# *Influence of Different Organic Matter Loading on Ambient Bacteria in Brackish "Challenge" Water Stored in Small-scale, Simulated Ballast Water Tanks*

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## *Abstract:*

Every day, thousands of ships travel the world's oceans and load and discharge ballast water as they conduct trade and commerce. Organisms indigenous to the waters where ballast water is loaded are transported to different waters where they are non-native. Invasions of aquatic nuisance species (ANS) transported via ballast water can have ecosystem and economic impacts. The efficacy of technology options proposed and developed for use by commercial and military shipping to mitigate ANS invasions under different environmental conditions must be evaluated. The U.S. Naval Research Laboratory (NRL 6130) in Key West, FL conducts research and development for the evaluation of ANS treatment technologies for ballast water management systems (BWMS). Organic matter content and ballast water can influence the growth, viability, and mortality of specific organisms in ballast water tanks and the efficacy of BWMS. There is a need to assess the effect of different organic matter (OM) additives on ambient organisms using specific "challenge" conditions for the evaluation of ANS treatment technologies. A controlled study using a series of scaled-down, simulated ballast water tanks was performed to evaluate the effect of OM additives on ambient bacteria in brackish ballast water over a fixed hold time. Results were inconclusive but provide a baseline assessment of the influence of different OM additives on ambient bacteria in brackish waters that will assist the NRL Code 6130 in conducting research and development related to ANS treatment and BWMS technologies.

## *Experimental Design/Methods:*


**Figure 1**: Map showing the location of the HOL study site on the Severn River in the mesohaline Chesapeake Bay (Google Earth).

**Table 1:** Overview of simulated ballast water tank study design (tanks shown below, right).

| Tank Numbers | Label | Treatment Description |
|---|---|---|
| 1-3 | Control | Ambient Severn Water (ASW) |
| 4-6 | Test #1 | ASW + POM |
| 7-9 | Test #2 | ASW + DOM |
| 10-12 | Test #3 | ASW + POM + DOM |

| Water Types | Minimum Water Characteristics |
|---|---|
| Fresh (Salinity <1 PSU) | DOM: 6 mg/L as DOC<br>POM: 4 mg/L as POC |
| Brackish (Salinity 10-20 PSU) | MM: 20 mg/L<br>TSS = POM + MM: 24 mg/L |
| Marine (Salinity 28-36 PSU) | Temperature: 4 – 35 °C |

**Table 2**: Water quality "challenge" matrix conditions (from Table 3, U.S. EPA, 2010).

A series of plastic buckets (**Table 1**; Control and 3 Test Treatments; 3 x each) were used to evaluate the growth rate of ambient bacteria under artificially modified POM and DOM conditions (**Table 2**) in water collected from the Severn River (**Fig. 1**) over a 7-day period simulating ballast water incubation/storage. Non-caffeinated iced tea was used as a DOM additive and humic micromate was used as a POM additive.

## *Results:*

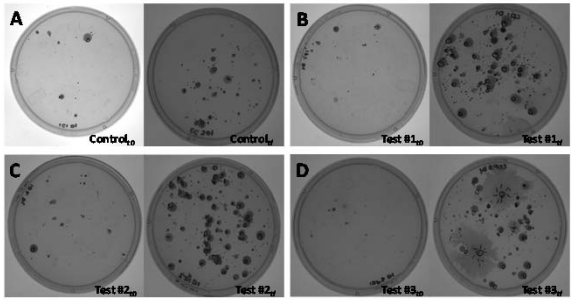


**Figure 2:** Example images of initial (t0 = 0 days) and final (tf = 7 days) bacteria plate cultures for treatments:(A) Control; (B) Test #1; (C) Test #2, and; (D) Test #3.

Bacterial cultures were prepared for each tank (**Table 1**) at t = 0 days and t =7 days using a 1 mL sub-sample of filtered (0.7µm GFF) tank water pipetted into 9 mL of deionized water. A 1 mL sample from a series of serial dilutions (10x, 100x, 1000x) of this solution was spread evenly onto pre-filled marine agar plates and allowed to incubate in a bio-clean fume hood at room temperature for ~ 6 days. A control plate with only deionized water was used to determine if there was any laboratory contamination. Plates were checked periodically during the incubation to verify growth was exhibited and the cover plates were marked. The final number of colonies per plate (non-specific) were quantified through visual observation and manual counting. **Figure 2** shows example images of initial and final bacteria plate cultures for the control and each tank test treatment (Test #1, Test #2, and Test #3).

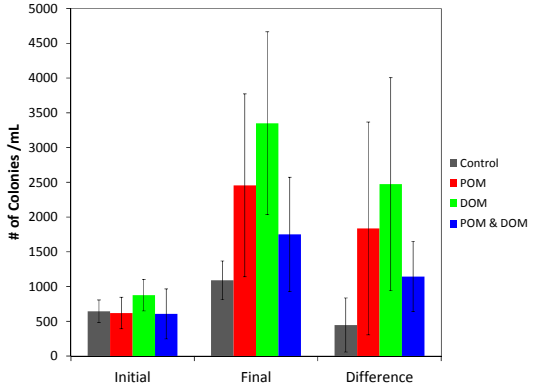


**Figure 3:** Comparison of average (n=3) initial, final, and difference between initial and final bacterial counts (# of colonies/ml) for the Control and each test treatment (Test #1, Test #2, and Test #4). Error bars indicate the standard deviation of the three tanks used for the control and each treatment.

**Figure 3** shows that there was little difference noted between the average initial bacterial counts (# of colonies/ml) in the Control and each treatment. Although the standard deviations of the average count values are large, there does appear to be a difference in the final counts (t =7 days) and in the difference between the initial and final counts in the Control and the three different treatments, especially in the DOM case.

## *Discussion:*

Although **Figure 3** suggests a possible difference between the initial (t = 0 days) and final (t =7 days) counts in the Control and the three different treatments (Test #1, Test #2, and Test #3), the results do not support a statistically-significant difference between them. **Table 3** shows the two-sided P-values for student t-tests (assuming equal variance) comparing final counts and the difference in counts in the Control tank samples and each treatment. None of the results support a significant difference (P value ≤ 0.05) between the control and the treatments at the 95% confidence interval. The difference between the final counts in the Control and the DOM treatment is, however, significant at the 90% confidence interval. Simple single factor ANOVA analyses (assuming equal variance) of final counts and the difference in counts yields two-sided P-values of 0.07 and 0.35, respectively, suggesting observed differences in bacterial counts between groups could have been due to chance alone.

| | P-Value (95% Confidence) | |
|---|---|---|
| T-Test | Final | Difference |
| Control - Test #1 | 0.15 | 0.16 |
| Control - Test #2 | 0.06 | 0.12 |
| Control - Test #3 | 0.25 | 0.13 |

**Table 3:** Two-sided P-values for student t-tests on bacterial counts (95% Confidence).

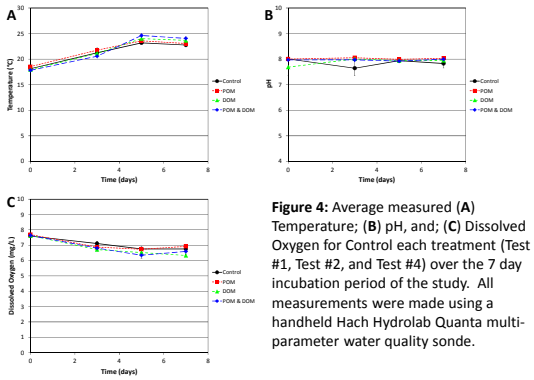


**Figure 4:** Average measured (**A**) Temperature; (**B**) pH, and; (**C**) Dissolved Oxygen for Control each treatment (Test #1, Test #2, and Test #4) over the 7 day incubation period of the study. All measurements were made using a handheld Hach Hydrolab Quanta multi-parameter water quality sonde.

Differences in individual tank conditions could have resulted in the observed differences in bacterial counts between the Control and test treatments. Tank conditions (Temperature, pH, dissolved oxygen) did vary slightly over the course of the experiment (**Fig. 4**) but variations were fairly consistent between tanks so they should have affected each tank in a similar manner. Outside contamination is another possible explanation for the observed differences in bacterial counts but it is more likely that there was simply not enough replication in the experimental design to allow for statistically-significant results. The DOM results do suggest a possible difference in ambient bacterial growth.

## *Conclusions:*

- Results were inconclusive but provide a baseline assessment of the influence of different OM additives on ambient bacteria in brackish waters that will assist the NRL 6130 in conducting R&D related to BWMS/ANS treatment technologies.
- Future studies should include bigger tanks, more replication, better controls, and organism-specific analyses.
- Pending results of dissolved and particulate organic carbon analysis may improve findings.

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