

# Comparisons of *E. coli* Uptake and Depuration in the American oyster *Crassostrea virginica* and the Suminoe oyster *Crassostrea ariakensis*.

Tanya J. Bean<sup>1\*</sup>, James A. Morris, Jr.<sup>2</sup>, Patricia K. Fowler<sup>3</sup>, Rachel T. Noble<sup>1</sup>, and William W. Kirby-Smith<sup>4</sup>

<sup>1</sup>University of North Carolina, Institute of Marine Sciences 3431 Arendell St. Morehead City, NC \*tbean@email.unc.edu

<sup>2</sup>National Centers for Coastal Ocean Science, NOAA, 101 Pivers Island Rd. Beaufort, NC

<sup>3</sup>NC DENR Shellfish Sanitation, PO Box 769, Morehead City, NC 28557

<sup>4</sup>Duke University Marine Lab, 135 Duke Marine Lab Rd., Beaufort, NC 28516



## Introduction

The eastern oyster, *Crassostrea virginica*, is among the most valuable shellfish in the US. Prized for its contribution to commercial fishing and restaurant revenue, this species is also widely revered for its reef building and water filtration capabilities. Unfortunately, this once plentiful commodity has been decimated by factors such as poor water quality, disease, and over-harvesting. The scenario becomes worse every year as the ecosystem becomes stuck in a negative feedback system: reduced water quality causes oyster mortality, which results in less water filtration and a further reduction in water quality.

The oyster industry on the west coast of the US has seen great success since the introduction of the non-native *Crassostrea gigas*. This species is commercially valuable and has had minimal adverse ecological impacts. In light of the successes of this introduction, several species have been proposed for introduction into the mid-Atlantic states. At present, the species thought to have the greatest potential for success in this area is *Crassostrea ariakensis*. This species is of particular interest due to its tolerance to oyster pathogens such as MSX and Dermo, its rapid growth, and its taste.

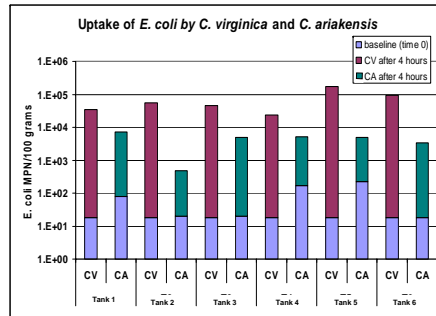
It is well documented that raw or undercooked shellfish can serve as vectors of human pathogens including *Vibrio vulnificus*, Hepatitis A virus, and norovirus. Currently, all regulations used to open and close shellfish beds to harvest are based on the uptake and depuration of fecal indicator bacteria in the native oyster *Crassostrea virginica*. Research is necessary to compare the response of *Crassostrea ariakensis* to bacteria from fecal contamination sources and other bacterial pathogens before public health officials can establish appropriate management strategies for this species.

## Method

- Oysters were purchased from aquaculture facilities and held in six separate recirculating tanks.
- Oysters were fed every 6 hours and a fluorometer was used to measure chlorophyll a to verify oyster feeding activity.
- Tanks were seeded with  $10^3$ - $10^4$  *E. coli* cells/100ml.
- Oysters were allowed to feed for 4 hours, then were harvested and analyzed for *E. coli* using MTF and IDEXX and *Vibrio* using TCBS agar.
- Oysters were randomly selected from tanks and Shellfish homogenates were created as written in Recommended Procedures for the Examination of Sea Water and Shellfish.
- Homogenate samples were split between 3 separate labs and tested for *E. coli* using MTF and IDEXX Colilert-18. Samples were tested for *Vibrio sp.* using membrane filtration on TCBS agar.
- Depuration was accomplished using UV irradiation and methods as above.

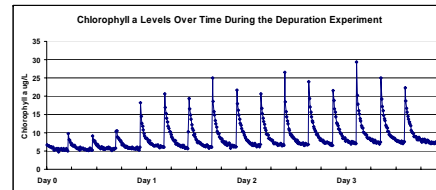
## Bacterial Uptake

- Compare bacterial uptake between *C. virginica* and *C. ariakensis*.



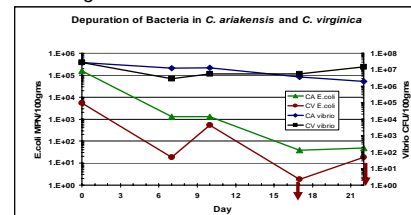
*C. virginica* uptake rate = 17000 *e. coli*/100gms/hr

*C. ariakensis* uptake rate= 1000 *e. coli*/100gms/hr



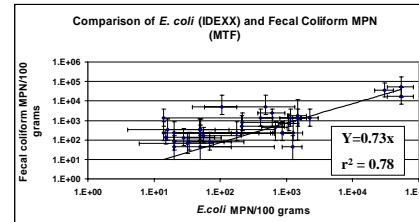
## Depuration

- Compare bacteria depuration rates between *C. virginica* and *C. ariakensis*.

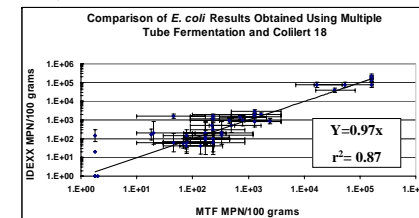


## Methods Comparison

- Compare available methods for the assessment of indicators in shellfish waters and meats.



The standard method required by the FDA for the assessment of bacterial loading in shellfish meat is Multiple Tube Fermentation (MTF). This assay can take up to an entire business week to acquire results and is dependent on skilled laboratory technicians to maintain sterility the entire time. This procedure is labor intensive and costly, therefore it would be advantageous for regulatory agencies to switch to another assay, assuming both the standard method and the new assay have the same sensitivity and accuracy.



## Colilert-18 Advantages



- Cost and labor associated with Colilert-18 is substantially less than that of other methods.
- Given the specificity of Colilert-18 for *E. coli*, quantification is directly related to the presence of bacteria stemming from fecal contamination.
- Colilert-18 takes 63% less time than MTF

## Conclusions

- Experimental design successfully compared bacterial uptake and depuration.
- Rate of *E. coli* uptake is an order of magnitude greater in *C. virginica*.
- Rates of *E. coli* depuration were similar for both species.
- Depuration using UV sterilization appears to have a negligible effect on *Vibrio*.
- Rates of depuration were similar for the two species, however levels of *E. coli* were consistently higher in *C. ariakensis*.
- There is a statistically significant correlation ( $r^2=0.78$ ) and 73% of fecal coliforms measured by MTF were determined to be *E. coli* as measured by IDEXX.
- There is a statistically significant correlation ( $r^2=0.87$ ) and 97% agreement between *E. coli* measured by MTF and IDEXX.

## Ongoing Research

- Compare post harvest bacteria levels between *C. virginica* and *C. ariakensis*
- Development of an *E. coli* Quantitative Real Time PCR Assay for shellfish meat samples
- Further analysis comparing *Vibrio sp.* uptake by both species.
- Assess the effect of salinity on uptake of bacterial species
- Assess uptake of *Vibrio sp.* using fluorescently labeled bacteria

## Acknowledgements

This project is an excellent example of interagency collaboration.

Special thanks to Kate Morrison (UNC-CH), Andy Haines (NC DENR), Diane Mason (NC DENR), Daniel Govoni (DUML), Jennifer Parker (UNC-CH), Ginger Kelly (UNC-CH) and Steve Fries (UNC-CH) for technical assistance and sample processing.