



NORTH SEA BALLAST WATER

The Interreg IVB  
North Sea Region  
Programme



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

## What to do with ballast water?!



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November 2013

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What to do with Ballast water?!

## 1. Introduction

Ballast water is essential for the safe operation of cargo vessels; it ensures the stability and manoeuvrability of un-laden ships. Because over 80% of the world's merchandise is moved by ships approximately 3 to 5 billion tons of ballast water is carried around the world each year<sup>1</sup>. This ballast water (sea water) contains billions of small organisms, eggs and larvae from the port of origin. After discharging ballast water some of these species can (and have) become invasive, causing serious ecological, economic and health threats<sup>1</sup>. In response to these threats the International Maritime Organisation (IMO) adopted the International Convention for the Control and Management of Ships Ballast Water & Sediments (or the Ballast Water Management Convention) in 2004<sup>2</sup>.

Although the reason for the adoption of the Ballast Water Management Convention was clear a lot of questions about the actual implementation still remained. Therefore the North Sea Ballast Water Opportunity (NSBWO) project was started in 2009 to answer these questions in a practical and scientific manner. Moreover, the NSBWO project 'aims to reach regional cohesion, innovation and develop future strategies in ballast water policies and ballast water management' (Ballast Water Times #1, 2011<sup>2</sup>).

In this booklet you will find the summaries of articles written during the NSBWO project. The focus of these summaries lies on the compliance with the D-2 standard (i.e. treatment of ballast water) of the IMO Ballast Water Management Convention, since the D-1 standard (i.e. midsea ballast water exchange) will be phased out in the future. The articles are arranged according to 5 topics: **indicative analysis**, **in-depth analysis**, **overview of analyses**, **risk assessment**, and the **NSBWO project** itself.

Recurring terms are alphabetically explained in a glossary.

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<sup>1</sup> Source: <http://globallast.imo.org/index.asp?page=problem.htm&menu=true>

<sup>2</sup> Source: <http://www.northseaballast.eu/northseaballast/1996/7/0/82>

## Indicative analyses

### 2. On board testing of three indicative analysis tools

#### Use for shipping companies

Three methods were selected and tested for their applicability on board commercial vessels for the analysis of phytoplankton (size range 10-50 µm). All tested methods are compact, easy to use and deliver results quickly. Therefore they are suitable to bring aboard by e.g. the Port State Control officers to check for compliance with the D-2 standard.

#### The article

Title: "**Onboard tests of the organism detection tools BallastCAM, FluidImaging, USA, Hach-PAM-fluorometer, USA, and Walz Water-PAM-fluorometer, Germany. Results and findings**"

Authors: **Stephan Gollasch** (GoConsult) and **Matej David** (Dr. Matej David Consult s.p.).

Date: **December 2012**

#### Detailed information

Gollasch, Stehouwer and David (2012) (fact sheet "Available indicative and in-depth analyses") listed several technologies in their report and from these methods three were selected for an on board performance test: the BallastCAM, the Hach-PAM-fluorometer, and the Walz-Water-PAM-fluorometer. The BallastCAM provides cell counts and additionally it photographs each organism, which enables identification of viable cells by an expert. However, this identification of viability by an expert is prone to errors. Both fluorometers determine the viability and the biomass of the organisms still present in the samples taken from the ballast tanks containing treated ballast water, but cannot deliver viable cell counts. All three methods have their weaknesses (see table below) but a combination of the BallastCAM and a fluorometer could provide an accurate count of viable cells in treated ballast water, hereby proving (non)-compliance with the D-2 standard. Therefore it seems prudent to develop a method that combines both BallastCAM and fluorometer technologies.

Method	Pro	Con
BallastCAM	Counts cells, individual cells photographed (i.e. enables viability determination by expert)	No viability measurement
Hach-PAM-fluorometer	Measures viability and biomass	No cell counts, does not detect low concentrations
Walz-Water-PAM-fluorometer	Measures viability and biomass, measures cells of all concentrations	No cell counts

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### 3. On board testing of viable algae in Ballast Water

#### Use for shipping companies

To test for compliance with the D-2 standard the viability of organisms in treated ballast water has to be determined. Determining the viability of cells is important since the D-2 standard of the IMO specifically focusses on viable organisms. A fluorometer can be used on board a vessel as an indicative measure of the viability of algae in treated ballast water. Since the viability of algae in ballast tanks starts decreasing after 2 weeks, this method is useful to check the viability of algae on board when a sea voyage is no longer than 2 weeks (to prove compliance is due to the treatment and not because of natural causes such as mortality during storage time of the sample).

#### The article

Title: "**Algae viability measurement over time**"

Authors: **Stephan Gollasch** (GoConsult) and **Matej David** (University of Ljubljana).

Date: **March 2010**

#### Detailed information

This study was initially performed to document algal survival over time. This becomes important when it is not possible to determine the number of viable cells immediately after taking samples of the treated ballast water. When viability is used as compliance control it is needed to know how long algae can survive in a sample before cell mortality affects the results of the compliance test. On board testing of the viability of organisms in the size range between 10 and 50  $\mu\text{m}$  (mainly phytoplankton) can be performed by a fluorometer (the Pulse-Amplitude Modulated fluorometer), which uses a (red) laser to measure the viability. This research showed that the viability of algae taken from a ballast tank is almost unchanged during the first two weeks of storage when the sample is kept cooler than ambient water conditions during sampling and in the dark. Thereafter their viability decreases over time.

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#### 4. Indicative detection of bacteria

##### Use for shipping companies

The Q-PCR method can be used to check for gross non-compliance in ballast water by measuring the concentration of pathogenic bacteria. This method cannot determine the viability of the bacteria nor can it count the number of colony forming units (CFU). CFU's are the units used in the discharge standard. The method requires a couple of hours and it needs to be performed by an expert with dedicated instrumentation, which makes it less suitable to use on board. Whether it is possible to use the method in a moving vehicle is unclear.

##### The article

Title: "**Demonstration and feasibility of Q-PCR and immune detection of human pathogens as candidate methods for compliance control and requirements for compliance control efforts**"

Authors: **Stephan Gollasch** (GoConsult) & **Louis Peperzak** (NIOZ)

Date: **June 2013**

##### Detailed information

The report addresses two organism detection methods and focusses on their suitability for compliance control of treated ballast water. The methods can be used to check gross non-compliance as presence / absence of bacteria and for compliance by enumeration. When bacteria are absent in the indicative compliance test the ballast water is in line with the D-2 standard. However, when organisms are detected a more detailed study by Q-PCR is needed. During land-based testing of ballast water treatment systems it is impossible to add *Vibrio cholerae* cells to the test water due to safety reasons. Therefore, NIOZ used non-toxic *Vibrio* bacteria instead of *Vibrio cholerae*. After this substitution a third (CARD-FISH) method was used to analyse treatment systems.

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## 5. Indicative bacteria testing in a laboratory

### Use for shipping companies

The 'DNA bead' method can be considered for an indicative analysis to check for compliance with the D-2 standard since it can detect the presence or absence of bacteria. It is impossible to use this method to check viability and whether as well as how many colony forming units are present in the ballast water sample. The method takes up to four hours and needs to be performed in a laboratory by experts.

### The article

Title: "**Feasibility of immune magnetic separation and detection of human pathogens in ballast water on board**"

Author: **Stephan Gollasch** (GoConsult), **Hans Flipsen** (Evers + Manders Subsidieadviseurs B.V.) & **Rob van Weeghel** (Formerly with Zebra Bioscience)

Date: **June 2013**

### Detailed information

In this report a comparison is made of the practicability of three bacterial detection methods. Of those three methods only one seemed probable for indicative bacterial analysis. With the DNA bead method it is possible to prove the presence/absence of the bacterial species listed in IMO standard D-2 (i.e. *Vibrio cholerae*, *E. coli*, and *Intestinal enterococci*). Further, determination of colony forming units of the three bacteria or viability of the detected bacteria (needed to prove compliance with the D-2 standard) is impossible. The DNA bead method presented here is a biomagnetic separation process that can isolate bacteria in fluids. It works as follows: a magnetic bead is combined with a species specific DNA strand. This strand attaches itself to the bacterial DNA, resulting in a combined bead-bacterial-DNA complex. The next step includes a magnet that attracts the bead-bacterial-DNA complex. The remainder of the fluid is washed away leaving the bead-bacterial-DNA complexes attached to the magnet. After removing the magnet and detaching the beads, the bacterial DNA can be released. Once bacterial DNA is isolated a method called Polymerase Chain Reaction (PCR) can be used to identify the bacteria. However, this method is not able to discriminate between dead and living bacteria so that colony forming units cannot be established. Therefore, compliance control with the D-2 standard is impossible.

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## **6. Algal Growth Detection from Remote Sensing.**

### **Use for shipping companies**

A Risk Index for Ballast Water Exchange has been developed for which, among others, information from satellites about the water quality is used. Risk is defined as the risk that an invasive species, which is released by ballast water exchange at a certain location, may survive and cause damage to the ecosystem. An online tool is developed in which the risk can be calculated. The Risk Index is determined by several factors, being the water depth and distance to coast and protected areas; transparency, chlorophyll concentration and concentration of suspended matter derived from satellite remote sensing; currents and the water salinity and temperature at the points of ballast water uptake and destination. The major part of the article describes the method of remote sensing, as well as its applications, limitations and opportunities. Remote sensing uses information from light that underlies the process of absorption and reflection by surfaces on earth and in the air. Data about water constituents can be disturbed by clouds and atmospheric processes. The analysis of water becomes more challenging when water is a mixture of suspended matter and absorbing substances – such as close to the coast – than in open oceans. This should be taken into account when analysing the data. Validation of the remote sensing data is needed and is done by comparing data from the field (in situ) with the satellite data.

### **The article**

**Title: “Algal Growth Detection from Remote Sensing – A Study on the applicability of satellite data for algal bloom detection in the scope of ballast water management issues”**

**Authors: Kerstin Stelzer (Brockmann Consult GmbH)**

**Date: August 2013**

### **Detailed information**

Ocean Colour Remote Sensing is a method that can be used to monitor the status and development of coastal and open ocean waters. Remote sensing is based on measuring the energy of light that is reflected from the earth's surface. In the ocean, algal bloom growth can only be detected if the patches have a certain extent. The validation of the remote sensing data is performed by comparing the concentrations with in situ information. The identification of phytoplankton species needs ground truth sampling. Furthermore, the data are influenced by disturbing aspects like atmospheric circumstances (haze, clouds), as well as complex waters which contain also suspended matter and coloured dissolved organic matter (CDOM). The article describes several examples in which blooms are detected. The studies were performed by the MERIS Sensor of the ENVISAT satellite and are available from 2002 to 2012.

The method of ocean colour remote sensing can be applied to the case of ballast water exchange risk analysis. A Risk Index for Ballast Water Exchange is developed for two different applications, namely the ship dependent (dynamic) Ballast Water Risk Index and the Average Risk Index. Risk is considered the risk that an invasive species, which is released by ballast water exchange at a certain location, may survive and cause damage to the ecosystem. The Risk Index is determined by several factors, being the water depth and distance to coast and protected areas; transparency, chlorophyll concentration and concentration of suspended matter derived from satellite remote sensing; currents derived from an operational prediction model and the water salinity and temperature at the points of uptake and destination of ballast water.



### 7. In-depth testing for bacteria

#### Use for shipping companies

There are standard methods to test if the bacteria mentioned in the IMO D-2 standard are present in ballast water. These methods are time consuming (due to incubation period) and need to be performed in laboratory circumstances by trained experts.

#### The article

Title: "**Testing of Ballast Water Treatment Systems performance regarding organisms below 10 micron in minimum dimension**"

Authors: **Stephan Gollasch** (GoConsult), **Allegra Cangelosi** (Northeast-Midwest Institute & Great Ships Initiative) and **Louis Peperzak** (NIOZ).

Date: **November 2012**

#### Detailed information

The report discusses various options for the detection of the bacteria identified in the IMO D-2 standard (i.e. < 1cfu of *Vibrio cholerae* per 100 ml; < 250 cfu of *Escherichia coli* & < 100 cfu of *Intestinal enterococci*). Additionally, other potentially harmful organisms below 10 µm were discussed (e.g. various viruses, bacteria, archaea, and small phytoplankton species smaller than 10 µm (e.g. dinoflagelates) which produce toxins). However, this fact sheet will not deal with these organisms since they are not addressed by the D-2 standard. As it turned out, most ballast water test facilities (i.e. GoConsult, GSI, IMARES, MERC, NIOZ, NIVA) use comparable methods to test Ballast Water Management Systems (BWMS) for bacteria mentioned in the IMO D-2 standard. However, due to the low concentrations of these bacteria in test water it is difficult to detect them and thereby determine the effectiveness of BWMS in this regard. Since adding pathogens to the test water is not possible (due to health, safety and environmental reasons) effectiveness of treatment systems for these organisms has to be determined in another way. Therefore several ideas, on how these tests should be performed, are being explored. Ideas include using non-toxic *V. cholerae* as substitutes to the pathogenic *V. cholerae* to measure the effectiveness of a treatment system; or using fish pathogens as source of information relative to human pathogens. These prospects however, are still in a developmental stage.

## 8. In-depth testing for algae

### Use for shipping companies

While most in-depth tests have to be carried out by specialists in a lab, the test discussed here (OVIZIO's 'oLine') is an easy detection method aimed at users with little to no academic training. The method is most suited for the detection of organisms in the range of 10-50  $\mu\text{m}$  (mainly phytoplankton: small algae). The costs of the 'oLine' system are €85,000. This does not include the fluorescence option that enables determination of viable cells. Since the IMO specifically mentions viable organisms it is crucial to incorporate fluorescence in the 'oLine' when compliance needs to be proven. The system is not sufficiently tested yet. In 2012, developments were undertaken to make the 'oLine' more portable so it can be used on board by e.g. the Port State Control.

### The article

Title: "**Demonstration of the OVIZIO organism detection technology, the 'oLine', for Compliance Enforcement with the Biological Standards of the IMO BWMC**"

Authors: **Stephan Gollasch** (GoConsult), **Eva-Maria Zetsche** (Vrije Universiteit Brussel) and **Joël Henneghien** (OVIZIO Imaging Systems).

Date: **June 2012**

### Detailed information

The report discusses the characteristics and applications of a method for the detection of organisms in the 10-50  $\mu\text{m}$  range (mainly phytoplankton). The method can be used to detect organisms in all sorts of size ranges, although it is most suited for the detection of organisms in the range of 10-50  $\mu\text{m}$ . In combination with the 'OsOne' software the 'oLine' can detect and count particles in a fluid and when properly trained these particles can be differentiated and classified automatically. The processing of information will be largely automatic by the 'OsOne' software. The 'oLine' is a largely automatic, simple and accurate method to check for compliance with the D-2 standard using Digital Holographic Microscopy. In this method a laser is split into an undisturbed light beam (the reference beam) and a light beam that passes through the sample (the object beam). By recombining these two light beams a 3-D image (or a so-called hologram) of the organism is formed by a digital camera. The method enables a fast, easy, and largely automatic analysis. However, the current 'oLine' does not use fluorescence and it can thereby not deduce whether a counted cell is dead or alive. **It is crucial** to determine the viability of cells because the IMO D-2 standard specifically focusses on cells that are viable due to their ability to grow after ballast water is released in the environment. To determine the viability of organisms in the ballast water samples, additional features (e.g. fluorescence) have to be incorporated in the 'oLine'. These features have been developed separately and can be included in the system. The system is not sufficiently tested yet. For instance, the time it takes for the 'oLine' to get a result is not properly tested, however it is known that a 1ml sample takes approximately 15-20 minutes to analyse.

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## 9. Harmonization of ecotoxicity testing

### Use for shipping companies

When a Ballast Water Management Systems (BWMS) uses chemicals during treatment, toxic products may be discharged. To prove that discharged ballast water does not pose a risk to the receiving environment, toxicity tests are needed. A lot of discussion is still on-going about what procedures are best used for land-based toxicity testing after ballast water has been treated according to the G8 guideline (i.e. the guideline for the approval of BWMS). IMARES (Institute for Marine Resources And Ecosystem Studies) hosted an international workshop to discuss the procedures used for toxicity testing of ballast water samples.

### The article

Title: "**Minutes NSBWO project workshop ecotoxicity testing during land-based testing**"

Author: **Klaas Kaag** (IMARES)

Date: **May 2012**

### Detailed information

The goal of the NSBWO project meetings was to discuss and harmonize toxicity testing procedures among research facilities (i.e. NeoEnBiz, Golden Bear Facility, NIVA, MERC, and IMARES) during land-based testing for type-approval of BWMS. Since research facilities all seemed to use various methods to test ecotoxicity this seemed prudent. During the workshop the G9 guideline of the IMO was used as an instruction on which toxicity tests should be performed. In general, the test facilities used the same type of tests, but the exact test protocols varied. Important issues to harmonize are sample (pre-)treatment and QA/QC. The evaluation of test results should be transparent and consistent. Test species used for toxicity testing should be representative of different biological groups. According to the G8 guideline of the IMO, ballast water needs to be held for 5 days, but for risk analysis water can also be evaluated after only 24h or 48h holding time.

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## 10. Sampling of Ballast Water by NIOZ and GoConsult

### Use for shipping companies

This study focused on the assessment of the viability and number of coastal unicellular eukaryotes and protists in ballast water on board two ships in the port of Rotterdam. Additionally, the study points out the difficulties of planning an indicative on board ballast water sampling event. The experiment showed that both research teams and crews should be prepared for taking samples from various access points, i.e. sounding pipes, open manholes, on-deck overflow, or the engine room. When ballast water samples need to be taken from the engine room it is important for the scientific team to realize that it is impossible to sample large volumes of water without having a proper discharge of the sampled water.

### The article

Title: "**Ballast Water Sampling in Rotterdam with ROSCOFF Team**"

Author: **Stephan Gollasch** (GoConsult)

Date: **June 2010**

### Detailed information

Ballast water sampling took place on board of two vessels in the Port of Rotterdam as part of the Interreg IVB (NS)BWO project. The ballast water samplings are dependent on the cargo operation of the vessel, which means that the scientific team is often subject to waiting. After ballast water is taken in, the number of living organisms inside a ballast tank usually reduces exponentially due to the harsh environment of the ballast water tanks. This means that when there are still viable organisms present in ballast water after a specific holding period (5 days in this case), the number of organisms in the port of intake is probably high and the risk of transferring organisms to the receiving port is increased. Various analyses (microscopy, PCR, FISH molecular analyses of DNA, RNA, and cellular material) were used to group the genetic data of the organisms found during testing of the ballast water. Additionally, a PAM-fluorometer was used to check viability of the cells present in the ballast water. As expected, after 5 days in a tank without light, the viability of algae was low. However, there were indications that living phytoplankton cells were still present in the untreated ballast water. This indicates that storage of ballast water in tanks is not enough to comply with the D-2 standard of the IMO.

## 11. Ballast Water: Risky Business

### Use for shipping companies

When a ship transports ballast water between ports with comparable aquatic environments it may be exempted from the BWMC. Before a vessel qualifies for exemption it should prove that its discharge of ballast water does nothing to harm the environment of the receiving port. This needs to be determined according to the G7 guidelines of the IMO, which provide standards for the risk assessment of discharging untreated ballast water. Discussion is still on going about when the risk of ballast water discharge can be considered sufficiently low for a ship to be exempted from BWM requirements. Since a risk assessment has cost and legal implications for shipping companies it is important to be sure that input data used for the process is reliable. Presently, this input data is still subject to uncertainties so that without comprehensive port baseline surveys which would deliver reliable data no exemption can be granted.

### The article

Title: "**Ballast water risk assessment for intra North Sea shipping**"

Authors: **Matej David** (University of Ljubljana) & **Stephan Gollasch** (GoConsult)

Date: **June 2010**

### Detailed information

This report describes three risk assessment approaches as contained in the guidelines for risk assessment of ballast water (G7): 1) environmental matching risk assessment compares the environmental characteristics (e.g. temperature and salinity) from the areas of ballast water origin and discharge. These characteristics are considered to be surrogates for the species ability to survive in the new environment, 2) species biogeographic risk assessment identifies overlapping species in the donor and recipient area, these are used as indications of similarity of environmental conditions. Overlapping species in donor and recipient ports are an indication of similarity posing a low risk for the receiving port, and 3) species specific risk assessment assesses the potential invasiveness of selected individual species and anticipates the harm this species could cause in the new environment. The report considers the possible use of these risk assessment approaches in the North Sea. A risk assessment can determine the danger that ballast water might pose to a receiving area. A risk assessment of the introduction of non-native species is based on the likelihood that a new organism will establish itself in the new environment, and on the potential danger this species can pose to the new environment. A species can become harmful when: the organism is present at the donor harbour, when it survives the transport, when it survives the discharge into a new environment, when it can survive in the new environment, if it can establish itself in the new environment, and if it can spread and harm the new environment. The aim of a risk assessment is to reflect the real situation as accurately as possible. However, since the ballast water issue has not been extensively researched, the likelihood of error is high. Hereby the precautionary approach is adopted, with the primary emphasis to avoid an underestimation of the danger that a species can pose when introduced to a new environment. The risk assessment should be based on reliable data

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(i.e. port baseline surveys), which are currently lacking, but need to be conducted before an exemption may be considered.

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## **12. Workshop on counting zooplankton larger than 50µm**

### **Use for shipping companies**

This article gives an insight in what happens with collected ballast water samples at the test facilities. The IMO G8-guideline regards approval of Ballast Water Treatment Systems (BWTs). However, the in G8 formulated guidelines for sampling and measuring viable organisms larger than 50µm during land-based tests of BWTs are relatively undetailed and therefore may be implemented differently among the BWT test facilities. These differences were explored during a workshop held in 2010. The workshop resulted in recommendations to the authorities and to IMO with regard to sampling, measuring and analyzing data and with regard to the comparability of the results of different testing facilities. On top of that, recommendations on quality management and assurance and reporting results are included. If you are interested in the procedures that are followed at the different test facilities, this report would be interesting to read.

### **The article**

**Title: "Report on the workshop on aspects of certification and compliance enforcement purposes: counting zooplankton larger than 50µm"**

**Authors: Frank Fuhr, Isabel M. van der Star, Louis Peperzak (all NIOZ at the time the workshop was held)**

**Date: November 2013**

### **Detailed information**

The aim of the document is to achieve a consistent quality in land-based testing of BWTs around the world. It includes recommendations on the topics that were discussed during the workshop, being sampling and sample handling, sample analysis, test conditions, quality management and assurance and reporting results. The workshop participants stressed the importance of three recommendations in particular as being of interest for authorities and the IMO requirements, because these could change their opinion. First, they suggest to lower the IMO requirement that inlet water should contain 100,000 organisms per cubic meter to a minimum range of 50,000 until 80,000m<sup>-3</sup>. On the other hand, they suggest to increase the required survivorship in the control tank to at least 1,000 but possibly better 10,000 living organisms bigger than 50µm m<sup>-3</sup>. Second, they state that it is not required to take ambient samples at every run. They suggest annually carrying out a facility validation instead. Third, every procedure in the testing facilities should be verified by peer reviewed publications or else be described in such a way that the approval authority can verify it. When room for interpretation is left, this should be clearly mentioned in the reports. In that case, the decision lies with the authorities.

Appendix 1 contains a list of participants to the workshop including their addresses and e-mails.

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### **13. Ballast water workshop 10-50µm**

#### **Use for shipping companies**

The G8 guideline defines ballast water discharge standards (BWDS). A workshop was organised on the enumeration of organisms between 10 and 50µm, related to the BWDS. Currently, different measuring methods are used by the different workshop participants which represented several research institutions. Each of the participants was actively involved in the testing of ballast water management systems. They explored the differences between their methods and opportunities to use comparable protocols for enumerating the 10-50µm organisms.

#### **The article**

Title: **"Ballast water workshop 10 - 50µm: phytoplankton-microzooplankton. Final report."**

Authors: **Nick Welschmeyer, Nicole Bobco, Jules Kuo, Brian Maurer** (Moss Landing Marine Laboratories)

Date: **March 2011**

#### **Detailed information**

In March 2011 a four-day workshop was held at Moss Landing Marine Laboratories, California State University. The document contains a list of all the workshop participants, the agenda of the workshop and a summary of the discussion exchanges that were recorded among participants during the first three workshop days. The subject of the workshop was the enumeration of 10-50µm organisms related to the ballast water discharge standards (BWDS) defined in the IMO G8 guidelines. The participants were each actively involved in the testing of ballast water management systems (BWMS). They shared their experiences in an overarching attempt to evaluate live numeric counts of 10-50µm organisms related to ballast water test procedures. Hands-on demonstrations of counting procedures by flow cytometry and epifluorescence microscopy were performed in the laboratory. They listed the methods of the respective research institutes which varied (slightly) for each institute. Discussions of indirect, non-numeric biochemical methods of viability determination were considered. The group agreed that it is important to take time-integrated samples when testing for 10-50µm organisms. Furthermore, the group critically reviewed the methods for determining live organism concentrations and discussed special treatment of the 'treatment' sample. The important issues of Quality Assurance and Quality Control were discussed, focussing on the consistency of data credibility and quality among facilities. The last topic that is described is the determination of the organism size in which, among others, the standards for taking minimum and maximum dimension were discussed. On the final day a tour was given of the Golden Bear Facility (GBF), a ballast treatment test facility at the California Maritime Academy (CMA) Vallejo, California; GBF is operated by CMA in a joint partnership with scientists from Moss Landing Marine Laboratories.



## Overview of analyses

### 14. Available indicative and in-depth analyses

#### Use for shipping companies

Several detection methods to test for compliance (both indicative and in-depth) with the D-2 standard are available. To check for gross non-compliance a rapid analysis may be done. When there is reason to doubt a vessels compliance to the D-2 standard a more detailed analysis needs to be performed. As per today, there is not a single method available that can identify all organism groups mentioned in the D-2 standard at once. Additionally, testing for colony forming units (cfu) of bacteria needs to be performed by experts in a lab because of the difficulty of identifying cfu and due to safety measures.

#### The article

Title: "**BWO technical outline and requirements for organism detection systems for establishing compliance enforcement**"

Authors: **Stephan Gollasch** (GoConsult), **Peter Paul Stehouwer** (NIOZ) and **Matej David** (Dr. Matej David Consult s.p.).

Date: **December 2012**

#### Detailed information

This report summarizes potential detection technologies, including their 'pros' and 'cons', to prove (non)-compliance with the BWM Convention. Both D-1 and D-2 standards were discussed but the focus of this fact sheet is on testing the compliance with the D-2 standard since the D-1 standard will be phased out over time. Methods used for compliance testing need to be accurate and reliable, practicable (i.e. short time to a result, simple to use, and portable) and cost efficient. The accuracy of the compliance test to detect organisms is hereby of prime importance. For bacterial analysis (i.e. *Vibrio cholerae*, *Escherichia coli* & *Intestinal enterococci*) standard tests exist but for analysing whether viable phyto- and zooplankton are present in ballast water new detection methods need to be developed or existing methods need to be adapted. The report describes detection tools to prove the presence/absence of organisms by DNA, RNA, ATP, Chl a, and measurements of increased oxygen content (i.e. presence of algae). These descriptions are followed by more specific methods available for detecting the three groups as referred to in the D-2 standard (i.e. >50 µm, organisms >10 µm or <50 µm, and the bacteria *V. cholerae*, *E. coli* & *I. enterococci*). The following methods seem to be the most promising approaches to test compliance with the D-2 standard:

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<b>Organism group</b>	<b>Indicative analysis on board</b>	<b>Detailed D-2 compliance test</b>
> 50 µm	Stereomicroscopy (viable organism count), flow cameras	Stereomicroscopy (viable organism count),
>10 µm or <50 µm	PAM/FDA/flow cameras, ATP, flow cytometry (semi-quantitative)	Stains, flow cytometry (viable cell count)
<i>Escherichia coli</i>	Fluorometry (semi-quantitative)	Möller & Schmelz/IDEXX selective bacteria media (colony forming units)
<i>Intestinal enterococci</i>	Fluorometry (semi-quantitative)	Möller & Schmelz/IDEXX selective bacteria media (colony forming units)
<i>Vibrio cholera</i>	Enzyme detection (presence/absence)	Traditional TNBC agar plating with selective media (colony forming units)

## Risk assessment

### 15. The risks of treating Ballast Water

#### Use for shipping companies

From the moment when the Ballast Water Management Convention enters into force roughly 50,000 ships need to be equipped with Ballast Water Treatment Systems (BWTS) to comply with the D-2 standard of the Convention. Among the articles discussed in the report are some that consider different risk assessments (e.g. human and environmental health), available treatment systems, the function of land-based testing, the sustainability of treatment systems, and the procedure for Type Approval of BWTS according to guidelines G8 and G9. Before BWTS gain Type Approval it is important to prove that these systems do not produce products that are harmful to the environment or human health.

#### The article

Title: "**Emerging risks from Ballast Water Treatment**"

Authors: **Barbara Werschkun, Thomas Höfer & Matthias Greiner**. (Federal Institute for Risk Assessment)

Date: **August 2012**

#### Detailed information

This report contains a collection of various articles. As a whole it considers all risks concerning Ballast Water Treatment Systems (BWTS). Before commercialisation a BWTS has to prove that it is safe to use, i.e. that it does not produce harmful, toxic or reactive products that can in some way have an adverse effect on the environment or on human health. Even when systems do not make use of an active substance certain harmful products can come into being. Therefore it is of the utmost importance to make sure (through a risk assessment) that treatment systems do not produce such harmful or toxic products. When a treatment system makes use of an active substance (e.g. chlorine) the Marine Environment Protection Committee of the IMO decides whether it is safe to use or not. After their decision Type Approval can be issued by the applying flag state. In 2012 there were 16 treatment systems that had Type Approval. Many of those Type Approved systems used active substance (e.g. chlorine) to rid the ballast water of (viable) organisms.

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## **16. Biofouling: a means of aquatic species transfer**

### **Use for shipping companies**

Biofouling is defined as the accumulation of aquatic organisms to a surface that is immersed in or exposed to the aquatic environment. It includes both micro- and macro-organisms and both sessile and mobile species. Biofouling of ships is a concern for safety, economic and environmental reasons. Antifoulings that are currently used against biofouling either contain biocides or are biocides-free. The use of organotin compounds, such as TBT (tributyltin), is prohibited due to the damaging effects it had on marine life. Management measures currently in force are the voluntary "Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species".

### **The article**

Title: **"Biofouling: a means of aquatic species transfer"**

Authors: **Anne Bouyssou** (World Maritime University)

Date: **August 2013**

### **Detailed information**

Biofouling has been a little recognized means of species introduction that is induced by the shipping industry compared to ballast water. Congregation of organisms takes place in a five- stage process. Several factors, both operational and environmental, influence this attachment of organisms to a ship's hull. In earlier stage the organisms are still easy to remove. When time passes, they will stick fast and build up. Congregation of marine organisms also takes place inside the ship. Biofouling of ships is a concern for safety, economic, and environmental reasons. Safety can be compromised because biofouling can hamper proper functioning of pipework and associated appliances. Biofouling can reduce a ships' fuel efficiency and can increase maintenance costs, thereby creating an economic impact. Compared to ballast water, biofouling imposes an equivalent or higher risk to transfer species. Another environmental impact of biofouling concerns an increase in CO2 emissions due to frictional resistance when the vessel moves through the water. Reduction of biofouling can take place with antifouling coatings with or without toxic agents. Antifouling no longer contains organotins, because they had a damaging effect on marine life. Instead, copper is often used associated with booster biocides. Since 2011, voluntary "Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species" are adopted by the Marine Environment Protection Committee (MEPC) of the IMO. This represents the first international action addressing ships' biofouling.

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## **17. Comparison and environmental impact of different dechlorination compounds**

### **Use for shipping companies**

During operation of ballast water management systems based on electrolysis Active Substances can be generated which react with seawater and form substances that are called total residual oxidants (TRO). These TROs can cause harm for the marine environment and therefore the TRO concentration in the discharged ballast water should be kept below a concentration of 0.2 mg/L. Lowering the concentration of TROs can be done by adding compounds that can initiate a neutralisation process called dechlorination. This article looks into the environmental impact of five compounds that can be used for the dechlorination process. It studies the effect of changes in the pH, of the end product sulfate and of oxygen depletion on the environment. The effect on the pH is not significant as the ocean has a buffering capacity. The researchers found that the released sulfate does not harm the environment because of the high sulfate concentrations in the seawater. In order to prevent that oxygen levels are altered, it is said that the best dechlorination compound is either sodium thiosulfite or sodium metabisulfite.

### **The article**

**Title: "Comparison and environmental impact of the different dechlorination compounds sulphur dioxide, sodium sulphite, sodium metabisulfite, sodium bisulfite and sodium thiosulfate"**

**Authors: Brigitte Behrends (Marena Ltd.)**

**Date: September 2013**

### **Detailed information**

This article compares the environmental impact of the five different dechlorination agents mentioned in the title. The researchers tested three effects of the agents, being the effect on the pH, on sulfate concentrations and on dissolved oxygen concentrations in the ocean. All five agents lowered the pH of the discharge water. However, this will not change the pH of the ocean significantly as the ocean has a buffering capacity due to its alkaline characteristics. Acidification would thus not be an issue for the agents. Second, sulfate is formed when the neutralisation reaction takes place. The amount that is generated is considered not concerning as the background concentrations of sulfate in the ocean are high. Therefore, no significant environmental impact related to sulfate is expected. Last, the agents influence the amount of dissolved oxygen (DO) in the water as they are reducing agents that consume DO in the water. It is said that sodium thiosulfate can best be used as a neutralising agent, since it consumes less oxygen than other compounds. Considering theoretical calculations would suggest that sodium metabisulfite demands the least oxygen compared to the other agents. However, sodium thiosulfate might be recommended because of side-reactions that may slow down the oxidation process.

## NSBWO project

### 18. Prolongation of the Ballast Water Opportunity Project

#### Use for shipping companies

The extension of the Ballast Water Opportunity (BWO) project has to bring about certain developments, among which: the development of testing methods for both land and shipboard testing of ballast water, the development for compliance and enforcement methods, and the evaluation of errors in ballast water sampling methods.

#### The article

Title: "**BWO Extension to the budget or alternative grant applications**"

Authors: **Stephan Gollasch** (GoConsult), **Hans Flipsen** (Evers + Manders Subsidieadviseurs B.V.) & **Jessica van der Laak** (Evers + Manders Subsidieadviseurs B.V.).

Date: **June 2013**

#### Detailed information

This report summarizes a list of successful new projects of the BWO. The budget of the BWO project has been extended to June 2014 after approval of the BWO Steering Committee in the annual meeting of 2013.

Since the start of the BWO project, more than 20 projects were successfully applied for by many facilities and project partners. These projects addressed ballast water management, ballast water related risk assessments, ballast water sampling, compliance control and enforcement aspects, and alien species in general, and include: NIOZ and IMARES-WUR that are involved in developing a management action plan for alien species in the Wadden Sea (i.e. WIASAP); IMARES-WUR has developed a standard method for testing ballast water discharges for gross non-compliance with the IMO's BWMC; BSH that participates in a project using satellite technology aiming to evaluate the risks associated with ballast water exchange, and gathers information on how to take a representative ballast water sample; WMU is involved in developing a project about alien species control and marine life protection in the Mediterranean Sea; CaTO is involved in supervision of students at the Groningen University that explore what can be learnt from species survival in BWM systems; GoConsult is testing several ballast water sampling methods on board commercial vessels to evaluate which methods deliver representative results, and undertakes a risk assessment regarding ballast water management in intra-Baltic shipping, among others; Cytobuoy is participating as an expert in a couple of projects; and finally Brockmann Consult GmbH is involved in the implementation of a risk assessment tool and a data base for alien species within harbours. The projects just mentioned are only a few within which these facilities and project partners participate.

## 19. Proceedings of NSBWO Europort 2011 conference

### Use for shipping companies

The goal of the conference was to raise awareness amongst the maritime world of the challenges and options posed by Ballast Water Management. Topics touched upon included: US regulations for Ballast Water Management (BWM); the phasing schedule of IMO's Ballast Water Management Convention (BWMC) once it enters into force (see glossary); risk assessment and environmental acceptability of Ballast Water Management Systems according to the guidelines G9 and G8 (IMO). It also informed the audience on present solutions for BWM on-board ships and their installation.

### The article

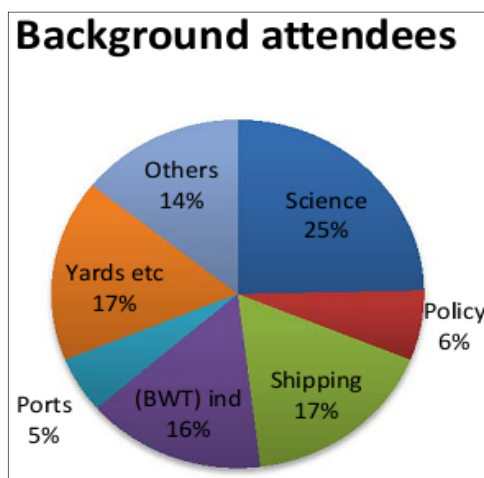
Title: "**Proceedings NSBWO Europort 2011 Conference 'Threat or Treat'**"

Authors: **Cato ten Hallers-Tjabbes** (CaTO Marine Ecosystems) & **Etienne Brutel de la Rivière** (MEA-NL)

Date: **November 2011**

### Detailed information

Ballast water is used for maintaining draught, stability and manoeuvrability of ships. Ballast water contains billions of small organisms (plants, animals, larvae and eggs) that are present in the port of intake. When such organisms are released into the port of destination they can develop into a plague, causing ecological and economical damage. To prevent this from happening the IMO (International Maritime Organization) adopted the Ballast Water Management Convention (2004). The Convention enters into force 12 months after the ratification of 30 states, and when 35% of the global tonnage is represented<sup>3</sup>. One of the topics discussed during the conference was the intent of the US to impose more stringent measures regarding ballast water discharge than the D-2 standard of the IMO. At present BWM systems are capable of achieving the IMO standard of Regulation D-2. The US Coast Guard adopted D-2, with a view of a future more stringent standard. The IMO's guidelines G8 and G9 were highlighted, in particular in view of the environmental acceptability of BWM systems. Guideline G8 acknowledges that a Ballast Water Management System not using active substances (physical treatment, such as UV irradiation) needs to be evaluated for the possibility that harmful end or by products may be formed. To



ensure that harm to the receiving environment is not to be expected, an evaluation of environmental acceptability has been incorporated in G8. Such evaluation is the core of G9. To inform about the options available for ships the different types of BWM systems were highlighted during the conference, followed by information on strategies for mounting systems on ships. The conference was attended by 266 participants, representing the maritime industry (shipping and ports), technology, policy makers, scientists and many others.

<sup>3</sup> At 31 July 2013, the Convention was ratified by 37 States, representing 30,32% of world merchant shipping tonnage.

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## 20. **Biofouling: a means of aquatic species transfer**

### **Use for shipping companies**

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### **The article**

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Authors: **Anne Bouyssou** (World Maritime University)

Date: **August 2013**

### **Detailed information**

Biofouling has been a little recognized means of species introduction that is induced by the shipping industry compared to ballast water. Congregation of organisms takes place in a five- stage process. Several factors, both operational and environmental, influence this attachment of organisms to a ship's hull. In earlier stage the organisms are still easy to remove. When time passes, they will stick fast and build up. Congregation of marine organisms also takes place inside the ship. Biofouling of ships is a concern for safety, economic, and environmental reasons. Safety can be compromised because biofouling can hamper proper functioning of pipework and associated appliances. Biofouling can reduce a ships' fuel efficiency and can increase maintenance costs, thereby creating an economic impact. Compared to ballast water, biofouling imposes an equivalent or higher risk to transfer species. Another environmental impact of biofouling concerns an increase in CO2 emissions due to frictional resistance when the vessel moves through the water. Reduction of biofouling can take place with antifouling coatings with or without toxic agents. Antifouling no longer contains organotins, because they had a damaging effect on marine life. Instead, copper is often used associated with booster biocides. Since 2011, voluntary "Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species" are adopted by the Marine Environment Protection Committee (MEPC) of the IMO. This represents the first international action addressing ships' biofouling.



## 21. Glossary

This glossary contains a summary of all terms that are used in the following fact sheets. Since the D-1 standard of the IMO (exchange of ballast water) will be phased out in the near future the focus of all fact sheets will be on compliance with the D-2 standard (treatment of ballast water). To check whether a vessel is in compliance with the D-2 standard two analyses can be used: an indicative analysis, and an in-depth analysis. On the next page you may find the D-2 discharge standards of IMO and a timetable that describes when certain vessels need to comply with the D-2 standard once the Ballast Water Management Convention is entered into force.

- The **Ballast Water Management Convention (BWMC)** came into being in 2004 to reduce the rapidly growing problem of unwanted effects of global shipping. It formulates two ballast water performance standards: the D-1 and D-2 standard are described below
- **Colony forming unit (cfu)** is an estimate of the viable bacterial cells
- The **D-1 standard** concerns the exchange of ballast water in mid sea to reduce the amount of non-native species in ballast water during ballast water discharge
- The **D-2 standard** concerns the treatment of ballast water before it is discharged. For the discharge standards see table 1 on the next page
- **Fluorometer** is a method used to count viability and biomass of cells that are present in a water sample
- **G7:** Guidelines for the risk assessment of ballast water. When a ship transports ballast water between ports with comparable environments it may be exempted from the BWMC. Before a vessel qualifies for exemption it should prove that its discharge of ballast water does nothing to harm the environment of the receiving port. This needs to be determined by a risk assessment
- **G8:** Guidelines for the approval of Ballast Water Management Systems
- **G9:** Guidelines for the approval of Ballast Water Management Systems that make use of active substances
- An **in-depth analysis** is used when the indicative test results in doubts whether or not IMO's D-2 standard was met. This analysis provides more detailed information on the (non-) compliance of the vessel. This type of test is time consuming (> 8 hours to several days) and needs to be performed by experts in a laboratory. The analysis is carried out with the highest possible accuracy
- An **indicative analysis** is a rapid analysis of the ballast water that can be used on board to confirm gross non-compliance with the D2-standard. It delivers results in a matter of minutes, but with a certain level of inaccuracy (it is a 'quick and dirty' test). To perform this test no extensive training is needed, i.e. anyone can interpret the results
- **Indicator microbes** are the bacteria mentioned in the D2-standard: *Vibrio cholerae*, *Escherichia coli*, and *Intestinal enterococci*
- **Phytoplankton:** small algae present in sea water (most in the range of 10-50 µm)
- **Ship board testing** is an indicative analysis (see above)
- **Zooplankton:** small animals present in sea water (most larger than 50 µm)

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**Table 1: D-2 discharge standards of the IMO considering VIABLE cells. Source: Discharge standards Lloyd's register – BWT technologies, SEP 2012**

Organism category	Regulation
Plankton, >50 µm in minimum dimension	< 10 cells / m <sup>3</sup>
Plankton, 10-50 µm	< 10 cells / ml
Toxicogenic Vibrio cholera (O1 and O139)	< 1 cfu* / 100 ml or less than 1cfu /g (wet weight)
Escherichia coli	< 250 cfu* / 100 ml
Intestinal Enterococci	< 100 cfu* / 100 ml

Table 1: IMO 'D2' standards for discharged ballast water

\* colony forming unit

**Table 2: Timetable for Ballast Water Treatment in the Convention for the Control and Management of Ships Ballast Water & Sediments from the IMO after the MEPC 65 meeting. Source: Lloyd's Register 2013 – IMO MEPC 65 Summary**

**Reschedule for ships constructed before the entry into force (EIF)\* of the Convention**

Ballast capacity	Constructed before 2009	Constructed in or after 2009 but before 2012	Constructed in or after 2012
Less than 1500m <sup>3</sup>	EIF before 2016: by 1 <sup>st</sup> IOPP** renewal survey after the anniversary of the delivery of the ship in 2016 EIF after 2016: by 1 <sup>st</sup> IOPP renewal survey	By 1 <sup>st</sup> IOPP renewal survey after EIF	
Between 1500m <sup>3</sup> and 5000m <sup>3</sup>	EIF before 2014: by 1 <sup>st</sup> IOPP renewal survey after the anniversary of the delivery of the ship in 2014 EIF after 2014: by 1 <sup>st</sup> IOPP renewal survey		
Greater than 5000m <sup>3</sup>	EIF before 2016: by 1 <sup>st</sup> IOPP renewal survey after the anniversary of the delivery of the ship in 2016 EIF after 2016: by 1 <sup>st</sup> IOPP renewal survey	By 1 <sup>st</sup> IOPP renewal survey after EIF	

- \* "EIF" means entry into force of the BWM Convention. This occurs 12 months after the date when condition for entry into force is met by sufficient number and tonnage of ratifications
- \*\* The IOPP renewal survey indicated in the table below refers to the renewal survey associated with the IOPP Certificate required under MARPOL Annex I

Ships constructed after the entry into force of the Convention are required to comply on delivery.



NORTH SEA BALLAST WATER

European Union  The European Regional Development Fund

**The Interreg IVB  
North Sea Region  
Programme**



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

