



BALLAST WATER

BALLASTWISE: BALLAST WATER ANALYZER

Test report

BALLASTWISE

BallastWISE is a rugged, portable equipment that can quantify live organisms in the size ranges: 10-50 μm and $\geq 50 \mu\text{m}$ in samples from treated ballast water. BallastWISE automatically counts and provides the results within 15-20 minutes depending on the sample type. Compliance or non-compliance with the ballast water discharge standard is displayed immediately after the analysis. BallastWISE uses a unique motion analysis and auto fluorescence detection. The method is based on image analytical detection of individual organisms as well as fluorescence from chlorophyll a content in individual organisms. Combining the two methods ensures detection of a very large fraction of live organisms given the fact that most heterotrophic organisms without chlorophyll a are motile.

RESULTS FROM TESTS WITH ORGANISMS

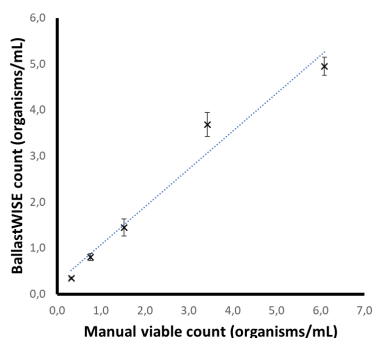


Figure 1. Two species of zooplankton $>50 \mu\text{m}$ counted by BallastWISE and compared to microscopy counts.

Actively swimming organisms (*Tigriopus californicus* and *Branchedionus plicatilis*) larger than $50 \mu\text{m}$ analysed by BallastWISE revealed a significant correlation with manual viable counts made in a microscope ($r^2=0.97$, $P<0.05$, Figure 1), and showed that BallastWISE was able to detect very low concentrations of organisms larger than $50 \mu\text{m}$.

A large range of $>50 \mu\text{m}$ organisms from freshwater, brackish and marine water samples from DHI's land-based test facility in Denmark has been analysed by BallastWISE and excellent agreement between results from BallastWISE and by microscopy counting were found ($r^2=0.92$, $P<0.05$, $n=53$, Figure 2).

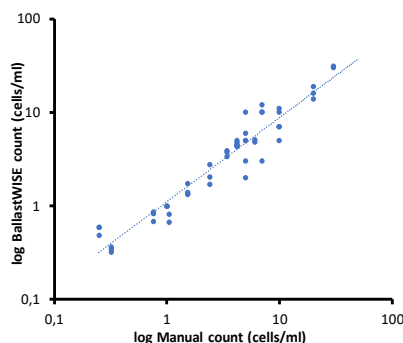


Figure 2. Natural samples of organisms $>50 \mu\text{m}$ counted by BallastWISE and compared to microscopy counts.

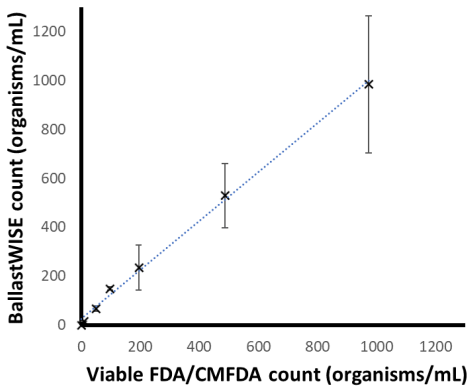
TEST DETAILS

METHODS

Samples used for the tests all consisted of live plankton samples either from monocultures, natural seawater samples or ballast water samples from DHI's test facility. BallastWISE was tested against the recognized methods in accordance with ETV protocol /1/. DHI performs the analysis as part of type approval testing in accordance with IMO G8 guidelines /2/ and U.S. Coast Guard standards /3/.

The 10-50 μm fraction were stained with CMFDA/ FDA and organisms are considered viable when they show a bright green fluorescence with or without red autofluorescence by use of an inverted epifluorescence microscope. Furthermore, motile unstained organisms in the size range 10-50 μm , were included in the countings. The $>50 \mu\text{m}$ fraction was counted as live organisms using standard movement and response to stimuli techniques.

The samples were analysed with BallastWISE parallel to the manual viable counts with 3-5 replicates to determine a mean concentration of each sample. For the 10-50 μm fraction 22,5-45,0 mL sample was determined, while for the $>50 \mu\text{m}$ fraction 0,73-1,2 L sample was counted for the determination of the mean concentration of organisms. All samples were analysed within 5 hours after sampling by a trained staff in DHI's laboratories.



Average counts by BallastWISE of a *Tetraselmis sp.* culture (cell size 10-15 µm) prepared in 6 dilutions with filtered seawater. The results obtained by BallastWISE showed excellent correlation with CMFDA/FDA viable counts of the samples in the microscope ($r^2 = 0.99$, $P < 0.05$, Figure 3).

Figure 3. Dilutions of *Tetraselmis sp.* culture counted by BallastWISE and compared to CMFDA/FDA viable cell microscopy counts.

Mixed samples of natural organisms in the 10-50 µm fraction also showed excellent correlation ($r^2 = 0.99$, $P < 0.05$) between BallastWISE measurements and CMFDA/FDA viable counts, Figure 4. However, BallastWISE generally detected more organisms than found by CMFDA/FDA counts. The reason for this deviation between the methods was caused by algal cells that, although alive and proliferating, did not stain by CMFDA/FDA and consequently, they were not counted in the microscope. Replicated, quantitative measurements have previously demonstrated that the staining method *cannot* accurately discriminate living from dead cells of *all* species of phytoplankton. Recently it was shown that only 33-42% of 24 different algae species tested gave acceptably accurate results from viability testing /4/.

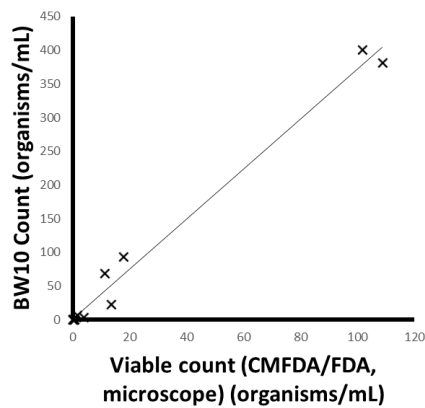


Figure 4. Mixed natural samples in the size range 10-50 µm counted by BallastWISE and compared to CMFDA/FDA microscopy counts.

Viable organism concentrations in discharge water (<10 organisms/mL)	
Total count (BallastWISE, org./mL)	Viable count (CMFDA/FDA, microscope, org/mL)
3.64	3.83
0	0.50
0	0.50
0	0.17
0	0.17
0	0
0	0
6.17	1.50

When evaluating treated ballast water samples by viable cell count in microscope showing less than 10 cells per mL, which is the required density of live organisms where compliance with the ballast water discharge standard is achieved (/1//2/), the results from BallastWISE were accurately reflecting this, see results in the table.

CONCLUSIONS

The test results show that BallastWISE can quantify live organisms of phytoplankton and zooplankton in the fractions 10-50 µm and > 50 µm. Being automatic and fast, BallastWISE is well suited for compliancy testing in connection to ballast water purification.

REFERENCES

- /1/ United States Environmental Protection Agency, Environmental Technology Verification Program. Generic Protocol for the Verification of Ballast Water Treatment Technology (ETV protocol). EPA/600/R-10/146, September 2010
- /2/ Marine Environment Protection Committee (MEPC). Guidelines for Approval of Ballast Water Management Systems (G8). Resolution MEPC. 279(70). Adopted 28 October 2016
- /3/ United States Coast Guard. Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters. Federal Register, Vol. 77, No. 57, 23 March 2012
- /4/ MacIntyre, H.L., & Cullen, J.J., 2016. Classification of phytoplankton cells as live or dead using the vital stains fluorescein diacetate and 5-chloromethylfluorescein diacetate. J.Phycol. 52:572-589

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