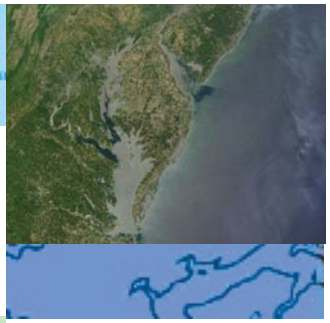

NOAA CHESAPEAKE BAY OFFICE

Non-native Oyster Research

Research Topic:

Evaluating Human Health Risks:

Uptake, depuration, and post-harvest levels of water-borne human pathogens in Crassostrea ariakensis *and* C. virginica

Quarterly Review
Winter 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*.

Evaluating human health risks: Uptake, depuration, and post-harvest levels of waterborne human pathogens in *Crassostrea ariakensis* compared with *C. virginica*

Why is this research important for the EIS?

As filter feeders, oysters can concentrate pathogens within their tissues at levels much higher than those of the surrounding water. These pathogens can be transmitted to humans when oysters are eaten. Waterborne pathogens that may occur in oysters and can cause human illness include:

- Bacteria (Examples: *E. coli*, *Vibrio*, *Mycobacterium*, *Salmonella*, *Campylobacter*)
- Protozoans (Examples: *Cryptosporidium*, *Giardia*)
- Viruses (Examples: Norovirus, enterovirus, adenovirus, hepatitis A virus)

Risk of illness is greatest when oysters are eaten raw, but some viruses and protozoans can even survive certain cooking methods if cooking is incomplete.

Some human pathogens, notably *Vibrio vulnificus* and *V. parahaemolyticus*, are naturally present in coastal waters and typically occur in highest concentrations during the summer when water temperatures are warm. *Vibrio vulnificus* causes 95% of all seafood related illnesses in the United States, and about 50% of human infections result in death. Another environmental bacterium, *Mycobacterium marinum*, is a pathogen of both humans and fish. Other human pathogens occur as a result of fecal pollution from wastewater treatment plants or other land-based sources of animal or human waste. Levels of pathogens released from these sources are often related to how thoroughly wastewater is treated before discharge or weather patterns such as high rainfall events.

In the United States, human health risks associated with the consumption of bivalve shellfish are managed at the state level under guidance from the National Shellfish Sanitation Program (vm.cfsan.fda.gov/~ear/nss2-toc.html) and the Interstate Shellfish Sanitation Conference (www.issc.org). States typically perform periodic water quality testing and apply threshold levels of indicator pathogens (e.g., *E. coli*) to issue consumption advisories, require shellfish bed closures, or certify areas as safe for shellfish harvest. Also, shellfish retailers are required to obtain training and implement Hazard Analysis Critical Control Point (HACCP www.cfsan.fda.gov/~lrd/haccp.html) plans for post-harvest handling procedures to ensure product safety.

A key question for the risk analysis of the EIS is whether current water quality and product handling standards based on experience with native shellfish will be sufficiently protective of human health in the case of non-native species. Specifically, will the Asian oyster, *C. ariakensis*, pose a greater, lesser, or similar risk for human health compared with the native oyster, *C. virginica*? Answering this question depends upon knowing whether there are differences in the accumulation, depuration (release of pathogens), or post-harvest decay of human pathogens between *C. ariakensis* and *C. virginica*. This topic was identified as one of the "essential" research priorities described in 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*.

Presentations:

James Morris (NOAA/NCCOS) - Comparative microbial dynamics in *Crassostrea virginica* and *Crassostrea ariakensis* (PIs: Morris, Bean, Noble, Fowler)

Thaddeus Graczyk (Johns Hopkins) - Recovery, bioaccumulation, and inactivation of human waterborne pathogens by the non-native oyster *Crassostrea ariakensis* driven by water salinity (PIs: Graczyk, Schwab)

Kim Reece (VIMS) - Comparing microbiological characteristics of *C. ariakensis* and *C. virginica* with respect to uptake and elimination of fecal contamination indicators and *Vibrio* species (PIs: Reece, Kator)

Gerardo Vasta (COMB) - Evaluation of *Crassostrea ariakensis* as a potential sink or reservoir for pathogens of humans, finfish, and shellfish (PIs: Vasta, Schott, Alavi, Cheng, Breitburg, Hines)

Carys Mitchelmore (UMCES) – Does *C. ariakensis* accumulate more microbial pathogens than *C. virginica* increasing the pathogenic risk for human consumption? (PI: Mitchelmore)

NOTE: Several of these research projects are just getting underway. Therefore, results are either not yet available or very preliminary for much of this work. Additional results will become available as more of this work is completed in 2006 and 2007.

Some preliminary findings:

- Preliminary results from initial field and laboratory experiments indicate that *C. ariakensis* and *C. virginica* exhibit differences in their relative rates of uptake and depuration of certain human pathogens. However, it is too early to draw any firm conclusions because of confounding factors and variability in initial test results. For example:
    - In one controlled laboratory experiment, market size diploid *C. virginica* and triploid *C. ariakensis* were exposed to known concentrations of *E. coli* and *Vibrio* species. *C. virginica* both accumulated and depurated *E. coli* faster than *C. ariakensis*. Rates of *E. coli* accumulation were ten times faster, and rates of depuration were two times faster in *C. virginica* relative to *C. ariakensis*. Depuration rates for *C. virginica* in this experiment were consistent with those observed in previously published research for this species. There was no difference between the two oyster species in the rates of uptake and depuration of *Vibrio* species.
    - In a preliminary field experiment, triploid *C. virginica* and triploid *C. ariakensis* were deployed near a sewage treatment plant in the James River. Initial results from these in situ uptake experiments suggest a difference in uptake and retention of both fecal indicator bacteria and FRNA coliphage when *C. virginica* and *C. ariakensis* are compared. Although differences in uptake and elimination from experiment to experiment will show variability due to changing environmental

conditions, shellfish activity, size, condition, and feeding, overall the current data are consistent with an emerging hypothesis that *C. ariakensis* will at times concentrate and retain fecal indicators to a greater extent than *C. virginica* over a 14-day relay period.

- What is clear is that pathogen uptake and depuration processes are greatly affected by a suite of factors, including environmental conditions and physiology of the oysters themselves.
  - One series of laboratory experiments with market size diploid *C. ariakensis* examined protozoan pathogens at three salinity levels (20 ppt, 12 ppt, 8 ppt). Recovery efficiency, depuration, bioaccumulation, and inactivation of protozoan pathogens by *C. ariakensis* were strongly influenced by salinity. The highest rates for all these processes occurred at salinity levels of 20 ppt and 12 ppt.
  - Ploidy status (i.e., diploid vs. triploid) of oysters may affect many physiological attributes, which could in turn affect uptake and depuration of pathogens. Much of the work with *C. ariakensis* involves triploids, which are effectively sterile, for biosecurity purposes. Work underway will test differences in pathogen uptake and depuration of diploids versus triploids.
- Because of the many complicating factors, it will be important to obtain results from multiple experiments employing a variety of approaches. Laboratory studies will be able to control environmental factors and expose oysters to known concentrations of specific pathogens. Field studies, on the other hand, will serve to test rates of uptake and depuration under real world conditions. However, the natural variability of environmental conditions will make it difficult to determine which factors are responsible for any observed patterns in field studies. Thus, a combination of laboratory and field studies will likely be necessary to establish robust conclusions about the degree to which *C. ariakensis* differs from *C. virginica* in the accumulation and elimination of human pathogens.
- In a study of post-harvest pathogen levels, market size diploid *C. virginica* and triploid *C. ariakensis* were held in mesh bags and stored at 4^{o} C for 12 days. Over that period, *Vibrio* species levels declined 2.5 times faster in *C. virginica* (92% decrease in *Vibrio* sp.) than in *C. ariakensis* (69% decrease in *Vibrio* sp.). *E. coli* levels were highly variable, with no significant differences between the two oyster species. During this study, it was observed that *C. ariakensis* desiccates more quickly than *C. virginica* during post-harvest storage. Shell gaping and rapid desiccation, possibly leading to decreased shelf-life, of *C. ariakensis* has also been observed in other studies highlighted in previous Quarterly Review reports.
- There is a need for improved diagnostic tools to detect, identify, and quantify waterborne human pathogens in shellfish. Some of the research currently underway involves developing, optimizing, or validating probes for individual strains, species, or groups of pathogens that occur in oysters and can cause human illness, or diseases in finfish or other shellfish species.

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