OZONE, SEAWATER and AQUATIC NONINDIGENOUS SPECIES:
Testing a Full-Scale Ozone Ballast Water Treatment System on an American Oil Tanker

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1 EXECUTIVE SUMMARY

1.1 Background

The transport of microscopic organisms found in the ballast water of marine vessels can be a major worldwide threat to the environment and public health. Many of these organisms are not resident to the environment into which they are transported in ballast waters, and thus are collectively referred to as “non-indigenous species (NIS).” Because they are not adapted to local ecosystems, NIS can substantially disrupt the structure and function of coastal marine waters into which they are introduced. The U.S. Coast Guard also estimates that NIS introductions cause approximately $6 billion in economic damage in the United States annually. For example, the U.S. government estimates that, over the past 10 years, it has cost nearly $4 billion to repair damage caused by the non-indigenous zebra mussel to shorelines, water treatment, and power generating stations in and around the Laurentian Great Lakes.

Although many transfer mechanisms (or vectors) have contributed historically to the invasion of coastal habitats by aquatic NIS, shipping has been the vector responsible for many of the known invasions. Ballast water exchange, or mid-ocean exchange, occurs when ships replace coastal waters in their ballast tanks with open ocean water to reduce the abundance of coastal NIS. Ballast water exchange is currently the only management strategy available for ships to reduce the quantities of non-indigenous coastal plankton in ballast water, but is generally viewed only as a temporary measure to reduce the risk of invasions. It is a management strategy that many ships can implement immediately, and which does not require retrofitting or development of new technology. However, ballast exchange has some significant limitations. First, it is not always possible to safely conduct an exchange, because of risks to the structure and safety of vessels (especially in heavy seas). Second, even when performed, ballast exchange still leaves a residual of coastal organisms.

Therefore, efforts are now underway to develop and implement technological alternatives to ballast water exchange. Although many treatment possibilities are being explored, their evaluation is at an early stage, and no alternative treatments have yet been approved by state, regional, or federal regulatory authorities. At the present time, the U.S. Coast Guard (as directed by the National Invasive Species Act of 1996) requires that alternative treatments be, at a minimum, as effective as ballast water exchange. However, no specific guidelines or minimum standards of efficacy presently exist to assess the performance of these alternative treatments.

1.2 Testing the Effectiveness of Ozone as a Potential Treatment Technology

In 1998, BP Alaska and Nutech O3, Inc. undertook the development and testing of ozone gas as a potentially effective alternative method of decontaminating ballast water infested with NIS. A full-scale prototype ozonation system was recently installed and tested on board the BP-affiliate ship the *S/T Tonsina* (Alaska Tanker Company).

BP and Nutech subsequently partnered with several prominent academic and industrial research institutions to design and implement a rigorous, independent scientific investigation in the ozone system’s ability to remove NIS from marine ballast waters. The study described in this report represents the first of what is hoped will be several experimental phases designed to provide a full evaluation of the efficacy of the prototype Nutech O3, Inc. ozone system that is currently installed on the *S/T Tonsina*. The primary goal of this present (Phase 1) study was to conduct a field-scale test of the operation and effectiveness of this ballast water treatment system for removal of a wide range of coastal marine organisms. While earlier studies suggested that the process was quite effective on bacteria, its performance with respect to higher organisms at the field scale was untested prior to the present study.

The specific objectives of the present study were to:

1) determine the disinfection effectiveness of a full-scale ozone system in comparison with ballast water exchange efficiency.

2) determine the acceptability of discharging treated ballast water using whole effluent toxicity testing, and to determine the latent toxicity of the subsequent ballast water discharge.

3) obtain operational experience with the prototype ozone system in order to implement further system improvements.

1.3 Experimental Design

The *S/T Tonsina* is an 869-foot, double-hull oil tanker on which a prototype ozonation system has been installed and connected to the ship's 12 segregated ballast water tanks, each of which has a capacity of approximately 850,000 gallons. This initial Phase I study relied upon Niskin bottle grab samples and vertical net tows to collect samples from the ballast water tank using several access points (manways or Butterworth® openings) on the deck of the ship to two of these tanks: No. 3 wing port (O_3 treatment) and No. 3 wing starboard (air-sparged control).

One preliminary and three full experiments were conducted over the course of one year. The preliminary test, designed to provide data for the full scale testing, provided information on the formation of reaction by-products and bacterial effectiveness. Experiment 1 closely mimicked the ozone dosage that could be achieved on the *S/T Tonsina* during routine operations. During a typical 3.5-day voyage, the ozone system would apply 0.62 mg/L/hr ozone to the 2,849,537 L of each segregated ballast water tank in the vessel for a duration of five hours. This would be achieved by treating the 12 segregated ballast water tanks separately. During experiment 1, the ozone-loading rate was 0.59 mg/L/hr and lasted 5 hours. Experiment 2 achieved an ozone-loading rate of 0.86 mg/L/hr that resulted from improved operation of the ozone generator. In experiment 3, where only the vertical portions of the tanks were treated and the experiment lasted for 10 hours, an ozone-loading rate of 1.35 mg/L/hr was achieved. In Experiments 2 and 3, much larger amounts of ozone were purposely directed to the tank compartment that we sampled which translated in higher ozone loading rates and higher kill rates.

In addition to the three ozone experiments, two ballast water exchange experiments were conducted using ballast tanks that had been filled at the same time using the same seawater as those used in the ozone experiments to obtain a direct comparison between the effectiveness of exchange and ozonation. Grab samples and net tow samples were collected for water chemistry, and microbial and plankton community composition. Samples were collected immediately prior to the ozone experiments, then again prior to and following an open water ballast exchange event using standard *S/T Tonsina* protocols.

1.4 Results

1.4.1 Ballast Water Exchange

Two ballast water exchange experiments were conducted during this study. The organisms that were used to evaluate the effectiveness of ballast water exchange were phytoplankton and zooplankton. Benthic organisms such as amphipods and shore crabs that were included in the ozone studies were not included in the exchange experiments. This was because ballast tanks have large structural elements that attenuate flow and thus provide refuge for these organisms. Furthermore, large organisms tend to be more rare in ballast tanks than small organisms, and those in benthic habitats are difficult to sample. Although it is believed that ballast water exchange will be less efficient at removing organisms from benthic habitats, this remains largely untested. Figure 1.1 summarizes those results.

Results from the first two exchange experiments suggested that the average removal of marine organisms using ballast water exchange on the *S/T Tonsina* was 64 %, which is

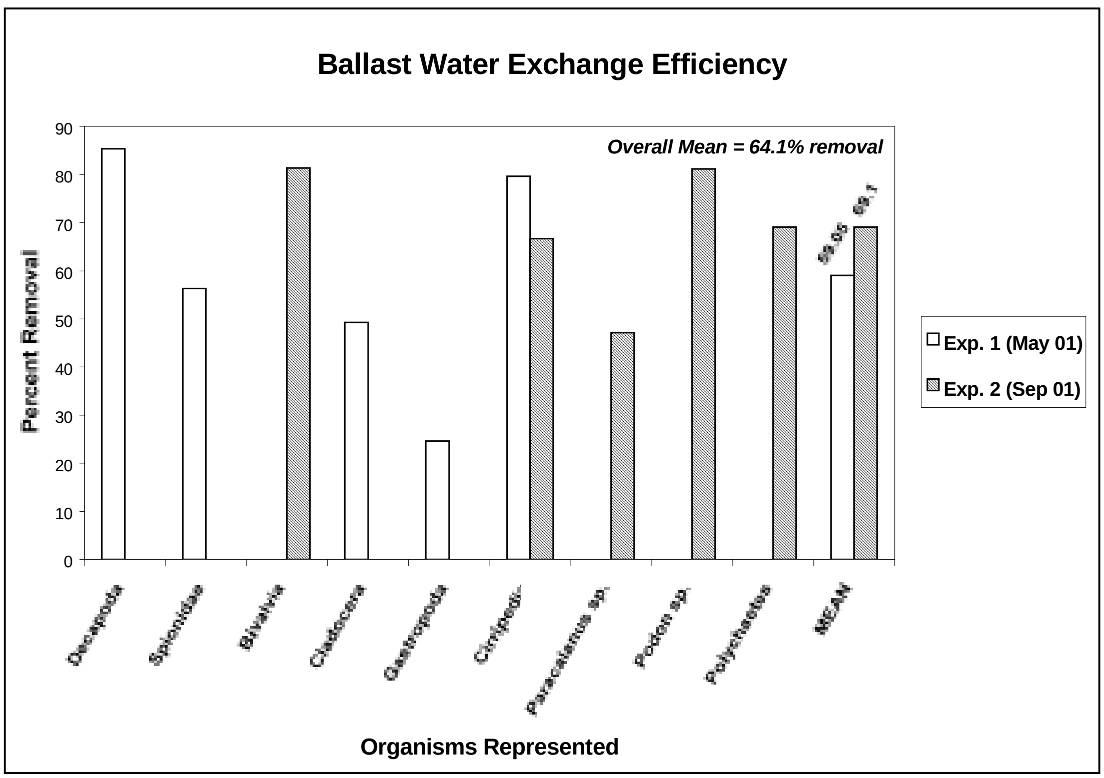

Figure 1.1: Summary of percent removal of marine organisms following ballast water exchange

considerably less efficient than proposed regulatory targets of 95%. This is also less efficient than ballast water exchange efficiencies sometimes measured on other vessels. The direct comparison of ballast water exchange and ozone treatment on the same vessel is critical in evaluating the ozone treatment effectiveness. Moreover, our results (1) underscore the variation the can exist within ship type, and (2) suggest the level of "kill" needed for ozone treatment to surpass ballast water exchange aboard the *S/T Tonsina* may be lower than that for other vessels.

1.4.2 Ozone Chemistry

In seawater where there is a significant concentration of bromide ion (Br^-), ozone is catalytically destroyed with a half-life of five seconds. As expected, there was no ozone observed in any of the ballast water samples we analyzed. Therefore, ozone *per se* can be considered a good oxidant for the disinfection of marine ballast water because it is not chemically persistent.

Bromate ion (BrO_3^-) was never observed in the samples, suggesting that the lower pH of the coastal water favored the formation of hypobromous acid (HOBr). Ozone and its residuals apparently did react with naturally occurring organic matter that resulted in the formation of modest concentrations of bromoform in our experiments. The appearance of bromoform and the fact that no bromate ions (or chloroform) were detected in any of the experiments indicates that bromine (represented by hypobromous acid/hypobromite ions, or $HOBr/OBr^-$) was formed in significant quantities during the ozonation process.

Concentrations of ozone-produced oxidants (i.e., bromine) were measured in ballast waters using an electrode measurement of Oxidation-Reduction Potential (ORP), and a chemical measurement for Total Residual Oxidants (TRO). Ozonation increased ORP levels up to a plateau of ca. 700-800 millivolts (mV), which is consistent with seawater disinfection targets used by commercial marine exhibit aquaria. TRO levels exceeded limits of analytical detection (4 mg/L as chlorine equivalents) in most of the experiments on board the *Tonsina*. The scientific literature suggests that even 4 mg/L TRO should exceed concentrations known to be acutely toxic (e.g., 1-2 mg/L) to many marine organisms.

1.4.3 Ozone Kill Efficiency in Ballast Water Tanks

Tables 1.1 and 1.2 summarize the efficiency of kill for the different organisms, for the different experiments and time of ozonation at the time of sampling. The percent kill is compared to the 64 % exchange efficiency (i.e., percent removal) as measured for the *Tonsina* above. The percent removal for each group is indicated, followed by an indication of whether percent removal (i.e., kill) of that particular organism was greater than (pass), or was less than (fail) ballast water exchange.

The results indicate that:

1. 99.9 % of the culturable bacteria were killed.

2. In separate experiments, not shown in Tables 1.1 and 1.2, no bacterial re-growth was observed after 30 days storage in the dark in the laboratory.

3. Up to 99 % of the zooplankton were killed or near death using the ozone process.

4. Between 92 – 100 % of the phytoplankton were killed using the ozone process (except for diatoms, for which results were inconclusive).

5. Sheepshead minnows appeared somewhat more resistant to the ozone treatment, but in the latter two tests when both dead and near-dead organisms percentages were combined, 98 and 100 % treatment was achieved.

6. Mysid shrimp were effectively removed in one experiment where 78 % were killed or near dead.

7. The benthic organisms studied (shore crabs, amphipods) were not effectively killed or rendered moribund by the ozonation process.

8. These results were consistent with experiments conducted using known numbers and species of marine organisms suspended in the ballast water tanks in mesh cages.

Table 1.1. Ozone **mortality** compared to 64 % *S/T Tonsina* ballast water exchange efficiency. The percent removal is followed by an indication of whether removal of that particular organism was better than ballast water exchange (pass), or was not as good as exchange (fail).

Experiment Number (Sample Time)	Suspended Organisms						Benthic Organisms	
	Bacteria	Zooplankton	Phytoplankton		Sheepshead Minnow	Mysid Shrimp	Amphipods	Shore Crabs
			Dinoflagellates	Microflagellates				
Exp. 1 (5 hrs)	>99.9 % pass	67 % fail	N/A	N/A	2% fail	30 % fail	0 % N/C[1]	0 % N/C
Exp. 2 (5 hrs)	>99.9 % pass	43 % fail	94 % pass	92 % pass	N/A	N/A	N/C	N/C
Exp. 2 (10 hrs)	>99.9 % pass	82 % fail	N/A	N/A	8 % fail	77 % fail	15% N/C	10 % N/C
Exp. 3 (5 hrs)	>99.9% pass	85 % fail	100 % pass	97 % pass	N/A	N/A	N/A	N/A
Exp. 3 (10 hrs)	>99.9% pass	94 % fail	N/A	N/A	100 % pass	69 % fail	7% N/C	0 % N/C

[1] N/C = No comparison possible (i.e., benthic organisms not sampled during ballast water exchanges)

Table 1.2. Ozone **mortality + moribund** compared to 64 % S/T Tonsina ballast water exchange efficiency. The percent removal is followed by an indication of whether removal of that particular organism was better than ballast water exchange (pass), or was not as good as exchange (fail).

Experiment Number (Sample Time)	Suspended Organisms						Benthic Organisms	
	Bacteria	Zooplankton	Phytoplankton		Sheepshead Minnow	Mysid Shrimp	Amphipods	Shore Crabs
			Dinoflagellates	Microflagellates				
Exp. 1 (5 hrs)	>99.9 % pass	79 % pass	N/A	N/A	47 % fail	45 % fail	3 % N/C	0 % N/C
Exp. 2 (5 hrs)	>99.9 % pass	71 % pass	94 % pass	92 % pass	N/A	N/A	N/A	N/A
Exp. 2 (10 hrs)	>99.9 % pass	87 % pass	N/A	N/A	98 % pass	54 % fail	20 % N/C	10 % N/C
Exp. 3 (5 hrs)	>99.9 % pass	95% pass	100 % pass	97 % pass	N/A	N/A	N/A	N/A
Exp. 3 (10 hrs)	>99.9 % pass	99% pass	N/A	N/A	100 % pass	78 % pass	21 % N/C	0 % N/C

[1] N/C = No comparison possible (i.e., benthic organisms not sampled during ballast water exchanges)

1.4.4 Laboratory Toxicity Tests

Median lethal concentrations (i.e., [1]LC50) for all but one species exposed to ozonated artificial seawater in the laboratory ranged from 698 - 768 mV ORP, and from 1.29 - 2.93 mg/L TRO. 50% mortality was never achieved for the amphipod, *Leptocheirus plumulosus*. These data were consistent with results from the caged organism studies in which mortality (at least for mysids) also was strongly correlated to ORP measurements. Therefore, ORP measurements ranging from 700-800 mV appear to be associated with significant acute mortality in a variety of marine species both in the field and in the laboratory.

Furthermore, the relative sensitivity of test species exposed to ozone (as measured by ORP) was similar in both the field and lab experiments. In the caged studies, the sheepshead minnow *C. variegatus* was the most sensitive species, followed by mysids (*A. bahia*) and amphipods (*R. abronius*). In the laboratory, LC50 values for *C. variegatus* were indeed lower than *A. bahia*, suggesting that the sheepshead minnow was slightly more sensitive with respect to ORP exposure. One of the amphipod species tested in the laboratory (*L. plumulosus*) was less sensitive to ORP than either sheepshead or mysids. Thus, laboratory studies provided a realistic indication of ozone toxicity to various species.

1.4.5 Toxicity of Ballast Water Following Ozonation

A major concern following treatment of ballast waters with any biocide is the discharge of potentially toxic chemicals to the environment. For ozonated seawater, bromine is the residual

[1] LC50 represents the concentration of a chemical that causes 50% mortality in an acute toxicity test

oxidant most likely to exist for reasonable periods of time in concentrations of potential concern to marine organisms. Therefore, we conducted a series of laboratory tests with ozonated seawater generated either from the main *Tonsina* experiments, or using a similar laboratory-scale ozone generator. The goals of these studies were to evaluate whether ozone residuals may be toxic in seawater, and whether this toxicity may persist over time.

1.4.5.1 Whole Effluent Toxicity Tests

As part of the regulatory process for the approval of a ballast water chemical treatment process, the treated water will likely need to be screened for potential toxicity using standard whole effluent toxicity (WET) tests. WET tests are widely conducted as part of routine monitoring of wastewater discharges regulated under the federal Clean Water Act. Results of the WET tests using ozone-treated ballast water, with the mysid shrimp *Americamysis bahia* and the topsmelt *Atherinops affinis*, indicated that ozonation byproducts were stable enough to cause toxicity (30-80% ozonated ballast water causing acute mortality) in ballast waters even 1-2 days after ozonation. However, no chemical measurements were conducted in these tests to quantify concentrations of ozone-produced oxidants.

1.4.5.2 Latent Toxicity Tests

To validate these results, mysid shrimp also were exposed to ozone (using 4-5 hours of ozonation) in the laboratory using experiments of similar design to the WET tests. We initiated tests with ozonated waters that were stored for 0, 24, or 48 hours, and measured toxicity along with ORP and total residual oxidant (TRO) over time. As expected from the WET tests, residual oxidants did not disappear from ozonated waters held in the dark 24 or 48 hours in a sealed container at 12 ºC. All organisms died when exposed to 50, 75, or 100% ozonated water that was stored either 0, 24, or 48 hours. In treatments where 100% mortality occurred by 24 hours, the oxidation-reduction potential (ORP) was greater than 720 mV, and TRO greater than 1.76 mg/L.

We also evaluated whether relatively short-term ozonation might generate sufficient oxidant (i.e., bromine) to cause acute mortality to mysid shrimp transferred to clean seawater 1-2 days following ozonation. Limited mysid mortality (30-60%) occurred within the 1.5 hours of ozone exposure in laboratory experiments where TRO concentrations exceeded 4.0 mg/L. However, 100% mortality was observed in those survivors 48 hours after transfer to clean seawater. No mortality was observed within 1.5 hours of ozonation, or at 24 hours post-exposure when TRO measurements were less than 1.0 mg/L, but 60% mortality occurred in these same treatments by 48 hours post-exposure. Therefore, it appears that sufficient amounts of bromine oxidants built up in the ozonated water over 1.5 hours to have induced both immediate and, to an even greater extent, delayed mortality after transferring organisms to clean water (up to 48 hours later).

The presence of bromine thus may cause both immediate and delayed toxicity to marine organisms even after relatively short periods of ozonation. Preliminary experiments suggested, however, that this residual bromine may be easily removed using commonly available reducing agents such as sodium thiosulfate, and thus could remove toxicity from ozonated ballast waters prior to discharge. Bromine also is likely to be quickly destroyed (i.e., chemically reduced) upon discharge into marine surface waters, and so may be of only limited environmental/regulatory concern for ballast water discharge. Additional study is warranted, however, to verify this conclusion.

1.5 General Conclusions and Recommendations

This set of preliminary studies using the prototype system on board the *Tonsina* suggested that ozonation has the potential for being an effective and safe technology for removal of non-indigenous species from ballast water. Some of the primary conclusions of our study include:

1. Using this prototype system, 5-10 hours of ballast water ozonation resulted in 71-99% removal of most marine phytoplankton, zooplankton, and bacteria depending on the amount of ozone gas delivered to individual ballast water tanks over time. Benthic organisms (e.g., crabs, amphipods), however, appeared to be relatively resistant to ozone treatment. It is possible, however, that these experiments could have underestimated overall system effectiveness because of the longer-term residual toxicity of bromine, which is likely the most important and toxic oxidant produced by ozone in seawater. Additional study under field conditions is warranted to verify this conclusion.

2. This organism removal efficiency was greater than that achieved (64% on average) using empty-refill ballast water exchange on the same vessel. Other vessels may achieve greater ballast water exchange efficiency, but studies are few and highly variable.

3. Both field and laboratory experiments suggested that significant organism mortality can be achieved once concentrations of ozone-produced oxidants reach 1 – 3 mg/L (as chlorine equivalents), or when oxidation-reduction potential reaches levels of 700 – 800 mV. Once further validated, such toxicity thresholds could be used to help develop control targets for aiding the routine operation of ozone systems.

4. Our preliminary results suggested that bromine was the ozone-produced oxidant that was most likely responsible for organism mortality. Furthermore, bromine may persist at toxic concentrations in ballast waters 1-2 days following ozonation depending on storage conditions and exposure to sunlight. However, bromine may easily be eliminated (i.e., chemically reduced) prior to, or quickly following, ballast water discharge. Additional study will be required to further evaluate these conclusions.

While these results suggest that the ozonation of ballast water may be a useful treatment technology for prevention of non-indigenous species introductions, uncertainties remain in our scientific understanding of system efficacy. To address these uncertainties, additional studies will soon be underway that will help refine our scientific understanding of ozone system effectiveness and environmental safety. Federal research grants have already been approved for additional study using this system on board the *Tonsina* over the next two years, and are planned to address some of the following issues/uncertainties:

- It will be important to better quantify the spatial and temporal variability of ozone effectiveness within and between different ballast water tanks.
- The length of time bromine residuals persist following ozonation under field conditions will need to be verified.
- The toxicity of bromine residuals to marine organisms will need to be evaluated further in order to determine whether this residual toxicity can be used to increase overall organism removal efficiency over longer periods of time.
- Results from the present study can be used to refine system operations to maximize organism removal efficiency, either via enhanced delivery of ozone gas, use of bromine residuals, enhanced mixing of these oxidants, or a combination of all three.
- Given the current regulatory focus on ballast water exchange as a benchmark technology for comparison to alternative treatments, we will to continue comparisons of ozone treatment to ballast water exchange organism removal efficiencies on this same vessel to improve statistical confidence in our conclusions.
- To ensure environmental and regulatory acceptance of this treatment system, it will be critical to further evaluate removal/destruction of bromine residuals prior to ballast water discharge (e.g., using chemical reducing agents). It will also be important to evaluate the extent to which bromine concentrations will naturally attenuate following discharge to surface waters (e.g., mixing with natural water, photochemical decomposition, etc.).

1.6 Acknowledgements

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