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A shipboard comparison of analytic methods for ballast water compliance monitoring

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ABSTRACT

Promising approaches for indicative analysis of ballast water samples have been developed that require study in the field to examine their utility for determining compliance with the International Convention for the Control and Management of Ships' Ballast Water and Sediments. To address this gap, a voyage was undertaken on board the RV Meteor, sailing the North Atlantic Ocean from Mindelo (Cape Verde) to Hamburg (Germany) during June 4–15, 2015. Trials were conducted on local sea water taken up by the ship's ballast system at multiple locations along the trip, including open ocean, North Sea, and coastal water, to evaluate a number of analytic methods that measure the numeric concentration or biomass of viable organisms according to two size categories (≥50 μm in minimum dimension: 7 techniques, ≥10 μm and <50 μm: 9 techniques). Water samples were analyzed in parallel to determine whether results were similar between methods and whether rapid, indicative methods offer comparable results to standard, time- and labor-intensive detailed methods (e.g. microscopy) and high-end scientific approaches (e.g. flow cytometry). Several promising indicative methods were identified that showed high correlation with microscopy, but allow much quicker processing and require less expert knowledge. This study is the first to concurrently use a large number of analytic tools to examine a variety of ballast water samples on board an operational ship in the field. Results are useful to identify the merits of each method and can serve as a basis for further improvement and development of tools and methodologies for ballast water compliance monitoring.

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1. Introduction

The International Maritime Organization adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments in 2004 to minimize the transfer of harmful aquatic organisms and pathogens in ships' ballast water (IMO, 2004). Regulation D-2 restricts the concentration of viable organisms ≥ 50 μm in minimum dimension at discharge to <10 viable organisms per cubic meter, and organisms < 50 μm and ≥10 μm in minimum dimension (hereafter, 10–50 μm) to <10 per millilitre (IMO, 2004). Now that the International Ballast Water Management Convention has been fully ratified, and will enter into force on September 8, 2017 (IMO, 2016), there is a pressing need for ships to plan installations of ballast water treatment systems, and for regulators to plan the implementation of the Ballast Water Management Convention into their national legislation and Port State Control inspection programs.

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Compliance monitoring and enforcement must be consistent, rigorous, and efficient (IMO, 2008); it can be divided into two main parts: ballast water sample collection and sample analysis, each of which is challenged by several difficulties (Gollasch et al., 2003; Gollasch and David, 2013; IMO, 2013; Gollasch and David, 2015). A number of tools and technologies are in development for both sampling and analysis, and recent studies have shown promising results for sampling devices (First et al., 2012; Bradie, 2016). For sample analysis, two types of methods may be employed: 'indicative' or 'detailed' analyses. 'Detailed' analyses, such as microscopy, provide a direct and precise measurement of the number of viable organisms in a sample that typically requires extensive scientific expertise, costly equipment, and a timeframe generally too long for a compliance enforcement scenario. In contrast, 'indicative' analysis methods should be rapid and easy to operate, typically measuring biological, physical, or chemical parameters that can be related to the number of viable organisms in a sample to provide an indication of potential non-compliance [gross exceedance] with Regulation D-2 (Bailey, 2015; Frazier et al., 2013; IMO, 2013). Indicative methods rely on various indicators to assess the viable biomass and/or viability of organisms in samples collected, including adenosine triphosphate (ATP) methods that detect cellular energy (Wright, 2012), fluorescence measurements that rely on the natural photosynthetic activity of chlorophyll-containing cells (phytoplankton) (Veldhuis et al., 2006; Wright, 2012), and fluorescein diacetate (FDA) methods that detect enzymatic activity (non-specific intracellular esterases or enzymes; Welschmeyer and Maurer, 2011) (Gollasch and David, 2010). Some indicative methods use calibration curves to convert the measured parameter to an estimated organism concentration. Several promising approaches have been developed but require further study in the field to understand their methodological differences and to assess their comparability, accuracy, and precision (Gollasch and David, 2010; Gollasch et al., 2012; Gollasch et al., 2015).

In this paper, we report the results of a series of trials that were undertaken to compare methods for ballast water sample analysis by conducting replicated, comparative testing on marine water samples collected onboard the research vessel 'Meteor' in transit from Mindelo, Cape Verde to Hamburg, Germany. Water samples were collected from the ship's ballast water system and analyzed in parallel by multiple analytic methods for the ≥50 μm and 10–50 μm size classes (7 and 9 techniques, respectively) to assess comparability between methods, with particular reference to microscopic analysis as the standard method. In so doing, we evaluated the sensitivity and precision of the different methods and provide an overview of the benefits and drawbacks of each method (i.e. costs, training requirements, processing time, and interpretability of output). To our knowledge, this is the largest study to date to assess the comparability and reliability of analytic tools for ballast water compliance management under field conditions. Results offer insight into the benefits and limitations of each method, and support ongoing efforts to establish reliable uniform analytic methods for compliance monitoring under the International Ballast Water Management Convention.

2. Methods

2.1. Sample collection

Samples were collected during ballast water uptake of sea water while in transit (sea chest intake positioned at 2.5 m depth), except one trial where samples were collected during discharge of Mindelo harbour water that had been held in a ballast tank for three days. The main ballast line of the RV Meteor is equipped with multiple sampling points to allow simultaneous collection of paired samples of untreated sea water in the engine room. During the voyage, we used three different sample collection devices (plankton net, SGS Ballast Water Sampler 1 (BWS1), and Triton skid NP 6007 TG 18) to run 20 paired trials, collecting a total of 40 samples. The plankton net (50 μm diameter mesh) is the traditional method of concentrating ballast samples, whereas sampling skids are compact devices that have been developed to enable filtration and concentration of large volumes of water in a small space. During each trial, ~1000 L of water was concentrated for analysis of organisms ≥ 50 μm, using the 'cod' end (50 μm mesh, plankton net) or inbuilt filter (50 μm mesh, sampling skids) of each sample collection device, to a final volume of 1 L (some exceptions; see Table A2). The volume of water filtered was quantified using a magnetic flow meter (Seametrics WMP104-100) for net samples and built-in flow meters for the sampling skids. For each sample, between 10 and 16 L of water was taken for analysis of 10–50 μm organisms by collecting approximately 500 mL of the filtrate produced by each sampling device every minute.

All rinse water used during sample collection (and later analyses) was prepared by sequentially filtering local sea water taken through the ship's scientific sea water tap system through a series of meshes (1000, 500, 35, and 8 μm, nominal pore sizes) followed by filtration through a 0.2 μm passive (gravity-fed) filter cartridge (Whatman Polycap TC150). Rinse water was prepared prior to the start of each trial, so that the rinse water was sourced from the same geographic location as the samples being tested. Table A1 in Appendix A contains detailed trial information including salinity, temperature, sampling time and positions, sample collection devices used, ballast water flow rate, and total volume of water that passed through the ship's ballast system during the trial.

2.2. Sample preparation

All sample collection and further handling, like sample splitting and sieving, were completed in a uniform way, so that observed variability is more likely explained by analysis method rather than by sample handling. Water samples containing organisms and particles ≥ 50 μm were concentrated during sample collection, so post-collection processing was not required. Individual subsamples for each analysis method were taken by mixing each 1 L condensed sample by inversion five times, half-filling each subsample bottle (7 bottles, total volume 35–300 mL depending on analysis requirements), and repeating this procedure until bottles were topped up to the required volume. This splitting procedure (5× inversion of the sample bottle, half fill, 5× inversion, fill remainder) was used to fill all subsample bottles detailed below.

Water samples containing organisms and particles < 50 μm (10–16 L filtrate samples) were processed to generate the fractions required for the remaining analyses (<50 μm for flow cytometry, 10–50 μm for all other methods). The subsample bottle for flow cytometry was filled first, and the remaining water was filtered on a 10 μm (pore diameter) Sterlitech polyester track etch (PETE) membrane filter. The retained particles were resuspended in filter-sterile sea water with a final concentration up to 16× the original concentration (see Table A2). The concentrated sample was split into subsample bottles for analysis of the 10–50 μm size class (8 subsamples, volume 25–350 mL). For most analytic methods, there was no further assessment of the size of organisms in the size-fractionated samples (i.e. all organisms contained in a given sample were considered to be within the relevant size class). However, the Satake Pulse Counter uses pulse strength to estimate organism size (see Appendix B for details), and microscopists used photomicrographs of 50 μm calibration beads (≥50 size class) and Sedgewick-Rafter grid widths (10–50 size class) as size references.

After each trial, all sampling gear, sample carboys, and subsample bottles were cleaned in a dilute (100–200 ppm) or concentrated (2500 ppm) bleach solution (depending on equipment robustness) made using the ship's potable water supply to prevent cross-contamination of living organisms between tests. After bleaching, all equipment was rinsed with MilliQ water three times; plankton nets were rinsed with potable water three times before being rinsed once with MilliQ water and hung to dry. Prior to re-use, all sample carboys and

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