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#### Abstract

The effects of contaminated river water on the filtration rate of zebra mussels from a clean reference site were studied. After a 48-h exposure period to filtered water from the rivers Rhine, Meuse and Amstel (The Netherlands), the filtration rate was measured. It was demonstrated that water from contaminated locations inhibited the filtration rate. Inhibition was higher during low water levels in the rivers Rhine and Meuse than during high water levels, suggesting that contaminants are diluted during high water levels. It is concluded that the shortterm filtration-assay with *D. polymorpha* can be used for assessing water quality.

#### Introduction

The freshwater mussel, *Dreissena polymorpha*, has been used in several kinds of ecotoxicological studies. The valve movement of the mussels is used to monitor acute changes in water quality (Kramer *et al.*, 1989), while the accumulation of metals and organic chemicals in the tissues of mussels has been measured to assess the bioavailability of these substances in the field (Karbe *et al.*, 1975; Kraak *et al.*, 1991; Van Hattum *et al.*, 1992). Furthermore, *D. polymorpha* has been used as a test organism in ecotoxicological laboratory experiments (Kraak *et al.*, 1992; Bleeker *et al.*, 1992; Fisher *et al.*, 1993).

Biomonitoring studies demonstrated high concentrations of metals in mussels from the river Meuse and elevated concentations from the river Rhine (Kraak *et al.*, 1991). Their occurence in these contaminated rivers and the accumulation of metals suggests that the zebra mussel is a tolerant species. In contrast, laboratory studies demonstrated that metals reduce the filtration rate of *D. polymorpha* (Kraak *et al.*, 1992). Because of this discrepancy, it seems worthwhile to study whether effects on the filtration rate take place in the field. To this purpose, the effects of contaminated river water on the filtration rate of zebra mussels from a relatively clean reference site (Lake Markermeer) were examined. These experiments were designed to address the following hypotheses: 1) The filtration rate of zebra mussels is inhibited by water from contaminated rivers, and 2) This inhibition can be measured in a short-term laboratory assay.

#### Materials and methods

Zebra mussels (*Dreissena polymorpha* (Pallas)) and water were collected from Lake Markermeer (The Netherlands), a relatively contaminant-free location (Kraak *et al.*, 1991). The water was sieved ( $25 \mu$ m) and kept in a storage barrel from which it was pumped continuously over a sand filter. The mussels were sorted by length (1.6–2.0 cm) and distributed over the experimental units. The average length did not differ between treatments. An experimental unit consisted of 25 mussels placed in a plastic aquarium (12,51), containing 31 of filtered (0.45 m) lake water. Water temperature was kept at 15 °C, hardness was 150 mg CaO/I and pH was 7.9. The water was aerated continuously and always saturated with oxygen. Evaporation of water was limited by covering the aquaria with sheets of glass. The light/dark regime was synchronized with the natural day/night regime.

Four experimental units were run simultaneously, one of them being a control (Lake Markermeer water). Water from the following locations was tested: Eijsden, on the river Meuse at the Belgian/Dutch border (km 620): Grave, more downstream on the river Meuse (km 790); Lobith, on the river Rhine at the German/Dutch border (km 863), and three locations in the river Amstel, all located within the city limits of Amsterdam: 1) near the Amstel park, 2) the Berlage Brug and 3) the Magere Brug. To check the influence of uncontaminated water with different physical-chemical characteristics than that of Lake Markermeer, a control experiment was performed in which mussels were exposed to water from the relative clean, oligo-mesotrophic Lake Maarsseveen I. Water from all locations was tested twice; in the case of the locations in the rivers Rhine and Meuse once during high water levels and once during low water levels. These experiments were carried out during the autumn of 1990 and spring 1991. The locations in the river Amstel were tested in the spring of 1992 and water levels did not differ between the two sampling dates.

Filtrated (0.45  $\mu$ m) surface water was added to the aquaria on the day after collection of the water and the mussels. After 24 and 48 hours, the water in the aquaria was renewed. After 48 hours, after renewal of the water, the filtration rates were measured. To determine the filtration rate, the mussels were fed with Scenedesmus acuminatus (30000 cells/ml). The algal concentration decreased, due to the filtration activity of the mussels. Pilot experiments showed that in this experimental setup, sedimentation and cell division of algae played an insignificant role. Algal grazing was determined by taking 4 water samples from each aquarium at 0, 10, 20, and 30 minutes after addition of the algae. The algal concentrations in the water samples were measured using a Coulter Counter. The filtration rate was calculated from the decrease in algal concentration, according to Coughlan's formula (1969):

$$m = \frac{M}{nt} ln \frac{C_o}{C_t},$$

in which

m = filtration rate in ml/mussel/hour, M = volume of the test solution (3000 ml), n = number of animals/aquarium (25),

t = duration of the filtration measurement in hours,

- $C_0$  = algal concentration at the beginning of the determination of the filtration rate, and
- $C_t$  = algal concentration at time t.

The filtration rates of the experimental treatments were expressed as a percentage of the filtration rate in the corresponding control.

To determine the metal concentrations in the mussels, the soft tissues, without byssus threads, of mussels collected in the field were placed individually in 2.2 ml polyethylene tubes, freeze dried, weighed and dissolved by wet digestion using nitric acid and hydrogen peroxide. The animals were analysed for metals by flame or furnace AAS following Timmermans et al. (1989). Quality control of metal analysis was performed using digestion blanks and reference material (IAEA shrimp MA-A-3/TM and IAEA simulated freshwater W-4). The measured values were in good agreement with the certified values (<10% deviation). To test if the metal concentrations in mussels from the test locations differed from the metal concentrations in the controls, Bartlett's test for homogeneity of variances, one-way analysis of variance and Scheffé's test for a posteriori comparison of means were used.

#### **Results and Discussion**

Most treatments decreased the filtration rate of the zebra mussels; water from the river Meuse at Eijsden had the strongest effect: the filtration rate was reduced to 12% and 58% (Fig. 1). Meuse water sampled at Grave, 170 km downstream, was less inhibitory. In the river Rhine, a reduction to 57% was observed in the first experiment, but no effect was observed in the second experiment. In the river Amstel a decreasing inhibition was observed from the Amstelpark to the Magere bridge (Fig. 2), i.e. from the outer city to the inner city. Water from the oligotrophic Lake Maarsseveen I had no effect on the filtration rate of zebra mussels from Lake Markermeer, so the negative effects of water from the test locations could indeed be attributed to differences in water quality. This is also indicated by the differences between the two observations in the rivers Rhine and Meuse. The different responses on the two sampling dates can be explained by differences in water discharge: during autumn the drainage in the rivers was lower (Lobith 1,073 m<sup>3</sup> sec<sup>-1</sup>; Eijsden 28 m<sup>3</sup> sec<sup>-1</sup>) than during spring (Lobith 1,516  $m^3$  sec-1; Eijsden



*Fig. 1.* Filtration rate of zebra mussels exposed for 48 h. to water from the rivers Rhine and Meuse. The filtration rate is expressed as a percentage of that from mussels in Lake Markermeer water.



*Fig.* 2. Filtration rate of zebra mussels exposed for 48 h. to water from the river Amstel. The filtration rate is expressed as a percentage of that from mussels in Lake Markermeer water.

546 m<sup>3</sup> sec<sup>-1</sup>). The different degrees of inhibition of the filtration rate were corroborated by differences in physicochemical water variables: in general, concentrations of contaminants were higher during low water levels than during high water levels (RIWA, 1993).

In the river Amstel, a water quality gradient from the outer to the inner city could be observed. The mouth of the river Amstel is in Amsterdam, where its water is divided over the city's system of canals. These canals are regularly flushed with water from Lake IJ, a part of the relatively clean Lake Markermeer, which may explain the better response of mussels to water from the inner city.

In addition to the filtration rate experiments, the concentrations of Cu, Zn, Cd and Pb were determined in mussels originating from the water sampling locations in the rivers Rhine and Meuse (Fig. 3). Mussels from Eijsden contained the highest Cu, Zn, Cd and Pb concentrations, all differing significantly (p < 0.05) from mussels from Lake Markermeer, the reference area. The Cu, Zn, Cd and Pb concentrations in mussels from Grave and the Cu and Cd concentrations in mussels from Lobith were also significantly (p < 0.05) higher than in mussels from Lake Markermeer. In the case of Cd and Pb, the concentrations in mussels from Eijsden were significantly (p < 0.05) higher than in mussels from Grave and Lobith. The results of the internal metal concentrations in mussels from the water sampling locations confirm the results of the filtration-assay: figure 3 shows that the highest metal concentrations were measured in mussels from Eijsden, where the most inhibitory water sample was collected. Nevertheless, animals were present at the contaminated locations. This suggests that those populations are developing more slowly than at clean locations or that populations may have been adapted to local contamination levels.

Our study demonstrated that the filtration rate of zebra mussels is affected by water from contaminated rivers, suggesting that effects on in situ populations are possible. It can be concluded that the short-term filtration assay can be used for assessing water quality. This expands the number of systems already used in Dutch rivers, like those based on daphnids, fish and Microtox tests (De Zwart and Folkerts, 1990). In the years 1975-1977 water from the river Rhine caused acute effects on daphnids (Slooff, 1983). Since then, the water quality of this river increased markedly, and 10 years later no acute effects on daphnids were observed (Canton et al., 1987). In the first experiment of this study however, water from the location Lobith caused a 40% reduction of the filtration rate. Since the water quality of the river Rhine remained the same or has even improved further, this suggests that the mussel filtration bio-assay may be more sensitive than the acute daphnid test. When testing Rhine water in 1988 with the Microtox test, De Zwart and Folkerts (1990) had to concentrate the water 100-fold on XAD-columns before about 50% inhibition was observed. This also indicates that the filtration bioassay, using clean reference populations of the zebra mussel ranks high in sensitivity among the available techniques for assessing water quality.



*Fig. 3.* Metal concentrations in mussels (mean  $\pm$  s.d.) from the rivers Rhine and Meuse and in mussels from Lake Markermeer (n = 10).

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