

COMPARISON OF LAND-BASED TEST SETUPS FOR A BALLAST WATER MANAGEMENT SYSTEM

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SUMMARY

Two land-based setups were tested at different locations using the same combined treatment technologies, to assess the effect of different control and treated tanks condition as well as overall effectiveness of a ballast water treatment system. The test procedure included a five day storage period of organisms in the control and treated tanks as specifically advised in the type approval procedure for shipboard and land-based tests described in the IMO Guideline 'G8'. The configurations and materials of control and treated tanks used in each test location were different resulting in invalid test results at one testing location.

1. INTRODUCTION

In 2004 the International Maritime Organisation (IMO) adopted the Ballast Water Management Convention in order to minimise the spread of aquatic organisms across the globe via shipping. Two regulations (D-1 and D-2) were defined in the Convention to be used to prevent viable organisms from being discharged from ballast tanks. Any ballast water management system must meet the requirements of one of these regulations prior to discharge of ballast water. Regulation D-1 is the Ballast Water Exchange Standard which states that ships must exchange a minimum of 95% ballast water volume during exchange. Regulation D-2 is the Ballast Water Performance Standard and states the limit of the allowable number of viable organisms which can be discharged from vessels when a treatment system is used (IMO 2004), these levels are:

- Less than 10 viable organisms $\geq 50 \mu\text{m}$ in minimum dimension per m^3
- Less than 10 viable organisms $\leq 50 \mu\text{m}$ and $\geq 10 \mu\text{m}$ in minimum dimension per ml
- And discharge of indicator microbes is specified as follows:
 - Less than 1 colony forming unit (cfu) per 100 ml or less than 1 cfu per 1 g (wet weight) zooplankton samples of toxicogenic *Vibrio cholerae* (O1 and O139),
 - Less than 250 cfu of *Escherichia coli* per 100 ml,
 - Less than 100 cfu of intestinal *Enterococci* per 100 ml.

D-1 was the minimum standard to be imposed while systems capable of meeting D-2 were developed. The determination and publication of D-2 was extremely important in terms of developing new ballast water treatments as the industry was finally given a standard which had to be met before the IMO will approve and certify systems for sale. By October 2010 ten treatment systems have received IMO Type Approval certification (MEPC 2010). A further eleven systems are expected to gain Type Approval by 2012 (Lloyds Register 2010; MEPC 2010). Published as part of the Convention the

guidelines 'G8' have set out the procedure for administrations or their designated bodies (e.g. classification societies) to be used for assessing ballast water management systems. This paper provides additional guidance to manufacturers and ship owners on the evaluation procedure. A ballast water management system should undergo land-based and shipboard testing and receive type approval upon achieving successful results in accordance with Regulation D-2. In both test cycle procedures (land-based and shipboard) as described in G8, the storage of ballast water prior to final discharge has been emphasised and considered to be part of the test procedure.

In this study two series of tests were performed using the same treatment system, which consisted of a combined filtration and UV system, at two different testing locations with different storage conditions. The aim of this study was not to follow the exact land-based procedure as laid out in G8, but to assess the effect of different control and treated storage conditions on the validity of the test cycle.

2. MATERIALS AND METHODS

2.1 TEST SETUP

Two series of tests were performed using the combined filter and UV treatment system. In both series of tests the effectiveness of the combined treatment system on the brine shrimp, *Artemia salina*, and the single cell green alga, *Tetraselmis suecica*, was assessed. All tests included initial treatment on Day 0, five days storage (in accordance with G8) of treated and untreated (control) seawater and repeated UV treatment on Day 5 prior to final discharge (Figures 1 and 2). UV treatment was reapplied on Day 5 to kill any organisms which had survived initial treatment. Table 1 summarises the similarities and differences between the two test series.

2.1(a) Test Series 1

Two tests were performed at the Dove Marine Laboratory (DML), UK in May 2009. The storage tanks

used were rectangular and made of reinforced concrete, with the top open to the atmosphere (Figure 3). The outlet valves from the tanks were 30 cm above the base and so it was not possible to completely empty the tanks via the outlet valves. There was also 5 – 10 cm of sludge/mud at the bottom of both tanks.

A tank was filled with seawater from Cullercoats Bay to which the test organisms, *A. salina* and *T. suecica* were added. To ensure the organisms were distributed throughout the tank an aquarium type submersible pump was used to re-circulate the water. The seawater used in the storage tank had been passed through a sand-filter, and so kaolin was added to lower the UV transmittance of seawater in the storage tank to natural levels. The seawater was pumped firstly through the treatment system into the Treated tank, with samples collected at each sampling point for biological assessment (Figure 1). The remaining seawater in the storage tank was pumped into the Control Tank untreated. The water was stored for five days. On Day 5 seawater from the Treated tank was discharged after a second UV treatment, and samples were collected before and after UV treatment for biological assessment (Figure 2). Samples were taken directly from the Control tank to evaluate the effect of five days storage on the test organisms without treatment.

2.1(b) Test Series 2

Two similar tests were conducted at the Port of Blyth (PoB), UK in August 2009. In these tests cylindrical tanks made of steel were used to mimic, as far as practicable, a ship's ballast tanks (Figure 4). Unlike the tanks used in Series 1 these tanks were sealed and access was limited to a manhole located on the top surface of each tank. The discharge valves on the tanks were installed in the base, which made it possible to rinse and completely drain them. The tanks were cleaned at the end of each test to avoid any interference from one test to another.

Tanks were filled with seawater from the North Sea to resemble a location from which ships would take their ballast water. The test organisms were added to the tank in quantities which aimed to meet the G8 input requirement, and the same sampling procedure was followed as described in Series 1.

2.2. EQUIPMENT

The filter used in this study contained a fine screen which consisted of a multilayered sintered stainless steel mesh with a nominal pore size of 40 µm, and had the capability of auto-flushing without stopping the flow rate. The UV reactor used in this study had eight 3.5 kW medium pressure UV lamps, which were located perpendicular to the flow of the water (Figure 5). To calculate the average UV dose delivered the 'calculated dose approach' was adopted (Bolton, 1999). This was recommended by the manufacturer and the UV reactor

used was validated for this approach. In the calculated dose approach, the UV dose delivered is a function of the flow rate, lamp status, UV intensity and UV transmission of the water. All operational parameters were measured online during the treatment process for each individual test. Table 2 shows the measured and calculated parameters of the tests conducted in both series.

2.3 SAMPLING METHODS

A sampler (Figure 6) was designed to collect simultaneous samples in triplicate and installed at each sampling point. Each sampler had three valves and a sample tube with a 90° bend towards the direction of the flow, located in the centre of the main piping system. The use of these samplers provided a means for continuous sampling throughout the test rather than a discrete sampling (top, middle and bottom of tank) method. The volume of samples collected at each of the sampling points for biological analysis of *A. salina* and *T. suecica* were based on the G8 guidelines and are shown in Table 3. All samples were collected in triplicate and were assessed within six hours of collection, as required by the G8 guidelines.

2.4. BIOLOGICAL ASSESSMENT OF TETRASELMIS SUECICA

In both test series *T. suecica* samples were assessed using a FlowCAM using a 100 µm depth flow cell and a 10 x objective. A live/dead assessment was made using the fluorescent vital stain fluorescein diacetate (FDA), which was prepared as described by Jochem (1999). This stain enters live cells and cellular metabolism causes a fluorescent particle to be released, thereby allowing the identification of live. A primary stock of 5 mg FDA per ml DMSO was made and stored at 5 °C. This primary stock was diluted 100-fold to make a working stock which was kept on ice in the dark for the duration of use. Samples were stained with 100 µl FDA per 3 ml water sample and analysed using the FlowCAM Trigger mode under dark conditions. When using the Trigger mode, samples were diluted with freshly filtered (0.45 µm filter) seawater to enable the accurate detection of cells. FlowCAM detects only live cells and then calculates the number of live cells per ml taking into account the sample volume, size of flow cell used and the dilution of the sample

2.5. BIOLOGICAL ASSESSMENT OF ARTEMIA SALINA

In both series of tests, *A. salina* samples were concentrated using a 50 µm mesh and rinsed with filtered (0.45 µm filter) seawater before analysis. The entire sample was examined using Meiji dissection microscopes at 10 – 45 x magnification. All *A. salina* in samples were classed as either live or dead by visually observing the organisms for internal or external movement. If no movement was observed initially then

an organism was 'prodded' to determine whether movement could be induced. If an individual still showed no movement it was then classed as dead. The total number of live organisms per 1000 L was calculated.

2.6. STATISTICAL ANALYSIS

The live cell counts for *T. suecica* and *A. salina* were assessed for normality (Anderson-Darling test) and equal variance (Levene's Test). The data were then analysed for differences in live cell concentrations before and after treatment using the one way ANOVA statistical test.

3. RESULTS

3.1 ARTEMIA SALINA RESULTS

Filtration in all tests demonstrated significant removal of *A. salina*, which left few organisms ($\geq 50 \mu\text{m}$) for the UV system to treat (Figures 7 and 8). The overall effectiveness of the treatment system for *A. salina*, including five days storage in a treated tank, was significantly different in all tests (Table 4). A significant difference between the number of live *A. salina* before and after the filter was observed in each test (Table 5).

3.2 TETRASELMIS SUECICA RESULTS

Unlike the organisms $\geq 50 \mu\text{m}$, filtration did not show a significant reduction in the number of live organisms in the $\geq 10 \mu\text{m} < 50 \mu\text{m}$ size category (Figures 9 and 10). The treatment system for *T. suecica* was effective in all tests and it significantly reduced the total number of live organisms before and after treatment (Table 6). The filter was not responsible for this reduction (Figures 9 and 10) and a significant reduction of live *T. suecica* before and after UV treatment was observed (Table 7).

4. DISCUSSION

The number of live organisms in a Control tank is an important factor in any ballast water treatment experiment, and is used to validate the results obtained to determine the effectiveness of a treatment system. According to the IMO G8 Guidelines if in any test cycle the average discharge from the control water is a concentration of less than or equal to 10 times the values in Regulation D-2.1, the test cycle is invalid', i.e. ≤ 100 viable organisms per ml or per m^3 . The distinctive difference in the biological results of these two series of tests is the number of live organisms in the Control tank. Figures 6 to 9 clearly demonstrate this and reveal that the number of live organisms in the Control Tank in Series 1 tests (DML) is below the IMO standard, i.e. less than 100 viable organisms per m^3 or per ml. In the second test series (Figures 8 and 10) a high number of live organisms were present in the Control tank after the five day storage period. A low concentration of organisms in the Control tank for any test, e.g. Series 1 tests carried

out at the Dove Marine Laboratory, shows that the five days storage had a detrimental effect on the organisms and resulted in mortality. However, for the tests performed at the Dove Marine Laboratory this may not be the only explanation for the observed decrease in organism numbers. The position of the discharge valve, which is situated 30 cm from the base of tank means that the tanks could not be completely drained and some water remained in the tank after each test. This remaining water, together with sediment at the bottom of tank, could have provided a shelter for the organisms in which to settle during the five day storage period. Sediment at the bottom of ballast tanks has been reported to be species rich with organisms forming stable communities which avoid discharge with ballast water (Gollasch et al., 2000).

The results obtained in this study showed that the significant reduction in live *A. salina* was attributed to filtration. The number of live *A. salina* which entered the UV reactor was too low to establish any solid evidence of the UV systems effectiveness. Nonetheless, filtration alone did not reduce the number of live *A. salina* sufficiently to meet the D-2 discharge standard in any of the tests performed, therefore it is possible to conclude that UV treatment was complementary to filtration for organisms $\geq 50 \mu\text{m}$. The UV system was required for the inactivation of organisms in the size range of $\geq 10 \mu\text{m} < 50 \mu\text{m}$, as filtration, due to the size of organisms, cannot substantially reduce the total number of live organisms.

In any ballast water treatment test setup due care has to be taken to eliminate any element of doubt, which could reduce the credibility of the results. This can be as simple as the position of the discharge valve on the tank, which may allow organisms to shelter below its level and become inaccessible during sampling. While the Series 1 tests performed at the Dove Marine Laboratory resulted in a significant biological reduction of live organisms (shown in Figures 7 and 9) they are considered invalid in respect to the IMO G8 guidelines.

After exposure to UV light organisms are able to repair the damage caused and there are two main processes used for this: photo-repair and dark-repair (United States Environment Protection Agency 2006). Both processes can be performed in the presence of light and it was expected that the exposure of organisms in the Treated tank to sunlight during Series 1 tests would aid the recovery of organisms during the storage period. However low numbers of organisms existed in both the control and treated tanks after the five days storage period which did not support the hypothesis of possible re-growth.

The number of live organisms present in the Control tank of the tests conducted at the Port of Blyth was above the requirement stated in the IMO Guideline G8 (Figures 8 and 10). Based on these two tests it is possible to conclude that the combined filtration and UV system

could effectively treat seawater containing live organisms of both size categories to meet the D-2 discharge standard.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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Table 1: Test conditions in both Test Series completed. (Test Series 1 – Dove Marine Laboratory, Test Series 2 – Port of Blyth).

	Parameters	Test Series 1	Tests Series 2
Control & Treated Tanks	Shape	Rectangular	Cylindrical
	Material	Cement	Metallic with internal coating
	Internal condition	Sludge/ mud at the bottom	Clean (some rusty area)
	Enclosed	No (open to atmosphere at top)	Yes
	Capacity (m ³)	60	55
Seawater	Source	North Sea (sand-filtered)	North Sea (Harbour area)
	Salinity ‰	30	30
	Temperature (° C)	15.5 ~ 16.3	13.8 ~ 14.5
	pH	8.0	8.0
Pump and piping arrangement	Adding organisms to the tank	Aquarium pump with flexible plastic hose	Aquarium pump with flexible plastic hose
	For treatment purpose	Centrifugal pump with reinforced flexible hose	Centrifugal pump with reinforced flexible hose
Organism	Zooplankton	<i>Artemia salina</i>	<i>Artemiasalina</i>
	Phytoplankton	<i>Tetraselmis suecica</i>	<i>Tetraselmis suecica</i>
Tests conducted	Month	May 2009	August 2009
	Air Temperature (° C)	14	18

Table 2. Measured (Flow rate, UV transmission, UV lamp power) and calculated (UV dose, UV exposure time) parameters of the tests conducted in both series.

Test Series	Test	Day	Flow rate (m ³ hr ⁻¹)	UV Transmission (%)	UV lamp power (%)	UV dose (mWScm ⁻²)	UV Exposure time (s)
1	1	0	38	82.9	75	745	1.95
		5	69	86.8	75	488	1.07
2	2	0	66	92	100	930	1.12
		5	60	89	100	844	1.23
	1	0	43.5	83.6	50	477	1.68
		5	64.6	70.5	100	371	1.14
	2	0	91.8	78.5	100	350	0.8
		5	127	73.3	100	213	0.58

Table 3. Volume of samples collected at each sampling point in all tests for *Artemia salina* and *Tetraselmis suecica*. (Volumes are in L).

Day	Sampling point	<i>Artemia salina</i>	<i>Tetraselmis suecica</i>
0	Before Filter	20	1
	After Filter	100	1
	After UV	1000	10
5	Control Tank	100	1
	Before UV	20	1
	After UV	1000	10

Table 4. Statistical results of the 1-way ANOVA test.

Test Series	Test	P-value	F Value	Degrees of freedom (d.f)
1	1	<0.001	71.74	4
	2	<0.001	49.43	4
2	1	<0.001	16.35	4
	2	<0.001	30.08	4

Table 5. Statistical results of the 1-way ANOVA test.

Test Series	Test	P-value	F Value	Degrees of freedom (d.f)
1	1	0.001	71.69	1
	2	0.002	49.26	1
2	1	0.016	16.33	1
	2	0.005	30.07	1

Table 6. Statistical results of the 1-way ANOVA test.

Test Series	Test	P-value	F Value	Degrees of freedom (d.f)
1	1	<0.001	83.74	4
	2	<0.001	74.65	4
2	1	<0.001	21.83	4
	2	<0.001	22.07	4

Table 7. Statistical results of the 1-way ANOVA test.

Test Series	Test	P-value	F Value	Degrees of freedom (d.f)
1	1	0.003	41.34	1
	2	0.004	38.07	1
2	1	0.0013	17.94	1
	2	0.05	7.37	1

Day 0 procedure

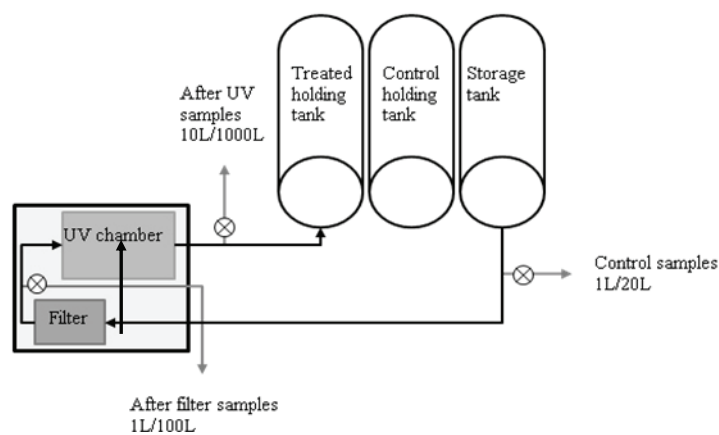


Figure 1: Schematic diagram of test procedure in Day 0, showing three sampling points.

- ⊗ indicates the sampling valves in the diagram.

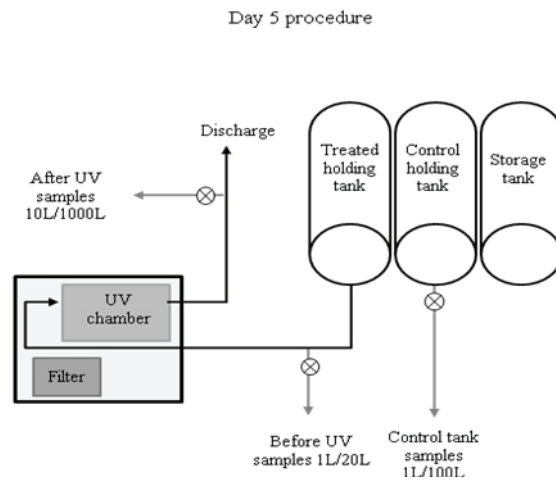


Figure 2: Schematic diagram of test procedure in Day 5, showing three sampling points
 . ⊗ indicates the sampling valves in the diagram.



Figure 3: Control and treated tanks at Dove Marine Laboratory



Figure 4: Control and treated tanks at the Port of Blyth

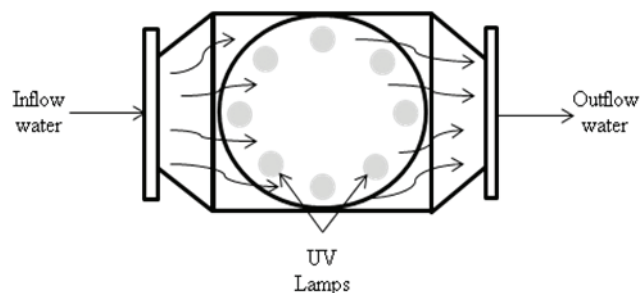


Figure 5: Cross-flow inline UV treatment system with 8 UV lamps

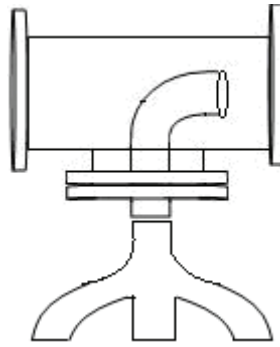


Figure 6. Sampler designed to obtain three simultaneous samples.

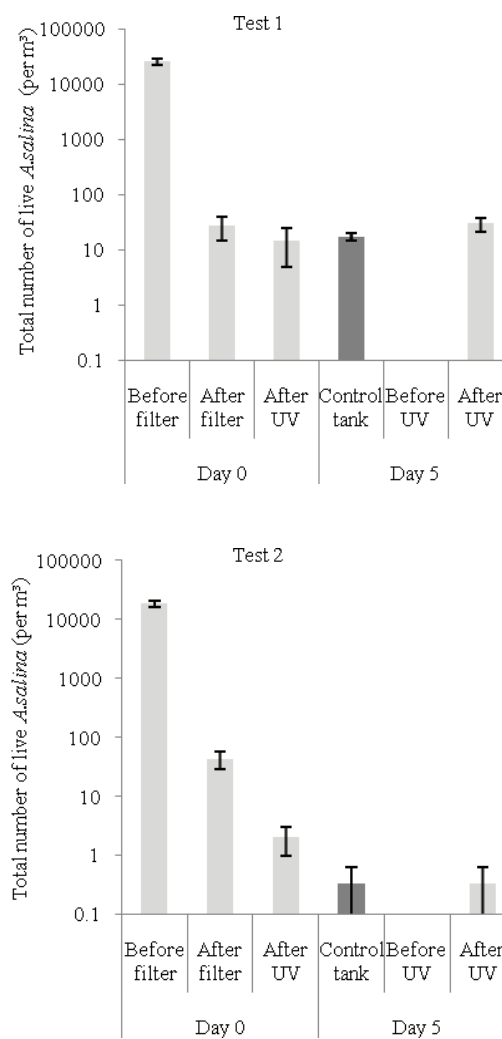


Figure 7: The total number of live *Artemia salina* ($\geq 50\mu\text{m}$) in 1000L for the tests conducted in Series 1 (Dove Marine Laboratory). All data are the average of three replicates \pm standard error. (Note: scale is logarithmic).

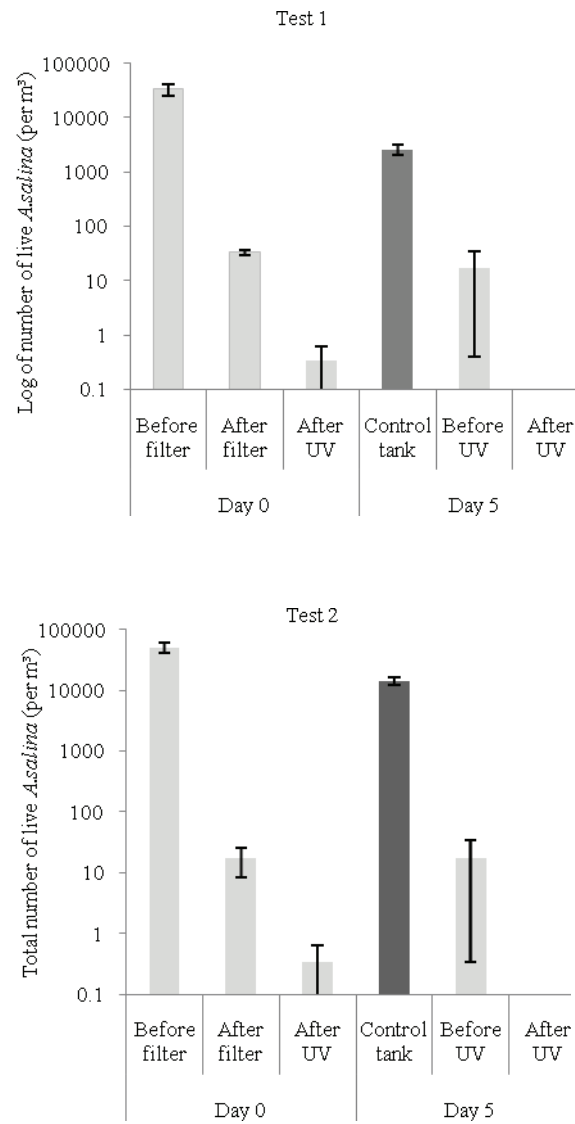


Figure 8: The total number of live *Artemia salina* ($\geq 50\mu\text{m}$) in 1000 L for the tests conducted in Series 2 (Port of Blyth). All data are the average of three replicates \pm standard error. (Note: scale is logarithmic).

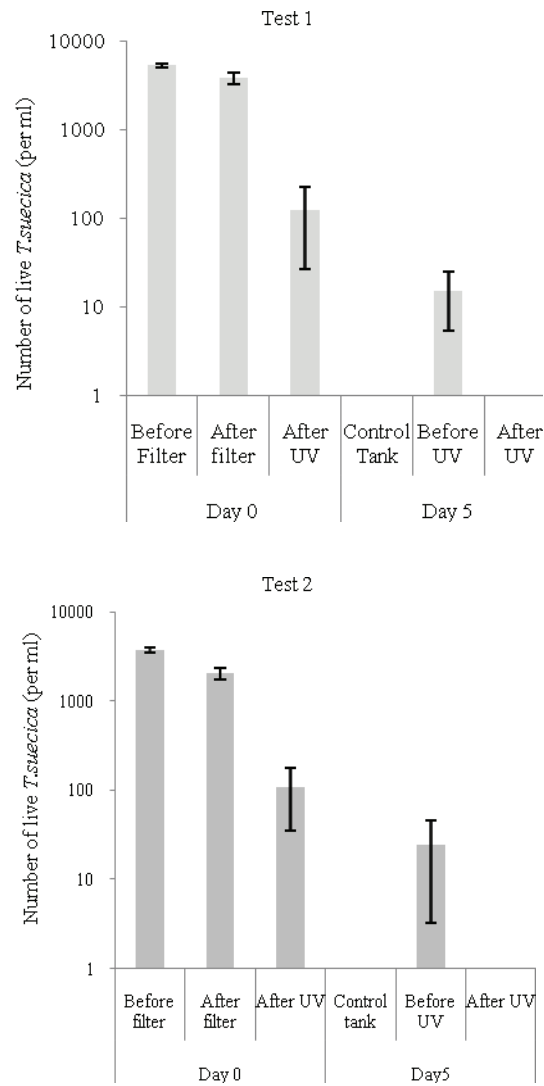


Figure 9: The total number of live *Tetraselmis suecica* ($\geq 10\mu\text{m} < 50\mu\text{m}$) in 1 ml for Series 1 tests (Dove Marine Laboratory). All data are the average of three replicates \pm standard error. (Note: scale is logarithmic).

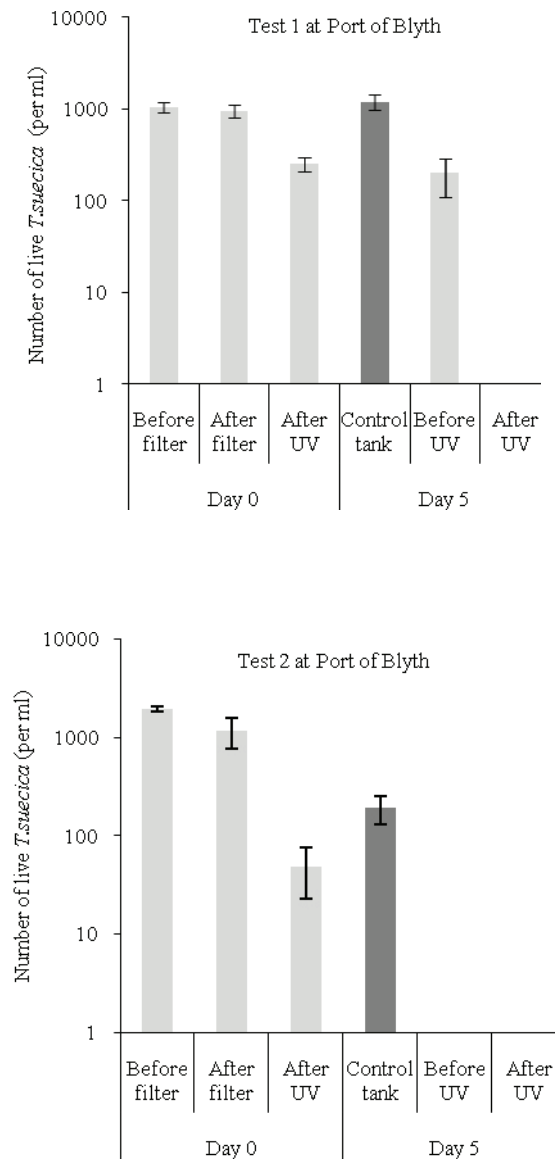


Figure 10: The total number of live *Tetraselmis suecica* ($\geq 10\mu\text{m} < 50\mu\text{m}$) in 1 ml for the Series 2 tests (Port of Blyth). All data are the average of three replicates \pm standard error. (Note: scale is logarithmic).