ON BOARD TESTS OF THE ORGANISM DETECTION TOOLS BALLASTCAM, FLUIDIMAGING, USA, HACH-PAM-FLUOROMETER, USA, AND WALZ-WATER-PAM-FLUOROMETER

RESULTS AND FINDINGS

Stephan Gollasch
GoConsult
Grosse Brunnenstrasse 61
22763 Hamburg
Germany

Matej David
Dr. Matej David Consult s.p.
Korte 13e
6310 Izola
Slovenia

Reporting Period 7, March 2012 to August 2012
18-12-2012

This report should be quoted as follows
Gollasch S, David M, 2012. On board tests of the organism detection tools BallastCAM, FluidImaging, USA, Hach-PAM-fluorometer, USA, and Walz-Water-PAM-fluorometer. Results and findings. Prepared for Interreg IVB North Sea Ballast Water Opportunity project: 10 pp.

TABLE OF CONTENTS

1 BACKGROUND

In Ballast Water Opportunity (BWO) one of the duties in WP4 are to check the applicability of potential organism detection technologies onboard of commercial vessels. A screening of technologies was completed and a comprehensive report was submitted in an earlier reporting period (Gollasch & Stehouwer 2012) which benefitted from the authors work in another study (Gollasch & David 2010). This report is an additional contribution of BWO Deliverables

- D4-3 Identification of additional techniques for Compliance Enforcement and Monitoring based on outcome WP2 and
- D4-14 Technology assessment for compliance enforcement the relation between the BWMC policies, enforceability and technology.

One of the challenges when biologically analyzing ballast water samples, e.g. for compliance control purposes with the D-2 standard, is the prompt and accurate enumeration of viable organisms per size class found in the samples.

From the candidate technologies listed in Gollasch, Stehouwer & David (2012), three methods were selected for a closer consideration to analyse phytoplankton organisms in the size range below 50 and above 10 micron in minimum dimension, i.e. for their applicability on board:

1. the BallastCAM of FluidImaging, USA,
2. the Hach-PAM[1]-fluorometer (AquaPen Version 1.2.1.1), USA, and
3. the Walz-Water-PAM-fluorometer.

FluidImaging developed the BallastCAM as an instrument to analyse ballast water samples with a focus on phytoplankton organism less than 50 micrometres and greater than or equal to 10 micrometres in minimum dimension. Its suitability for zooplankton organisms in this size class or above 50 micron in minimum dimension needs to be tested. However, the system was never used and tested on board of a commercial vessel, but this is likely the environment such analytical tools need to be operated in should in the future port state control officers plan to check on the spot if the ballast water is in compliance with the D-2 standard. It should be noted that the system does not distinguish between viable and dead particles, but it delivers particle counts and photos of each particle counted so that a later analysis may reveal numbers of viable organisms, although the accuracy level of such an approach is questionable. To familiarize the authors with the use of the BallastCAM, Kevin Stewart of FluidImaging met the authors and demonstrated the use of the BallastCAM in a hotel room in New York. For this purpose an ocean water sample containing a natural plankton assemblage was used.

The Hach-PAM-fluorometer, which was provided by Bio-UV (see below), was developed as test kit for compliance control with the D-2 standard and it works in a similar way as the Walz-Water-PAM-fluorometer (Gollasch et al, 2012). The Hach system tested here is a pre-prototype unit which will be further modified to better fit for purpose. This especially refers to increase its sensitivity to detect lower algal cell concentrations (Harbridge (Hach) pers. comm.).

[1] PAM = Pulse Amplitude Modulated (fluorometry)

Both systems deliver a bulk measurement of viable algae, i.e. no organism count. However, the stronger the measured viability signal (for the Walz-Water-PAM-fluorometer in combination with a biomass measurement), the higher seems to be the number of viable cells in the sample.

1.1 ONBOARD USE OF THE SYSTEMS

The voyage during which all three systems were tested was undertaken on the container vessel MV MARFRET SORMIOU between New York, USA and Monzanillo, Panama in July/August 2012. This voyage was undertaken to challenge the Bio-UV (France) ballast water treatment system´s performance as a consultancy work of GoConsult. Ballast water was taken up in the Port of Kingston, Jamaica and later discharged in the Monzanillo port, Panama.

After all sampling and sample processing to document the performance of the Bio-UV ballast water treatment system was completed remaining samples were analyzed with the FuidImaging BallastCAM (Figure 1) and a Hach-PAM-fluorometer. The measurements of the Walz-Water-PAM-fluorometer were measured as part of the standard sample processing and was considered here for comparison.

In addition a second voyage was undertaken in October 2012 with ballast water uptake in Rotterdam, the Netherlands. Here the Hach and Walz Water PAM were used to analyse the samples.

On both voyages, untreated ballast water pumped onboard as well as treated ballast water, when discharged from the vessel, was analyzed.

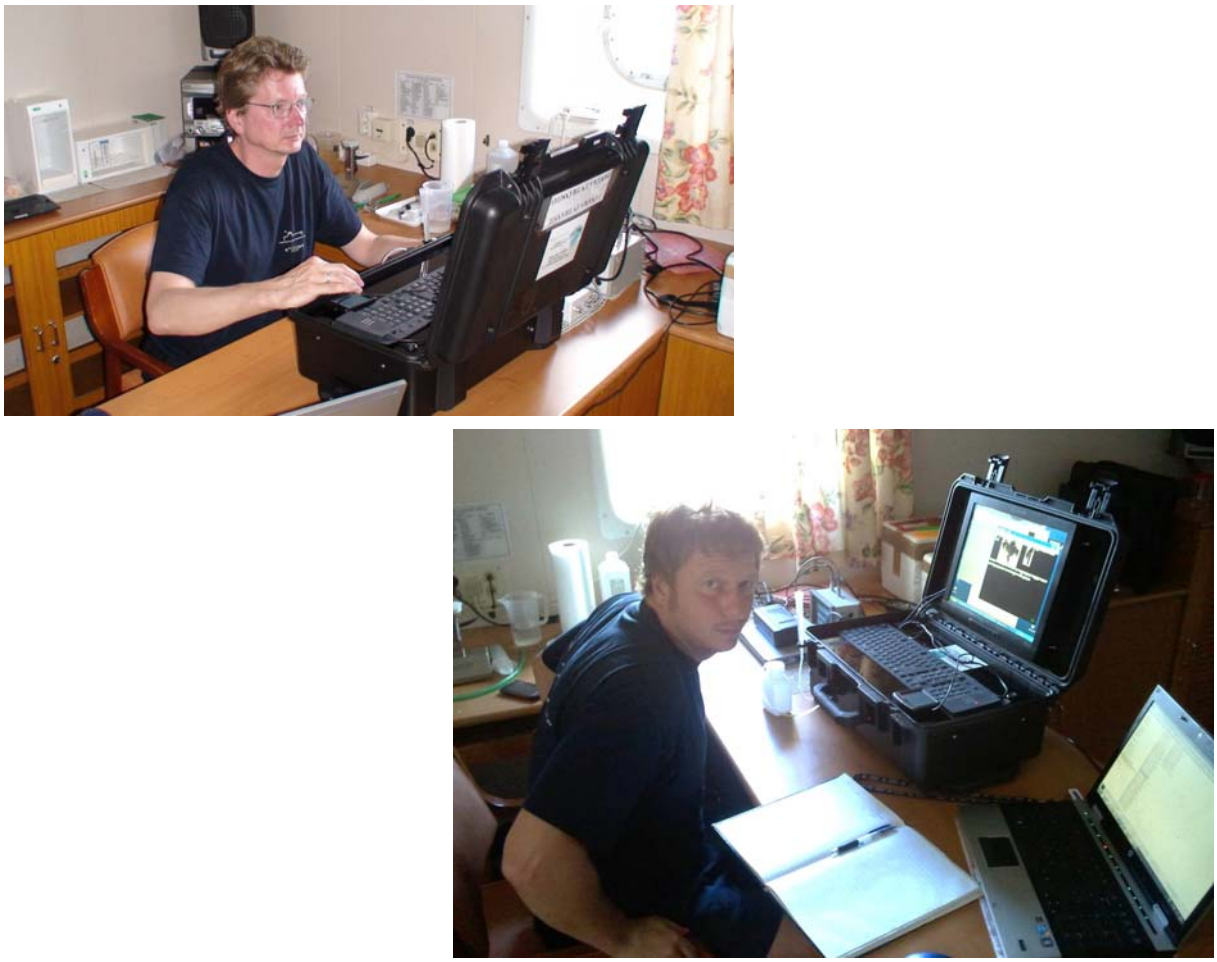

Figure 1: The BallastCAM of FluidImaging in on board use by Stephan Gollasch (top) and Matej David (bottom).

2 FINDINGS AND RECOMMENDATIONS

2.1 BALLASTCAM

The FluidImaging BallastCAM is a compact instrument with a moderate weight of ca. 20 kg. Due to this design it seem feasible that port state control officers, when planning to undertake ballast water requirements compliance checks, may bring such an instrument on board a vessel to analyze for organism numbers on the spot, i.e. during the onboard sampling event.

The BallastCAM is easy to use is and is designed to be operated by a “non-biologist” so that port state control officers can use it after a short training session. However, we noted that the keyboard is not easy to use and the software does not allow the copy/paste function for names when saving files or creating folders. Further, it is difficult to replace the flow cell because this is obstructed and not at all conveniently available. A flow cell replacement may be needed in cases when particles become stuck in the observation field of view, which happened during our test. Possibly a rubber layer on the outer screw to fix the flow cell could be helpful not to damage the cell by tightening the screw too much. The size dimension of this flow cell (200 microns) further limits its use for organisms in size ranges > 200 microns.

The rapid sample processing time would promptly enable port state mitigation measures should the ballast water samples indicate non-compliance with the D-2 standard. However, although the BallastCAM rapidly counts objects and their sizes in a water sample, a weakness of the BallastCAM is that, in its current design, it cannot distinguish between living and dead organisms/particles, which is a key feature to proof compliance with the D-2 standard. The use of stains to overcome this situation may be investigated.

To proof that the D-2 standard was met, in addition to the viability of organisms, their size needs to be documented in minimum dimension. During a zooplankton workshop (organisms above 50 micron in minimum dimension), held at NIOZ, Texel, The Netherlands in Fall 2010, as an event of the Interreg IVB Ballast Water Opportunity project, it was agreed that for the visual measurement of the minimum dimension the smallest visible axis of an organism should be chosen and the widest point on this axis be measured (see examples in Figure 2). If organisms are near the 50 um minimum dimension, efforts should be made to measure the third (less visible) dimension.

For organisms in the smaller size class of the D-2 standard, targeted to be analyzed by the BallastCAM, the same principle to measure the minimum dimension as outlined above should apply. The BallastCAM has several features to analyze the size of the objects identified, but it remains unclear how close these measurements get to identify the true minimum dimension. Tests may be undertaken by using organisms of different shapes to identify how close the BallastCAM measurements are to identify the minimum dimension in comparison to a visual observation by an expert.

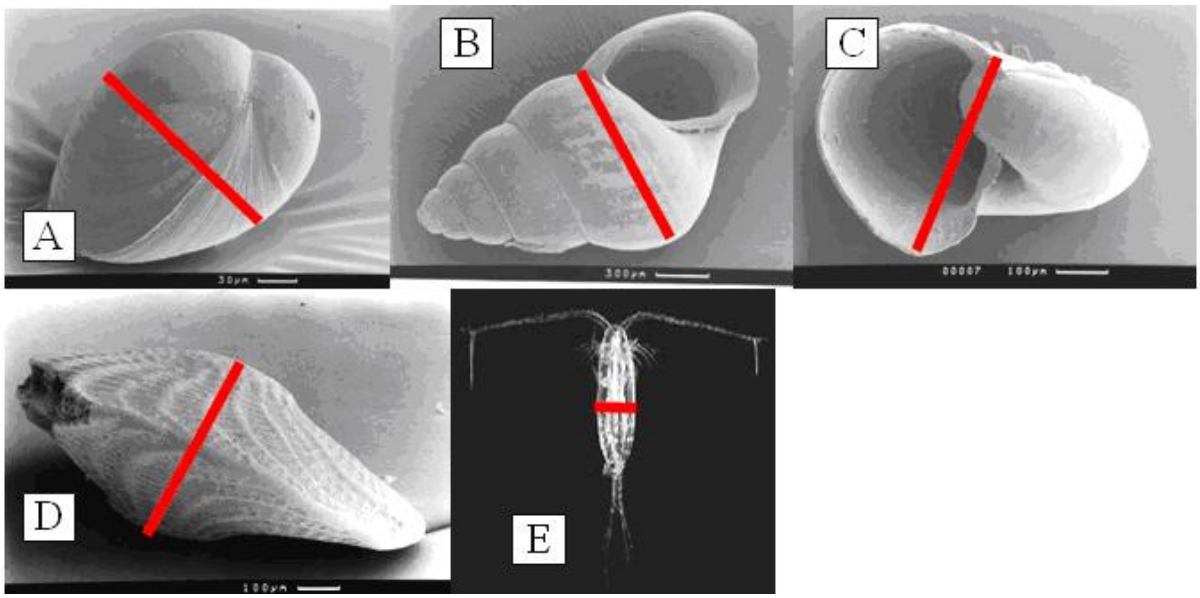

Figure 2: Minimum dimension measurements (red line) for selected organism types: A = mussel larvae, B and C = gastropod larvae, D = foraminifera (phytoplankton) and E = copepod. (Photos A - D: Stephan Gollasch, H: www.wikipedia.org).

2.2 ACTIVE FLUOROMETERS

The Hach-PAM active fluorometer is of very compact design (pocket size). It is battery-powered and therefore it can independently be used also at any sampling point. The simple use allows a non-biologist to work out the samples and the results are available promptly.

Figure 3: The Hach-PAM active fluorometer operated by Stephan Gollasch.

The Walz-Water-PAM-fluorometer is also of compact design, but it is bigger than pocket size and a laptop computer is recommended to operate the system, hence it should be operated on a desk. The use is simple so that a non-biologist can work out the samples and the results are available promptly. In addition to the viability measurement, the biomass is measured and this gives additional information regarding the concentration of cells in the sample.

A comparison of the performance of the two active fluorometers used is only enabled on a very limited basis due the low number of samples analysed. However, when comparing the viability measurements of the HACH and Walz instruments, in the first set of samples the HACH instrument measured lower values compared to the Walz instrument. In the second set of samples the reverse situation was observed. In the last test run the measured Fv/Fm values showed the highest difference (Hach 0,343 and Walz 0,466) (Figure 3). In conclusion, both instruments show the same result pattern, but are different in sensitivity and accuracy, especially in low value measurements.

It is recommended to repeat such comparative studies with a larger number of samples possibly also containing different algae and sediment concentrations.

Figure 4: The Walz-Water-PAM-fluorometer operated by Matej David.

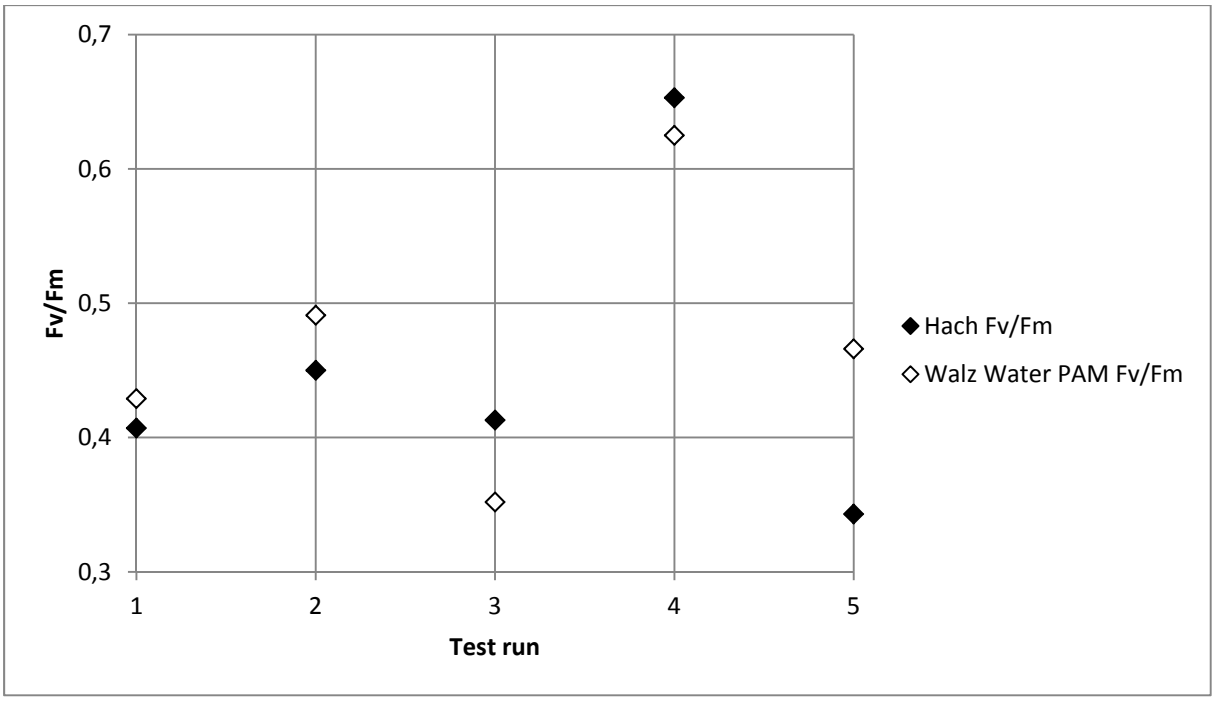

Figure 5: Comparison of algae viability measurements (Fv/Fm) of the Hach and Walz Water PAM.

The HACH instrument cannot be adjusted to enable measurements with very low or very high load of algae and it is unclear in which range of algal concentrations the instrument can be operated. We observed in measurements of treated water at discharge that the instrument reads “low value”. In contrast the Walz instrument can be adjusted by manipulating the gain according to the measurements, so that also algae in very high and very low concentrations can be measured.

In Figure 4 results of performance comparison experiments of the Hach and Walz Water PAM are shown. A very good correlation between the two tools is documented at higher concentrations of organisms (Harbridge pers. comm.).

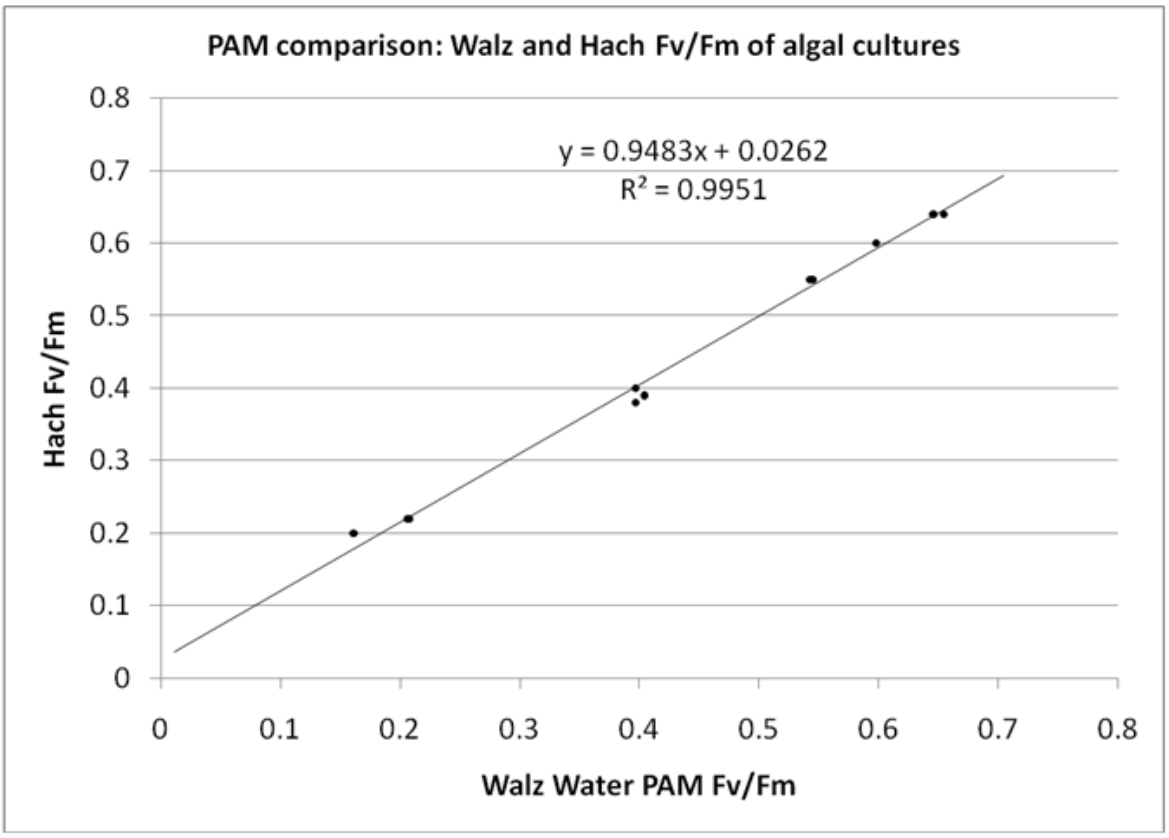

Figure 4: Comparison of algae viability measurements (Fv/Fm) of the Hach and Walz Water PAM made by N. Welschmeyer (Moss Landing Marine Laboratories). Measurements were made on 9 species of algal cultures reared to different levels of nutrient stress to create a wide range of Fv/Fm values.

2.3 INDICATIVE VS. IN-DEPTH SAMPLE ANALYSIS

According to the IMO BWM Convention two sample processing approaches are available. One is an indicative check of a sample, which likely delivers results promptly, but with a certain level of in-accuracy. In contrast a detailed sample analysis may be carried out with the highest possible accuracy.

The currently constructed BallastCAM cannot analyze the viability of objects, and therefore a detailed compliance control check with the D-2 standard is not enabled. The indicative sample analysis also needs to show the presence/absenceof viable organisms in a ballast water sample which is not possible with the current BallastCAM version. However, all objects identified by the BallastCAM are individually photographed by a color camera. For an indicative check of the ballast water, the objects photographed may be analyzed and those being undamaged and also containing chlorophyll may be assumed as viable. The filters included in the BallastCAM operation software enable sorting of objects by color so that all green, i.e. chlorophyll containing objects, can be counted per water volume. This result may be taken as an indicative compliance check. The errors of such a method need to be carefully evaluated.

The active fluorometers tested here measure the chlorophyll content in living cells by triggering the phytoplankton electron chain to respond. Such a response only exists in living cells thereby assessing photosynthesis activity by utilizing the relationship of chlorophyll fluorescence and photosynthesis to describe cell ‘health’. These instruments enable semi-quantitative cell density estimations with a greater level of precision compared to standard fluorometers because responding cells are measured and not only the chlorophyll content.

Active fluorometers may have a similar ability as the BallastCAM for compliance checks. The weakness of the active fluorometers is that they measure viability but do not deliver counts of cells, whereas the BallastCAM can deliver counts, but it cannot measure viability. However, the active fluorometers deliver additional information which seems essential for compliance checks, e.g. biomass, and some can also measure very low cell concentrations. It seems reasonable to investigate the possible combination of both instruments (active fluorometers and flow cameras) to enable counts of viable objects.

In cases where chlorophyll containing algal cells can be identified by the BallastCAM in the sample or the active fluorometers do identify viability, it may indicatively be assumed that living algae are present in the sample so that the ballast water treatment process was unsuccessful and the D-2 standard was met. If a high biomass and a strong response of viable organisms is identified when measuring with active fluorometers, this indicates gross exceedence of the D-2 standard. Similarly, the gross exceedence could also be documented if the BallastCAM would identify a high number of chlorophyll containing objects.

ACKNOWLEDGEMENTS

We gratefully acknowledge the permission of the ballast water treatment systems vendor Bio-UV, France, allowing us to operate the BallastCAM during the onboard performance tests of their ballast water treatment system and especially Ronan Cadet who enabled the on board sample taking. We further thank the crew of the test vessel MV MARFRET SORMIOU for their support during the ballast water operations. This work was partly undertaken to support the Interreg IVB Ballast Water Opportunity project where Gollasch is responsible for organism detection tools in ballast water.

REFERENCES

Gollasch S, David M 2010. Testing Sample Representativeness of a Ballast Water Discharge and developing methods for Indicative Analysis. Tender N° EMSA/NEG/09/2010. Final Report prepared for European Maritime Safety Agency, Lisbon, Portugal. 124 pp.

Gollasch S, Stehouwer PP, David M 2012. BWO Technical Outline and Requirements for Organism Detection Systems for Establishing Compliance Enforcement. Final Report. Prepared for Interreg IVB Project “Ballast Water Opportunity”. Submitted with 6th project reporting period. 88 pp.