Operational efficiency of ballast water biocides at low water temperatures.

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Summary

In the period 2013-2015 the effect of two biocides used for the treatment of ballast water has been evaluated at low ambient temperatures. Peraclean® Ocean and sodium hypochlorite were used as biocides.

Most of the tests were conducted during winter and early spring at the laboratories of IMARES in Den Helder, using outdoor cultures from which phytoplankton and zooplankton (as communities) were collected for the tests. In summer 2013, tests were also conducted at Svalbard in the Arctic using natural zooplankton. Here only Peraclean® Ocean was tested.

Based upon estimated levels of Peracetic Acid (PAA), the main active ingredient in Peraclean® Ocean, EC_{50}-values for phytoplankton varied between 0.68 and 1.65 and those for zooplankton taken from the cultures between 0.17 and 0.93 mg/l and for zooplankton collected at Svalbard between 0.42 and 1.90 mg/l. For zooplankton this is comparable to standard toxicity tests reported by De Lafontaine *et al.* (2008), but the phytoplankton communities appear less sensitive.

Based upon measured levels of free chlorine shortly after dosing, the EC_{50}-values for phytoplankton varied between 0.04 and 0.90 mg/l and for zooplankton between 1.21 and 3.18 mg/l. Within the tests, no effect of temperature was seen in the phytoplankton tests (temperatures ranging from 2°C to 20°C), while rotifers seemed more sensitive at 10°C, compared to 2°C. The toxicity valeus found were, however, much lower than those reported by the UK Environmental Agency (Sorokin *et al.* 2007), suggesting that toxicity is higher at low temperatures.

1 Introduction

Roughly half of the ballast water management systems (BWMS) currently listed (Lloyds, 2012) make use of an active substance to treat the ballast water. Research on efficacy of BWMS is conducted on land-based test facilities that are in majority located in temperate areas. Certification testing is conducted in spring and summer when production is high enough to meet the requirements for the challenge water as specified by IMO (G8). The assessment of environmental risk of the active substance is based upon standardised bio-assays with temperate or even tropical test organisms. The test temperatures characteristically vary between 20-30°C.

Shipping activities –and thus the use of ballast water- are not restricted to temperate waters in spring and summer. In the Port of Rotterdam for instance, more than 6 million m^3 ballast water was taken in February 2008 (Haskoning, 2008). Obviously, the organism load in February will be considerably lower than later in the season, but organisms are not absent and during favourable conditions winter blooms may occur at water temperatures <5°C. This is even more so in the Arctic region. Here relative high organism levels may be produced, enhanced by 24h of sunshine during the Arctic summer. As the sea ice retreats rapidly, shipping intensity in the Arctic increases at the same speed. Even though part of the shipping is only passing through, the use of ballast water with temperatures close to zero will increase in the next decades.

At the moment, the consequences of low water temperatures for the efficacy and environmental impact of active substances is not clear. A review by Heugens et al. (2001) indicates that in most cases toxicity increases with increasing temperature. However, this is based upon studies where temperature was increased to the upper tolerance levels and ranging between 15-30°C. Only few studies address the effects of contaminants at (Arctic) temperatures between zero and 5°C and these are either related to oil & gas production and mining (De Hoop et al., 2011; Chapman & McPherson, 1993; Chapman et al., 2006). No information is available on the effect of low temperatures on the toxicity of readily degradable biocides as are used in BWMS.

IMARES has started research on the toxicity/efficacy of biocides used for treatment of ballast water. The aim is to clarify whether ballast water treatment at very low temperatures (<5°C) as may be encounters in Arctic waters during the summer and in temperate and sub-Arctic water during the winter period. For the laboratory based studies, standardized ecotoxicity test protocols were modified to simulate ballast water treatment: i.e., all testing is conducted in the dark.

The first set of tests was conducted during winter and spring in the Netherlands (2012-2013), using marine and freshwater organisms cultured in outdoor ponds. A second set of tests was conducted during summer 2013 at Ny Alesund, Svalbard (above the Arctic Circle), using marine organisms collected in the field.

The tests were financed by Evonik Degussa, DFO Canada, IMARES R&D (KB14-005)

2 Assignment

The aim of the project is to determine the influence of low water temperatures (<5°C) on the functioning of BWMSs. These conditions are characteristic for Arctic waters and for the Great Lakes during winter and early spring. Functioning of a BWMS is defined here by two indicators: 1) its ability to remove organisms from the ballast water taken in (efficacy), and 2) the residual toxicity of active substances at discharge (environment risk).

In an exploratory study, organisms used for standardised ecotoxicity testing were hatched at or adapted to low water temperatures. The results of these test were not satisfactory, because hatching was slow and incomplete. Therefore, outdoor whole-community cultures were set-up during winter time from which unsorted samples representing zooplankton and phytoplankton communities were tested. The tests with these natural communities were conducted under laboratory conditions, but at the same temperatures as the water in the outdoor cultures. Peraclean® Ocean (PO) and sodium hypochlorite (NaOCl) were used as biocides in the tests. In summer 2013, tests were conducted with natural zooplankton at Svalbard, using Peraclean® Ocean at temperatures equal to the water they were collected from.

Algal growth in ecotoxicity tests is routinely assessed by measuring chlorophyll-a content and activity of the photosynthetic processes by means of fluorescence. The efficacy of ballast water treatments to remove phytoplankton is routinely measured by cell counts using vital staining, which is a far more elaborate and labour intensive method. The applicability of fluorescence measurements to assess differences in algal mortality was also explored.

3 Materials and Methods

3.1 Winter tests Netherlands – Culturing and harvesting organisms

Outdoor cultures
Type: Freshwater (local ditch), Sea water (Eastern Scheldt)
Volume: 4000 L
Conditions:
-Continuous light
-Mixing by aeration
-Added nutrients
FW: full ISO, or N,P, Si
SW: F2, N,P, Si
-Weekly monitoring WQ parameters and Chl-a
-Weekly counts of zooplankton and phytoplankton cell density

Organisms
Separation by sieving 50µm: ± zoo- vs phytoplankton
Zooplankton: Rotifers (Testudinella, Brachionus), ciliates (Paramecium), copepods
Phytoplankton: Diatoms (Melosira, Attheya, Nitzschia), Euglenidae (Trachelomonas), Greenalgae (Ankistrodesmus)

Concentration of organisms
Zooplankton by sieving
Phytoplankton by sieving or by increasing pH to 10.5 using 8N NaOH, and restoring pH in concentrate using HCl.

3.2 Arctic tests Ny Alesund

The Arctic research station at Ny Alesund, Spitsbergen was visited in July 2013. Field samples were taken to collect test organisms. It appeared to be too late in the season for phytoplankton to bloom and even concentration steps did not yield measurable amounts of phytoplankton. The effort was, therefore, directed at testing zooplankton, which could be collected in low numbers from Kongsfjorden.

3.3 Analyses

3.3.1 Water quality

The basis water quality parameters pH, temperature, salinity and oxygen saturation in the cultures was measured using handheld instruments with electrodes.
The temperature in the test cabinets was automatically registered, using Escort Junior temperature loggers.

3.3.2 Chemical concentrations

All test concentrations reported are nominal concentrations based upon volumetric calculations. Chlorine levels were analysed using a Spectroquant® Chlorine Cell Test on a WTW S6 Photometer. This test is based upon the DPD-method.

3.3.3 Phytoplankton

A BBE-Moldaencke Algal Lab Analyser (ALA) was used for the assessment of bulk chlorophyll-a (µg/l) and genty (%activity) using fluorescent properties of pigments (e.g., chlorophyll). Minimal 25 ml of the sample is put in a glass cuvette with a stirring magnet. This is placed in the measuring chamber. The ALA gives back readings for chl-a and activity in different algal classes and the concentration of yellow substances. These algal classes (greenalgae, diatoms, cryptophyta and cyanobacteria) are based upon emission spectra and are only indicative. The limit of detection of the ALA is approx. 3 µg/l chl-a, which in standardised cultures of algae used for ecotoxicity testing corresponds to 10.000 to 50.000 cells/ml.

Handheld fluorometers have been developed especially for use in ballast water assessment. It may be expected that these have a much higher level of sensitivity as they claim to discriminate at the level of 10 viable cells/ml. For part of the tests, the Turner Ballast Check (BC) could be used in parallel with the ALA. The BC uses 3 ml plastic cuvettes, that are placed into the measuring chamber. It gives the chl-a concentration in µg/l and the yield as measure of photosynthetic activity.

For the assessment of surviving phytoplankton cells after a ballast water treatment, vital staining using FDA is prescribed in the ETV protocols (ETV, 2010). This dye is an indicator for membrane stability based on intracellular esterase activity, resulting in green fluorescence inside the living cell. Experience with the phytoplankton communities evaluated is necessary, as stationary cells may show less fluorescence (Peperzak & Brussaard, 2011). A solution of 2.5 mg FDA in 10 ml dimethyl sulfoxide (DMSO) was used for staining. Aliquots of 500 µl were stored at -20°C until use. To 2 ml of sample with phytoplankton, 5 µl of the FDA solution was added. This was mixed and after 5 min storage in the dark, living cells were counted on a fluorescence microscope. As the FDA solution leaks out of the cells, the sample should be counted within 30 min.

3.3.4 Zooplankton

Zooplankton was counted using a dissection microscope with variable magnification. Organisms were considered dead when they were not showing any movement, even after tactile stimulation.

3.4 Test description

3.4.1 Phytoplankton tests

Phytoplankton was tested using PO and NaOCl using slightly different procedures. Basically, test water was collected from the ‘phytoplankton’ culture. One Control (C0) and 6 test concentrations (C1-C6) were prepared, by adding increasing amounts of the test substance from a concentrated stock solution in milli-Q. The test substance was thoroughly mixed through the culture using a plunger and replicates were poured in separate Erlenmeyer flasks for the exposure period.

The tests were prepared and conducted in a climate chamber at the same temperature as the outdoor cultures, used to extract the organisms from. In some tests, parallel series were exposed to a different temperature. As the tests simulate a ballast water treatment, the flasks remained in the dark during the full exposure period. During test initiation and subsampling dimmed light was used.

Subsamples for organism counting were taken at different moments in time to see whether low temperatures induced delayed effects. In all PO-tests, samples were taken after 1 day and 3 days. In some tests samples were taken after 2 or 6 days exposure. In the chlorine-tests, initially cell densities and chlorophyll were assessed directly after initiation and after 24 and 48h. Later only 24h assessments were conducted.

Zooplankton tests

Tests were conducted at the IMARES laboratories (Den Helder, the Netherlands) using zooplankton concentrated from outdoor cultures and at Svalbard using zooplankton concentrated from local sea water.

Zooplankton was concentrated using a 50 µm mesh sieve. As variable and low numbers of copepods were present in the outdoor cultures, the concentrate was sieved using a 100 µm mesh sieve to remove these, resulting in organisms between a size of 50 and 100 µm. The zooplankton test was, therefore, effectively a test with rotifers. From a stock with a density of approx. 10,000 org/l (10 org/ml), 14 beakers were filled with 500 ml of test water containing the organisms. The water was continuously stirred to keep the organisms homogeneously distributed. The test substance was directly added to each beaker and immediately mixed through the full contents using a plunger, in order to avoid local over-dosing. A control (C0) and 6 test concentrations (C1-C6) were made in duplo (replicate A and B).

The tests were prepared and conducted in a climate chamber with the same temperature as the outdoor cultures, used to extract the organisms from. As the tests simulate a ballast water treatment, it remains in the dark during the full exposure period. During test initiation and subsampling dimmed light was used.

Subsamples for organism counting were taken at different moments in time to see whether low temperatures induced delayed effects. The exact day of sampling depended on the relation of starting day and weekends. Day 2, 3 5, 6 and 7 were sampled in different tests.

For the countings, 6 subsamples of 1 ml each were taken from a test beaker after thorough homogenisation using a plunger. These subsamples were counted separately using a binocular microscope.

At Svalbard, the number of organisms appeared to be too low to follow this procedure. Therefore, organisms were individually added into the test solution in 24-well plates. Each well contained 3 organisms and survival was assessed directly in the plate without further manipulations.

4 Results

4.1 Phytoplankton analysis procedures

In all tests where vital counts were completed, they consistently reflected a dose-response relationship: With increasing dose, a lower number of living cells was counted. For chlorophyll-a concentration and photosynthetic activity, the relationship was not consistent (Figure 1). In the test conducted Febr 26th, the different analyses showed comparable dose response curves, although the genty parameter of the ALA (chl-a%) showed a strange peak at 9.9 mg/l. A week later (March 4th), chlorophyll-a levels measured with ALA and BC strongly increased until 1 mg/l before dropping to levels below the detection limit at the highest concentration. The genty parameter and yield followed the counts, but remained at a high level (40% of control values). The chl-a% showed increase to 60% of the control values at 10mg/l and then disappeared, while the BC-yield further increased to 80% of the control values at 31mg/l. In another test (not shown), chlorophyll-a levels showed a slight decline to 50% of control values, while genty and yield remained more or less stable (never getting below 80% of control values), but cell counts indicated no survival in the two highest concentrations.

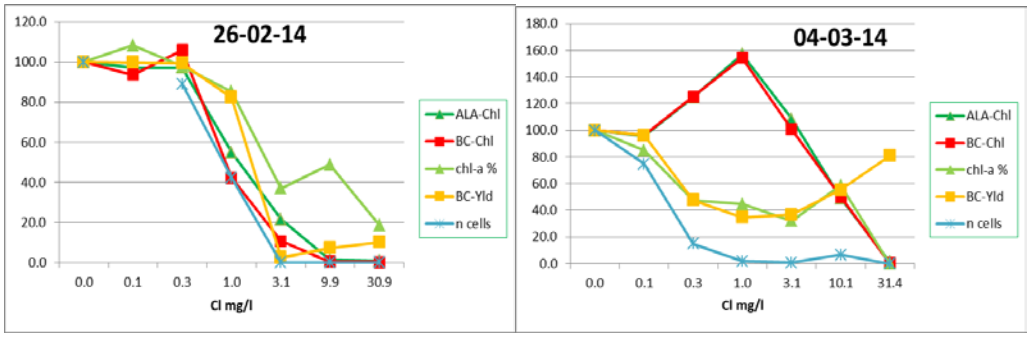

Figure 1 *The effect of NaOCl on a marine phytoplankton community, comparing different analyses (see main text for explanation).*

Similar effects were seen for tests with marine and freshwater phytoplankton exposed to PO (Figure 2). Where the cell counts showed clear dose-response curves with no survival in the highest concentrations, the chlorophyll-a concentrations (only ALA used) showed a very variable response to the PO, showing sometimes even significant increase. The genty parameter showed a better dose-response pattern, but never reached zero at the highest concentrations.

Fluorescence analyses are developed for in-situ measurement of chlorophyll-a in living plants, giving an indication of the health status of plants. When phytoplankton is treated with biocides, the cells and chloroplasts disintegrate and the chlorophyll-a disperses in the water. The fluorescence methods are not developed to analyse free chlorophyll-a. The apparent increases are artefacts caused by the free chlorophyll-a and possibly other pigments. It may be that this free chlorophyll-a disappears in time (during a voyage of the ship), but within the duration of the current tests it was not seen. For a good interpretation of the effects of temperature on the mortality of phytoplankton, vital staining is a more reliable method and will be used for the further analyses.

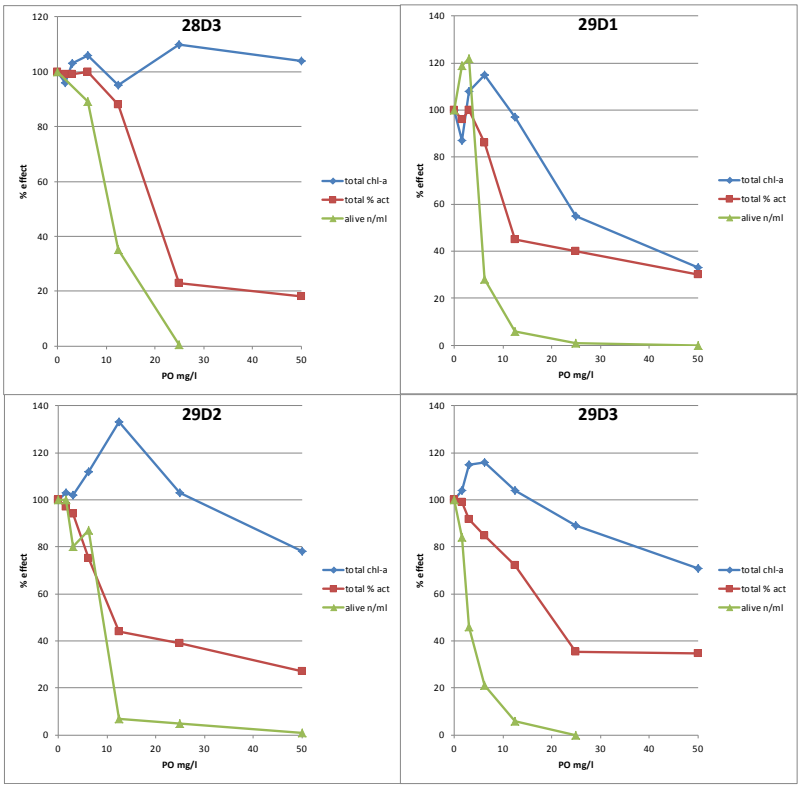

Figure 2 *Comparison of dose-response curves for total chlorophyll-a (blue lines and markers), activity % of photosynthetic system (genty) (red lines and markers) and FDA cell counts (green lines and markers) in one marine (28D3) and 3 freshwater tests.*

4.2 Outdoor communities

In winter and early spring of 2013, cultured phytoplankton and zooplankton communities were challenged with Peraclean Ocean. The results for the tests with phytoplankton are based upon cell counts using FSA as vital stain. The EC50-values for different exposure times are given in Table 1. Due to a rapid increase in effect over a short concentration range, sometimes EC_{50}-values could only be approached (indicated with ~). The EC_{50}-values do not show a constant difference over time, indicating that the effects are rather instantaneous. This is supported by the constancy of NOEC, the concentration needed to kill 100%, and the hill slope parameter of the sigmoidal curve (Table 6 in Appendix). The results do not show a difference between marine and freshwater tests.

Table 1 EC_{50} values (mg/l) for Peraclean Ocean tested with natural phytoplankton communities derived from freshwater (FW) and marine (SW) cultures. Calculations are based upon FDA cell counts.

Code	Water type	Temp (°C)	EC_{50} 1d	EC_{50} 2d	EC_{50} 3d	EC_{50} 6d
1D29	FW	5.5	~ 11.3	-	~ 6.12	~ 6.24
2D29	FW	5.5	16.5	-	~ 21.77	8.43
3D29	FW	5.3	5.50	-	6.41	3.11
1D28	SW	3.5	6.79	6.48	7.40	5.92
2D28	SW	3.5	7.41	6.89	6.71	-
3D28	SW	4.2	~13.5	-	#	-

Not able to calculate an EC50; - not analysed

Rotifers exposed for varying periods of time showed comparable responses, indicating that for these organisms the effect is instantaneous too. The response of marine and freshwater rotifer communities seems very comparable (Table 2).

One community (taken from culture D30) was tested against 2 temperatures. At the time, the temperature in the culture was 12°C. One series was tested at 4.2° and the other at 15°C. Rotifers and crustaceans (exposed separately) showed different results. The rotifers showed the same EC_{50}-value at both temperatures. The copepods on the other hand appeared to be more sensitive at the higher temperature (Table 2).

Table 2 EC_{50} values (mg/l) for Peraclean Ocean tested with natural rotifer and copepod (C) communities derived from marine (SW) and freshwater (FW) cultures.

Code	Water type	Temp (°C)	EC_{50} 1d	EC_{50} 2d	EC_{50} 3d	EC_{50} 4d	EC_{50} 6d	EC_{50} 7d
1D28	SW	4.2	2.20		2.15			1.72
2D28	SW	3.9	8.80			9.21		9.59
3D28	SW	3.7	1.66	1.51	~ 3.34			~1.50
4D28	SW	3.5	4.53			2.11	2.54	
1D29	FW	4.1	5.77	3.22	5.03		3.62	
2D29	FW	4.2	5.34			7.60		5.50
1D30	FW	4.2	2.40	1.98				
2D30	FW	15.0	1.81	2.01				
1D30-C	FW	4.2		12.5-50				
2D30-C	FW	15.0		9.32				

Marine phytoplankton communities exposed to NaOCl show EC_{50}-values varying between 0.04 and 0.93 mg/l nominal Cl. The NOECs varied from <0.1 (lowest test concentration) to 0.5 mg/l. The NOECs were not directly related to the concentration needed to obtain 100% effect (Table 3). Temperature did not seem to have much effect on the toxicity of chlorine. It was observed that when in the exposure with 10 mg/l Cl more than 1 mg/l free Cl was left after 24h, no living cells were counted (test 2 and 6, 7 not analysed).

Table 3 Results of exposure of cultured marine phytoplankton communities to NaOCl. Calculations are based on FDA counts after 24h exposure. Effect concentrations in mg free Cl/l nominal.

Sequence	Temp °C	NOEC mg/l	100% mg/l	EC_{50} mg/l	-95% mg/l	+95% mg/l	r^2	Hill slope
1	8.6	0.32	>10	0.82	0.78	0.86	0.998	2.9
2	7.4	0.3	<9.9	0.85	0.78	0.94	0.991	2.4
3	7.5	<0.1	31.4	0.16	0.13	0.19	0.954	2.5
4	2.5	0.1	31.1	0.27	0.25	0.28	0.997	2.4
5	20	<0.1	31.7	0.25	0.19	0.32	0.942	1.5
6a	3.1	<0.1	9.8	0.10	0.10	0.10	1.000	2.4
6b	7.5	<0.1	<9.8	0.04	0.01	0.24	0.996	4.1
7	2	0.5	10	0.93	<	>	0.983	-

Naturally occurring barnacle larvae were used in a test with NaOCl. Due to circumstances the surviving organisms could only be counted directly after initiation (<30min) and after 3 days. Between 30 min and 3 days the effects of chlorine markedly increased. This was most obvious at nominal concentrations of 5 and 10 mg Cl/l. At 50 mg /l the effect was maximal within 30 min, whereas at 1 mg/l, the effect size increased from 29 to 35 %. This is shown in the steepening of the dose-response curve (Figure 3), which is quantified by the hill-slope parameter increasing from 0.91 to 3.75, and the marked decrease of the EC_{50}-value (Table 4). Note that the concentration needed to kill 100% of the organisms remained the same, because even after 3 days still a surviving larvae was found in the 5 mg/l, as well as in the 10 mg/l exposure.

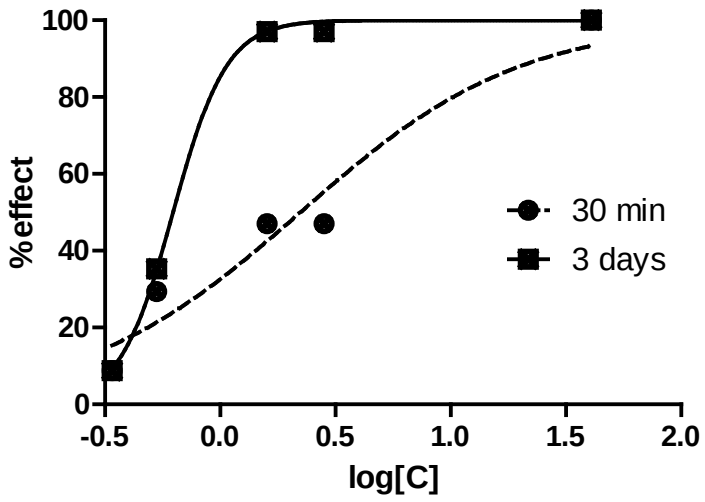

Figure 3 Dose-response curve of barnacle larvae exposed to chlorine.

Further zooplankton tests were conducted with communities collected from the outdoor cultures. Here only the freshwater cultures developed sufficient organism densities to allow testing. These tests are summarized in Table 4 (test 2 to test 6). For practical reasons, only the size fraction between 50µm and 100µm was used, mainly consisting of rotifers. A maximum treatment dose of 10 mg Cl/l was used, as rotifers are relatively sensitive for chlorine compared to the larger crustacean species. Because the first tests indicated only a very limited difference in effect size between 24h and 48h exposure, accompanied by increased mortality in the controls, only 24h data are used.

The EC_{50}-values were consistently higher for the rotifers compared to the barnacle larvae, but in general 5 or 10 mg Cl/l was sufficient to kill all organisms in the test. Three tests were conducted at 10°C, compared to 2°C for the other tests. In these tests, the EC_{50}-values appeared lower than at 2°C, except for Test 3, which showed an extremely low Hill slope. When the results for rotifer are plotted, the curves for the 10°C tests are clearly to the left (lower effect concentrations) compared to the 2°C tests (Figure 4).

Table 4 Results of exposure of natural marine barnacle larvae (test 1, 66h exposure) and cultured freshwater rotifer communities to NaOCl (24h exposure). EC_{50}-values in mg free Cl/l measured within 30 min; NOEC and 100% effect exposure nominal. Tests denoted a and b were initiated simultaneously with the same community.

Sequence	Temp °C	NOEC mg/l	100% mg/l	EC50 mg/l	-95% mg/l	+95% mg/l	r^2	Hill Slope
1 (30 min)	2	0.5	50	2.23	0.97	5.13	0.947	0.91
1 (3d)	2	0.5	50	0.62	0.58	0.67	1.00	3.75
2	2	0.05	10	2.23	1.53	3.24	0.87	3.38
3	2	0.05	10	1.51	0.63	3.61	0.84	1.20
4a	2	0.5	>10	3.07	1.42	6.67	0.83	1.84
4b	10	<0.05	5	1.21	0.75	1.95	0.93	3.11
5a	2		10	3.18	0.02	498	0.97	13.92
5b	2		10	2.81	1.57	5.03	0.84	2.16
6a	10	<0.56	5.6	1.58	1.26	1.98	0.94	13.41
6b	10	<0.56	5.6	1.35	1.04	1.74	0.88	5.68

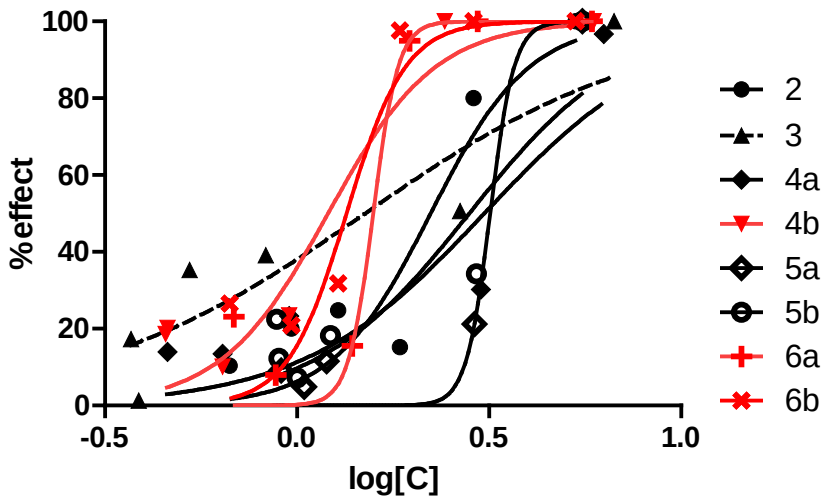

Figure 4 Combined dataset rotifer tests; in red 10°C tests

The 10°C tests were the only tests where less than 10 mg Cl/l was sufficient to kill all organisms. A direct comparison of NOEC-values is more complicated, due to the high variation in effect size at the lower doses. This variation is partly a methodological artefact, because the test animals are not quantitatively added to the test concentrations at the start. It is also a characteristic of chlorine and other biocides of which the effects are based upon the activity of free radicals. Chlorine will react directly with all organic material in the test water and even though the water is directly homogenized after addition of the stock solution it is a matter of change how many organisms are killed at first shot.

It is the remaining chlorine radicals that cause the continued killing as long as there is sufficient left. This is called TRO (Total Residual Oxidants). It is this reaction that is responsible for the relatively steep dose-effect curves.

4.3 Zooplankton Arctic

At Svalbard only larger zooplankton (marine copepods) could be collected for testing. These were quantitatively exposed to PO. Fourteen separate tests could be conducted (Table 5). The toxicity consistently increased from 24h to 48h, indicating that the effect of PO was not maximal yet after 24h.

The zooplankton at Svalbard seems less sensitive to PO than the rotifers cultured in winter in the Netherlands, but comparably sensitive to the copepods (only 1 test).

Table 5 Overview of test results for Arctic zooplankton Svalbard exposed to Peraclean Ocean.

Test code	EC_{50} 24h	EC_{50} 48h
SV002	7.85	4.78
SV003	9.46	5.22
SV004	8.47	7.37
SV005	6.61	4.18
SV006	23.10	8.65
SV007	12.24	6.56
SV008	7.35	5.76
SV009	7.96	6.16
SV010	13.25	7.96
SV011	13.94	9.54
SV012	10.46	6.48
SV013	24.92	18.85
SV014	22.81	18.95
SV015	15.03	11.69
Average	13.10	8.73
Min	6.61	4.18
Max	24.92	18.95

5 Discussion

The toxicity of the biocides Peraclean Ocean and sodium hypochlorite, has been tested at low ambient temperatures as may occur in the Arctic during summer, but also in temperate areas during winter.

The efficacy of PO is based upon Peracetic acid (PAA), which could not be measured during the current research project. PAA has, however, been analysed during other tests with PO. In these tests, PAA levels were 10-11% of the nominal PO levels directly after dosing. Using 10% as correction for the toxicity data, the EC_{50}-values based upon PAA vary from 0.68 to 1.65 mg/l for phytoplankton, 0.17 to 0.93 mg/l for zooplankton in winter and 0.42 to 1.90 mg/l for Svalbard zooplankton. For zooplankton these values are in the same range as have been reported by De Lafontaine *et al.* (2008). Phytoplankton shows higher toxicity values.

The toxicity of chlorine is based upon nominal values (most phytoplankton data) as well as measured concentrations of free chlorine shortly after dosing. Toxicity values for chlorine varied from 0.04 mg/l to 0.90 mg/l for phytoplankton and 1.21 to 3.18 mg/l for zooplankton. These values are much lower than those summarised by the UK Environmental Agency (Sorokin *et al.* 2007). This suggest that temperature may cause higher efficacy, as well as a higher environmental risk. This is, however, not directly supported by the results of the current tests. The phytoplankton tests span a temperature range of 2°C to 20°C without a temperature-related difference in toxicity, while the rotifers tested at 10°C seem to show higher toxicity than at 2°C.

6 Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 1th of April 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

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Justification

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The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: Drs. P. de Vries
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Signature: 

Date: 27 March 2015

Approved: Drs. F.C. Groenendijk
Head of Department Maritime

Signature: 

Date: 27 March 2015

Appendix A

Table 6 *Test results for cultured phytoplankton communities exposed to Peraclean Ocean. Nominal values used.*

Marine	T(°C)	Day	EC50	-95%	95%	r2	Hill	NOEC	100%
1D28	3.5	T1	6.79	6.35	7.26	0.9915	4.9	3.1	>50
		T2	6.48	5.37	7.83	0.9468	3.4	3.1	>25
		T3	7.40	6.61	8.28	0.9848	3.8	3.1	>12.5
		T6	5.92	5.25	6.67	0.9809	6.0	3.1	>12.5
2D28	3.5	T1	7.41	5.97	9.19	0.9989	3.6	<6.3	>25
		T2	6.89	3.30	14.4	0.9917	3.8	3.1	>12.5
		T3	6.71	5.77	7.80	0.9959	4.0	3.1	50
3D28	4.2	T1	~13.54			0.9581	~16.24	6.3	25

Freshwater	T	Day	EC50	-95%	95%	r2	Hill	NOEC	100%
1D29	5.5	T1	~11.32			0.5938	~20.83	3.1	>50
		T3	~6.124			0.924	~20.04	3.1	50
		T6	~6.242			0.9217	~112	3.1	50
2D29	5.5	T1	16.48	5.95	45.7	0.7361	8.9	12.5	>50
		T3	~21.75			0.4314	~19.11	12.5	>50
		T6	8.43	5.95	11.9	0.8642	6.3	3.1	>50
3D29	5.3	T1	5.50	0.58	0.90	0.8693	1.5	3.1	>50
		T3	6.41	5.20	7.91	0.9435	2.6	3.1	50
		T6	3.11	2.43	3.99	0.9169	2.3	<1.6	25
1D30	*4.2*	*T3*	*<NOEC*	*0.49*	*29.5*	*0.776*	*2.5*	*<12.5*	*50*

Table 7 Overview of test results for rotifer communities exposed to PO. D28 marine; D29 and D30 freshwater.

Test code	Temp (°C)	Day	EC50	95-	95+	r2	Hill Slope	n	DF
Zp1D28	4.2	1	2.20	1.63	2.98	0.881	2.05	10	8
Zp1D28	4.2	3	2.15	1.54	3.01	0.858	2.05	10	8
Zp1D28	4.2	7	1.72	1.08	2.77	0.701	2.14	10	8
Zp2D28	3.9	1	8.80	6.14	12.60	0.833	1.47	14	12
Zp2D28	3.9	4	9.21	5.02	16.88	0.592	2.16	14	12
Zp2D28	3.9	7	9.59	7.14	12.87	0.869	5.57	14	12
Zp3D28	3.7	1	1.66	1.23	2.23	0.848	3.24	8	6
Zp3D28	3.7	2	1.51	1.20	1.91	0.912	10.77	8	6
Zp3D28	3.7	3	~ 3.335			0.793	~ 17.49	10	8
Zp3D28	3.7	7	~ 1.496			0.989	~ 28.81	10	8
Zp4D28	3.5	1	4.53	3.11	6.59	0.890	1.80	12	10
Zp4D28	3.5	4	2.11	1.28	3.48	0.740	1.19	12	10
Zp4D28	3.5	6	2.54	1.60	4.04	0.796	1.20	12	10
Zp1D29	4.1	1	5.77	3.69	9.01	0.993	1.80	4	2
Zp1D29	4.1	2	3.22	2.48	4.19	0.928	2.11	12	10
Zp1D29	4.1	3	5.03	3.44	7.35	0.881	1.48	12	10
Zp1D29	4.1	6	3.62	2.97	4.41	0.959	1.95	12	10
Zp2D29	4.2	1	5.34	4.15	6.86	0.917	3.18	12	10
Zp2D29	4.2	4	7.60	6.21	9.32	0.928	3.28	12	10
Zp2D29	4.2	7	5.50	4.57	6.61	0.960	2.40	12	10
Zp1D30	4.2	1	2.40	2.06	2.78	0.964	2.72	12	10
Zp1D30	4.2	2	1.98	1.43	2.73	0.874	5.07	12	10
Zp2D30	15.0	1	1.81	0.08	43.14	0.953	13.76	10	8
Zp2D30	15.0	2	2.01	1.38	2.91	0.943	9.35	10	8

Table 8 Overview of test results Arctic zooplankton Svalbard. Nominal concentrations.

Test code	Test day	EC10	EC50	EC90	-95%	+95%	r^2	HillSlope	n	DF
SV002	1	2.95	7.85	31.84	7.06	8.73	0.996	2.245	6	4
SV003	1	4.79	9.46	39.81	8.82	10.14	0.998	3.23	6	4
SV004	1	5.01	8.47	32.6	8.23	8.72	1.000	4.19	6	4
SV005	1	4.47	6.61	173.7	6.22	7.02	0.996	5.632	7	5
SV006	1	2.86	23.10	104.8	7.97	67.01	0.502	1.052	7	5
SV007	1	4.89	12.24	106.3	10.16	14.75	0.987	2.394	6	4
SV008	1	2.96	7.35	20.89	6.05	8.91	0.987	2.416	6	4
SV009	1	4.25	7.96	18.67	5.08	12.45	0.891	3.5	6	4
SV010	1	5.52	13.25	14.31	11.93	14.73	0.996	2.507	6	4
SV011	1	4.89	13.94	9.763	10.91	17.83	0.981	2.095	6	4
SV012	1	3.36	10.46	186.5	7.32	14.94	0.959	1.933	6	4
SV013	1	3.58	24.92	30.66	17.34	35.82	0.959	1.132	6	4
SV014	1	4.96	22.81	18.24	19.56	26.59	0.991	1.441	6	4
SV015	1	2.12	15.03	14.9	11.77	19.18	0.981	1.123	6	4
SV002	2	1.73	4.78	14.83	3.19	7.18	0.934	2.157	6	4
SV003	2	1.71	5.22	30.81	4.00	6.81	0.977	1.964	6	4
SV004	2	3.07	7.37	23.46	5.44	9.98	0.967	2.507	6	4
SV005	2	1.63	4.18	133.1	3.59	4.86	0.977	2.333	7	5
SV006	2	0.15	8.65	135.8	1.53	49.05	0.346	0.5434	7	5
SV007	2	1.06	6.56	110.9	4.43	9.70	0.955	1.207	6	4
SV008	2	1.97	5.76	13.25	4.76	6.97	0.987	2.043	6	4
SV009	2	2.79	6.16	15.97	4.40	8.61	0.951	2.773	6	4
SV010	2	4.27	7.96	17.7	7.27	8.72	0.997	3.532	6	4
SV011	2	2.95	9.54	10.72	7.09	12.82	0.975	1.873	6	4
SV012	2	1.79	6.48	493.1	4.44	9.45	0.957	1.707	6	4
SV013	2	2.67	18.85	40.49	11.99	29.65	0.936	1.124	6	4
SV014	2	2.65	18.95	16.87	12.67	28.34	0.951	1.116	6	4
SV015	2	1.23	11.69	13.6	9.50	14.37	0.987	0.9763	6	4