

Potential of Venturi Oxygen Stripping to Stop Ballast Water Invasions in Freshwater Environments

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Abstract:

Invasive species are considered to be one of the most destructive environmental problems facing the world today. They can alter habitats, cause extinction of native species, and have enormous related economic costs. Because ballast water is the primary source of aquatic invasions, the International Maritime Organization has recently passed regulations that will require ocean-going vessels to treat water prior to discharge. It has proven challenging, however, to find an environmentally friendly treatment that is effective at reducing the potential for invasions and yet also acceptable to the shipping industry in terms of safety, time and cost. This study examined a ballast water deoxygenation treatment system called Venturi Oxygen Stripping because it has been shown to remove estuarine and marine plankton while also reducing ballast tank corrosion. The specific focus was to examine the efficacy of this treatment on lake organisms to determine if VOS is appropriate for vessels operating freshwater environments. Results suggest that VOS can be an effective ballast water treatment option.

Introduction:

Invasions by non-native aquatic species are increasingly common worldwide in coastal habitats (Cohen and Carlton 1998, Ricciardi 2001). For instance, over 150 non-indigenous species have been documented in both the Chesapeake Bay (Ruiz et al. unpub. data) and Great Lakes systems (Grigorovich et al. 2003, Nicholls and MacIsaac, et al. 2003). Although the effects of many invasive species on habitats and communities remain unknown, some have had demonstrably strong and negative impacts. One of the most obvious examples is found in the freshwater environment of the Great Lakes. The zebra mussel (*Dreissena polymorpha*) introduction into the Great Lakes region has resulted in massive ecological changes and billions of dollars in economic costs associated with damage and control (Johnson and Carlton 1996, MacIsaac et al. 2002a).

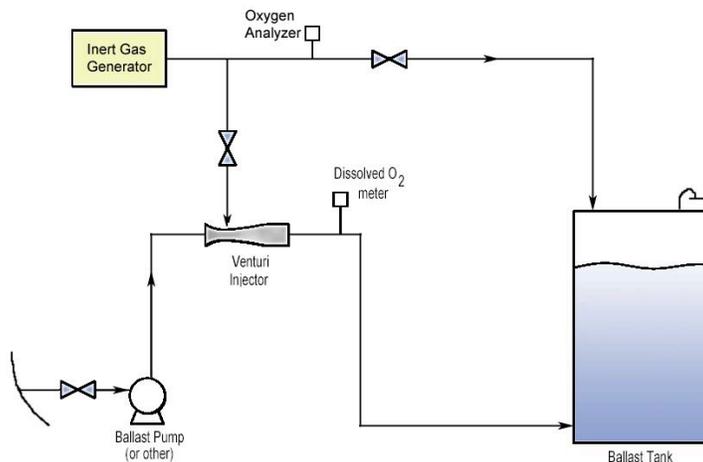
Global shipping, which moves 80% of the world's commodities and is fundamental to world trade, inadvertently transports many aquatic organisms (see review by National Research Council 1996). In particular, ballast water is considered the most important vector responsible for transporting and introducing non-native aquatic species to new biogeographic regions (Carlton and Geller 1993, Ricciardi 2001). Vessels commonly pump in water at one port and discharge it at another. Many planktonic organisms captured in ballast waters survive even lengthy journeys onboard ships. Examination of ballast water upon arrival of vessels has revealed living and viable bacteria (McCarthy and Khambaty 1994, Ruiz et al. 2000), protists (Galil and Huelsmann 1997), dinoflagellates (Hallegraeff and Bolch 1991), diatoms, zooplankton, benthic invertebrates, and fish (Williams et al. 1988, Carlton and Geller 1993 and Wonham et al. 2000). Increasing international traffic by increasingly large vessels translates into ever more enormous amounts of water, and planktonic organisms, being moved around the world by ballast water transport (e.g., the largest oil tankers can now have up to 40,000,000 gallons of ballast water capacity).

Since transport in ballast tanks is a major source of introductions, attention has focused recently on means of treating ballast water. However, it has proved challenging to find an environmentally friendly technique that is effective at reducing introductions and yet is also acceptable to the shipping industry in terms of safety, time, and cost. For instance, offshore exchange of ballast water is currently required for ships entering US ports from overseas to reduce introductions but the process is time-consuming (thus costly), cannot be performed in rough sea conditions, and has limited effectiveness in some environments and for certain vessel designs (e.g., Cooper et al. 2002, Ruiz et al. in prep.).

In an attempt to address this ballast water invasions the International Maritime Organization (IMO) has agreed upon a set of standards for organisms released in ballast water when a vessel arrives in port. The February 2004 International Convention for the Control and Management of Ships' Ballast Water, states that ships conducting ballast water management shall discharge less than 10 viable organisms per cubic metre greater than or equal to 50 μm in minimum dimension and less than 10 viable organisms per milliliter less than 50 μm in minimum dimension and greater than or equal to 10 μm in minimum dimension; and discharge of the indicator microbes shall not exceed the specified concentrations. The indicator microbes, as a human health standard, include, but are not be limited to: A) Toxicogenic *Vibrio cholerae* (O1 and O139) with less than 1 colony forming unit (cfu) per 100 ml or less than 1 cfu per 1 gram (wet weight) zooplankton samples; B) *Escherichia coli* less than 250 cfu per 100 ml; C) Intestinal *Enterococci* less than 100 cfu per 100 ml.

Dr. Tamburri and colleagues have been evaluating a system called Venturi Oxygen Stripping (VOS, developed and patented by NEI Treatment Systems, LLC) that appears to be an answer to the invasive species problem. VOS limits the number of aquatic organisms surviving transport in ballast tanks, while simultaneously giving the ship owners an economical advantage by significantly reducing ballast tank corrosion rates. (Tamburri et al. 2002, Tamburri et al. 2003).

Fig. 1. Basic schematic of the VOS system onboard a vessel.



VOS is a rapid, in-line system that mixes inert gas (mostly nitrogen with small amounts of carbon dioxide and only trace levels of oxygen) directly into ballast water as it is drawn into the vessel (Figure 1). The gas is mixed with the

ballast water using a venturi injector manifold that creates a micro-fine bubble emulsion where dissolved oxygen quickly diffuses out of the water into the gas. Because adding carbon dioxide in solution forms both carbonic and carboxylic acid, the pH of treated water is also reduced to between 5.5 and 6.

Dr. Tamburri's laboratory, pilot-scale and full-scale prototype results to date demonstrate that this system can meet IMO regulations with holding times of greater than four days for estuarine and marine organisms (Tamburri et al. 2003). Although it is clear that the hypoxic conditions alone are toxic to the majority of planktonic organisms, it is the combination of reduced oxygen levels (between 0.2 and 1.0 mg/l), CO₂ saturation with lowered pH (between 5.5 and 6.0), and mechanical disruption as organisms pass through the venturi injector that is responsible for the elimination of potential invaders. Therefore, VOS is not simply a deoxygenation treatment but a single unit or method that produces a combination treatment.

While previous studies have found very promising results, the efficacy of the VOS system has yet to be evaluated for freshwater organisms. Given the enormous problem of ballast water invasive species found in freshwater environments, the focus of this study was to determine if VOS can eliminate planktonic lake organisms in a series of laboratory experiments.

Hypothesis: 1) There will be significantly fewer live Zooplankton, Phytoplankton and Bacteria in freshwater treated with VOS system after treatment than before. 2) There will be significantly fewer live Zooplankton, Phytoplankton and Bacteria in the freshwater treated with VOS than in controls overtime.

Methods:

Natural water was collected from Lake Lariat, Maryland, for use in all experiments. Lake Lariat is located 6 miles north of Chesapeake Biological Laboratory (CBL) and is part of the Patuxent Watershed. The surface area of this lake is approximately 86-100 acres, and is an impoundment (man made lake) created in 1965. All experiments were carried out at CBL during July and August 2005. For each experiment, lake surface water was collected from a dock in three 16-L pails. Lake water was then transported to the laboratory and used in experiments within 2 hours of collection.

For each holding time experiment (described below), approximately 40 L of lake water was placed in a large tank containing a submerged centrifugal pump. (Figure 2). Using a series of valves and tubes, water would either be pumped directly to three 1 L flasks open to air (controls) or through a small-scale VOS system before entering a second similar set of three 1 L airtight flasks (treated). The small-scale VOS introduced a mixer of nitrogen and CO₂ through a half-inch venturi injector as micro-fine bubbles, which in turn lowered oxygen levels to hypoxia and lower pH to approximately 5.5 (see results). After the water is pumped into the three control and three treated flasks, they were immediately placed in the dark to mimic the light environment in ballast tanks onboard vessels. Dissolved oxygen, temperature, pH, and salinity of control and treated water was monitored before and after the trials with a multi-parameter YSI water quality instrument.



Fig. 2. (A) Small-scale VOS system with associate tubing and valves. (B) Light-tight container with the six identical 1-L flasks. The three control flasks are open to air during experiments, while treated flasks are sealed with a ball valve.

From previous work, 48 hours is commonly required for significant mortality of zooplankton and 96 hours for phytoplankton held under VOS conditions (see Tamburri et al. 2002, 2003). Therefore different holding times of 24, 48, 72, 96, and 120 hours were examined for freshwater organisms in separate experiments. Prior to all holding time experiments, the planktonic community of initial water was quantified using the methods for the three IMO categories (described below).

To quantify zooplankton both before and after the various holding times, the entire known volume of the containers was passed through a 50 µm screen and examined under a dissecting microscope. Numbers of live and dead organisms were score by general taxonomic group. Living individuals were identified by examining reactivity or movement.

Because of the inherent difficulties in identifying live versus dead protests (organisms between 10 and 50 µm in size), this project focused quantifying changes in chlorophyll concentrations (µg/L) as an indicator of viable phytoplankton using mainly in-vivo fluorometry and in one experiment extractive chlorophyll fluorometry. In addition to determining chlorophyll concentrations prior to beginning the experiments, 4 ml samples of water were collected from each container (control and treated), after a stirring and analyzed for in vivo chlorophyll concentrations immediately after the experiment ended. Finally a 100ml sample was taken from each container and placed under grow-lights, with algae growth nutrients, for 48 hours. Samples for in vivo chlorophyll were taken after a 24 and 48 hour intervals. Although precise abundances of cells/ml cannot be determined for diverse communities of phytoplankton using this regrowth approach, this appears to be the only feasible method to determined presence/absence of living organisms.

The final biological component monitored was the bacterial indicators identified in the IMO standards, excluding *Vibrio cholerae* because the cost and hazards of working with this particular pathogen. One 100 ml sample of water from each treated and control container was analyzed for concentrations of culturable *E. coli* and *Enterococci* using a commercially available chromogenic substrate, most probable number method (IDEXX Laboratories, Inc.; Noble et al. 2003).

Results:

Physical Conditions – Table 1 described the conditions of Lake Lariat water used in the experiments. Dissolved oxygen in initial lake water was at normal levels. The water in three control containers after various holding times had decreases but remained well above lethal levels for aquatic organisms. The water treated with VOS however, remained at hypoxic levels through out the experiments, regardless of holding time. Similarly, pH was normal in controls throughout the experiments and dropped to slightly acidic levels in treated water.

Table 1. Range in values for dissolved oxygen (DO), pH, temperature and salinity measured in ambient lake water, post control and post VOS.

	Lake Lariat (ambient)		Control		VOS	
	Average	SD	Average	SD	Average	SD
DO (mg/l)	8.44	0.559	6.64	0.406	0.38	0.127
pH	7.57	0.155	7.61	0.19	5.29	0.169
Temp (°C)	28.62	2.181	28.45	2.521	28.92	2.521
Salinity (ppm)	0.05	0	0.05	0	0.05	0

Zooplankton (> 50 μm) – The preliminary counts of living zooplankton in initial water collected from Lake Lariet ranged from 101,000 upwards to 402,000/m³. There was one day (120 hr experiment shown above) where the numbers were high compared to the other days. Under control conditions organisms showed a slow expected drop in abundance. This is most likely due to being held in darkened laboratory conditions. However under VOS-treated conditions, organisms quickly died (Figure 4). There were virtually no living organisms alive in any of the holding time after treatment with VOS. The one exception was in the 24-hour run where one nematode (total out of the three replicates) was found alive. Figure 3 displays the values for each holding time (666.7/m³ corresponds to the one live nematode).

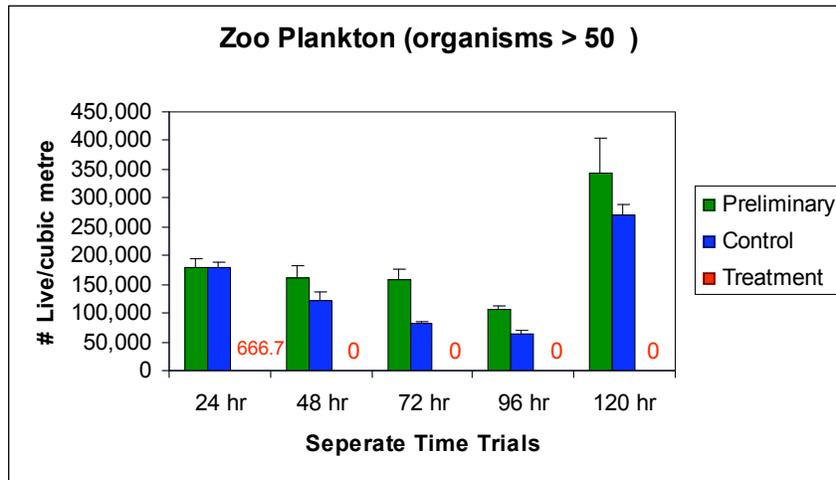
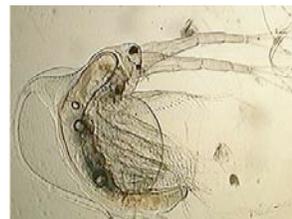


Fig.3. Number of live organisms > 50 μm per cubic meter of water after replicate experiments (Control n=3 and VOS n=3) for 1, 2, 3, 4, 5 days holding times. Preliminary ambient lake samples were also displayed as a reference for starting conditions.

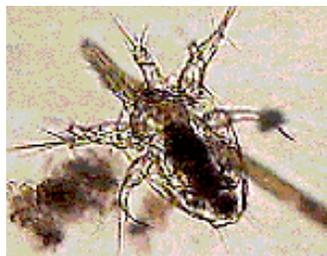
Zooplankton species diversity was fairly low in the natural lake water. Some of the most common zooplankton found are shown below in Figure 4.



Copepod *Crustacea*



Holopedium *Crustacea*



Nauplius *Crustacea*
(copepod larvae)



Hexarthra *Rotifera*

Phytoplankton (10 - 50 μm) – Initial in vivo chlorophyll values suggested abundant and healthy phytoplankton when all experiments were begun. However, while there were decreases in chlorophyll levels after the various holding times, no clear trend is apparent after regrowth experiments (Figure 5). The impact of VOS on freshwater algal is therefore difficult to discern using this fluorometric regrowth technique.

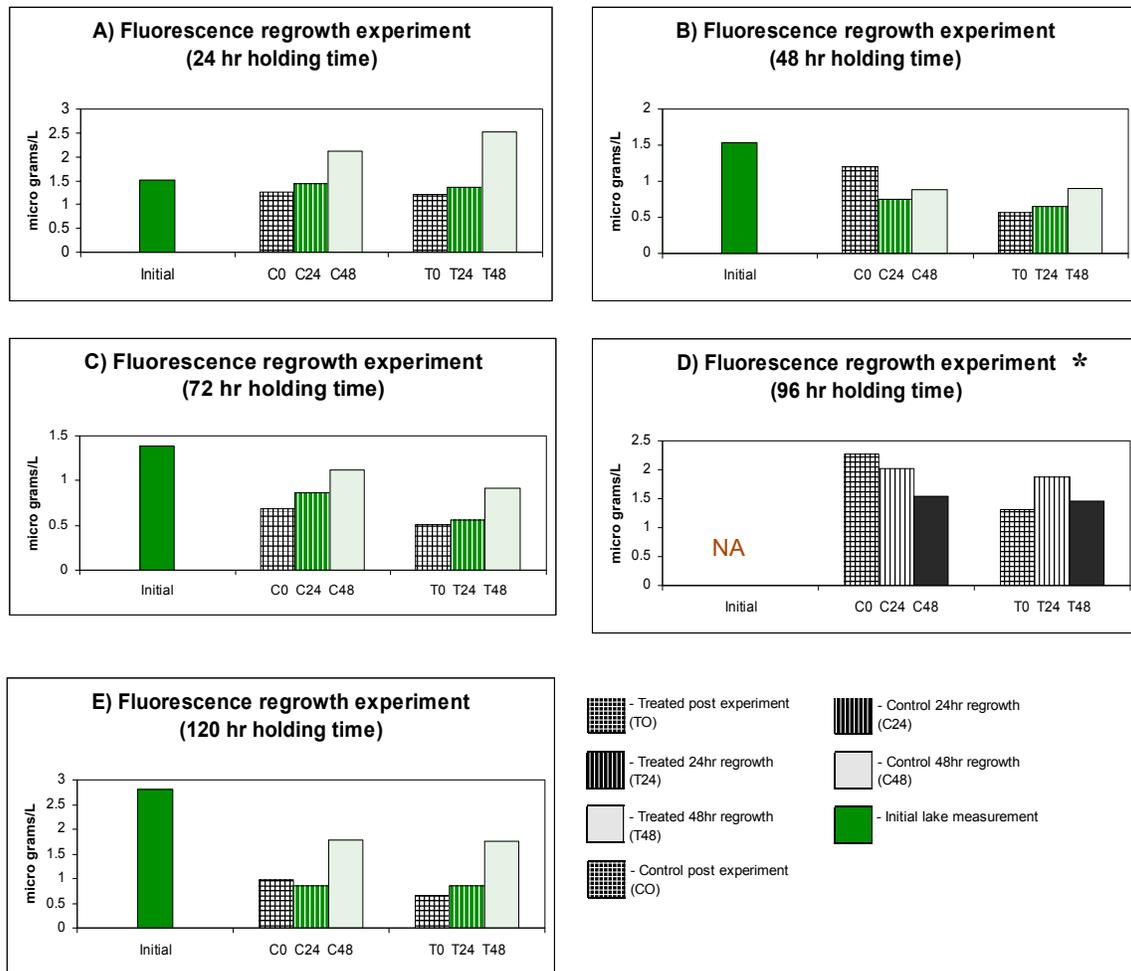


Fig.5. Results from in vivo or extractive* chlorophyll analyses of lake water just prior to starting the experiment are plotted (green). Values for Control (blue, n=3) and VOS treated water (red, n=3) are shown for various holding times (A, B, C, D and E) at completion of experiment, after 24 hr regrowth, and after 48 regrowth periods. Initial value for 96 hr was not taken (NA).

Bacteria – Mixed results were found when examining response of *Escherichia coli* to the VOS treatment (Figure 6). When comparing treated vs. control the expectation was to have lower colony forming unit (cfu)/100ml in treated and higher in controls. While not significant, the trend is for slightly more in treated. There are higher values in the 72 hour lake water sample (treatment was at 132.2/100ml and control at 87.6/100ml) but the amounts in all other samples generally is below 10/100ml. It is important to note that all levels were extremely low and are significantly below IMO standards. Similarly, *Enterococci* showed very low counts, highest being 3.1/100ml in the controlled 72-hour trial. *Enterococci* therefore was also well below IMO standards.

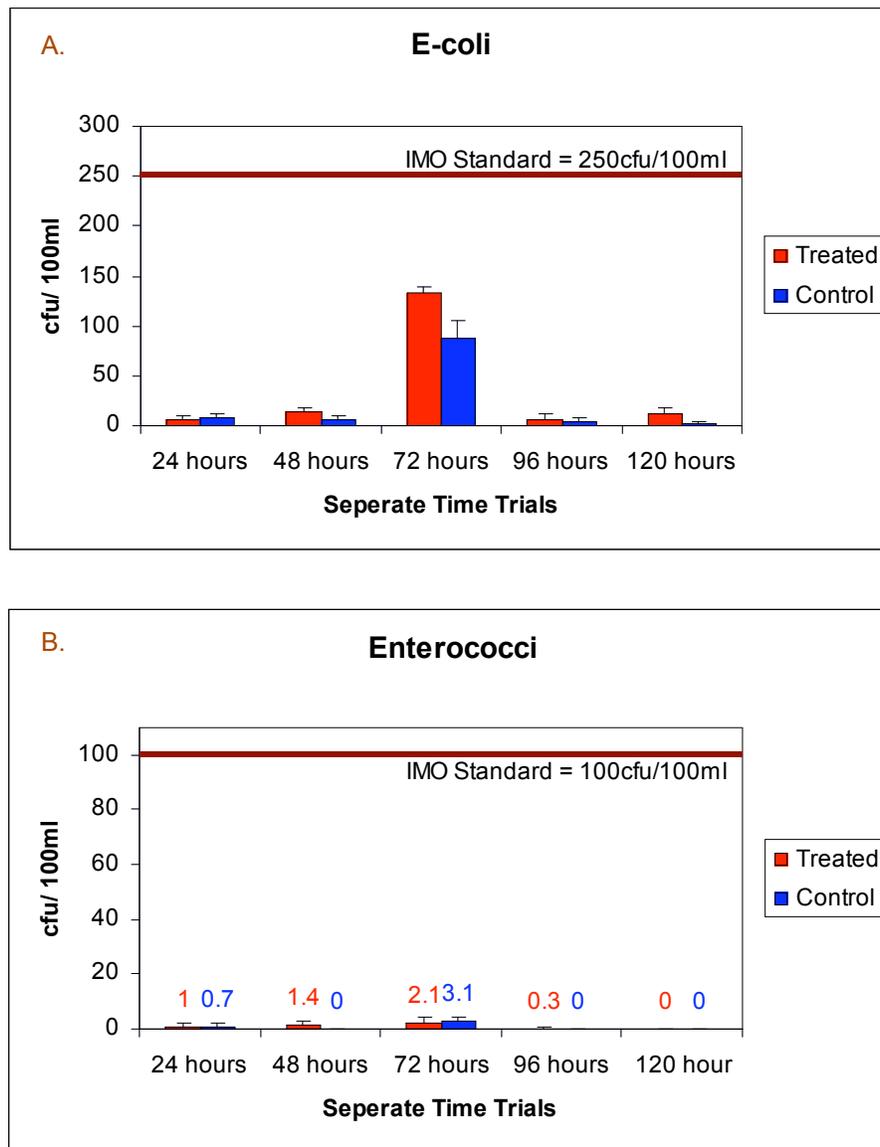


Fig.6. Abundances of *E. coli* (A) and *Enterococci* (B) in Control (n=3) and VOS-treated (n=3) lake water were quantified after 24, 48, 72, 96 and 120 holding periods. 100 ml water samples were analyzed with a commercially available chromogenic substrate method (IDEXX Laboratories, Inc.; Noble et al. 2003).

Discussion

The basic goal set at the beginning of this research project was to evaluate the efficacy of the VOS system to kill freshwater organisms. Some results were clear and congruent with the hypothesis but some were less so. The first section of study was zooplankton. There will be significantly fewer live Zooplankton in 1) freshwater treated with VOS system after treatment than before, and 2) freshwater treated with VOS than in controls overtime. This hypothesis was found to be accurate on both accounts, in all the trails there were virtually no zooplankton alive after undergoing VOS treatment. There was however one nematode still alive after a 24hr trial in one of the beakers. Unfortunately because numbers found were extrapolated up to a 1m^3 volumes, that one nematode equivocates into 666.7 org/m^3 (Fig.4). A possible explanation is that this organism is by nature a benthic inhabitant, therefore conditioned to hypoxic conditions. However the basic hypotheses are verified and the data correlates with findings from others previous work; 48 hours is commonly required for significant mortality of aquatic organisms held in hypoxia to be found (Tamburri et al. 2002, 2003). A high reading of organisms/ m^3 in the 120-hour trial is most likely due to a hotspot

The hypotheses for the phytoplankton portion were the same as zooplankton, but more difficult to interpret. The first hypothesis was proven inaccurate because the first time trial of 24 hours produced higher control and treated than that of initial numbers. This however only applies to the 24hr trial possibly meaning VOS was not given enough time to effectively kill off enough phytoplankton. The other lengthier time trials show definite drops in phytoplankton quantities across the board when comparing initial to control and treated. The mixed results made the next step of comparing quantities of treated vs. control difficult. The second hypothesis was proven inaccurate because quantities in treatments were not always lower than that of controls. While the 96 and 120 hour trials did show a lower rate of regrowth in treated water vs. control levels, the 24 and 48 hour trials actually show increased growth in treated water. The results are mixed however still positive, showing that generally over time things are dying.

The bacterial portion of this experiment produced results which do not argue against, nor validate the hypotheses. The reason is due to the extremely low natural levels of bacteria, both *E. coli* and *Enterococci* alike. There is a trend showing that there are more bacteria in treated containers. A possible explanation for this is the fact that all the treated containers have dead and decomposing zooplankton remains, therefore thrive more in this type of environment. However the important message is that all bacteria levels in all trials are significantly and desirably lower than the IMO standards.

In conclusion –While results for some of the specific biological parameters examined are difficult to interpret, responses of freshwater organisms appear to be very similar to those of estuarine and marine plankton (Tamburri et al. 2002, 2003). With additional replication and refinement of experimental approaches, it is likely that VOS will demonstrated to meet IMO regulations after four to five days of ballast water holding time and to greatly reducing the risk of potential aquatic invasions in freshwater environments.

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