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Research Article

Examination of additives used to augment "challenge water" used in verification testing of ballast water management systems: mass yields and biological impacts

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Abstract

Rigorous evaluation of ballast water management systems (BMWSs) at land-based test facilities requires that water used in testing meets minimum concentrations of dissolved and particulate material, for example, using the criteria in the U.S Environmental Technology Verification (ETV) Program's protocol for testing of BWMSs. Here, uptake water ("challenge water") can be augmented with compounds to meet these benchmarks. In this study, we evaluated materials used to supplement dissolved organic matter (DOM), particulate organic matter (POM), and mineral matter (MM) used to achieve challenge water criteria. To determine the additives' contributions to DOM and POM pools, the mass yields of *Camellia sinesis* (decaffeinated iced tea) extract and humic matter were calculated at different temperature and salinities. Additionally, the response of ambient organisms to these additives was measured in mesocosm experiments, in which changes in organism concentrations were measured after a 5-d holding time. Living organisms were grouped into three size classes: ≥50 µm (nominally zooplankton), ≥10 to <50 µm (nominally protists), and <10 µm (measured as culturable, aerobic, heterotrophic bacteria). Significant differences in concentrations between control and treatment mesocosms after 5 d were not detected for organisms in the ≥10 to <50 µm or the ≥50 µm size classes. However, bacterial concentrations increased significantly in mesocosms augmented with exogenous materials. Thus, direct impacts (or indirect impacts through increased bacterial concentrations) were not apparent among organisms in the two largest size classes. Finally, a literature review of DOM, POM, and total suspended solids concentrations in coastal waters was conducted. It revealed that the challenge water concentrations outlined in the ETV protocol are at the middle to upper range of concentrations observed in coastal and estuarine water. The mean DOM and POM concentrations in this data set typically fell short of the ETV minimum requirements, and more data are needed to fully assess the suitability of these requirements.

Key words: invasive species, protists, turbidity, verification testing, water quality, zooplankton

Introduction

Water—and living organisms within it—is taken aboard ships into ballast tanks to manage the draft, stability, trim, and stress on the structure of the vessel. As awareness grew that organisms in ballast water may become nuisance species in the waters into which they are discharged, regional, national, and international attention became focused on the issue of ship-mediated biological invasions. In 2004, the International Maritime Organization (IMO) adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments, which establishes standards for the discharge of living organisms in ballast water and has not yet been ratified sufficiently to enter into force (IMO 2004). Subsequently, the U.S. Coast Guard (USCG) issued a final rule with essentially the same standard (USCG 2012), which is in agreement with the United States Environmental Protection Agency's (US EPA) Vessel General Permit (EPA 2013). To meet the

standard, most commercial ships will install a ballast water management system (BWMS) to treat ballast water upon uptake, upon discharge, by in-tank dosing, or using some combination of these approaches. Prior to obtaining flag state Type Approval to be installed aboard vessels, BWMSs must undergo land-based and shipboard verification testing to determine their efficacy.

To generate robust and reliable testing data, the US EPA, in cooperation with the USCG, and with the input of stakeholders and researchers, finalized the Environmental Technology Verification (ETV) Program "Generic Protocol for the Verification of Ballast Water Treatment Technology", which provides procedures for conducting verification tests of BWMSs at land-based test facilities (US EPA 2010). The protocol prescribes the "challenge" (uptake water) conditions for testing, including the thresholds for water-quality characteristics and the concentrations of living organisms in three size classes: ≥50 μm (nominally zooplankton), ≥10 to <50 μm (nominally protists), and <10 μm (measured as culturable, aerobic, heterotrophic bacteria). These minimum levels were selected to represent "challenging, but not rare, water quality conditions representative of the natural environment" (US EPA 2010). Dissolved compounds and particulate matter in the test water will decrease the treatment efficacy, especially for BWMSs using ultraviolet (UV) radiation or chemicals (e.g., chlorine or other oxidants) or to treat organisms. For example, many systems use UV radiation to treat ballast water (Lloyd's Register 2011). Because dissolved organic matter (DOM) and particulates will absorb and attenuate or reflect UV light (Qualls et al. 1983), unless the water flow through the BWMS is decreased or the UV fluence (dose) is increased, high DOM loads will decrease the efficacy of this approach. Further, total suspended solids (TSS), which are the sum of particulate organic and mineral matter (POM and MM, respectively), can potentially shield aquatic organisms from UV treatment. Additionally, TSS may reduce the efficiency of filters and hydrocyclones that are used to physically remove organisms. Likewise, organic matter will react with halogenated compounds (e.g., sodium hypochlorite), consuming the oxidant and shielding, perhaps, living organisms (LeChevallier et al. 1981).

Challenge water quality conditions may be met by augmenting ambient concentrations of dissolved and particulate matter with commercially available substances. Because coastal and estuarine environments receive organic and mineral matter input from riverine sources that introduce terrigenous materials (e.g., clay minerals) and plant-derived organic matter into nearshore waters (Wang et al. 2004; Cai et al. 2012), the most desirable additives will reflect these terrestrial inputs of dissolved and particulate materials. For example: *Camellia sinesis* (decaffeinated iced tea mix), which primarily dissolves into DOM; pulverized humic matter, which is primarily composed of POM; and finely-powdered clay minerals, which contributes exclusively to MM may be used. Both *C. sinesis* and humic matter, which were suggested for use in ETV testing (US EPA 2010), are derived from plant matter, and they contain a complex assemblage of organic molecules ranging in molecular mass, solubility, and reactivity. How these materials dissociate when introduced into waters used in verification testing, however, is unclear. Challenge water in ETV testing may vary in salinity (<1 to 36 psu) and temperature (4–35°C), and thus, these compounds may react differently based upon characteristics of the test water. For example, salinity can affect the solubility of organic matter, and compounds soluble in freshwater may precipitate more readily in higher salinity waters (Sholkovitz 1976). Consequently, the additives could contribute differently to the pools of dissolved and particulate organic matter based upon characteristics of the water.

The response of ambient, planktonic organisms to these substitute compounds also warrants investigation. Additives, if used, should not drive changes in organism concentrations and dynamics. Because microbial communities can change rapidly (minutes to days; e.g., Suttle 1994; Bratbak et al. 1996; Kim et al. 2011), amending the test water with additives could be apparent in the relatively short hold times (<5 d) for verification tests.

Overall, the goal of this work was to determine the suitability of using substitute compounds for achieving minimum concentrations of dissolved and particulate matter. The objectives of this study were to: 1. Determine the mass yields of *C. sinesis* and humic matter in dissolved and particulate organic matter pools in waters with varying temperature and salinity, 2. Expose ambient organisms to these additives in mesocosm experiments to test the hypothesis that the addition of carbon substrate will stimulate bacterial growth and respiration and, in turn, affect higher trophic levels, and 3. Gauge, using published reports, whether the concentrations of DOM, POM, and TSS in coastal waters are in accord with concentrations specified in the ETV protocol.

Methods

Description of additives

The additives examined in this study, which were used as examples in the ETV Protocol, were all commercially available. Powdered *C. sinesis* extract was supplied as decaffeinated, instant iced-tea mix (Lipton®; Unilever; Glasgow, Scotland), and humic matter was supplied as pulverized, fine-grained humates (Mesa Verde Resources; Placitas, New Mexico). Inorganic minerals were supplied as ISO 12103-1 Ultrafine Arizona Test Dust (Powder Technology, Inc.; Burnsville, Minnesota), which was composed primarily of silicon dioxide and aluminum oxide.

Dissolution of additives: Temperature and salinity dependence

While *C. sinesis* and humic matter are added to contribute to the dissolved and particulate organic matter pools, respectively, the contribution of the compounds to each of these pools may depend upon the temperature and salinity of the water. To determine the effect of temperature and salinity on dissolution, in a laboratory study, additives were added to two water types (0 and 35 psu), each at two temperatures (4° and 35°C). The source of water was either deionized and reverse-osmosis purified water (Type I water; 0 psu) or ambient seawater (35 psu). Both water types were filter-sterilized (0.22 µm) prior to transferring 1 L into opaque, high-density polyethylene containers sealed with a screw cap. The containers were not completely filled; approximately 200 mL of headspace remained in the containers. The water temperature was adjusted by holding the containers in a refrigerator (4°C) or a heating oven (35°C) until the target temperature was reached (typically, containers were held overnight). Temperature and salinity were measured prior to the addition of the additives. The two temperature and two salinities generated four treatment types: 1. 4°C and 0 psu, 2. 4°C and 35 psu, 3. 35°C and 0 psu, and 4. 35°C and 35 psu. Three additive types were tested at all temperature and salinity treatments: 1. *C. sinesis* only (100 mg), 2. Humic matter only (100 mg), 3. Both *C. sinesis* (100 mg) and humic matter (100 mg). These masses, which were greater than needed to meet challenge water criteria, provided quantities sufficient for gravimetric measurements. The containers were mixed by manually shaking for 30 s prior to sample processing. Three independent trials were performed for all three additive types, and in each, three replicate samples for each of the four treatments was prepared. Samples were subsequently analyzed for DOM, POM, MM, and TSS concentrations as described below.

Response of ambient organisms to additives

The response of ambient, marine organisms to additives was examined by quantifying the planktonic community in mesocosms (3 m^3, aboveground, fiberglass tanks) amended with additives (treatment) compared to unamended (control) tanks in three replicate trials conducted from September to October 2012. Over this time, the temperature of seawater at this location (24.586 N, 81.794 W) ranged from 24 to 29°C, and salinity ranged from 35 to 37 psu. Two mesocosms were concurrently filled with 3 m^3 of ambient seawater from a seawater pumping and transferring system described elsewhere (First et al. 2012). The treatment tank was amended with *C. sinesis*, humic matter, and inorganic minerals to achieve the specified concentrations of DOM, POM, and MM, respectively (Table 1). The control tank was not amended. The mesocosms were not amended with additional organisms to reach the challenge water criteria for organism concentrations required for ETV testing (US EPA 2010). Rather, ambient concentrations, reflective of the carrying capacity of the environment, were used to test the response of organisms to additives. Both tanks were mixed by stirring with an oar (ten rotations in each direction) and covered with a cloth tarp to attenuate ambient sunlight. Sampling was conducted by uncovering a portion (~1 m^2) of the tank, mixing as described above, and transferring sample water into containers using a hand-held rotary pump. For each sample type, three subsamples were collected to examine the variability within each tank (described below). All samples were collected immediately (day 1) and at the termination of the hold period (day 5).

Additionally, interim samples were collected daily to mix the water and monitor temperature and salinity (via a hand-held refractometer). The process of mixing and sampling was completed within 20 min, after which, tanks were covered again.

To analyze organisms ≥50 µm, 20 L of seawater was collected from each tank and concentrated by sieving the water through a 35-µm mesh (with a nominal diagonal distance of 50 µm). To rinse the sieve, filtered seawater (<0.22 µm) at the same temperature as the sample water was used. Approximately 20 mL of the concentrated sample was retained and transferred into a pre-weighed

Table 1. Water quality parameters examined in mesocosm experiments. Target concentrations are set within the ETV protocol (US EPA 2010). Values for the control and treatment mesocosms at the start of trials are the mean of three trials with three subsamples per trial. Concentrations of DOC were converted to DOM using the carbon mass ratio of *Camellia sinesis* extract (36%, see text for details); for completeness, both measurement units are displayed.

		Concentrations (mg L^{-1})	
Parameter	ETV Protocol	Control (mean ± 1SD)	Treatment (mean ± 1SD)
Dissolved organic carbon (DOC)	6	1.8 ± 0.1	5.3 ± 0.3
Dissolved organic matter (DOM)	17	5.1 ± 0.3	15.0 ± 0.8
Particulate organic matter (POM)	4	1.9 ± 0.3	8.2 ± 5.0
Mineral matter (MM)	20	5.3 ± 0.8	22.7 ± 3.3
Total suspended solids (TSS)	24	7.3 ± 1.0	30.8 ± 2.9

centrifuge tube. The exact sample volume was determined by measuring the temperature and salinity of the sample water to determine density, from which sample volume was calculated. To analyze organisms ≥10 to <50 µm, 5 L was collected and concentrated on a 7-µm sieve (with a nominal diagonal distance of 10 µm) to 50 mL. To analyze bacteria (culturable, aerobic, heterotrophs) and water quality parameters (DOM, POM, MM, and TSS), water samples were collected but not filtered (50 mL and 1000 mL, respectively).

Determining organism concentrations

Organisms ≥50 µm were quantified by adding sample water to a Bogorov counting chamber and scanning the entire chamber with a brightfield microscope (20x magnification). Briefly, organisms ≥50 µm (measured by comparing to 50 µm microbeads) were scored as living or dead and classified into general categories (e.g., crustacean nauplii, adult copepods, annelids, etc.). Motility was used to differentiate living from dead organisms, and, when necessary, unmoving organisms were gently prodded to stimulate movement (US EPA 2010). Non-motile organisms (e.g., diatoms) were scored as living if cellular structures, such as chloroplasts and frustules, were intact.

Organisms ≥10 to <50 µm were quantified by labeling 1 mL of sample water with a combination of chloromethyl fluorescein diacetate (CMDFA) and fluorescein diacetate (FDA) and counting fluorescent organisms using a epifluorescence microscope (Steinberg et al. 2011). Briefly, fluorescing organisms were counted from portions of a 1-mL Sedgewick-Rafter counting chamber, which was scanned at 100x magnification using the optical filter sets for blue light excitation and green emission. Typically, >100 organisms were counted and identified to broad taxonomic category (e.g., ciliates, flagellates, diatoms). Heterotrophic bacteria were quantified using heterotrophic plate counts. Samples were serially diluted with 0.22 µm filtered, heat-sterilized (121°C, 15 min) seawater, and a range of sample dilutions (10^1 to 10^5) were introduced to the nutrient agar plates to account for the range of potential bacterial concentrations. Here, 0.1 mL aliquots were spread on the surface of nutrient agar plates (BD DIFCO Marine Agar 2216; BD; Franklin Lakes, NJ) using sterile applicators. The agar plates were incubated at 25°C for 5 d, at which time the number of colony forming units (CFU) were counted on the surface of the agar. Ideal sample dilutions yielded from 5 to 50 CFU per 0.1 mL, and the sample dilutions with CFU counts in this range were used to estimate concentrations. Typically, sample dilutions 10^1 to 10^3 yielded CFU counts within this optimal range; only a few samples (~10% of the total) required greater dilutions to yield CFU counts within this range.

Water quality analysis

Water quality analyses were performed following standard methods (US EPA 1983). Briefly, total suspended solids (TSS) were measured by filtering 500 mL of sample water through a pre-combusted, glass fiber filter (GF/F, effectively 0.7 µm). The filtrate was collected and analyzed to measure DOM (as below). The filter was then rinsed three times with Type I water (50 mL) to remove residual dissolved salts, after which, the filter was dried at 104°C for 3 h, which was sufficient to achieve a constant mass (unpubl. data). The difference between the final dry filter mass and the dry mass of the filter prior to

filtration was used to estimate TSS, expressed in units of mg L^{-1}.

The dry TSS filter was retained and then heated to 550°C in a combustion oven and held for 20 min (which was sufficient to achieve a constant mass in preliminary experiments). After cooling the filter to room temperature, the filter was weighed to determine the mass of non-combustible material (i.e., MM). Particulate organic matter (POM) was defined as the difference between TSS and MM, that is, the combustible component of TSS. Analyses of TSS, MM, and POM were completed within 1 day after each trial was completed.

A total organic carbon analyzer (Shimadzu TOC-VCHS; Shimadzu, Kyoto, Japan), which measured the concentration of non-purgeable organic carbon via the combustion catalytic oxidation method, was used to quantify DOC in samples, which was several orders of magnitude greater than the limit of detection for the approach (0.5 ppm). As above, samples were filtered through a GF/F filter, which removed particulate matter and allowed only dissolved compounds (i.e., <0.7 μm) in the sample. After filtration to remove particulates, the sample water was acidified and kept refrigerated prior to analysis, which occurred within 1 week of collection. Readings were reported in ppm DOC and were converted to mg L^{-1} based upon the density of the sample water, which varied with temperature and salinity. Concentrations of DOC were adjusted by subtracting the baseline concentration of DOC in the test water (i.e., prior to the addition of additives) from the measured value. To convert concentrations of DOC to DOM, DOC concentrations were adjusted to the contribution of carbon to total mass of *C. sinesis*: this mass contribution was determined by creating a series of samples with known DOM concentrations, which were made by dissolving a measured mass of *C. sinesis* in Type I water (ranging from 100 to 1,000 mg L^{-1}), and measuring the DOC concentrations. The mass contribution of carbon was determined by the linear relationship between the known DOM concentrations of *C. sinesis* and the measured DOC concentrations. The mass yield (*Y*, %) of materials (DOM, POM, and total organic matter [combined DOM and POM]) was measured as the mass recovered (*R*, mg) divided by the mass added (*A*, mg) times 100.

Data analysis

Analytical results from two subsamples were averaged to yield a mean value for each sample, and a two-way ANOVA with temperature and salinity as factors was used to detect significant differences ($p < 0.05$) in mass yields of the three additives types. Pairwise t-tests were performed for all samples when significant differences in mass yields were observed.

In experiments assessing the effects of additives on ambient organisms, concentrations of organisms were log normalized prior to calculations and statistical comparisons (Sokal and Rohlf 1995). A one-way ANOVA was used to detect significant differences in organism concentrations between control and treatment mesocosms. Because the independent experiments occurred on three separate weeks, the starting concentrations of ambient organisms varied among experiments. Therefore, the relative response of organisms was used to gauge the response of organisms to treatment, for example, the concentration of living organisms on day 5 was normalized to the concentration of living organisms on day 1. Data analysis and plotting was performed using SigmaPlot (V11.2; Systat Software, Inc.; San Jose, CA).

Results and discussion

Dissolution of additives

Carbon represented 36% of the total mass of *C. sinesis* (linear regression, $R^2 = 0.999$, $p<0.05$; data not shown). Because DOM was not measured directly (rather, via DOC concentrations), this value was used to convert all DOC measurements to DOM. Across all temperatures and salinities, total mass yields of *C. sinesis* and humic matter averaged 98 ± 2% and 96 ± 2%, respectively (mean ± 1 SD, n = 4 treatments; Figure 1A and 1B, respectively). When combined, the total mass yield of *C. sinesis* and humic matter was significantly lower than yields of a single additive (92 ± 3%; ANOVA, $p<0.05$; Figure 1C). In this instance, the interactions between the two additives and, perhaps, the higher concentration of total mass in these treatments (200 vs. 100 mg L^{-1}) potentially led to higher rates of organic matter volatilization. This complex mixture of organic compounds from both sources may contain compounds that volatilize at higher rates in saturated solutions. While not measured directly in this study, volatilization could account for some of the mass lost.

Differences among percent yields, although significant in some cases (ANOVA, $p < 0.05$), were small—the percent differences among the treatments were <6%. Minor, but statistically

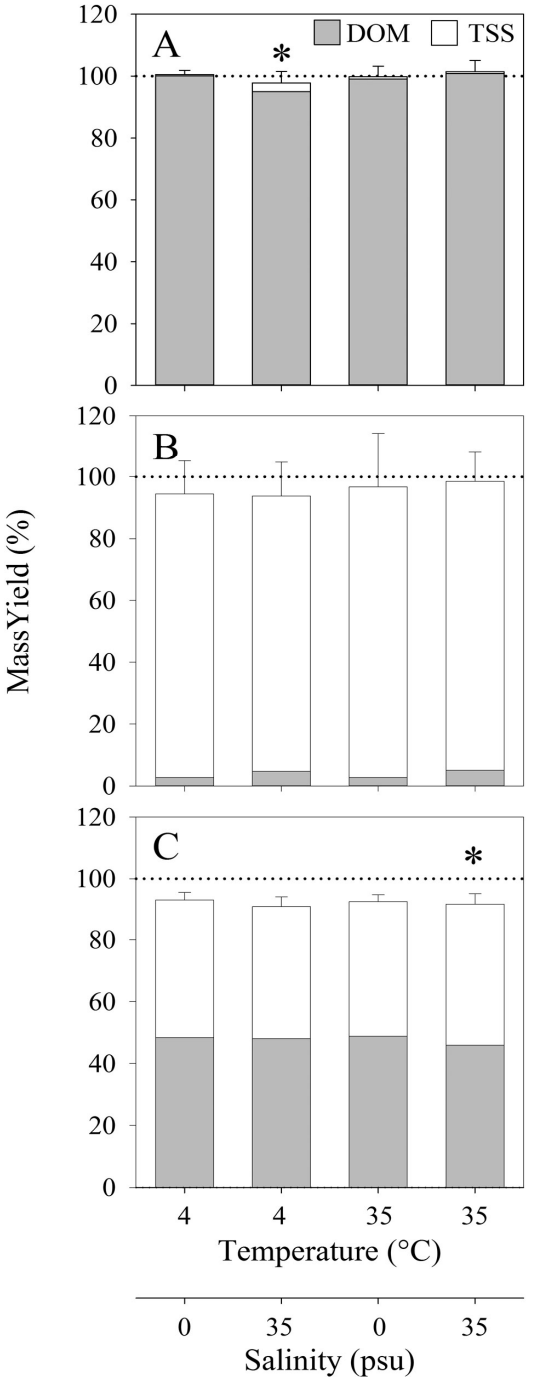

Figure 1. Mass yield of organic compounds added to water at different temperatures (4° and 35°C) and salinities (0 and 35 psu). Shown are the mass yields of **A**. *C. sinesis* only, **B**. humic matter only, and **C**. both *C. sinesis* and humic matter. Total yields, relative to the mass added, are shown with error bars (mean +1 SD, n = 3 independent trials), and the DOM and TSS (which is primarily composed of POM) are shown as relative portions of the total yield. The dotted line identifies a 100% mass yield. The asterisk marks significantly different mass yields (ANOVA, $p>0.05$)

significant differences occurred in two treatments with *C. sinesis*. For *C. sinesis* alone, DOM yield was 93 ± 2% at 35 psu and 4°C, which was significantly lower than other treatments (range: 96 – 99%, n =3, Figure 1A). In trials with both *C. sinesis* and humic matter, DOM yield was 46 ± 2% at 35 psu and 35°C, which was significantly lower than in other treatments (range: 48–49%, Figure 1B). These mass yields indicate that *C. sinesis* and humic matter contributed almost exclusively to their intended organic matter pools: DOM and POM, respectively. Because different compounds can be used to meet challenge water criteria (US EPA 2010), and because different compounds will likely show dissimilar mass yields based upon temperature and salinity, studies such as this one are useful.

Mesocosm experiments

The three trials were completed within 50 days, and the composition of the planktonic community varied among trials. For example, initial concentrations of organisms in the ≥50 µm size class ranged from 20 ± 5 ind. L^{-1} (mean ± SD of two samples: the control and treatment mesocosms at day 1) to 95 ± 11 ind. L^{-1} among the trials, which corresponds to log concentrations of $10^{1.3}$ to $10^{2.0}$, respectively (Figure 2A). No significant differences were observed among treatments or sampling days in either the ≥50 µm or the ≥10 to <50 µm size classes (Figure 2A and 2B). However, bacterial concentrations were significantly greater in the treatment mesocosms at day 5 relative to other treatments (Figure 2C).

No consistent pattern in relative concentrations of organisms in the ≥50 µm or the ≥10 to <50 µm size classes was observed among the three trials (Figure 3). Likewise, changes within subcategories of organisms (e.g., crustacean nauplii, annelids, ciliates, etc.) were neither statistically significant nor consistent among replicate trials (data not shown). Therefore, additive compounds did not appear to restructure the assemblages within the two largest size classes. Mortality of organisms ≥50 µm (i.e., the relative concentration of dead organisms observed in samples), did not vary significantly between control or treatment tanks or between initial and final sampling (ANOVA, $p>0.05$) and was <3% in all samples (data not shown), which is in accord with the baseline mortality observed in ambient communities in this location (~2.6%; First et al. 2012). The baseline mortality rate likely represents both *in situ* mortality (organism natural death and senescence) and *in vitro*

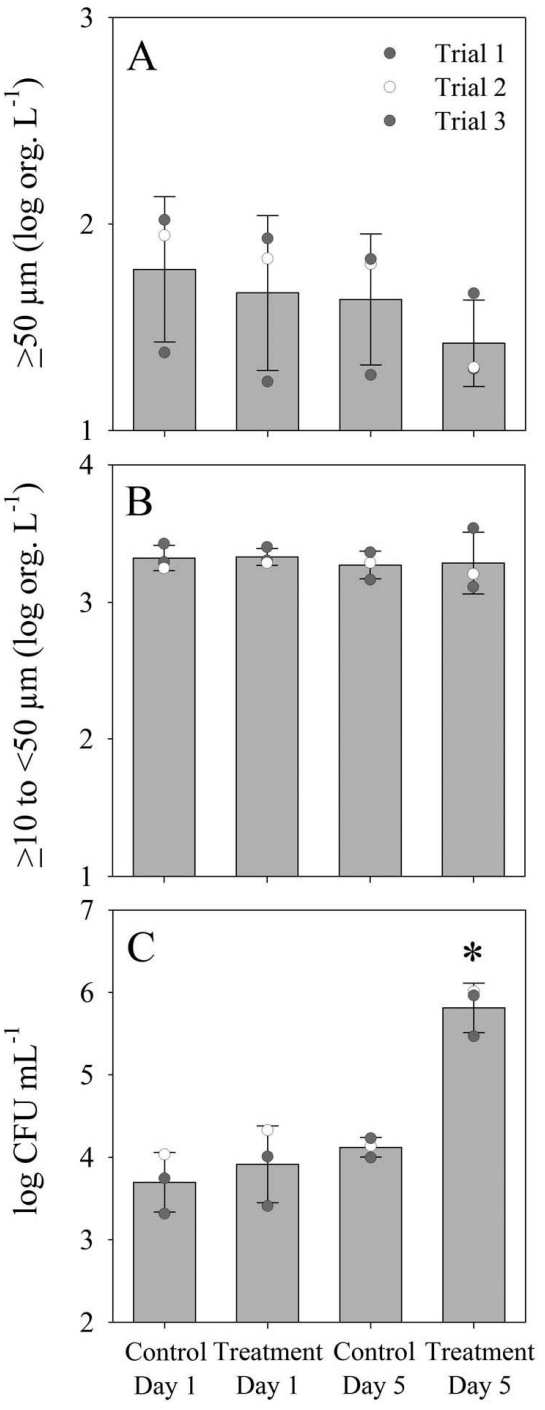

Figure 2. Log concentrations of living organisms in the A. ≥50 µm, **B**. ≥10 to <50 µm, and C. <10 µm size classes. Organisms in the <10 µm size class were culturable, aerobic, heterotrophic bacteria. Bars show the mean concentrations (±1 SD, n = 3) for the control and treatment mesocosms initially (day 1) and at the end of the experiment (day 5). Symbols show the concentrations from each of the three independent trials. The asterisk marks significantly different concentrations (ANOVA, $p>0.05$), which only occurred in bacterial concentration in the treatment tank on day 5.

mortality, which could include, for example, damage during sample processing and autogenous mortality during containment. Regardless, the low mortality rate—while not reflective of total organism loss—indicates that conditions within the amended mesocosm were suitable to these organisms. If organisms in the ≥50 µm and the ≥10 to <50 µm size classes did react to the additives, their response was not apparent or observable within the changes in concentrations. Initial concentrations of organisms in the mesocosms were less than the challenge water concentrations specified for the ≥ 50 µm and ≥10 and <50 µm size classes (10^5 m^{-3} and 10^3 mL^{-1}, respectively; US EPA 2010). However, organisms were not added to the mesocosms to meet these criteria, as the goal of these experiments was to examine the direct effects of the additives. The addition of organisms to the experimental mesocosms would potentially cause greater and more stochastic fluctuations in organism populations, complicating the comparison between the response of organisms in the control and treatment mesocosms.

The only significant response was the consistent increase in bacterial concentrations in amended mesocosms after 5 d incubations (ANOVA, $p<0.05$; Figure 2C and Figure 3). The increase in bacterial concentrations was coincident with the large relative decline in the DOM concentration in amended mesocosms. On average, DOM concentrations in amended mesocosms declined from 15.0 ± 0.8 mg L^{-1} (Table 1) to 10.1 ± 0.1 mg L^{-1}. Relative concentrations, which demonstrate the change in concentration over the experimental incubation, were significantly lower for DOM in the treatment tank relative to the control tank (Figure 4).

The infusion of organic matter predictably stimulated heterotrophic bacteria, as bacterial rapidly respond to increases in carbon availability in estuarine environments with episodic and periodic inputs of terrestrial carbon (e.g., Iriarte et al. 2003). The secondary impacts on larger organisms and higher trophic levels, however, are less predictable. Bacteria are generally consumed by nanoflagellates and ciliates, which are in turn available to larger consumers (e.g., phagotrophic protists and microinvertebrates). Protists can also respond rapidly to exploit high prey concentrations, and therefore, rapid shifts in the microbial food web can occur in short time periods (~1 d; Agis et al. 2007; Kim et al. 2011). Regardless, over the time scale of these trials, no such changes were evident.

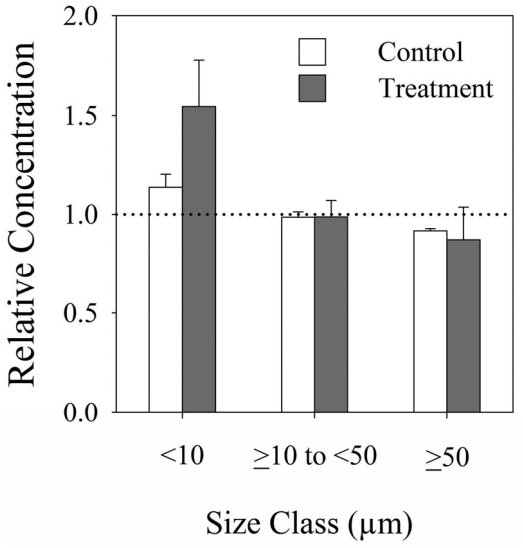

Figure 3. Relative changes in the concentrations of organisms in the three size classes measured in the field experiments. Relative concentrations are calculated as the log-normalized concentrations measured at day 5 compared to day 1 and display the concentration changes within size classes throughout the experiment: the horizontal dotted line marks unity, indicating no change in concentration, and relative concentrations above and below this line indicate increases and declines in concentrations, respectively. Bars show the mean (+1 SD, n = 3) for both the control and treatment mesocosms.

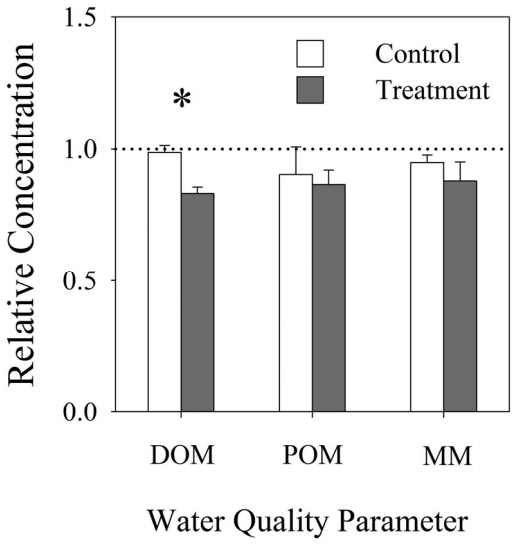

Figure 4. Relative changes in the concentrations of water quality parameters: dissolved organic matter (DOM), particulate organic matter (POM), and mineral matter (MM). The figure is arranged as Figure 3 (see the caption for Figure 3 for additional details). Bars show the mean (+1 SD, n = 3) for both the control and treatment mesocosms. The asterisk marks significant differences in the relative concentration between the control and treatment mesocosms (ANOVA, $p>0.05$), which only occurred for DOM concentrations.

Increased activity of heterotrophic bacteria can also affect the water chemistry by increasing O_2 demand and assimilating dissolved nutrients. Additionally, bacterial growth in high DOM water can lead to aggregation of particles and, in turn, the sedimentation of large aggregates (Alldredge and Silver 1988). Finally, viral infection of bacteria represents a consistent source of mortality—approximately 20–40% of the community is lysed daily—which affects the cycling of organic matter in surface waters and subsequent burial in sediments (Suttle 2007).

Prevalence of challenge water concentrations

A search of published reports was undertaken to determine if the minimal concentrations of dissolved and particulate matter used in land-based testing following the ETV Protocol are in accord with concentrations found in coastal waters. Values reported were primarily from sampling locations in North America, including along the Atlantic and Pacific coastlines, in the Gulf of Mexico, and in freshwater locations. For consistency, concentrations of DOC and POC were converted to DOM and POM using a single conversion factor (40%, representing the mass contribution of carbon to the generic formula for organic matter: CH_2O). ETV challenge water conditions were within ranges observed in the nearshore and estuarine environments (Figure 5). Concentrations of DOM ranged from 5–10 mg L^{-1} (2–4 mg L^{-1} of DOC). The maximum concentration of DOM observed was 31 mg L^{-1}, which is equivalent to 12 mg L^{-1} of DOC. The ETV minimum concentration for DOC is 6 mg L^{-1}. Concentrations of POM ranged from 1–4 mg L^{-1} (0.4–1.6 mg L^{-1} of POC; the ETV minimum concentration of POC is 4 mg L^{-1}). The highest POM concentration observed was 17 mg POM L^{-1} (7 mg L^{-1} of POC). Concentrations of TSS were, of the three parameters examined, the most variable, ranging from 1 to 90 mg L^{-1}; concentrations ranged from 10 to 40 mg L^{-1}. The ETV minimum concentration is 24 mg L^{-1}, which is within the middle to lower range of TSS concentrations. Thus, the concentrations of water quality parameters prescribed in the ETV Protocol are reflective of the upper range of typical concentrations of DOM and POM reported for near-shore and estuarine environments. The DOM and POM values, in particular, seem to warrant more attention: the ETV value is, in most cases, higher than the mean recorded values from the literature.

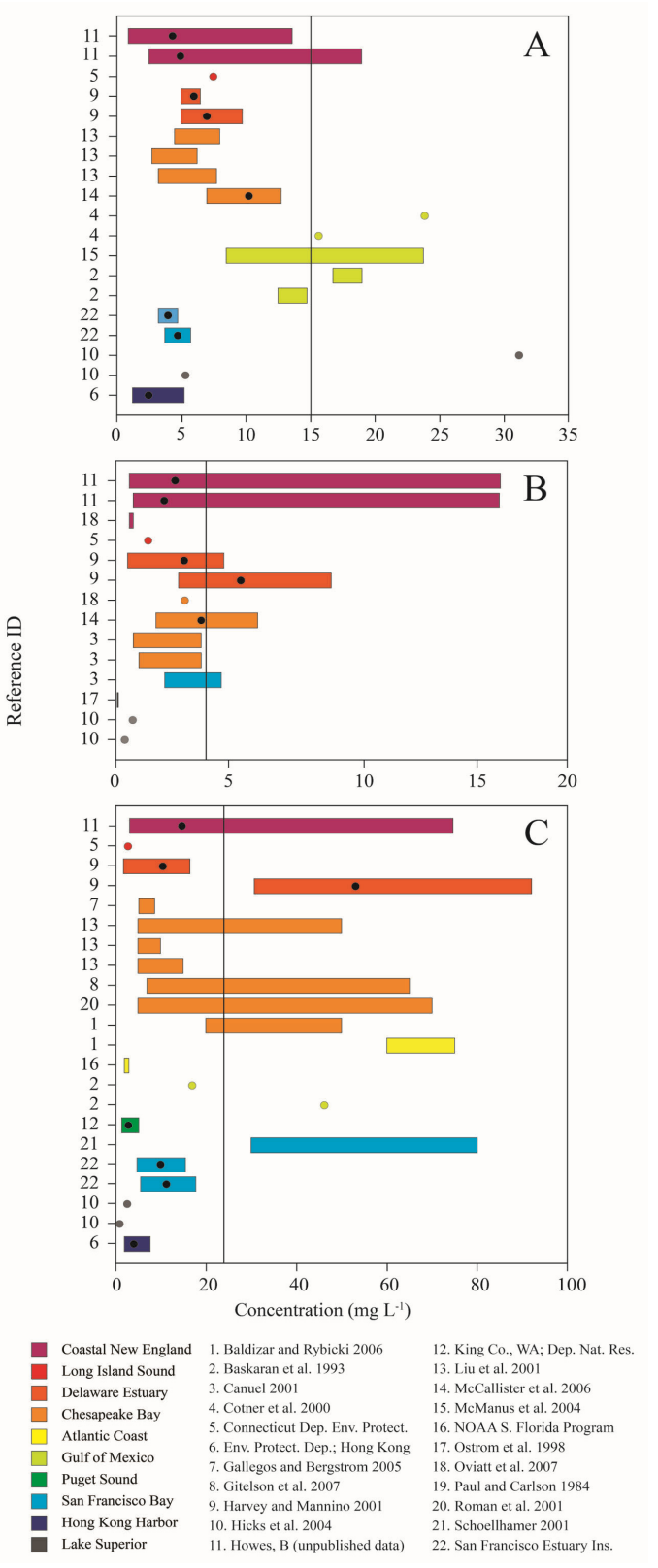

Figure 5. Summary of values of A. DOM, B. POM, and C. TSS in coastal environments, as reported in peer-reviewed publications, government websites, and personal communications. Sources of the data identified by a reference number, which refers to the numbered citations on the right (full citations are in the literature cited). The colors indicate the general geographic range, with the references grouped by location. Colored bars indicate data ranges, which show the minimum and maximum values reported. Symbols show the mean concentration reported. When both ranges and mean values were reported, the mean values are shown as black symbols. Vertical lines represent the minimum concentrations set in the ETV Protocol (US EPA 2010).

Conclusions

Amending challenge water with additives has the potential to directly restructure the community of organisms, and potentially, affect the response of organisms to ballast water treatment. Although concentrations of heterotrophic bacteria consistently and significantly increased in water augmented with additives relative to control water, the additives used in this study did not significantly or systematically affect the concentration of living organisms in the ≥10 to <50 µm or the ≥50 µm size classes. It is possible that secondary impacts of amending the test water—such as a depletion of O_2 and concomitant mortality of micro-invertebrates or an increased dominance of heterotrophic microorganisms—could cascade to higher trophic levels. No such effects were evident, however, on the time scale used in this study (5 d), which represents the longest hold times currently used in land-based testing of BWMSs. Therefore, the use of additives described in the ETV Protocol does not appear to affect the concentration of living organisms in the two largest size classes.

The minimum requirements for DOM, POM, and TSS concentrations in the ETV Protocol were set to provide "challenging, but not rare, water quality conditions representative of the natural environment" (US EPA 2010). A search of published data showed that TSS values listed in reports and peer-reviewed publications appeared to meet those criteria. The reported DOM and POM concentrations, however, usually fell short of the ETV Protocol minimum values, warranting further scrutiny.

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