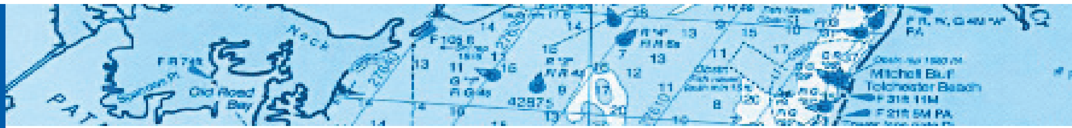
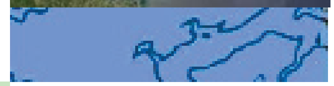




NOAA CHESAPEAKE BAY OFFICE

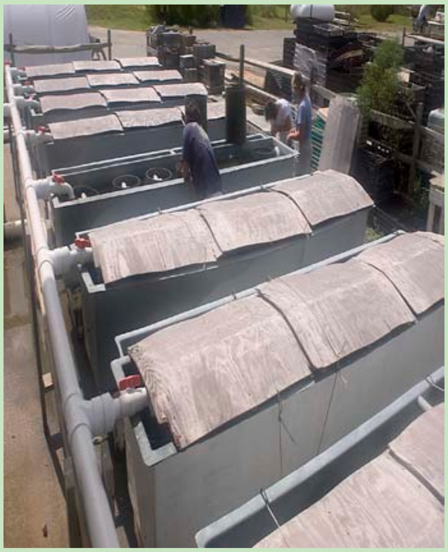
Non-native Oyster Research

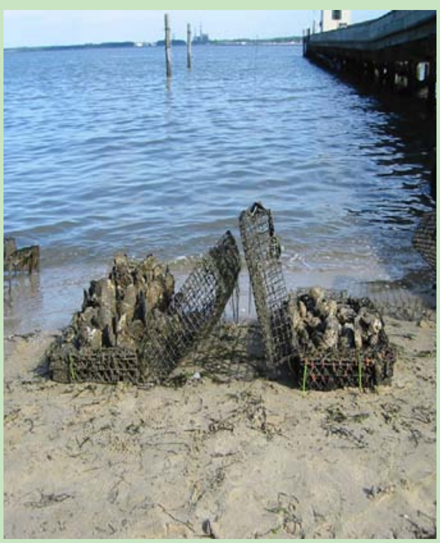
Research Topic:

Abstracts from posters and presentations given at the 98th Annual National Shellfisheries Association Meeting

March 26-30, 2006

Monterey, CA





Quarterly Review
Spring 2006

NOAA CHESAPEAKE BAY

Background

Decline in abundance of the native oyster, *Crassostrea virginica*, in the Chesapeake Bay has led to the collapse of a formerly productive fishery and the loss of significant ecological services. Two oyster diseases, MSX and Dermo, have contributed at least in part to the decline, and continue to challenge oyster restoration efforts. In response to this situation the State of Maryland and Commonwealth of Virginia have proposed to intentionally introduce a non-native oyster species, *Crassostrea ariakensis*, which has greater resistance to the pathogens responsible for MSX and Dermo. Considerable controversy exists over the proposed course of action and many questions remain about the implications of such an introduction.

In 2003 the U.S. Congress authorized the Army Corps of Engineers to prepare an Environmental Impact Statement (EIS) to examine both the risks and benefits of introducing this species to the Chesapeake Bay. The EIS is being conducted by the Corps as the lead federal agency, with the Maryland Department of Natural Resources (MDNR) and the Virginia Marine Resources Commission (VMRC) serving as lead state agencies. The U.S. Environmental Protection Agency (EPA), National Oceanic and Atmospheric Administration (NOAA), and Fish & Wildlife Service (FWS) are cooperating agencies on the EIS.

In 2004 the NOAA Chesapeake Bay Office (NCBO) initiated a 3-year Non-native Oyster Research program funded at $2M annually to obtain the scientific information needed to evaluate the proposed Asian oyster introduction. The program is aimed at research priorities recently identified by the National Research Council (NRC) and the Scientific and Technical Advisory Committee of the Chesapeake Bay Program (STAC), as well as guidance from the International Code of Practice on the Introductions and Transfers of Marine Organisms.

Research findings are reviewed quarterly at meetings or web conferences sponsored by NCBO and hosted by the Chesapeake Research Consortium. Invitees include scientists conducting research relevant to the EIS, representatives from federal and state agencies, and other interested management groups. These quarterly review sessions are designed to facilitate timely discussions of research results among scientists and managers, and speed the transfer of information to the EIS evaluation process. It must be emphasized that the findings of ongoing research are preliminary. Additional time will be required for the projects to be completed, and the results to be peer reviewed.

Summary reports of all Quarterly Reviews and additional information on NOAA's Non-native Oyster Research initiative are available at http://chesapeakebay.noaa.gov/.

Spring 2005	Overview of research topics: Taxonomy, genetics, disease, human health, ecology, interspecific interactions, ecosystem services and functions
Summer 2005	Aquaculture options: Biological and economic factors affecting aquaculture production of native and non-native oysters in the mid-Atlantic
Fall 2005	Potential for *Crassostrea ariakensis*-*C. virginica* interactions: Larval substrate selection, post-settlement competition, and fertilization interference
Winter 2006	Evaluating human health risks: Uptake, depuration, and post-harvest levels of waterborne human pathogens in *Crassostrea ariakensis* compared with *C. virginica*.
Spring 2006	Special session on *Crassostrea ariakensis* at the 98th Annual National Shellfisheries Association meeting

Abstracts from Posters and Presentations Given at the 98th Annual National Shellfisheries Association Meeting*
March 26-30th
Monterey, California

*These abstracts were also printed in the *National Shellfisheries Association Program and Abstracts of the 98th Annual Meeting* and many will also have full papers published in a future special edition of the *Journal of Shellfish Research*.

Introduction

NCBO sponsored a day and a half long special session on *C. ariakensis*, at the 98th annual meeting of the National Shellfisheries Association (NSA). The conference, held March 26th- 30th in Monterey, CA, was the most well attended NSA conference ever, with over 350 people present. Many of the projects featured in the *C. ariakensis* special session were funded through the NCBO Non-native Oyster Research Program. The session included a plenary talk that discussed the history of non-native oyster introductions elsewhere, 20 presentations, and nine posters. The posters and presentations covered a wide range of topics including the genetics, disease tolerance, possible human health risks, reproduction, and ecology of the Asian oyster, as well as interactions with the native Chesapeake Bay oyster species, *C. virginica*. Many papers presented during the session will be published in a special edition of the *Journal of Shellfish Research*—facilitating the timely delivery of this important information through the peer-reviewed literature. The abstracts from these posters and presentations constitute this Spring 2006 Quarterly Review Report. They are broken out roughly by the topic areas Oyster Diseases and Parasites, Harmful Algae, Human Health, Aquaculture, Ecological Interactions, Growth, Metabolism, and Reproduction, Genetics, and Modeling.

<u>Oyster Diseases and Parasites</u>

ASSESSMENT OF THE ABILITY OF *CRASSOSTREA ARIAKENSIS* HEMOCYTES TO KILL *PERKINSUS MARINUS* IN VITRO

Mohammad R. Alavi, José A. F. Robledo, Eric J. Schott, Keiko Saito, Satoshi Tasumi, and Gerardo R. Vasta (UMBI).

The American oyster, *Crassostrea virginica*, has been reduced to such low numbers in the Chesapeake Bay that the introduction of the Asian oyster *C. ariakensis* is being considered by the industry, and state and federal agencies. To assess the susceptibility of *C. ariakensis* to *Perkinsus marinus* (Dermo) we compared the capacity of *C. ariakensis and C. virginica* hemocytes or plasma to kill or inhibit proliferation of *P. marinus* trophozoites,. *P. marinus* trophozoites were exposed in vitro to oyster hemocytes or plasma for 24 and 72 hours and the parasite's ability to proliferate was assessed by culture in nutrient medium for four weeks, with weekly measurements of cell densities. Although both *C. virginica* and *C. ariakensis* hemocytes reduced the viability of most of the exposed parasites, even at the ratio of 16:1 (hemocytes:parasites) a small proportion of the parasite survived and proliferated upon addition of culture medium. To rigorously discriminate between adherent trophozoites from those phagocytosed by the oyster hemocytes, we developed a method based on the lipophilic dye CM-DiI which remains highly fluorescent when incorporated into cell membranes. Using this method, both degraded and intact parasites could be detected within hemocytes 24 hours post-exposure, confirming our previous results.

Potential microbial pathogens are also among the targets of oyster hemocytes. By the application of both PCR-based assays and culture enrichment for *Vibrio* spp., *V. parahaemolyticus, V. vulnificus, Mycobacterium* sp., and enterobacteria, we will compare the pathogen content of *C. ariakensis* and *C. virginica* from similar sites in the Chesapeake Bay. (Supported by grants from the Maryland DNR and NOAA)

POSTER: SHORT-TERM EVALUATION OF MORTALITY FROM *BONAMIA SP.* INFECTIONS IN *CRASSOSTREA ARIAKENSIS* AT TWO LOCATIONS IN SOUTHEASTERN NORTH CAROLINA

Troy Alphin (UNC), Ryan B. Carnegie (VIMS), and Martin H. Posey (UNC)

Crassostrea ariakensis (the Suminoe oyster) has been proposed as a potential aquaculture species for several states along the Atlantic coast of the United States, including Maryland, Virginia, and North Carolina. A 2003 trial of *C. ariakensis* in Bogue Sound, North Carolina, resulted in high mortality due to infection by *Bonamia* sp. The presence of *Bonamia* sp. may represent a significant impediment to the future culture of *C. ariakensis* along the east coast. As part of a field study evaluating the presence of *Bonamia* sp. in coastal North Carolina, *Ostreola equestris*, a species that may serve as a reservoir host, was sampled from a variety of locations from Bogue Sound to Wilmington. *Bonamia* sp. was detected in the Wilmington area in July 2005 and juvenile triploid *C. ariakensis* were deployed at two Wilmington locations in late September to determine if parasite transmission to this non-native species would occur. The first site was a research bottom lease, at the mouth of Hewletts Creek, NC and the location where *Bonamia* sp. was observed in *O. equestris*, while the second site was located 3 km south along the ICW at a site where O. *equestris* had not been noted. *C. ariakensis* were deployed for eight weeks with weekly monitoring of *Bonamia* sp. prevalence and mortality, and biweekly monitoring of growth. *Bonamia* sp. infections in *C. ariakensis* were noted at both locations, with a higher *Bonamia* sp. prevalence at the research lease site. Peak mortality approached 40% during the 5th week of the study.

POTENTIAL IMPACT OF *BONAMIA SP.* ON *CRASSOSTREA ARIAKENSIS* IN CHESAPEAKE BAY AND NORTH CAROLINA

Ryan B. Carnegie, Nancy A. Stokes, Corinne Audemard, and Eugene M. Burreson (VIMS), Melanie J. Bishop, Charles H. Peterson, Ami E. Wilbur, Troy D. Alphin, and Martin H. Posey (UNC)

A *Bonamia sp*. parasite emerged in 2003 as the cause of mortality (> 85%) among experimental seed *Crassostrea ariakensis* in Bogue Sound, North Carolina. With introduction of this oyster to Chesapeake Bay proposed, this epizootic gave urgency to investigations into the nature of bonamiasis in the mid-Atlantic: its annual timing and range, environmental limitations, and the identity and distribution of parasite reservoirs. Field studies included *Bonamia* sp. monitoring in *C. ariakensis* deployments along a salinity gradient from Bogue Sound, and at distant coastal sites; in serial deployments of seed *C. ariakensis* to upwellers on Bogue Sound; and among crested oysters *Ostreola equestris* from Bogue Sound and Masonboro Sound (Wilmington), NC. Laboratory trials have explored effects of reduced salinity on existing *Bonamia sp.* infections. Epizootics recurred in 2004/2005, generating infections for laboratory use and allowing an initial portrait of *Bonamia* sp. to be developed. This parasite causes acute disease and mortality in smaller/younger *C. ariakensis* (< 40 mm) in higher salinity coastal waters during warmer months. Infections may regress in winter. It is known to occur only at Bogue Sound and also in Masonboro Sound, > 100 km to the southwest. It occurs in *O. equestris* in both places, but this oyster may not be a functional reservoir for *Bonamia sp*., or its only other host. In the laboratory, infections were generally, but not always completely, purged at 20 and 10 psu, further suggesting that lower estuarine salinities (such as in Pamlico Sound, where *Bonamia* sp. has been notably absent from a mesohaline *C. ariakensis* nursery site, or Chesapeake Bay) may limit *Bonamia* activity.

OBSERVATIONS OF ASIAN *PERKINSUS* SPECIES AND *PERKINSUS MARINUS* IN THE SUMINOE OYSTER, *CRASSOSTREA ARIAKENSIS*

Jessica A. Moss and Kimberly S. Reece (VIMS)

Decline of *Crassostrea virginica* populations has led to an interest in using the non-native oyster species, *Crassostrea ariakensis*, for aquaculture development, fishery resource enhancement and habitat restoration in the Chesapeake Bay region. Previous field studies comparing the performance of *C. ariakensis* and *C. virginic*a in the region suggested that the non-native oyster was substantially more tolerant of the endemic protozoan parasite, *Perkinsus marinus*. We conducted a disease survey of *C. ariakensis* in China, Japan and Korea using molecular diagnostics. PCR-based assays revealed two *Perkinsus* species in Asian Suminoe oyster populations that currently are not found in US waters; *Perkinsus olseni* and, based on DNA sequence data, an undescribed *Perkinsus* species. We will report on progress towards molecular characterization of this undescribed *Perkinsus* species. Quarantine protocols for *C. ariakensis* brought to the US from Asia should limit introduction of exotic pathogens, however, it is important to understand the impact that Asian *C. ariakensis* pathogens could have on local bivalve species in case of accidental introduction via the host or through an indirect source such as ballast water, in which case the non-native oyster could act as a pathogen reservoir. We will report results of challenge experiments being conducted with Asian *C. ariakensis* pathogens to examine the impacts on the local Chesapeake Bay bivalve species, *C. virginica* and *Mercenaria mercenaria,* as well as on *C. ariakensis.* During these experiments, we unexpectedly observed the acquisition of moderate and heavy intensity infections of *P. marinus* in *C. ariakensis* oysters being maintained in the laboratory.

POSTER: A HISTOLOGICAL INVESTIGATION OF OYSTER PARASITES AND PATHOLOGY IN CHINA

Emily Scarpa, Susan Ford, Ximing Guo, Lisa Ragone Calvo, and David Bushek (Rutgers)

The proposed introduction of *Crassostrea ariakensis* into Chesapeake Bay as a means to restore oyster populations presents a number of potential risks, such as pathogens and pathological conditions that require careful examination and research before approval. Parasites occurring, even at low prevalence, in *C. ariakensis* within its native distribution may seriously impact this or other species in a different environment. Pathogens present in oyster species coexisting with *C. ariakensis* in its native habitat may also present problems if they are able to use *C. ariakensis* as a host or reservoir. In this survey, 27 samples were collected from 8 provinces along China’s coastline. Cross-sections of individual oysters were preserved in Davidson’s fixative. Genetic analysis of ethanol preserved gill samples was conducted to identify species of individual oysters.

Nine samples were selected based on presence of *C. ariakensis*. These oysters, representing 6 sites and consisting of *C. ariakensis* and a number of coexisting species, were processed by normal histological procedures and examined. Individual *C. ariakensis* were also examined using a fluorescence immunostaining technique to identify presence of *Perkinsus* sp. infections. Observed parasites included ciliates, such as *Sphenophyra*-like ciliates and trichodinids; crustaceans, including intestinal copepods; coccidians, including *Nematopsis* and a coccidian-like organism; rickettsia/chlamydia-like organisms; and trematodes. Overall prevalence of any particular parasite did not reach above 3% and averaged less than 1%. However, prevalences of coccidian-like and *Nematopsis* species were as high as 45% and 64%, respectively, at specific sites. To date, no evidence of a significant pathological impact has been observed.

PERKINSUS SPP. AND *BONAMIA* SPP. INFECTIONS IN *CRASSOSTREA ARIAKENSIS* MAINTAINED IN A FULLY CONTAINED AQUACULTURE SETTING.

Eric J. Schott, José A. F. Robledo, Mohammad R. Alavi, Keiko Saito, Satoshi Tasumi, and Gerardo R. Vasta. (UMBI)

In the Chesapeake Bay, diseases caused by *Perkinsus marinus* and *Haplosporidium nelsonii* (Dermo and MSX, respectively) have contributed to drastic declines of populations the native oyster, *Crassostrea virginica*. The Asian oyster, *C. ariakensis*, which grows readily to market size in Dermo-endemic Chesapeake Bay waters, is being considered for introduction to restore oyster populations. While apparently tolerant to *P. marinus, C. ariakensis* exposed to Bay waters may harbor the parasite at prevalences of up to 80%, raising the possibility that it could serve as a reservoir in which the parasite could increase its virulence. A crucial question is whether *P. marinus* can be transmitted from *C. ariakensis* to naïve *C. virginica*. We conducted cohabitation experiments with *Perkinsus*-infected *C. ariakensis* and *Perkinsus*-free *C. virginica*. The prevalence of *Perkinsus* infection in both 'donor' and potential 'recipient' populations was assessed at 2 and 4 weeks by PCR-based methods. After 4 weeks, *Perkinsus* was present in *C. virginica*.

A potential protozoal disease of *C. ariakensis, Bonamia ostraea*, has been associated with mortalities of experimental populations of *C. ariakensis* in Pamlico Sound of NC. The potential exists for *Bonamia* sp. to be present in Chesapeake Bay. We previously detected PCR amplicons indicative of *Bonamia* spp. in *C. ariakensis* reared in the Chesapeake Bay. We have conducted cohabitation studies in which *B. ostreae*-infected *Ostraea edulis* were co-cultured with *C. ariakensis.* After 4 weeks, we detected no *Bonamia* sp. in the recipient *C. ariakensis* using PCR-based methodologies. (Supported by grants from the Maryland DNR and NOAA)

<u>Harmful Algae</u>

EFFECTS OF HARMFUL ALGAE ON FEEDING AND GROWTH OF *CRASSOSTREA ARIAKENSIS*

Jeff Alexander, Donald Meritt, Stephanie Alexander, Angela Padeletti, Diane Stoecker, and Patricia Glibert (UMCES).

Introduction of the Asian oyster, *Crassostrea ariakensis,* to Chesapeake Bay is of considerable interest in the broader plans for Bay restoration. Overfishing, loss of habitat, and disease have caused dramatic losses in the native oyster, *Crassostrea virginica*, and it is thought that the Asian oyster may succeed where the native oyster has not because of its high growth rates and resistance to disease. One of the manifestations of poor water quality in Chesapeake Bay is the development of harmful algal blooms. Critical to an understanding of the potential success of the Asian oyster in Chesapeake Bay is knowledge of the impact of common harmful algae on oyster growth. In feeding experiments, oysters were exposed to algal mixtures containing *Isochrysis sp.* (a standard hatchery feed organism) paired with one of the harmful algal test species in varying proportions: dinoflagellates *Prorocentrum minimum* and *Karlodinium veneficum*, and raphidophytes *Heterosigma akashiwo* and *Chattonella subsala*. All harmful algae were cleared from the water by the oysters. Feces and pseudofeces were sampled for 18 hrs and examined for the presence of harmful algal cells, and the presence of indicator pigments of those cells. In the case of *P. minimum* intact cells and indicator pigments were found in both pseudofeces and feces. In *K. veneficum* indicator pigments were found in both pseudofeces and feces. In the case of *H. akashiwo* and *C. subsalsa*, however, feces and pseudofeces had a very low abundance of intact harmful algae or their indicator pigments, and feces had proportionately more sloughed off oyster gut cells, suggesting differential digestion of these cells relative to the dinoflagellates. Experiments were also conducted to determine the effect of *P. minimum* on growth. Effects of exposure to *Prorocentrum minimum* were compared to that of *Isochrysis sp.*, one of the standard hatchery feed organisms, and a non-fed control treatment. Valve height of cultchless test oysters, prepared by grinding away a portion of the shell periphery following guidelines from the EPA Oyster Acute Toxicity Test, was measured before and after 96 hrs exposure to each treatment. Growth on *Prorocentrum minimum* was equivalent to that on *Isochrysis sp.*

CRASSOSTREA ARIAKENSIS AND *C. VIRGINICA* RESPONSES TO *ICHTHYOTOXIC KARLODINIUM VENEFICUM*

Emily F. Brownlee (Calvert High School), Allen R. Place, Hirofumi Nonogaki, and Jason E. Adolf (UMBI), Stella G. Sellner (Morgan State), and Kevin G. Sellner (CRC)

The Eastern oyster *Crassostrea virginica* and the Asian oyster *C. ariakensis* are native and potentially introduced oysters, respectively, in the Chesapeake Bay and as such, will be exposed to the natural phytoplankton assemblages including harmful species throughout their life cycles. Recent work suggests that at least one of these prey items, the ichthyotoxic dinoflagellate *Karlodinium veneficum*, occurs frequently throughout the growth period for the oysters, thereby insuring frequent exposures for all life stages of the bivalve. *Karlodinium* produces linear polyketide toxins (karlotoxins) which elicit toxicity through sterol-dependent, non-specific pores in biological membranes. Spat and juvenile oysters of each species were exposed to a moderately toxin level [18.5 ± 6.2 ng ml^{-1}] of the dinoflagellate at environmentally-relevant cell densities and growth contrasted with rates observed on other phytoplankton species, including the spring bloom former *Prorocentrum minimum* and a phytoplankton mixture routinely used in oyster hatcheries. Growth for spat and juveniles of both oysters was significantly reduced when feeding on *Karlodinium*, relative to the other prey species. Experiments are continuing to identify the importance of food quality versus toxin content in oyster responses. These initial results suggest that the cosmopolitan *Karlodinium veneficum* inhibits growth and would potentially curtail oyster production within the tidal Bay and its tributaries throughout the oysters' growth periods and considering its global distribution, potential impact throughout temperate areas should be assessed.

Human Health

RECOVERY, BIOACCUMULATION, AND INACTIVATION OF HUMAN WATERBORNE PATHOGENS BY *CRASSOSTREA ARIAKENSIS*

Thaddeus K. Graczyk, Autumn S. Girouard, Leena Tamang, Sharon P. Nappier, and Kellogg J. Schwab (JHU)

Introduction of non-native oysters (i.e., *Crassostrea ariakensis*) into the Chesapeake Bay has been proposed as necessary for restoration of the oyster industry; however, nothing is known about the public health risks related to contamination of these oysters with human pathogens. Commercial size *C. ariakensis* triploids were maintained in large marine tanks with low (8 ppt), medium (12 ppt) and high (20 ppt) salinity water spiked with 1.0×10^5 of transmissive stages of the following human pathogens: *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts and microsporidian spores (i.e., *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Enterocytozoon bieneusi*). Viable oocysts and spores were still detected in oysters on day 33 post water inoculation (pwi), and the cysts on day 14 pwi. The recovery, bioaccumulation, depuration, and inactivation rates of human waterborne pathogens by *C. ariakensis* triploids were driven by salinity, and were optimal in medium and high salinity water. The concentration of human pathogens from ambient water by *C. ariakenis* and retention of these pathogens without (or with minimal) inactivation and a very slow depuration rate, provides evidence that these oysters may present a public health threat upon entering the human food chain if harvested from polluted water. This conclusion is reinforced by the concentration of waterborne pathogens used in the present study which was representative of levels of infectious agents in surface waters including the Chesapeake Bay. Aquacultures of non-native oysters in the Chesapeake Bay will provide excellent ecological services in regards to efficient cleaning of human infectious agents from the estuarine waters.

COMPARISON OF *CRASSOSTREA ARIAKENSIS* AND *C. VIRGINICA* IN THE DISCHARGE AREA OF A NUCLEAR POWER PLANT IN CENTRAL CHESAPEAKE BAY

Richard I. Mclean (Maryland Department of Natural Resources) and George R. Abbe (Morgan State)

The potential for introduction of the non-native *Crassostrea ariakensis* into Chesapeake Bay prompted a 5-mo comparison study with *C. virginica* conducted near the Calvert Cliffs Nuclear Power Plant in Maryland, with the primary goal being a comparison of uptake rates of radionuclides released by the plant. The study began with approximately 200 market size triploid *C. ariakensis* (1-yr old) and 200 diploid *C. virginica* (3-yr old) held in secure stainless steel cages 0.5 m off bottom. Half were deployed from July to September 2004, and half from July to December. Although quantities of radionuclides released by the CCNPP were insufficient to yield detectable concentrations in either species, other data were obtained.

From July to December *C. ariakensis* increased its initial shell length by nearly 20% compared to 9% for *C. virginica*. Although mean shell sizes of both species were similar, meat weights and condition indices for *C. ariakensis* were significantly greater ($p<0.001$) than for *C. virginica*. *C. ariakensis* had lower overall mortality even though nine were lost to blue crab predation during the first exposure period. After 5 mo of exposure, prevalence of dermo disease in *C. virginica* was 65% with a relative intensity of 1.50 on a 0-7 scale; *C. ariakensis* showed no sign of infection.

Although the non-native *C. ariakensis* may have much to offer with high meat yield and disease resistance, rapid growth of triploids results in very thin shells, which may make them more susceptible to blue crab predation than the thicker-shelled *C. virginica*.

COMPARISON OF BACTERIA UPTAKE AND DEPURATION BETWEEN *CRASSOSTREA ARIAKENSIS* AND *CRASSOSTREA VIRGINICA*

James A. Morris Jr. (NOAA), Tanya J. Bean and Rachel T. Noble (UNC), and Patricia K. Fowler (NC Department of Environment and Natural Resources)

Considerations to introduce the Suminoe or Asian oyster *Crassostrea ariakensis* along the East Coast have raised many questions regarding ecology, economics, and human health. To date, research has focused primarily on the ecological and socioeconomic implications of this initiative, yet few studies have assessed its potential impact on public health. Our work compares the rates of bioaccumulation and depuration of indicator organisms (such as *E. coli)* and *Vibrio sp.* between *Crassostrea virginica* and *Crassostrea ariakensis* in the laboratory. Preliminary results suggest that the rates of bioaccumulation of *E. coli* in *Crassostrea ariakensis* were significantly lower than those for *Crassostrea virginica* and that depuration was variable between the two species. This research provides coastal managers with insight into *Crassostrea ariakensis* response to bacteria, an important consideration for determining appropriate management strategies for this species. Further field-based studies will be necessary to elucidate the mechanisms responsible for the differences in rates of bioaccumulation and depuration.

Aquaculture

RISKY BUSINESS: PROSPECTIVE LOOK AT TRIPLOID *C. ARIAKENSIS* AQUACULTURE

Standish K. Allen Jr. (VIMS)

Among the options under consideration by the current Environmental Impact Statement (EIS) process on the introduction of *C. ariakensis* to Chesapeake Bay is that of aquaculture of triploid *C. ariakensis*. The premise for this option is that sterile triploids can eliminate or lessen the risk of inadvertent introduction. Triploidy is produced by crossing tetraploid with diploid brood stock, yielding high proportions of triploids – but not 100%. Our experience with certifying 8 batches (average sample size for each certification: 3558) of triploids produced in this way indicates that, on average, batches are 99.86 0.094% (95% CI) triploid. Essentially, the risks posed by triploid aquaculture boils down to the risk of reproduction from the 1 in 1000 diploids scattered among the crop. We have overseen two major industry aquaculture trials with triploid *C. ariakensis*. Because of Federal permitting requirements by the Army Corps of Engineers for these trials, various other Federal agencies, as well as States and NGOs, have weighed in on provisions for permitting. Risk has been quantified by a simple probability/ demographic model to estimate the number of surviving spat that result from various deployment options. Moreover, these risk calculations have been deemed cumulative, such that those risks posed in a previous industry trial with triploids are summed with those for subsequent ones. Clearly, this approach leads to spiraling impossibility to continue these trials, and seemingly, excludes the opportunity for commercial scale aquaculture. Instead, a broader approach is suggested. Specifically, while the risk model currently in use effectively estimates risk of reproduction it does not effective address risk of naturalization, which is ultimately where the problem lies. The approach now taken with the models developed by University of Maryland and Versar for diploid introduction, where larval transport estimates feed into demographic models to predict population growth is much more realistic and can be done for the prospective industry overall, or for specific sites proposed for large scale triploid aquaculture of *C. ariakensis*.

OYSTERS AND OYSTER FARMING IN CHINA: A REVIEW

Ximing Guo (Rutgers), Guofan Zhang (Institute of Oceanology, Chinese Academy of Sciences, PRC), Lumin Qian (Third Institute of Oceanology, Oceanic Administration, PRC), Haiyan Wang (Rutgers), Xiao Liu (Institute of Oceanology, Chinese Academy of Sciences, PRC), and Aimin Wang (Ocean College, Hainan University, PRC)

In an effort to survey *Crassostrea ariakensis* populations in China, we conducted literature reviews and visited over 37 sites along China's coast. Here we present our findings about oysters and oyster farming in China in light of recent taxonomic revisions. Seventeen species of oysters have been reported along China's coast. Many of the species occur in southern China and are relatively rare. There is considerable confusion about the classification of *C. ariakensis* and three other species. Numerous oyster reefs, both ancient and living, exist along the coast, and *C. ariakensis* was present in all four live reefs that we saw. In three of the reefs, *C. ariakensis* is the dominant or founding species, where large oysters on the bottom are *C. ariakensis* and small oysters on top are other species. Oyster farming is primarily for *C. hongkongensis* in the south (Guangxi and Guangdong), *C. gigas* in the north (Shandong and Liaoning), and *C. angulata* in the middle (Fujian and Zhejiang). *C. hongkongensis*, also known as the white oyster or *C. rivularis*, is one of the most important species cultured in China. *C. hongkongensis* culture is based on natural seeds with one estuary in Guangxi supplying about 2.5 billion. Most of the published literature on *C. rivularis* from southern China is for *C. hongkongensis*, not for *C. ariakensis*, which is not intentionally cultured. It is present at low frequencies in natural *C. hongkongensis* seeds and selected against by farmers. Pollution has devastated oyster populations in at least two estuaries.

ECONOMIC CONSIDERATIONS REGARDING THE RESTORATION OF CHESAPEAKE BAY OYSTER POPULATIONS USING NATIVE OR NON-NATIVE SPECIES

Douglas Lipton (UMD), Tom Murray (VA Sea Grant), and James Kirkley (VIMS)

The stated goal of the Environmental Impact Statement related to *Crassostrea ariakensis* introduction in the Chesapeake Bay is to restore the population to a level that could sustain a fishery equivalent to average landings from 1920-1970. Given the changing market for oysters on both the supply and demand side, it is unlikely that a fishery of this size, about 4.9 million bushels per year, is economically feasible. We suggest that a fishery is more likely sustainable at about 2.6 million bushels. In addition to the commercial oyster fishery there are a variety of other costs and benefits related to a restored oyster population. The benefits of a restored oyster population, given current knowledge, appear to be similar whether they are based on the native *C. virginica* or the introduced *C. ariakensis*. Thus, the major differences between the competing options of allowing or not allowing the introduction of a reproducing population of *C. ariakensis* appears to be a risk-time trade-off in terms of market and non-market benefits and costs.

THE FEASIBILITY OF USING TRIPLOID *CRASSOSTREA VIRGINICA* FOR ON BOTTON CULTURE IN THE CHESAPEAKE BAY

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The Federal Register, Vol. 69, No. 2, dated Monday, January 5, 2004 provided notice of "DEPARTMENT OF DEFENSE, Department of the Army; Corps of Engineers Intent [To Prepare a Programmatic Environmental Impact Statement] for the Proposed Introduction of the Oyster Species, *Crassostrea ariakensis*, Into the Tidal Waters of Maryland and Virginia To Establish a Naturalized, Reproducing, and Self-Sustaining Population of This Oyster Species" and described seven alternatives to be evaluated in the EIS process. Alternative 4 addressed the issue of using the native oyster (*Crassostrea virginica)* species in the development and expansion of aquaculture in the Chesapeake Bay. In the past, the majority of Virginia's commercial oyster production came from leased oyster ground. Historically, lease holders would either purchase or harvest oyster seed from rivers such as the Great Wicomico, Piankatank or James, and plant on their leases throughout the Virginia portion of the Bay. We describe a project to examine the feasibility of using triploid native spat on shell in the same manner as oyster planters have used wild oyster seed from seed Rivers. Approximately forty million triploid larvae were set on 400 bushels of shell and planted on an oyster bed owned by Bevan's Oyster Company in the Yeocomico River, Virginia in summer 2005. We present here early results of setting success, growth, mortality and condition index.

Ecological Interactions

OYSTER SEX WARS: EVIDENCE FOR A 'GAMETE SINK' IF *CRASSOSTREA VIRGINICA* AND *CRASSOSTREA ARIAKENSIS* SPAWN SYNCHRONOUSLY

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Available data indicate spawning seasons for the Asian oyster *Crassostrea ariakensis* and the eastern oyster *C. virginica* overlap. Hybrids can form, but the larvae are not viable. If *C. ariakensis* are introduced into Chesapeake Bay and synchronous spawning occurs with *C. virginica*, hybridization could reduce the production of viable larvae (= gamete sink). We examined the effects of gamete age, sperm concentration, and ratios of heterospecific gametes on fertilization rate and hybridization of the two species. Hybrid fertilization rates were consistently lower than pure crosses. Fertilization rate decayed with gamete age, but occurred in gametes up to 8 hrs old. Fertilization rate also decayed with decreasing sperm density in both pure and hybrid crosses. Finally, fertilization rate declined by as much as 60% when sperm were (1) given a choice of eggs from each species to fertilize or (2) required to compete to fertilize eggs from a single species. Hence, a gamete sink will likely occur if these two species spawn synchronously. The magnitude of the gamete sink will depend in part on proximity of the two species, on gamete concentrations in the water column, and on the proportion of hybrids that form. Current efforts are enumerating the proportion of hybridization that occurred during these experiments. Molecular genetic methods to amplify ITS regions of the rRNA gene have been validated and yield two bands in pure crosses and four bands in hybrid crosses. Individual larvae are being typed to determine the proportion of hybrids formed under the various gamete mixtures.

COMPARISONS OF POST-SETTLEMENT SURVIVAL AND GROWTH IN *CRASSOSTREA VIRGINICA* AND *C. ARIAKENSIS* IN RELATION TO TIDAL EMERSION

Peter Kingsley-Smith and Mark W. Luckenbach (VIMS)

In many high salinity regions of the mid-Atlantic *C. virginica* is limited to the intertidal zone where it achieves a partial refuge from predation. Persistence in such intertidal habitats necessitates tolerance of both desiccation and extremes of temperature during periods of aerial exposure. Such capabilities in *C. ariakensis* have yet to be determined.

The effects of aerial exposure by tidal emersion on comparative survival and growth rates of diploid *C. ariakensis* and diploid *C. virginica* were investigated using oysters set on plastic tiles and grown in a flow-through quarantine system. Four tidal regimes were simulated; 1) *high intertidal* (3.5 hrs emersion), 2) *mid tide* (2 hrs emersion), 3) *low intertidal* (1 hr emersion) and 4) *subtidal* (constant immersion). Vertical ("North-facing" and "South-facing") and horizontal ("Up" and "Down") tile orientation treatments were also incorporated in the experimental design. Tiles were individually photographed on a weekly basis between June and August 2005 and image analysis software was used to gather survival and growth data.

The high intertidal treatment exceeded the physiological tolerances of both species; complete mortality occurred within the first 2 weeks. In the mid tide and the low intertidal treatments *C. ariakensis* and *C. virginica* exhibited intermediate growth and survival. Growth rates were highest and levels of mortality were lowest in the subtidal treatment. Determination of the tolerance of *C. ariakensis* to aerial expsoure will improve our understanding of its potential to colonize intertidal habitats, to compete with native species for resources, and to become a fouling nuisance if introduced.

CHANGES IN SHELL STRENGTH OF *CRASSOSTREA VIRGINICA* AND *CRASSOSTREA ARIAKENSIS* IN RESPONSE TO CRAB PREDATORS FROM CHESAPEAKE BAY

Roger I. E. Newell, Victor S. Kennedy, and Kristi S. Shaw (UMCES)

If *C. ariakensis* were to be introduced to Chesapeake Bay it is unknown if its abundance would be controlled by the same predators that affect *C. virginica* populations. In replicated laboratory studies, we compared the relative susceptibility of juvenile diploids (shell height < 25 mm) of both oyster species to five species of potential crab predators. We tested two species of mesohaline mud crab, *Rhithropanopeus harrisii* [carapace width 6-16 mm] and *Eurypanopeus demissus* [6-18 mm]), two species of polyhaline mud crab *Panopeus herbstii* [9-29 mm] and *Dyspanopeus sayi* [8-20 mm], and the euryhaline blue crab, *Callinectes sapidus* [35-65 mm]. We found that all four species of mud crab and the blue crab significantly ($P<0.05$) preferentially consumed *C. ariakensis* compared to *C. virginica*. We hypothesized that this differential predation may stem from differences in shell strength between the two species of oysters. Using an Instron load compression instrument we found that the strength of the upper valve of C. ariakensis was ~50% lower than for comparably sized *C. virginica*. Oysters of both species grown for 54 d in the presence of (but protected from) blue crabs exhibited different responses. *C. ariakensis* grew denser shells (total weight/shell area) that approximately doubled their compression strength compared to same species control oysters. The shell strength of *C. virginica* also approximately doubled, but these stronger shells had a significantly lower density than their controls. We conclude that both species of oysters respond morphologically to the presence of predators, although the response differs markedly between species.

POSTER: SETTLEMENT OF *CROSSOSTREA ARIAKENSIS* LARVAE UNDER CONDITIONS FOUND IN THE CHESAPEAKE BAY

Mario N. Tamburri (UMCES), Mark W. Luckenbach (VIMS), Denise L. Breitburg (SERC), and Stephanie M. Bonniwell (VIMS)

The Asian oyster (*Crassostrea ariakensis*) is being considered for introduction into the Chesapeake Bay. However, our current understanding of the biology and ecology of *C. ariakensis* is insufficient to predict whether an introduction will be successful, provide desired benefits, or have adverse impacts. Behavior of native oyster (*C. virginica*) pediveligers has been studied for many years and it is well established that they use a variety of habitat characteristics when selecting a site for colonization. Perhaps the most important of these are chemical cues emitted by adult conspecifics, which can lead to gregarious larval settlement and dense, persistent reef communities. Conversely, almost nothing is known about how larvae of *C. ariakensis* respond to conditions found in Chesapeake Bay or about the critical life history processes of settlement and metamorphosis.

We have examined how the behavior and substrate preference of two *C. ariakensis* strains ("south China" and "west coast" at the time of settlement compares with that of *C. virginica*. Results demonstrate many similarities but also a few important differences. For example, both species and strains of larvae greatly prefer natural substrates (e.g. shell) covered with biofilms for colonization but the west coast strain of *C. ariakensis* exhibited greater attachment onto manmade substrates (e.g. fiberglass) than *C. virginica*. Waterborne chemical cues emitted by adult oysters were also found to enhance substrate attachment for all larval forms, whereas initial data suggests hypoxia inhibits larval attachment with the south China strain of *C. ariakensis* perhaps most sensitive to low oxygen conditions.

<u>Growth, Metabolism, and Reproduction</u>

AGE AND GROWTH OF WILD *CRASSOSTREA ARIAKENSIS* AND *C. GIGAS* FROM LAIZHOU BAY, CHINA

Juliana M. Harding and Roger Mann (VIMS)

Shell height at age estimates from Suminoe (*Crassostrea ariakensis*) and Pacific (*Crassostrea gigas*) oysters from a natural oyster reef in Laizhou Bay, China were compared with estimates from triploid *C. ariakensis* of known age from the Rappahannock River, Virginia. Both *C. ariakensis* and *C. gigas* reach shell heights in excess of 76 mm (3 inches) within two years after settlement regardless of the source location. This fast growth appears to continue through at least Age 4 or Age 5 in wild individuals as the growth trajectory for both species had not begun to flatten in the oldest individuals collected. Fitted growth curves were not significantly different between species within the same habitat, within species in different habitats or between species in different habitats.

METABOLIC RATES OF *CRASSOSTREA ARIAKENSIS* AND *CRASSOSTREA VIRGINICA* AT TWO TEMPERATURES AND THREE SALINITIES

Nicole Harlan and Kennedy Paynter (UMD), and Donald Meritt (UMCES)

Maryland and Virginia have proposed to replace the native oyster, *Crassostrea virginica*, with the suminoe oyster, *Crassostrea ariakensis*, in Chesapeake Bay. *C. virginica*, is highly tolerant of hypoxic conditions and can survive emersion or nearly anoxic seawater for days to weeks depending on the temperature. In order replace *C. virginica*'s ecological niche of establishing vast benthic reefs in Chesapeake Bay, *C. ariakensis* may require similar tolerances. However, when the oysters were placed in sealed jars of anoxic water, *C. ariakensis* lived for an average of four days, while *C. virginica* persisted for more than fourteen days. Studies at 22oC have shown that the metabolic rate of *C. ariakensis* (1.96 +/- 0.102 O2/hr/gdw) is significantly higher than that of *C. virginica* (1.15 +/- 0.079 mg O2/hr/gdw; p=0.0244). In order to better understand the aerobic requirements of these two species under different conditions, standard metabolic rates of each species were determined at two temperatures, 10 and 20oC, and three salinities, 5, 15, and 25 psu. At both temperatures and all three salinities, the metabolic rate of *C. ariakensis* was higher than that of *C. virginica*. Upon immersion in the test chambers, *C. ariakensis* gaped and began using oxygen within minutes, while *C. virginica* kept their valves shut for much longer. These data may influence the decision to use *C. ariakensis* as an ecological substitute for *C. virginica* in the Chesapeake Bay.

POSTER: COMPARATIVE GROWTH AND SURVIVAL OF DIPLOID AND TRIPLOID SUMINOE OYSTERS, *CRASSOSTREA ARIAKENSIS*, IN MULTIPLE QUARANTINE SYSTEMS

Heather D. Harwell and Standish K. Allen Jr. (VIMS)

Much of the research on *Crassostrea ariakensis* has revealed superior growth rates and resistance to disease compared to the native oyster, *C. virginica*. All field studies of growth and survival have utilized sterile triploid oysters for reasons of biosecurity. Thus, triploids are serving as a surrogate for diploid performance in these field trials. A direct, simultaneous comparison of the growth and survival of diploid and triploid *C. ariakensis* is needed to refine population growth models based on triploid field data. Three replicate lines of diploid and triploid *C. ariakensis* were placed at four quarantine systems in Virginia and Maryland in December 2004. Individual repeated measures of subsets of oysters were gathered from monthly data on percent survival, wet weight, and shell length. In addition, quarterly estimates of condition index were obtained. Data gathered in this fashion will be used to determine a correction factor(s) that can then be applied to results from past studies of triploid *C. ariakensis* in order to refine models of potential population growth.

TRIPLOID *CRASSOSTREA ARIAKENSIS* AND *CRASSOSTREA VIRGINICA* GROWN AT FOUR SITES IN CHESAPEAKE BAY

Kennedy Paynter (UMD), Donald Meritt (UMCES), Stan Allen (VIMS), Jake Goodwin and Marcy Chen (UMD)

Triploid suminoe oysters, *Crassostrea ariakensis*, and triploid eastern oysters, *Crassostrea virginica*, were produced nearly simultaneously in the hatchery and deployed in cages at four sites in Chesapeake Bay in April 2004. The four sites represented the broad range of salinities present in the Bay. The 'high' salinity site was in the York River near the Virginia Institute of Marine Science (VIMS; mean salinity 19 psu). The Patuxent River site had a mean salinity of 11 psu and the Choptank River site of 9 psu. The 'low' salinity site in the Severn River had a mean salinity of 6 psu. Growth of both species was positively correlated with salinity. As of September 2004, no significant differences in size or disease prevalence existed between species at any site. Dermo, the disease caused by *Perkinsus marinus*, was detected in both species in July but only at the York River site. However, *C. ariakensis* grew more later in the year than did *C. virginica* and appeared to begin growing earlier in the following Spring. Thus, *C. ariakensis* became significantly larger by June 2005. During the summer of 2005, *C. virginica* at the Choptank and York river sites grew more slowly than *C. ariakensis*, presumably due to *P. marinus* infections. Mean shell height of *C. ariakensis* at the York River site in October 2005 (deployed 19 months) was 140 mm compared to 105 mm in *C. virginica*. Physiological differences between the species apparently allow *C. ariakensis* to grow more actively at lower temperatures.

Species Identification and Genetics

POSTER: DEVELOPMENT OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN *CRASSOSTREA ARIAKENSIS* AND RELATED *CRASSOSTREA* SPECIES

Hyungtaek Jung (UDE), Woo-jin Kim (National Fisheries Research & Development Institute), and Patrick Gaffney (UDE)

As a candidate for deliberate introduction into Atlantic waters, the Suminoe or jinjiang oyster *Crassostrea ariakensis* has recently been the subject of intense interest, with particular focus on its ecology, taxonomy and population genetics. Because the taxonomy of Asian *Crassostrea* is still incomplete, and identification on morphological grounds difficult, a suite of genetic markers suitable for identifying species and subspecies is needed. Such markers will allow screening of candidate source material, allow development of genetic signatures of hatchery strains for evaluating introductions, and ultimately provide tools for selective breeding and pedigree monitoring. We have tested primers originally designed to amplify fragments of known genes (Type I markers) in *C. gigas* for their ability to amplify putative homologs in the related Asian species *C. angulata*, *C. ariakensis* and *C. hongkongensis*. Of 16 loci developed for *C. gigas*, all amplified successfully in *C. angulata*, 15 amplified in *C. ariakensis* and 14 amplified in *C. hongkongensis.* In contrast, only five loci could be amplified in the Atlantic species *C. virginica*. Direct sequencing of amplicons revealed multiple intraspecific and interspecific candidate polymorphisms, providing tools for genetic identification, linkage mapping, pedigree monitoring, and phylogenetic analysis.

DISTRIBUTION OF *CRASSOSTREA ARIAKENSIS* IN CHINA

Haiyan Wang (Rutgers), Lumin Qian, Guofan Zhang, and Xiao Liu (Institute of Oceanology, Chinese Academy of Sciences, PRC), Aimin Wang and Yaohua Sh i (Ocean College, Hainan University), Nianzhi Jiao (Center for Marine Environmental Sciences, Xiamen University), and Ximing Guo (Rutgers)

It is commonly assumed that *Crassostrea ariakensis* is synonymous with *C. rivularis* that, according to the literature, is abundant and widely distributed in China. However, at least three species, *C. gigas, C. hongkongensis* and *C. ariakensis*, have been reported as *C. rivularis* in China, creating uncertainties about the distribution of *C. ariakensis*. To determine the distribution of true *C. ariakensis* in China, we collected and classified 2,624 oysters from 50 locations along China's coast using species-specific DNA markers. *C. ariakensis* was found at 10 sites ranging from northern Shandong to Guangxi. While *C. ariakensis* had a wide geographical distribution, its occurrence within its range is patchy or scarce. Overall, *C. ariakensis* accounted for only 9.5%

of all oysters collected. Large *C. ariakensis* populations were found in only three areas: Jiulong River in Fujian, Dongzao Harbor near Yangtze River and Yellow River basin in Bohai Sea, with none observed in-between. In Guangxi and Guangdong, *C. ariakensis* was present in all samples collected at low frequencies (0.5 – 17.5%). All three major populations are found in or near large rivers, and the absence of rivers may be a factor contributing to the fragmented distribution. At all sites, *C. ariakensis* co-existed with other species: *C. gigas* in Bohai Sea; *C. sikamea* at Dongzao Harbor; and *C. hongkongensis, C. angulata, C. sikamea* and *Saccostrea* species in southern China. *C. ariakensis* tended to occur subtidally, while other species were often found intertidally.

POSTER: IDENTIFICATION OF *CRASSOSTREA* SPECIES FROM CHINA USING SNP-BASED MARKERS

Haiyan Wang and Ximing Guo (Rutgers)

China is home to 17 species of oysters and among them, five *Crassostrea* species, *C. hongkongensis, C. angulata, C. gigas, C. sikamea* and *C. ariakensis* are most common and commercially important. These five species often co-exist in the same estuary, and their identification using morphological characteristics is problematic. Genetic markers are needed for rapid and reliable identification of these oysters. Single nucleotide polymorphisms (SNPs) are simple and powerful markers for various genetic analyses. In this study, we developed species-specific SNP markers for the identification of common oysters from China. The mitochondrial cytochrome oxidase I (COI) gene and the nuclear 28S ribosomal RNA gene were used for marker development. DNA sequences from different species were either obtained by direct sequencing or downloaded from GenBank. Sequences were aligned, and species- and genus-specific SNPs were identified. Primers were designed for species/allele-specific amplification to generate fragments of different sizes in each species. A multiplex set of species-specific markers from COI was able to distinguish all five *Crassostrea* species in a single-tube PCR. It also separated *Ostrea* and *Saccostrea* species from *Crassostrea* species with the exception of *C. virginica* and *C. rhizophorae*. The 28S primer set was able to separate *C. hongkongensis, C. ariankensis* from other species, as well as *Saccostrea* and *Ostrea* species from *Crassostrea* species (except *C. virginica* and *C. rhizophorae*). The SNP-based markers do not require fluorescence-labeling or post-PCR digestion, providing a simple, fast and reliable method for oyster identification.

POSTER: IDENTIFICATION OF *CRASSOSTREA ARIAKENSIS* USING ITS LENGTH POLYMORPHISM

Yongping Wang and Ximing Guo (Rutgers)

Oysters cannot be reliably identified using morphological characteristics alone. In an effort to develop genetic markers for oyster identification, we studied length polymorphism in internal transcribed spacers (ITS) between ribosomal RNA genes in 12 common species of Ostreidae: *Crassostrea virginica, C. rhizophorae, C. gigas, C. angulata, C. sikamea, C. ariakensis, C. hongkongensis, Saccostrea echinata, S. glomerata, Ostrea angasi, O. edulis,* and *O. conchaphila.* We downloaded and aligned ribosomal RNA sequences from all oyster and some other bivalve species to identify conserved sequences flanking ITS1 and ITS2. We designed two pairs of primers and optimized PCR conditions for simultaneously amplification of ITS1 and ITS2 in a single tube. Amplification was successful in all 12 species, and PCR products were visualized on high-resolution agarose gels. ITS2 was longer than ITS1 in all *Crassostrea* and *Saccostrea* species, while they were about the same size in three *Ostrea* species. No intraspecific variation in ITS length was detected. Among species, the length of ITS1 and ITS2 was polymorphic and provided unique identification of eight species or species pairs: *C. ariakensis*, *C. hongkongensis*, *C. sikamea*, *O. conchaphila*, *C. virginica/C. rhizophorae*, *C. gigas*/*C. angulata*, *S. echinata*/*S. glomerata*, and *O. angasi*/*O. edulis*. Two species within a pair were not distinguishable by ITS length. The ITS assay provides simple, rapid and effective identification of *C. ariakensis* and several other oyster species. Because the primer sequences are conserved, the ITS assay may be useful in the identification of other bivalve species.

POSTER: GENETIC DIFFERENTIATION AMONG FOUR *CRASSOSTREA ARIAKENSIS* POPULATIONS IN ASIA BY MICROSATELLITE POLYMORPHISM

Jie Xiao, Jan F. Cordes, Kimberly S. Reece (VIMS)

Crassostrea ariakensis is being considered for introduction into Chesapeake Bay to help revive the declining native oyster industry and bolster the local ecosystem. Little is known, however, about wild populations of *C. ariakensis* in its native region, including native distributions, overall genetic diversity, genetic structure, and levels of gene flow. Several discontinuous natural populations have been identified along a wide geographic range in the western Pacific. It's still unclear whether these all are natural populations or whether some are a result of anthropogenic transportations. We studied the genetic variance among 4 wild populations of *C. ariakensis* from Ariake Bay, Japan, Kahwa River, South Korea, the Yellow River basin in northern China, and Beihai in southern China, using novel microsatellite makers. Initial results are reported for 3 loci (CarG110, Car119-6a, Car11-70) developed from *C. ariakensis* partial genomic libraries and screened for the presence of null alleles in 8 family crosses. Estimated multilocus F*st* (0.0168) values were highly significant for all samples, indicating heterogeneous populations exist in these regions. Single locus F*st* and *P* values are quite variable among these loci, and additional markers are being developed to further test the null hypothesis of population homogeneity. These preliminary results indicate genetic structure exists among populations of *C. ariakensis* in its native region and suggest that microsatellite markers could serve as efficient genetic tags for monitoring *C. ariakensis* introduced into the Chesapeake Bay from different native gene pools.

Modeling

STOCK-RECRUIT RELATIONSHIPS FOR *CRASSOSTREA VIRGINICA* AND *C. ARIAKENSIS* IN CHESAPEAKE BAY DEVELOPED FOR A DEMOGRAPHIC OYSTER POPULATION MODEL

Jon H. Vølstad and Jodi R. Dew (Versar)

A demographic population model is being developed to support an ecological risk assessment (ERA) of the proposed introduction of *Crassostrea ariakensis* and restoration alternatives of *C. virginica* in Chesapeake Bay by the states of Maryland and Virginia. As part of the model, a stock-recruit relationship was developed and is defined as the ratio of number of spat recruited to October (when the annual Fall survey is preformed by the Maryland Department of Natural Resources (DNR)) to number of standardized (to 77mm) female oysters. Stock-recruit relationships were estimated using empirical data collected from 1980 to 2004 by the DNR in annual surveys of oyster beds in Maryland, shell length data, sex ratio by size estimates, and fecundity relationships between shell length and number of eggs produced.

R-square value for *C. virginica* stock-recruit relationship was 0.45 with 2.6 spat produced per standardized female oyster. The number of spat produced was 2.6, 2.8, and 2.1 per standardized female oyster when the stock-recruit relationship was examined per average, dry, and wet rainfall year, respectively. This stock-recruit relationship for *C. virginica* is used for *C. ariakensis* to predict the number of spat produced per standardized *C. ariakensis* stock to a 77mm female *C. virginica* oysters, under the assumption of similar mortality rates for spat of both species to October. The stock-recruit relationship for both species can also be altered to include gamete loss from inviable hybrids in cross-fertilization when species are reproducing in close proximity to each other.

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