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HARMFUL AQUATIC ORGANISMS IN BALLAST WATER

Summary of currently available ballast water indicative analysis instruments

Submitted by IMarEST

SUMMARY

Executive summary:	This document presents a summary of the currently available indicative analysis instruments for ballast water testing as an informational resource for interested stakeholders
Strategic direction, if applicable:	1
Output:	1.14
Action to be taken:	Paragraph 30
Related documents:	MEPC 73/19; resolutions MEPC.173(58); MEPC.252(67); MEPC.290(71); A 30/Res.1120; BWM.2/Circ.42/Rev.1 and BWM.2/Circ.70

Introduction

1 The Harmonized System of Survey and Certification (HSSC) Guidelines (resolution A.1120(30)) include procedures for the installation and commissioning of ballast water management systems (BWMS). Paragraph (BI) 1.1.2.19 of the HSSC Guidelines requires that "…an operational test of the ballast water management system was carried out based on the installation commissioning procedures and that documented evidence is provided which shows compliance of treated discharge ballast water is compliant with regulation D-2 through sampling and analysis based on applicable guidelines developed by the Organization."

2 The *Guidance for the commissioning testing of ballast water management systems* was approved during the seventy-third session of the Marine Environment Protection Committee (MEPC) and published as BWM.2/Circ.70, and states "…representative samples should be analysed for all size classes included in the D-2 standard using indicative analysis methods...."

3 Other documents relevant to port State control and *The Experience Building Phase associated with the BWM Convention* (resolution MEPC.290(71)) that reference indicative analysis include:

.1 *Guidelines for ballast water sampling* (G2) (resolution MEPC.173(58)) as a first step prior to performing detailed analysis to establish if a ship is potentially in compliance with the BWM Convention;

.2 *Guidelines for port State control under the BWM Convention* (resolution MEPC.252(67)) as part of the four-stage inspection procedure; and

.3 *Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)* (BWM.2/Circ.42/Rev.1).

4 Indicative analysis can also be useful in BWMS performance monitoring. For instance, shipowners and/or crew members may want to perform indicative analysis to proactively confirm normal BWMS operation prior to arrival in port. Indicative analysis can also be used to evaluate the effectiveness of a contingency measure that has been implemented or used by BWMS manufacturers that may want to verify system performance during technical service visits.

5 A variety of indicative analysis instruments have been developed and are currently available. These instruments can differ in aspects such as their portability, sampling and analysis capabilities, measurement principles, and the organism size classes detected.

6 There is currently a lack of widespread familiarity about indicative analysis and the available technology. IMarEST prepared this document as a resource to summarize relevant technical aspects and considerations of currently available indicative analysis instruments for ballast water testing. This review only collects available information into one location as a first step in understanding the currently available options and does not assess aspects such as suitability, reliability, and accuracy or aim to verify manufacturer claims; reviewing these aspects may be an important next step.

7 The review identified a total of 12 indicative analysis instruments that can detect organisms in the D-2 standard; one instrument detects all three size classes, four instruments detect two size classes, and seven instruments detect one size class.

Indicative analysis vs. detailed analysis

8 The D-2 standard includes three organism size classes: ≥ 50 µm (generally, zooplankton), ≥ 10 µm and ≤ 50 µm (generally, phytoplankton) and indicator microbes (*E. coli*, Enterococci and *V. cholerae*). While these generally accepted size class divisions of zooplankton and phytoplankton are useful, it is recognized that organisms in a sample may not fall into these size classes exactly (e.g. the ≥ 10 µm and ≤ 50 µm size class may include both zooplankton and phytoplankton).

9 Organism concentrations can be evaluated using detailed analysis or indicative analysis. Detailed analysis provides direct measurements of organism concentrations and robust results but involves complex sampling and analysis procedures that require time and the expertise of a scientific team. Indicative analysis methods often rely on proximal measurements (i.e. chlorophyll fluorescence, ATP, etc.), but can yield results in less time with less complex sampling and analysis procedures.

10 The *Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)* (BWM.2/Circ.42/Rev.1) provides definitions of indicative analysis and detailed analysis, which are copied below to help with understanding the focus and information presented in this document:

".1 An *indicative analysis* means a compliance test that is a relatively quick indirect or direct measurement of a representative sample of the ballast water volume of interest:

.1 an indirect, indicative analysis may include measurements whose parameters do not provide a value directly comparable to the D-2 standard, including biological, chemical, or physical parameters (e.g. dissolved oxygen levels, residual chlorine levels, Adenosine triphosphate (ATP), nucleic acid, *chlorophyll a*, and that by variable fluorescence, etc.) The practicalities, applicability and limitations of these methods should be understood before they are used in compliance testing;

.2 a direct measurement, which is directly comparable to the D-2 standard (i.e. the determination of the number of viable organisms per volume) may also be indicative if it has:

.1 a large confidence interval, or

.2 high detection limits.

.2 A *detailed analysis* means a compliance test that is likely to be more complex than indicative analysis and is a direct measurement of a representative sample used to determine the viable organism concentration of a ballast water volume of interest. The result of such measurement:

.1 should provide a direct measurement of viable organism concentration in the ballast water discharge which is directly comparable to the D-2 standard (number of viable organisms per volume);

.2 should be of sufficient quality and quantity to provide a precise measurement of organism concentration (+/- [X] organisms per volume) for the size category(ies) in the D-2 standard being tested for; and

.3 should use a measurement method with an adequate detection limit for the purpose for which it is being applied."

11 According to the definitions above, a detailed analysis is a direct measurement of the viable organism concentration. An indicative analysis is either an indirect measurement, or a direct measurement with a large confidence interval, a statistically low sample size, or high detection limits. However, there are no defined values for how large the confidence interval or detection limit needs to be for a direct measurement to be considered indicative instead of detailed. For this reason, and also recognizing that there are currently no standardized methods for verifying indicative analysis instruments, this document focuses on instruments that have been identified as capable of performing indicative analysis by the manufacturer.

Onboard analysis challenges and usefulness of indicative analysis instruments

12 As noted in paragraph 9 above, ballast water compliance can be assessed using detailed or indicative analysis. Detailed analysis to verify compliance with the D-2 biological discharge standard requires a high level of precision; performing this on board a ship is complex with several limiting factors that need to be considered, including, inter alia:

.1 Time – ships are only in the shipyard or in port for a limited amount of time, and of this time, only a limited amount is available for sampling and analysis of ballast water;

.2 Space – there is a limited amount of space available on a ship to accommodate ballast water sampling and analysis equipment and personnel;

.3 Power – power is a practical consideration for onboard testing (i.e. availability of outlets near the sampling location, voltage fluctuation);

.4 Operational restrictions – ballast water discharge may be of limited duration or volume due to ship stability and/or draft requirements; and

.5 Cost – the personnel and equipment required can be cost prohibitive.

13 Indicative analysis instruments that provide a measurable indication of compliance with the D-2 standard and less complexity have been developed to help overcome many of the onboard testing limitations. Indicative analysis instruments can be useful tools in supporting practical implementation of the HSSC Guidelines installation commissioning procedures, port State control compliance inspections and/or BWMS performance monitoring.

Currently available indicative analysis instruments

14 As previously stated, this paper focuses on indicative analysis instruments that use indirect measurements (as defined in BWM.2/Circ.42/Rev.1) and are identified by the manufacturer as indicative analysis instruments for ballast water testing. This summary includes indicative analysis instruments that are available from the original equipment manufacturer (OEM) and excludes "kits" comprised of re-packaged OEM instruments offered by third-parties. Further, the instruments included were those that IMarEST experts had knowledge of and/or were identified through online research, and the manufacturer indicated the instrument was available as of January 2019. Other indicative analysis instruments are currently under development and are anticipated to be available in the future.

15 Information presented was gathered by contacting the manufacturer directly, utilizing information publicly available on the manufacturers' website, and relying upon information that was supported by the available product literature. Information that could not be located or was not provided by the manufacturer is marked as "not indicated". A brief description of each indicative analysis instrument identified is presented alphabetically by manufacturer below and summarized in table 1.

.1 bw monitor, from Ballast Water Monitoring A/S in Denmark, is an automated in-line system permanently installed onboard providing real-time performance monitoring of ballast water treatment systems using fluorescence. Information: http://www.bw-monitor.com/

.2 10cells, from bbe Moldaenke GmbH in Germany, is a field instrument for the quantification of living cells in ballast water. The measurement is based on the natural fluorescence of chlorophyll *a* in algal cells. Information: https://www.10cells.com/en/

.3 FastBallast, from Chelsea Technologies Group in the United Kingdom, is a portable system which performs testing based on fluorescence. Information: https://www.chelsea.co.uk/

.4 Ballast Water Checker, from Euro Tech in China, is a handheld instrument based on PAM fluorescence technology measuring abundance of chlorophyll *a*. Information: http://www.euro-tech.com/en/

.5 BW680, from Hach in the United States, is a handheld fluorometer which tests for photosynthetically active chlorophyll like that found in living phytoplankton. Information: https://www.hach.com/

.6 Colilert-18 & Enterolert-E, from IDEXX in the United Kingdom, uses proprietary Defined Substrate Technology (DST) nutrient indicators and microbial enzyme activity to detect *E. coli* and Enterococci. Using the Quanti-Tray System, results are based on the Most Probable Number (MPN) method. Information: https://www.idexx.com/en/

.7 B-QUA, from LuminUltra in Canada, uses an ATP second generation method of analysis. This technology relies on the quantification of a molecule called Adenosine Triphosphate (ATP), an energy carrier found in all living organisms. Information: https://www.luminultra.com/b-qua/

.8 BallastWISE, from MicroWISE in Denmark, analyses the organisms in ballast water by monitoring their movements and fluorescence. The method is based on image analytical detection of individual organisms as well as fluorescence from chlorophyll *a* content in individual organisms. Information: https://microwise.eu/ballastwise/

.9 P. Counter, from Oceantech Co., Ltd. in the Republic of Korea, is a portable counter which uses fluorescence and image processing to detect organisms. Information: http://www.oceantech.co.kr/

.10 Ballast Eye, from Satake in Japan, estimates the number of organisms using a reagent stain, Fluorescein Diacetate (FDA). Information: https://satake-group.com/

.11 Integrated Ballast Testing, from SixSenso Technologies in Spain, uses Image Cytometry for detection and integrated labelling via microfluidics. Information: www.sixsenso.com

.12 Ballast-Check 2, from Turner Designs in the United States, is a handheld fluorometer which displays estimates for both cells/mL and cell viability. Information: https://www.turnerdesigns.com/ballast-check-2

16 In table 1 below, each indicative analysis instrument is denoted as in-line (I) or portable (P). In-line instruments are considered as those that are permanently installed on board. The primary measurement principle(s) and the organism size class(es), as defined in the D-2 standard, that the instrument is capable of detecting are listed. Minimum sample

analysis volume is the minimum volume the instrument requires to perform sample analysis. The time to obtain results is the time required for analysis by the instrument and does not consider the time needed to collect and/or process the ballast water sample. The available information was reviewed for each instrument to determine if consumables and/or reagents are required as part of the standard analysis protocol.

17 An approximation of equipment cost is provided to assist stakeholder evaluation. Four cost categories (in USD) were established to allow ranking: < $5,000, $5,000 to $10,000, $10,000 to $20,000, and > $20,000. Costs for any consumables required to perform analysis with the instruments are not included.

18 Indicative analysis instruments can require varying levels of operator skill depending on the ballast water sample collection and/or preparation procedures prescribed by the manufacturer and operation of the indicative analysis instrument itself. The number of personnel required to perform sampling and analysis can also vary. The IMarEST endeavoured to include this aspect more comprehensively; however, without having complete information on the standard operating procedures for all instruments, it was not possible to clearly define skill levels that would result in a fair ranking. Therefore, operator skill level required to perform indicative analysis can only be noted as an important aspect that stakeholders should consider.

Summary of findings

19 As summarized in table 1, a total of 12 currently available indicative analysis instruments were identified. One instrument is available for permanent in-line installation, while the majority (11) of the instruments are described by the manufacturer as portable. Review of instrument specifications indicated that some of the instruments described by the manufacturer as portable require an onboard power connection.

20 Nine of the indicative analysis instruments have measurement principles based on fluorescence, while the remainder rely on molecular, chemical, and/or imagery to generate results for the differing organism size classes. Prior to sample analysis with an indicative analysis instrument, the use of additional equipment, such as sample concentration skids and/or phytoplankton nets, may be required. The manufacturer literature should be consulted to determine the complete sampling and analysis procedures.

21 There are indicative analysis instruments available for all three D-2 standard organism size classes: ≥ 50 µm, ≥ 10 µm and ≤ 50 µm and indicator microbes. Of the instruments listed, three are able to detect the ≥ 50 µm organisms, 11 are able to detect the ≥ 10 µm and ≤ 50 µm organisms, and three are able to detect one or more of the indicator microbes. Four of the instruments are capable of detecting more than one D-2 standard organism size class. Three instruments detect two size classes, and eight instruments detect a single size class. One instrument detects all three size classes; however, the analysis for indicator microbes estimates the total bacteria concentration in a sample to provide an indication of potential *E. coli*, Enterococci and *V. cholerae* presence (i.e. *E. coli*, Enterococci and *V. cholerae* are not specifically measured for comparison to the D-2 standard).

22 Minimum sample analysis volumes varied across instruments, ranging from 1 drop to 1.2 litres. It is important to note that these values represent the volumes required for the instruments to analyse the sample and the total volume of ballast water discharge that needs to be collected during sampling may be significantly larger; however, enough data was not identified to provide further details for this aspect. Additionally, each manufacturer may have sampling procedures and sample volumes specified in their operating procedures that may not be considered "representative" of the whole ballast water discharge for differing purposes (i.e. port State control, commissioning testing). While the topic of appropriate sample volume

is outside the scope of this document, the International Standards Organization (ISO) is developing ISO 11711, of which Parts 2 and 3 will include guidance for ballast water sampling and analysis (PPR 5/INF.5). The sampling and analysis procedures that have been developed by the International Council for the Exploration of the Sea (ICES) (PPR 5/5/2) also provide a useful reference.

23 For the instruments identified, the time to obtain the indicative analysis test results ranges from less than 1 minute to 24 hours. As noted in paragraph 12.1 above, the time allocated for ballast water sampling and analysis when a ship is in the shipyard or in port can be a limiting factor. With respect to BWMS commissioning testing, BWM.2/Circ.70 indicates that the ballast water discharge sample should be collected "…after the full treatment has been applied." This means that any treatment holding time and/or treatment at discharge indicated on the BWMS Type Approval Certificate should be applied before the ballast water discharge sample is collected. Therefore, the time needed to perform BWMS commissioning testing must consider the full BWMS treatment process, the time required for sample collection and analysis, and the time required to obtain results from the indicative analysis instrument(s) used.

24 Five instruments require the use of consumables and/or reagents to perform analysis. Seven manufacturers describe their instrument as not requiring consumables and/or reagents; however, in some cases the product literature indicated there are instances where secondary procedures may require the use of ancillary supplies (i.e. disposable filters, calibration solutions, etc.). Therefore, an indicative analysis instrument that does not require consumables and/or reagents to perform each test does not imply that no ancillary supplies are needed to operate or maintain the instrument.

25 Four equipment cost categories (< $5,000, $5,000 to $10,000, $10,000 to $20,000 and > $20,000, in USD) were established and each manufacturer was requested to indicate the category applicable to their instrument. Instrument costs were confirmed by 10 out of 12 manufacturers. Equipment costs were primarily in the < $5,000 USD (three instruments) and > $20,000 USD (four instruments) categories. The equipment cost ranking used in this summary is only intended to provide stakeholders with a general idea of the cost range and does not represent a detailed cost evaluation. Aspects such as the instrument type (i.e. permanent in-line installation or portable) and analytical capabilities can influence costs and require full consideration; manufacturers should be contacted to ascertain actual costs. Instruments may also have additional costs associated with consumables, instrument calibration and/or regular maintenance.

26 This review revealed that there are a variety of options available to perform indicative analysis and the instruments differ in features such as capability, portability, sampling and analysis requirements, time to obtain results, and cost. Similar to ballast water management systems, there may not be a "one size fits all" solution appropriate for all scenarios and stakeholders have a variety of aspects to consider when evaluating indicative analysis instruments.

Table 1 – Ballast water indicative analysis instruments summary

Technology manufacturer	Product brand name	In-line (I) or Portable (P)	Measurement principle(s)	D-2 standard organism size class(es) detected			Minimum sample analysis volume	Time to obtain results	Consum-ables and/or reagents required? (Yes/No)	Equipment cost (USD)
				≥ 50 µm	≥ 10 µm - ≤ 50 µm	indicator microbes				
Ballast Water Monitoring A/S	bw monitor	I	Active Fluorescence, Light scattering		✓		Flow through	< 3 seconds	No	> $20,000
bbe Moldaenke GmbH	10cells	P	Active Fluorescence		✓		1-50 mL	< 1 minute	Yes	$5,000 – 10,000
Chelsea Technologies Group	FastBallast	P[1]	Fluorescence, Single Turnover Active Fluorometry		✓		20 mL	< 10 minutes	No	Not indicated
Euro Tech (Far East) Ltd.	Ballast Water Checker	P	Active Fluorescence		✓		Not indicated	Not indicated	No	Not indicated
Hach	BW680	P	Active Fluorescence		✓		2 mL	< 3 minutes	No	< $5,000
IDEXX[2]	Colilert-18 & Enterolert-E with Quanti-Tray System	P[3]	Microbial enzyme activity and MPN			✓[4]	100 mL	18-24 hours	Yes	< $5,000
LuminUltra Technologies	B-QUA	P	ATP	✓	✓	✓[5]	1 L, 200 mL - 1L, 100 mL	40 minutes	Yes	$5,000 – 10,000
MicroWISE	BallastWISE	P[3]	MFA	✓	✓		1.2 L, 150 mL	22 minutes, 12 minutes	No	> $20,000
Oceantech Co., Ltd.	P. Counter	P	Fluorescence & Image Processing		✓		1 drop – 500 mL[6]	< 1 min	No	$10,000 - $20,000

Technology manufacturer	Product brand name	In-line (I) or Portable (P)	Measurement principle(s)	D-2 standard organism size class(es) detected			Minimum sample analysis volume	Time to obtain results	Consum-ables and/or reagents required? (Yes/No)	Equipment cost (USD)
				≥ 50 µm	≥ 10 µm - ≤ 50 µm	indicator microbes				
Satake	Ballast Eye	P[3]	Pulse Counting FDA	✓	✓		100 mL, 5 mL	16 minutes	Yes	> $20,000
SixSenso Technologies	Integrated Ballast Testing	P[3]	Cytometry		✓	✓	250mL, Flow through	< 1 hour, 2-4 hours	Yes	> $20,000
Turner Designs	Ballast-Check 2	P	Fluorescence, Active Fluorescence		✓		3 mL	< 1 minute	No	< $5,000

[1] Product for permanent in-line installation under development.

[2] IDEXX also offers a presence/absence method, the method that allows for numerical quantification for comparison to the D-2 standard is presented.

[3] Instrument requires onboard power connection.

[4] Analyses for *E. coli* and Enterococci only, does not include *V. cholerae*.

[5] Estimates the total bacteria concentration in a sample as an indication of potential *E. coli*, Enterococci and *V. cholerae* presence.

[6] Two measurement options: glass plate (1 drop of sample) or instrument submersion in sample water (500 mL).
µm – micron; mL – millilitre; L – litre; ATP – adenosine triphosphate; FDA – Fluorescein Diacetate; MFA – Motility and Fluorescence Assays; MPN – Most Probable Number.

Discussion

27 Incorporation of ballast water testing using indicative analysis into the guidance approved at MEPC 73 (BWM.2/Circ.70) has resulted in an increased focus on the available technology and how to practically implement the guidance.

28 This summary presents 12 indicative analysis instruments that are currently available to support implementation of the BWMS commissioning testing procedures, port State control compliance inspections, and/or BWMS performance monitoring. However, there is currently a lack of industry-wide experience and knowledge about indicative analysis instrument capabilities, how they work and are used. There are also currently no common methods to evaluate the technical readiness or perform validation of indicative analysis instruments.

29 Standardized protocols and/or guidance on the above aspects would facilitate practical implementation of ballast water testing by indicative analysis methods. Development of such guidance is anticipated as stakeholders move forward and indicative analysis experience is gained. This is evidenced by the proposal submitted by Denmark to PPR 6 for development of a verification methodology for indicative ballast water monitoring systems (PPR 6/4).

Action requested of the Committee

30 The Committee is invited to note the information provided in this document.