

2017

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Wier, Timothy P.; Moser, Cameron S.; Grant, Jonathan F.; Riley, Scott C.; Robbins-Wamsley, Stephanie H.; First, Matthew R.; and Drake, Lisa A., "Optimal approaches for inline sampling of organisms in ballast water: L-shaped vs. Straight sample probes" (2017). *U.S. Navy Research*. 118.

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Optimal approaches for inline sampling of organisms in ballast water: L-shaped vs. Straight sample probes



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ARTICLE INFO

Keywords:

Invasive species
Protists
Microorganisms
Water quality
Ballast water

ABSTRACT

Both L-shaped (“L”) and straight (“Straight”) sample probes have been used to collect water samples from a main ballast line in land-based or shipboard verification testing of ballast water management systems (BWMS). A series of experiments was conducted to quantify and compare the sampling efficiencies of L and Straight sample probes. The findings from this research—that both L and Straight probes sample organisms with similar efficiencies—permit increased flexibility for positioning sample probes aboard ships.

1. Introduction

Regulations that limit the concentrations of living organisms in ballast water discharge have been established to minimize the transfer and introduction of aquatic invasive species. To accurately estimate organism concentrations, samples must be representative of the volume of interest. From these samples, counts of living organisms may be used to determine a ship's compliance with the discharge standard (USCG, 2012). Representative samples are also necessary in verification testing of ballast water management systems (BWMS), which are installed aboard ships to meet the discharge standard (IMO, 2004, USCG, 2012). Ships often have ballast flow rates which vary during uptake and discharge operations, making it especially important that sampling systems are designed with the flexibility to collect representative samples over these changes. That is, changes in the flow rate through the sample probe must mirror changes in the flow rate in the main ballast line from which it draws samples.

Representative samples of ballast water will have physical characteristics and biological assemblages similar to the water in the main ballast line. Specifically, concentrations of living organisms in each of three size classes of regulated organisms ($< 10 \mu\text{m}$ [bacteria, here, three indicator species and a pathogen], ≥ 10 and $< 50 \mu\text{m}$ [nominally protists], and $\geq 50 \mu\text{m}$ [nominally zooplankton]) must be representative of in situ concentrations in the ballast water. Thus, sampling equipment and methods should not selectively retain or exclude

certain organisms based upon size or morphology, nor should they induce organism mortality during sampling operations.

Ballast water sampling includes: 1) a sample port (“door”), which is the opening in the main ballast pipe, 2) a sample probe, which is the tube or pipe that collects the sample, and 3) sample flow control, collection, and processing equipment. The design of the sample probe, which encompasses the probe shape, the operational flow rates it can accommodate, and its location in the ballast piping system, may affect sampling efficiency. This paper focuses on configurations for sample probes.

In a study using computational fluid dynamics (CFD) to examine different configurations of sample probes, a probe with an opening facing upstream into the flow was shown to minimize turbulence near the probe opening (Richard et al., 2008). Additionally, it was recommended that the sample probe opening should be located in the center of the ballast main line, with the probe situated in a section of straight piping that has sufficient distance from any obstructions to achieve fully developed turbulent flow (Richard et al., 2008). An important consideration in sample probe design is the isokinetic diameter (D_{ISO}), which when sized correctly, the diameter where the flow velocity in the sample probe is equal to flow velocity in the main line. Formerly, the guidance was to use sample probes sized to $1.5\text{--}2.0 \times D_{ISO}$, to allow flow to slow upon entering the sample probe. Subsequently, empirical experiments measuring the capture efficiency (CE) of living organisms along a range of isokinetic ratios showed the CE did

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not vary significantly among sample probes, which ranged from 1.0 to $2.0 \times D_{ISO}$ (Wier et al., 2015). These findings suggested that wider ranges of sample probe configurations are acceptable for obtaining representative samples than previously recommended (i.e., $1.5\text{--}2.0 \times D_{ISO}$; EPA, 2010).

During these trials, another important question arose regarding the configuration of the sample probe: does the choice of L-shaped (L) or straight (Straight) sample probe affect the representativeness of the sample? This question is relevant because the placement of the sample probe in the ballast piping system often depends upon—and is limited by—the ship's upstream and downstream piping configurations and space constraints. The current guidance indicates that the sampling probe “should be oriented such that the opening is facing upstream and its entrance leg is parallel to the direction of main pipe flow and concentric to the larger pipe, which may require sampling pipes to be “L” shaped with an upstream facing leg, if installed along a straight section of discharge pipe” (EPA, 2010). Following this guidance, the sample probe may also be straight and inserted into the main line at a bend or elbow.

Given the two probe configurations, the differences, if any, between the performances of the sample probe geometries have been questioned. Initially, empirical experiments address this question by measuring the CE of fluorescent, polystyrene microbeads (unpublished data). In a series of trials, no difference in CE was observed between microbead concentrations in samples collected using the L or Straight sample probes. While demonstrating that neutrally buoyant, inert objects were not affected by the geometry of the sample probe, these studies were not able to verify that the different sample probe geometries (and potentially different fluid dynamics resulting from the different sample probes) do not affect CE and mortality of organisms.

To examine if sample probe geometry affects the CE and mortality of organisms, a series of trials was performed using both laboratory- and field-scale test platforms in Key West, FL, USA. The goal of these experiments was to determine if the two sample probes were equivalent for shipboard sampling. That is, are the CE and mortality rates of both probes comparable?

Underpinning the idea of representative sampling is that representative samples must be collected over the operational range of ballast flows. To understand the variability in real-world conditions, 15 companies were surveyed to determine the uptake and discharge ballast flow rates. From these data, the variability in flow rates was calculated, and it may be used for future studies.

2. Materials and methods

Two sets of trials were designed for this study: 1) laboratory-scale trials and 2) field-scale trials. In laboratory-scale trials, ambient organisms in the ≥ 10 and $< 50 \mu\text{m}$ size class were examined, while field-scale trials focused on ambient organisms in the $\geq 50 \mu\text{m}$ size class. In both experimental designs, which were not run concurrently, ambient source water was used.

2.1. Laboratory-scale experiments for organisms ≥ 10 and $< 50 \mu\text{m}$

The test platform to conduct the laboratory-scale experiments was modified from a platform used in a previous study (Wier et al., 2015). Briefly, the source tank—a 380 L (100 gal.), cylindrical tank with a conical bottom—was connected to a positive displacement pump (Fig. 1). Clear piping (nominally, 2.5 cm [1 in.] diameter; 2.67 [cm actual inner diameter] transferred the source water through a piping circuit, which contained openings for the L and Straight probes. Sample probes, with an inner diameter of 0.49 cm [0.19 in.] were positioned downstream of a length of straight piping at least 10 times the diameter of the pipe. This distance of 10 times the pipe diameter provides geometric similitude (similarity) and fully developed flow (Cengel and Cimbala, 2006) between the upstream and downstream locations. To

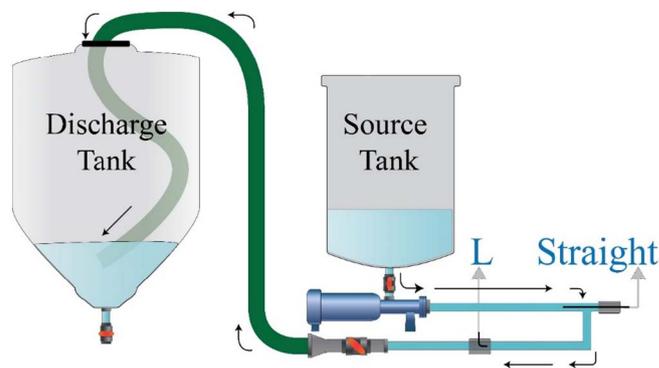


Fig. 1. Diagram of the laboratory-scale test platform. Water in the source tank was pumped through the main line to the discharge tank. Water was sampled from either the L or the Straight sample probe during the operation and collected in a sample tank that was positioned on a mass scale (not shown). Arrows indicate the direction of water flow. The figure is not drawn to scale.

avoid possible confounding effects of using two probes in a given trial (e.g., increased turbulence downstream of a probe), only one probe was inserted in the main line for each trial; the opening for the other probe was plugged. Each sample probe collected a time-integrated sample through a flexible tube leading into the sample tank, a ~ 4.3 L (1.1 gal.) opaque polypropylene jug with a screw cap. Flow from the sample probes was controlled with pinch valves and set so that volumetric flow was approximately isokinetic (i.e., $1.0 \times D_{ISO}$). The same make and model of pinch valve was used for flow control for both the L and straight apparatus, therefore, the effects of this valve on organism mortality were assumed to be similar. The actual flow through the sample probe was determined by continuously recording the sample mass. A laboratory balance (Ohaus Corporation, Pinebrook, NJ; 5 g resolution) transmitted the mass of the sample tank (positioned on top of the balance) every second to a custom computer interface (designed in LabVIEW 12.0, National Instruments, Inc.; Austin, Texas) that logged the measurements throughout the sampling operation. Flow through the main pipe was determined using a GF Signet magnetic flow meter (George Fischer, Ltd.; El Monte, CA).

Due to a problem in the interface between the voltmeter (which was connected to the flow meter) and the logging software on the computer, automatic logging was not possible. Instead, a 3 min video of the flow meter's digital display (on the face of the flow meter) was captured. After the analysis was completed, the digital display was transcribed into a spreadsheet at 1-s intervals (180 data points were collected for each trial).

2.1.1. Sampling operations

Supply water: A sampling event consisted of filling a 380 L (100 gal) tank with seawater and flowing the seawater through a piping loop with a sample probe (either the L or Straight probe) into a discharge tank. An experimental trial consisted of two sampling events: one with each probe. The order of sampling—whether using the L probe first or second—was alternated between trials. Each sampling event yielded seven samples: three from the source tank collected prior to flowing water through the pipe loop, one from the sample port throughout the 3-min sampling event, and three from the discharge tank upon completion of the sampling event. Water in the source and discharge tanks was well mixed with a wooden oar (10 \times rotations in both directions, interspersed with strokes across the center of the tank) period to collecting three, discrete samples (4.3 L each) using a hand-turned rotary pump.

The volume of each sample was calculated by weighing all of the sample vessels to the nearest 0.5 g to determine the mass of sampled water. Temperature and salinity (determined by a thermometer and refractometer, respectively) of the sample water were used to determine

the density of seawater following standard equations (Fofonoff, 1985; calculated using <http://fermi.jhuapl.edu/denscalc.html>), and the mass of seawater was converted to volume using the calculated density.

2.1.2. Sample processing and analysis

Sample processing began immediately and required 30 min to complete. The entire sample volume, which ranged from 3.3 to 4.3 L, was screened through a 7 μm mesh sieve. The sieve retained organisms $\geq 10 \mu\text{m}$, and the entire sample volume was concentrated to approximately 20 mL. The actual volume was determined by measuring the mass of the sample and converting mass to volume as described above. Sample analysis, consisted of microscope counts and measurements of variable fluorescence. Briefly, for microscope counts, water samples were incubated with a set of vital fluorochromes: fluorescein diacetate and chloromethylfluorescein diacetate (FDA and CMFDA; final concentrations of 5 and 2.5 μM , respectively). After a 10 minute incubation, the labeled sample was then transferred into a gridded chamber (a Sedgewick Rafter slide), and seven randomly chosen rows (each with 50 grids, equivalent to 50 μL) were visually scanned. The fluorescent (i.e., living) organisms were categorized into general taxonomic categories and tallied (Steinberg et al., 2011). As a data quality indicator, blind, duplicate counts were performed by a second analyst on one of the analytical replicates for each sample in the ≥ 10 and $< 50 \mu\text{m}$ trials.

Because dead organisms were not easily detected using light microscopy, and because only living cells would display fluorescence, concentrations of dead organisms were not measured. Rather, variable fluorescence was used to estimate the relative status of the population of organisms ≥ 10 and $< 50 \mu\text{m}$. Variable fluorescence was measured using a pulse amplitude modulated (PAM) fluorometer (Water PAM, Walz, Effeltrich, Germany). Detailed descriptions of the instrument and the analysis protocol are described elsewhere (Wier et al., 2015). Briefly, an aliquot of concentrated sample (3 mL) was placed in a quartz cuvette and the initial (F0) and maximum (FM) fluorescence were measured using a standard approach, and variable fluorescence (FV) was the difference between FM and F0 (Schreiber, 1998). The photochemical yield (FV/FM) was used as a relative indicator of the physiological status of the phytoplankton community (Genty et al., 1989).

2.2. Field-scale experiments for organisms $\geq 50 \mu\text{m}$

The field-scale test platform was used to conduct experiments comparing the L and Straight sample probe configurations during sampling of ambient seawater for organisms $\geq 50 \mu\text{m}$. The field-scale experiments were performed using the 20 cm (8") diameter main pipe capable of pumping seawater at a rate of approximately $200 \text{ m}^3 \text{ h}^{-1}$ (880 gpm). In contrast to the laboratory-scale experiments, both sample probes were used simultaneously in the field-scale experiments. The sample probes were placed at least 10 straight diameters downstream from the nearest obstruction. Additionally, there were two places to install the L sample probe within the piping, and these positions were alternated upstream and downstream from the Straight sample probe between trials to ensure the downstream sample did not realize any adverse effects from the positioning of the upstream probe. The sample probes each led to separate sample tanks with a maximum capacity of 3.2 m^3 (840 gal). Each sample tank contained a plankton net with a mesh size of 35 μm (nominal diagonal opening of 49 μm) and a mouth diameter of 0.5 m (19.7"; SeaGear, Co.; Melbourne, FL). Prior to sampling, both tanks were filled with approximately 150 L (40 gal) of seawater, which was sufficient to submerge the cod ends and the opening of the hoses from the main pipe.

2.2.1. Sample probe design

Both L and Straight sample probe diameters were 1.5" nominal pipe size (NPS) pipes (Diameter Nominale [DN] 40) with an internal diameter of 1.61" (4.1 cm). English were used in these trials, but for

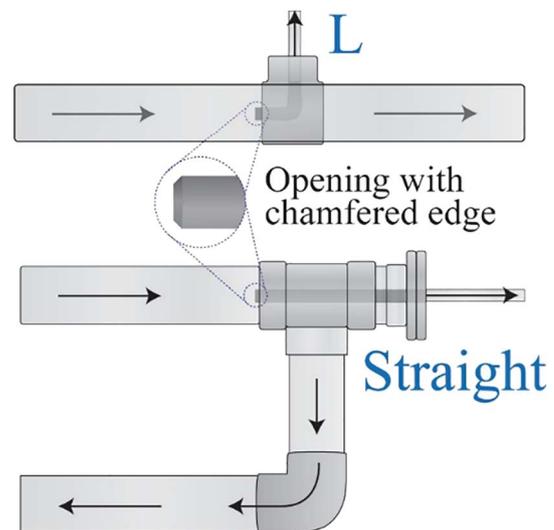


Fig. 2. Diagram of the L and Straight sample probes. The arrows indicate the direction of water flow. The figure is not drawn to scale.

clarity, both English and metric units are given for the pipe sizes throughout this paper. The experiments compared the concentration of living and dead organisms $\geq 50 \mu\text{m}$ collected from the two sample probe configurations. The L and Straight sample probes were arranged as shown in Fig. 2 facing into the main ballast flow stream and in the center of the pipe.

The Straight sample probe was designed and installed in the piping system (Fig. 2). This sample probe was designed using a straight section of 1.5" [DN 40] NPS schedule 40 PVC that was installed at the elbow flow transition in the piping. The installation required removing an existing 90° elbow and replacing it with an 8" (20.3 cm) socket weld tee and a section of 8" (20.3 cm) piping, and gluing it to an 8" (20.3 cm) flange. An 8" (20.3 cm) blind flange was fabricated to accept the 1.5" [DN 40] NPS sample probe.

The experiment was conducted with sample probes at three locations in the piping system labeled as SP-2, SP-3 and SP-4 (Fig. 3). The three locations allowed for the position of the probe to be changed, so the L sample probe was not always upstream of the Straight sample probe, which was always located at SP-3. To prevent any bias due to location, the position of the L sample probe was changed randomly between experiments—half the experiments had the L probe upstream of the Straight sample probe and in the other experiments, the order was reversed.

2.2.2. Sampling operations

Ambient seawater was pumped through the piping system to a serpentine mixing loop where the sample probes were located. Both sample probes collected samples at the same volumetric flow rate for all trials. Sample flow was monitored by an in-line flow sensor (Signet Magmeter) using the facility supervisory control and data acquisition system (Honeywell Experion PKS; Morris Township, NJ). This same system also controlled the ambient seawater flow in the main water supply pipe.

The target flow through the main pipe was 1045 gpm ($237 \text{ m}^3 \text{ h}^{-1}$). Once this flow was stabilized, the valves for both sample probes were opened to maintain the target flow rates of 25 gpm ($5.7 \text{ m}^3 \text{ h}^{-1}$). This flow rate was selected, as it allowed the trials to run for 32 min, and it allowed approximately 3 m^3 (793 gal) of seawater to be collected from each sample probe through its plankton net. The final volume was verified by measuring the depth of water in the sample tanks. The flow rate through the sample probes was controlled using a pneumatically actuated diaphragm valve for each probe, and the ratio of the sample flow velocity to the main flow velocity was 1.2 (i.e., sub-isokinetic).

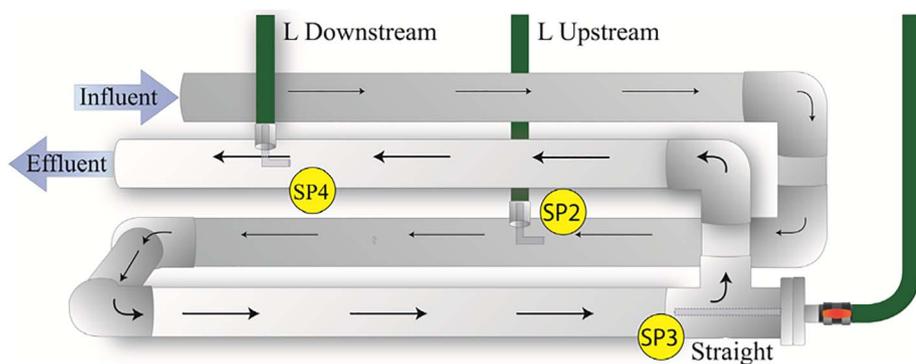


Fig. 3. Diagram of the field-scale test platform. The “mixing loop” contains three sampling positions: sample port 2 (SP2), SP3, and SP4. Water is supplied to the mixing loop with two seawater intake pumps, and the effluent is routed to the control tank (not shown in image). The arrows indicate the direction of water flow, and green tubes indicate the flow of water sampled from L-shaped or straight sample probes. The figure is not to scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Typical Values for dilutions and sample volume for the two size classes (≥ 10 and $< 50 \mu\text{m}$; $\geq 50 \mu\text{m}$). A = aliquot volume, C = concentrated sample volume, D = dilution, and S = total sample volume.

Parameter	≥ 10 and $< 50 \mu\text{m}$	$\geq 50 \mu\text{m}$
D	1.015 ×	10–20 ×
C	20 mL	1 L
A	1 mL	5 mL
S	4300 mL	3000 L

After the sampling operation was completed, the sample supply hoses were removed from the plankton nets, and the plankton nets were rinsed using a pressurized spray bottle (8 L [2.1 gal]) filled with filtered seawater (FSW; GF/F; Whatman) Concentrated samples from the plankton net were approximately 1 L (0.26 gal), and the volume was determined by measuring sample mass as described above.

2.2.3. Sample processing and analysis

Briefly, the concentrated sample, once transferred into a 1 L Pyrex™ bottle and weighed to measure sample mass, was mixed by slowly inverting the bottle five times. The bottle was then uncapped and 1 mL of sample was aspirated from the center of the well-mixed water using a serological pipette. The aliquot was transferred into a 15 mL centrifuge tube. This process was repeated an additional four times to yield a 5 mL subsample. Five subsamples were collected following this routine. The subsamples were not independent replicates, rather, they were considered analytical replicates and were used to measure the variability of organism concentrations measured within the sample. A rapid, initial analysis was performed to determine whether the subsample aliquots

required dilution. If the concentrations of organisms $\geq 50 \mu\text{m}$ were too high to count accurately (e.g., $> 30 \text{ Ind. mL}^{-1}$), the sample was diluted with FSW. Dilution factors ranged from 10 to 20 ×.

A 5 mL aliquot of the diluted sample was transferred into a Bogorov (counting) Chamber, and 50 μm microbeads were added as a size reference so that ~10 microbeads were visible in each field of view. Analysts scanned the entire chamber using a stereomicroscope at 20–30 × magnification. Moving organisms, organisms responding to physical stimulus (e.g., prodding with a metal probe), and algal cells with intact cellular structures were categorized as living, binned into general taxonomic categories, and tallied. As a data quality indicator, duplicate counts were performed by two analysts on 20% of the samples. The threshold value for percent variation was set at 20%, and the target value for the percent coefficient of variation (CV) among all replicate counts was $\leq 20\%$. All analyses were completed within 6 h of sample collection.

2.3. Variability in flow rates

With the help of the USCG, BWMS vendors that had conducted shipboard testing on operating vessels were contacted. They were given a survey that addressed the operational aspects of the ballast system by requesting data on a variety of parameters: ballast main pipe diameters, maximum observed flow rates, minimum observed flow rates, duration of shipboard sampling events, and any additional notes or comments. These data were solicited for both the uptake and discharge ballast operations. 14 BWMS vendors responded to the survey. The size and description of the ships were not included in the survey responses to keep the results anonymous.

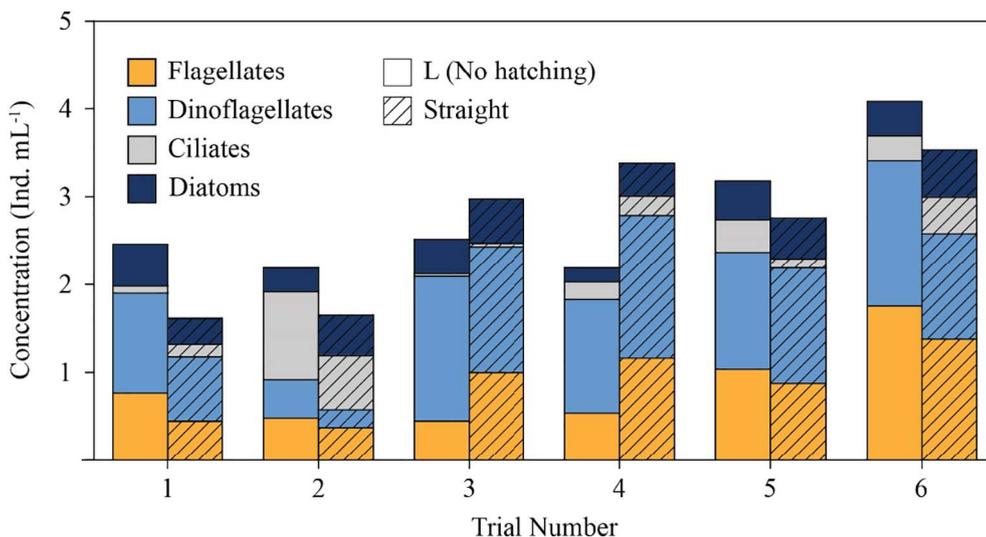


Fig. 4. Community composition of organisms ≥ 10 and $< 50 \mu\text{m}$. Concentrations of living flagellates, dinoflagellates, ciliates, and diatoms were measured via epifluorescence microscopy after incubating the samples with two fluorochromes.

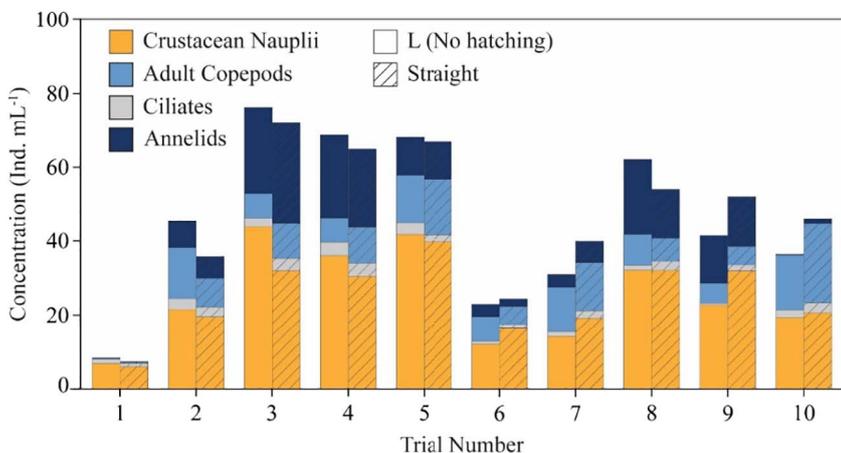


Fig. 5. Community composition of organisms $\geq 50 \mu\text{m}$ measured via light microscopy.

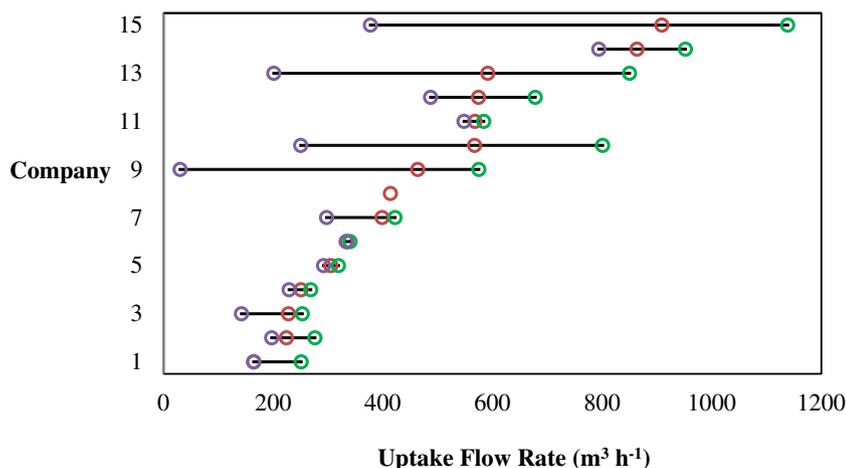


Fig. 6. Results of a survey on the variability in ballast water flow rate during ballast water *uptake* operations for 15 ships. Red circles show the average uptake flow rate, purple circles show the minimum uptake flow rate, and green circles show the maximum uptake flow rate. The black, horizontal lines represent the range in ballast flow rates. Ship 8 only provided the average observed flow rate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

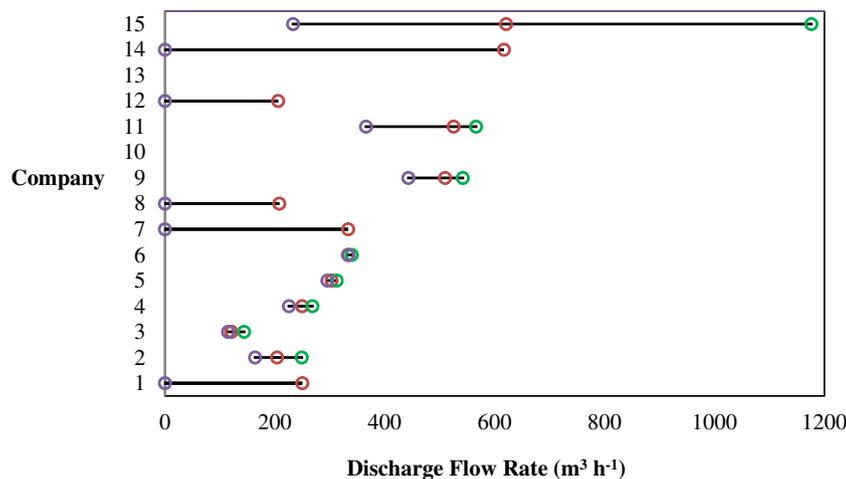


Fig. 7. Results of a survey on the variability in ballast water flow rate during ballast water *discharge* operations for 15 ships. Red circles show the average discharge flow rate, purple circles show the minimum discharge flow rate, and green circles show the maximum discharge flow rate. The black, horizontal lines represent the range of ballast flow rates. Discharge flow rate for ships 10 and 13 was not supplied. Maximum flow rate data for ships 1, 7, 8, and 12 was not provided. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.4. Data analysis

Concentrations of the populations of organisms (P) were calculated from the total number of living individuals (I) counted in the aliquot volume (A). This concentration was adjusted to account for the sample dilution (D), the concentrated sample volume (C), and the total sample volume (S) (Eq. (1)):

$$P = \frac{I \cdot D \cdot C}{A \cdot S} \tag{1}$$

This equation applied to both size classes of organisms. For organisms ≥ 10 and $< 50 \mu\text{m}$ from the laboratory-scale experiments, the sample was diluted by the addition of fluorochrome labels: $15 \mu\text{L}$ was added to 1 mL of sample, which is equivalent to a dilution of $1.015 \times$ (Table 1). Organisms $\geq 50 \mu\text{m}$ from the field-scale experiments were diluted with FSW, and dilution factors ranged from 10 to $20 \times$. Typical dilutions and sample volumes measured for the two size classes are shown in Table 1.

Because organisms were tallied in general taxonomic categories, the total number of organisms was partitioned into subcomponents (e.g.,

crustacean nauplii, ciliates, annelids, etc.), allowing the concentrations of the constituent groups to be calculated.

2.4.1. Calculating capture efficiency

Organisms' concentrations were log-normalized prior to conducting calculations. Concentrations of ambient organisms varied among the trials, so concentrations in samples from the sample probes were normalized to the concentrations in the source tank. This process allowed for a comparison across different trials. For laboratory-scale trials, the *CE* (%) of living organisms ≥ 10 and $< 50 \mu\text{m}$ was calculated from the concentrations in the sample tank or the discharge tank (P_{SP} or P_{DT} , respectively; Ind. mL^{-1}) relative to concentrations in the source tank (P_{ST} , Ind. mL^{-1}) for each sampling operation (Eq. (2)):

$$CE_{10-50}(\%) = 100 \cdot \frac{P_{SP \text{ or } P_{DT}}}{P_{ST}} \quad (2)$$

For organisms $\geq 50 \mu\text{m}$, obtaining accurate estimates of concentrations in the source water or the discharge tank was not feasible. However, it was possible to collect samples simultaneously through the L and Straight sample probes. In this case, the concentrations of organisms in the Straight sample probe were normalized to concentrations in the L sample probe, as this configuration has a long history of use. Thus, *CE* for the $\geq 50 \mu\text{m}$ size class was calculated using the following equation (Eq. (3)):

$$CE_{\geq 50}(\%) = 100 \cdot \frac{P_{\text{Straight}}}{P_{L-\text{Probe}}} \quad (3)$$

2.4.2. Variable fluorescence of organisms ≥ 10 and $< 50 \mu\text{m}$

Similar to the concentrations of organisms, F_V/F_M measured in samples from the sample and discharge tanks were normalized to allow for comparisons among trials, each varying in the composition of the ambient community. The F_V/F_M values from both the L and Straight sample probes were normalized to values measured in samples from the source tank, which were collected at the start of each sampling operation. If a sample probe induced physiological damage to the algal community, F_V/F_M should decrease from values measured in the source tank.

2.4.3. Mortality of organisms $\geq 50 \mu\text{m}$

The mortality of organisms $\geq 50 \mu\text{m}$ (*M*, %) was measured as the relative contribution of dead organisms (P_{DEAD}) to total organisms, which was the sum of living (P_{LIVE}) and dead organisms (Eq. (4)):

$$M(\%) = 100 \cdot \frac{P_{\text{DEAD}}}{(P_{\text{DEAD}} + P_{\text{LIVE}})} \quad (4)$$

2.4.4. Statistical analysis

Statistical differences were measured using a *t*-test ($\alpha = 0.05$), which was used to compare *CE* and relative F_V/F_M of organisms in samples from L and Straight sample probes. A one-sample *t*-test was used to determine whether *CE* measurements were significantly different from 100%. Prior to analysis, a Shapiro-Wilk test was used to verify that data conformed to a normal distribution, validating the use of the parametric *t*-test.

3. Results

3.1. Organisms ≥ 10 and $< 50 \mu\text{m}$

The capture efficiency (*CE*) of organisms ≥ 10 and $< 50 \mu\text{m}$ was not significantly different between the sample probe types. For both L and Straight sample probe trials ($n = 6$), the *CE* of organisms in the sample tank was $120 \pm 22\%$ and $107 \pm 25\%$, respectively. These values were higher, but not significantly greater, than *CE* in the discharge tanks ($110 \pm 13\%$ and $107 \pm 10\%$), respectively.

Relative values of variable fluorescence (F_V/F_M) were not significantly different in either sample probe. For both L and Straight sample probe trials, the relative F_V/F_M in the sample probe tank was $101 \pm 0.6\%$ and $100 \pm 0.8\%$, respectively. These values were not significantly different from relative F_V/F_M values in the discharge tanks ($100 \pm 0.2\%$ and $100 \pm 0.4\%$, respectively).

The community of organisms ≥ 10 and $< 50 \mu\text{m}$ was dominated by flagellates, dinoflagellates, ciliates, and diatoms (Fig. 4). For all laboratory-scale trials, relative abundance of organisms in these categories was comparable in samples from both the L and Straight sample probes.

On one of the analytical replicates for each sample in the ≥ 10 and $< 50 \mu\text{m}$ trials, blind, duplicate counts were performed by a second analyst. In all cases, the percent difference between the duplicate counts was $< 20\%$ (data not shown).

3.2. Organisms $\geq 50 \mu\text{m}$

The calculated mean *CE* for all live organisms $\geq 50 \mu\text{m}$ was $99.8 \pm 0.5\%$ ($n = 10$), and *CE* ranged from 93.2 to 107% in all of the trials ($n = 6$). The mean *CE* was not significantly different from 100% (*t*-test, $p > 0.05$, $n = 10$), indicating that, overall, the two sample probes performed comparably. The location of the L sample probe (relative to the Straight sample probe) did not affect the sampling efficiency: *CE* for both downstream ($99.7 \pm 0.6\%$) and upstream ($100 \pm 0.4\%$) locations were not significantly different from each other or significantly different from 100% (*t*-test, $p > 0.05$, $n = 5$).

The data quality indicators met the target: in all cases, the percent difference between the duplicate counts was $< 20\%$ (data not shown). Examining the mesh netting of the plankton nets following the field-scale trials did not reveal mangled organisms on the mesh, which would have prompted an investigation into the procedure for rinsing the plankton nets.

Mortality was measured for each sample, and although observed mortality ranged from 0.3 to 15% in the trials, there were no significant differences in mortality resulting from the sample probe configuration within a trial. The community of organisms $\geq 50 \mu\text{m}$ was dominated by crustacean nauplii, adult copepods, ciliates, and annelids. For all trials, the relative abundance of organisms in these categories was qualitatively comparable in samples from both the L and Straight sample probes (Fig. 5).

3.3. Variability in flow rates

As expected, the flow rates varied greatly among ships. Across ships, flow rates during ballast water uptake operations were as low as $30 \text{ m}^3 \text{ h}^{-1}$ and as high as $5179 \text{ m}^3 \text{ h}^{-1}$, with a mean of $468 \text{ m}^3 \text{ h}^{-1}$ and a median of $411 \text{ m}^3 \text{ h}^{-1}$ (Fig. 6).

Ballast water discharge operations among the ships yielded a mean of $338 \text{ m}^3 \text{ h}^{-1}$ and median of $251 \text{ m}^3 \text{ h}^{-1}$ (Fig. 7). For a given ship operation, the range was as great as $943 \text{ m}^3 \text{ h}^{-1}$, with the maximum discharge flow rate of $1176 \text{ m}^3 \text{ h}^{-1}$ and minimum of $229 \text{ m}^3 \text{ h}^{-1}$.

4. Discussion

In both laboratory- and field-scale trials, the configuration of the sample probe—whether L or Straight—did not affect the sampling efficiency of organisms ≥ 10 and $< 50 \mu\text{m}$ or $\geq 50 \mu\text{m}$. This result is consistent with the findings of a previous study, which examined the sampling efficiency of microbeads and found no differences in the capture efficiency of small ($27\text{--}32 \mu\text{m}$ in diameter) or large ($150\text{--}180 \mu\text{m}$ in diameter) microbeads in L or Straight sample probes (unpublished data). For living organisms, mortality resulting from differential pressure gradients and fluid dynamics resulting from the different configurations of the sample probe could hypothetically result in differential rates of mortality. No such differences were observed in

these experiments. Further, the concentrations of organisms in the sample tanks and the discharge tanks showed similar concentrations of living organisms and community assemblages.

The variability between maximum, average, and minimum flow rates was high across ships, and in some cases, within a given ship. The greatest range on discharge operations (the time in which compliance with the discharge standard would be determined) was $943 \text{ m}^3 \text{ h}^{-1}$. The variations result in variations in flow velocities within ballast pipes, and these quantitative data illustrate the criticality of designing sample probes (or having a series of sample probes with different diameters available during sampling operations) to allow the flexibility to obtain representative samples across a range of flow rates and flow velocities.

The findings that L or Straight sample probes perform comparably may broaden the operational guidelines for testing and provide greater flexibility to shipboard testing organizations. The results indicated that there was no adverse effect on the organism capture efficiency or mortality for either size class of organisms ($\geq 50 \mu\text{m}$ and ≥ 10 and $< 50 \mu\text{m}$). Depending on the ship's architecture, sample probes located at bends and elbows in the ballast main line may be easier to access or closer to ideal locations for positioning sampling equipment than probes inserted in straight sections of pipe. Further, the different geometries available will assist designers and ship-owners with flexible options for ballast water sampling design.

Acknowledgements

This work was supported by the U.S. Coast Guard (USCG) Environmental Standards Division (CG-OES-3, contract HSCG23-14-X-MMS010, Task 5.1), although it does not represent official policy of the USCG or the U.S. Navy. We are grateful to Richard Everett and Regina Bergner (USCG) for their advice and programmatic guidance. We appreciate the input from the following ballast water management system vendors: Ecochlor, Inc., Headway, JFE Engineering Corporation,

Jiangsu Nanji, Optimarin AS, and DeNora Water Technologies and the other anonymous vendors that participated in the voluntary survey of their ballast systems and flow rates. This research was supported by Diane Lysogorski (Section Head, Naval Research Laboratory Code 6136 and Director, Corrosion and Marine Engineering, Key West, FL). The reviews of this paper by Edward Lemieux (Director, Center for Corrosion Science and Engineering, Code 6130), Warren Schultz (Acting Superintendent, Chemistry Division, U.S. Naval Research Laboratory), and three anonymous reviewers improved it—thank you.

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