Summary of NOAA-Funded Non-native Oyster Research

Understanding *C. ariakensis* within its native range: Taxonomy, pathogens, and ecology

A histological investigation of oyster parasites and pathology in three Chinese estuaries containing varying mixtures of coexisting oyster species including *Crassostrea ariakensis*
Grantee: Rutgers
Principal Investigators: David Bushek (Rutgers HSRL), Susan Ford (Rutgers HSRL), Ximing Guo (Rutgers HSRL)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: December 2004-November 2006
Summary:
(This is a companion project to NOAA award NA04NMF4570424: Genetic and ecological structures of oyster estuaries in China and factors affecting success of *Crassostrea ariakensis*: clues from a reclassification.)

Year 1:
a) To survey adult *C. ariakensis*, *C. gigas*, and *C. hongkongensis* from ten estuaries in China for the presence of potential pathogens
b) To survey oyster spat in three Chinese estuaries

Year 2:
c) To continue to document parasites in three species of oysters along the coast of China via standard histology. Molecular genetic and immunological assays are added as recommended by reviews from year one. During year two we will (a) survey a growing cohort of *C. ariakensis*, *C. gigas*, and *C. hongkongensis* during spring and fall 2006 from three estuaries for parasites and pathologies using histological, molecular genetic and immunological methods, and (b) disseminate results for the development of the EIS.

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
Grantee: Virginia Institute of Marine Science
Principal Investigators: Jan Cordes (VIMS), Kimberly Reece (VIMS)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: October 2004-March 2007
Summary:
Use nuclear, mitochondrial, and microsatellite markers to:
a) assess the amount of intraspecific genetic variation within and among existing native populations of *C. ariakensis*;

b) determine the genetic relationship of current U.S. hatchery stocks of *C. ariakensis* to native populations to aid in determining the strain of *C. ariakensis* best suited for any introductions that may become approved; and
c) provide molecular tools that can be used to identify the source population(s) of any unapproved *C. ariakensis* introductions into Chesapeake Bay.

Genetic and ecological structure of oyster estuaries in China and factors affecting success of *Crassostrea ariakensis*
Grantee: Rutgers
Principal Investigators: Ximing Guo (Rutgers HSRL), Aimin Wang (Hainan University), Guofan Zhang (Institute of Oceanology Chinese Academy of Sciences), Haiyan Wang (Institute of Oceanology Chinese Academy of Sciences)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: December 2004-November 2007
Summary:
Crassostrea ariakensis is poorly understood at this time largely due to taxonomic confusions in China where this species is widely reported. The overall objective of this project is to redefine *C. ariakensis* populations in China in light of a recent taxonomic correction, and to understand factors that affect the distribution and relative abundance of *C. ariakensis* under species competition in its native habitat. Specifically we propose to:
1) Develop a set of diagnostic markers for identification of common oysters from China;
2) Reclassify oyster populations along China's coast using molecular markers;
3) Study recruitment and competition between *C. ariakensis* and its competitor species; and
4) Collect and correlate ecological data with relative abundance at each site.

Analysis of genetic variation in *Crassostrea ariakensis*: Evaluation of germplasm resources for broodstock development
Grantee: Virginia Institute of Marine Science
Principal Investigators: Kimberly S. Reece, Standish K. Allen Jr.
Grantor: NOAA, Virginia Graduate Marine Science Consortium
Funding Period: February 2001-June 2003
Summary:
Genetic variation in wild populations and hatchery stocks of Suminoe oyster (*Crassostrea ariakensis*) was examined by means of PCR amplification of the mitochondrial COI gene and nuclear ITS-1 region followed by restriction fragment length polymorphism (RFLP) analysis and using three microsatellite DNA markers. Collections from China, Japan and hatchery-propagated stocks from the USA were examined. Hierarchical analysis of molecular variance (AMOVA) and pairwise comparisons revealed a significant differentiation between the samples from northern China and Japan, and those from southern China ($P < 0.05$) with RFLP patterns that were unique for the northern and southern groups. Microsatellite assignment tests also assigned 97.6% and 99.0% of oysters from either the northern (Yellow River, China and Itoki River, Japan) or southern (Zhuhai and Yangjiang) samples to their putative groups as identified first by

PCR-RFLP. Approximately half of the individuals in a sample from the Beihai region of the Guangxi province in southern China had the RFLP pattern characteristic of the oysters collected at the northern sites. In addition, the microsatellite profiles were used to assign each individual oyster from Beihai to either the northern or southern groups and assignment test results were consistent with the PCR-RFLP results. Surprisingly, no individuals from the Beihai sample were heterozygous for the nuclear ITS-1 region alleles. Overall, results of this study suggest that the northern and southern groups of *C. ariakensis*, apparently sympatric, are different species with some degree of reproductive isolation. The parental population for one hatchery stock was the Beihai sample which initially was composed of both the northern and southern genetic types. After hatchery spawns, however, most (>97%) of the progeny fell into the northern genetic group based on both PCR-RFLP and microsatellite analysis, indicating that the individuals with the southern genotype contributed very little to the spawn due either to gametic incompatibility, differential larval survival, or a difference in timing of sexual maturity. In addition to evaluation of variation within *C. ariakensis* samples, we used the sequence information obtained during this study to develop a molecular genetic key for the identification of 13 species of oysters (2 species of *Saccostrea* and 11 species of *Crassostrea*). The key is based on RFLP analysis of the ITS region and COI gene. We are able to unambiguously distinguish 11 of the 13 species using the ITS marker and two restriction enzymes. The COI locus is currently being developed as a backup marker and has so far proven promising by distinguishing 10 of the 13 species based on a single enzyme digestion.

Potential pathogens of *Crassostrea ariakensis* in its native range in China and in established populations in Washington, USA
Grantee: Virginia Institute of Marine Science
Principal Investigators: Eugene M. Burreson, Standish K. Allen Jr., Kimberly S. Reece
Grantor: NOAA/NMFS, NCBO Fisheries Research Program
Funding Period: October 2002-September 2004
Summary:
Project results are complementary to those of: Screening of *Crassostrea ariakensis* from its native range in Japan and China for herpes virus and *Perkinsus spp.* infections

Crassostrea ariakensis oyster samples from China, Japan, and from the hatchery at VIMS in Virginia were surveyed for oyster Herpes-like virus (OsHv) and for *Perkinsus* species using molecular diagnostics. Oysters were also examined for parasites and potential pathogens by routine histological examination. Very few parasites were found by histopathology and most of those observed were common gill ciliates or larval cestodes that occur in mollusks worldwide. Two cases of *Perkinsus* spp. were found at Beihai, in southern China, by histopathology. PCR, a more sensitive technique, revealed many additional *Perkinsus* infections and the presence of Herpes virus as well. Of 10 Asian sites surveyed, 6 showed evidence of OsHv infection and 9 evidence of *Perkinsus* spp. None of the hatchery samples screened showed infection with OsHv, but local *Perkinsus* spp. were detected in two of three hatchery groups examined. Sequencing of PCR products from Asian individuals positive for *Perkinsus* showed 2 species of *Perkinsus*

were present—*P. olseni*, which is known to be widely distributed in mollusks in the Pacific Ocean and the eastern Atlantic Ocean, and a new, undescribed species.

Life history and ecology

Long-term mesocosm studies of competitive interactions between diploid *Crassostrea virginica* and *C. ariakensis*
Grantee: University of Maryland
Principal Investigators: Roger Newell (UMCES Horn Point Lab), Denise Breitburg (SERC), Mark Luckenbach (VIMS), Chris Dungan (MDNR Oxford Lab)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: March 2005-Febuary 2008
Summary:
This project will:
1. Determine the ecological interactions between diploid *C. ariakensis* and *C. virginica* at all life stages from larval settlement through juvenile and to three year-old fully grown oysters.
2. Compare the growth rate and gametogenic cycle of diploid *C. ariakensis* and *C. virginica* under ambient annual temperature and salinity cycles typical of the upper Chesapeake Bay.
3. Determine the susceptibility of diploid *C. ariakensis* to native parasites and pathogens under mesohaline conditions typical of the Chesapeake Bay.
4. Compare the reefs formed by multi-age cohorts of *C. ariakensis* and *C. virginica* growing either separately or sympatrically and the relative utilization of these reefs by various invertebrates and vertebrates.
5. Synthesize date obtained in this study on settlement, survival, growth, and reproduction to develop an age-based matrix population model to predict the potential for population growth of *C. ariakensis* and *C. virginica* growing either alone or sympatrically.

Comparative performance of triploid *Crassostrea ariakensis* and *C. virginica* in bottom habitats in Virginia and Maryland
Grantee: Virginia Institute of Marine Science
Principal Investigators: Mark Luckenbach, Stan Allen Jr., and Peter Kingsley-Smith (VIMS), Kennedy Paynter, and Donald Meritt (UMCES)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: July 2005-December 2007
Summary:
Our overall objective is to provide a better understanding of how *C. ariakensis* will grow and survive in bottom environments. Specifically, our objectives are to:
1. Determine growth and survival rates of *C. ariakensis* in different bottom environments.
2. Compare the growth and survival of *C. ariakensis* and *C. virginica* within and across bottom environments.

3. Compare the growth and survival of each species under intra- and interspecific competition.
4. Characterize the growth forms of *C. ariakensis* and *C. virginica* grown in dense assemblages on the bottom and quantify the associated habitat complexity.
5. Investigate disease dynamics associated with cohabitation by *C. ariakensis* and *C. virginica* within experimental reefs.

Caged *Crassostrea ariakensis* deployment in the Chesapeake Bay: Growth, disease, *Polydora* infestation, and mortality in three and four-year-old non-native oysters
Grantee: University of Maryland, College Park
Principal Investigator: Kennedy Paynter
Grantor: NCBO Non-native Oyster Research Program
Funding Period: July 2005-June 2006
Summary:
This project will measure the growth, disease acquisition and mortality of the non-native oyster *Crassostrea ariakensis* in the field. The project extends the experimental grow out of native and non-native oysters at four sites in the Chesapeake Bay for two additional years.

Comparative post-settlement growth and survival in the Suminoe oyster *Crassostrea ariakensis* exposed to intertidal emersion
Grantee: Virginia Institute of Marine Science
Principal Investigators: Mark Luckenbach (VIMS), Peter Kingsley-Smith (VIMS)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: July 2005-March 2006
Summary:
The overall objective of this work is to understand *C. ariakensis'* growth and survival capabilities over a range of tidal exposures in order to assess both its potential to persist as intertidal populations and to pose a fouling nuisance. Our specific objectives are:
a) To compare the post-settlement survival and growth of the non-native Suminoe oyster, *C. ariakensis*, compared to the native Eastern oyster, *C. virginica*, held under a range of simulated tidal emersion regimes; and
b) To investigate the effects of the vertical and horizontal orientation of the substrate on the post-settlement survival and growth of *C. ariakensis* and *C. virginica* held under a range of tidal emersion regimes.

Metabolic differences between *Crassostrea ariakensis* and *Crassostrea virginica* at varying temperature and salinity – is *C. ariakensis* sensitive to low oxygen?
Grantee: University of Maryland, College Park
Principal Investigator: Kennedy Paynter
Grantor: NCBO Non-native Oyster Research Program
Funding Period: July 2005-June 2006
Summary:

1) to characterize the metabolic rate of native and non-native oysters; 2) to determine the ability of *C. ariakensis* to tolerate periods of anoxia or hypoxia; and 3) to determine the release of metabolic byproducts during hypoxic or anoxic exposure.

Predation by polyhaline invertebrate predators on young non-native oysters, *Crassostrea ariakensis*, in Chesapeake Bay
Grantee: University of Maryland
Principal Investigators: Victor Kennedy (UMCES Horn Point Lab), Roger Newell (UMCES Horn Point Lab)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: March 2005-March 2007
Summary:
1. Determine effects of polyhaline and marine invertebrate predators on comparative survival of *Crassostrea ariakensis* and *Crassostrea virginica*.
2. Provide these results to population modelers and federal and state personnel responsible for an EIS assessment.

Will predation mortality differ for larvae of native and nonnative oysters?
Grantee: Smithsonian Institution
Principal Investigators: Denise Breitburg and Richard Fulford (SI), Mark Luckenbach (VIMS), and Roger Newell (UMCES)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: March 2006-February 2008
Summary:
Demographic models and other means of predicting the potential for population increase of native *Crassostrea virginica* vs. non-native *C. ariakensis* require information on relative mortality rates of the two species. Predation mortality is likely the major source of mortality during the larval stage. There are large and potentially important differences in coloration, size, and swimming behavior between *C. ariakensis* and *C. virginica* veligers, and among *C. ariakensis* veligers from different source populations, that are likely to result in differences in their encounter rates with predators and capture rates, and hence their mortality rates. The ability to predict differences in survival through the larval stage is critical to any attempt to model larval dispersal, model the potential for population growth, predict which strain is suitable for introduction, or predict differential settlement of native and non-native oyster species in Chesapeake Bay.

Behavior, substrate selection and survival of *Crassostrea ariakensis* pediveliger larvae and juveniles to variation in environmental conditions
Grantee: University of Maryland Center for Environmental Science
Principal Investigators: Mario Tamburri (UMCES CBL), Mark Luckenbach (VIMS), Denise Breitburg (SERC)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: March 2005-Febuary 2007

Summary:
Our overall objective is to determine how the behavior, substrate preferences, and survival of *C. ariakensis* at the time of settlement differs from or resembles that of *C. virginica* and how these traits will impact recruitment, the likelihood of successful introduction into the Chesapeake Bay, and the potential for becoming a fouling nuisance species. We will:

a) continue evaluations of the effects of various substrates, chemical cues, and oxygen levels on settlement and metamorphosis;
b) continue examining behavior of pediveligers in response to intra- and inter-specific chemical cues and varying dissolved oxygen levels;
c) evaluate the roles of sedimentation on settlement and metamorphosis; and
d) evaluate the effects of hypoxia and sedimentation on the survival of newly metamorphosed juveniles.

Quantifying the response of different strains of *Crassostrea ariakensis* larvae to environmental change under spatially realistic conditions
Grantee: University of Maryland
Principal Investigators: Roger Newell (UMCES Horn Point Lab), Victor Kennedy (UMCES Horn Point Lab), Joan Manuel (UMCES Horn Point Lab)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: March 2005-Febuary 2006
Summary:
a) Determine whether larvae of two strains of *C. ariakensis* occupy the same vertical position in the water column as larvae of *C. virginica*, if these responses differ with ontogenetic development or diurnally, and if larvae aggregate either near the bottom, the surface of the water column, or at a halocline.
b) Determine the behavioral responses that create such distributions by examining if larvae swim away from or toward the bottom (i.e., are negatively or positively geotactic) or respond positively or negatively to a halocline, and if acute salinity changes alter these responses.
c) Measure mean and maximum swimming and sinking speeds, which define the extent to which larval behavior influences position in the water column.
d) Provide these parameters to modelers so they can account for larval movement, in addition to passive dispersal, when attempting to predict the dispersal of larvae from spawning events at any given place or time.

The use of non-native oysters in the restoration of Chesapeake Bay oyster populations and the potential threats posed by harmful algae
Grantee: University of Maryland, Center for Environmental Science
Principal Investigators: Patricia Glibert, Donald Meritt, and Diane Stoecker
Grantor: NCBO Non-native Oyster Research Program
Funding Period: October 2005-September 2007
Summary:
Evidence is mounting that harmful algae, particularly species common to the

Chesapeake Bay, have detrimental effects on oysters. We thus aim to compare the impacts of two common harmful algal (HAB) species on *Crassostrea ariakensis* and *C. virginica* in terms of: 1) the impact of HABs on spawning success; 2) larval growth and survival when exposed to HABs; 3) larval settlement when exposed to HABs; and 4) the rate of spat growth. There is strong evidence to date that some HABs common in the Chesapeake Bay contribute to shellfish toxicity and/or mortality, induce physiological stress, inhibit oyster spawning, and reduce larval survival and settlement. Experiments will be undertaken in the quarantine laboratory of the Horn Point Laboratory. Experiments will be conducted at different life stages of both oyster species under controlled, 'standard' algal diet, and with a diet of, or exposure to, one of the harmful algal species of interest. Spawning success, fecundity, larval growth and survival, settlement success, and shell growth will be measured and compared for the standard diet controls and the harmful algae-exposed treatments.

Developing a Relationship between Gamete Concentration, Turbulent Mixing, and Fertilization Efficiency in *Crassostrea*
Grantee: Virginia Institute of Marine Science
Principal Investigators: Mark Luckenbach, Roger Mann, Elizabeth North
Grantor: NCBO Non-native Oyster Research Program
Funding Period: July 2006-June 2007
Summary:
Current efforts to model population growth rates for *C. ariakensis* and *C. virginica*, especially at low population density, involve uncertainty in estimating fertilization efficiency that spans several orders of magnitude. This research seeks to reduce that uncertainty by developing and testing relationships between gamete concentration, turbulent mixing, and fertilization success. This project will conduct a series of laboratory experments using *Crassostrea* gametes to determine critical gamete characteristics and obtain body size-specific estimates of sperm production. Researchers will then employ two types of models--a fertilization kinetics model and a turbulence-based model to estimate fertilization success as a function of gamete concentration and (in the latter model) turbulent mixing. Predictions from the models will then be tested using *Crassostrea* gametes in grid-stirred chambers under controlled turbulent mixing levels. The results of this work will provide experimentally validated formulations for predicting fertilization success as a function of gamete concentrations under realistinc turbulent mixing regimes. Coupled with body size-specific male and female fecundities, these formulations will improve our ability to estimate fertilization success as a function of oyster density.

Fertilization interference between *Crassostrea ariakensis* and *Crassostrea virginica*: Supplement for distance experiments
Grantee: Rutgers, The State University of New Jersey
Principal Investigators: David Bushek, Ximing Guo, Gregory DeBrosse, and John Quinlan
Grantor: NCBO Non-native Oyster Research Program

Funding Period: December 2004-November 2006

Summary:

a) To compare gamete longevity between *C. virginica* and *C. ariakensis* under environmentally relevant temperatures and salinities;
b) to determine and compare effects of sperm density on fertilization rate for each species in both pure and hybrid crosses;
c) to determine the extent of hybridization that occurs under varying mixtures of gametes;
d) to determine the species composition and yield of viable larvae that successfully reach settlement when produced from varying mixtures of gametes; and
e) to use annular flume experiments to determine the effective fertilization distance for inter- and intraspecific fertilization of these two oyster species.

Growth and Reproduction of the Suminoe Oyster in a U.S. Subtropical Environment: EIS Ramifications?

Grantee: Harbor Branch Oceanographic Institution, Inc.

Principal Investigators: John Scarpa, Roger Newell

Grantor: NCBO Non-native Oyster Research Program

Funding Period: October 2006-September 2007

Summary:

Newell and colleagues have reported that diploid *C. ariakensis* grew significantly greater than *C. virginica* at low water temperatures (3-15 degrees C) in the Chesapeake Bay. This major finding raises the question whether *C. ariakensis* would grow year-round in warmer waters, such as along the southern Atlantic or Gulf Coasts, and how that would potentially affect interactions with *C. virginica* in those areas. Therefore, this project will complement the current growth/reproductive/disease studies by Newell et al., in the Chesapeake Bay by assessing, under quarantine mesocosm conditions (i.e., triplicate 700-L tanks supplied with ambient flowing seawater with all effluent treated by chlorine and neutralized before release), growth, reproductive capability, and disease susceptibility of *C. ariakensis* in Florida waters. Environmental factors (temperature/salinity) will be measured daily, and chlorophyll a levels will be measured weekly. Growth, as determined by shell size, will be measured monthly on the same oysters throughout the study. Reproductive capability will be assessed by examining and describing histologically gametogenesis in samples of each oyster species. Parasite and other potential pathogens will be examined using histological and molecular techniques. This will provide data at the opposite extreme of temperature for potential impacts of diploid *C. ariakensis* on subtropical estuarine ecosystems by planned or inadvetent transfers. The project will transfer to Florida some of the one- and two-year-old diploid *C. ariakensis* and *C. virginica* currently being grown at the Horn Point Laboratory in Maryland, which will allow for direct comparison of all data between the two studies. This approach will allow this study to be completed within the necessary timeframe to coincicde with the completion of other research funded by NCBO.

Competitive interactions between *Crassostrea virginica* and *C. ariakensis*

Grantee: Virginia Institute of Marine Science

Principal Investigators: Mark Luckenbach, Gene Burreson
Grantor: Sea Grant, Oyster Disease Research Program
Summary:
To develop a high-capacity quarantine system that can be used to:

(1) evaluate the growth form and growth rate of *C. ariakensis* in space-limited conditions (i.e., intraspecific competition);
(2) assess the outcome of competition between *C. ariakensis* and *C. virginica*;
(3) investigate interactions between adults and new recruits of each species; and
(4) draw inferences based upon (2)-(4) about the potential of *C ariakensis* to form biogenic reefs and to coexist with *C virginica*.

Assessing the potential for natural predators to control the spread of the Suminoe oyster, *Crassostrea ariakensis*
Grantee: University of Maryland
Principal Investigators: Roger Newell, Victor Kennedy (UMD Horn Point Lab)
Grantor: Sea Grant, Aquatic Nuisance Species Program
Summary:
We will investigate if *C. ariakensis* spat are as vulnerable as *C. virginica* spat to predators in the first weeks to months after larval settlement and metamorphosis. We will quantify mortality rates of different sized *C. ariakensis* when exposed to the dominant predators in the mesohaline region of estuaries in the mid-Atlantic region. In concurrent studies we will measure the mortality of similarly sized eastern oyster *C. virginica* exposed to the same predators. By statistically comparing rates of mortality between the two species exposed to the same predators under identical conditions we will ascertain if natural predators can control feral populations of *C. ariakensis* to the same degree as they control Eastern oyster populations. *C. ariakensis* and *C. virginica*, ranging from 0.5 to 50 mm in shell height, will be exposed under strict quarantine conditions in the laboratory to invertebrate (flatworms and 3 species of crab) and vertebrate (fish) predators that have been shown to feed actively on *C. virginica*.

Characterizing performance of the Suminoe oyster, *Crassostrea ariakensis*, in Maryland waters
Grantee: University of Maryland
Principal Investigator: Ken Paynter
Grantor: Sea Grant, Oyster Disease Research Program
Summary:
This project examines the potential for growth, disease susceptibility, survival, and reef habitat creation of *C. ariakensis* in Maryland waters. Sterile, triploid juvenile *C. ariakensis* and *C. virginica* will be deployed in biosecure cages at three secure sites in Maryland (UMD Horn Point Laboratory, UMD Chesapeake Biological Lab, U.S. Naval Academy) and one site in Virginia (VIMS). Two different sets of cages will be deployed. For growth, disease, and mortality studies, approximately 800 oysters of each species will be deployed in two separate, replicate subtidal cages of 400 each. Cages will be on racks underwater standing 0.5 meters off the bottom. They will be secured, underwater, to

pilings near the research piers of each site. For reef studies, 2 cages for each species, each containing 250 oysters, will be deployed. Therefore, 8 cages will be deployed at each site: 2 cages of *C. virginica* for sampling, 2 cages of *C. virginica.* for reef development, 2 cages of *C. ariakensis* for sampling, and 2 cages of *C. ariakensis* for reef development. Oysters will be monitored until November 2005.

Oyster Diseases: Susceptibility of *C. ariakensis* to known disease-causing parasites and pathogens

Pathogenic *Bonamia* sp. from North Carolina
Grantee: Virginia Institute of Marine Science (VIMS)
Principal Investigators: Eugene Burreson (VIMS), Ryan Carnegie (VIMS), Corinne Audemard (VIMS), Charles Peterson (UNC IMS)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: October 2004-September 2006
Summary:
Year 1:
The overall objective of this proposal is to determine the risk to *Crassostrea ariakensis* of the *Bonamia* spp. recently discovered in North Carolina. This will be accomplished with four sub-objectives:
a) to describe the seasonal prevalence and intensity of both newly identified *Bonamia* spp. from North Carolina in both oyster hosts, *C. ariakensis* and *Ostrea equestris*, in Bogue Sound, NC, and to determine the infection acquisition window for the *Bonamia* sp. pathogenic in *C. ariakensis*;
b) to determine, at the time of peak summer prevalence and intensity, the distribution of the *C. ariakensis Bonamia* sp. along a salinity gradient from the mouth of the Newport River at Bogue Sound up-river into Core Creek and the Neuse River estuary;
c) to determine if the risk of *Bonamia* sp. infections varies with proximity to North Carolina international marine terminals; and
d) to determine the temperature and salinity tolerance of the *C. ariakensis Bonamia* sp.

Year 2:
The overall objective of this project is to provide finer focus to the threat *Bonamia* spp. hold over *C. ariakensis—where* in terms of estuarine salinity profiles, *when* in terms of oyster size/age, and *from what source* in the local environment. We will address this through pursuit of five sub-objectives:
1) To determine the threshold salinity below which *Bonamia* sp. pathogenicity in *C. ariakensis* declines.
2) To determine the threshold size or age above which *C. ariakensis* resists acute bonamiasis.
3) To identify potential non-oyster sources of *Bonamia* sp. in Bogue Sound.
4) To determine if *B. ostreae* can be transmitted to *C. ariakensis* by cohabitation with infected *O. edulis*.

5) To determine if *C. ariakensis* can become infected by *B. ostreae* through injection, and if it can persist at salinities typical of the Chesapeake Bay.

Year 3:
The overall objective of the third year of this project is to close critical gaps in understanding of the risk Bonamia spp. poses to *C. ariakensis*: the source of Bonamia sp. in the environment, the role *C. virginica* plays in epizootics, and the nature of the prepatent period. This project will address this through pursuit of three sub-objectives: 1) To identify the reservoir for Bonamia sp. within the UNC-IMS upweller system. 2) To determine the susceptibility of *C. virginica* to Bonamia sp. infection. 3) To determine the prepatent period for Bonamia sp. in *C. ariakensis*.

Susceptibility of *Crassostrea ariakensis* to *Bonamia* species: Potential for increased disease transmission between oyster species
Grantee: University of Maryland, Biotechnology Institute
Principal Investigators: Gerardo Vasta and Jose Robledo
Grantor: NCBO Non-native Oyster Research Program
Funding Period: July 2005-July 2007
Summary:
In order to gain further insight into the risks of introducing exotic oyster species (*C. ariakensis*) into Chesapeake Bay, we propose to: 1) investigate in whole animal studies the ability of Bonamia species to cause disease in *C. ariakensis* and *C. virginica*; 2) examine *in vitro* the possibility that even without developing disease, *C. ariakensis* and *C. virginica* may become reservoirs for *Bonamia* species; and 3) develop novel specific tools for parasite diagnosis and cell separation that will further contribute to advancement in this field.

Potential for *Crassostrea ariakensis* to serve as a vector for exotic pathogens in Chesapeake Bay
Grantee: Virginia Institute of Marine Science
Principal Investigators: Kimberly Reece (VIMS), Ryan Carnegie (VIMS), Eugene Burreson (VIMS), Chris Dungan (MDNR Oxford Lab)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: October 2004-March 2006
Summary:
a) To determine the capacity of *C. ariakensis* to transmit the oyster herpes-like virus vertically from broodstock to progeny.
b) To determine the capacity of *C. ariakensis* to transmit the oyster herpes-like virus to native Chesapeake Bay bivalves.
c) To determine the pathogenicity of Asian *Perkinsus* spp. to *C. ariakensis*, *C. virginica*, and *Mercenaria mercenaria*.
d) To determine the potential for *C. ariakensis* infected with Asian *Perkinsus* spp. to transmit the parasites to *C. virginica* and *M. mercenaria*.

Potential for Crassostrea ariakensis to harbor pathogens of humans and shellfish: Potential for increased disease transmission between oyster species
Grantee: University of Maryland, Biotechnology Institute
Principal Investigators: Gerardo Vasta and Eric Schott (COMB), Denise Breitburg, and Anson Hines (SERC)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: July 2005-July 2007
Summary:
It remains unknown if *Crassostrea ariakensis* may harbor, transmit, or alternatively kill, pathogens of humans, or pathogens of fish and shellfish to a different degree than native oysters. The proposed study will 1) survey the presence of a series of human and shellfish pathogens (bacterial, viral, protozoal) in *C. ariakensis* and *C. virginica* deployed in Chesapeake Bay waters 2) study the ability *in vitro* of hemocytes of both *C. ariakensis* and *C. virginica* to either kill or harbor the pathogens under study, and 3) investigate the ability of experimentally-infected oysters to transmit pathogens to conspecifics and heterospecific oysters co-cultured in aquaculture or in a field setting.

Human Consumption Risk

Does *Crassostrea ariakensis* accumulate more microbial pathogens from *Crassostrea virginica*, increasing the pathogenic risk for human consumption?
Grantee: University of Maryland, Center for Environmental Science
Principal Investigator: Carys Mitchelmore
Grantor: NCBO Non-native Oyster Research Program
Funding Period: July 2005–June 2007
Summary:
This study will determine if *C. ariakensis* accumulates human pathogens to a greater or lesser degree than *C. virginica* and whether this may be complicated by ploidy status. It is known that *C. virginica* serves as a reservoir and vector of bacterial pathogens; the extent to which *C. ariakensis* plays a similar role is unknown. This is a situation that might affect the economic viability of the fishery and have implications as to the suitability of applying safe-fishing zones as an umbrella for all oyster species.

Comparing microbiological characteristics of *C. ariakensis* and *C. virginica* with respect to uptake and elimination of bacterial and viral pathogens *in situ*
Grantee: Virginia Institute of Marine Science
Principal Investigators: Howard Kator, Kimberly Reece, Kennedy Paynter
Grantor: NCBO Non-native Oyster Research Program
Funding Period: October 2006-September 2007
Summary:
The overall objective of this project is to characterize the uptake and elimination of indicator microorganisns and pathogens from *C. ariakensis* and *C. virginica* contaminated in situ. There is very little information of this nature, and preliminary

results suggest that *C. ariakensis* concentrates and retains microorganisms to a greater extent than *C. virginica*. To close gaps in our understanding of the potential human disease risk that might be associated with consumption of raw *C. ariakensis*, the project includes the following subobjectives: 1) to measure the uptake and elimination of fecal indicators and pathogens following in situ contamination of both oyster species in Virginia and Maryland; 2) to measure and compare the densities of selected Vibrio spp. in both oyster species harvested from growout areas in both Virginia and Maryland; 3) to measure changes in Vibrio spp. concentrations during storage of both oyster species under typical harvesting and commercial refrigeration and assess shelf life.

Environmental tolerance-dependent competition between adult *Crassostrea ariakensis* and *C. virginica* in recovering and retaining waterborne disease agents in relation to water salinity
Grantee: Johns Hopkins University
Principal Investigators: Thaddeus Graczyk (Johns Hopkins University, Bloomburg School of Public Health)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: April 2005-March 2006
Summary:
To characterize water salinity-dependent competition between commercial size diploid stages of *C. ariakensis* and *C. virginica* in the recovery efficiency and retention, depuration, and inactivation rates of human waterborne viral and protozoan pathogens such as Hepatitis A Virus (HAV) and Noroviruses (NoVs), and *Cryptosporidium parvum* and *Giardia lamblia*, respectively.

Aquaculture

Comparative Economic Evaluation of Triploid *C. ariakensis* and Triploid Disease *C. virginica*: Companion Trial to 2005 Virginia Seafood Council Deployment
Grantee: Virginia Institute of Marine Science
Principal Investigators: Standish K. Allen Jr., Ph.D., Karen Hudson, Robert Fisher
Grantor: NCBO Non-native Oyster Research Program
Funding Period: October 2005-September 2007
Summary:
This project will address the following objectives: 1) Compare relative commercial potential of triploid *C. ariakensis* with triploid *C. virginica* using commercial grow-out techniques from the ten farms in the Virginia Seafood Council trial, to include measurements of size, weight, and mortality. 2) Determine the extent to which triploids are responsible for disease resstance by comparing diploid and triploid *C. virginica* from same stocks at the ten farms. 3) Provide a platform for continuation of ODRP study on shell disease and initiate new investigation to determine the effect of hardening on incidence of Polydora and shell stock shelf life.

The potential for using triploid *Crassostrea virginica* for on-bottom culture in the Chesapeake Bay
Grantee: Virginia Institute of Marine Science
Principal Investigators: Melissa Southworth, Roger Mann, A. Thomas Leggett Jr., AJ Erskine
Grantor: NCBO Non-native Oyster Research Program
Funding Period: July 2005-December 2007
Summary:
The overall objective of the project is to monitor the feasibility of using triploid *Crassostrea virginica* spat on shell in the same manner as oyster planters have used wild oyster seed from seed rivers, grown out to market size. The specific objectives are: (1) To measure the growth, mortality, and disease prevalance of triploid *Crassostrea virginica* spat on shell grown on the bottom in the same manner as oyster planters have used using wild oyster seed from seed rivers. (2) Compare the costs versus yields of growing diploid and triploid strains. (3) Compare the condition index of triploid *C. virginica* grown in bags and wild diploid *C. virginica* grown on the bottom. The includsion of bag culture in the design matrix is new for the second year. It represents a significant data addition at very modest costs.

Biosecurity and comparative field trials of triploid *Crassostrea ariakensis* with *C. virginica*
Grantee: Center for Innovative Technology with subcontract to Virginia Institute of Marine Science
Principal Investigators: Standish K. Allen Jr., Kimberly S. Reece, Eugene M. Burreson
Grantor: NOAA, Chesapeake Bay Office, earmark
Funding Period: October 2003-September 2004
Summary:
Monitoring results, site locations, deployment methods, and other information can be found at www.vims.edu/vsc

I) In summer 2003, the Virginia Seafood Council will begin deployment of about one million triploid *C. ariakensis* see for "economic analysis of triploid *C. ariakensis* aquaculture." Funds will be used to apply biosecurity monitoring and data collection to the industry trial. These activities will consist of documentation throughout the study period of reversion, ploidy, gonad/gametic development, growth rates and condition indices, disease occurrence for *C. ariakensis* and genotyping. In addition, the project will enable the parallel deployment of triploid disease resistant *C. virginica* (DEBY strain) under the same conditions as a comparison to *C. ariakensis*.

II) To ensure continuing high levels of biosecurity in research with non-native species, funds will be used for installation of two quarantine systems for VIMS. The first is for the Kauffman Aquaculture Center (KAC), a biosecurity facility now under construction for VIMS from private fund donations. KAC is designed for maintaining fertile broad stock in isolation from natural waters of the Chesapeake. The second application of funds for biosecurity will be devoted to

non-native nursery system at Gloucester Point, VA, where non-native spat are reared to sizes to be transported to KAC.

III) Microsatellite markers are being developed to increase resolution for genotyping *C. ariakensis.* Microsatellite enriched libraries have been developed and primers are being designed to target these simple sequence repeat regions of the genome. A bank of 24 *C. ariakensis* samples from hatchery stocks and geographically distinct locations are being used to screen for polymorphisms at the microsatellite loci and to optimize PCR conditions for high throughput genotyping on our automated systems. The final step in the screening process will be to screen the loci with *C. ariakensis* families to ensure that the loci are following the rules of Mendelian inheritance and that they do not exhibit evidence of null (non-amplifying) alleles. These markers will be used for differentiating among hatchery stocks and natural populations.

Non-native oyster trials for aquaculture
Grantee: North Carolina Division of Marine Fisheries, Fisheries Management Section
Project Manager: Mike Marshall
Grantor: NOAA, Southeast Region, Federal Hurricane Grant
Summary:
Our study aimed to fill voids in the existing knowledge of Suminoe oyster performance in North Carolina by: (1) assessing growth and survivorship of the non-native oyster under a wide range of environmental conditions and at different times of the year; (2) incorporating rates of growth and mortality into an economic model which assesses the likely economic benefits to be reaped through the culture of the Suminoe oyster; and (3) assessing the environmental risk of release of the proposed introduction through a review of published and unpublished literature on the ecology of the Suminoe oyster in its native waters in Asia.

Economics

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
Grantee: Main Street Economics
Principal Investigator: Robert Wieland
Grantor: NCBO Non-native Oyster Research Program
Funding Period: August 2005-July 2006
Summary:
Year1:
Determine harvest and production costs in the oyster fishery and analyze net returns in the industry over the recent past to inform prospects for a public *C. ariakensis* fishery and alternatives. Examine publicly funded oyster restoration and management efforts and specific management initiatives and analyze these for comparisons of net benefits to the introduction and its alternatives.

Year 2:
The objective of the proposed study is to provide economic information and analysis focusing on the prospective costs and returns to the action and its alternatives. Building on the current study of the net returns in the fishery and public costs of oyster restoration with respect to *C. virginica*, the proposed study will identify the range of feasible technical and organizational alternatives for the introduction and its alternatives and estimate their prospective costs and returns. The study will also take account of short-term and long-term resource constraints (i.e., planting stock and habitable substrate) facing the action and its alternatives. To the extent that new habitat restoration techniques are tested for effectiveness over the period of the project, the study will assess their costs and potential changes in those costs, given changes in scale or scope. The study seeks to better establish the private and public benefits that might accrue to the action or its alternatives. To the extent that reliable projections are obtainable for projected stocks of either *C. ariakensis* or *C. virginica*, these will be used to estimate total and net benefits for both commercial harvesters and the wider public. Based on biological research on bivalve biodeposition and its influence on sediment nitrogen dynamics (Newell, et. al., 2002), a simple calculation will be undertaken of the value of the nitrogen removal venefits attending various estimated stock outcomes.

Support

Biological material support for studies on *Crassostrea ariakensis*
Grantee: Virginia Institute of Marine Science
Principal Investigators: Stan Allen (VIMS)
Grantor: NCBO Non-native Oyster Research Program
Funding Period: October 2004-September 2007
Summary:
Provide biological materials for studies on *C. ariakensis i*n association with funded projects from NCBO non-native oyster research program. Project objectives: 1) Maintain brood stock of *C. ariakensis* and *C. virginica* of sufficient diversity to produce a variety of test material for lab and field studies; 2) maintain overlapping year classes of diploid and triploid *C. ariakensis* and *C. virginica* adults for distribution as needed to projects in 2004, 2005, and 2006; and 3) produce new year classes of diploid and triploid *C. ariakensis* and *C. virginica* larvae, seed and juveniles for distribution as needed to projects from 2004-2007.