	1,440	0.000442	0.6	Less Than 1%	None	N/A
91	FWS	Research	Denise Breitburg, SERC (MD Location)	Not Provided	2007	Rhode River	2,880	0.000442	1.3	Less Than 1%	None	N/A
92	FWS	Research	Howard Kator, VIMS (VA Location)	Not Provided	2007	Eastern Shore	100	0.00031	0.0	Less Than 1%	None	N/A
93	FWS	Research	Howard Kator, VIMS (VA Location)	Spring	2007	James River	400	0.00031	0.1	Less Than 1%	None	N/A
94	FWS	Research	Howard Kator, VIMS (MD Location)	Spring	2007	Severn River	400	0.00031	0.1	Less Than 1%	None	N/A
95	FWS	Research	Howard Kator, VIMS (MD Location)	Summer	2007	Severn River	500	0.00031	0.2	Less Than 1%	None	N/A
96	FWS	Research	Howard Kator, VIMS (MD Location)	Fall	2007	Severn River	400	0.00031	0.1	Less Than 1%	None	N/A
97	FWS	Research	Howard Kator, VIMS (VA Location)	Spring	2007	York River	400	0.00031	0.1	Less Than 1%	None	N/A
98	FWS	Research	Howard Kator, VIMS (VA Location)	Summer	2007	York River	500	0.00031	0.2	Less Than 1%	None	N/A
99	FWS	Research	Howard Kator, VIMS (VA Location)	Summer	2007	York River (*Vibrio* study)	100	0.00031	0.0	Less Than 1%	A cage was found to be missing in March 2008.	The Principal Investigator searched the area and found no trace of the cage or the lines and buoys that had been attached to the cage. It is believed that the cage with the oysters was stolen.

Order	Source(s) of information	Data Sources	Principal Investigator or Grower (Company Name) / Landside Location	Season	Date of Cage Deployment	Cage Locations	*C. ariakensis* # allowed at site (per permit) or actual # of oysters deployed at site	Probability Diploid	Estimated Total Number of Diploids	Estimated Rate of Triploid to Diploid Reversion	Accidental *C. ariakensis* Oyster Release Incidents	Evaluation of the likelihood that these oysters could establish a viable population
96	North Carolina Division of Marine Fisheries (NC DMF)	Research	Eugene Burreson, VIMS (NC Locations)	Fall	9/2004	Various (Neuse River Estuary to mouth of Newport River - Bogue Sound; Oregon, Ocracoke and Cape Fear River Inlets; 12	6,000	Not Provided	Not Provided	Not Provided	800 oysters (one floating rack) were lost from Core Creek (Newport River) and suspected stolen after a search did not recover debris from the float.	N/A
97	NC DMF	Research	Eugene Burreson, VIMS (NC Locations	Summer	8/2005	UNC-IMS upwellers, Hoop Pole Creek in Bogue	1,000	0.40%	4	Not Provided	None	N/A
98	NC DMF	Research	Eugene Burreson, VIMS (NC Locations	Fall	9/2005	UNC-IMS upwellers, Bogue Sound, NC; Hewletts Creek,	5,000	0.10%	5	Not Provided	None	N/A
99	NC DMF	Research	Eugene Burreson, VIMS (NC Locations)	Fall	10/2005	UNC-IMS upwellers, Bogue Sound, NC	800	0.10%	0.8	Not Provided	None	N/A
100	NC DMF	Research	Eugene Burreson, VIMS (NC Locations)	Summer and Fall	7, 8 and 9/2006	Bogue Sound, NC	5,500	0.06%	3.3	Not Provided	None	N/A
101	NC DMF	Research	Eugene Burreson, VIMS (NC Locations)	Summer	2007	Bogue Sound, NC	4,500	0.30%	13.5	Not Provided	None	N/A

Order	Source(s) of information	Data Sources	Principal Investigator or Grower (Company Name) / Landside Location	Season	Date of Cage Deployment	Cage Locations	*C. ariakensis* # allowed at site (per permit) or actual # of oysters deployed at site	Probability Diploid	Estimated Total Number of Diploids	Estimated Rate of Triploid to Diploid Reversion	Accidental *C. ariakensis* Oyster Release Incidents	Evaluation of the likelihood that these oysters could establish a viable population
102	NC DMF	NC Field Trials	UNC IMS	Summer	2003	Pamlico, Roanoke and Bogue Sounds, NC	120,000	4:29983	Not Provided	Not Provided	A hurricane caused four (4) racks (20 cages) to wash out at a nursery area in Swanquarter Narrows. During the recovery effort, one cage broke and spilled 1,500 oysters out into the open-water. Divers were unable to recover these at the time due to poor visibility. An additional dive 10 days and 2.5 hours of dredging in the area 10 months later did not recover any *C. ariakensis* .	No apparent survival on-site.
											750 oysters were found to be missing from a floating culture in Roanoke Sound on Jan. 5, 2004. The cause was thought to be weather (high winds) and remaining floats were removed. 73 live *C. ariakensis* were recovered from the site in March 2004.	Recovered oysters showed good survival.
											7,750 oysters were lost due to floating culture bags breaking from mooring lines on Jan 22, 2004 near Buxton, NC. Plans were made to recover the oysters by hand. A totals of 156 live seed were recovered during two (2) search efforts; other bags of *C. ariakensis oysters* were thought to have moved into the open sound.	Survival in area is expected to be poor.
											Although grow-out bags were intact, 45 live and 40 dead *C. ariakensis* seed were found on the bottom below floating bags in Buzzards Bay on Feb. 20, 2004.	
105	NC DMF	NC Field Trials	UNC IMS	Fall	2003	Pamlico and Bogue Sounds, NC	300,000	2:988 incomplete test	Not Provided	Not Provided	None	N/A

APPENDIX D

THE EFFECTS OF SUSPENSION FEEDERS ON BENTHOS AND SEDIMENT

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The Effects Of Suspension Feeders On Benthos And Sediment

Suspension feeding bivalves can influence food web structure through feeding and biogeochemical processes through excretion, biodeposition, and bioturbation. Such effects may occur in both the water column and in the sediment. Although little work has examined the effects of *C. ariakensis* on water quality and benthos, much work has focused on the Pacific oyster, *C. gigas*. Additional studies have considered the effects of eastern oyster, *C. virginica*, as well as the blue mussel (*Mytilus edulis*) and the Manila clam (*Venerupis philippinarum*).

Pacific Oyster (*C. gigas*)

The Thau lagoon in France has been studied extensively to explore how the farming of Pacific oysters (*C. gigas*) has influenced water column and sediment characteristics. Thau lagoon is a semi-enclosed marine lagoon where it takes ~3 months to turn the water over. Oysters are farmed on 4m-long lines which are suspended from 5 m tall, 50 X 12 m metal frames. Souchu et al. (2001) examined the effect of *C. gigas* farming on water column biogeochemistry in the Thau lagoon. Compared to reference sites, oyster sites had 44.4% reduced chlorophyll a and 26% reduced particulate organic carbon. Oxygen concentrations were only slightly lower at oyster farming sites. The presence of oysters was also associated with abundant pico-phytoplankton, which have rapid growth rates. Oysters were able to control phytoplankton except during the summer when both phytoplankton production and oyster growth were greatest. Oyster farming areas also had elevated concentrations of nutrients including 73% greater ammonium, 36% greater phosphates, and 19% greater silicate. Chapelle et al. (2000) developed a model to study the effect of *C. gigas* farming on biogeochemical cycling in Thau lagoon. In the model, oysters affected nitrogen cycling by excreting ammonium, which supports phytoplankton (pico- and nano-) growth, and through biodeposition which drives sediment release of nitrogen and leads to oxygen reduction. Oysters are also associated with a decrease in both phytoplankton levels and zooplankton levels. Reduced oxygen levels through respiration were also linked to oysters. Mazoni et al. (1998) examined the impact of suspended oyster culture plus associated epifaunal fouling community (sponges, bryazoans, ascidians, polychaetes) on water quality characteristics. They found no effect of the suspension-feeding community on water column chlorophyll a levels. However, dissolved oxygen and nitrogen were significantly affected. The species composition of the biofouling community explained differences in biogeochemical fluxes at the interface between the epifauna and the water column; when polychaetes were abundant, phosphate fluxes were high whereas when ascidians dominated, nitrate-nitrate fluxes were great. Fouling epifauna were also associated with reduced water column oxygen. De Casabianca et al. (1997) studied sediment and vegetation characteristics along a eutrophication gradient associated with *C. gigas* farming in the Thau lagoon. At more eutrophied sites, silt and shell fragments composed a greater fraction of the sediments. In the region of the lagoon where shellfish tables were located, the macroalgae, *Gracilaria bursa-pastoris* dominated inside the shellfish tables whereas *Ulva rigida* dominated outside. In the portion of the lagoon where farming was less concentrated, eel grass communities (*Zolstera marina* and *Z. noltii*) were common.

Schmidt et al. (2007) studied vertical profiles of naturally-occurring, short-lived radio-nuclides to determine the effect of *C. gigas* farming on bioturbation of surface sediments in the Thau lagoon (France). Bioturbation is the physical re-working of sediment particles through the activities of the benthic community which can affect the distribution of organic matter and contaminants. Schmidt et al. hypothesized that particularly high rates of bioturbation (5-35 cm^2 yr^{-1}) are linked to biodeposition from oysters.

The impacts of *C. gigas* have also been studied in man-made experimental ponds in France (Dupuy et al. 2000). *C. gigas* was placed at densities of 20 m^{-2} in one experimental pond and the bacterioplankton communities were compared to those in a reference pond. Dupuy et al. (2000) found that oysters altered the bacterioplankton community through size-selective consumption of particles that were >5µm in diameter. Flagellates and ciliates beyond the 5µm threshold contributed to the oyster diet and the authors suggest that this is an important carbon connection between the microbial loop and secondary biomass production.

Forrest and Creese (2006) compared areas in a New Zealand estuary where oysters (*C. gigas*) had been farmed continuously for 8 years to reference locations. Oysters are grown on 1m-wide racks that are spaced 5-6 m apart. Samples were collected from beneath oyster racks, between racks, and at several distances away from the racks. Beneath the racks, there were greater rates of sedimentation, more sediment organic matter (6-7% vs. 3-4%), and reduced seabed elevation. There was also more silt/clay (5-7% vs. 3-4%) and reduced redox potentials (100-200 vs. 400 to 450 mV at 20 mm depth) in sediments under racks. Oyster racks appeared to have no effect on species richness. Benthic communities away from the oyster racks were characterized by low densities of a wide variety of large-bodied invertebrates which are typical of undisturbed communities. The reduced seabed elevation beneath racks was attributed to an interaction between local hydrodynamics and the racks on which oysters were grown.

Eastern Oyster (*C. virginica*)

The effects of on-bottom *C. virginica* on water quality characteristics were examined in 13,000 L mesocosms with environmental parameters similar to those in Narragansett Bay, RI (Pietros and Rice 2003). Two hundred caged oysters were present in each mesocosm and it was estimated that the oyster community was able to filter all of the water in the mesocosm within 3 days. Several factors did not differ between oyster and reference tanks including nitrate, chlorophyll a, and ammonia-N. Sediment traps found greater particulate organic matter (~20.0 vs. 5.3 g/m2/d) and particulate inorganic matter (7.2 vs. 2.3 g/m2/d) in oyster tanks compared to controls. The alga, *Nitzchia striata*, dominated oyster tanks whereas *Skeletonema costatum* was dominant in reference tanks. Ammonium excretion increased exponentially with oyster biomass and was rapidly taken up by phytoplankton production.

Other Bivalves (Blue mussel, *M. edulis*; Manila clam, *Venerupis philippinarum*)

Grant et al. (1995) compared areas below suspended mussel (*M. edulis*) culture lines to reference sites in Nova Scotia, Canada. Compared to reference sites, areas below culture were characterized by twice the rate of sedimentation due to biodeposition and rates of ammonium release were up to an order of magnitude greater. Oxygen consumption was 0.1 to 0.4 mm mol greater at cultivation sites indicating greater rates of anaerobic metabolism there. Abundance of polychaetes was 10-40% lower but abundance of scavenging snails that preyed on fallen mussels was 10-20% greater under culture lines.

Bendell-Young (2006) compared biological and sediment characteristics on three beaches in British Columbia (Canada) that had been exposed to 0, 3, and 5 years, respectively, of on-bottom

Manila clam (*Venerupis philippinarum*) farming. She found that clam farming beaches had decreased species richness (Jack-knife estimates, 25-45 species vs. <35 species) and altered bivalve community structure. Manila and little-neck clams dominated the reference beach and were concentrated at high tidal elevations whereas Manila clams were dominant and evenly distributed across tidal heights on farmed beaches. This is attributed to the presence of the moon snail, *Euspira lewisii*, which is present and feeds at lower tidal elevations at the reference beach but which is excluded with mesh netting on the oyster beaches. Farmed beaches also had greater accumulations of organic matter (1-2% vs. <0.5%) presumably due to biodeposition and of silt (2.5-4% vs. ~1%).

Summary and Implications for Aquaculture

The aquaculture of oysters may be expected to have noticeable but localized effects on water quality, sediment, and benthos. Most of these effects may occur for either on-bottom or off-bottom forms of aquaculture. However, because off-bottom aquaculture can be implemented in areas without existing oyster cultch, the changes in the existing environment would be greater for that aquaculture mode than for on-bottom aquaculture, where and increase in oysters on existing beds would simply alter the magnitude of existing ecosystem responses. Greater rates of sedimentation and enriched organic content in sediments underneath or near aquaculture units due to biodeposition are likely to increase benthic microalgal production in the sediments. Although reduced oxygen availability in the sediment is possible, current evidence for Eastern oysters indicates that aquaculture does not cause sediment anoxia. However, that response would vary depending on the density of organisms being farmed. Greater percentages of finer grain substrate types that are associated with aquaculture may make those locations more prone to erosion or sediment redistribution by wave energy. Reductions in the amount of phytoplankton present through feeding may occur locally but some studies have reported no change. Enrichment of Chesapeake Bay waters through excess nutrient loadings may limit the occurrence of such an effect. Still other studies have reported that aquaculture is associated with increased phytoplankton in small size classes because oysters remove larger cells from the water column through feeding, leaving smaller, rapidly-growing cells to proliferate. Similar effects could be expected on bacterioplankton communities with smaller cells becoming dominant in the vicinity of aquaculture units. Reduced local oxygen levels through respiration are likely, but current evident suggests altered levels would not be detrimental to other respiring organisms. Increased concentrations of dissolved nitrate and ammonia nitrogen could be expected through resuspension of biodeposits and excretion. Changes in water clarity and nutrient concentrations may also impact the local macro-vegetation community. While an increase in SAV biomass and other algal species would be expected to occur in response to increase in water clarity, some studies have found decreases or changes in species composition. Increased levels of bioturbation induced by benthic community responses to enhanced biodeposition could redistribute oxygen, nutrients and other materials throughout the sediments. Any fouling organisms that colonize the shells of oysters could also contribute to changes in oxygen, algae and nutrient concentrations in the water column. Such effects would depend on the characteristics (i.e. species, mode of feeding) of the fouling community. Oysters that fall from off-bottom aquaculture units could attract predators as could aggregates of oysters growing unconfined on the bottom. While this has been shown only for mussels in aquaculture, similar patterns are possible for oysters.

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ATTACHMENT E

PROSPECTIVE LOOK AT TRIPLOID AQUACULTURE WITH RESPECT TO RELATIVE SAFETY

Attachment E-2

Prospective look at triploid aquaculture with respect to relative safety

S.K. Allen, Jr.

Introduction

At present, the oyster industry for shucked product in Chesapeake Bay is almost entirely dependent on imports from other states. By some accounts, the standing stock of oysters in the entire Bay is about equivalent to three times the annual production by the major shucking houses in Virginia, i.e., approximately three billion oysters. This situation impelled the Virginia General Assembly in 1995 to direct the Virginia Institute of Marine Science (VIMS) to conduct research on non-native alternatives to "determine whether species not native to Virginia water could play a role in the shellfish industry" (Allen 2005). In 1996, trials with triploid *C. gigas* were deployed, with disappointing results overall (Calvo et al. 1999). In 1998 trials began with *C. ariakensis* that were more encouraging (Calvo et al. 2001), where *C. ariakensis* displayed high survival, fast growth and low disease susceptibility. Seemingly, this is what the Legislature and industry had been waiting for. Beginning in 2000 and since then, the vast majority of triploid *C. ariakensis* deployed in the Bay have been for industry-based trials: 3,000 in 2000; 30,000 in 2001; 1,000,000 in 2003; 1,200,000 in 2005; 800,000 in 2006; and 695,000 in 2007. After the second successful deployment of triploid *C. ariakensis* by industry in 2001, the Virginia General Assembly raised the stakes with House Joint Resolution No. 164 showing their "support for the continuation of efforts to establish commercial aquaculture production of genetically sterile *Crassostrea ariakensis*".

Note that there are a couple of distinctive features of House Joint Resolution No. 164, indicating an evolution of general mindset by the Legislature and their constituency. First is the mention of aquaculture, not restoration or fisheries, as a solution to production problems. Second is the mention of "genetic sterility," aka triploidy, a relatively higher tech approach to the non-native aquaculture opportunity. In fact, Virginia – including the Legislature, its investigative body VIMS, and the oyster industry represented by the Virginia Seafood Council – has embraced the option for triploid non-native aquaculture almost since it was posed by the National Academy of Sciences report, "Non-native oysters in the Chesapeake Bay" (NRC 2004). Today this option is represented in the current Environmental Impact Statement process by "Alternative 5: Aquaculture: Establish State-assisted, managed or regulated aquaculture operations in Maryland and Virginia using suitable triploid, non-native oyster".

What does triploid non-native aquaculture mean? How will it work? How big would it be? What are the risks, the advantages? And how can the risks be mitigated in a large scale industry? These are all essential questions underpinning Alternative 5, which may or may not be addressed in a concerted focused manner by the EIS. This paper is an attempt to examine these issues.

Background

Triploids for population control

Triploid oyster culture is enabled by the development of tetraploids (Guo and Allen 1994b). Tetraploids have four sets of chromosomes. Since the complement of chromosomes in a tetraploid is divisible by two, which is essentially what meiosis accomplishes during gamete formation, tetraploids are fertile. Moreover, gametes produced from tetraploids contain two sets of chromosomes. (Normal reproduction in diploids yields gametes with a single set of chromosomes.) Therefore, one highly efficient method of making triploid oysters is to breed tetraploids with diploids in the hatchery (Guo et al. 1996). Triploids created in this way are referred to as *genetic* or *natural* triploids. The major manifestation of triploidy in oysters is the disruption of normal reproductive physiology, rendering triploids functionally sterile (Allen 1986; Allen and Downing 1990; Guo and Allen 1994a).

It is the disruption of reproductive potential that lies at the heart of the Alternative 5, a strategy that I will call *population prevention*, as distinguished from population control (limiting the spread of established species) or eradication (removing introductions). I define population prevention as a strategy to prevent, or at least severely handicap, the ability of an introduced species to establish itself in a new environment. The utility of triploid *C. ariakensis* for population prevention has been exploited throughout the EIS process in projects funded by the NOAA Chesapeake Bay Office in their Non-native Oyster Research Program. That is, a number of deployments of triploid *C. ariakensis* have been used to gather data to assess the biological and ecological characters of *C. ariakensis* for the purpose of evaluating the risk of introduction, without making an introduction. It is this same principal of population prevention that allows us to consider an intermediate course between “no introduction” and “complete introduction,” i.e., aquaculture using triploids.

Population prevention for limiting the ability of a species to naturalize has several examples in the fish world. In freshwater, the U.S. Fish and Wildlife Service (FWS) deemed in 1985 that triploid grass carp, *Ctenopharyngodon idella*, were an environmentally safe, weed-controlling fish, and at the same time, established a ploidy inspection program for commercial sales of triploids, allowing their shipment around the country. Triploids are produced in this species by heat or pressure treatments, or in combination. That is, they are induced triploids. FWS offers a triploid grass carp inspection service for natural resource agencies in the United States and in other countries. The inspection program provides assurance that shipments of grass carp alleged to be all triploid, do not, within the confidence limits of the inspection program, contain diploids. Between 1985 and 2005, more than 7 million triploid grass carp were shipped throughout 45 states (Mitchell and Kelly 2006). The likelihood that triploid populations of grass carp could naturalize was estimated by evaluating the distributions and ploidies of gametes (Allen et al. 1986).

A number of states have programs to stock triploid, instead of normal diploid, salmonid species for population prevention, in this case to prevent both *de novo* populations and guard against interbreeding. Fish stocking is stringently regulated in Alaska, and stocking outside of a range of a species is only allowed using sterile fish (ADFG, unpublished). For rainbow trout, *Onchorynchus mykiss*, the most predominantly stocked fish, "non-native" genotypes (meaning of different population origin) are stocked as all-female, triploid (*cf.* Chourrout and Quillet 1982). ADFG is currently devising a new policy in which other salmonid species (e.g., Arctic grayling, Arctic char) will also have to be triploid if they are to be stocked in areas where they do not originate (Chris Habicht, ADFG, personal communication). Certification requires a minimum of 99% triploids with a 95% confidence interval. In fact, a total of nine other states have ongoing programs for sterilizing hatchery-reared salmonids (Kozfkay et al 2006), including Idaho, Washington, Oregon, Montana, Utah, Nevada, and California as well as at least two eastern states – Vermont and North Carolina. The purpose of these programs is to conserve the genetic integrity of native stocks, i.e., prevent interbreeding between hatchery and wild fish. However, certification standards for the programs in the lower 48 states are uneven, sometimes with none, and all (except Alaska) use "mixed-sex" sterile fish. That is, they do not use all-females (Kozfkay et al 2006). In all cases above, triploid are produced by either heat or pressure treatements, so-called triploid induction. Tetraploid x diploid production is unavailable, like it is for oysters.

Triploid fertility

The case for the sterility of triploid fish is strong, with the general observation that triploid females are nearly completely sterile (Benfey, 1999). For oysters, things are not that simple. Despite the effectiveness of creating triploids using tetraploids, the process is not perfect, triploids produce some gametes, and triploids are unstable, to some degree. Therefore, there are four aspects of the biology of triploids that engender some risk for establishing reproductive populations: (1) fidelity of the tetraploid x diploid cross that produces the triploids, (2) fertility of triploids, (3) outcome of spawning among triploids and between diploids and triploids, and (4) stability of the triploids. These parameters are discussed below.

Fidelity of 4n x 2n crosses

Theoretically, the creation of genetic triploids by crossing tetraploids with diploids should result in 100% triploids. We have had a number of opportunities to test this empirically in *C. ariakensis*. Until recently, we have not been able to realize perfect 100% triploidy in these spawns – there have been, in 14 spawns, 49 diploids in 60,061 putative triploids examined, or 99.92%. This is slightly better than 1 in 1000, overall, but of course there is variation, so that some spawns are better than 1 in 1000, and some worse. There are three possible explanations for the presence of diploids. First, diploids could have come from contamination by diploid cultures in the hatchery at the same time, although this is entirely avoidable. Second, diploids might arise through uniparental inheritance, either gynogenesis or androgenesis. For gynogenesis to occur, a tetraploid sperm would fertilize the egg, but not participate in embryogenesis. Normally, lack of sperm incorporation would yield a haploid embryo, unless there was subsequently a spontaneous retention of a polar body in this egg. If so, diploidy would be restored. Gynogenesis was suggested as the cause of diploids in 4n x 2n crosses of *C. gigas* (McCombie et al. 2005). For androgenesis, tetraploid sperm, which is di-haploid (2n), would activate the diploid egg, but development would proceed without participation from the female chromosome, again yielding a diploid embryo. Evidence for this was presented by Guo and Allen (1997). Spontaneous gynogenesis or androgenesis of this sort will be rare in 4n x 2n crosses because the survival of gynogens and androgens is exceedingly low (*cf.* Wada 2000). There is nothing we can do about this type of spontaneous "mutation." A third possible source of diploids among the triploids is low level hermaphroditism among the diploid females used for the spawn. Even a small amount of sperm, co-produced in the female, could lead to diploids among the triploids. Neither can we control this natural phenomenon in hatchery spawns.

Given that diploids might be an "expected" aberration in 4n x 2n crosses, we have developed hatchery protocols to eliminate diploids from the seed population (Allen et al, in prep.). In short, oyster larvae reach a terminal size at the end of the larval period, and that size is determined by a number of factors, environmental and genetic. Under the same culture conditions, triploid larvae obtain a large terminal size than diploids: about 380µm versus 350µm. Consequently, by choosing an appropriately sized screen to harvest eyed larvae, it is theoretically possible to exclude diploids, while harvesting only triploids. These eyed larvae then become seed source. We have done this exercise twice, once in both 2006 and 2007. In 2006, we found 0 diploids out of 3042 examined for certification of a VSC triploid *C. ariakensis* trial. In 2007, for the same purpose, we found 0 out of 10,560. In other words, we could find *no* diploids using this screening procedure.

Fertility of triploids

Triploids produce gametes in all *Crassostrea* species studied to date (*virginica* – Lee 1988; *gigas* – Allen and Downing 1990, Eudeline 2004; *ariakensis* – Erskine 2003, S.K. Allen, Jr., VIMS, unpublished). Females are generally more sterile than males, but on occasion, can produce millions of eggs. (Fertility of triploid females is, in fact, the foundation of producing tetraploids [Guo and Allen 1994 b]).Gametes from mature triploid individuals do not have a normal genetic constitution, that is, instead of being haploid (as if from diploid), the gametes have various levels of chromosomal imbalance. The analysis of triploid gametogenesis as it pertains to chromosome imbalance and reproductive probabilities was examined by Allen et al (1986). As a consequence, their reproductive potential must be gauged in probabilistic terms (see discussion below).

Establishing precise fecundity for triploids is difficult. Histological studies provide only a relative measure of fecundity. Data from experiments to manipulate the spawning of triploid includes only data on females, not males. Two estimates of female fertility were provided for *C. gigas* triploids: 1.1 and 2.2 million eggs per female (Gong et al 2004 and Allen and Guo 1994a, respectively. These numbers can be deceiving however. In the case of Allen and Guo's study, only one triploid female had high fecundity (21.5 million eggs), whereas in the other 18 females where eggs were counted, all were less than 4.4

million, with the average of those, 1.2 million eggs per female. Compare this to an average fecundity of like-sized diploids of about 50 million eggs per female. Thus, average fecundity in triploid females is largely controlled, it would seem, by relatively few, exceptional females. In our work to develop tetraploids in the genus *Crassostrea*, we have observed this phenomenon in three different species, where exceptional triploids range between about 1 in 50 for *C. virginica* to 1 in 20 for *C. gigas* and *C. ariakensis*. Interestingly, triploids made from tetraploids – genetic triploids – seem to have higher fecundity than triploids made by induction techniques (*gigas* – Eudeline and Allen 2000; *ariakensis* – Zhou 2002 v. Erkine 2003).

A final consideration for fertility of triploid populations is sex ratio. Sex ratio of triploids, compared to diploids, can be highly skewed, often favoring males over females and with a high ratio of hermaphrodites (*gigas* – Allen and Downing 1990; *ariakensis* – Zhou 2002, Erskine 2003). However, sex ratio and fecundity varies by environment. It is not possible to characterize each environment for its ultimate effect on fecundity and sex ratio. For estimation of reproductive potential, then, a 50:50 sex ratio will be assumed.

Outcome of spawning among triploids and between diploids and triploids

Some studies have surmised, based on retrospective analysis by histology, that triploids can spawn, i.e., release gametes on their own during the spawning season (*gigas* – Allen and Downing 1990; *ariakensis* – Erkine 2003). For triploids, even immature (unripe) cells are eliminated, i.e., spermatocytes or oocytes, versus spermatozoa and mature eggs. Such a release of gametes raises the possibility that triploids may a reproductive impact over and above the potential impact of diploids among them.

Reproductive potential of triploid *Crassostrea gigas* has been studied extensively for a number of reasons, ranging from documentation of their sterility for commercial purposes (Allen and Downing 1990) to estimation of their reproductive capacity for population prevention (Guo and Allen 1994a; Gong et al. 2004). Comparable studies on triploid *C. ariakensis* triploids for reproductive potential have not been reported. However, *C gigas* is probably a good surrogate for *C. ariakensis* because they have similar reproductive characteristics (S.K. Allen, Jr., VIMS, unpublished data) as triploid *C. gigas*.

Estimation of reproductive likelihood in triploid oysters was not quite as simple as the case for grass carp (*cf.* Allen et al. 1986), where females are completely sterile and therefore, triploid x triploid matings are implausible. Triploid Pacific oysters of both sexes produce gametes. Theoretically, there are two types of crosses that might result from a population of genetic triploids: triploid x triploid – the obvious one, and triploid x diploid – if diploids do in fact exist in the population. To be completely thorough, there also is a possibility that gametes from spawning triploid *C. ariakensis* could co-mingle with gametes from normal diploid *C. virginica*. These three issues will be examined below.

For this treatment, we need to define "reproductive potential." As used by Guo and Allen (1994a) originally, it was the product of relative fecundity times relative survival of a triploid cross. For labeling, we will use T for triploid and D for diploid, and list the female first, so that, TD is a triploid female x a diploid male.

For example, if survival of diploids was 20% and survival of a triploid cross was 1%, then the relative survival would be 5%, and if relative fecundity of triploids was 5%, then the reproductive potential would be 5% x 5% = 0.25%. In reality, male fecundity of triploids has never been measured, so there is an assumption that it is equal to female fecundity. This is probably not true, but since eggs, and not sperm, will be the limiting gamete for reproduction – and therefore, establishment of a population – then female fecundity is probably a reasonable estimation of for reproductive potential. Also, as reported by Allen and Guo (1994a), survival is calculated to 2 months old, so effectively, survival to spat stage.

In addition to the actual survival of larvae to spat stage (e.g., 2 months), the reproductive potential of a cross emanating from triploids must account for the *types* of larvae produced. For example, if a TT (or TD or DT) cross yielded diploids, this would be a different consequence than if it yielded triploids, or some other "dead end" ploidy that would fail to contribute to a sustained population. In other words, if triploid x triploid crosses yield only triploids, then so what? In fact this is *not* the case, and so these crosses among triploids and between diploids and triploids need to be considered.

Finally, all survival estimates were obtained in the hatchery, versus those that would be encountered in the wild.

<u>Triploid x triploid</u> – An analysis of reproductive potential in triploid *C. gigas* revealed that although gametes from triploids were fully capable of fertilization, aneuploid progeny resulted (Guo and Allen 1994a). When triploids were crossed with themselves, the ploidy of resulting embryos was 2.88n on average (versus 3n for a true triploid), that is, *hypo*triploid. Within individual spat from 3n x 3n crosses, there was substantial variation in chromosome numbers, about ± 6 chromosomes. For example, a single individual oyster might have between 22 and 32 chromosomes in different cells. Among progeny from 3n x 3n crosses, there was very little variation in the average number of chromosomes (range: 28.6-29.1). After one year, ploidy analysis of surviving TT juveniles was obtained by flow cytometry, and 4% were "diploid," although there may have been substantial aneuploidy. Flow cytometry is not able to detect small levels of aneuploidy.

Arguable, the resulting progeny from 3n x 3n crosses were non-viable from the sense of a sustaining population, i.e., they were highly aneuploid and also nearly triploid. In addition, survival of fertilized eggs through metamorphosis and settlement (2 months) was 0.0085%, compared to about 21% for the diploid cross. Therefore, the relative survival of 3n x 3n crosses was estimated to be 0.04% of diploids. The approximate fertility of triploids was 1.2 million per female compared to about 50 million for diploids – about 2%. Therefore the reproductive potential calculated by Guo and Allen for this cross was 0.0008% of diploids. (0.04% relative survival x 0.02% relative fecundity). Factor in that of the expected survivors, only a fraction are diploid – putatively capable of further reproduction – and the revised reproductive potential is roughly 0.000032%. (Table 1).

Diploid x triploid – Triploids may serve as either the male or the female portion of this cross, and the outcome is different depending. Below I will refer to either TD or DT crosses, where the female is listed first: TD = triploid female x diploid male. Again, we rely on data from *C. gigas*, and there have been two papers addressing the reproductive potential of these crosses. That of Guo and Allen (1994a) dealt with crossing triploids that were produced from inducing triploidy with cytochalasin B (*cf.* Allen et al. 1989). The work by Gong et al. 1994 examined crosses made with triploids produced genetically, by 4n x 2n cross. (Gong et al. did not attempt a TT cross, or a diploid control, since the question they were addressing was not primarily concerning reproductive potential of triploids.)

For DT crosses, survival to two months in the Guo and Allen study was 0.0007% and for Gong et al., an order of magnitude higher – 0.007%. Taking diploid survival into account, the relative survival of DT crosses in these studies was 0.003% and 0.033%, respectively. After at least nine months, the percentage of diploids among those sampled was determined to be 100% for Gou and Allen and 98% for Gong et al. Therefore the revised reproductive is 0.00007 or 0.00438% for crosses made from either chemical or natural triploids (Table 1). Clearly the latter is the more relevant to our discussion. The Gong et al. estimates of reproductive potential using genetic triploids was about 65 times higher than that of Guo and Allen using chemical triploids.

TD crosses are fundamentally different genetically, which both papers discuss, but are outside of our purview. Relative survival is higher in TD crosses, about 45-75 times higher than DT. The proportion of diploids remaining among the survivors after at least 9 months is lower, 33% for Guo and Allen and 47% for Gong et al. Therefore, the revised reproductive potential for TD (chemical) is 0.00148% that of the diploid; for TD (genetic), it is 0.09417% of the diploid (Table 1).

One could average the reproductive potential of DT and TD crosses to obtain an estimate for overall reproductive potential of a triploid population. It seems that the more conservative approach would be to take the highest figure – i.e., that for RRP of genetic TD: 0.09% of the diploid reproductive potential.

One final point regarding the difference between the two studies needs to be highlighted. The fecundity of genetic triploids as reported by Gong et al. exceeds that estimated from chemical triploids. This observation has been reported before (Eudeline and Allen 2000, Eudeline 2004). Genetic triploids seem to be about 6-7 times more fecund than chemical triploids. Reasons for this have been discussed by Gong et al, 2004, Erskine 2003, and Eudeline 2004. Erskine (2003), working with genetic triploids, also clearly showed that fertility varies with environment. In his work, he found that lower salinity environments seemed to favor males and moderate to high salinities favored females.

Table 1: Summary of factors contributing to reproductive potential (RP – as an estimated percent of diploid capability) in crosses made between triploid and diploid *C. gigas*. Two different types of triploids were used for these studies: chemical (induced) and genetic (natural – 4n x 2n). Revised RP (RRP) takes into account the ploidy of survivors, i.e., only survivors with approximately diploid constitutions will reproduce "normally." TT: 3n x 3n; TD: 3n♀ x 2n; DT: 2n♀ x 3n. Data from chemical triploids taken from Guo and Allen 1994a; data from genetic triploids taken from Gong et al. 1994.

	PERCENT					
Triploid type	**Survival (2 mos)**	**Relative survival**	**Relative fecundity**	**RP (% of 2n)**	**diploids (> 9 mo)**	**RRP (% of 2n)**
TT – chem	0.0085	0.040	2	0.0008	4	0.000032
DT – chem	0.0007	0.003	2	0.00007	100	0.00007
DT – genet	0.0070	0.033	13.4	0.0045	98	0.00438
TD – chem	0.0463	0.225	2	0.0045	33	0.00148
TD – genet	0.3140	1.49	13.4	0.2004	47	0.09417

C. ariakensis x *C. virginica* interbreeding – There seems to be little barrier to fertilization of eggs of either species by the sperm of the other. However, the resulting embryos develop only to the straight-hinge stage and die (Allen et al. 1993). Therefore, the major concern regarding the interaction of triploid *C. ariakensis* gametes with those of *C. virginica* is one of gamete interference. Clearly for this to happen, two predisposing conditions need to occur. First, populations of the two species – that is, the triploid *C. ariakensis* farm and the natural *C. virginica* oyster population – must be in close proximity, because the effective distance of fertilization for sperm, especially if it is abnormal triploid sperm, is limiting. Second, ripeness and spawning of diploid *C. virginica* and triploid *C. ariakensis* must be simultaneous. Erskine (2003) compared the development of gonads between triploid *C. ariakensis* and diploid *C. virginica*, and reported a significant delay in the development of *C. ariakensis* gonads compared to *C. virginica* in all sights, something that he referred to as gametogenesis lag.

Stability – reversion and mosaics

The process and implications of reversion for triploid *C. ariakensis* deployment (e.g., aquaculture) is probably generally misunderstood, or at least incompletely understood. I will describe this phenomenon and its implications in some detail.

The first observation confirming the process of reversion occurred during field tests of triploid *C. gigas* in 1993 (Allen 1993, Allen et al. 1996). Certified triploid (chemical) *C. gigas* were deployed in Delaware and Chesapeake Bays. After about 9 months of exposure, we found a relatively high proportion of mosaics – that is, oysters with both diploid and triploid cells in the somatic tissue – among our triploid oysters. The occurrence of mosaics themselves is not particularly surprising since the triploid induction process (i.e., chemical tripoloids) effectively poisons newly dividing embryos. Abnormal progeny, such as mosaic individuals with two cell types, might be expected as a matter of course. The surprising result was that the frequency of mosaics in several triploid populations increased over time, suggesting that some triploids were losing chromosome sets, a process dubbed “reversion.” Starting from a completely triploid animal, reversion results in an oyster with two cell populations – the original, in this case triploid, and the mutating population, in this case diploid. The resulting creature is called a mosaic.

The classic definition of mosaicism is the presence of two or more cell types in the same organism. In our case, it is the presence of triploid and some other cell type(s) within the same oyster. This other cell type is generally diploid, although (*i*) whether or not the “diploid” cells contain balanced sets of chromosomes is unknown; (*ii*) there can be more than one other cell type, as has been found in our lab among tetraploid oysters; and (*iii*) some mosaic conditions, like that found in the gonad of triploids, is natural because of the process of meiosis. The presence of mosaics among triploid populations is generally unappreciated for two major reasons. First, it requires some level of sophistication in ploidy analysis, for example, flow cytometry (FCM), to find mosaics. With FCM, the frequency distribution histograms of mosaics appear as distinct ploidy types, usually triploid and something else. The second reason mosaics have gone unnoticed is that they generally occur in very low frequency (e.g., <1% in genetic triploids).

The manifestation of reversion can be regarded in the same sense as we regard animals affected with disease. There are two metrics to describe its progression: incidence and intensity. Incidence refers to the number of animals in a population that have begun the process of reversion. This can be described by a percentage, is a population metric, and in our experience is low (see below). Intensity refers to the degree to which an animal has undergone reversion, that is, what percentage of its original triploid cells has begun to lose chromosomes. This can vary from a very low percentage for animals just starting the process, to high percentages for those that have a “bad case” of reversion. The metric of intensity applies to an individual, and is hard to apply to a population in a meaningful way.

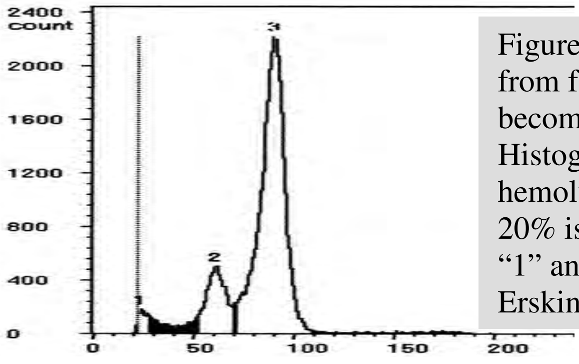


Figure 1: Left – Frequency distribution histogram from flow cytometry of a triploid oyster that has become a mosaic through the process of reversion. Histogram shows that 80% of cells of this tissue – hemolypmph – is triploid (peak labeled 3), while 20% is diploid (peak labeled 2. The incidence is “1” and the severity is 20%. (Histogram from Erskine 2003).

Incidence – Incidence of reversion is probably related to inherent genetic features in the triploids. The strongest evidence for this is that chemical triploids have a higher rate of reversion than genetic triploids, and this genetic difference persists across environments (Allen et al. 1999). For *C. ariakensis* the process of reversion is quite slow, taking a year or so to begin affecting the population (Zhou, 2002, Erskine 2003, and data below). We have relatively strong estimates of reversion in genetic triploid *C. ariakensis*.

Data on the process of reversion in triploid *C. ariakensis* come largely from our experience with VSC trials, especially the first one in 2003. Through the years we have also continued to examine and document reversion. Two of the more useful studies are summarized below and represent the incidence(s) of reversion. As a general statement, we can say that reversion occurs in about 1% of the population of triploid *C. ariakensis* up to market size, or a little bigger. For some reason, the frequency is lower in *C. virginica*. However, one of the hypotheses for explaining reversion has to do with growth rate, such that reversion is more apt to occur during periods of rapid growth. It makes sense, then, that a higher level of reversion would obtain in a more rapidly growing species, like *C. ariakensis*, than *C. virginica*.

Table 2: Incidence of reversion in previous field trials of triploid *C. ariakensis* and *C. virginica*. Ca = *C. ariakensis*; Cv = *C. virginica*. Incidence of reversion is calculated by [no. mosaics/ (total sampled – no. diploids)], since diploids can not give rise to mosaics of diploid and triploid cells.

Project	**Species**	**Total sampled**	**No. sites**	**No. mosaics**	**Incidence of reversion (%)**
Erskine 2003	Ca	930	6	11	1.20
2003VSC	Ca	8604	8	67	0.78
2003VSC	Cv	5554	8	10	0.18

Intensity – Intensity of reversion dictates risk. That is, the worry about reversion is that it will progress to a state that allows a formerly triploid oyster to regain full (diploid) reproductive potential. Diploid reproductive potential means that the gametes a mosaic makes will be normal haploid eggs and sperm.

As previously mentioned, reversion is progressive such that, over time, both the incidence and severity increases. Increased incidence in 1993 drew attention to the phenomenon originally, and we have seen increased incidence in every population. Incidence is greater in chemical triploids than genetic ones (Allen et al. 1999) although, again, only the latter are pertinent to this discussion.

We demonstrated progression of intensity in the first VSC trial in 2000-2002 by following individual oysters and sampling them over time, non-destructively. About quarterly, we carefully withdrew hemolymph and subjected the sample to flow cytometry, which characterizes the DNA contents of the cells. There data are shown in Table 3, below. The first entry represents the sampling period that the mosaic was first discovered, which was over a year after deployment.

These data illustrate several points. First, most of the triploids undergoing reversion accumulate diploid cells gradually over time, although there may be seasonal effects when mitotic indices are high. Second, the rate of reversion is quite variable, with some "streakers" (nos. 5, 9, 12) that seem particularly unstable, and others that languish (nos. 2, 4). Third, there are some inconsistencies in the trend for progressive reversion (highlighted boxes) and we are not certain whether this represents a sampling phenomenon or a biological one. Finally, there is one individual that reverted all the way back (no. 9) after 2+ years. It is the first mosaic we have followed all the way back to the diploid state.

Table 3: Progression of intensity of reversion in 13 individually monitored triploid *C. ariakensis* from the 2000 year class, and deployed for VSC trials of that year. Shown is the percentage of diploid cells over time. Sampling started in December 2000 but we detected no mosaics until September 2001. The date when data are first entered indicates when we detected the mosaic from random samples and began to follow those individuals. ns = not sampled; oyster 7 died.

	Sample date				
Oyster no.	Sept2001	Dec2001	Mar2002	Jun2002	Aug2002
1	4	6	3%	76%	0%
2	5	5	2%	0%	0%
3	4	5	7%	15%	26%
4	16	21	16%	10%	12%
5	64	70	45%	77%	84%
6	10	17	47%	52%	70%
7	--	4	8%	dead	
8	--	46	52%	61%	63%
9	--	82	91%	93%	100%
10	--	8	13%	33%	54%
11	--	--	7%	ns	69%
12	--	--	9%	ns	82%
13	--	--	--	--	18%
Mean	*17.2%*	*26.4%*	*24.9%*	*46.2%*	*48.2%*

So, what is the fate of triploids that revert? To determine this, we have tried to sample the gonad tissue in mosaics when possible. Mosaics are rare, so our sample size is limited to those that turn up. Determining the ploidy of gametes is further limited by whether or not we find a mosaic in the reproductive season, or not. Finally, we are generally limited to assessing males only, because males produce sperm that is a much more discreet tissue to analyze by FCM than oocytes. That said, the data we have on mosaic *C. ariakensis* are presented in Table 4. The bottom line is that we have never observed haploid (i.e., normal gametogenic) cells in a mosaic.

Table 4: The gonad of mosaic *C. ariakensis* were sampled for evidence of haploid gametes in different projects. Various levels of mosaicism were encountered, and the most severe case (with the highest percent diploid cells) is listed in the last column. The final row shows the results of sampling pure triploids, which showed no signs of mosaicism, as a "check" on triploid gametogenesis.

Project	**No. gonads sampled**	**No. samples with 1n sperm**	**Highest % diploid cells**
Erskine 2003	11	0	66%
VSC 2003	38	0	51%
On-bottom experiment	4	0	59%
Triploids sampled	3377	0	none

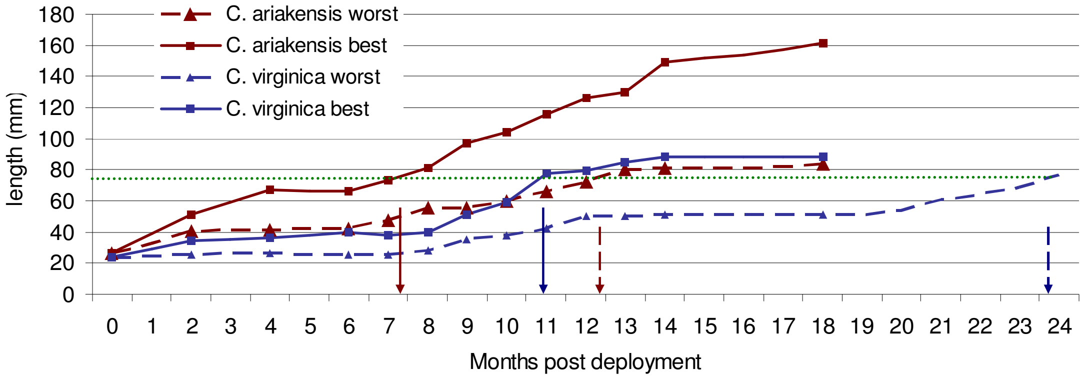
Why wouldn't haploid cells obtain in a mosaic? It may not be that they will never obtain, so much as we have not sampled any mosaic at a sufficiently old age that is *has* obtained. We do know that different tissues of the same mosaic oyster will show different levels of mosaicism, and we believe that is due to differential rates of mitosis, such that, rapidly growing cells revert more quickly than slower growing ones. For the gonad, it is active only part of the year, and accordingly, the levels of diploid cells found in gonad are always lower than in gill or hemolymph tissue (Chandler 1999, Erskine 2003). Therefore, perhaps the inevitable march toward producing haploid cells, in the gonad of a mosaic, is quite protracted. It seems that for animals that will dwell for a limited time (i.e., < 2 years), as aquaculture animals, the overall risk from reproduction is nil.

Potential value of triploid *C. ariakensis* aquaculture

<u>Half shell vs. shucked market</u>

<u>Biological advantage over *C. virginica*</u>

We have monitored the growth and survival of VSC trials since 2003, and results are consistent among all of them. Triploid *C. ariaksensis* grows quickly (more so than triploid *C. virginica*), is apparently unaffected by major Bay diseases, and has shown marketability, especially in for shucking trade.



Definition and management of critical control points

Why "critical"

Reproduction

Disease

Definition and management of critical control points

Diploid brood stock
Tetraploid brood stock
Biosecurity of brood stock
Control of hatchery processes
    Spawning –
    Larval rearing (primary certification) and setting –
    Post-set certification –
    Nursery –
Grow out

Modeling the risks to grow out

Setting goals – phases of growth

Phase I – caged aquaculture with monitoring
Phase II – expansion of caged aquaculture
Phase III – spat on shell, on-bottom culture
Economic development support

Discussion

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