

## 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 into 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 rather, a significant volume of water is replaced.*

*During spring 2011, such an experiment was conducted in 4-m<sup>3</sup> 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 degree of impact. Effects seen in bioassays are not directly copied in mesocosms. Results might be affected by physical characteristics like pH, oxygen, DOC and nitrogen or phosphorus levels. However, high risk indicated by the toxicity tests corresponded with high levels of disturbances in 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 to De Jong et al. (2008). The mesocosms are a useful tool for assessment of treatments, including the side effects, in discharged ballast water, by integrating effects as well as recovery of multiple interacting species.*

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### Introduction

For ballast water treatment systems that use active substances, there is a need to test their system under IMO guideline G9. This guideline asks for estimating the ecological risk of the

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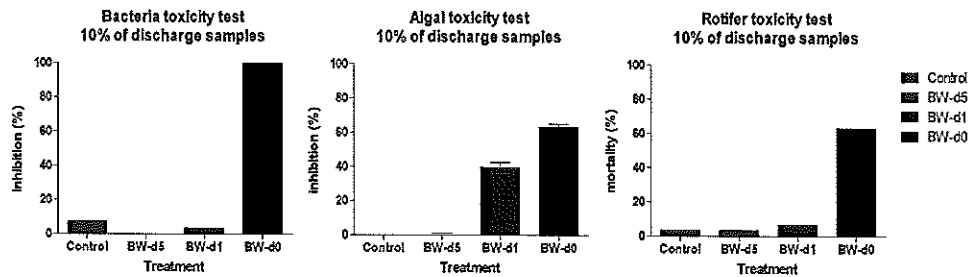
tests. These mesocosm tests are all performed in stagnant fresh water systems and dosed with an active substance. More recently, IMARES developed marine stagnant systems and tested these systems with additions of substances. Applicability for use with effluents like ballast water discharge was not investigated yet. The replacement of a portion of water may, in itself, already cause multiple effects. Therefore, as part of the InterregIVb project called "North Sea Ballast Water Opportunity" (NSBWO), the applicability of mesocosms for use in whole effluent testing was investigated. This pilot study was designed to try to answer two research questions:

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

### **Materials and Methods**

The mesocosms that were used for this study were intended 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, whereas the "real" ecosystem is characterised by a high rate of water refreshment. It was decided, however, not to use flow-through mesocosms for this test in order to allow a good determination of the fate of the treatment. In total, eight tanks were selected for this pilot study. Each circular tank had a volume of 4 m<sup>3</sup>. The tanks were filled with a sediment layer and a water compartment. Phyto- and zooplankton were introduced with the test water at the start of the establishment phase. Lists 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 their primary food resource. For the 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. Deeply burrowed in the sediment, the lugworm can be found in its habitat 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 microflora, 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.

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

Figure 2 shows a selection of the results for the mesocosm study. The line graphs present the number of days on the x-axis 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 a 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 during the first ten days and stimulation effects for about three to four weeks. BW-d0 reduced during the first ten days and then remained stimulated for about five weeks. After six weeks, all systems show very low concentrations of chlorophyll, 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 a 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 BW-d1 and BW-d0 when compared to the control system 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.

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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 remained lower when compared to the other treatments.

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

### Conclusions

In Table 2, results are summarized by comparing the control situation with the three different treated ballast waters. If there was stimulation seen in the analysis for the treated water compared to the control, this is shown in green, whereas red indicates negative effects and yellow indicates that no clear effects were visible. If the effects were clearly observable, but only for a short period, this is shown as dashed. The three toxicity tests are presented first.

Even though no toxicity was found for BW-d5, the mesocosm study does reveal some effects. These effects can be a result of replacing the water and also a result of physical changes caused by the treatment (e.g., 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 an important characteristic of the effluent and, thus, have a potential 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 has shown negative results. It should be recalled, however, that phytoplankton was seriously reduced the first ten days after treatment. All toxicity tests revealed negative effects for the treatment. In the mesocosms negative effects for many of the species were seen, but stimulating effects were also seen, such as for the algae after the first drop, the harpacticoid copepods and the large amounts of cocklespat.

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,

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