

Onboard Testing of Ballast Treatment Efficiency: Summary Report

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Abstract

The OptiMar Ballast System (UV/hydrocyclone) was tested on five cruises made between October 2001 and July 2003, on board the cruise liner *Sea Princess* and container vessel *RJ Pfeiffer*. At-sea trials were evaluated independently by two scientific teams focusing on the analysis of microbiota (viruses, bacteria, phytoplankton, bulk particles <200 μm) and zooplankton. Experiments compared biological activity in UV treated and control samples collected directly in ship ballast tanks and also in independent small scale plastic enclosures. On two cruises, three parallel treatment tanks (each in duplicate) consisting of control, UV and open-ocean exchange treatments were analyzed. Generally, UV ballast treatment resulted in detectable sterilizing effects when referenced to untreated ballast control samples. However, prolonged ballast tank containment (48-96 h) resulted in reduced biomass and survivorship in both controls and UV treatments. That is, prolonged ballast containment per se introduced significant 'kill' of its own. Most importantly, the collective assays for plankton metabolism and survivorship, when measured after 96 h of tank containment, showed that the final dispositions of ATP/POC, bacterial colony growth, phytoplankton photochemical efficiency (fluorescence yield) and zooplankton survivorship were comparable in UV treatment tanks and open-ocean exchange tanks; UV treatment samples and open-ocean exchange samples yielded lower biological activity than found in parallel 96 h controls. Thus, UV treatment, coupled with prolonged tank containment (96 h), yielded reductions in biological activity comparable to the current ballast management practice of open-ocean exchange. Results showed that UV treatment, coupled with 96 h ballast tank containment, resulted in live counts of zooplankton (>73 μm) that met the ballast water performance standards recently adopted by the International Maritime Organization; that is, <10 viable organisms per m^3 . The volumetric open-ocean exchange efficiencies, computed from chl *a* concentrations, also exceeded the newly adopted ballast water exchange standard of >95% efficiency (IMO 2004). The fate of UV treated organisms in ballast water held for short term periods (<48 h), before environmental discharge, remains unclear.

Introduction

California Assembly Bill 703, which outlines California's Ballast Management Program, calls for the onboard testing of newly designed ballast treatment technologies. In 2001, the California State Water Resources Control Board (SWRCB) in conjunction with the California State Lands Commission (CSLC), initiated test trials of ballast treatment systems installed on 'volunteer' commercial vessels in support of California Assembly Bill 703. Testing took place on five cruises made between October 2001 and July 2003. The first three cruises were made on the cruise vessel *Sea Princess* (Princess Cruises Ltd.); the final two cruises were aboard the container vessel *RJ Pfeiffer* (Matson Navigation Co.). The *Sea Princess* was subsequently transferred to P & O Cruises and renamed the *Adonia* in 2003. The research team was composed of scientists from Moss Landing Marine Laboratories (Principal Investigators: Rusty Fairey and Nick Welschmeyer) and the Romberg Tiburon Center for Environmental Studies (Principal Investigator: Stephen Bollens); both institutions are members of the California State University. The treatment technology under evaluation was the OptiMar Ballast System designed/installed by OptiMarin AS (Norway); US sales representative, Hyde Marine Inc., Cleveland. The treatment system consisted of combined UV/Hydrocyclone components, similar to the system installed on the sister Princess cruise liner, *Regal Princess* (see McDowell 2000). This report summarizes scientific findings regarding the performance of onboard ballast treatment with the OptiMar Ballast System.

Objectives

Two primary objectives were considered under the scope of the present work. The first objective, ostensibly the most straightforward, was to provide a quantitative evaluation of the fate of entrained planktonic organisms in treated vs. untreated ballast samples. The second objective, a subset of the first, was to provide quantitative comparisons of treated

ballast water vs. ballast water that had undergone open-ocean exchange (Drake et al. 2002). The latter objective derives from current regulatory practices regarding aquatic non-indigenous species and the management of ballast water. Open-ocean exchange, also referred to as ‘mid-ocean exchange’ and ‘ballast water exchange (BWE)’ (Oemcke and Van Leeuwen 2003; Jeroen et 2002; Waite 2002), is currently the only mechanism of ballast treatment approved by the State of California. The technique calls for volumetric exchange of coastal-derived ballast water with open-ocean water taken outside of the coastal zone (ideally, greater than 200 miles from land). Deballasting is permissible in California ports only for ballast water that had previously been subjected to open-ocean exchange

During this project, all cruises aboard the *Sea Princess* were made in coastal waters with no opportunity to conduct open-ocean exchange. Experiments during those cruises focused specifically on achieving comparisons between ‘control’ and ‘treatment’ samples. The transit route between Honolulu and Oakland aboard the *RJ Pfeiffer* provided ideal circumstances to include comparisons of ballast treatment to open-ocean exchange. The design of experiments aboard *RJ Pfeiffer* were given careful consideration regarding the achievement of the best possible evaluation of treatment vs. open-ocean exchange under conditions of real-time ship operations. This study benefited from guidance from the US Coast Guard and the Volpe Institution, who highlighted the community interest in addressing the issue of ballast treatment vs. ballast exchange.

Cruise Logistics

Cruise dates and routes were as follows:

- 1) *Sea Princess* 1: October 9, 2001 – October 12, 2001; Los Angeles, CA to Mexico (various ports).
- 2) *Sea Princess* 2: October 29, 2001 – November 2, 2001; Los Angeles, CA to Mexico (various ports).

- 3) *Sea Princess* 3: September 26, 2002 – September 26 – September 30; Los Angeles, CA to Mexico (various ports).
- 4) *RJ Pfeiffer* 1: July 3, 2003 – July 7, 2003: Honolulu, HI to Oakland, CA.
- 5) *RJ Pfeiffer* 2: July 17, 2003 – July 21, 2003: Honolulu, HI to Oakland, CA.

The three *Sea Princess* cruises and two *RJ Pfeiffer* cruises are identified in this report as SP1-SP3 and RJP1-RJP2, respectively.

Ballast treatment testing was conducted under routine ship operating conditions. Therefore, some compromises between vessel safety and scientific sampling design were unavoidable. Availability of ballast tanks was determined by ships' crew such that vessel maneuverability and safety were not compromised. In the case of the *Sea Princess*, two ballast tanks were available for testing purposes. Cruise itinerary on *Sea Princess* included departure from Los Angeles followed by tightly scheduled port stops in Cabo San Lucas, Mazatlan and Puerto Vallarta, Mexico. Time spent in port was typically less than one day. Initial planning meetings discouraged commencement of ballast experiments in the port of Los Angeles due to the heavy work load associated with security and passenger loading. The initial sampling plan, implemented during SP1 and SP2, was to collect ballasted water from each Mexican destination port, such that multiple evaluations of treated vs. untreated source water could be made during each cruise. Water from each harbor was ballasted onboard into control and treatment ballast tanks and held for ca. 20 h before deballasting and testing (see Sampling). Harbor conditions in terms of planktonic biomass varied widely (forty-fold range in chlorophyll *a*), providing a range of biological/optical conditions for the OptiMar Ballast System. However, tests for biomass and viability in ballast tanks were generally completed in one day, thus limiting the length of time allowed for 'kill' to set in.

Special comments are warranted here for cruises SP1 and SP2. Experimental results for the first two cruises were suspect for two primary reasons, which were not fully evident until post-cruise analyses were completed. 1) Anti-galvanic corrosion and gray water contamination, associated with installation plumbing and residual tank fouling, likely

resulted in reduced delivery of necessary UV dosage. 2) Short term (<1 d) biological evaluation of UV effects, employed in the early scientific sampling plan, may not have permitted adequate time for 'kill' to take effect, even if the UV dose delivery had been correct. After inspection of data from SP1 and SP2, our initial tentative conclusion was that UV/Hydrocyclone treatment had relatively little effect on planktonic organisms, relative to control samples. Results to this effect were presented at conferences (Welschmeyer et al. 2002; Welschmeyer and Bollens, 2002) highlighting the need for careful control in engineering and testing procedures. Engineers from OptiMarin AS verified that over the course of the initial 6 month trial up to 4 mm of stainless steel had undergone corrosion in the inner surface of the UV reactor. Inspections showed accumulation of rust and black sludge in the UV reactor. Additionally, during SP1 and SP2, ship's vibrations resulted in frequent breakage of the medium-pressure UV lamps within the UV reactor. Lamps were replaced as needed during the scientific test cruises, but this resulted in down-time during the ballast experiments.

In response to the disappointing early results, Princess Cruises refit the ballast water pipes, isolating them from the gray water sources, and the OptiMar Ballast System was redesigned and installed anew on *Sea Princess* in 2002, resulting in a one year delay between SP2 and SP3. However, the performance of the system was notably enhanced (discussed herein). Moreover, with the benefit of previous experience on SP1 and SP2, the scientific work plan was also modified. 1) Experiments were included with longer observation periods for temporal resolution of sterilization effects within the ballast tanks. 2) Parallel experiments were set up, in addition to the 'ballast tank' experiments, using shipboard plastic enclosures. The enclosures provided samples without the introduced variability of 'tank effects', which might include sources of variability such as settling/resuspension within the tank and valve/plumbing contamination upon sampling. It is important to note that the primary caveat in the use of the small enclosures was the omission of a second UV exposure which normally occurs during the deballasting phase in ballast tank treatment experiments. Thus, our independent plastic enclosures received one half the UV dose expected under normal ballast treatment procedures. Treatment experiments drawing from the ballast tanks per se received the double dose of UV

radiation expected under normal operations; that is, water was UV-irradiated on the inflowing ballasting operation and was also UV-irradiated on the outflowing deballasting operation.

This report focuses entirely on results obtained for the final three cruises, SP3, RJP1 and RJP2, in which 1) OptiMar Ballast System installation and stability was corrected, 2) scientific sampling protocol was modified and 3) opportunities were provided for quantitative evaluation of ballast treatment vs. open-ocean exchange. Results from SP1 and SP2 are not detailed in this report for reasons cited above.

METHODS

The primary question at the core of ballast treatment evaluation is simple: does the treatment remove or sterilize plankton entering and leaving the ship's ballast tanks? Unfortunately, the practical path to answering that question is complex, because planktonic organisms constitute a diverse biotic assemblage, representing autotrophic and heterotrophic organisms from most phyla known to inhabit the ocean. Viruses, bacteria, phytoplankton, seaweeds, invertebrates and fish have all been documented in discharged ballast water; invasive scenarios have been associated with each organism group (Carlton and Geller 1993; Ruiz 2000). To our knowledge there is no single test assay which can be applied to verify removal or sterilization of all biota in a bulk sense. To provide as thorough an evaluation as possible, we assembled an array of test assays directed towards most of the expected organismic groups. The assays included biochemical, physiological and microscopic techniques. Wherever possible we utilized modern techniques, such as nuclear fluorescence staining and pulse-amplitude-modulated (PAM) fluorescence. However, we included older methods, such as streak plating of cultivable bacteria, to evaluate bacterial 'growth' potential which is otherwise difficult to evaluate from 'direct' cell enumeration alone (Azam 2001). Labor intensive visual inspections of live/dead status on fresh samples were mandatory for larger metazoan zooplankton, resulting in some limitations on the number of samples that could be processed in real-time.

The methodological assays could be coarsely divided into two categories: those that measured concentrations per se, e.g., zooplankton density (individuals/vol) and chl a concentration (ug/L), and those that reflected physiological/metabolic activity, e.g., zooplankton survivorship and PAM-based photochemical quantum efficiency. Common radioisotope methods (^{14}C , ^3H) for determining planktonic rate processes such as photosynthesis and bacterial production were not utilized for our work on commercial vessels. However, application of these methods to verify inactivation of metabolic processes will certainly require consideration in future studies.

Tank sampling

The method of ballast tank ‘sampling’ was a non-trivial aspect of the overall evaluation protocol. Internal characteristics of ballast tanks (shape, baffling, corrosion, sludge etc.) are unique for each ship (and each tank). Earlier reports have used a wide range of methods for accessing and sampling water within ballast tanks (Carlton and Geller 1993; Ruiz 2000). Recent observations suggest that considerable patchiness is expected in the concentrations of biota, particularly mesozooplankton, within ballast tanks (Murphy et al 2002). Our research team benefited from numerous meetings and shipboard site visits with representatives from SWRCB, CSLC, Princess Cruises and Matson Navigation Co., made in advance of the actual sea trials. As a group, we collectively evaluated the sampling limitations. It was decided, on practical terms, that the best real-world evaluation of treatment efficacy would be given in the measured biological characteristics of the water ultimately discharged by the ballast pumping system. Accordingly, the OptiMar Ballast System was installed with in-line sampling ports along the ballast piping system that allowed water to be sampled before and after UV/Hydrocyclone exposure during the onboard ballasting phase, as well as during the offboard de-ballasting phase. Thus, all sampling was confined within the engine room, with no need for direct access to individually tested ballast tanks through deck lids, sounding tubes, etc. (cf. Drake et al. 2002). As will be seen below, the ability to measure water entering ballast tanks,

followed by measurements of the same water immediately withdrawn from the same tank (after mixing), provided significant information on the internal variability of each tank.

Analytical Techniques

Viruses. Samples were preserved with 0.02 μm filtered glutaraldehyde (2% final concentration) and stored at refrigerator temperature for filter processing at the lab. Quantitative aliquots (1-5 mL, depending on biomass) were filtered onto 0.02 μm aluminum Anodisc filters (Whatman), stained with SYBR Gold (Molecular Probes) as per Chen et al (2001) and mounted under a cover slip with one drop Slo-Fade (Molecular Probes); microscope slides were frozen until counting under 1250x magnification, usually within one week (Olympus BH-2, 50W blue excitation).

Bacteria. Epifluorescence bacterial counts were made on the same SYBR Gold stained microscope preparations used for viruses. Cultivable bacteria were enumerated onboard ship on sterilized marine agar (Difco) Petri plates (5cm) after a 24 h room-temperature grow-out in the dark; streak volume was 50 μL .

Phytoplankton. Chlorophyll *a* was determined onboard ship in acetone extracts using single-step fluorescence (Welschmeyer 1994). Samples for HPLC analysis of chlorophylls and carotenoids were filtered onboard ship and stored at liquid nitrogen temperature until laboratory extraction and analysis. Chromatographic pyridine-based elution on a C8 column followed that of Zapata et al (2000). PAM fluorometric measurements of photochemical quantum yield based on variable fluorescence (F_v/F_m) were made with a Walz Water-PAM fluorometer. Samples were dark adapted for two minutes in the PAM instrument chamber prior to commencement of a 10 minute fluorescence light curve, with dark recovery. All estimates of fluorescence yield (F_v/F_m) reported here were taken from the initial dark adapted measurement.

ATP/Particulate Organic Carbon. Adenosine Triphosphate (ATP) was analyzed using luminescence technique. General extraction protocol onboard ship followed that outlined

by Karl (1993). Samples captured on Whatman GF/F filters were extracted for 5 minutes in boiling 20mM Tris buffer (5mL) and immediately frozen onboard ship. ATP concentrations were determined at the laboratory on a Turner Designs TD 20/20 luminometer using firefly extract, HEPES buffer and ATP standards obtained from Turner Designs, Sunnyvale, CA. Samples for particulate organic matter analysis were collected on 25 mm Whatman GF/F filters, dried on board ship and stored in a desiccator under vacuum until analysis at the lab. Blank subtractions were based on parallel filters placed on the same filtration devices but rinsed only with 5 mL milli-Q water. Particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined by combustion on an CEC CHN analyzer using cystine as a standard.

Zooplankton. Quantitative zooplankton samples were collected as follows. A 165L plastic container was placed directly under the closed sampling valve. A 0.5m, 73 μ m mesh plankton net was placed over the valve and nested inside the container where filtered water was captured to measure the absolute sample volume; the technique also buffered the zooplankton from physical harm. During sample collection, water was allowed to run through the net at a constant rate. In addition to recording the time elapsed to fill the 164.5L container, we also recorded the flow rate of water through both the UV main and sludge pipes, and percent UV irradiance as measured by the OptiMar system. After the sample was collected in a 'live' 1L cod-end, we recorded the salinity (parts per thousand) and temperature ($^{\circ}$ C) of the water in the zooplankton container.

Zooplankton were counted and distinguished as live or dead in each sample using a dissecting stereomicroscope (6.3-40x). Zooplankton were considered dead when unresponsive to being poked with a probe. Subsamples were collected until a target number of approximately 100 specimens were assessed. Survivorship and density was determined by enumerating live zooplankton in Stemple pipette sub-samples taken from the seawater or ballast water samples. During enumeration, zooplankton were separated into five taxonomic groups: Copepod nauplii, calanoid copepods, cyclopoid copepods, harpacticoid copepods, and "other" (those zooplankton that did not fall into the other four groups). The counting analysis was performed within 1 hour of collecting the sample.

Subsamples were preserved with 10% Formalin and retained separately from the rest of the sample for later analysis, if needed.

General experimental design

Sea Princess Cruise SP3

Ballast Tank Experiments

Two ballast tank time series experiments (treatment vs. control) were run to compare the effect of the OptiMar system on plankton density and survival over time. Both tanks were similar, with a capacity of 82 m³. One tank was filled with UV treated seawater, while another was filled with untreated (control) seawater. The tanks were sampled at intervals of 0h (while the tank was filled), 24h, and 48h (first experiment) and 0h, and 24h (second experiment). Before filling the ballast tanks, seawater was run through the ballasting system for 5 minutes to ensure that the pipes were clean. Prior to collecting samples, both the ballast pipes and sampling ports were purged with seawater from the harbor or water from the ballast tank itself depending on whether we were filling (t = 0) or deballasting (t = 24 and 48h).

Enclosure Experiments

To avoid the added variability that is inherent in using ballast tanks (Murphy et al 2002), we incorporated the use of enclosures to study the effects of the OptiMar system. Three experiments were performed using water from the harbors of Cabo San Lucas, Mazatlan, and Puerto Vallarta, collected on the dates of Sept 26, 27, and 28, 2002, respectively. Microbial samples were collected in 10L plastic carboys (control/treatment) and held in darkened conditions in the lab area where room temperature was ca. 2 C higher than that of ambient seawater. Samples were taken nominally at 0h, 24h, 48h and 72h through the time series. Zooplankton were collected in two 165L enclosures, one with treated seawater and one with non-treated seawater. Valving permitted simultaneous collection of treated and untreated pairs such that similar water was collected in each container; the water for both microbial and zooplankton enclosures thus did not come into contact with

ballast tanks. Note, however that the treated samples in these experiments were only exposed to UV treatment once, during the filling operation.

Microbial enclosure experiments were mixed thoroughly prior to each sampling point and dispensed into aliquots for lab processing. The large volume zooplankton enclosures were fully drained via a ¾ in. siphoning hose into a submerged, 73µm plankton net with a non-filtering cod end to reduce damage to the zooplankton. Samples were analyzed in the shipboard laboratory using methods identical to those in the ballast tank experiments.

RJ Pfiesser Cruises RJP1 and RJP2

Ballast Tanks Experiments

In order to compare the effects of the OptiMar UV system and open-ocean exchange on the biological activity of plankton, six ballast tanks were filled with seawater from the Sand Island Harbor of Honolulu, HI on July 3, 2003. All of the tanks were similar double bottom ballast tanks with capacities ranging from 377 to 512 m³. Two tanks (duplicates) were filled with treated seawater, while the remaining four were filled with untreated seawater (duplicate control ballast tanks and duplicate open-ocean exchange ballast tanks).

All ballast tanks were sampled at 1h, 48h, and 96h. The open-ocean exchange procedure was commenced midway between Honolulu and Oakland, after the 48h sampling point; the two exchanged tanks were sampled thereafter at 72h and 96h. Sampling of the ballast tanks was carried out in the same manner as described for SP3 samplings except that the sampling valves were of a different design and the Tygon tubing ran 4m for the non-treated port and 12m for the treated port instead of the 1m length used in the SP3 cruise. Analyses of the samples were identical to the methods outlined in the SP3 cruise methods.

Enclosure Experiments

During the RJP cruises, we conducted enclosure experiments similar to those on SP3. Microbial enclosure procedures were identical to those practiced on SP3; however, we

increased the number of zooplankton enclosures for RJP1 and RJP2. Five 165L zooplankton enclosures were filled with treated seawater while the other five containers were filled with non-treated water. Seawater was again taken from sampling ports that were installed in the main piping before (untreated) and after (treated) the OptiMar system. Enclosures were filled in treated and untreated pairs at the same time to collect similar water masses in each. The zooplankton containers remained in the engine room during the full course of the time series (the size of the containers prevented transport to a location elsewhere on the ship). A temperature recorder, taking measurements every minute for the duration of the experiment, was placed in one of the enclosures. One untreated and one treated enclosure was sacrificed daily to yield a five day time series of the zooplankton densities and survivorships. These samples were collected and processed in the manner described above in the methods for the SP3 enclosure study.

Grow-Out Experiments

Samples of both treated and untreated (control) zooplankton were collected aboard the RJ Pfeiffer for use ashore in grow-out experiments, post-cruise. On July 3, 2003 the double-bottom number 3 ballast tank was filled with 450m³ (from a level of 50m³ to 500m³) seawater (35.7ppt, 27.2 deg C) from the Sand Island Harbor of Honolulu, HI. The ballast tank was filled with untreated seawater that bypassed the OptiMar system. After the tank was filled, it was sampled to determine the density of live and dead zooplankton and additionally was sampled as above for the other shipboard experiments. During each sampling approximately 50m³ was removed from the ballast tank resulting in a final volume of 244m³ on July 7.

On July 8, the number 3 ballast tank was emptied through the ballast system, including passage through the OptiMar system. As the ballast tank was being emptied, seawater was collected from the sampling ports upstream (untreated) and downstream (treated) of the OptiMar UV system. Duplicate treated and non-treated samples were collected. Although each pair of treated and untreated samples was taken concurrently to ensure similar water masses were being sampled, the two duplicates were taken ca. 15 minutes apart. To determine the volume of water that was to be filtered in order to achieve the

target number of 200 zooplankton, we used the last known (July 7, 2003) density of live zooplankton (485 m^{-3}). Seawater was sampled with a net as described previously. The net was submerged in water of the same temperature and salinity as the sampled water (16.6 deg C and 35.3ppt) to protect the zooplankton from damage. Once the filtering was complete, the net was rinsed clean and the contents rinsed out of the cod end into 4L plastic jars and kept in a cooler with ice packs to reduce the metabolism and activity of the zooplankton during the 1h transport from the port of Oakland, CA to the laboratory in Tiburon, CA. It may be significant to note that although we defined these samples as ‘treated’ and ‘untreated’, we later realized that on this particular pumping operation, the deballasted seawater was pumped at high rates of flow (up to $495 \text{ m}^3\text{h}^{-1}$) through the OptiMar ballast system. The high flow reduced the net UV exposure below the contracted sterilizing UV dose of 90 mJoules/cm^2 associated with specified nominal flow rates of $350 \text{ m}^3\text{h}^{-1}$; the effective dose was ca. 60 mJoules/cm^2 calculated from linear scaling of flow rate. It should also be clear that the treatment samples received UV irradiation only once in this experiment.

At the laboratory, presoaked 165L enclosures were filled with $25 \mu\text{m}$ filtered San Francisco Bay water ($17.5 \text{ }^\circ\text{C}$ and 27.1-29.6 ppt). These enclosures were placed in a large circular tank (1m high and 3m in diameter) filled with circulating water to maintain the temperature of the enclosures with that of the Bay. Treated and untreated zooplankton from the four, 4L jars were then introduced into separate enclosures. After 72 hours, one enclosure each of treated and untreated (control) zooplankton was siphoned off through a 73 micron mesh and into 1L containers to be counted as described previously.

Results

Results will be presented separately for microbial and zooplankton analyses. The two groups of samples are distinguished largely by sampling method. All microbial samples

were collected in 10L plastic carboys during engine room sampling; they were screened through 200 μm nylon mesh to remove larger organisms, and then dispensed into aliquots for processing (i.e., filtration, preservation, streaking). Large volume zooplankton samples were volumetrically concentrated using nylon mesh zooplankton nets for visual microscopic analyses as discussed above.

Microbial Samples

Figs. 1-7 show results for time series analysis of UV-treated, and untreated, samples. In most cases, the data format is the same for Figs 1-7; ballast tank experiments are shown on the left side of each figure, enclosure experiments are on the right (filled circles for control samples; unfilled circles for UV treated samples). During SP3, only two ballast tanks were available for experiments; a treatment tank and a control tank. In this, case error bars (± 1 standard deviation) are based on triplicate analysis of each analytical parameter. Six ballast tanks were available for experiments on RJP1 and RJP2; triplicate analyses (nominally) were made for all analytical parameters within each tank, means were computed and the grand means for replicate ballast tanks are plotted in Figs. 1-7. Four tanks served as controls up to, and including, the 48 h sampling on RJP1 and RJP2. After 48 h, two of the control tanks were sampled, drained (deballasted) completely, and refilled as open-ocean tanks; the exchange occurred approximately half way between Honolulu and Oakland. Thus, on RJP1 and RJP2, four control tanks were analyzed at 0 h and 48 h, and only two control tanks remained at 96 h.

In general, most parameters decreased over time inside both control and treatment tanks. Photochemical quantum yield, chl *a*, particulate organic carbon (POC) and ATP showed decreasing trends in ballast tanks (Figs. 1-4, left side) throughout most of time-series experiments. Photochemical yield was reduced in all UV treated samples, relative to corresponding control samples (Figs 1a-g). The reduction in photochemical yield was evident even in the time zero samples. The sensitivity of the Walz PAM fluorometer was adequate for all samples except the open-ocean exchange seawater. Typical initial values of the fluorescence yield parameter, F_v/F_m , were 0.6, as would be expected for

physiologically competent phytoplankton (theoretical maximum is ca. 0.7; Genty (1989)). In some UV treatments the final dark-adapted yield value decreased to 0.2, a condition associated with moribund algal cells and/or irreversibly stressed cells. We were unfortunately not able to corroborate negatively impacted photosynthetic performance on board ship. The effect of UV exposure on phytoplankton photochemical efficiency was near instantaneous (1-2 h typically lapsed between engine room sampling and lab analyses) and F_v/F_m decreased continuously with time. Parallel decreases in F_v/F_m were also noted in some control experiments but the UV treated samples always showed greater reductions in F_v/F_m . No signs of recovery in photochemical yield were evident from any of the experiments (Fig. 1). Among the analytical microbial techniques used in this study, the PAM fluorometric technique provided the most consistent results, with best precision (many error bars in Fig. 1 are smaller than the symbol). It is important to note that the PAM fluorometric technique is the only density-independent technique used in our microbial assays. The instrument operates by interrogating the variable fluorescence response of phytoplankton. The yield parameter, the ratio of variable fluorescence (F_v) to maximum fluorescence (F_m), is expected to be the same for samples of widely varying chlorophyll concentrations, but equivalent physiological state, as long as suitable blanks are applied (Cullen and Davis 2003). The density-independent nature of the measurement was invaluable in this study where variability in organism abundance, both in the source harbor water and in the ballast tanks, was high (see below). The only caveat in the PAM measurements was the lack of sensitivity for open-ocean samples which had low chl *a* concentrations < 0.01 ug chl *a*/L; we were not able to reliably determine photochemical yield on most open-ocean exchange samples. However, on RJP2 the mean of 6 replicates from the 96 h exchange tank was 0.8 ± 0.3 . Measurements of photochemical quantum yield made with fast-repetition-rate fluorometry in the central Pacific show photochemical yield values of 0.4-0.6 (Behrenfield et al. 1996). Thus, based on results summarized in Fig. 1, it is likely that the low photochemical yield of UV treated Honolulu harbor water held in ballast tanks for 96 h (Fig. 1) is lower than that of untreated open-ocean exchange water held for half as long in the ballast tanks.

Concentrations of chl *a* and ATP showed reductions in all UV treatments conducted in ballast tanks (Figs. 2a-d and 3a-d). However, the results were not always paralleled in enclosure experiments (Figs. 2g and 3e,f). Importantly, after 96 h of ballast tank containment, the ATP and chl *a* levels in UV treatment tanks approached those of the open-ocean exchange water (filled triangles in Figs 2c,d and 3c,d, RJP1 and RJP2). We expected ATP to quickly decrease in killed cells (Karl 1993) and although this trend is consistent in ballast tanks, we note that some enclosure experiments did not support this observation (Fig. 3, right side).

Bulk particulate organic carbon (POC) also showed decreases in ballast tanks for both controls and treatments (Fig 4a,b), however, the reduction was dampened in corresponding enclosure experiments (Figs 4e,f). The rationale for measuring POC was to characterize the general standing crop of particulate organic material, both living and dead, in each experimental treatment. The bulk POC concentration serves as a normalizing factor in computing the ratio of living carbon to dead carbon from ATP/POC ratios (see below). We were not expecting significant decreases in POC from UV treatment per se. However, the consistent drop in POC within ballast tanks (Figs 4a,b) deserves some discussion.

At least three possible alternatives can be considered in explaining the ballast tank POC reductions. 1) The UV treatment may have succeeded in killing biota, which then decomposed. However, decreases in POC were seen in both treatment and controls, inconsistent with this hypothesis. 2) The tank itself (through accumulated chemical toxicity and/or resident heterotrophic predators/decomposers) could have reduced the POC concentration, thereby affecting both treatments and controls. 3) The bulk particulate material stirred in during the filling (ballasting) operation may have settled below the ballast pump outlet over the course of the 96 h experiment, again affecting POC in both treatment and control; the initial high POC measurements at time zero could have reflected resuspended sediments from the shallow harbor water as well as resuspended sediments already present in the bottom of the ballast tanks.

Some insight into the consistently observed POC decreases inside *RJ Pfeiffer* tanks can be gained by examining the time course of C/N (g/g) ratios of the particulate material. The C/N ratios in the ballast tanks at 0, 48 and 96 h were 10.6, 8.5 and 7.0, and 9.7, 9.5 and 7.0 (g/g) for control and UV samples respectively on RJP1; on RJP2 the same C/N temporal series was 11.8, 9.9 and 9.0, and 12.7, 8.2 and 7.9. Thus, all C/N values decrease over time in both control and UV treatment tanks. If the bulk POC decreased as a result of ingestion/decomposition as considered in #2 above, we would expect the C/N ratio to rise since nitrogen is preferentially remineralized; we observed the opposite. The observed decrease in C/N could have been associated with the chemical signature of differential particle settling and suspension. For instance, if resuspended detritus (from the tank bottom and/or harbor source water) is mixed in during the filling (ballasting) operation, the initial C/N would be expected to be high (a C/N of 10-15 is typical of detrital sediments); the apparent C/N ratio would decrease as large, non living particles settle out leaving smaller suspended biota with lower C/N ratios approaching Redfield values (ca. 7). We observed such a trend in C/N in ballast tank experiments. However, C/N in enclosures remained high with no declining trend (C/N ranged from 9-12). The microbial enclosures were mixed thoroughly before each sampling, thus settling would not have occurred. Although we did not collect POC/PON samples on SP3 we did observe marked color changes in the material collected on filters from seawater flowing into the tanks compared to seawater immediately drained out of the tanks in Cabo San Lucas (the first tank filling operation of the cruise) (Fig. 8a). Thus, it is reasonable to suspect that detrital material from the harbor and/or tank bottoms could have been suspended in ballast tanks during the earliest sampling times, contributing to the wide variation in concentration-based parameters among tanks. The general consensus is that ballast tanks are less than ideal test tubes.

The ratio of ATP/POC ($\mu\text{g/g}$) is shown in the lower half of Fig. 4. Although both ATP and POC each decreased coincidentally in RJP1 and RJP2 tanks (in both controls and treatments; Figs. 3a,b and 4a,b) it appears that ATP concentrations decreased disproportionately, as evidenced by decreasing ATP/POC ratios (Figs. 4c,d). The final ATP/POC ratios were generally less than 20 ($\mu\text{g/g}$) after 96 h of ballast tank containment.

Living carbon is generally assumed to express a POC/ATP ratio of 250 (g/g) (Karl 1993). In reciprocal form, to match the units used in this study, that is equivalent to an ATP/POC ratio of 4000 ($\mu\text{g/g}$). Thus, as little as 0.5% of the total POC (20/4000) might have been present as living carbon at the end of 96 h ballast tank containment. This calculation excludes larger zooplankton which were removed with a 200 μm mesh net prior to POC and ATP filtering.

The microscopic enumeration of stained viruses and bacteria and visual enumeration of bacterial grow-out colonies were highly variable. However, bacterial colony counts at times resulted in impressive visual verification that UV treated samples were impacted relative to controls (see Figs. 8b,c). Depressed colony growth in UV treated samples, relative to controls, was noted at some point in 6 of the 9 tank and enclosure experiments. However, the comparative trend in control/treatment colony counts did not necessarily remain consistent through the full time course (Fig 5). Results unfortunately varied widely and in one case, enclosure RJP2 (Fig 5), the UV treated sample produced bacterial colony counts that were always higher than controls, and at times too numerous to count. (We ruled out the possibility of accidental mislabeling of enclosures on RJP2 using corroborative data from PAM fluorometry Fig. 1). It should be noted that we were not working under sterile conditions aboard ship and it is possible that bacterial plates were subject to random contamination, though triplicate streak plates often made the results convincing (Fig. 8c,b). While the variability is troublesome, it is significant to note that after 96 h, the treated ballast tanks on RJP1 and RJP2 showed bacterial colony counts comparable to those from open-ocean exchange treatments (Figs. 5c,d); importantly, treatment and exchange results were reduced relative to controls.

Epifluorescence enumeration of bacteria and viruses (termed virus-like particles, VLP) were variable, with no clear pattern emerging through analysis of time-series enclosures (Figs. 6 and 7). The microscopic assay is laborious and somewhat ambiguous since direct cell counting does not distinguish between live and dead cells. Thus, we concentrated our counting efforts only on selected samples such as enclosure experiments during SP3, and final 96 h tank analyses of the ocean exchange experiments on RJP1 and

RJP2. Bacterial concentrations after 96 h were similar in control and UV tanks; both were ca. 3 times higher than exchange tanks (Fig. 6). Variability in virus counts between replicate tanks was too high to yield meaningful comparisons among control, UV and exchange treatments (Fig. 7).

A summary plot of microbial assays that were indicative of metabolism and physiological state is given in Fig. 9 for each pair of ballast tanks used in the comparison of control, UV and exchange treatments on RJP1 and RJP2. The data in Fig. 9 are from the final deballasting operation after 96 h. We reasoned that the 96 h sampling point represents water that which would be ultimately deballasted at the end of a typical Honolulu-Oakland run on *RJ Pfeiffer*. The results for ATP/POC, bacterial colony growth and PAM-based photochemical yield, showed that UV treatments and open-ocean exchange samples were lower than controls in almost all cases (anomalously high ATP/POC in one UV treatment of RJP1 was noted; Fig 9a). Interestingly, UV treatments approached the conditions found in open-ocean exchange tanks. Photochemical yield was not determined on exchange samples but, as discussed, previously, it is likely that ocean-exchange (held for only 48 h) exhibits a *higher* photochemical yield than UV treated water held for 96 h. .

Zooplankton

SP3.

Ballast Tank Experiments

Total density of live zooplankton decreased over the first 24 hrs, in both control and treatment (UV/Hydrocyclone) tanks, but did so more dramatically in the treatment tanks (Fig. 10a). Survivorship after 24 h, referenced to the density of live organisms established at time zero, was ca. 2% in the treatment, and ca. 16% in the control tanks (Fig. 10b).

Enclosure Experiments

Survivorship at 20 hours was consistently lower in the treatment enclosures compared to the control enclosures (ca. 68% vs. 92%; Fig. 10c). In absolute terms, however, survivorship in the enclosures during SP3 was always quite high, i.e., well above the survivorship seen in the tank experiments above (Fig 10a,b). Why the tanks themselves would contribute so much additional mortality to the zooplankton is unclear, but presumably is related to water quality and/or interactions with the sides and bottom of the tanks.

Composition of the zooplankton varied over time (0 hr vs. 24 hr) in both the control and treatment enclosures, but to a greater degree in the treatment enclosures (Fig 11). For instance, the proportion of nauplii in the community tended to decrease, and the proportion of “other” increased with time (Fig 11). Overall though, the effect of treatment on taxonomic composition of zooplankton was more subtle than the effect on overall survivorship.

RJP1 and RJP2

Ballast Tank Experiments

Just as with the SP3 results, total density of live zooplankton decreased over the course of the RJP1 and RJP2 experiments, in both control and treatment (UV/Hydrocyclone) tanks, but did so more dramatically in the treatment tanks (Fig 12). By 96 hours, differences between control and treatment tanks were quite small. Indeed, total densities in the open-ocean exchange (OOE) treatment were similar to both the UV treatment and control densities. Final 96 h abundances of live zooplankton for control, UV and OOE tanks during RJP1 were 263.4, 10.0 and 1.4 animals m^{-3} ; final live abundances during RJP2 were 258.1, 6.2 and 6.5 animals m^{-3} , respectively. Similar results were seen in the survivorship data (Fig 13), i.e., decreased survivorship over time, but with a greater effect resulting from UV treatment. In the case of OOE, however, the two experiments (RJP1 and RJP2) yielded different results: on RJP1, OOE survivorship was similar, or lower, than UV (or control) treatments, whereas on RJP2 OOE survivorship was greater than that of UV treatment and about the same as that of the control (Fig. 13).

Variability in the composition of the zooplankton was high in both RJP1 (Fig. 14) and RJP2 (Fig. 15), e.g., between times, between duplicate tanks, and between treatments, such that no obvious patterns or trends were evident.

Enclosure Experiments

Survivorship was reduced in the UV treatment enclosures compared to the control enclosures for all time points greater than or equal to 24 hours (the lone exception being at 48 hrs in RJP2, which we consider to be an outlier; Fig. 16). In these experiments the compositional shifts in the zooplankton over time were clearer, and consistent with the SP3 enclosure experiments: nauplii tended to be replaced by “other” zooplankton (Fig. 17). No overall treatment effect was seen in the compositional data, however.

Grow-out experiments

The grow-out experiments yielded interesting and dramatically different results from the ship-board experiments described above: there was no effect of treatment, with both control and treated zooplankton experiencing very high survivorship (>80%), even after 72hrs (Fig. 18). In other words, treatment effects observed on zooplankton survivorship in the tanks and ship-board enclosures were absent when the zooplankton were reared in SF Bay water at ambient conditions. In these experiments, time zero abundance of live organisms was established at the time of the 96 h tank sampling; thus, absolute values of survivorship are higher than those noted in Figs. 13 and 16. This implies one of two possibilities. First is that the treatment effect on zooplankton survivorship in the ship-board experiments was mediated through the zooplankton food and/or water; that is, once placed in untreated water (e.g. grow-out experiments), even treated zooplankton were able to survive at very high rates. The second possibility is that the reduced mortality (effectiveness) in the grow-out experiments relative to the shipboard tank and enclosure experiments was due to the reduced UV dosage experienced during the single passage through the OptiMar system. Distinguishing between these two possibilities should be a high priority for future grow-out experiments.

If the first interpretation (possibility) of the results proves to be the correct one, this would have important implications for treatment and subsequent release of ballast water zooplankton. Unless held for a sufficiently long period of time (≥ 48 hrs) to allow the zooplankton to interact with the treated water and suspended particles (food), treated zooplankton may survive at high rates upon their release into SF Bay, irrespective of whether they were treated with UV/Hydrocyclone or not.

Discussion

Independent analyses of microbiota and zooplankton by two scientific teams resulted in similar findings regarding the effects of the OptiMar Ballast System. Generally, UV treatment resulted in greater sterilizing effects than measured in controls (Figs. 1, 3,4,9,10,12, and 13). However, prolonged 96 h tank containment resulted in reduced survivorship and biological concentrations in both controls and UV treatments. Most importantly, the collective indices of plankton metabolism and survivorship, when measured after 96 h of tank containment, showed that the final dispositions of ATP/POC, bacterial colony growth, phytoplankton photochemical efficiency (yield) and zooplankton survivorship were comparable in UV treatment tanks and open-ocean exchange tanks (Figs. 9 and 13). Moreover, both UV and exchange treatments were lower than controls (Figs. 9 and 13).

There appears to be inherent toxicity associated with the ballast tanks per se and this has been observed previously (Wonham et al 2001). At least three factors can be suggested to contribute to the apparent tank-toxicity. 1) Organisms are held in complete darkness; for phytoplankton, reductions in chl a and photochemical yield were observed simply as a result of darkened conditions 2) The incoming ballast water is chlorinated in the main sea chest of the RJ Pfeiffer to prevent fouling; no control of this parameter was monitored. 3) Unknown resident tank fauna may consume and decompose newly ballasted organisms. These factors (save darkness) will vary between tanks and between ships; no effort was made to control these variables.

The significant finding in the study was that UV treatment worked above and beyond the sterilization caused by control tank containment alone in most cases during both *Sea Princess* and *RJ Pfeiffer* cruises. The extensive literature on UV disinfection (US EPA 2003) cites UV effectiveness in log reductions of target organisms (log 2 and log 3 reductions leave 1% and 0.1% of the original standing crop respectively). Our tests were not generally characterized by such large kill factors, especially over the short term (<24 h). However, in several bacteria colony growth experiments we observed zero colonies in UV treated samples (Fig. 5). At the end of 96 h, UV treated ballast tanks showed roughly 1% zooplankton survivorship (Fig. 13). UV treated enclosure experiments on RJP1 and RJP2 yielded no live animals after all sampling times >48 h (Fig. 16). ATP/POC, a proxy for living biomass to total biomass, decreased roughly four-fold in UV treated tank and enclosure experiments (Figs. 4c,d,h). Phytoplankton photochemical efficiency dropped ca. three-fold in UV treated ballast tanks on all cruises (Figs. 1 a-c).

Recently, the International Convention for the Control and Management of Ships Ballast Water and Sediments was adopted by the International Maritime Organization (IMO 2004). Regulations outlined in the Convention call for ballast treatments and exchange treatments to meet minimum performance standards. Specifically, ballast treatment should yield no greater than 10 viable organisms m^{-3} for larger plankton (>50 μm) and open-ocean exchange must exceed 95% volumetric exchange efficiency. It is of some interest to note that both ballast treatment and open-ocean exchange conducted during this study met those criteria. Final UV treated ballast tanks yielded less than 10 live zooplankters m^{-3} on SP3, RJP1 and RJP2 (Figs. 10,12). Open ocean exchange efficiencies can be calculated as $E(\%) = (C_0 - C_e)/(C_0 - C_i)$, where C_0 is the initial concentration of tracer in the ballast tank, C_i is the concentration of tracer in the incoming exchange water and C_e is the final concentration of tracer in the ballast tank after mixing and/or flushing. Using chl *a* as a natural tracer (Fig. 2c,d) we compute greater than 99% volumetric exchange efficiency for RJP1 and RJP2.

Our finding is that UV treatment increased the kill factor above what would be expected from prolonged tank confinement alone. Importantly, the combination of UV treatment and long (96 h) tank confinement yielded conditions similar to those found in open-ocean exchange treatments. It remains to be seen whether UV treatment and short term tank confinement (<48 h), followed by discharge into natural waters results in continued deterioration of plankton survivorship or eventual recovery.

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