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Difficulties in obtaining representative samples for compliance with the Ballast Water Management Convention

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ABSTRACT

As implementation of the Ballast Water Convention draws nearer a major challenge is the development of protocols which accurately assess compliance with the D-2 Standard. Many factors affect the accuracy of assessment: e.g. large volume of ballast water, the shape, size and number of ballast tanks and the heterogeneous distribution of organisms within tanks. These factors hinder efforts to obtain samples that truly represent the total ballast water onboard a vessel.

A known cell density of *Tetraselmis suecica* was added to a storage tank and sampled at discharge. The factors holding period, initial cell density and sampling interval affected representativeness. Most samples underestimated cell density, and some tanks with an initial cell density of 100 cells ml^{-1} showed <10 cells ml^{-1} at discharge, i.e. met the D-2 standard. This highlights difficulties in achieving sample representativeness and when applied to a real ballast tank this will be much harder to achieve.

1. Introduction

In 2004 the International Convention for the Control and Management of Ships' Ballast Water and Sediments ('the Convention') was adopted by the IMO. This Convention aims to reduce the transportation of species across the globe in ballast water by eliminating viable organisms prior to discharge. As part of the Convention, threshold levels, also known as standards, were set to state the allowable number of viable organisms and indicator microbes within the ballast water discharged at port (IMO, 2004). These levels are stated in Regulation D-2 (the 'Ballast Water Discharge Standard') and when the Convention enters into force will become the standard vessels have to meet to legally discharge their ballast water. Crew on any ship found to violate this level could face prosecution, costing port authorities, port state controls, ship operators, ship owners and cargo owners millions of pounds. The convention will enter into force 12 months after ratification by 30 states which represent 35% of the world's merchant shipping tonnage (IMO, 2004), and at the time of writing (October 2012) 36 states representing 29.07% of the worlds tonnage had ratified the Convention. As the ratification date comes closer, researchers and regulators need to start deciphering the meaning and handling of the representativeness of the samples.

Compliance with standards is essential for effective implementation of any environmental regulation. Sampling, as well as adequate inspection and monitoring, are equally essential in any environmental pollution control or prevention policy (RCEP, 1998). To ensure ballast discharges meet Regulation D-2, sampling is required to determine the number of viable organisms present (Pazouki et al., 2009). In addition, according to the G2 guideline, those samples used to determine a ship's compliance must be 'representative' of the 'whole' ballast water to be discharged (IMO, 2008). Representativeness of ballast water samples has not, however, yet been discussed clearly and while G2 guideline states that representative samples are required it does not provide clear guidelines on how to obtain these samples.

To define representativeness of samples the following definition from the Royal Commission on Environmental Protection (1998) can be considered:

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"any numerical environmental standard needs to be robust, recognise scientific assessment and should be specified in a way that takes full account of the nature of the substance to which it relates, the extent of statistical variation in the parameter to which it relates and the requirements for verification".

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From this definition two types of sample 'representativeness': biological and statistical, can be identified. To obtain biological representativeness samples should 'take full account of the nature of the substance to which it relates', and so in ballast water sampling this should show a true account of the diversity and living status of organisms contained within ballast tanks. For statistical representativeness samples should 'take full account of the extent of statistical variation in the parameter to which it relates', hence, in ballast water studies it refers to the number of organisms. The idea of samples being both statistically and biologically representative is recent in ballast water research (i.e. Pazouki et al., 2009). Therefore to try and comply properly with G2 samples must satisfy both aspects of representativeness.

Statistical representation enables findings to be generalised to a larger population, and so if a sample is being used to generalise a population it must truly represent it (Stuart, 1984). To obtain data which is statistically representative of an entire ship the volume of ballast water to be sampled must first be determined. This has been addressed by Basurko and Mesbahi (2011) and Miller et al. (2011) using different statistical approaches to determine the volume of water required for statistical representation. The results obtained in each study varied widely, highlighting the difficulty in determining a standard approach. Even once statistical representation is determined there is a further hurdle: how do we know that the samples collected are biologically representative of the entire contents of the ballast water? The distribution of organisms within ballast tanks is known to be heterogeneous (Murphy et al., 2002; Gollasch and David, 2010) and this will hinder the collection of samples which are biologically representative of an entire ship. Further problems encountered while trying to obtain biologically representative samples from ballast tanks include: the large volume of ballast water present in vessels, differential locations of ballast water uptake, the presence of sediments and irregular shapes of the tanks (Murphy et al., 2002).

The frequency of collecting samples from the tank discharge will affect the accuracy of the data obtained. Ideally, samples would be taken at frequent intervals, e.g. 2 min, throughout the whole discharge of the tank to obtain an accurate idea of organism distribution. In doing this the number of samples which require analysing would be very high and the time required to do this would cause 'undue delay to the ships operation, departure or movement' (IMO, 2008). By reducing the sampling frequency the accuracy of data decreases, but sampling a vessel for compliance would be feasible, this is the compromise which must be made. However, the required sampling frequency is not conclusively determined in the G2 requirements. G8 details the sampling required in shipboard approval testing as: 3 replicate samples at the 'beginning', 'middle' and 'end' of discharge for each influent water, control discharge and treated discharge water. A total of 27 samples (9 sampling points $\times$ 3 replicates) would be collected and require processing, with 9 samples collected per water type (3 sampling points $\times$ 3 replicates). This number of samples is much more feasible, and the results could be determined within 1 day with sufficient biological expertise, equipment and biologists available to perform the analysis. The effect that this reduction in sample collection has on the accuracy of the data obtained will be addressed in this study.

In order to demonstrate the difficulty in obtaining samples which are statistically representative of a whole tank this study performed tests to assess the variability in the abundance of the alga *Tetraselmis suecica* during complete discharge of a 1 m^3 storage tank. The distribution of organisms throughout the tank could be affected by the length of holding time e.g. increased holding duration could allow organisms to settle to the bottom of the tank or attach to the walls. This was investigated by assessing organism distribution in tanks which had been stored for 1, 3 and 5 days. The effect of sampling frequency was investigated by considering 4 'scenarios': sample collection every 2, 6, 12 and 18 min throughout the duration of the tank discharge, i.e. from 'continuous' to reduced sampling intervals. The tanks used were regularly shaped and contained a known inoculation density of the test organism.

2. Methods

2.1. Test organism and inoculation concentration

The test organism *T. suecica*, a single celled green alga representative of the $\geqslant 10 < 50$ μm size class (defined in Regulation D-2 of the Convention), was used in tests. This organism was used due to its size, ease of culture and due to its common use as a test species for ballast water treatment system testing in G8 approval tests. The experiments were conducted in two sets and a known concentration of *T. suecica* (Set 1: 10 cells ml^{-1}; Set 2: 100 cells ml^{-1}) was inoculated into a 1 m^3 storage tank. The seawater was filtered by 50 μm prior to inoculating with *T. suecica*.

The concentrations 10 cells ml^{-1} and 100 cells ml^{-1} were chosen due to their applicability in compliance testing. 10 viable cells ml^{-1} is the borderline level for a vessel to fail to comply with the D-2 Discharge Standard. 100 cells ml^{-1} was subsequently identified as it was 10 times above the level at which a vessel would fail to meet the standard. The aim was to determine whether it would be possible for a vessel known to be substantially over the discharge standard to 'pass' compliance testing.

2.1.1. Test setup

Three identical, replicate tanks were used (Tanks 1, 2 and 3). The tanks were square, 1 m^3, plastic storage tanks with a discharge valve located at the base of the tank. The interior design of the tanks was sloped in order to direct all water to the valve, facilitating full discharge.

The tanks were covered for different holding periods (1, 3 and 5 days) in the dark mimicking ballast tank conditions. A total period of 5 days was used as that is the time period specified in the G8 guidelines for ballast water testing. Samples were also taken on days 1 and 3 to look at the trends observed within this period.

After the required holding time the seawater was discharged at a constant flow rate (1 m^3 h^{-1}) from the bottom of the tank for the three replicate tanks. This flow rate was the fastest flow rate that could be sustained for the entire discharge period to ensure constant conditions throughout.

Samples (70 ml) were collected from each tank simultaneously, every 2 min, to perform as near to continuous (or 'on-line') sampling as was feasible and to monitor the discharge as closely as possible, taking into account the time required between samples to collect, fix and seal each sample. The sampling size was determined based on a down-sizing calculation for the scaled-down version of our experiment (1 tonne) relative to the volume specified in the G8 guidelines (200 tonnes, sample size 10 L). After collection Lugols iodine was added to preserve samples for analysis.

2.1.2. Data analysis

Analysis was completed using FlowCAM, an automated particle analyser used to count and identify plankton in seawater samples. The samples were run in AutoImage mode to capture images of all