ECOLOGICAL RISK OF TREATED BALLAST WATER:
A MESOCOSM EXPERIMENT

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ABSTRACT

As a consequence of the IMO Ballast Water Convention, in the near future large amounts of water treated with an active substance will be discharged in harbours and coastal areas. With regard to the ecological risk assessment of active substances used in ballast water treatment systems, mesocosms may be applied. Routinely, mesocosms are applied as 'higher tier tests' in the ecological risk assessment of pesticides. For ballast water testing, adaptation of the test set-up is necessary, as not a small amount of a toxic substance is added, but a significant volume of water is replaced instead.

During spring 2011, such an experiment was conducted in 4-m^3 outdoor marine mesocosms with PERACLEAN Ocean® as the active substance. Three different treatment levels were created by replacing 10% of the volume of test systems with treated ballast water aged for 1 hour (BW-d0), 24 hours (BW-d1) or 5 days (BW-d5). Two control systems did not receive any treatment. At the same time, the toxicity of the ballast water was tested with standard laboratory bioassays confirming earlier test results. During the 69 day exposure period, the water compartment was sampled weekly. At the end, the test systems were drained and the bottom compartment was sampled.

The results show that replacement of water without remaining active substances is not free from effects. However, the level of toxic substances present in the treated water corresponded with the amount of effects. Effects seen in bioassays are not directly copied in mesocosms. Results might be affected by physical characteristics like pH, oxygen, DOC, N/P. However, high risk indicated by the toxicity tests corresponded with high level of disturbances of the ecosystem. Mesocosms can be used in higher tier assessment of whole effluents, such as ballast water. Even when as much as 10% of the water volume is replaced by treated water, treatment effects are obvious. Moreover, clear recovery of some systems was observed within the test period enabling to assess the No Observed Ecological Adverse Effects Concentration (NOEAEC) conform De Jong et al. (2008). The mesocosms are a useful tool for assessment of treatments including the side effects in discharge ballast water, by integrating effects as well as recovery of multiple interacting species.

INTRODUCTION

For ballast water treatment systems that use active ingredients there is a need to test their system under IMO guideline G9. This guideline asks for estimating the ecological risk of the active substance used in the BWMS for the receiving environment. Toxicity tests, the so-called bioassays, need to be conducted to estimate the ecotoxicological impact of the treatment to the environment. A bioassay is a test in which an organism is exposed to a concentration series of a substance or to whole effluents like discharged ballast water (WET-testing). A batch of these tests including different trophic levels of organisms like algae, crustacean and fish are used to assess the risk of treated ballast water in a harbor. There is a big difference between the little organisms used in bioassays compared to the hugeness and complexity of a harbor. Not to speak about all different harbors around the world. Therefore, results from these single species tests need to be used with caution. In the risk assessment this is done by using safety or assessment factors (Table 1). If not much information is available the uncertainty is very high and for the translation to ecosystem levels a high safety factor is used. The more information you gather, the lower the safety factor can be. However, as long as you look at only single species tests, it is hard to translate the effects into an ecosystem where populations interact.

Table 1 Assessment factors for risk assessment of ballast water under IMO G9 as presented by GESAMP in the 38th meeting.

Assessment factor	GESAMP 38th meeting (PNEC general)
10,000	Lowest short-term L(E)C50 from 1-2 fresh/marine species from one or two trophic levels
1,000	Lowest short-term L(E)C50 from 3 fresh/marine species representing three trophic levels
100	Lowest short-term L(E)C50 from 3 fresh/marine species representing three trophic levels + 2 additional marine species
100	1 chronic NOEC from fresh/marine species but not algae
50	2 chronic NOEC from fresh/marine species including algae representing two trophic levels
10	3 chronic NOEC from fresh/marine species including algae representing three trophic levels

For (non-) agricultural biocides this problem was recognized and experimental ecosystems (mesocosms) with multiple species have been developed. These mesocosms allow the fate and impact of a treatment on the ecosystem to be examined under longer-term controlled, but realistic (semi-natural) conditions. In the legislation procedure of biocides, mesocosms are well accepted tools and data can overrule toxicity data derived from single species laboratory tests. These mesocosm tests are all performed in stagnant fresh water systems and dosed with an active substance. More recent, IMARES developed marine stagnant systems and tested these systems with addition of substances. Applicability for use with effluents like discharge ballast water was not investigated yet. The replacement of a portion of water may in itself already cause multiple effects. Therefore, as part of the Interreg IVb project "North Sea Ballast Water Opportunity" (NSBWO), the applicability of mesocosms for use in whole effluent type of testing was investigated. This pilot study was set-up to try to answer two research questions:

- How to discriminate between effects caused by replacement of water and effects of toxic substances?
- How predictive are toxicity test results (i.e. bioassays) for effects of treated ballast water on ecosystems?

MATERIALS AND METHODS

The mesocosms that were used for this study intent to mimic a shallow soft sediment ecosystem as much as possible. This type of ecosystem is common along the whole European coast. The mesocosms, however, are static where the "real" ecosystem is characterised by a high rate of water refreshment. It was, however, decided not to use flow through mesocosms for this test in order to allow a good determination of the fate of the treatment. In total 8 tanks were selected for this pilot study. Each circular tank had a volume of 4 m3. The tanks were filled with a sediment layer and a water compartment. Phyto- and zooplankton was introduced with the test water at the start of the establishment phase. A list of species representative for various taxonomic classes that are commonly present in shallow soft sediment coastal ecosystems were introduced deliberately. Sponges and bivalves both use phytoplankton as main food resource. As bivalve species the sediment dwelling cockle was selected. Two gastropod species were introduced; the small mudsnail and the larger periwinkle. Both species feed mainly on benthic algae, but the mudsnails live on the sediment surface while periwinkles prefer the solid substrate of the mesocosm sides. As a representative of the group of crustaceans larger than zooplankton, the mudshrimp was introduced. This shrimp lives in the top layer of the sediment where it feeds on organic material. Deeper burrowed in the sediment the lugworm can be found where it lives in U-shaped burrows. Lugworms are very important sediment bioturbators in many shallow coastal ecosystems. For stabilization of the ecosystems the water fraction was re-circulated for one month. This creates a stable community of pelagic invertebrates and micro-flora, as well as similar water quality conditions in all mesocosms at the start of the application of the test substance (chlorophyll-a, pH, dissolved oxygen concentration, salinity and nutrient concentrations). Just before the start of the exposure phase, each mesocosm unit became static. Within each system water circulation was created by continuous aeration. The salinity in the mesocosms was kept at the initial value 30±2‰. Evaporation losses were replenished with demineralised water. Each mesocosm was covered with a transparent lid to minimise the influence of rainfall.

Seawater was treated on several days to mimic different discharge and concentration circumstances. The following discharge water treatments were created: 5 days old (BW-d5), 24 hours old (BW-d1) and freshly treated (BW-d0). Each treatment was dosed into two mesocosms. Two control tanks (Control) did not receive any ballast water. Dosing into the mesocosms was performed on the same day by replacing about 10% of the total water volume. The ballast water was treated with PERACLEAN Ocean® provided by Evonik Degussa GmbH. This treatment consists of two main active substances Peracetic acid (PAA) and hydrogen peroxide (H_2O_2). To check the dosing concentrations the discharge water was measured before and after dosage in the mesocosm tanks. No H_2O_2 and PAA could be measured for the control and BW-d5. H_2O_2 was still present in the BW-d1 and BW-d0 treated water and about 10% could still be measured after dosing into the mesocosms. Hardly any PAA could be measured in BW-d1 and none after dosing in the mesocosms. Only BW-d0 showed the presence of PAA. The dosed mesocosms were monitored for another 69 days to monitor the effects. An extensive list of analyses were performed during the exposure period. This includes water quality parameters like oxygen and pH but also sampling of zoo- and phytoplankton communities. After the deployment period, the systems were emptied and also the sediment compartment was sampled intensively.

As results from the study were still being processed at the moment of the presentation, only observations were presented. No statistical analysis had been done on the data.

RESULTS

Toxicity of the ballast water was tested at the start of the exposure in the mesocosm experiment. In total three bioassays were selected: a bacteria test (ISO, 2007), an algae test (ISO, 2006) and rotifer test (MicroBioTests Inc.). Each bioassay tested all the different treated ballast waters and a sample of untreated ballast water. The samples were diluted in a concentration series according to the test procedures of each bioassay. At 10% dilution, the expected effects of the treated ballast water samples in the mesocosms could be derived (Figure 1). For the Control tanks and for the BW-d5 tanks no toxicity was found. The algal toxicity test showed inhibitory effects of ~40% for the BW-d1. No effects was found for the bacteria and the rotifer test. All three bioassays showed effects for the freshly prepared ballast water (BW-d0), ranging from 100% inihibition for the bacteria to ~60% effect for the algae and rotifers.

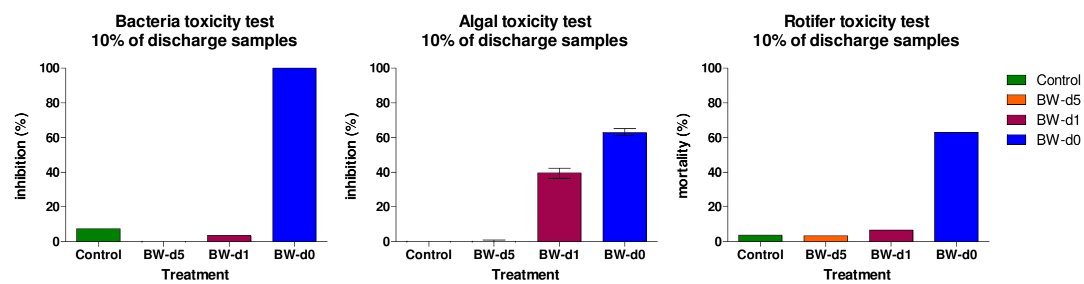

Figure 1 Results of the bioassays at 10% of the discharge ballast water samples. Presented are results from a bacteria, an algae and a rotifer test.

Figure 2 shows a selection of the results for the mesocosm study. The line graphs present on the x-axis the day numbers before and after dosing. For the bar graphs the treatment is presented on the x-axis. In all graphs the error bars are the ranges of the different treatments. For the line graphs the ranges of the control are accentuated with the green color.

The biomass of the phytoplankton community is presented as total chlorophyll-a concentration. The before period, is the stabilization time for the systems. The graph shows that the systems were following similar patterns. After dosage a short stimulation is seen for the five day old ballast water (BW-d5). After about three weeks the pattern is similar to the control again. BW-d1 showed negative effects the first ten days and stimulation effects for about three to four weeks. BW-d0 reduced the first ten days and then kept stimulated for about five weeks. After six weeks all systems show very low concentrations of chlorophyll-a which is normal for summer conditions. Due to the very low concentrations it is uncertain whether full recovery took place.

The zooplankton community was sampled weekly and biweekly samples were selected for analyses. Calanoid copepods seem to show stimulation for BW-d5 and BW-d1, but not for BW-d0. This effect is seen more often in mesocosm research and is often referred to as a classic mesocosm result. As response to effects on other species a population is stimulated until the dose becomes toxic. Stimulation of the harpacticoid copepods is seen for BW-d1 and BW-d0. BW-d5 follows the control system. After 42 days, the zooplankton populations collapse in all systems.

Bivalvia larvae produced by the introduced cockles were more numerous in the BW-d1 and BW-d0 compared to the control and BW-d5. After a short period, the larvae settled and disappeared from the water column. However, sampling the benthic community at the end of the study confirmed the higher amounts of juvenile cockles in BW-d1 and BW-d0.

The population of *Corophium volutator* amphipods in the mesocosms seemed to be able to cope with the stress of the dosing with BW-d5 and BW-d1. The population was reduced for the mesocosms which were dosed with BW-d0. However, another amphipod (*Microdeutopus gryllotalpa*) showed up in the BW-d0 discharge and not in the other systems. Still total amphipod counts were still lower compared to the other treatments.

The polychaeta *Polydora ciliata* shows the classic mesocosm graph, wherein the species population compared to the control is stimulated for BW-d5, inconclusive for BW-d1 and reduced for BW-d0.

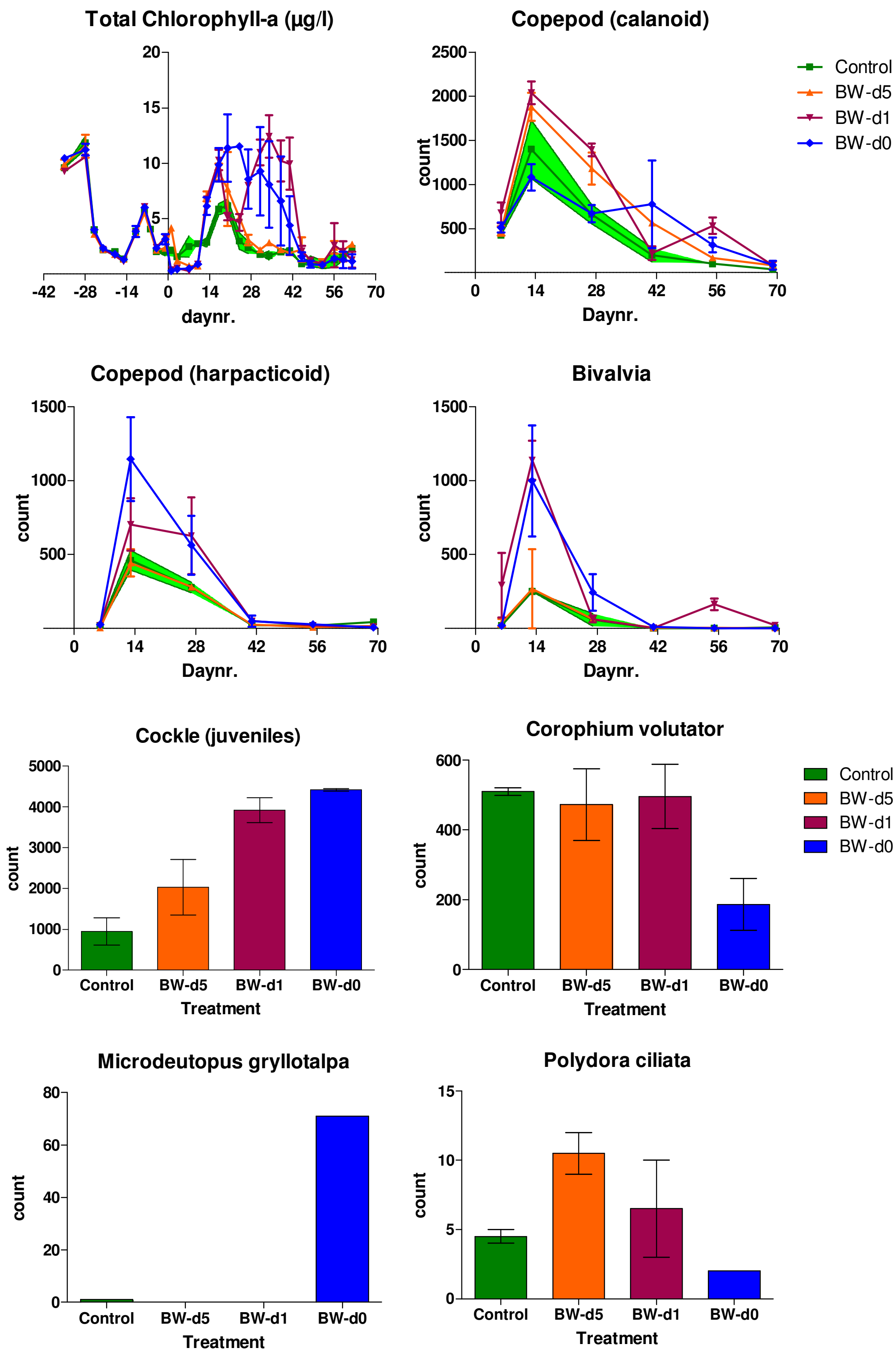

Figure 2 Graphs of a selection of the ballast water mesocosm results. Presented are phytoplankton (chlorophyll-a), zooplankton (copepods and bivalvia), amphipods (*Corophium volutator* and *Microdeutopus gryllotalpa*) and polychaeta (*Polydora ciliate*).

CONCLUSIONS

In Table 2 results are summarized by comparing the control situation with the three different treated ballast waters. If there was a stimulation seen in the analysis for the treatment compared to the control this is shown as a green color, red color shows negative effects and yellow if no clear effects were visible. If the effects were very well present, but only visible shortly this is shown dashed. The three toxicity tests are presented first.

Even though no toxicity was found for the BW-d5 the mesocosm study does reveal some effects. These effects can be a result of replacing the water but also physical changes caused by the treatment (pH, oxygen). In toxicity tests the aim is to look at chemical effects and not physical effects even though it is part of a treatment. In a mesocosm study and at discharge in a harbor the physical changes will be part of the effluent and thus effect. One toxicity test revealed negative effects for BW-d1, the algae test. The phytoplankton biomass, however, was stimulated in the mesocosm study and not hampered. This is opposite to what is expected to happen. More parameters seem to be stimulated by the treatment and only one is showing negative results. Remember, however, that phytoplankton was seriously reduced the first ten days after treatment. All toxicity tests revealed negative effects of the treatment. In the mesocosms negative effects for a lot of the species was seen, but also stimulating effects like for the algae after the first drop, the harpacticoid copepods and the large amounts of cockle spat.

It is concluded that replacement of water without remaining active substances is not free from effects. However, the level of toxic substances present in the treated water corresponded with the amount of effects. Effects seen in bioassays are not directly copied in mesocosms. Results might be affected by physical characteristics like pH, oxygen, DOC, N/P. However, high risk indicated by the toxicity tests corresponded with high level of disturbances of the ecosystem. Mesocosms can be used in higher tier assessment of whole effluents, such as ballast water. Even when as much as 10% of the water volume is replaced by treated water, treatment effects are obvious. Moreover, clear recovery of some systems was observed within the test period enabling to assess the No Observed Ecological Adverse Effects Concentration (NOEAEC) conform De Jong et al. (2008). The mesocosms are a useful tool for assessment of treatments including the side effects in discharge ballast water, by integrating effects as well as recovery of multiple interacting species.

Table 2 Summary of a selection of results for the pilot ballast water mesocosm study. Presented is the effects for the control system versus each treatment for a list of type of tests (toxicity test, organism and water characteristics) including the type of output (C=concentration, N=numbers/counts and G=growth). The effects are presented in colour where Yellow=no clear effects, Red=negative effects, Green=stimulation effects, dashed=temporary effects.

Type of test		**Control versus**		
		BW-d5	**BW-d1**	**BW-d0**
Bacteria test	-			
Algae test	-			
Rotifer test	-			
Total Chlorophyll-a	C			
Copepod (calanoid)	N			
Copepod (harpacticoid)	N			
Bivalvia larvae	N			
Cockles (juveniles)	N			
Corophium volutator	N			
Microdeutopus gryllotalpa	N			
Polydora ciliate	N			
Halichondria panicea	G			
Mytilus edulis	G			
Ctenodrilus serratus	N			
Cockles (adults)	N			
Littorina littorea	N			
TOC	C			
DOC	C			
Acidity	C			
Oxygen	C			
Ortho-phosphate	C			
Oligochaeta sp.	N			
Arenicola marina	N			
Ammonium	C			

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