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Modeling sampling strategies for determination of zooplankton abundance in ballast water

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ABSTRACT

Ballast water has been a major source of non-indigenous species introductions. The International Maritime Organization has proposed performance standard that will establish an upper limit for viable organisms in discharged ballast. Here we test different sampling efforts for zooplankton in ballast water on a commercial vessel. We fit different probability density functions to find the most representative and evaluated sampling efforts necessary to achieve error rates (α, β) of <0.05. Our tests encompassed four seasonal trials and five sample volumes. To estimate error rates, we performed simulations which drew from 1 to 30 replicates of each volume (0.10–3.00m^3) for mean densities ranging between 1 and 20 organisms m^{-3}. Fieldwork and simulations suggested that >0.5 m^3 samples had the best accuracy and precision, and that the Poisson distribution fit these communities best. This study provides the first field test of a sampling strategy to assess compliance with the future IMO standard for large vessels.

1. Introduction

Ballast water is one of the world's largest vectors for non-indigenous species (NIS) transfer (Molnar et al., 2008). Efforts to control this vector in the Great Lakes began in 1989 with voluntary mid-ocean ballast water exchange (BWE) for vessels entering with filled ballast-water tanks, which was followed by mandatory regulations in 1993. Regulations were extended to vessels with 'empty' ballast-water tanks in 2006 and 2008 in Canada and the USA, respectively. Ballast water management (BWM) has become a standard procedure worldwide, and is overseen by the International Maritime Organization (IMO). Current IMO best management practises request vessels with full ballast tanks conduct exchange on the open ocean to ensure that 95% of the ballast volume has been exchanged, to achieve an in-tank salinity of at least 30‰ (IMO, 2008a). While this procedure is effective in preventing the movement of NIS between freshwater ports that are connected by transoceanic routes (Bailey et al., 2011), it is less effective when both origin and destination ports are marine (Wonham et al., 2001). In 2004 the IMO proposed new performance standards (IMO D-2) (IMO, 2004). This agreement sets numerical limits on the density of two plankton size groups (<10 viable organisms m^{-3} for minimum dimension >50 μm and <10 viable cells mL^{-1} for organisms between 10 and 50 μm) as well as for three bacteria indicators (IMO, 2004). The IMO D-2 convention has yet to be ratified and implemented (IMO, 2004).

Many companies and research groups are testing technology devices and processes to ensure compliance with IMO D-2 standards. Initial steps for approval include testing of devices by an independent third party at verification facilities designed to provide bench-scale estimations, usually referred to as land-based testing. Verification centers also must replicate treatment trials as part of the bench-scale evaluation. Sampling strategies and sampling effort are intended to be easily replicable (IMO, 2008b). Model ballast tanks must be ≥200 m^3. For shipboard sampling, control and treated samples need to be collected in triplicate, that uptake and final densities be determined for control tanks, and that viable organism density be assessed before discharge of treated ballast water (IMO, 2008c). However, current guidelines provide no guidance on sample volumes or how they are collected.

Current technology devices have been tested primarily using land-based tests, though a subset has also used shipboard testing (Gollasch and David, 2010). However, no clear method exists for sampling onboard vessels, particularly for sampling directly from ballast tanks. Thus, an imbalance exists in the prescribed sampling process for land-based versus shipboard testing. Onboard sampling poses a major challenge as the IMO D-2 standard requires very low densities of zooplankton, and estimating live density of organisms requires large sample volumes, even under the best case (and unrealistic) scenario that

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organisms are randomly distributed (Lee et al., 2010; Miller et al., 2011; Frazier et al., 2013). Moreover, random dispersion of zooplankton in ballast tanks cannot be assumed, as organisms may aggregate and thus may exhibit a patchy distribution (Murphy et al., 2002; First et al., 2013).

Given that access to tanks is often limited, one important question researchers seek to answer is the relationship between sampling method and sample representativeness (Gollasch and David, 2011). Zooplankton sampling in ballast tanks may be done using plankton nets via hatches (Briski et al., 2013; Simard et al., 2011) or, less commonly, by pumping a known volume from the tank into a plankton net (McCollin et al., 2008; Veldhuis et al., 2006; Gollasch and David, 2010). Sampling a ballast tank is complicated as access is limited while in port and very difficult while en route (Wright and Mackey, 2006). Samples must be representative of the entire population, easy to replicate, and unbiased. Another consideration is inherent stochasticity associated with low population densities, with concerns regarding both accuracy and precision (Lemieux et al., 2008). In addition, the sampling strategy must allow inferences to be made regarding densities of viable zooplankton in treated water.

Another important element is to determine the minimum water volume adequate for representative sampling (Gollasch and David, 2011). Several studies have addressed the effects of low organism density and sample volume on estimating the true density of zooplankton, using both Poisson and negative binomial distributions (Lee et al., 2010; Miller et al., 2011; Frazier et al., 2013; Costa et al., 2015). The validity of this theoretical approach has not yet been affirmed empirically. The Poisson distribution is suitable under the assumption of a centralized outflow that can be sampled entirely or in equal time intervals (First et al., 2013). A key challenge is access to the entire water column of a tank. Net tows likely introduce bias as only the upper portion of the tank is typically sampled.

In this study, we tested different sampling volumes using three in-tank sampling points to sample the full depth of a ballast tank on a working cargo vessel. Our goal was to identify the sampling efforts that will provide accurate density estimations of zooplankton at the very low abundances that the IMO D-2 standard requires for compliance. We also designed a simple model to contrast common distributions that have been examined theoretically to provide a sample volume that managers can utilize to verify compliance with the IMO D-2 standard.

2. Methods

Ballast samples were collected during voyages by the Federal Venture, between 2012 and 2013 [see Paolucci et al. (2015)]. The vessel transited from three ports (Saguenay, Trois Rivières, and Bécancour) in Quebec, Canada to two ports (Vila do Conde and Sao Luis) in Brazil. A single trial was conducted during each voyage where samples were taken and analyzed. Samples were collected from the largest ballast tank (Tank 2) on the starboard side, with 25 mm diameter inlet pipes (Alfagomma 266GL Water S&D PVC Standard Duty) installed at three depths (4.5, 14.5 and 16.0 m below top deck level) to account for vertical variation in organism distribution (Fig. 1). We selected those depths based on the geometry of the tank: 4.5 m is the middle section of the attached wing tank, 14.5 m is the highest open space in the double-bottom tank, and 16.0 m is just above the baffle line in the deepest portion of the tank. Each inlet pipe contributed one third of the total sample volume. To assess sampling effort, triplicate samples totalling 0.10, 0.25, 0.50, 1.00 or 3.00 m^3 were collected. Samples were collected two days after ballast-water exchange was performed in the North Atlantic region using a pneumatic, self-priming diaphragm pump. Ballast water was transferred from the tank to the forepeak of the vessel where it was filtered through a 35 μm plankton net. Water volume sampled was measured with a Seametrics flowmeter (WMP-Series Plastic-Bodied Magmeter). In-line valves were used to keep water flow rate

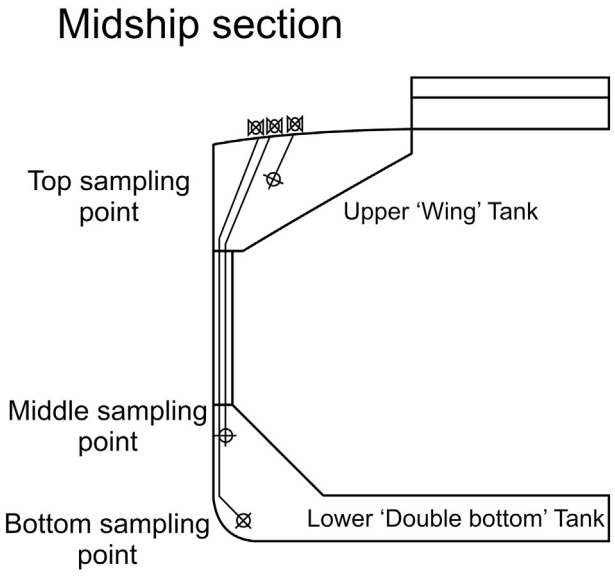

Fig. 1. Location of sampling ports inside the ballast tank.

to 40 L min^{-1} in order to avoid mortality due to strong currents. Samples were then fixed in 95% ethanol for microscope counting. We assumed that all intact individuals encountered when processing under the microscope were alive at the time of capture. Each sample was counted entirely to assess population density. The order in which sample volumes were collected was randomized using a random number generator in Excel (Microsoft Inc.).

We conducted basic descriptive statistics (mean and standard deviation) for our four trials. Variance was grouped for fall and spring as those samples were not statistically different and mean densities were similar. Our first goal was to determine the best volume for sampling. Since the true density of organisms in the ballast tank was not known, we assumed that the mean density of organisms over all sample volumes in each trial was an accurate estimate of true density. Preliminary analysis of variance (ANOVA) revealed that volume sampled had a large impact on the density of organisms in the tank ($p = 0.0056$). We estimated density based on the data points collected from the same volume. We assumed that if we sampled at the same volume repeatedly inside the tank, the density of organisms would follow a given probability distribution function (PDF). We performed the following analysis on each of five PDFs (Poisson, Weibull, Negative binomial, Gamma, and Log-normal) with respect to each volume individually. We estimated the parameters of each PDF by maximum likelihood estimation (MLE). Then, we created random number generators based on the estimated PDFs to sample more data points (i.e. one thousand data points) for the density of organisms for each volume, and calculated the mean square error (MSE) based on our assumption that the true density was the average of density estimates in all trials for each volume (Walther and Moore, 2005).

2.1. Modeling PDF for distribution of zooplankton

Our second goal was to determine how altering the spatial distribution of zooplankton would affect the sampling error rate. Specifically, our objective was to identify the number of samples of a particular volume that would be required to confidently state that a vessel was compliant with the IMO D-2 limit of <10 viable organisms m^{-3} for zooplankton-sized organisms while keeping the rate of Type I and II errors below 5%. In other words, the cumulative sample number of each individual density (from 1 to 20 organisms m^{-3}) required in each scenario was constrained to no more than a 0.05 error rate for both false positives and false negatives.

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