Ministry of Environment of Denmark
Environmental Protection Agency

BallastWISE Inline Automated monitoring of microorganisms in ballast water

MUDP Report

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Miljøteknologisk Udviklings- og Demonstrations-program

Projektet, som er beskrevet i denne rapport, er støttet af Miljøteknologisk Udviklings- og Demonstrationsprogram (MUDP) under Miljøministeriet, der støtter udvikling, test og demonstration af miljøteknologi.

MUDP investerer i udvikling af fremtidens miljøteknologi til gavn for klima og miljø i Danmark og globalt, samtidig med at dansk vækst og beskæftigelse styrkes. Programmet understøtter dels den bredere miljødagsorden, herunder rent vand, ren luft og sikker kemi, men understøtter også regeringens målsætninger inden for klima, biodiversitet og cirkulær økonomi.

Det er MUDP's bestyrelse, som beslutter, hvilke projekter der skal modtage tilskud. Bestyrelsen betjenes af MUDP-sekretariatet i Miljøstyrelsen.

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1. Introduction

The International Convention for the Control and Management of Ships' Ballast Water entered into force in September 2017, and by September 2024 all ships must conform to the D2 standard, which specifies the maximum number of viable organisms allowed to be discharged. Non-compliance with the D2 standard can be costly and may potentially be harmful to the environment through the release of non-native invasive species into the marine environment. Furthermore, non-compliance with the D2 standard may cause delays if detected by port state authorities.

In order to ensure compliance with the D2 standard and proper function of the ballast water management system, monitoring of the ballast water discharge is essential. An inline system that automatically monitors live organisms in ballast water discharge would be a useful tool for shipping companies, shipowners and port state authorities.

MicroWISE has previously developed a portable system for automated analysis of live organisms in ballast water (BallastWISE portable). In this project a built-in system for automatic measurement of the number of live organisms between 10-50 μm and ≥50 μm according to the D2 standard was developed, BallastWISE Inline is based on the BallastWISE patented measurement technology (se Appendix 2).

Development of the BallastWISE analysis system for quantification of organisms in ballast water samples has been supported by the Danish Maritime fund (2015, 2017), the Danish Environmental Protection Agency (2017), the Danish Innovation Fund (2019), and the Danish Market Development Fund (2018).

2. Summary

The International Convention for the Control and Management of Ships' Ballast Water entered into force in September 2017, and by September 2024 all ships must conform to the D2 standard, which specifies the maximum number of viable organisms allowed to be discharged.

To ensure compliance with the D2 standard and proper function of the ballast water management system, monitoring of the ballast water discharge is essential. In this project a built-in system for automatic measurement of the number of live organisms between 10-50 µm and ≥50 µm according to the D2 standard was developed, BallastWISE Inline. BallastWISE Inline is based on the unique BallastWISE measurement technology. The system is fully automated and does not require the addition of chemicals or manual intervention by the crew during normal operation.

3. Opsummering af projekt

Den internationale ballastvandkonvention trådte i kraft i september 2017, og senest i september 2024 skal alle skibe overholde D2-standarden, som angiver det maksimale antal levedygtige organismer, der må udledes.

For at sikre overholdelse af D2-standarden og korrekt funktion af ballastvandrensesystemer er overvågning af ballastvandsudledningen afgørende. I dette projekt er der udviklet et indbygget system til automatisk måling af antallet af levende organismer mellem 10-50 µm og ≥50µm i henhold til IMO's D2-standard, BallastWISE Inline. BallastWISE Inline er baseret på den patenterede BallastWISE måleteknologi. Systemet er fuldautomatisk og kræver ikke tilsætning af kemikalier eller manuel indgriben fra besætningen under normal drift.

4. Development of BallastWISE Inline

4.1 The IMO Ballastwater convention

Aquatic invasive species present a major threat to marine ecosystems, and ballast water has been identified as a major pathway for the introduction of invasive species to new environments. According to IMO estimates, ships carry between 3 to 5 billion tons of ballast water around the globe each year.

In order to prevent the spread of invasive species in aquatic environments the "The International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004" (Ballast Water Management Convention) was adopted in 2004. The Convention entered into force in September 2017, and by September 2024 all ships must conform to the D2 standard, which specifies the maximum number of viable organisms allowed to be discharged (Table 1).

The US Coast Guard has adopted the USCG treatment discharge standard, which is the same as the IMO BWM Convention D-2 Standard.

TABLE 1. Ballast water performance standard (Regulation D-2).

Organisms	Discharge limits
Viable organisms, ≥10 - <50 µm (mainly phytoplankton)	<10 cells/ml
Viable organisms, ≥50 µm (mainly zooplankton)	<10 organisms/m^3
Indicator microbes:	
Toxicogenic *Vibrio cholera*	<1 colony forming unit (cfu)/100ml
Escherichia coli	<250 cfu/100ml
Intestinal Enterococci	<100cfu/100ml

Compliance with the D2 standard can only be ensured by frequent monitoring of the ballast water discharge. A built-in system for automatic measurement of the number of live organisms between 10-50 µm and ≥50 µm according to the D2 standard (BallastWISE Inline) was developed to enable automatic monitoring of these organisms, and to ensure proper function of the ballast water management system.

Exceedances of the D2 standard have mainly been observed for organisms ≥50 µm (zooplankton) in the "Experience building phase" (MEPC 78/4/1), which makes this size fraction especially important to monitor.

BallastWISE Inline automatically generates test reports showing average number of live organisms per volume. The report can be sent to the Port State Control (PSC) thus facilitating the work of the PSC. For PSC testing of ballast water poses a challenge, therefore automatic control is a significant improvement

4.2 Development of the BallastWISE Inline hardware components

The BallastWISE Inline is a complex system consisting of hardware components and software, which are equally important, and were developed in parallel. The prototype system is shown in FIGURE 1.

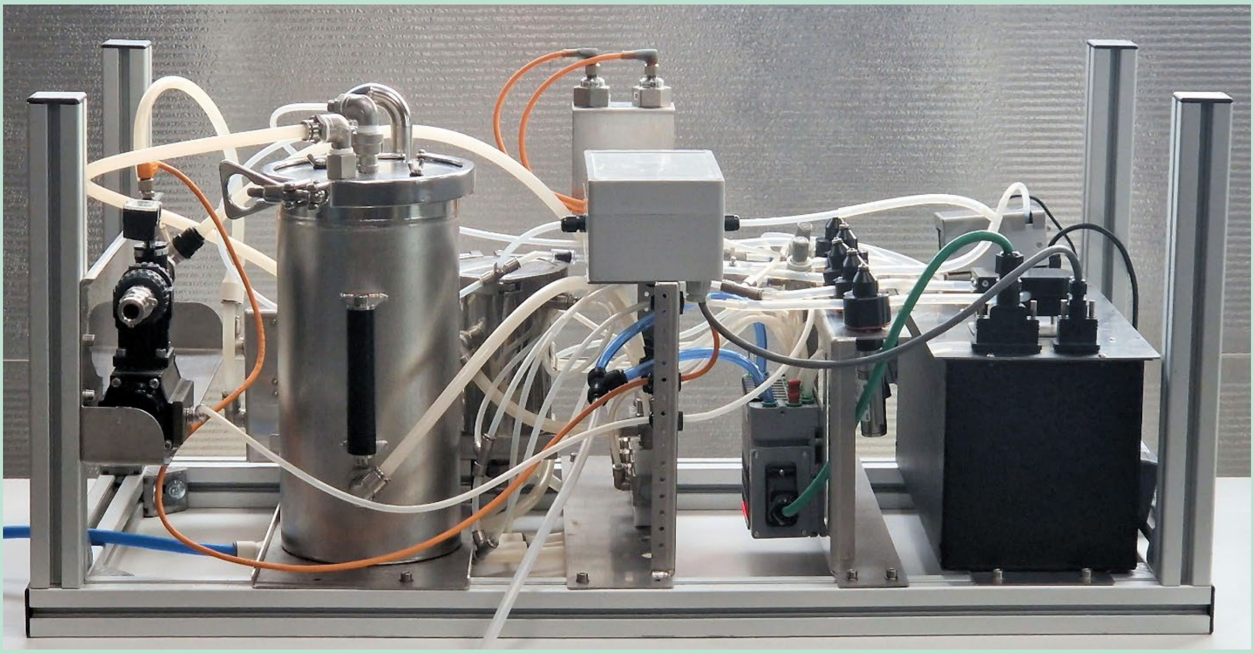

FIGURE 1. BallastwiSE Inline prototype.

4.2.1 Testbench for prototype testing and development of the inline filter unit

The testbench was used to test the preliminary prototype, optimization of flow rates, and identification of the need for a filter component. The test bench was used extensively during the filter design.

The testbench consisted of a 1000 L tank on a trailer (FIGURE 2), which was filled from the top via a pump at a rate of 40 L per minute. The pump was powered via an inverter and 12V battery. A compressor was set up to supply pressure simulating the pressure from a ship's ballast water tank. It was thus possible to experiment with different pressure levels and water flow rates. Flow was measured electronically, and the test bench was used optimize the filter design and test different flow and pressure regimes.

FIGURE 2. The testbench during a sampling event.

4.2.2 BallastWISE Inline hardware components

The BallastWISE Inline overview (Fig. 3) shows the inlet of ballast water from the ship, water flow, sensors, and pressure valves. The system is pressure-controlled, and water flow is regulated using pressure-controlled valves.

The principal components are:
- Valve controlled water inflow
- Tank for collection of water samples for the 10-50 µm fraction
- Filtering unit for collection of water samples for the ≥50 µm fraction
- Measuring unit for quantification of organisms between 10-50 µm and ≥50 µm
- Outflow to bilge or back into ballast water discharge

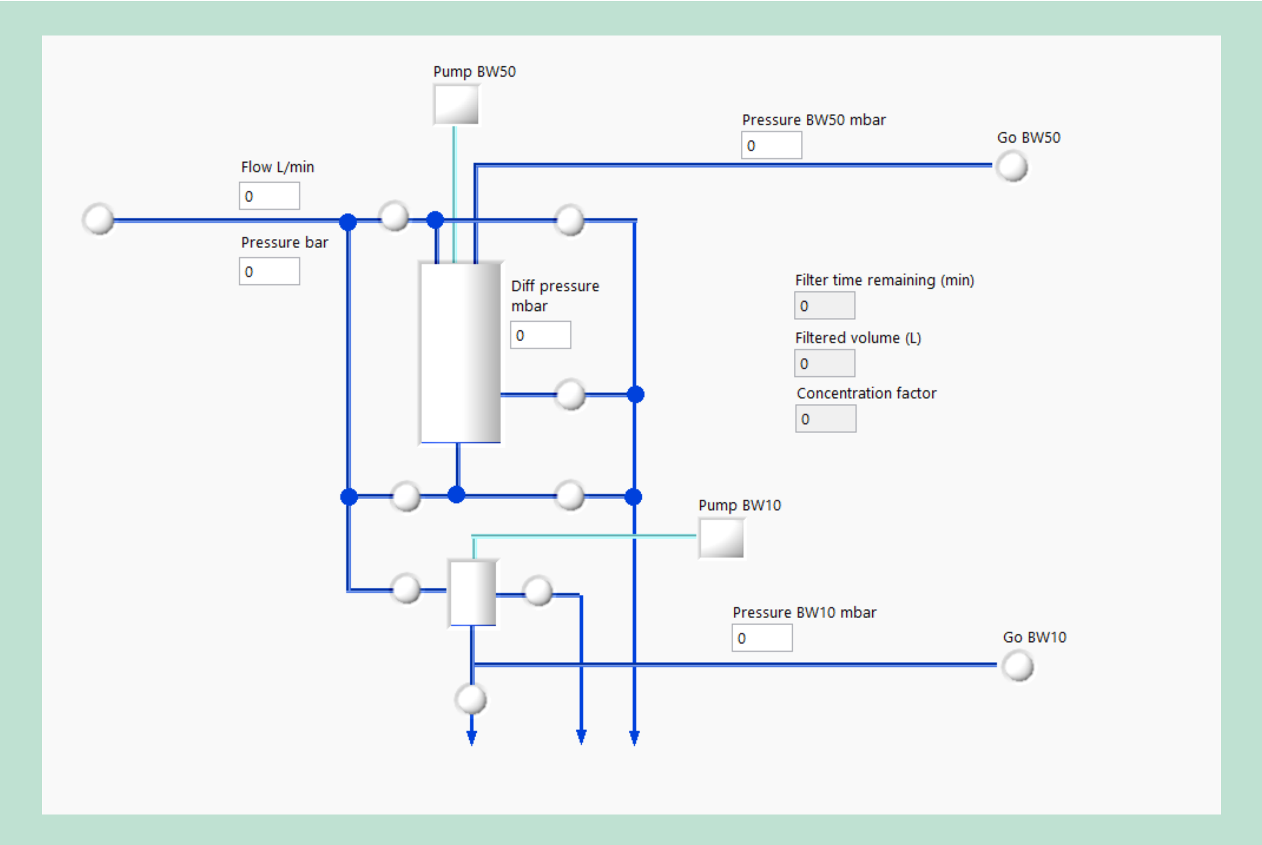

FIGURE 3. BallastWISE Inline overview: water flow (blue lines), pressure valves (round white symbols), the filter unit, pressure tank, pressure pumps and flow sensors are also shown.

4.2.2.1 Water in- and outflow

The system will be supplied with water from the ballast water system, when installed on a ship. Sampling will be from an isokinetic sampling point (EMSA 2019). The water inflow can be completely shut off by a high-pressure ball valve, which is normally closed unless activated (Fig. 4). The flow rate from the sampling point can be adjusted if need be via an external membrane valve to be within 6-10 L/min. The flow rate is measured via a vortex flow metre (Fig. 4). Outflow can be collected and pumped away.

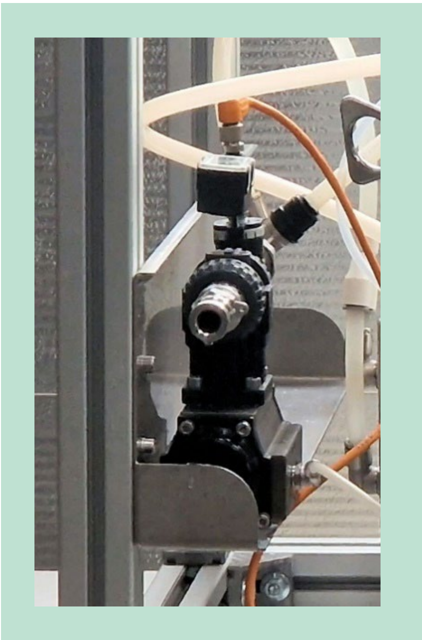

FIGURE 4. Water inflow valve with electronic flow metre.

4.2.2.2 Sampling of water for the 10-50 µm fraction

The samples for analysis of the 10-50 µm organisms size fraction are collected in a designated tank (Fig. 5). The water is sampled directly from the inflow pipe. The tank can be subsequently pressurised to create a flow into the optical measuring chamber. The pressure level is monitored by a pressure sensor and the pressure is programmable in software.

FIGURE 5. Sample container for the 10-50 µm size fraction, volume 1 L.

4.2.2.3 The BallastWISE filtering unit

The maximum number of viable organisms ≥50 µm in ballast water discharge must be less than 10 viable organisms per cubic metre. To be able to detect a low number of organisms per cubic metre, the samples need to be concentrated using a plankton filter that retains organisms ≥50 µm.

The BallastWISE Filter unit was designed to concentrate organisms ≥50 µm, using a plankton net. The filter unit was designed in collaboration with KC Denmark (Fig. 6).

The filter is placed within a cylinder, and water is led through the inflow valve into the Nylon filter (35 µm mesh size), where zooplankton organisms are concentrated.

After the filtration process, the concentrated sample is fed into the optical measuring chamber in the same way as with the small fraction by applying pressure to the filter housing. After each filtration the filter is automatically rinsed through a backflush cycle

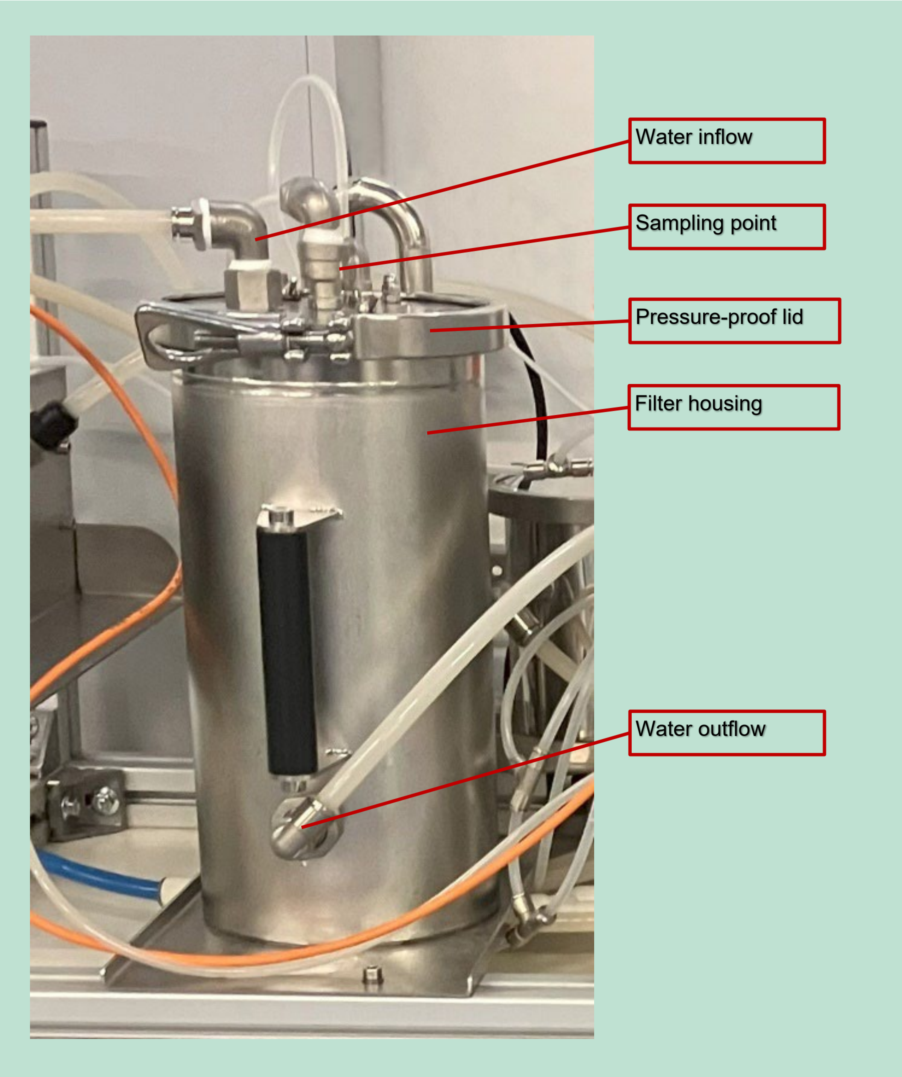

FIGURE 6. The BallastWISE filter unit.

4.2.2.4 The BallastWISE measuring unit

The method for quantification of living organisms has been rewarded patents in six countries/regions (Appendix 1).

The BallastWISE Inline measuring unit is similar to BallastWISE portable, with a few modifications, which enable the inline functionality.

The pumps in the portable system, which are used for filling and emptying the measuring chambers, have been replaced with pinch valves and a pressure system, which allows automation of the chamber filling and emptying with much less wear on the tubing and with less exposure to electronics, which can be difficult to get type approved for dangerous environments.

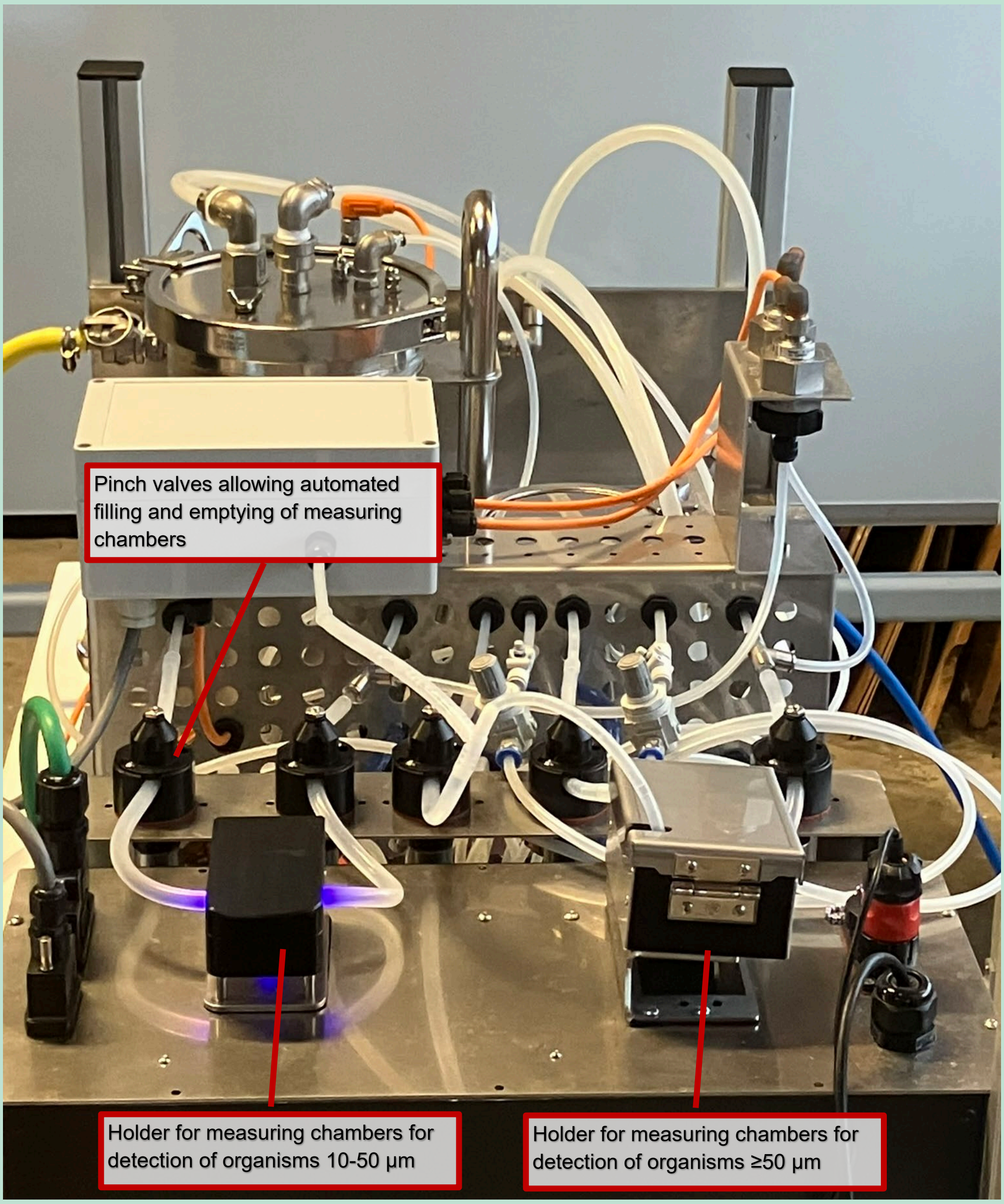

FIGURE 7. The BallastWISE Inline measuring unit with chamber holder for detection of organisms ≥50 µm and between 10-50 µm. Pinch valves for automatic filling and emptying the measuring chambers are mounted on a holder, visible behind the measuring unit.

4.2.2.5 Electronics and pressure control

A pneumatic valve manifold is used to turn pressure on and off to open and close pneumatic valves. 16 pneumatic valves control the flow in and out of the sampling vessels and in and out of the optical measuring chambers. Each port in the manifold is connected to an electronic relay and controlled by software via a microprocessor. Input signals to the control software consist of the flow metre, and a pressure sensor for each sampling vessel (Fig. 8).

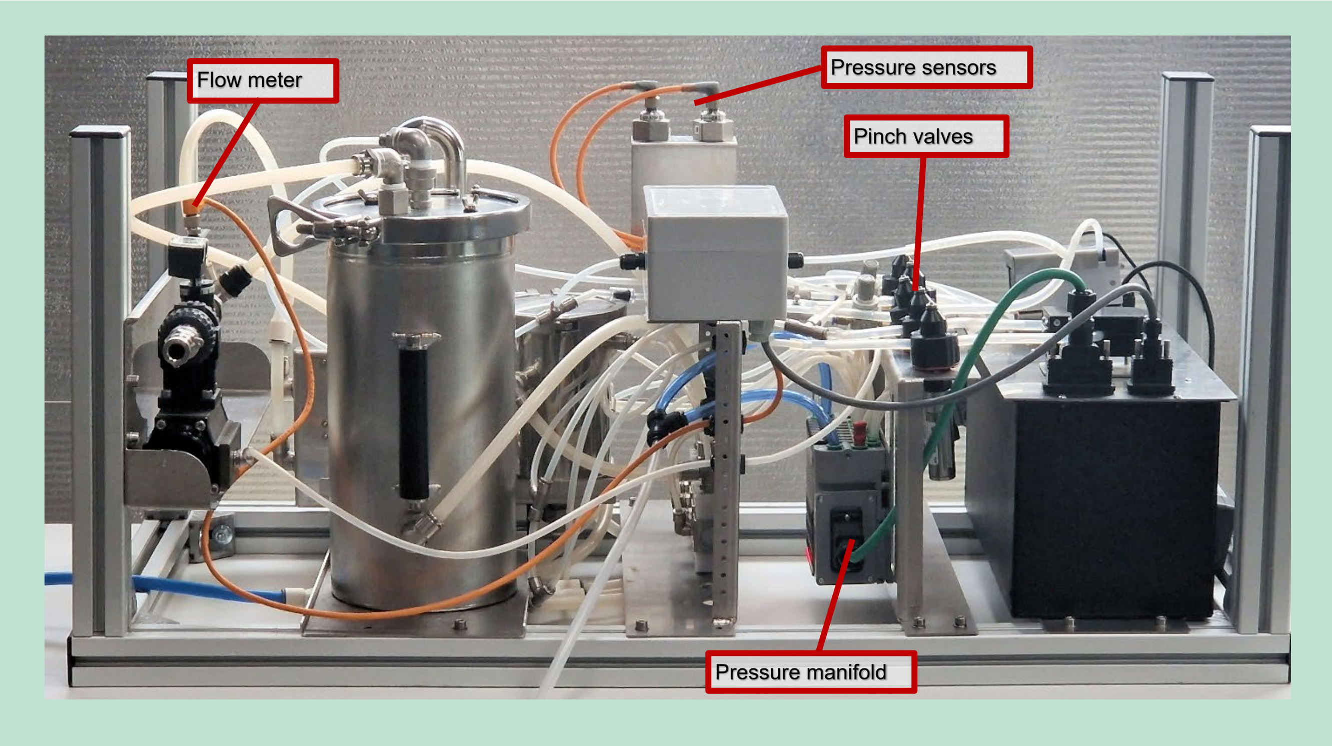

FIGURE 8. Input sensors are connected via orange cables, to the left is the flow meter and in the centre are the two pressure sensors. The pressure manifold can be seen mounted below and to the right of the pinch valves.

4.3 BallastWISE Inline software

The inline software application needs to control the collection of samples and the subsequent analysis. The front panel of the software application is shown in Figure 9. The front panel displays the state of the system, which valves are open, the pressure readings, and the flow rate, as well as the latest or ongoing test results. Other tabs can display live video feeds, and test result overviews. It is well suited for remote control and remote access, by which most problems with the system can be quickly identified. The computer running the software can be mounted on the inline framework or it can be placed remotely and connected via an optical fibre link or fast wired ethernet link.

Tools for fine tuning and calibrating the sensors, pressure levels, and flow timing parameters are available as icons on the left side of the panel.

A schedule can be set up to run automatically but it is expected that testing will be required to run during de-ballasting. This could be synchronised by way of a signal of some sort from the de-ballasting controller.

Results from tests are in the form of individual reports and a small database containing results. More in-depth documentation can be found in the form of swimming tracks (for heterotrophic organisms in both size classes) and fluorescent images for phytoplankton. Optional video recordings are also available. The reports and database can potentially be uploaded to a central server and merged with data from other ships or used as documentation towards proof of compliance.

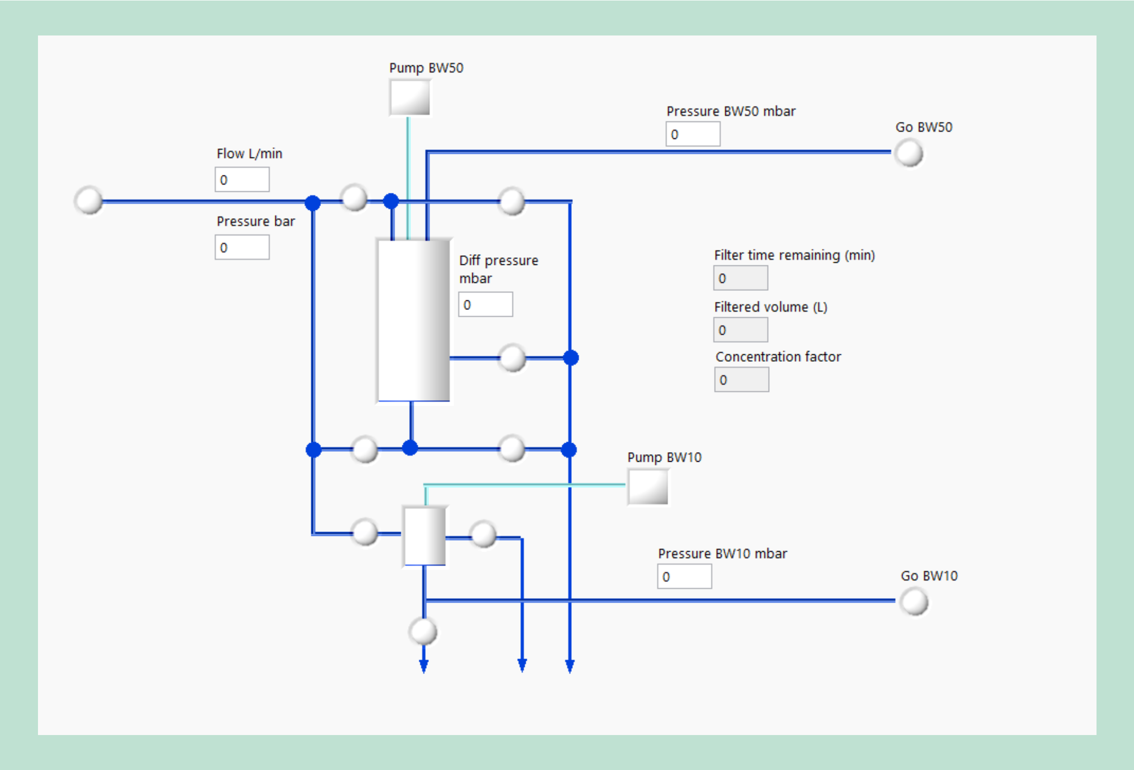

FIGURE 9. The BallastWISE Inline software front panel.

5. Test of BallastWISE Inline

BallastWISE Inline was tested during 3 weeks at the Royal Netherlands Institute for Sea Research (NIOZ). The aim was to test the integrated mechanical and software functions of BallastWISE Inline while running 2 daily test cycles autonomously during the testing period.

BallastWISE Inline performed uninterrupted during three weeks of continuous daily use. There were no upset conditions such as failure, breakdown, or malfunction (e.g., fouling) that could have led to incorrect functioning and conclusions on organism abundance, apart from one possibly false-positive signal. The full test report is included in Appendix 2.

6. Conclusion and future perspectives

5.1 Conclusions

The BallastWISE Inline project has shown that it is possible to carry out ongoing tests of ballast water and automatically count the number of viable organisms between 10-50 µm (mainly phytoplankton) and ≥50 µm (mainly zooplankton) without addition of chemicals. This has achieved by using the BallastWISE analysis method, paired with an inline filtering system for concentrating zooplankton samples and automatic pressure and sampling cycles. The patented technology for direct counting of organisms does not require the addition of chemicals and ensures that the IMO D2 requirements will be met in a sustainable and environmentally friendly way.

BallastWISE Inline was able to automatically collect, filter and analyse both algae (10-50 µm) and zooplankton (> 50µm) through automated sampling, filtering, analysis and documentation through video files and data points for 3 weeks. The software platform enabled automated collection, analysis, and reporting of the data.

5.2 Future perspectives

There are currently no automated and scalable technologies for documentation of compliance with the IMO D2 ballast water requirements. BallastWISE Inline will be the first fully automatic system for continuous analysis of organisms between 10-50µm and organisms larger than 50µm in ballast water on board ships.

The final outcome of the project is a complete system for monitoring and reporting the number of live organisms in ballast water discharge. The system will consist of a hardware part and a cloud-based software platform, and will enable automated collection, analysis and reporting to owners, authorities, and other stakeholders.

Overall, BWI will be able to streamline and automate what is currently a time-consuming and expensive process. With a system that continuously tests the number of live organisms in ballast water before discharge, errors in ballast water purification systems can be corrected. Continuous testing of ballast water on ships will thus prevent the spread of invasive organisms in the marine environment. For shipping companies continuous testing provides assurance on the performance of their ballast water treatment system, and it provides documentation which could be presented to port state authorities.

6. References

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Blackburn N., Haecky P., Jurgensone I., Griniene E., Bruge S.l, Andersson A., Carstensen J. (2022) The use of an automated organism tracking microscope in mesocosm experiments. Limnology and Oceanography Methods, Volume20, Issue12

BWM/CONF/36. The International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004

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MEPC 78/4/1. Report on the experience-building phase associated with the Ballast Water Management Convention. 4 March 2022

Appendix 1. BallastWISE patented measurement technology

MicroWISE has been awarded patents for “A METHOD AND A DEVICE FOR QUANTIFYING LIVING ORGANISMS AND USE OF A DEVICE” in the countries below:

The patent relates to a method for quantifying living organisms in a liquid sample by guiding the liquid sample into a chamber and analysing images and video sequences for automatic quantification of live organisms.

- European patent nr. EP3615920
- US Patent nr. 11,408,815
- Japanese Patent nr. 7095075
- Republic of Singapore Patent nr. 11201909619R
- China, Patent nr. ZL 201780089947.6

Appendix 2. Test of Report of first test performed at NIOZ (Texel, The Netherlands)

BallastWISE-in-line prototype test results in 2023
Report of first test performed at NIOZ (Texel, The Netherlands)
L. Peperzak, 11 August 2023

Introduction

MicroWise ordered Control Union to evaluate a BallastWISE-in-line prototype of 100x50x60 cm, 50 kg (Fig. 1). This instrument can continuously count 10-50 µm and ≥50 µm organisms in running seawater with relatively low organism concentrations.

Figure 1. The prototype in Denmark

The instrument is an extension of the standard BallastWISE that is described in detail in Blackburn et al. 2022[1]. Per measurement, the instrument performs 40 counts in a 0.024 mL cham-

[1] The use of an automated organism tracking microscope in mesocosm experiments. L&O Methods 20, 2022, 768-780.

ber (10-50 µm organisms) and 18 counts in a 20 mL chamber (>50 µm organisms). The extension comprises a.o. a filter to concentrate the ≥50 µm organisms, an electronic flow meter whose data are stored, and a robotic ball valve with manual override for isolating the system from the sea water source. One 10-50 µm and one ≥50 µm automatic in-line BallastWISE measurement per day was to be performed.

In addition, two non-compliant water samples for both fractions were intended to be tested but these were abandoned due to a lack of time.

Methods
MicroWISE delivered and installed the instrument at NIOZ in the Aquarium building on June 28 and 29 (Fig. 2).

Figure 2. Installation of the in-line BallastWISE on June 28. From right to left: Pia Haecky, Nick Blackburn, both MicroWISE, and Cees van Slooten (CU).

The flow was set at 7.5 L/minute. Used was sand-bed filtered Marsdiep-seawater with ambient salinity and temperature. Organism concentrations were low but unknown. The filtered water is not used directly but is stored in a tank in the NIOZ aquarium building from where it is distributed. The filtration is irregular, depending on the water volume in the tank, and independent of seawater tide, salinity, or temperature. Because of the darkness in that tank, autotrophic organisms were not expected but it is known that larger, heterotrophic organisms can survive.

After installation some time was spent to investigate why the volume of ≥50 µm sample water was less than anticipated. This problem could not be resolved and therefore the number of chamber fillings was reduced from 20 to 15. Via internet, the client had direct access from Denmark to the in-line BallastWISE and controlled the test.

Control Union and NIOZ staff checked the instrument on weekdays for any upset conditions such as failure, breakdown, or malfunction (e.g., fouling). The filter was checked regularly for fouling after removal from the filter house.

MicroWISE delivered the usual BallastWISE table with date/time and results, along with the other usual documentation such as videos, fluorescence images, and raw track data.
Control Union reviewed the data, put it in this report and added experiences that were gathered during the test period of ca. 1 month.

After the test had finished, the instrument was packed by Control Union and shipped back to Denmark. Several items (compressor, PC screen) remained on Texel.

Results

The test ran from June 9 to June 28, 2023. Samples were counted daily. The samples for 10-50 µm organisms were not concentrated. The 1000 L samples for ≥50 µm organisms were concentrated on the internal filter and 15 samples of the concentrate were counted. For reasons unknown, the volume of concentrate was insufficient to count 20 samples.

The concentration of 10-50 µm organisms was always near zero cells/mL, but ≥50 µm concentration was often >25 organisms/m3 with a maximum just over 400 organisms/m3 (Fig. 3).

It is probable that the ≥50 µm organisms counted were harpacticoid (bottom-dwelling) copepods that can survive the dark in the filtered-water storage tanks of NIOZ.

Some particles in the filtered water were expected. In a test with a FlowCAM conducted in winter 2019, total particle concentrations were measured. Observations of the FlowCAM's images revealed that nearly 100% of these particles were inert. Their concentrations ranged from 50 per mL (>10 µm) to 10 per mL (>20 µm) to <0.02 to 2 per mL (>100 µm). The >100 µm concentration would then have been in the range of 20,000 to 2,000,000 per m^3. The in-line BallastWISE counts are a factor of 100 to 10,000 lower because this instrument does not count inert particles, it only detects living (motile) organisms in this size range.

The track images of the ≥50 µm organisms (in .png format) were reviewed and in only one image tracks were observed that seem to be indicative of a false positive signal (nine parallel lines in a corner of the chamber, 27-7-2023). The video files would need to be reviewed to confirm false positive signals

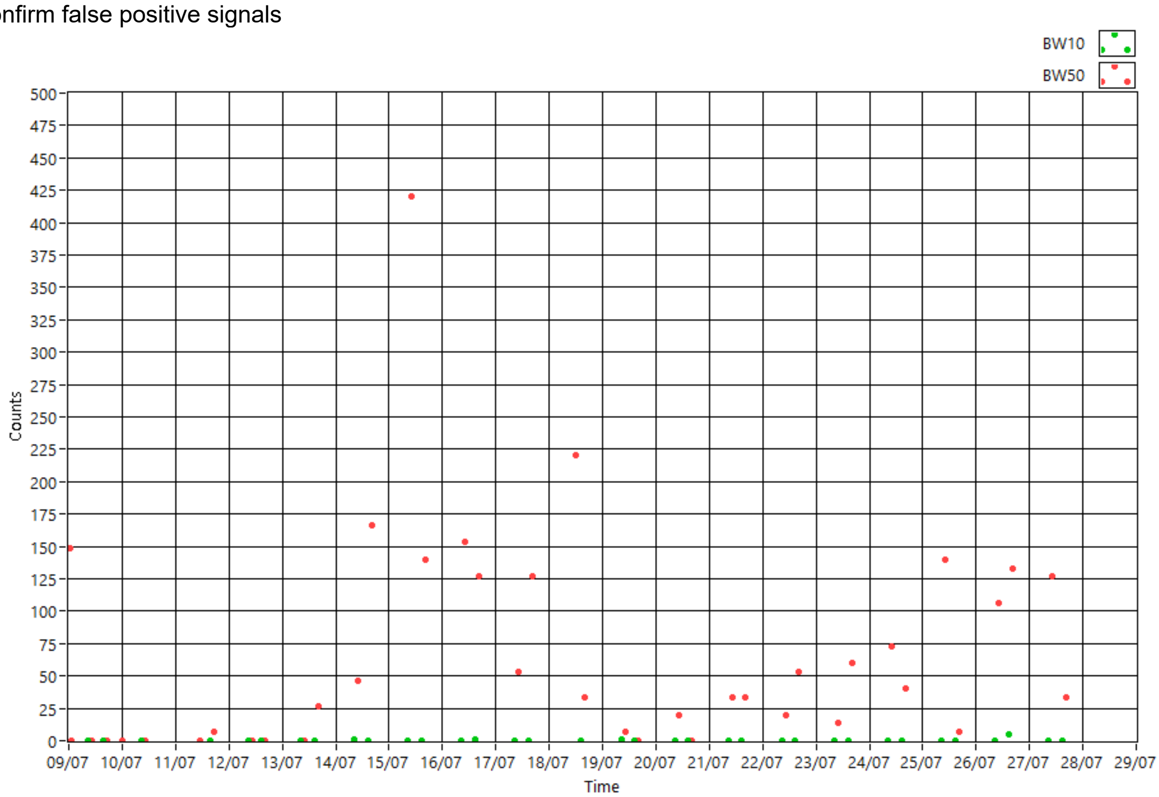

Figure 3. In-line BallastWISE counts of 10-50 µm cells/mL (BW10) and ≥50 µm organisms/m3 (BW50) organisms in NIOZ filtered seawater. The counts were performed in July 2023.

The following checks were made by NIOZ:

- Daily check on water supply and prototype functioning on weekdays (Monday to Friday). No failures to report.
- Weekly filter inspection: No failures to report. After the final test the filter appeared clean (Fig. 4). Ca. 350 mL of water was siphoned out of the filter house before packing the instrument for resending it to Denmark.

Figure 4. Filter appearance after the last test.

- Check of the pinch valve tubing after the final test: No failures to report (Fig. 5).

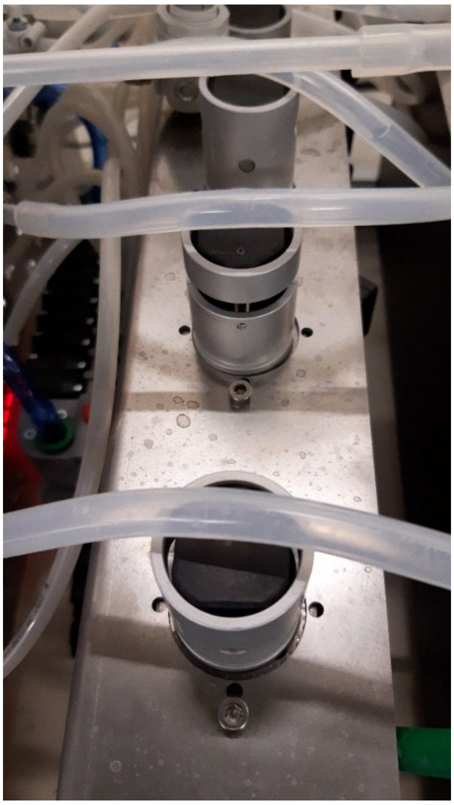

Figure 5. Pinch valve tubing after the final test.

- Check for fouling in the counting chambers after the final test: No failures to report (Fig. 6).

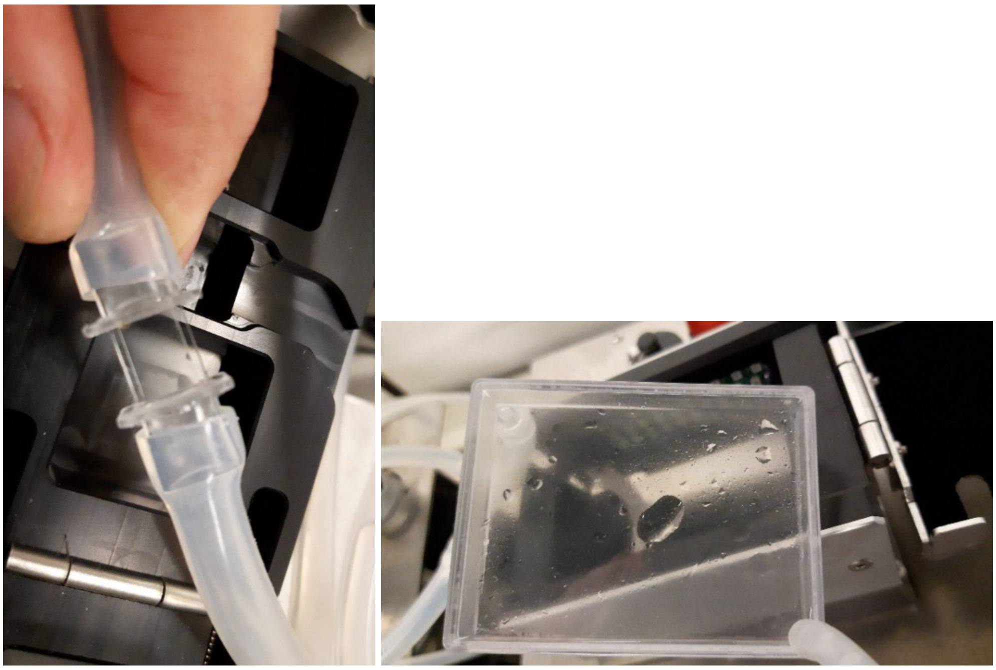

Figure 6. No apparent fouling in the counting chambers after the final test.

- The two tests for the 10-50 µm size fraction and the two tests for the ≥50 µm size fraction, where automated counts and manual counts were compared, were not performed due to time constraints.

Conclusions

The In-line BallastWISE performed uninterrupted during three weeks of continuous daily use. There were no upset conditions such as failure, breakdown, or malfunction (e.g., fouling) that could have led to incorrect functioning and conclusions on organism abundance, apart from one possibly false-positive signal.

The volume of concentrate for the ≥50 µm organisms should be increased to count 20 samples.

BallastWISE Inline

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Den internationale ballastvandkonvention trådte i kraft i september 2017 med henblik på at forebygge spredningen af akvatiske invasive arter. Senest i september 2024 skal alle skibe overholde den såkaldte D2-standard, som angiver det maksimale antal levedygtige organismer, der må udledes.

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