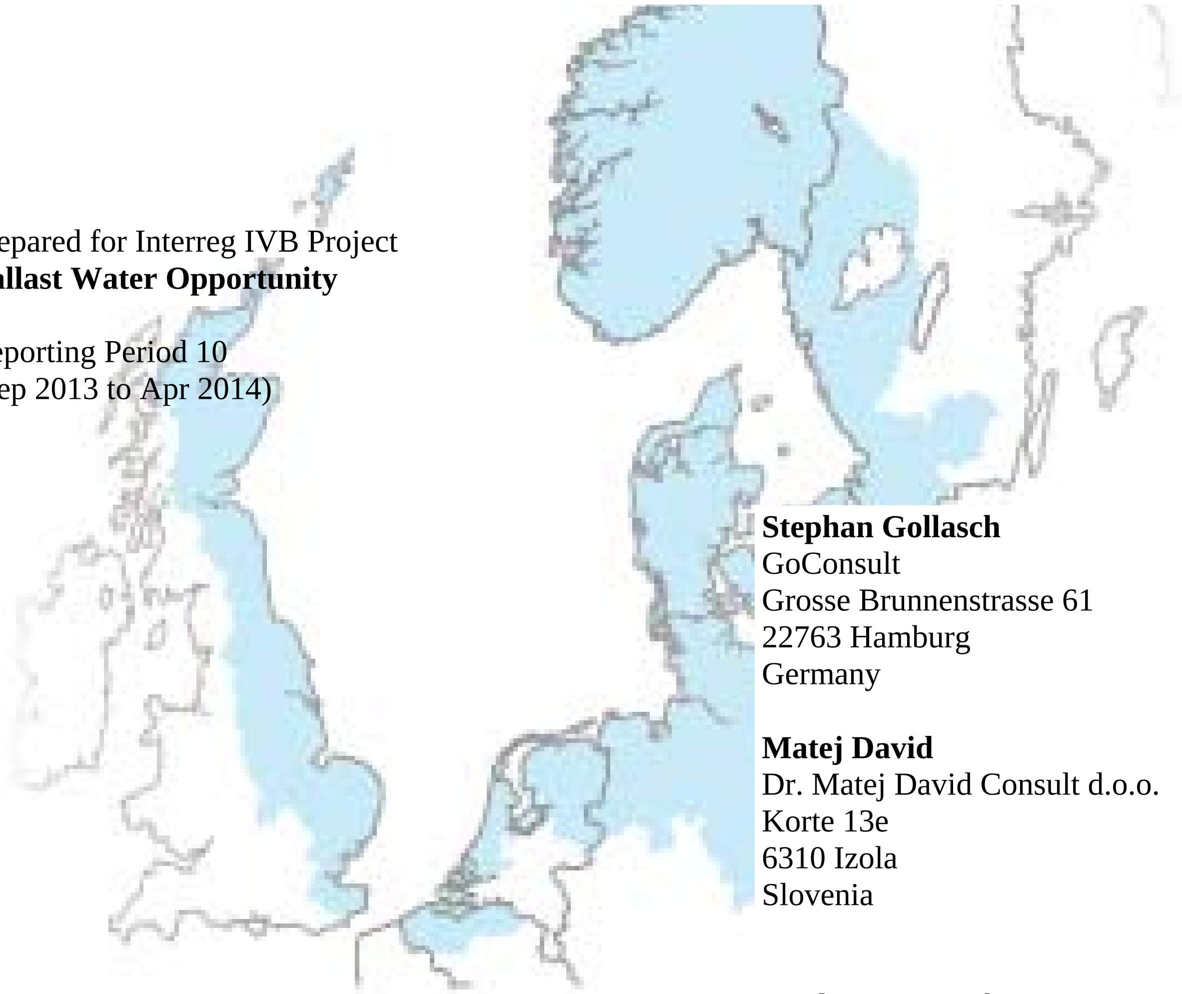
NORTH SEA BALLAST WATER

The Interreg IVB North Sea Region Programme

Investing in the future by working together for a sustainable and competitive region

Recommendations for IMO on the BWM Convention and the improvement of its supporting guidelines with an emphasis on the shipboard test aspects of the guidelines for approval of ballast water management systems (G8)



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Executive Summary

In Ballast Water Opportunity one of the duties in WP5 was to review the test requirements of ballast water management systems (BWMS) as stated in the "International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004" (BWM Convention) and its supporting guidelines, i.e., "Guidelines for approval of ballast water management systems (G8)" as WP5 deliverable "D5-12 Recommendations for IMO on the BWM Convention and the improvement of its supporting guidelines".

Due to the experience gained in approx. 100 shipboard tests of 18 different BWMS, Dr. Stephan Gollasch (WP4 leader) volunteered to take this task and to prepare a BWO report summarizing points for possible re-consideration when Guidelines G8 may be re-opened for a discussion at IMO. He invited Dr. Matej David with whom he jointly undertook almost all of the 100 shipboard test voyage. Dr. David's expertise covers the nautical and maritime sectors so that this report does not only consider biological aspects. Further, Dr. Gollasch and Dr. David attended the IMO meetings where the BWM Convention and the Guidelines G8 were drafted and adopted, and are members of the GloBalTestNet.

Guidelines G8 were originally adopted by IMO´s Marine Environment Protection Committee (MEPC) as Resolution MEPC.125(53) in 2005 and thereafter minor revisions were made and were adopted by Resolution MEPC.174(58) in 2008 (IMO 2008a).

The Guidelines G8 include general requirements concerning design and construction, technical procedures for evaluation and the procedure for issuance of the Type Approval (TA) certificate of BWMS. To satisfy the BWM Convention, a ballast water discharge must comply with the D-2 standard throughout the life of the vessel. Guidelines G8 were drafted to challenge BWMS so that their performance is not compromised even in high organism and/or sediment loads, or other challenging circumstances. Therefore, Guidelines G8 contain recommendations regarding the design, installation, performance, testing environmental acceptability and approval of BWMS.

It was concluded that the current Guidelines G8 requirements do not represent enough challenging test conditions where ballast water is taken up. Therefore, BWMS that have received TA under the present Guidelines G8 may be found non-compliant if operated in more challenging water conditions as the BWMS was exposed to during the TA tests. MEPC67 (October 2014) agreed that Guidelines G8 should be revised and the revision should commence as soon as possible.

The purpose of Guidelines G8 is to define test and performance requirements for the approval of BWMS which are split in land-based and shipboard approval requirements so that the required tests of a BWMS for TA are outlined in detail. This report will comment on possible Guidelines G8 improvements with a focus on the shipboard test requirements which are based on experiences gained by the authors.

Guidelines G8 test aspects we comment in this report and propose changes include: test duration; number of tests; challenge water conditions (abiotic and biotic); BWMS flow rates; normal ship operations; operation of the BWMS during the tests; control experiment; timing, number and volume of samples to take; subsample handing; sample storage conditions and sample handling; and how to define and measure minimum dimension of organisms. The proposed changes are believed to well strengthen mainly the shipboard TA tests of BWMS.

1 BACKGROUND

In Ballast Water Opportunity (BWO) one of the duties in WP5 was to review the test requirements of ballast water management systems (BWMS) as stated in the "International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004" (BWM Convention) and its supporting guidelines, i.e., "Guidelines for approval of ballast water management systems (G8)". Due to the experience gained in approx. 100 shipboard tests of 18 different BWMS, Dr. Stephan Gollasch (WP4 leader) offered to share his knowledge and to prepare a BWO report summarizing points for possible re-consideration when Guidelines G8 may be re-opened for a discussion at IMO. He invited Dr. Matej David with whom he jointly undertook almost all of the 100 shipboard test voyage. Dr. David's expertise covers the nautical and maritime sectors so that this report does not only consider biological aspects.

Further, both, Gollasch and David, attended the IMO meetings where the BWM Convention and the Guidelines G8 were drafted and adopted, so that they have clear insights of the purpose and original intention of this instrument. Gollasch and David are further members of the GloBalTestNet, which is an organization of all BWMS test facilities which has the objective to share BWMS test information with the aim to harmonize test procedures.

This report is a contribution of WP4 to the WP5 deliverable:

- D5-12 Recommendations for IMO on the BWM Convention and the improvement of its supporting guidelines.

2 INTRODUCTION

The Guidelines G8 were originally adopted by IMO´s Marine Environment Protection Committee (MEPC) as Resolution MEPC.125(53) on 22 July 2005 and thereafter minor revisions were made and the revised Guidelines G8 were adopted by Resolution MEPC.174(58) on 10 October 2008 (IMO 2008a).

The goal of the Guidelines G8 is to ensure uniform and proper application of the standards contained in the BWM Convention. The Guidelines G8 include general requirements concerning design and construction, technical procedures for evaluation and the procedure for issuance of the (Type Approval) TA certificate of BWMS. To satisfy the BWM Convention, a ballast water discharge must comply with the D-2 standard throughout the life of the vessel. Guidelines G8 were drafted to challenge BWMS so that their performance is not compromised even in high organism and/or sediment loads, or other challenging circumstances. Therefore, Guidelines G8 contain recommendations regarding the design, installation, performance, testing environmental acceptability and approval of BWMS.

The Guidelines G8 for approval of BWMS are aimed primarily at Administrations, or their designated bodies, in order to assess whether BWMS meet the standard as set out in Regulation D-2 of the BWM Convention. In addition, Guidelines G8 may be used as guidance for manufacturers and shipowners on the evaluation procedure of the performance of BWMS. Guidelines G8 further state that these guidelines should be applied in an objective, consistent and transparent way and their application and its content should be evaluated periodically by IMO to keep the instrument up-to-date.

During MEPC 63 (March 2012) it was noted that two type-approved BWMS were withdrawn from the market because the systems did not perform consistently under real world conditions although they went through the test requirements as outlined in Guidelines G8. It was voiced at MEPC by shipowners that the BWMS approved under the Guidelines G8 may not be challenged enough during the TA testing process to confirm that the BWMS will meet the D-2 standard under the various ballast water uptake conditions in the world´s ports. It was multiple times stated by the International Chamber of Shipping (ICS) that the experience gained to date during TA tests of BWMS should be used to improve the Guidelines G8 TA requirements so that these guidelines become "fit for purpose".

At MEPC67 (October 2014) it was agreed that Guidelines G8 should be revised and the revision should commence as soon as possible. A Correspondence Group was initiated to support the work and a lively email exchange occurred so that a list of possible items to be addressed when improving Guidelines G8 was prepared and priority was given to selected items on this list which are supposed to be easier to discuss and agree. Other items will need to be addressed later as it was felt that more data need to be generated to address these items in the sufficient level of detail. The Correspondence Group met face-to-face, but off official IMO working hours, at the January 2015 session of the MEPC Sub-committee on Pollution Prevention and Response (PPR). Gollasch and David are members of this Correspondence Group.

3 OBJECTIVE OF THIS REPORT

As the original version of Guidelines G8 was adopted approximately 10 years ago when no performance tests of BWMS were conducted yet the Guidelines G8 are to be updated as the state of knowledge and technology may require and at MEPC 67 (October 2014) it was agreed that the guidelines are to be reviewed by an expert correspondence group and the finding be communicated to MEPC68 (May 2015) (see above).

The purpose of Guidelines G8 is to define test and performance requirements for the approval of BWMS which are split in land-based and shipboard approval requirements so that the required tests of a BWMS for TA are outlined in detail. This report will comment on possible Guidelines G8 improvements with a focus on the shipboard test requirements which are based on experiences gained in approx. 100 BWMS TA test voyages completed by the authors to date.

4 COMMENTS ON GUIDELINES G8 SHIPBOARD TEST REQUIREMENTS

In general the shipboard tests of BWMS should focus on the practicability of the BWMS, its seaworthiness, and its biological efficacy. The objectives of the shipboard testing include verifying that the installed equipment continues to maintain effectiveness during shipboard operation.

The space for laboratory facilities, accommodation and life boat capacity for the BWMS test team is very limited on commercial vessels. Therefore it becomes challenging to fulfill the requirements of the Guidelines G8. Very creative scientific input is needed to develop suitable sampling protocols as well as suitable sample processing protocols for the purpose of the Guidelines G8 shipboard tests.

The specific comments regarding Guidelines G8 shipboard test requirements given in this report are structured that they start with more technical aspects like the test duration, number of tests, flow rates etc. and are followed by more biological aspects towards the end of this report.

4.1 Test duration

Guidelines G8 require that shipboard tests are to span over a test period of not less than six months. This requirement was added to evaluate the long-term durability of the BWMS and any possible aging effects of BWMS components (e.g. filters, UV-lamps). However, Guidelines G8 currently lack a requirement that the BWMS installed on board a vessel needs to be operated constantly for all ballast water operations during the 6 months testing period.

As written now the BWMS may only be used during the three shipboard tests, but not routinely. This is in contrast to the original intention of Guidelines G8 and needs to be changed to clearly state that the BWMS should be operated routinely during the entire six month test period.

4.2 Number of tests

Guidelines G8 require 3 shipboard tests to be conducted over a period of six months. It should be noted again that one of the main critical points stressed by the shipping industry is that the BWMS TA tests are not robust enough to provide confidence to ships to operate in the future in accordance with the BWM Convention requirements, what was also the original intention of the Guidelines G8. Furthermore, this point is certainly not only in the interest of the shipping industry, but is about achieving the prime goals of the BWM Convention, to protect the natural environment, human health and resources from the negative effects of the transferred harmful aquatic organisms and pathogens, as well as to continue providing safe and efficient shipping globally.

It should also be noted that vessels conduct very frequent ballast operations, practically during every port visit, hence in the future the BWMS will be run very frequently. Furthermore, vessels usually sail to many different ports all around the world and all-around the year, which results in loading ballast water of very different conditions , from fresh water (<0.5 psu) to fully marine water (>30 psu, even >40 psu), from temperatures around 0 to more than 30 °C (we had test water conditions with >38 °C), with very different organism compositions and concentrations, and with very different sediment loads from very clear water in some marine ports to frequently very high sediment load in ports situated in rivers or estuaries.

All the above very clearly indicates that with only 3 tests conducted it is practically impossible to get even close to different conditions that would be needed to really show that BWMS systems are able to perform well and achieve the D-2 standard requirements when exposed to different conditions. TA certificates may need to be limited to the shipboard tested conditions to avoid vessels non-compliance in some “specific” conditions, or more tests would need to be conducted in different salinity, temperature and sediment load conditions. Also is to be noted that different conditions tested during the land based tests cannot be a substitute the shipboard tests, as the complexity of different conditions, because the land based tests are conducted at the same spot, hence the composition of organisms is very much limited to that conditions and the preparation of challenging conditions, which is very much different in real world conditions when vessels sail around the world, e.g., not only the concentration of organisms is changing, but the composition of species which challenge the system.

With this it would be very important to have more shipboard tests conducted in different conditions. Regarding the number of tests we would recommend that the Guidelines G8 require at least 5 consecutive successful tests to be conducted over the period of operation of the BWMS for six months, and in different conditions as specified here below. This is also very much in line with the latest US BWMS test requirements.

4.3 Abiotic water conditions

It is recommended that Guidelines G8 include a paragraph to address that BWMS may only be certified for water conditions under those they were tested and performed well and the following sub-paragraphs list selected challenge water conditions, which may be considered for this purpose.

4.3.1 Total Suspended Solids

The total suspended solids (TSS) content of water has an impact on transparency and turbidity, both being relevant factors especially for BWMS using UV and also when active substances are used because chemicals may react with the TSS which may lower the impact on organisms.

The test requirements are meant to challenge BWMS. It was therefore agreed that the test water conditions should be representative of wide port water conditions. For freshwater and brackish water conditions, Guidelines G8 require a TSS concentration higher than 50 mg/l. However, in certain port environments the TSS content is much higher. As an example, in the Elbe River (Germany, between Brunsbüttel and Glückstadt) the TSS content reaches values >700 mg/l[1] in peak conditions with occasionally extremely high values above 900 mg/l and in Hamburg, a German fresh water port, the TSS content reaches values between 50 and 150 mg/l (Gaumert 2002, Bergemann 2004). Therefore a value near 50 mg/l does not reflect challenging conditions for BWMS and Guidelines G8 may be adjusted accordingly for landbased tests. Furthermore we recommend that the challenging conditions are specified also for the shipboard tests, i.e., at least two out of five shipboard tests should be conducted in the (new!) challenging conditions as specified for the landbased tests as appropriate.

4.3.2 Water salinities

Guidelines G8 require that BWMS are to be tested in at least two salinities separated by at least 10 PSU. One of the important differences in different challenging salinities is that BWMS that use electrochlorination BWMS need to have certain minimum salinity conditions to be able to generated enough active substance to treat the ballast water. Furthermore, freshwater environments are also known to have different species compositions than the

[1] Measured as “abfiltrierbare Stoffe” (= material, which can be filtered out of water, including biological material).

marine environment, as well as the indicator microbes stated in the D-2 standard may be expected in more challenging numbers, especially when the tests are conducted in warmer waters, while in the marine water environment these die faster.

It is further recommended that the internationally accepted Venice Salinity System (1959) is used to identify salinity limits, i.e. < ±0.5 ppt is freshwater, ±0.5 to ±30 ppt is brackish water (mixohaline) and > ±30 ppt is marine water.

Hence we would recommend that the Guidelines G8 specify that BWMS shipboard tests are possibly conducted in different water salinities, i.e., freshwater, brackish and marine water conditions, and it should be required that at least two out of five tests should be conducted in the freshwater (<0.5 psu) and at least two of the remaining three tests should be conducted in fully marine conditions (>30 psu). With this one test is recommended to be conducted in brackish conditions, or with salinity somewhere between 0.5 and 30 psu.

4.3.3 Water temperature

It is known that the water temperature has a certain effect on the performance of a treatment system, which in particular refers to the BWMS using active substances. Furthermore with seasonal and temperature changes of water also the organisms concentrations and diversity changes, e.g., in temperate and colder seas in winter conditions the concentrations of organisms are substantially lower compared to higher water temperatures, and in warmer water also the indicator microbes survive better, hence their concentrations in water are expected to be higher.

With this we recommended to conduct shipboard tests under different water temperatures, e.g. cold and tropical waters, which may be achieved by testing over different seasons or in different climate zones.

4.3.4 Climate zones

Different climate areas, e.g., tropical, temperate, cold areas, have different organisms compositions and concentrations. It is to be noted that it is not the same way challenging for the BWMS, e.g., if the majority of organisms >50 micrometres in minimum dimension are close to the 50 micrometres, or if the majority is in the range of 100 and above, as the filters easier filter out bigger organisms than those being close to the mesh size of the filter. Further, it is not the same challenge for testing the BWMS where small phytoplankton organisms are in majority in the range of 10-50 micrometres than in cases where the majority is below 10 micrometres, as the below 10 micrometres are not even accounted for because of the D-2 standard is (currently) limited to the 10-50 micrometres in minimum dimension.

4.4 Toxicogenic Cholera bacteria

Guidelines G8 require to test the challenge and discharge water from a BWMS for toxicogenic *Vibrio cholerae* (serotypes O1 and O139). Due to the toxic nature of these bacteria the analysis can only be done in a laboratory under certain safety conditions. These conditions cannot be implemented during the on board tests of BWMS so that samples for these bacteria need to be shipped to land-based laboratories.

It is understood that in certain world regions Cholera is of great concern and it was also documented that Cholera bacteria occur in ocean waters and are transported in ballast water of vessels. However, during the approx. 100 shipboard tests we have conducted these bacteria were never found, therefore we would recommend to change this requirement to be limited to the ballast water uptake areas where the World Health Organization (WHO) alert for the presence of Cholera is issued.

4.5 BWMS flow rates

All TA testing should realistically represent the flow rates the BWMS is designed for. This should be monitored appropriately during the tests. Should a treatment system be designed for a certain water flow, a deviation from this flow rate may result in an unrepresentative over- or underkill of organism as the treatment may be higher/lower than calculated. We are aware that the flow rate with filling and discharging tanks vary because of varying conditions, hence the BWMS should handle these in positive and negative way, i.e., about 20 % lower and 20% higher flow rates than the pump capacity/BWMS treatment rate capacity (TRC).

We recommend that Guidelines G8 include a definition of the range/limits of the flow rates during testing, i.e., the average flow rate during the test should not be lower than the 10% of the BWMS TRC. A flow rate documentation is currently not required for shipboard tests by Guidelines G8, and this should be added.

4.6 BWMS service and major component changes

After the six months test period began, the BWMS should only be serviced as per the operations and maintenance plans until the end of the testing period.

Major components of a BWMS, which may affect the efficiency of a BWMS, should not be changed during test runs. This requirement should be clearly stated in Guidelines G8. Major component changes may result in the requirement to repeat test runs.

4.7 Normal ship operations

Guidelines G8 require that tests are conducted during normal ballast water operations of the ship. As ships usually do ballast water operations in ports, the uptake and discharge tests should be conducted in ports and not at sea during navigation. In addition, vessels usually take up ballast water in one port and discharge it in another so that a vessel voyage becomes necessary between the uptake and discharge tests of a BWMS to fulfill the normal ship operation requirements. However, it is not explicitly stated in Guidelines G8 where the ballast water operations should be conducted during the test runs and that a vessel voyage should occur between ballast water uptake and discharge. This has to be added to Guidelines G8.

4.8 BWMS operation during tests

The BWMS developer has to ensure that for the shipboard test the ship's crew is familiar with the BWMS and equipment and that the crew is sufficiently trained to operate the BWMS and equipment. A representative of the BWMS developer may be attending the test voyage(s) to ensure that questions by the vessel crew regarding the BWMS and its equipment can be answered promptly that not the entire voyage is wasted due to missing information and due to the lack of communication when the vessel is outside cell phone or other communications tools reach. However, the representative of the BWMS developer should not at all interfere with the system operation. This was not yet addressed in Guidelines G8 and we recommend to add this aspect to guarantee independent test runs of fully developed BWMS.

4.9 Control experiment

To evaluate the BWMS performance it was agreed at IMO that in parallel to the test with the BWMS a control experiment needs to be conducted with untreated water during which a certain number of organisms need to survive. This is to document that the organisms in the treated water are treated by the BWMS and did not die because of the storage time and conditions between ballast water uptake and discharge. This requirement is implemented in both the land-based and shipboard tests.

However, this requirement causes problems in shipboard tests as certain countries today have regulations which do not permit the discharge of unmanaged ballast water so that the control test water cannot be discharged in ports of these countries. This will also be the case for the signatory countries of the BWM Convention once this instrument enters into force. As a consequence in these countries no shipboard test according to the current version of the Guidelines G8 would be possible and it is therefore recommend to add a waiver paragraph into Guidelines G8 for this aspect so that in consultation with the Administration the control experiment may either be dropped or that on an exceptional basis control water can be discharged in a port.

It should further be noted that on certain vessels the control experiment caused trouble because during the discharge experiments the living organisms in the control water “contaminated” the treated water so that living organisms where found in the treated water which very likely originated from the control experiment. Should this case be confirmed due to experiments on board of the test vessel and the ship pipework be the cause of this contamination problem, e.g. due to leaking valves, the Administration may be approached to waive the control experiment. A paragraph to address this may be added to Guidelines G8.

4.10 Sampling regime, i.e., sampling timing, number and volume of samples

All samples should be taken via an isokinetic sampling point. Guidance how to design an isokinetic sampling point is given in Guidelines G2 (IMO, 2008b) and we recommend to add a reference to this guidelines regarding the sampling point design into Guidelines G8.

The Guidelines G8 recommend sampling regime:
For the control tank:

- three replicate samples of influent water, collected over the period of uptake (e.g., beginning, middle, end); and
- three replicate samples of discharge control water, collected over the period of discharge (e.g., beginning, middle, end).

For treated ballast water:

Three replicate samples of discharge treated water collected at each of three times during the period of discharge (e.g., 3 x beginning, 3 x middle, 3 x end).

4.10.1 Control test

The control test was introduced as a parallel comparison test to confirm that organisms did not die because of hostile conditions in the tank between the uptake and discharge. Several countries around the world do not accept anymore the discharge of untreated ballast waters (Gollasch et al. 2015), hence control test cannot be applied. Furthermore, several shipboard tests we have conducted which included control tests, as well as different scientific studies, showed that zooplankton organisms well survive for 5 and more days between ballast water uptake and discharge, while phytoplankton even much longer (Gollasch & David 2010, Gollasch & David, own observations).

Based on this we recommend that control tests are not required anymore, but the treated test is sufficient for TA. This approach is also identical with the latest US BWMS test requirements.

4.10.2 Treated test

4.10.2.1 Treated uptake

At the uptake, instead of the control tests, the concentration of organisms should be tested before the BWMS to identify challenging concentration.

4.10.2.2 Treated discharge

The results of studies we conducted (Gollasch & David 2009, 2010, 2013) showed that different approaches in the sampling process influence the results regarding organism concentrations. The organisms in the discharge are affected in different ways, therefore the selection of the “wrong” sampling approach influences the TA result. The organism concentrations in the ballast water discharge may therefore be underestimated, and BWMS that does not perform well could be recognised as compliant with the D-2 standard.

It should be noted that a certain level of pragmatism is required during shipboard ballast water TA sampling especially when larger volumes of water need to be sampled. This is especially relevant to sampling for bigger organisms, and attempts should be made to avoid negatively impairing organism survival during the sampling process. Sampling teams are unlikely to have larger water collecting tanks (>1000 litres) available during the sampling event hence need to concentrate during the sampling procedure, and this process had shown to affect the viability of organisms filtered, i.e., those of 50 micrometres and above in minimum dimension.

During these studies it was observed that the sampling duration (i.e., length of the sampling process), timing (i.e., in which point in time of the discharge the sampling is conducted), the number of samples and the sampled water quantity are the main factors that influence the results regarding organism concentrations.

4.10.3 Recommended sampling duration

The shipboard tests results show that bigger organisms are negatively affected by longer sampling times. Considering that the results show that a shorter sampling time is still representative, the recommended sampling time of a sample taken during the tests in a sequential sampling is approximately 10 minutes. Longer sampling times result in an underestimation of the viable organism concentration in the discharge, especially for bigger organisms.

4.10.4 Recommended sampling timing

The shipboard tests results show that organism concentrations may vary considerably if the sampling is conducted at the very beginning or at the very end of the discharge process because of the patchy distribution of organism inside ballast water tanks. It is not recommended to take a sample at the very beginning (i.e., the first 5 min) or at the very end of discharge (i.e., the last 5 min), as an underestimation as well as an overestimation of organism concentrations may be expected. Based on this it is recommended that the sampling is conducted randomly anytime in the middle of the discharge, starting after 5 minutes from the start of discharge and ending 5 minutes before the end of the discharge.

4.10.5 Recommended number of samples

The shipboard tests results show that organism concentrations in all organism groups vary due to the patchy distribution of organisms inside the ballast water tanks, hence a single 10 minutes sequential sample may underestimate or overestimate the concentration of organisms being discharged. The results also show that an average of organism concentrations of 2 random samples in a sequential sampling procedure provide very similar results to the average of the 3 random samples. Based on this it is recommended that sampling is conducted by undertaking at least 2 random samples or better 3 samples to provide even more robust results, which are analysed immediately after each sampling event has ended, and that the organism concentration results are averaged.

4.10.6 Recommended sampled quantity

In the shipboard tests studies sequential sampling was conducted with an open method (i.e. net in a bigger bucket) over periods of 10 and 15 minutes, with flow rate averages ranging mainly between 30 and 45 litres per minute. To obtain most representative results it is recommended that:

- for the organisms greater than or equal to 50 micrometres in minimum dimension 350 to 500 litres should be filtered and concentrated;
- for the organisms less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension a "continuous drip" sample totalling to approximately 5 litres (i.e., collect about 0,5 litre of sample water every minute during the entire sampling time duration or collect about 0.5 litre of sample water every 50 to 100 litres depending on the flow rate) should be taken. The resulting 5 litres of sample water should be sub-sampled after mixing, and two sets of sub-samples are prepared, one alive and another preserved. We recommend sub-sample volumes of 60 to 100 ml;
- for bacteria, a sample of approximately 1 litre should be taken as a sub-sample after mixing from the 5 litre "continuous drip" sample.

4.10.7 Recommended sampling flow rate

It is also assumed that the sampling flow rates may influence the results. Lower flow rates obtained by partially closed valves of the sampling line may damage organisms, and a similar negative effect may be caused by to strong flow rates affecting mainly the filtering process of the bigger organisms. Hence, the flow rate, or "valve" effect, may cause an underestimation of the organism concentration as organisms may die during the sampling process. To avoid this negative influence it is recommended that the valve at the sampling point is opened as much as possible. However it should not exceed the flow rate of 50 litres/min so that the water pressure is not too high during sample concentration as this may impair organism survival. In general, minimum and maximum flowrates of the flow-meter(s) used need to be respected to enable accurate flow meter readings.

4.11 Organism concentration and subsample handing

4.11.1 Larger organisms

When sampling larger volumes of water as outlined above for the organisms greater than or equal to 50 in minimum dimension the sample needs to be concentrated. This concentration should be done by using a sieve no greater than 50 micrometres mesh in diagonal dimension as Guidelines G8 currently state. Guidelines G8 do not go further, but there are more aspects to consider.

The sample should not be concentrated to a smaller volume than 100 ml to minimize negative effects on the organisms in the concentrated sample. From a statistical perspective it may be appreciated that the entire concentrated sample is analysed. However, this would be a time consuming process and we observed organism mortality during longer sample processing times. It is therefore recommended that the subsamples of the concentrated sample are processed until in maximum a 5 % mortality is observed. This may result in approx. 10 % of the concentrated sample to be processed or more. In case no mortality is observed during subsample processing or the situation is unclear we recommend that subsamples are processed for in maximum 1 hour.

4.11.2 Smaller organisms

Our experiments have shown that sample concentration for organisms less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension is not beneficial as the concentration process caused negative effects on the organisms. Therefore we recommend not to concentrate these samples.

4.12 Sample storage conditions and sample handling

One other way to overcome the 6 hour time limit is, as Guidelines G8 state, to treat the sample in such a way so as to ensure that proper analysis can be performed. This refers, e.g., to sample storage conditions and using e.g. stains to enable a later identification of living/dead analysis.

In several experiments, and especially for the zooplankton organisms above 50 µm in minimum dimension, we used stains and we never found an acceptable level of stain efficacy.

In many experiments 10% or more of the organisms that should have been stained (irrespective if a live or dead stain was used) did not take up the stain and we concluded this error is too high to enable proper sample analysis. For this reason these samples are processed on board directly after sampling as much as possible. Our extended experience has shown that bacteria (excluding tests for toxigenic strains of Cholera bacteria, which can only be done under certain laboratory conditions) and zooplankton can be analysed on board within 6 hours after sampling.

In contrast phytoplankton viability analysis and enumeration is difficult on board because of sensitive and heavy gear would be needed (e.g., epifluorescence microscope, flow cytometer). The difficulty is that most such analytical tools are large and heavy which limits air travel and further many systems are fragile and/or need to undergo a laboratory based calibration after transport. This is in most cases difficult to achieve before boarding a vessel. As a consequence, sample storage may become unavoidable until the samples arrive in a land-based laboratory for processing. However, sample storage conditions are not specified in Guidelines G8, but clearly, the sample storage conditions should at best have negligible influence on the organisms.

For phytoplankton sample storage we recommend cool and dark conditions. However, the storage temperature should not be selected to generate a cold shock of the algae. Therefore, in cold-temperate waters the cooling may be achieved by storing the samples in a fridge. In the tropics the ambient water temperature may be above 30 °C so that a storage at room temperature represents already a cooling effect (air-conditioned cabin on board). In these circumstances the samples should not be stored in a fridge as the organism may be negatively affected by the cold shock (temperature drop of more than 20 °C in a few hours). The negative impact of cold storage on algal survival are reported in scientific literature (Gupta & Agrawal 2005, Sorrosa et al. 2005, De Boer et al 2005, Thornhill et al. 2008, Wang et al 2011, Valedor et al 2013). This view was also confirmed by the phytoplankton experts at BWMS test facilities, i.e., Louis Peperzak (NIOZ, the Netherlands) and Nick Welschmeyer (MLML, USA). Our own studies when working with ambient water temperatures of 38 °C have shown that a strong phytoplankton mortality occurred when the samples were stored in a fridge so that the temperature difference of more than 30 °C caused a fatal temperature shock.

To document the potential impact on algae mortality during the holding time several experiments were done and a literature search was conducted which is summarized as follows:

- The best phytoplankton sample storage condition is in the dark with a temperature approx. 10 °C cooler than the ambient sample temperature to avoid biased counts as due to the sample storage conditions.
- A sample storage time of up to 10 days (stored in a dark and cool environment) has little influence on the organism viability. This indicates that a one week to 10 day time duration between phytoplankton sample taking and quantitative viability measurement in a land-based laboratory is not very critical.

To assess the possible storage and transport impact of a sample it is suggested that a fluorometer[2] will be used on board to measure the algae (phytoplankton) viability directly after sampling. The fluorometer is a robust and portable tool. Most fluorometers will deliver

[2] Fluorometer examples, which were tested for this purpose include Walz Water Pulse Amplitude Modulated (PAM) Fluorometer, Germany; modified PAM bbe 10 cells, Germany and traditional fluorometer Hach BW680, USA.

bulk viability data, i.e., only semi-quantitative counts (the higher the reading the higher is the organism number), while there are some which as the result produce also the number of 10 to 50 micrometers phytoplankton cells per millilitre. After the fluorometer measurement on board, the samples will be stored for later analysis. The sample storage temperature should be documented by temperature loggers.

In addition to the fluorometer used on board, after the samples have reached the land-based analytical laboratory, the fluorometer measurement, possibly with the same type of fluorometer, will be repeated. To assess the possible impact of the sample transport the fluorometer results from on board and the land-based measurement after sample storage and transport are compared. As a second step the land-based laboratory will process the samples to enumerate living phytoplankton cells with a microscope or flow cytometer.

However, in all cases, the samples should be transported to the laboratory as soon as possible and kept under proper temperature conditions.

It is recommended that Guidelines G8 should include paragraphs on sample storage conditions and maximum allowable storage times between sample taking and sample processing.

4.13 Challenge water organisms

For shipboard tests, a minimum number of organisms need to be present in the intake water and this is specified as per the two organism size groups in D-2. We found in our on board studies that the minimum number of organisms greater than 50 µm in minimum dimension, i.e. >90 organisms/cubic metre, is easy to meet so that this does not represent a challenging condition. In contrast, the required minimum concentration for the organisms less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension (predominantly phytoplankton), i.e. >90 organisms/millilitre, is a more challenging requirement as for the larger organisms. We therefore recommend that to challenge BWMS the intake water organism concentration for the larger organism is increased from now 10 times the numbers in the D-2 standard (i.e. >90 organisms) to 100 times (i.e. to >900 organisms).

It is further recommended to consider the organisms below 10 micrometres in minimum dimension, which are currently not addressed by the D-2 standard. Several phytoplankton species in this size class are known to cause unwanted effects, such as harmful algae blooms so that their transport in ballast water is a undesirable event. It was further observed that in many cases the majority of the phytoplankton cells in a sample are below 10 micrometres in minimum dimension. With today´s sample processing methods these organism can be documented so that the D-2 standard may need to be re-discussed.

4.14 Definition minimum dimension

For organisms above 10 micrometres, the minimum dimension needs to be measured according to Regulation D-2 of the BWM Convention. Such a measurement is complex as all possible shapes of organisms need to be addressed and also it leaves room for interpretation of what the minimum dimension is by different analysts.

We believe this measurement should be based upon an investigation of the organism "body", thereby ignoring sizes of thin spines, antenna etc. In e.g. flat worms or diatoms the minimum dimension should theoretically be the smallest part of their "body", i.e. the dimension between the body surfaces when looked at the individual from the side. However, this is not always possible to be investigated as not all smaller organisms found in a sample can be turned by hand to investigate all axes. In ball shaped organisms the minimum dimension should be the spherical diameter.

In summary, the smallest axis of an organism “body” visible to the analyst or counting machine will need to be identified and the smallest point on this axis will be measured. This approach is suggested also for practical reasons, each identified viable organism cannot be turned on the side to measure its minimum dimension, because many zooplankton organisms would be moving or swimming, while the phytoplankton would be mainly too small to do this. Should counting machines be used for counting viable organisms, the orientation of an organism when passing through the observation chamber cannot be manipulated. Therefore, we recommend that in the Guidelines G8 the definition for the “minimum dimension” is added and that it is to be the smallest visible axes of the organism body in the moment of observation.

4.15 Colony versus single cells

A question arose in which size category a colony falls when the single cell is below 50 micrometres in minimum dimension but the colony is above 50 micrometres in minimum dimension. A team of experts, i.e. the ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors, believes that in those cases the individual specimen size should be measured, i.e. not the colony. This group finding is based upon the D-2 standard as it refers to organisms and not to colonies. Further, viability assessments should address the smallest unit able to reproduce which is the individual and not the colony. Based on this conclusion the size of the individuals should be measured and not the colony. This may be considered to be clearly stated in Guidelines G8.

5 CONCLUSIONS

The current Guidelines G8 requirements do not represent enough challenging test conditions where ballast water is taken up. Therefore, BWMS that have received TA under the present Guidelines G8 may be found non-compliant if operated in more challenging water conditions as the BWMS was exposed to during the TA tests. This underlines that Guidelines G8 should be modified to better reflect the real world ballast water uptake conditions, and further that the TA certificates should clearly identify the limitations of the BWMS operation according to the tests conducted.

The above proposed shipboard test conditions are more challenging for the BWMS and hence they are expected to deliver better and more reliable results to consequently have more reliable BWMS installed on vessels in the future. This would result in better natural environment, human health and resources protection and on the other side it would avoid, in cases of non-compliance of a non-properly performing BWMS, disruption of vessels commercial activities. We are well aware that the above proposed conditions become more challenging also for finding adequate vessels to meet these requirements, but from the very vast experience with tests all around the world we can confirm that this is achievable, and we recommend that this is well taken into account when vessels are being selected for shipboard tests.

With stronger test requirements it is hoped that BWMS are tested at or near real world ballast water uptake conditions so that the performance of such systems meets the D-2 standard under a wide range of conditions. Additional and stronger test requirements should be in the interest of all involved, the ship-owner to have a better guarantee that the BWMS serves for purpose, the BWMS manufacturer to have a widely applicable BWMS and the Administration to have greater confidence in the issued certificates.

It should be noted that currently a MEPC correspondence group discusses possible re-negotiations, amendments and changes to Guidelines G8 and it is hoped that the content of this report may be considered during these debates.

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