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HARMFUL AQUATIC ORGANISMS IN BALLAST WATER

Testing the neutral red staining in Colombia: results from the project "Produce technic-scientific information for the Protection of the Marine Environment in marine and port zones"

Submitted by Colombia

SUMMARY

Executive summary: This document exposes the advances in the validation of an analysis method to evaluate the viability in the >50 µm size class. The use of neutral red to stain ballast water samples in Colombia showed a high potential to be used as detailed analysis assay in the size class mentioned

Strategic direction, if applicable: 1

Output: 1.24

Action to be taken: Paragraph 14

Related documents: BWM.2/Circ.42/Rev.2, BWM.2/Circ.61/Rev.1 and BWM.2/Circ.70/Rev.1; resolution MEPC.300(72)

Introduction

1 In the *2020 Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)* (BWM.2/Circ.42/Rev.2) vital stains and visual microscopic counting are proposed as detailed analysis methods for the >50 µm size fraction. In the same way, the *2022 Guidance on methodologies that may be used for enumerating viable organisms for type approval of ballast water management systems* (BWM.2/Circ.61/Rev.1) suggests the use of FDA/CMFDA mixed vital stain but remains open to new methodologies that meet the requirements of specific Administrations.

2 According to the *Code for approval of ballast water management systems* (BWMS Code) (resolution MEPC.300(72)), the total sample volume should be analyzed, or the average density can be estimated, from a well-mixed subsample analyzed using a valid method. However, the counting of the whole sample is a time-consuming activity implying visual fatigue for the analysts and delays in the analysis time.

3 This document contains the main results from Colombia in the project "Producing technical-scientific information for the Protection of the Marine Environment in marine and port zones", where testing of neutral red as a vital stain to determine the viability of zooplankton organisms with size > 50 µm in 2022.

Additional research

4 During 2022 and 2023 different experiments were carried out to probe the neutral red stain potential in the viability determination method for the > 50 µm size fraction. The experiments included both natural and ballast water treated on board.

5 The neutral red stain demonstrated to be a useful alternative being applied to different taxonomic groups (polychaetes, copepods, molluscs) and preserve its functionality around a year under refrigerated conditions.

6 In 2021 and 2022, staining experiments were developed to evaluate the behavior in different organisms. The results revealed that the dye absorption changes depending on the animal group. In this sense, the stain is selective, and the selectivity must be evaluated specially for organisms like mollusks, tintinnids and crustacea larvae. At this instance, the interpretation of colored organisms belonging to these groups should be handle carefully.

7 A different experiment was carried out to describe the behavior of stain absorption with respect to the time; in this experiment no fixation was performed. After five days from the sampling, stained aliquots evidenced a change in the coloration from red-rose color to brown one. Besides, detritus agglomeration was observed in samples without the addition of formaldehyde. The fixation seems no modify the stain absorption of the organisms and contribute to retaining the vital stain for a longer time.

8 Experiments to determine the false positives and negatives rates included the heating of samples to elevated temperatures (110 °C). The results showed that the neutral red is absorbed even by death copepods but there was a clear difference between the absorption patterns. In heat killed organisms, the neutral red absorption was restricted to the digestive area; in contrast, live organisms absorbed the dye in the appendices and the exoskeleton. The warming process allowed the obtention of low false positives rates.

9 The estimated detection limit was lower than the discharge limit allowed for the > 50 µm size fraction (6.4 org/m³). However, this detection limit could be improved with the collection of a higher volume sample and the analysis of a major fraction of the sample.

10 An experiment to evaluate the recovery of the samples after the concentration process thought a sieve was performed. This experiment only considered organisms with a conspicuous morphology and relatively resistant to the mechanical damage as tintinnids belonging to the Favella and Leprotintinnus genera. The data showed recovery percentages between 50 and 100%. Despite the process can be improved, the obtained information suggest that the sieving process is safety and can be included in the method to reduce the analysis time and optimize the results.

11 The comparison between the results for two different analysts with a Bray-Curtis index evidenced similarities higher than 75%. Nevertheless, the experience of the analyst is crucial to interpretate the results of stain application because for a non-expert analyst the natural color of some organisms (for example copepods) can be considered as viability indicator.

12 Based on obtained results, Colombia considers the neutral red stain a suitable method to perform a detailed analysis of the ballast water samples collected. This method works well with natural and preserved samples, allows the transportation of the samples to the lab and provides the analyst a wider time to perform the analysis.

13 It is important to continue with the research about this stain to clarify the method limitations and its application to the 10-50 μm size fraction. Besides, the sampling validation is required to optimize some parameters of the method as the detection limit.

Action requested of the Committee

14 The Committee is invited to take note of the information contained in this document.
