Commissioning testing of Ballast Water Management Systems – method of testing UV treatment

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International discharge standards for Ballast water

With the implementation of the international ballast water convention all larger ships must fulfill the requirements for discharge of ballast water stated in the IMO D-2 regulation [IMO, BWM.2/Circ.70/Rev.2]. Most ships install a type approved ballast water management system (BWMS) to treat the ballast water in order to live up to the requirements. Upon installation of the BWMS, a commissioning test must be performed to validate that the system's mechanical, physical and chemical processes are working properly. Biological analyses of the discharge water are used to verify that the BWMS meets the IMO D-2 discharge standard [IMO, BWM.2/Circ.70/Rev.2].

Requirements for the content of viable organisms in discharged ballast water in accordance with Regulation D-2.

Organisms		Discharge standard
≥50 µm	Zooplankton	<10 organisms/m^3
≥10 µm and <50 µm	Phyto- and zooplankton	<10 organisms/mL
Vibrio cholerae (serotype O1 and O139)	Bacteria	<1 CFU[1)]/100 mL
E. coli	Bacteria	<250 CFU[1)]/100 mL
Enterococci	Bacteria	>100 CFU[1)]/100 mL

1) CFU, colony-forming units (bacteria that grows in a specific medium)

Biological analyses of ballast water treated with UV light

The commonly used methods for quantifying living organisms ≥10 µm and <50 µm onboard ships include so-called *indicative methods* (based on, e.g., the measurement of fluorescence) and *detailed methods* (based on, e.g., vital staining and microscopy). Measurements of fluorescence and microscopy after vital staining show if the organisms are viable when the analysis is performed. These methods pose a challenge when a BWMS applies ultraviolet (UV) light as disinfection source. UV treatment of ballast water causes damage to the organisms' DNA. However, in some cases, the organism does not die immediately after exposure to the UV radiation, but the damage on the DNA means that they will not be able to reproduce (living but not viable). When organisms ≥10 µm and <50 µm in ballast water treated by UV radiation are quantified by use of indicative methods or microscopy after vital staining, there is a risk of misleading results where organisms are recorded as 'living' although they are unable to reproduce and do not present a risk of becoming invasive species.

To meet the before mentioned issues when testing UV-BWMS, there has been an interest in methods that can measure the organisms' ability to reproduce. Currently, the most suitable method is called 'Most probable Number' (MPN). It identifies the most probable number of algae (phytoplankton) that can reproduce. The MPN method therefore accounts for the case that some algae will survive the UV treatment but are not able to reproduce, and, thus, they will not pose a threat as invasive species in the water environment receiving discharged ballast water.

How ballast water samples are affected by transportation time and temperature

If the MPN method is to be used for commissioning test, it is necessary to transport the water samples from the ship to a laboratory. This means that the impact of transportation time and the temperature during transportation must be validated to make sure that the results of the MPN analyses done on water samples taken onboard the ship are reliable.

A validation study was performed with samples containing the green algae *Tetraselmis suecica* in two different concentrations. One concentration represented a sample that would pass the discharge standard (5 - <10 organisms/mL), and the other concentration represented a sample that would not pass the discharge standard (approx. 100 organisms/mL).

The study showed that it is possible to transport water samples for up to 144 hours at 4-20°C without affecting the results of the MPN method. It was concluded that the MPN method is applicable for analyses of organisms (size ≥10 µm and <50 µm) in water samples taken onboard a ship, when the transportation time is shorter than 144 hours, and the temperature is kept between 4-20°C.

The study is described in detail below.

Results from the MPN study

Two different concentrations of *Tetraselmis suecica* cultures (5 - <10 organisms/mL and approx. 100 organisms/mL) were prepared by diluting a concentrated *Tetraselmis suecica* culture with seawater. The concentration of organisms was analyzed by direct microscopy counting of six subsamples stained with CMFDA/FDA. This count verified that the concentrations of *Tetraselmis* in the two samples were 8 organisms/mL and 99 organisms/mL, respectively.

The effect of transportation time was investigated by MPN analysis of the samples immediately (0-6 hours) after sampling and after keeping the samples in the dark for 24, 48, 72 and 144 hours.

To investigate *the effect of temperature* the samples were kept at either 4°C or 20°C.

After the different storage times the samples were diluted according to the MPN method, and the samples were analyzed after 14 days incubation. The results of the MPN analyses for the low concentration (8 organisms/mL) of *Tetraselmis suecica* are presented in Figure 1 and Figure 2.

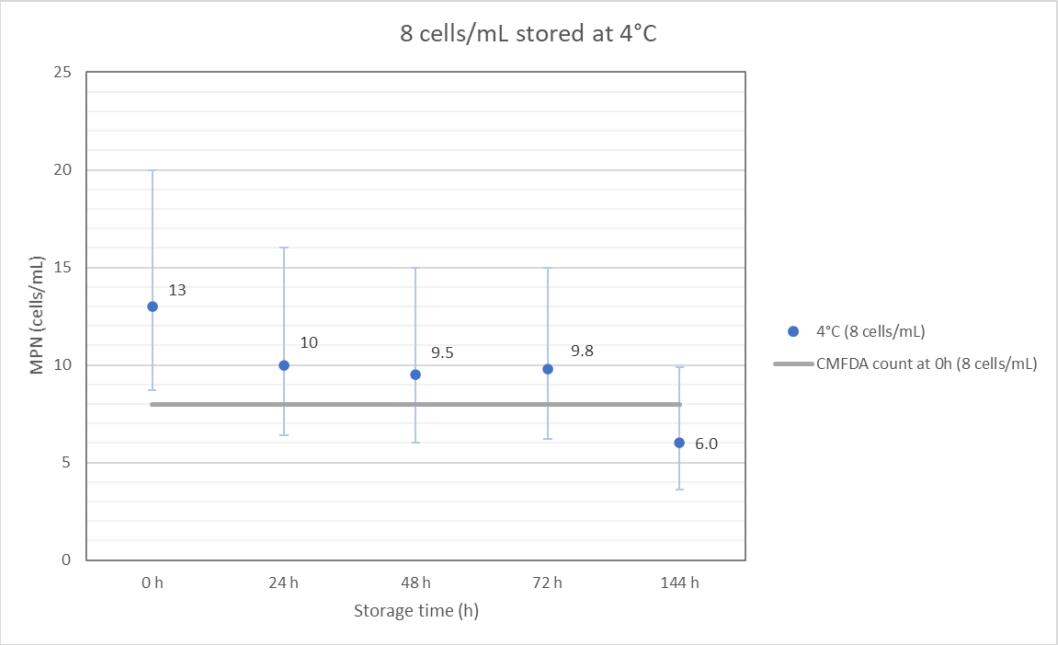

Figure 1 Results of the MPN assay of *Tetraselmis suecica* samples for the 8 organisms/mL concentration after 0; 24; 48; 72; and 144 hours storage at 4°C. The error bars show the 95% upper and lower confidence range based on the MPN calculations. The horizontal grey line describes the initial CMFDA/FDA count at 0h.

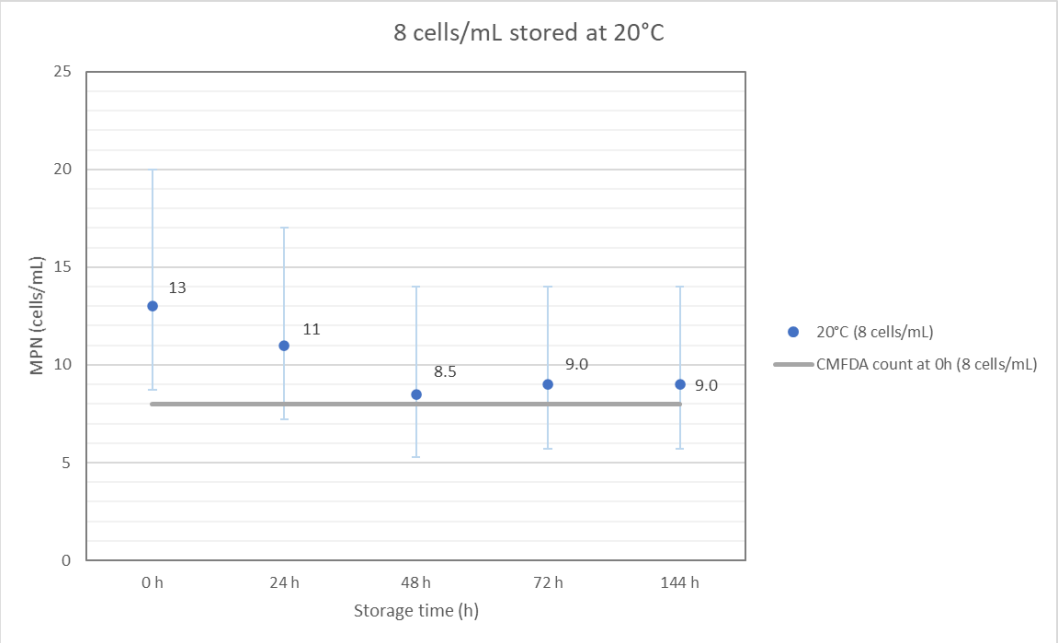

Figure 2 Results of the MPN assay of *Tetraselmis suecica* samples for the 8 organisms/mL concentration after 0; 24; 48; 72; and 144 hours storage at 20°C. The error bars show the 95% upper and lower confidence range based on the MPN calculations. The horizontal grey line describes the initial CMFDA/FDA count at 0h.

The sample without storage (0 h) and the low concentration of *Tetraselmis* was measured with MPN to 13 organisms/mL (95% confidence range: 8.7 – 20 organisms/mL) which was higher than the CMFDA/FDA direct microscopy count (8 organisms/mL, standard deviation: ±1.5). For all other MPN results the confidence range was consistent with the CMFDA/FDA results. The results of the MPN assay after storage (24 h – 144 h) did not differ significantly from the MPN result without storage (0 h) as shown by the overlapping confidence ranges. The results were similar for both the samples stored at 4°C and at 20°C.

The results of the MPN assay for the high concentration (99 organisms/mL) of *Tetraselmis suecica* are presented in Figure 3 and Figure 4.

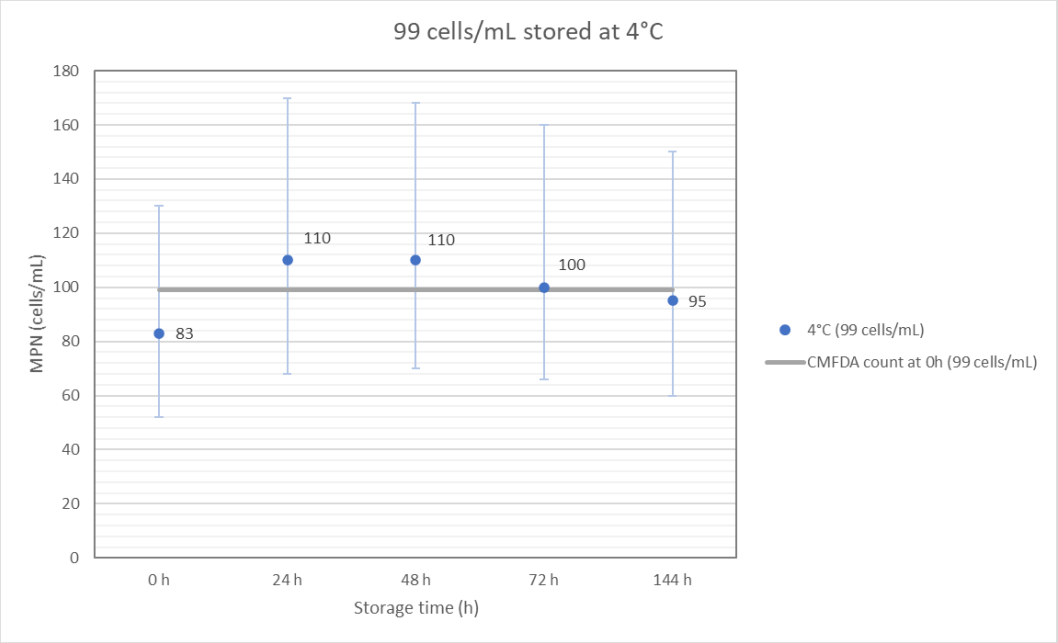

Figure 3 Results of the MPN assay of *Tetraselmis suecica* samples for the 99 organisms/mL concentration after 0; 24; 48; 72; and 144 hours storage at 4°C. The error bars show the 95% upper and lower confidence range based on the MPN calculations. The horizontal grey line describes the initial CMFDA/FDA count at 0h.

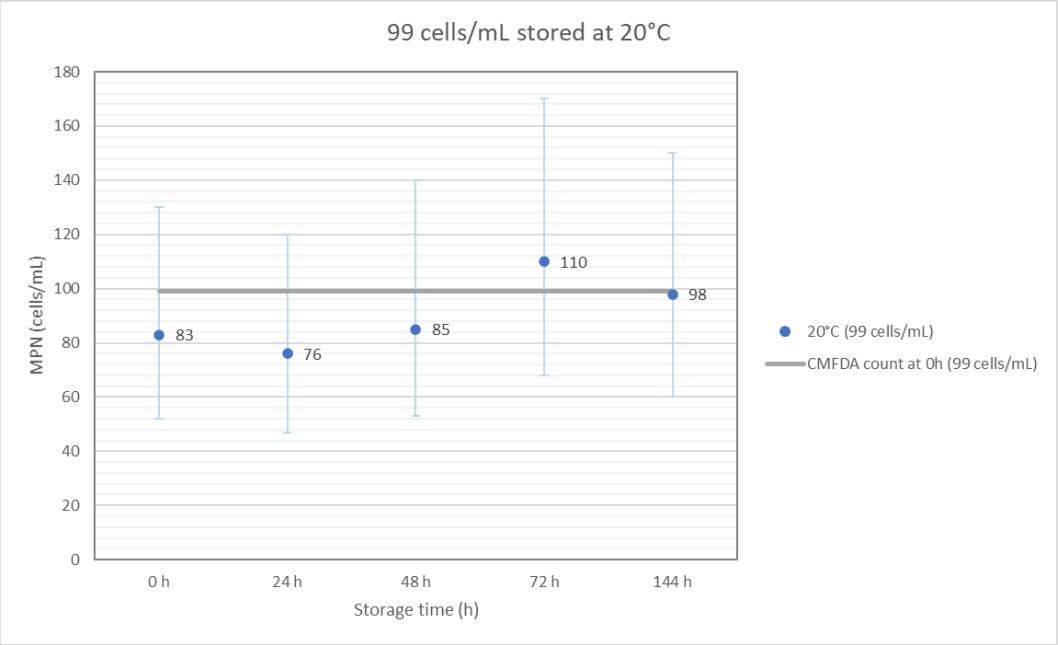

Figure 4 Results of the MPN assay of *Tetraselmis suecica* samples for the 99 organisms/mL concentration after 0; 24; 48; 72; and 144 hours storage at 20°C. The error bars show the 95% upper and lower confidence range based on the MPN calculations. The horizontal grey line describes the initial CMFDA/FDA count at 0h.

The sample without storage (0 h) and the high concentration of *Tetraselmis* was measured with MPN to 83 organisms/mL (95% confidence range: 52 – 130 organisms/mL) which was consistent with the CMFDA/FDA direct microscopy count (99 organisms/mL, standard deviation: ±11). The 95% confidence range for all other MPN results were also consistent with the CMFDA/FDA result. The MPN result without storage (0 h) was not significantly different from the MPN results for the samples stored for 24 to 144 hours as shown by the overlapping confidence ranges. The results were similar for both the samples stored at 4°C and at 20°C.