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Preliminary verification studies of the motility and fluorescence assay (MFA) for ballast water quality monitoring

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ABSTRACT

The International Maritime Organization (IMO) has adopted a ballast water convention to prevent spread of invasive species with ballast water and with it comes the need for fast and reliable tests to estimate the number of viable organisms found in ballast water.

During 2017 and 2018, computer-assisted automatic quantification of plankton was compared with direct microscopic counts. Analysed samples represented simulations of ballast water samples from Denmark. Samples consisted of live plankton monocultures, mixed cultures, or ambient organisms from local seawater. The aim was to validate the performance and efficiency of the automated Motility and Fluorescence Assay (MFA) system BallastWISE, which automatically counts motile and fluorescent organisms in the fractions ≥10 - <50 µm and ≥ 50 µm, according to the ballast water discharge standard.

Standardized counting methods for comparison were completed under microscope. Organisms in the ≥10 - <50 µm size range were analysed using an FDA (Fluorescein diacetate) and CMFDA (5-chloromethylfluorescein diacetate) staining method, and a standard movement and response to stimuli technique was used for the ≥50 µm size range.

BallastWISE results correlated well with direct microscopic counts, showing promising counting performance and efficiency when it comes to rapid analysis of ballast water plankton samples. Quantification of monoculture and mixed cultures using BallastWISE correlated well with manual viable counts (*Tetraselmis suecica* in size range ≥ 10 - <50 µm: Pearson correlation (r) = 1.0; *Tigriopus californicus* and *Brachionus plicatilis* in size range ≥ 50 µm: r = 0.99). BallastWISE counts also correlated well with viable microscopic counts of ambient organisms in natural seawater (size range ≥ 10 - <50 µm: r = 0.97 and size range ≥ 50 µm: r = 0.89). We propose that computer-assisted quantification of plankton provides a rapid and precise alternative to traditional counting and identification of organisms in water samples such as ballast water.

1. Introduction

The international maritime organization (IMO) has adopted a ballast water convention with the purpose of preventing spread of invasive species using approved ballast water treatment systems (BWTS) (IMO, 2018a, 2019b). The efficiency of a ships BWTS can be measured using laboratory analyses or ballast water test instruments. These tests should determine viable organism concentrations, which should be low after treatment in order to comply with the current regulations.

In this paper we focus on two organism size groups: ≥10 and < 50 µm (hereafter, 10–50 µm) and ≥ 50 µm, representing phytoplankton, microzooplankton and mesozooplankton. The threshold of an approved compliance test is a maximum of 10 viable organisms/mL for the 10–50 µm fraction and 10 viable organisms/m^3 for the ≥50 µm fraction. Standard practices in compliance tests the ≥50 µm fraction involve a filtration of 1 m^3 down to 1 L. Hence, concentrations are often referenced in organisms per L. Currently used compliance tests (manual counting by microscopy) are often resource and time consuming, and there is a need for both quicker and less costly methods with similar precision (IMO, 2018a, 2019b).

Available tools for analysis of organisms in the 10–50 µm fraction in ballast water include different methods: Variable fluorescence fluorometry, adenosine triphosphate (ATP) assays, vital markers, and/or imagery (IMO, 2019a). Variable fluorometry methods have proven very effective but are limited in that they are only able to detect cells containing chlorophyll (First et al., 2018; Vanden Byllaardt et al., 2018). ATP assays require minimal training but addition of chemicals (Lo Curto et al., 2017) and have so far proven less reliable in the 10–50 µm

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