INTERNATIONAL MARITIME ORGANIZATION

E

MARINE ENVIRONMENT PROTECTION COMMITTEE
71st session
Agenda item 4

MEPC 71/INF.25
28 April 2017
ENGLISH ONLY

HARMFUL AQUATIC ORGANISMS IN BALLAST WATER

Development of a versatile methodology using Motility and Fluorescence Assays (MFA) to count viable organisms

Submitted by Denmark

SUMMARY

Executive summary:	This document describes the principles and preliminary results of a method for evaluating the concentration of live organisms in a ballast water sample by detecting organisms that are motile and/or organisms which contain chlorophyll (MFA; Motility and Fluorescence Assay). The method which is entering its final evaluation is fully automated and can be used as a single assay by port State control officers to evaluate compliance with the D-2 standard. The same approach can also be implemented as part of BWMS to monitor efficiency during ballasting and deballasting operations
Strategic direction:	2
High-level action:	2.0.1
Output:	2.0.1.2
Action to be taken:	Paragraph 6
Related documents:	Resolutions MEPC.175(58), MEPC.252(67) and A.1088(28)

Introduction

1 The International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004 will enter into force in September 2017, and therefore the number of ships discharging ballast water that have to meet the D-2 standard will increase over the coming years.

2 Article 9 of the Convention stipulates that inspection of a ship may be carried out to verify the presence of an onboard valid Certificate; to inspect the ballast water record book, and/or sample the ship's ballast water in accordance with the Guidelines (G2). The Committee has subsequently developed the PSC Guidelines (MEPC.252(67)) to support the smooth

implementation of CME (compliance, monitoring and enforcement) activities. This document describes a method for estimating live organisms in the size fractions 10 to 50 µm and > 50 µm in accordance with the Ballast Water Management Convention.

3 The monitoring of the performance of a BWMS will become crucial because it raises the confidence that the treatment from type approved systems is adequate. Furthermore, the monitoring of this performance is crucial for the success of the implementation of the Convention.

4 MicroWISE has received support from the Danish Maritime Fund to combine existing technologies to finalize the development of a versatile monitoring system (BallastWISE, patent pending) which may be used for port State control as well as by technology developers/shipowners to evaluate compliance with the D-2 standard and treatment efficacy, respectively. BallastWISE was developed during two separate projects funded by the Danish Maritime Fund (Ballast Water Control System) and the Danish Nature Agency (BallastWISE). The projects were carried out by Bioras (Bioras.com), Unit-One (Unit-One.dk), Fishlab (Fishlab.dk) and DHI Denmark (https://ballastwater.dhigroup.com). The project results are available on the BallastWISE website (www.ballastwise.dk). MicroWISE is a company started by Bioras, Unit-One and Fishlab, which will carry out all activities related to BallastWISE.

Proposed supportive technology

5 The system is based on the expertise of MicroWISE to evaluate motility and fluorescence through assays for counting viable organisms in ballast water. The system described in the annex to this document has been tested through a series of studies carried out at DHI Denmark and is planned to be further evaluated by PML and DHI Singapore, all of which are members of the Global Ballast Water Test Organizations Network (GloBal TestNet). Preliminary results presented in the annex support the adequacy of the approach and confirm that combination of technologies can increase the reliability of testing ballast water for organisms in different size classes.

Action requested of the Committee

6 The Committee is invited to take note of the information contained in this document, in particular in paragraphs 4 and 5 and the annex.

***

ANNEX

OVERVIEW OF THE MFA METHOD

Chambers, optics, and resolution

1 The MFA method involves viewing organisms in optical chambers using suitably dimensioned optics. The fraction of 10-50µm requires a resolution of approximately 2µm in order to determine organism size with reasonable accuracy. As an example of a suitable camera and optical configuration, a camera with 1600x1200 pixels and a pixel size of 4.4µm would require a 2.2x lens and a field of view of 3.2mm x 2.4mm. A realistic depth of field is 0.7mm which gives a volume seen by the camera of 5µl. The detection limit is a single cell and the statistical outcome depends on the number of analysis of the chamber volume. The dimensions for an optical chamber for viewing the fraction >50µm are approximately 70mm x 50mm x 20mm (L x W x H) giving a volume of 70ml. Using the same camera resolution as above, the pixel size is 40µm, and the detection limit is also a single cell. In order to detect concentrations as low as 10 per m^3, a filtration through a 50µm mask towards achieving a 1000x concentration would be preferred, but this method still enables testing of unfiltered samples. For both size fractions, a number of chamber volumes would be analysed from the same sample to give a statistically sound result.

Physical framework and analysis time

2 Several chambers volumes need to be analysed to give an accurate result. In order to achieve this, pumps are connected between samples and chambers in order to fill and empty them. Synchronization between sample flow and analysis is performed by the computer. Approximately 60 seconds is suitable for each chamber analysis for the >50µm fraction including a waiting time for the liquid to settle after filling. Only 20 seconds is required for the 10-50µm fraction. 14 analyses for the large- and 50 analyses for the small fractions give a combined analysis time of less than 30 minutes. The filling and measurements are done automatically.

Motion detection

3 Most heterotrophic organisms, and virtually all flagellates and ciliates, are motile. This observation applies to both size fractions. The content of the chambers are analysed on a time base of 10 to 25 frames per second in real time by a computer to which the cameras are connected. Each frame is analysed and individual organisms are detected and measured. The movement of each organism is tracked over time from frame to frame by the computer. In this way, it can be established whether each organism is within the size range and whether it is motile or not. The sequences are also saved as video files for documentation.

Chlorophyll detection

4 Some autotrophic organisms contain chlorophyll and are non-motile while some contain chlorophyll and are also motile. The presence of chlorophyll alone is an indication of a live autotrophic organism, because chlorophyll disappears quite rapidly after cell damage. Chlorophyll within organisms can be detected by recording fluorescence after stimulation at a certain wavelength (violet light at approximately 420ηm). The insertion of a high pass filter of approximately > 500ηm between the chamber and the camera means that only fluorescence is observed by the camera. Chlorophyll is often evenly distributed throughout an organism so that organism size is determined by the overall size of the chlorophyll organelles, which merge into each other to form a single fluorescent object. There are relatively few autotrophic species that

are >50µm so this method is mostly relevant for the 10-50µm fraction. This method allows detection and sizing of organisms that are still against a dark background as well as detection of motile fluorescent organisms.

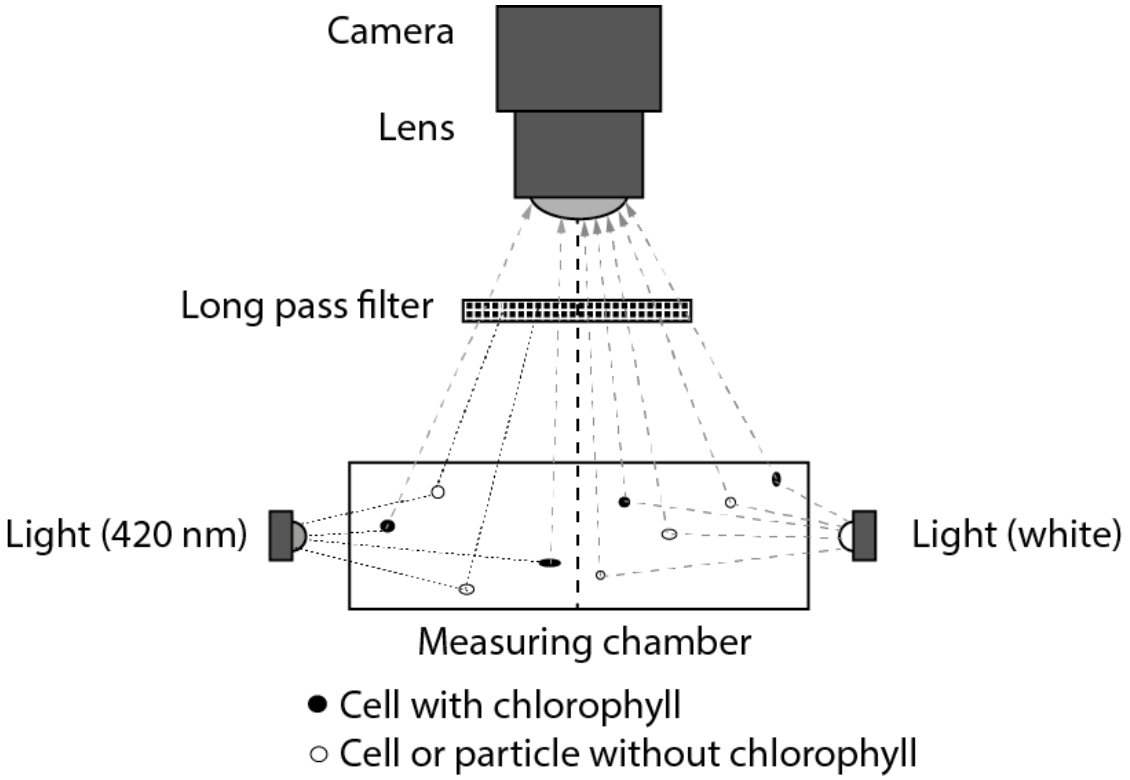

Figure 1: Camera, measuring chamber and lighting. The chamber is divided into two halves to show the two measuring principles. On the left side a 420 nm light shines into the measuring chamber, which excites chl*a*, resulting in emission of light at a higher wavelength from chl*a* containing organisms. This light is able to pass through the long-pass filter, and will be recorded by the camera. On the right side white light shines into the measuring chamber, and all particles and organisms that refract or reflect light are recorded by the camera. The long-pass filter only attenuates the white light marginally. The software processes these images to measure size, motility and presence of chl*a* for each individual organism.

Counting and detection limits

5 The system can detect single cells but the accuracy of counting depends on:

- the volume of the chamber
- the number of chambers that are analysed for a given sample
- the concentration of organisms that shall be detected

Simulations show that this accuracy for the 10-50µm fraction can be as high as 10 cells ± 30% with 95% confidence with a chamber volume of 30µl which is analysed 100x per sample. One litre or more of sample can be analysed for the >50µm fraction. The chamber is completely emptied between each fill and subsequent analysis, so the entire volume of the sample is actually analysed and the accuracy depends more on how the sample is collected and filtered.

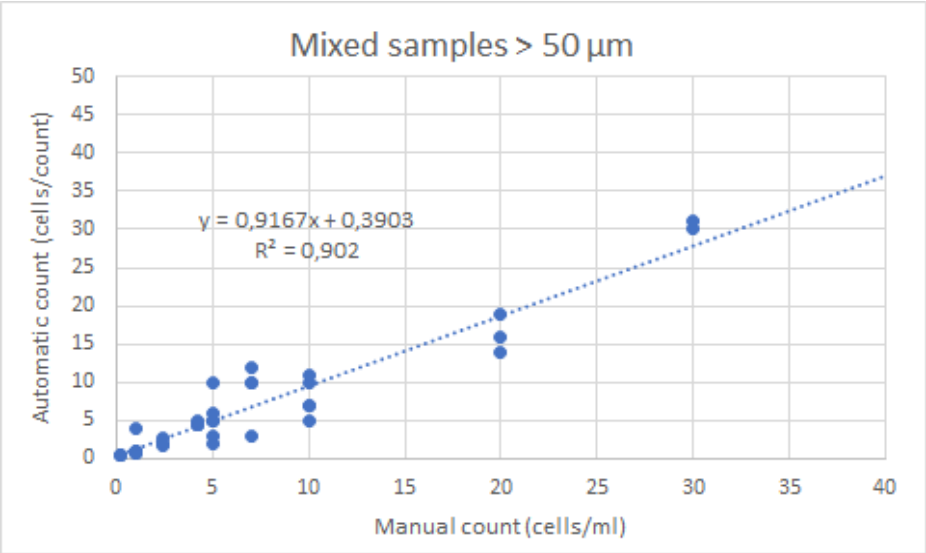

Figure 2: Results from the MFA method and manual counts of organisms > 50µm of specimens from cultures with daphnia, rotifers, ciliates and copepods.

Size determination

6 The resolution for the 10-50µm fraction is approximately 2µm and 40µm for the >50µm fraction, respectively, with current technology. Resolution can be improved with higher resolution cameras and optics so the configuration is a compromise between data bandwidth as well as cost. The relatively high resolution for the smaller fraction enables the system to determine which individual organisms lie within the given size limits with the given accuracy. Size determination becomes more accurate with increasing organism size so the lower resolution poses less of a problem for the larger fraction >50µm. Figure 3 shows size measurements with different species of organisms.

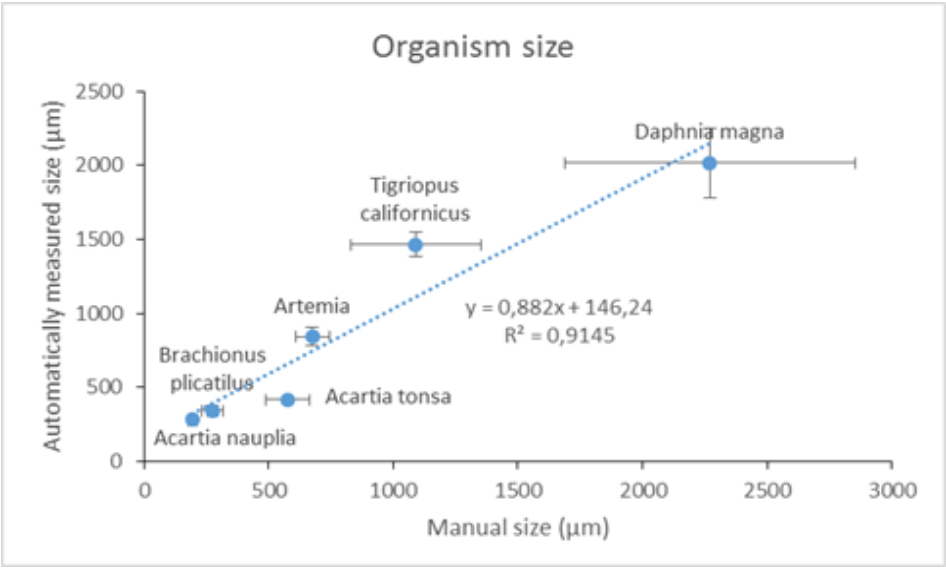

Figure 3: Comparison of manual and automatic measurements of organism sizes for different species and size classes in the range >50µm.