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Design and validation of a ballast water compliance sampling device for shipboard use

Cameron S. Moser[1,*], Matthew R. First[2], Timothy P. Wier[3], Scott C. Riley[3], Stephanie H. Robbins-Wamsley[3], Vanessa Molina[3], Jonathan F. Grant[4] and Lisa A. Drake[1]

[1]*Chemistry Division, U.S. Naval Research Laboratory, Code 6137, Key West, FL 33040, USA*
[2]*Chemistry Division, U.S. Naval Research Laboratory, Code 6137, Washington, DC 20375, USA*
[3]*Excet, Inc., Springfield, VA 22151, USA*
[4]*Battenkill Technologies, Inc., Manchester Center, VT 05255, USA*

**Corresponding author*

E-mail: cameron.moser@nrl.navy.mil

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Abstract

As international regulations governing the discharge of living organisms in ships' ballast water enter into force, port state authorities will require a device for compliance inspectors or their designees to potentially use onboard ships to conduct compliance testing. Importantly, the device must be easy to transport, quick to connect to the main ballast water pipe, and reliable in collecting a representative sample of ballast water flowing through the pipe. To that end, a pocket-sized Compliance Sampling Device (CSD) was designed, fabricated, and validated. The CSD incorporates a fixed-orifice flow meter and a valve for controlling flow. Experiments were conducted to evaluate the accuracy and precision of flow measurements using the CSD. To determine whether the restricted flow through the device causes loss or damage to organisms, concentrations of living organisms and photochemical yield measurements of microalgae collected from the CSD were compared to controls (samples of water freely flowing through an unobstructed hose). No significant differences in concentrations of organisms or photochemical yield measurements were observed between samples. Results from these experiments showed that the device would be acceptable to collect compliance samples aboard ships.

Key words: aquatic nuisance species, port state control, discharge standard, International Maritime Organization, invasive species, policy, shipboard sampling

Introduction

Separately, the International Maritime Organization (IMO) and the U.S. have established standards and procedures for the management and control of ships' ballast water to reduce the transfer of aquatic organisms and pathogens. In 2004, the IMO adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004), which entered into force on September 8th, 2017. Both the U.S. Coast Guard (USCG) and the Environmental Protection Agency (EPA) have effected similar requirements in the U.S. (USCG 2012; EPA 2013). These actions set a standard that limits the number of living organisms discharged in ballast water, allowing: (1) < 10 organisms ≥ 50 µm in size (typically dominated by zooplankton) per m^3, (2) < 10 organisms ≥ 10 µm and < 50 µm in size (typically dominated by protists, often phytoplankton) per mL, and (3) limits on indicator and pathogenic bacteria

per 100 mL (< 250 colony forming units [cfu] of *Escherichia coli*, < 100 cfu of intestinal enterococci, and < 1 cfu of toxigenic *Vibrio cholerae*).

To ensure that discharged ballast water meets these standards, port state authorities may need to rapidly collect and analyze a sample. This compliance testing differs from the extensive analyses performed as part of verification testing of ballast water management systems (BWMS, e.g., EPA 2010). This in-depth sampling and analysis required for verification testing is not practicable for rapid, shipboard analyses (e.g., King and Tamburri 2010). Thus, if the ballast water is to be evaluated quickly, an "indicative analysis" may be conducted (IMO 2008, 2009), where the groups of organisms prescribed by the discharge standard could be considered. In this regard, a representative whole water sample of the ballast water discharge can be collected to assess the number of living organisms per unit of volume. Organism concentrations in the ≥ 10 and < 50 μm size class are ideal for analysis, primarily because the sample volume needed is small (i.e., liters) compared to the larger size class (e.g., First et al. 2016). The ≥ 50 μm size class would require volumes on the order of 1 or more cubic meters (e.g., Miller et al. 2011) and specialized equipment to filter and concentrate organisms for analysis (e.g., Drake et al. 2014). Likewise, concentrations of organisms < 10 μm (with the exception of several enteric bacteria) are not specified in the discharge standard, and the regulated organisms typically do not exceed the discharge standard in ballast water; therefore, this smallest size class is not commonly considered for compliance sampling.

Some approaches and devices developed to collect whole (unfiltered) water samples of ballast water involve grab samples taken directly from the ballast tank through hatches or sounding tubes (Olenin et al. 2000; Gollasch et al. 2002; Hua and Liu 2007). Access to the ballast tank through these openings can be limited, making this approach impractical for widespread use (David and Perkovič 2004; Wright and Mackey 2006; Gollasch and David 2011). Furthermore, because organisms can be stratified within the tank (Murphy et al. 2002; First et al. 2013), grab samples must be collected from multiple depths in the water column. Regardless, in-tank samples are not considered representative of the volume of water sampled.

Other methods to collect samples are test apparatuses and skids with filters or plankton nets. These devices are connected to the main ballast pipe via tubing that leads to an in-line sample probe (i.e., a pipe inserted into the ballast line), and they return filtered water to the main ballast pipe (e.g., Schillak and Stehouwer 2013; Drake et al. 2014). In this manner, they can collect samples that are representtative of the discharged ballast water. Such devices are primarily used to filter and concentrate organisms in relatively large volumes of water (cubic meters), but they are valved with outlets to permit whole water samples to be collected as well. A drawback of these systems is that they can require sizeable filters or nets to reduce stress on organisms, and thus, the housings or tanks used to contain them can be large, heavy, and burdensome for a single person to carry onboard. The optimal device for compliance testing would be designed to draw whole water samples from the sample probe, but only in small volumes (liters), eliminating the need for bulky filter housings.

The device and the process used to collect a sample must not cause significant organism mortality. Sub-isokinetic and isokinetic sample flow velocities between 0.25 and 1 times the velocity in the main ballast pipe have been shown to minimize the mortality from the shear stress near the entrance to the sample probe. This equates to flows at between 1 and 2 times the isokinetic diameter (Wier et al. 2015). Therefore, the ideal compliance sampling device (CSD) must be capable of measuring and—perhaps more importantly—maintaining a given flow velocity. This requirement exists among other design constraints: the CSD must also be portable, easy to use, reliable, and sturdy enough for shipboard operations so that it can be transported, connected, and used by a single person. Samples collected with the CSD would be analyzed for living organisms aboard the ship or carried to an offshore location for analysis.

This paper describes the design and validation of a CSD for use by a port state control (PSC) officer or inspector (or his or her designee) sampling a vessel's ballast water discharge to determine compliance with regulations. The PSC officer conducts inspections of foreign ships arriving to ports, which can include checking relevant documents and certificates, ensuring that the vessel is crewed and operated in compliance with international regulations, verifying the competency of the ship's master and officers, and checking that the ship's overall condition and hygiene comply with international standards (Paris MoU 2017). If collecting a sample from a foreign or domestic ship was warranted, the inspector would carry the device onto a ship and connect it to a sample probe installed in the main ballast pipe of the vessel to collect an unfiltered, whole water sample.

Validation of the CSD was completed by assessing engineering and biological parameters. The engineering aspects were quantified by measuring the accuracy and precision of the sample flow. To evaluate the CSD's effect on organisms, the concentrations of living organisms and the physiological state of organisms

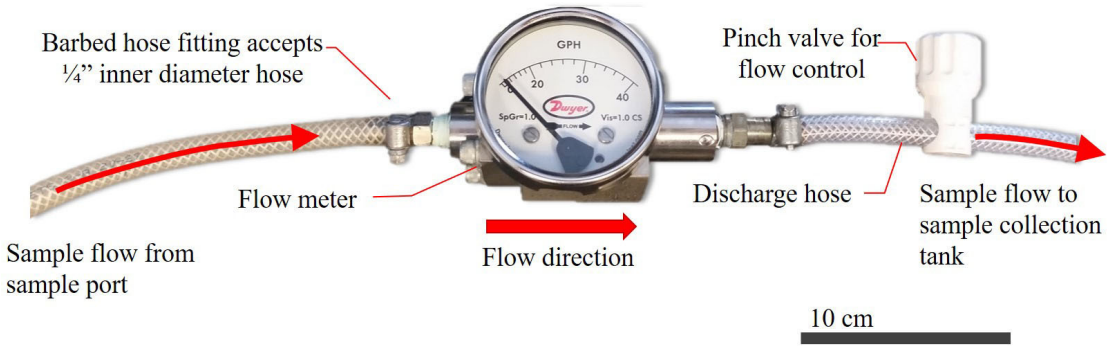

Figure 1. Photograph of the compliance sampling device (CSD), with components labeled.

that were collected using two configurations of the CFD were compared to organisms collected using a standard sampling technique (i.e., the control). Here, water flow was unobstructed by valves or diaphragms, flowing freely into the sample vessel.

Methods

Design of the Compliance Sampling Device

First, a set of performance objectives to obtain valid samples for compliance testing of ballast water was defined. Foremost, the sample must be of sufficient volume and aggregated over ample time to be representative of the water passing through the main ballast pipe at the time of sampling. The volume, and, by extension, the weight of the resulting sample should be suitable for a single person to carry, and the time for collection and processing must fit within the busy schedule of a PSC officer (i.e., it should take minutes, not hours). To meet these goals, the CSD was optimized to collect a time-integrated sample volume of 5 L over a minimum of 5 minutes, resulting in an average volumetric flow rate of 1 L min^{-1} over the sample period. At the discretion of the sampling party, the CSD can operate for any length of time and over a range of flow rates, offering flexibility to match the sample volume and time with ballast system particulars and directives of PSC. For example, if the sample probe were sized appropriately, the sample flow rate could be halved to 0.5 L min^{-1} to collect a 5 L sample over 10 minutes, as recommended by Gollasch and David 2017. The CSD can also be used to take multiple samples at separate times if analysis is needed at different stages of the discharge (e.g., to account for tank stratification if the ballasting event occurs over multiple hours). The samples will only be representative if the sample flow velocity is maintained at sub-isokinetic to isokinetic flows between 0.25 and 1 times velocity in the main ballast line (thus, the sample flow velocity does not exceed the velocity in the main ballast line).

A PSC inspector will typically bring aboard no more than a backpack for carrying essential equipment (J. Baeten pers. communication), and therefore, the CSD was designed to be small, portable, and lightweight for optimal handling. To save time, the CSD was designed to be simple, reliable, maintenance-free, and quick to set up and operate. Components were selected based on commercial availability and cost—the total cost of the device was < $1,000 USD. In addition, several assumptions were made for its use aboard a ship: 1) the device is connected to a pre-existing sample port installed with an appropriately sized sample probe, 2) electricity is not needed to power the device, 3) the device is used only to collect unfiltered, whole water samples from a pressurized pipe when deballasting is underway, and 4) compliance testing is based on analyzing only organisms in the ≥ 10 µm and < 50 µm size class. Once the sample is obtained, the inspector could use hand-held tools, which are commercially available for analyzing small volumes of water for compliance (e.g., Bradie et al. 2017; First et al. 2018), or carry the sample offsite for detailed analyses.

To achieve the above mentioned objectives and design considerations, the simplest and most economical configuration of the CSD was a fixed-orifice flow meter, plumbed in-line with a hose and pinch-valve for flow control (Figure 1). Beginning from the sample probe of the main ballast pipe, in this scenario, sample flow is drawn through a connected diameter nominal (DN) 8 mm (0.25") hose, which then enters the flow meter and exits through a hose of identical diameter, except where it is restricted from a pinch valve. The pinch valve acts like a diaphragm valve, where flow is controlled by contracting or expanding a bladder mechanism that minimizes shear

in the fluid (and minimizes mortality to organisms). The pinch valve is, however, much cheaper and more compact than diaphragm valves for similar-sized plumbing. The handle of the pinch valve can easily be adjusted to reach the desired flow rate. Sample flow is then directed into a collection container (Figure 2). Aside from the hoses, pinch valve, and indicator gauge, the material of the components used to build the CSD was 316 stainless steel for maximum durability and corrosion resistance.

Evaluation Approach

To evaluate the operational performance of the CSD, an experiment was conducted to verify the accuracy and precision of measurements using the fixed-orifice flow meter. Because the cross sectional area was sharply constricted at the orifice of the flow meter, to address the possible effect on organism mortality in relation to this potentially high stress location, an additional set of experiments were conducted. Both types of experiments are described in detail below. Statistical analyses were conducted using SigmaPlot (V11, Systat, San Jose, CA).

Accuracy and Precision of the CSD's Fixed-Orifice Flow Meter

Measurement accuracy of the fixed-orifice flow meter was evaluated by connecting the CSD to a municipal water supply at the U.S. Naval Research Laboratory in Key West, FL (NRLKW). Water flowed through the device and into a 20-L container (a carboy) for 3 minutes. A laboratory balance was used to measure the mass of the carboy as it filled, and sample (water) mass was logged every second to calculate the gravimetric flow rate. Next, this gravimetric flow rate was compared to the flow rate shown on the gauge of the fixed-orifice flow meter. Here, the accuracy of the CSD was calculated as the mean error between the two values. Precision of the flow output was assessed by calculating the coefficient of determination (R^2) of the mass of the carboy with respect to time via linear regression analysis. In this manner, the gravimetric measurements were compared to those of the CSD. This experiment was conducted five times at each of three flow rates spanning the measurement range of the flow meter: 20, 30, and 40 U.S. gal h^{-1} (1.26, 1.89, and 2.52 L min^{-1}, respectively).

Effects of the CSD on Organism Mortality

Testing was conducted at NRLKW, where a seawater pump and piping system was constructed to meet the criteria for BWMS test facilities (EPA 2010). Here, the main ballast pipe—15 cm (6") nominal pipe diameter

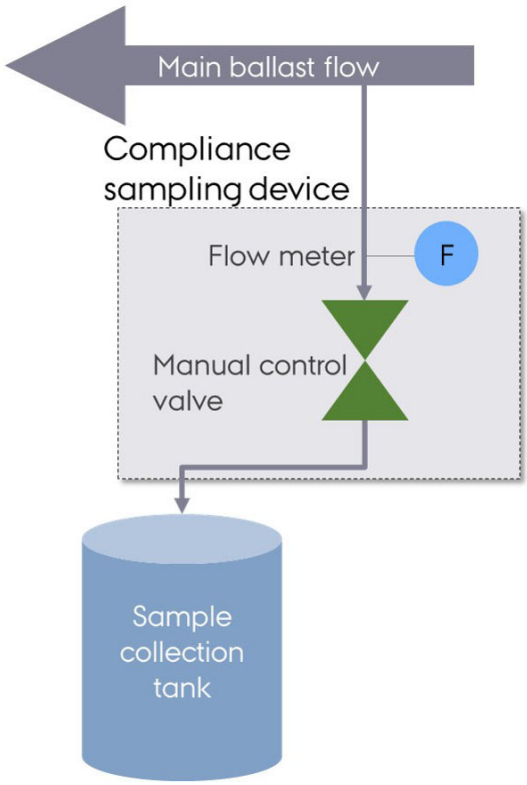

Figure 2. A schematic of the compliance sampling device (CSD) showing that it draws sample water from a main ballast line at a user-set flow rate and then water is collected.

(NPS) with an internal diameter of 20.3 cm (7.98")—included three 5 cm (2") NPS sample ports along the same straight length of pipe spaced 30 cm (~ 12") apart. The sample ports were fitted with 0.49 cm (0.19") inner diameter, 0.635 (0.25") outer diameter sample probes (Figure 3). The probes were stainless steel tubes bent into an "L" shape with the openings facing upstream into the flow (Richard et al. 2008; Wier et al. 2015). The probes, measured from the top of the "L" to the center axis of the bend, were approximately 30.5, 33, and 35.6 cm (12, 13, and 14") long — enough to be inserted through the sample port isolation valve and into the flow stream of the main ballast pipe. The distances of insertion of the three probes from the inner wall of the main ballast pipe were approximately 2.5, 5, and 7.6 cm (1, 2, and 3"). These offsets were calculated (NRL, unpubl. data.) such that each downstream probe opening was outside the boundary layer wake of the upstream probe (see calculation of turbulent boundary layer thickness, Schlichting 1968). The thickness of the tubes was selected such that drag force from the water

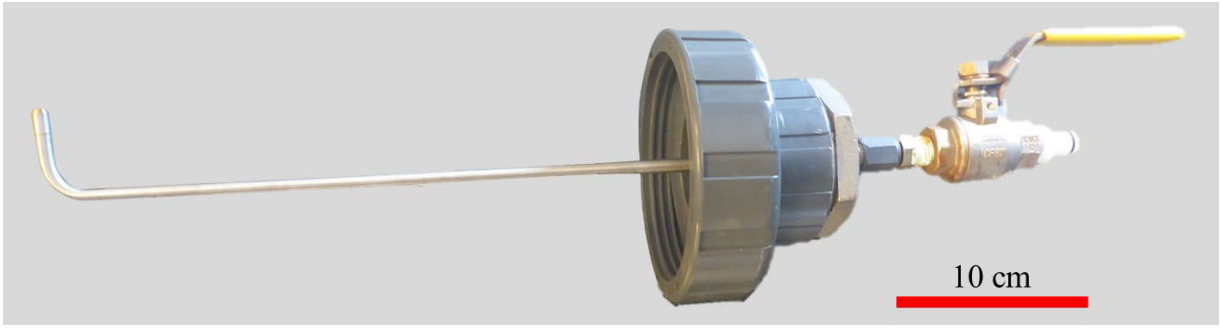

Figure 3. Photograph of one of three sample probes installed into the 5 cm (2") nominal diameter sample port of the 15 cm (6") main ballast pipe. Ballast water samples were collected simultaneously through the three of the probes using the CSD installed with a ball valve for flow control (CSD_b), the CSD installed with a pinch valve for flow control (CSD_p), and an open, unobstructed hose, i.e., the "free-flow" sample (control sample).

flowing over them in the main ballast line would result in deflection < 5 mm. The probes were used to take three whole water samples simultaneously during a ballast water sea-to-sea operation through 1) the CSD installed with a ball valve for flow control (CSD_b), 2) the CSD installed with a pinch valve for flow control (CSD_p), and 3) an open, unobstructed hose, i.e., the "free-flow" sample for control. The number of living organisms in the three samples was compared to determine any effects of the CSD on organism mortality.

Sample Collection

Prior to sampling, the three collection mechanisms (CSD_b, CSD_p, and control) were flushed using sea water from the main ballast line for at least 1 minute. The target volume for each sample collected was 5 L. The actual volumes of samples were determined gravimetrically by measuring the sample mass as described above and by using temperature and salinity to calculate the density of seawater from standard equations (Fofonoff 1985). Sample water was mixed by gently inverting the container five times before pouring the water through stacked 35-µm and 7-µm sieves used to size fraction organisms; the 7-µm sieve retained organisms larger than ≥ 10 µm and < 50 µm. The entire volume of each sample container (5 L) was concentrated, and the material retained (i.e., the filtrand) on the 7-µm sieve was washed into a 50-mL centrifuge tube using 0.22-µm filtered seawater. The resulting volumes were approximately 50 mL for each concentrated sample.

Quantifying Living Organisms

Living organisms ≥ 10 and < 50 µm were enumerated via epifluorescence microscopy following methods described elsewhere (Steinberg et al. 2011). Briefly, a 1-mL sample aliquot was labeled with fluorescein diacetate (FDA) and chloromethylfluorescein diacetate (CMFDA) at 2.5 and 5 µM final concentrations, respectively. Following a 10-min incubation period, the 1-mL sample was transferred into a gridded Sedgewick Rafter chamber, and between 5 and 7 rows of the chamber were randomly selected and scanned to detect living (i.e., fluorescing) organisms. Non-fluorescing organisms were classified as dead unless the object was moving. Organisms within the size class (as determined using microbeads with diameters approximately equal to the size thresholds) were categorized into general taxonomic groups and tallied.

Concentrations of living organisms were calculated using the following formula (Eq. 1):

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

Where P is the concentration of the population of living organisms ≥ 10 and < 50 µm, I is the count of the population within the sample aliquot (A), and C is the volume of concentrated sample, which was concentrated from the total sample volume (S). The addition of FDA and CMFDA resulted in a small dilution (D, 1.015x) of the sample. For size comparison, 10 and 50-µm microbeads with diameters approximately equal to the lower (10 µm) and upper (50 µm) boundaries of the size class were added as visual references for sizing organisms.

In addition to the epifluorescence microscope counts, organisms ≥ 10 and < 50 µm were analyzed using variable fluorescence fluorometry. A pulse amplitude modulated (PAM) fluorometer (Water-PAM; Walz, GmbH; Effeltrich, Germany) measured initial fluorescence (F_0) and maximum fluorescence yield (F_M) following the approach specified elsewhere

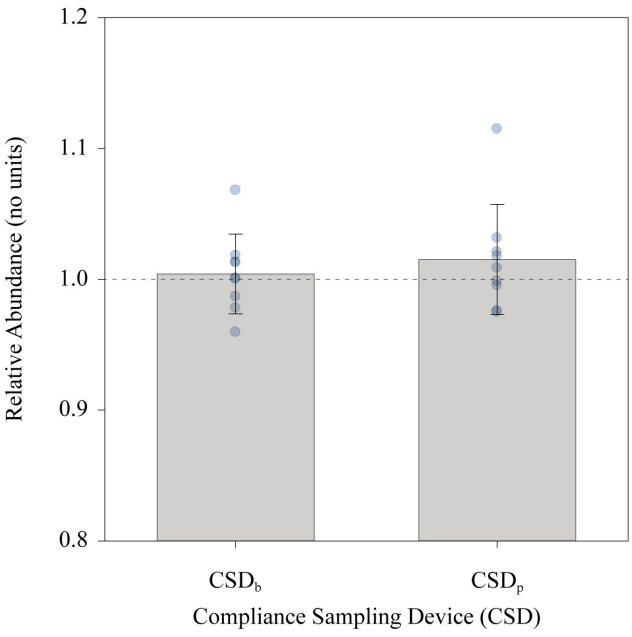

Figure 4. Relative abundance of living organisms ≥ 10 and < 50 µm in the two compliance sampling devices, the CSD installed with a ball valve for flow control (CSD_b), and the CSD installed with a pinch valve for flow control (CSD_p). Concentrations in samples from the compliance devices were log-transformed and normalized to concentrations sampled by the control method. The dotted horizontal line shows 1.0, where both the CSD and control samples are equal. Bars show the mean (with error bars marking standard deviation) of nine independent trials. Values for individual trials are displayed as circles.

(Genty et al. 1989; Schreiber 1998). From these two parameters, variable fluorescence ($F_V = F_M - F_0$) was used to calculate photochemical yield (F_V/F_M; no units; scaled from 0 to 1,000), a proximal measure of the physiological status of phototrophs (such as the microalgae) within the samples.

Results

For the CSD's fixed-orifice flow meter, the mean error, calculated based on percent difference between measured (CSD) and actual (gravimetric) flow rates, for 20, 30, and 40 gal h^{-1} was very low: 2.85 ± 1.43%, 0.66 ± 0.98%, and 1.19 ± 0.87%, respectively; in all cases, n = 5. As the carboy was filled with water, the relationship of the mass of the carboy to time was exceptionally strong in all trials ($R^2 > 0.99$), indicating that individual flow rates or CSD measurements of flow rates did not vary from the target fill rate (i.e., mass per time).

In experiments conducted to evaluate the CSD effect on organism mortality, the sample flow velocities using the CSD_b, CSD_p, and control methods were 36.5%, 37.2% and 109.5% of the velocity in the main ballast line, respectively (n = 9; data not shown). Sample flows using the CSD were within the target velocity range between sub-isokinetic and isokinetic flow, while the control samples were taken, on average, at slightly super-isokinetic levels. Comparing samples collected using the CSD and the control approach, the concentration of living organisms in the ≥ 10 and < 50-µm size range was not significantly different (Analysis of variance [ANOVA], $p > 0.05$). The mean living concentrations were 2.6 ± 0.45 mL^{-1}, 2.7 ± 0.57 mL^{-1}, and 3.0 ± 0.82 mL^{-1} for CSD_b, CSD_p, and control samples, respectively (n = 9; data not shown).

As the concentration of ambient organisms varied among trials, the relative abundance of living organisms was calculated as the ratio of the log-transformed concentration from the CSD (whether CSD_b or CSD_p) and the control approach, where flow was unobstructed (Figure 4). A one-sample t-test was used to test the hypothesis that relative abundances deviated significantly from 1.0, which would indicate that the CSD yielded samples with differing concentrations of organisms than the control approach. Differences between the CSD_b, CSD_p, and control were not significant ($p > 0.05$, n = 9).

Using the variable fluorescence fluorometer, no significant differences in photochemical yield were measured among samples collected with the CSD_b, CSD_p, and control approach (ANOVA, $p > 0.05$; Figure 5). The mean F_V/F_M was nearly identical among sample types, 489 ± 43 (no units), 504 ± 43, and 467 ± 37 for the CSD_b, CSD_p, and control samples, respectively. Values of F_V/F_M for living marine plankton communities were within ranges previously observed for living plankton at this location (First and Drake 2014).

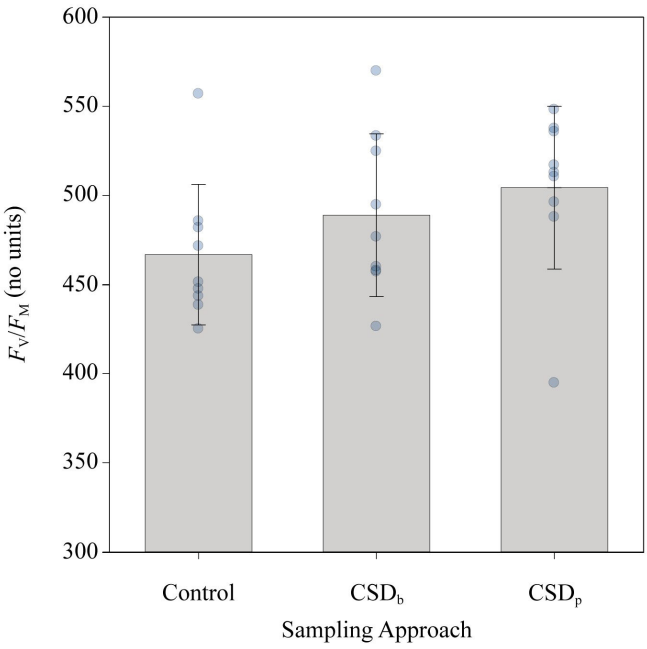

Figure 5. Photochemical yield (F_V/F_M) in samples collected with the control approach (free-flow) and both compliance sampling devices, the CSD installed with a ball valve for flow control (CSD_b), and the CSD installed with a pinch valve for flow control (CSD_p). Bars show the mean (with error bars marking 1 standard deviation) of nine independent trials. Values for individual trials are displayed as circles.

Discussion

These experiments suggest that the CSD is a practical shipboard compliance device. That is, the CSD met the design constraints described above and showed nearly identical biological results to control samples collected without the device. Flow rates measured by the CSD's fixed-orifice flow meter were highly accurate and precise when compared to gravimetric readings, and the extremely strong correlation between mass of the carboy and sampling time indicates good stability. Further, there was comparable organism mortality between the CSD (using two different flow control methods) vs. control samples.

Before the CSD can be considered for widespread use, several topics need to be explored. The fixed-orifice flow meter used in the CSD measures the highest maximum range of all models from the manufacturer, 0–40 U.S. gal (~ 150 L) h^{-1}. If a higher flow rate were needed, a model from a different manufacturer would have to be selected and validated. In addition to collecting samples for organisms ≥ 10 and < 50 µm, this device could collect a time-averaged, integrated sample that could be used to quantify the densities of indicator bacteria and pathogens, concentrations of dissolved or suspended matter, or concentrations of active substances and disinfection by-products from the BWMS's treatment processes. However, this approach would *not* be easily applied to collect samples for organisms ≥ 50 µm: large volumes (on the order of 1 or more cubic meters) are needed to collect a sufficient sample to predict, with confidence, that < 10 organisms m^{-3} are present. Additionally, water collected and concentrated would have to be either reinjected back into the ballast line or disposed. Either option requires extensive labor, equipment, and time. The CSD should also be tested in conditions with diverse ambient communities of organisms to ensure its widespread applicability for sampling ships around the world. The CSD was tested under specific conditions at NRLKW, and the assemblages of organisms and physical parameters of the ambient water may differ at other locations. Water with high particulate loads may also present complications, such as clogging. Thus, sampling in differing regions would provide a more complete assessment of the device. Likewise, shipboard trials to collect samples of treated ballast water are needed to determine the device's applicability in a working shipboard environment. Regardless, the results shown here indicate the CSD is a capable and promising device for compliance sampling under certain scenarios of ballast water monitoring.

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