Georgia Oyster Watch (GoOW)
Can Oysters be Utilized as Integrative Monitors of Bacteriological and Chemical Water Quality?

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Abstract
The use of bivalves as indicators of chemical and biological contamination is well established as a means to assess coastal water quality and shellfish safety for human consumption. However, little effort has been made to determine the relationship, if any, between chemical and biological contaminant accumulation in systems. In this study we investigate methods for estimating concentrations of bacteriological indicators in soft tissue of the Eastern oyster (Crassostrea virginica) and relationships between these indicators and corresponding measurements in ambient water. Samples for bacteriological enumeration were collected from 19 sites throughout coastal Georgia (USA). Total coliform (TC) and total fecal coliform (FC) bacteria were enumerated in oyster homogenates after dilution using modified most probable number techniques (EPA Methods 9212-8). Background concentrations of TC and FC bacteria in oyster homogenates ranged from 2 to 890 cfu/g dry wt and from 0 to 0.52 cfu/g dry wt, respectively. Tissue levels of Enterococci ranged between 66.4 to 809 cfu/ml tissue using a modified EPA method 1660. The ratio of bioluminescent to total bacteria (BLR) (0 to 0.07), a potential indicator of chemical and biological contaminant accumulation in oysters, was also enumerated in tissue homogenates. These data will be compared with PAH and total petroleum hydrocarbon contaminant levels to assess the overall utility of C. virginica as a biomonitor of human and marine ecosystem health.

Introduction

The eastern oyster, Crassostrea virginica, has long been used as an indicator of ambient water quality e.g. in NOAA’s National Status and Trends Manual Watch. Fecal coliforms (including E. coli) have been the most widely utilized indicator of fecal contamination (Cabelli et al., 1983).

There has been a recent switch to Enterococci as a more relevant indicator because of its persistence in saline waters (Rosen and Bulkin, 2001).

Marine bioluminescent bacteria has been shown to respond to a broad range of chemical contaminants (PCBs and mercury) in a concentration dependent fashion (Frischer et al., 2005).

This study reports on the optimization of bacteriological assays for use with oyster tissue homogenates and comparisons of these indicators in ambient water.

Materials and Methods

Ambient Water Quality using Standard Methods
- 400 ml of ambient water collected
- Total and Fecal Coliforms quantified using most probable number techniques (EPA Methods 9212-8)
- Enterococci quantified using EPA method 1660
- Bioluminescent bacteria enumerated by filtering aliquots (1.12 and 1.100 ml) onto the membrane (ambient and artificial) onto 47 mm diameter 0.22 μm polyethersulfone filters.
- Filtered on marine agar with tryptone (5 g/L) and yeast extract (5 g/L), incubated at 37°C for 24-48 h
- Bioluminescent bacteria = number bioluminescent forming units

Bacteriological Estimation in Oyster Tissues
- 16 g of oyster tissue blended with 52 ml of artificial sea water
- 2 ml tissue homogenate dried at 100°C for 24 h and water content determined gravimetrically
- Serial dilutions to 10^-4 trade with the homogenate, DI water, and artificial sea water
- Enterococci, Total and Fecal Coliforms

Bacteria quantified using previously described methods for assessing water

This study reports on the optimization of bacteriological assays for use with oyster tissue homogenates and comparisons of these indicators in ambient water.

Results

- Total coliforms in ambient water were not statistically correlated (p > 0.05) with total coliform concentrations in oyster tissue. (Figure 3)
- Enterococci and bioluminescent bacteria in water also did not show any statistical relationship with Enterococci and bioluminescent bacteria in oyster tissue (p > 0.05 and 0.79, respectively). (Figure 9 & 12)
- There was a statistically significant relationship between total fecal coliforms in ambient water and oyster tissue (p = 0.04 x 10^-3). (Figure 6)
- Marine-Whitney rank test showed a statistically significant difference between the mean levels of Enterococci in water and those in oyster tissue (p = 2.00 x 10^-3). (Figure 5 & 9)

Bacterial indicator levels enumerated from the oyster tissues will be compared with PAH and total petroleum hydrocarbon contaminant levels to elucidate any relationships between these contaminants and the aforementioned bacterial indicators, namely the bioluminescent bacteria. A control experiment to carry out toxicity to determine if systems are a source for Enterococci in ambient water. Preliminary results suggest that oysters are a source for the bacteria.

Future Work

Bacterial indicator levels enumerated from the oyster tissues will be compared with PAH and total petroleum hydrocarbon contaminant levels to elucidate any relationships between these contaminants and the aforementioned bacterial indicators, namely the bioluminescent bacteria. A control experiment to carry out toxicity to determine if systems are a source for Enterococci in ambient water. Preliminary results suggest that oysters are a source for the bacteria.

Literature Cited

Figure 1: Bioluminescence ratio from sampled sites throughout coastal Georgia (USA). Total coliform (TC) and total fecal coliform (FC) bacteria were enumerated in oyster homogenates after dilution using modified most probable number techniques (EPA Methods 9212-8). Background concentrations of TC and FC bacteria in oyster homogenates ranged from 2 to 890 cfu/g dry wt and from 0 to 0.52 cfu/g dry wt, respectively. Tissue levels of Enterococci ranged between 66.4 to 809 cfu/ml tissue using a modified EPA method 1660. The ratio of bioluminescent to total bacteria (BLR) (0 to 0.07), a potential indicator of chemical and biological contaminant accumulation in oysters, was also enumerated in tissue homogenates. These data will be compared with PAH and total petroleum hydrocarbon contaminant levels to assess the overall utility of C. virginica as a biomonitor of human and marine ecosystem health.

Figure 2: Bacterial indicator levels enumerated from the oyster tissues will be compared with PAH and total petroleum hydrocarbon contaminant levels to elucidate any relationships between these contaminants and the aforementioned bacterial indicators, namely the bioluminescent bacteria. A control experiment to carry out toxicity to determine if systems are a source for Enterococci in ambient water. Preliminary results suggest that oysters are a source for the bacteria.

Figure 3: Total coliforms in ambient water were not statistically correlated (p > 0.05) with total coliform concentrations in oyster tissue. (Figure 3)
- Enterococci and bioluminescent bacteria in water also did not show any statistical relationship with Enterococci and bioluminescent bacteria in oyster tissue (p > 0.05 and 0.79, respectively). (Figure 9 & 12)
- There was a statistically significant relationship between total fecal coliforms in ambient water and oyster tissue (p = 0.04 x 10^-3). (Figure 6)
- Marine-Whitney rank test showed a statistically significant difference between the mean levels of Enterococci in water and those in oyster tissue (p = 2.00 x 10^-3). (Figure 5 & 9)

The bioluminescent ratio for industrial and marina sites fell below the curve for pristine and recreational harvesting bed sites, indicating greater chemical contamination impact at the former sites. (Figure 3)

Conclusion

Total and fecal coliform, bioluminescent bacteria, and Enterococci can be enumerated in oyster tissue homogenates. Though there was not a statistically significant correlation between most of the bacterial indicators levels in water and oyster tissues, fecal coliforms in ambient water showed a weak correlation with levels in ambient water; however, more data are needed to properly confirm this correlation. The high concentration of Enterococci in water also suggests that the organism may be a source for the bacteria. The lower bioluminescent ratios for industrial and marina sites suggest that these sites have greater chemical contamination than the pristine and recreational harvesting bed sites.