



# NOAA CHESAPEAKE BAY OFFICE Non-native Oyster Research



## Research Topics:

*Oyster pathogens:  
Susceptibility and  
transmission;  
Effects of  
harmful algal  
blooms*



Quarterly Review  
Winter 2007



## 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/>.

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|--------------------|---|
| <b>Spring 2005</b> | Overview of research topics: Taxonomy, genetics, disease, human health, ecology, interspecific interactions, ecosystem services and functions                                 |
| <b>Summer 2005</b> | Aquaculture options: Biological and economic factors affecting aquaculture production of native and non-native oysters in the mid-Atlantic                                    |
| <b>Fall 2005</b>   | Potential for <i>Crassostrea ariakensis</i> - <i>C. virginica</i> interactions: Larval substrate selection, post-settlement competition, and fertilization interference       |
| <b>Winter 2006</b> | Evaluating human health risks: Uptake, depuration, and post-harvest levels of waterborne human pathogens in <i>Crassostrea ariakensis</i> compared with <i>C. virginica</i> . |
| <b>Spring 2006</b> | Special session on <i>Crassostrea ariakensis</i> at the 98th Annual National Shellfisheries Association meeting   |
| <b>Summer 2006</b> | Comparative growth and mortality of <i>Crassostrea ariakensis</i> and <i>C. virginica</i> : Providing additional data for the oyster demographic model                        |
| <b>Fall 2006</b>   | Understanding <i>Crassostrea ariakensis</i> within its native range in Asia   |
| <b>Winter 2007</b> | Oyster Pathogens: Susceptibility and transmission; Effects of harmful algal blooms  |

## Oyster Pathogens: Susceptibility and Transmission; Effects of Harmful Algal Blooms

The 2004 STAC report, *Identifying and Prioritizing Research Required to Evaluate Ecological Risks and Benefits of Introducing Diploid C. ariakensis to Restore Oysters to Chesapeake Bay*, identified research addressing *Bonamia* susceptibility, Herpes virus vertical transmission, and the potential for disease transmission between oyster species as high and essential priorities for the EIS. Susceptibility of *C. ariakensis* to harmful algal blooms (HABs) and *Perkinsus* species other than *P. marinus* were also noted as topics for further study.

### I. Oyster Pathogens: Susceptibility and Transmission

#### Why is this research important for the EIS?

*C. ariakensis* is being investigated for its susceptibility to, and potential to serve as a transmission vector for, a range of oyster pathogens. *Perkinsus marinus* is the parasite responsible for Dermo, one of the diseases that have devastated native oyster populations in the mid-Atlantic. Questions relevant to the EIS include the degree to which *C. ariakensis* is susceptible to *P. marinus* and other *Perkinsus* species, and whether *C. ariakensis* could serve as a disease reservoir or transmission vector to further exacerbate the impact of *Perkinsus* on *C. virginica* or other native shellfish in Chesapeake Bay.

*Bonamia*, an oyster pathogen in other parts of the world, was unknown from the mid-Atlantic region until 2003 when mass mortalities of triploid *C. ariakensis* were observed in experimental deployments in North Carolina. Although *Bonamia* infections have not been confirmed in triploid *C. ariakensis* deployed in Chesapeake Bay to date, it is possible that the pathogen would eventually find its way to the Bay and become established if a suitable host were present. Research is underway to investigate the geographic distribution and environmental tolerances of the *Bonamia* species discovered in North Carolina, in order to understand how *C. ariakensis* may be limited by *Bonamia* at salinities and temperatures characteristic of Chesapeake Bay and other mid-Atlantic coastal waters.

#### Presentations:

Kim Reece (VIMS) – Characterization, transmission and pathogenicity studies of *Perkinsus* spp. and oyster herpes-like virus in *Crassostrea ariakensis* and Chesapeake Bay native bivalves (PIs: Moss, Brown, Dungan, Carnegie, Burreson, Reece)

Chris Dungan (MD DNR) – Diseases among diploid native and Suminoe oysters reared in mesohaline Choptank River waters: 2004-2006 (PIs: Dungan, Newell, Luckenbach, Breitburg)

Gerardo Vasta (UMBI) – *Perkinsus* spp. and *Bonamia* spp. infections in *Crassostrea ariakensis* (PIs: Alavi, Robledo, Schott, Saito, Tasumi, Vasta)

Ryan Carnegie (VIMS) – *Bonamia* sp. parasitism of *Crassostrea ariakensis* (PIs: Carnegie, Audemard, Burreson, Peterson, Bishop)

Some preliminary findings:

***Perkinsus* species in Asia**

- Throughout the confirmed range of *C. ariakensis* in Asia (Japan, South Korea, China), *C. ariakensis* and other oyster species have now been screened for the occurrence of *Perkinsus* species using PCR-based molecular diagnostics and histology. Results indicate that *Perkinsus* is widespread in the region. *Perkinsus olseni* was found in oysters (*C. ariakensis* and *C. hongkongensis*) from Japan to lower China.
- In southern China there appears to be a newly discovered *Perkinsus* species that infects both *C. ariakensis* and *C. hongkongensis*. Efforts to characterize this undescribed species are currently underway.
- Laboratory experiments are also underway to test whether this new *Perkinsus* sp. can be transmitted to *C. ariakensis*, *C. virginica*, and *Mercenaria mercenaria* (native Chesapeake Bay clam) via cohabitation with infected *C. ariakensis* from Asia.

***Perkinsus marinus* (Dermo disease)**

- Although *C. ariakensis* is known to contract light to moderate infections of *P. marinus*, no heavy infections have ever been observed in field trials with this species in the Chesapeake Bay. In laboratory exposures however, *C. ariakensis* did develop moderate to heavy *P. marinus* infections with resulting mortality. Additional laboratory experiments found higher *P. marinus* infection levels in stressed *C. ariakensis* relative to unstressed individuals. Although cumulative mortality did not exceed 25%, all of the dead *C. ariakensis* in this experiment were infected with *P. marinus*. This work has recently been published (Moss et al. 2006).
- Seasonal *P. marinus* infections were observed in both *C. virginica* and *C. ariakensis* exposed to Choptank River waters during 2004 to 2006, despite low salinity conditions that kept ambient *P. marinus* levels down during this period. Infection prevalence and intensity declined with age among *C. ariakensis* oysters, but increased with age among *C. virginica* in this study.

***Perkinsus olseni***

- *Perkinsus olseni* is endemic to Asia, but is not currently known to occur in North America. As mentioned above, *P. olseni* frequently infects *C. ariakensis* in Asia. Challenge experiments in which *C. ariakensis*, *C. virginica*, and *Mercenaria mercenaria* were inoculated with *P. olseni* resulted in the presence of *P. olseni* DNA in all three shellfish species over a 10 week post-inoculation sampling period. Tissue

staining assays yielded infection classifications ranging from rare to very light. Histological analyses to confirm active infections are underway.

### ***Haplosporidium nelsoni* (MSX disease)**

- No *Haplosporidium nelsoni* infections were observed in diploid *C. ariakensis* or *C. virginica* exposed to Choptank River water between 2004 -2006. Due to low salinity, *H. nelsoni* infections were also extremely rare among wild native oysters in the Choptank River during this same period.

### ***Bonamia***

- In 2003, an undescribed species of *Bonamia* caused up to 100% mortality in triploid *C. ariakensis* deployed in Bogue Sound, North Carolina. The disease was most prevalent in smaller oysters (< 50 mm), and at peak summer temperatures (> 25°C). Results from this work have been published (Burreson et al. 2004; Bishop et al. 2006).
- The distribution of this newly discovered *Bonamia* species was initially thought to be limited to Bogue Sound and Newport River in North Carolina; however, recent re-analysis with a more sensitive PCR assay has found this parasite from Cape Hatteras, North Carolina to Hilton Head, South Carolina.
- Molecular phylogenetic analysis indicates the undescribed *Bonamia* sp. in Bogue Sound is most closely related to species found in the southern hemisphere, suggesting that it may be an introduced species (Burreson et al. 2004).
- Laboratory experiments with *C. ariakensis* found that both pathogenicity and infection intensity (i.e., leading to mortality) of this *Bonamia* sp increased with salinity, reaching maximum levels at or above 25 psu. While infections subside at lower salinities, the depressive effect of lower salinities is expected to have little effect in warmer months due to high parasite activity at higher temperatures.
- Work is ongoing to identify the endemic host for the undescribed *Bonamia* sp. in North Carolina waters, and to further test *C. virginica* susceptibility to this parasite.
- A second *Bonamia* species, *Bonamia perspora*, has also been discovered in North Carolina. *Bonamia perspora* occurs at prevalences as high as 62% in *Ostreola equestris* – a small, non-commercial oyster species known as the crested oyster, which occupies higher salinity waters in North Carolina. *Bonamia perspora* was found in *O. equestris* at high levels of prevalence up to 62%, but heavy infections were rare. Observation of heavy infection without disease suggests that *O. equestris* may be an effective reservoir and possible vector for *B. perspora*. These findings have been published recently (Carnegie et al. 2006).
- Despite annual histological disease screening (1,700 oysters/year) by the Maryland Department of Natural Resources since 1989, no *Bonamia* infections have ever been detected in wild oyster populations within Maryland. Similarly, no *Bonamia* DNA was found in PCR-based assays of either Choptank River native oysters or *C.*

*ariakensis* and *C. virginica* held in the laboratory with ambient water from the Choptank River between 2004-2006.

## II. Effects of Harmful Algal Blooms

### Why is this research important for the EIS?

The occurrence of harmful algal blooms (HABs) is increasing in Chesapeake Bay, and intense blooms – reaching densities of  $10^5$  to  $10^6$  cells/ml – can have profound impacts on shellfish adults and larvae. Two algal species that commonly bloom during oyster spawning season in the Chesapeake Bay are *Prorocentrum minimum* and *Karlodinium veneficum*. Although *P. minimum* is not toxic, it may impact shellfish feeding. Blooms of this species are most prevalent in lower salinity areas during spring. *Karlodinium veneficum*, which is toxic and can cause fish kills, blooms in the summer and fall.

### Presentation:

Pat Glibert (UMCES) – The impacts of harmful algal blooms on *Crassostrea virginica* and *Crassostrea ariakensis* (PIs: Glibert, Stoecker, Alexander, Meritt)

### Some preliminary findings:

- The impacts of *K. veneficum* on oyster larvae do not appear to differ significantly between *C. virginica* and *C. ariakensis*. Embryos and larvae of both oyster species were deformed within 24 hours of being exposed to this alga, although fewer deformities were observed if the oysters were more than 24 hours old at their first exposure. No deformities were observed from exposure to other algae species.
- *K. veneficum* appears to have significant impacts on larval survival and may thus impact oyster recruitment. Larval survival was significantly impaired at less than bloom-level cell densities, and there was complete mortality after three days at bloom-level densities. The high larval mortality caused by *K. veneficum* blooms has implications for both native oyster restoration, as well as any potential *C. ariakensis* introduction.
- *K. veneficum* also caused mortality in adult *C. ariakensis*. Results of oyster feeding experiments with various HAB species suggest that *K. veneficum* is digested and assimilated differently than *P. minimum* or control food.
- Impacts of the harmful algae *P. minimum* appear to be less significant than *K. veneficum* on oyster larvae, although effects of exposure to *P. minimum* over longer durations are unknown.

### **Recent Publications on the Work Reported in this Quarterly Review:**

Bishop, M.J., R.B. Carnegie, N.A. Stokes, C.H. Peterson, and E.M. Burreson. 2006. Predicting the outcome of a non-native oyster introduction: facilitation of an enzootic parasite. *Marine Ecology Progress Series* 325: 145-152.

Burreson, E.M., N.A. Stokes and R.B. Carnegie. 2004. *Bonamia* sp. (Haplosporidia) found in nonnative oysters *Crassostrea ariakensis* in Bogue Sound, North Carolina. *Journal of Aquatic Animal Health* 16: 1-9.

Carnegie, R.B., E.M. Burreson, P.M. Hine, N.A. Stokes, C. Audemard, M.J. Bishop, and C.H. Peterson. 2006. *Bonamia perspora* n. sp. (Haplosporidia), a parasite of the oyster *Ostreola equestris*, is the first *Bonamia* species known to produce spores. *Journal of Eukaryotic Microbiology* 53(4): 232-245.

Moss, J.A., E.M. Burreson, E.M., and K.S. Reece. 2006. Advanced *Perkinsus marinus* infections in *Crassostrea ariakensis* held under laboratory conditions. *Journal of Shellfish Research* 25(1):65-72.

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