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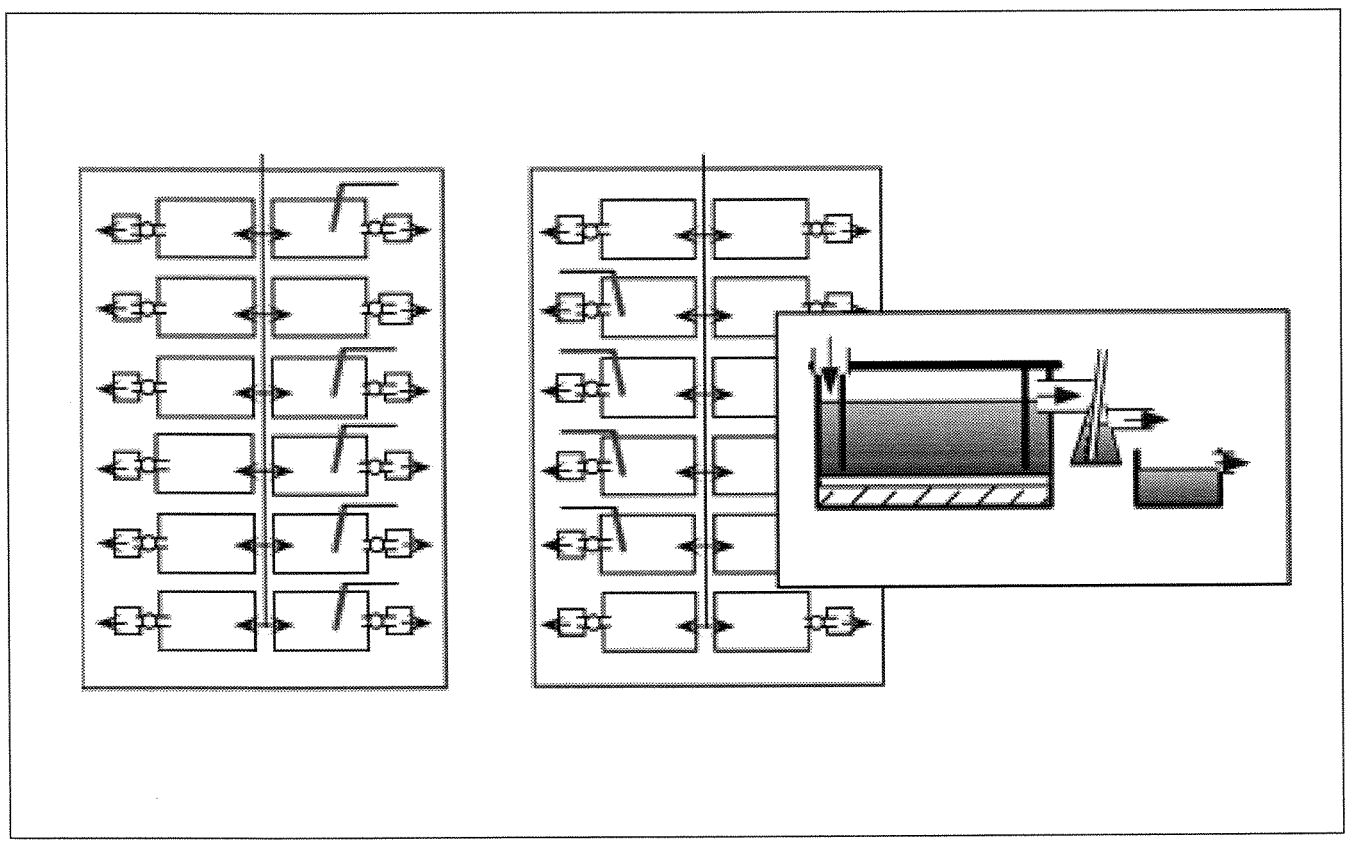
Interactions between eutrophication and contaminants

partitioning, bioaccumulation and effects on sediment-dwelling organisms



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REPORT

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Abstract

To study interactions between eutrophication and contaminants in marine sediments an experiment was designed and set up at NIVA's Marine Research Station, Solbergstrand. The experiment was performed in 24 continuously flushed glass aquaria within which three sediment-dwelling species were kept in a marine sediment. A filter-feeder, blue mussel, was kept in downstream aquaria. The experiment combined three environmental factors: oxygen availability, the presence or absence of contaminants, the addition of organic matter. The objectives of the project were: (1) to quantify differences in the partitioning of contaminants between sediment, pore water and biota as a result of the treatments, (2) to quantify effects of treatments and interactions between treatments on sediment-dwelling organisms, (3) to identify differences, if any, in the release of contaminants from the sediment as the result of treatments. All three contaminants bioaccumulated to higher levels in sediments with increased levels of organic material. Whereas feeding directly or indirectly appeared to be the major route for bioaccumulation of benzo(a)pyrene and mercury (Hg), was cadmium (Cd) also controlled by the concentration in pore water. Furthermore, sediment in enriched aquaria released more contaminants than sediment with low organic content. Organic enrichment had strong effects on growth in the three sediment-dwelling organisms. Growth was less affected by decreased oxygen availability. The presence of contaminants had only minor effects on the three sediment-dwelling species at the concentrations used here (4.8 mg/kg Cd, 0.2 mg/kg Hg, 1.3 mg/kg benzo(a)pyrene). Some biomarkers in *N. diversicolor* and *A. filiformis* reflected tissue contaminant concentrations, whereas there was no response in biomarkers in *A. alba* to any treatment or contaminant.

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1. eutrofiering	1. eutrophication
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Interactions between eutrophication and contaminants

partitioning, bioaccumulation and effects on sediment-dwelling organisms

Preface

The project was initiated by Jens Skei, and the final design was the result of numerous discussions within a group consisting of Jens Skei, Morten Schaanning, John Arthur Berge, Ketil Hylland from NIVA and Jonas Gunnarsson, Mattias Sköld from Kristineberg Marine Research Station (KMF). Dag Ø. Eriksen at the Institute for Energy Technology (IFE) provided much-needed input on the use of radioactive tracers. Dag Ø. Eriksen and Tone D. Bergan, IFE, did all analyses for gamma-emitters and some of the analyses for beta-emitters. The main part of the beta-analyses were done at KMF by Jonas Gunnarsson. Geochemical work was mainly done by Morten Schaanning with the assistance of Oddbjørn Pettersen. Measurements for biological effects were done at NIVA and at KMF by Ketil Hylland, Mattias Sköld and Jonas Gunnarsson. The project was jointly funded by SFT and NIVA. Jens Skei has been project manager and the contact at SFT for the project has been Per-Erik Iversen. The staff at Solbergstrand Marine Research Station (MRSS), Einar Johannessen, Håkon Oen and Oddbjørn Pettersen are thanked for their efforts to keep experimental conditions according to our specifications. Thanks are also due to Joanna Maloney, Ole Øystein Aspholm and Torild Nissen-Lie, who all contributed at different stages during the project.

Oslo, 15 September, 2006

Ketil Hylland

Contents

Summary

In marine environments influenced by human activities, the seabed is a major recipient of both contaminants and organic matter. To assess the importance of interactions between eutrophication and contaminants in marine sediments an experiment was designed and set up at NIVA's Marine Research Station, Solbergstrand (MRSS). The experiment was performed in 24 continuously flushed glass aquaria within which three sediment-dwelling species (*Nereis diversicolor*, *Amphiura filiformis* and *Abra alba*) were kept in a bottom layer of marine sediment. A filter-feeder, blue mussel *Mytilus edulis*, was kept in downstream aquaria. The experiment consisted of two levels of three environmental factors: oxygen availability (2.4-3.5 mg/l compared to approximately 7 mg/l), the presence or absence of contaminants (radiolabelled Cd (4.8 mg/kg), Hg (0.2 mg/kg) and benzo(a)pyrene (1.3 mg/kg) compared to no contaminants), with or without addition of organic matter (approximately 40 g C/m^2 compared to no addition). The objectives of the project were: (1) to quantify differences in the partitioning of contaminants (sediment, pore water, biota) as a result of the treatments, (2) to quantify effects of treatments and interactions between treatments on sediment-dwelling organisms, (3) to identify differences, if any, in the release of contaminants from the sediment as the result of treatments.

There was an initial retention of a labile fraction of cadmium (Cd) in the carbon-enriched sediments. Mercury (Hg) and benzo(a)pyrene was retained to the same extent in all treatments and no significant loss from the sediment was observed during the experimental period. In the pore water, Hg and benzo(a)pyrene were not detectable, whereas the concentration of Cd was consistent with the levels predicted from low sulphide ion activities recorded on Ag|AgS electrodes and the solubility of $CdS_{(s)}$.

The bioccumulation of Hg appeared to be predominantly controlled by ingestion of food particles and was significantly stimulated by organic enrichment. Particularly high metal concentrations in *A. alba* may have resulted from efficient feeding on added organic material containing adsorbed metals. Both food and water appeared to be involved in the bioaccumulation of Cd. Oxygen level affected accumulation of Cd in *A. filiformis* and *M. edulis*. This effect appeared primarily to occur via increased immobilisation of metals as sulphides under conditions of low oxygen availability. Bioaccumulation of benzo(a)pyrene was higher in the organically enriched compared to non-enriched aquaria (*N. diversicolor* and *A. filiformis*). In addition, more benzo(a)pyrene accumulated in blue mussels kept downstream of enriched aquaria compared to mussels kept downstream of aquaria not receiving organic material. Hypoxia did not appear to affect bioaccumulation of benzo(a)pyrene, in contrast to the results found for Cd.

Effects of organic enrichment and low oxygen availability were additive for growth in *N. diversicolor* and *A. filiformis*, whereas there was an interaction between hypoxia and organic enrichment in their effects on growth in *A. alba*, reflecting a decreased ability of this species to utilise the available organic material under hypoxic conditions. Sediment-bound contaminants had minor effects on growth and arm regeneration. Some biomarkers in *N. diversicolor* and *A. filiformis* reflected tissue contaminant concentrations, whereas there was no response in biomarkers in *A. alba* to any treatment or contaminant.

1. Introduction

1.1 General introduction

Interactions between eutrophication processes and the cycling of contaminants may be of major importance when it comes to evaluate and predict the bioavailability and fate of contaminants in the aquatic environment (Gunnarsson et al., 1995). Traditionally eutrophication processes have been studied separately from contaminant dynamics and only a few studies, mainly in limnic systems, have focused on possible interactions between these two environmental problems (Larsson et al., 1993; Lozano & Pratt, 1994; Taylor et al., 1991). Eutrophication is a complex process and has the potential to cause major changes in the ecosystem. One major effect is the decreased oxygen content in bottom waters and sediments commonly observed in eutrophic systems (Rosenberg et al., 1991). Decreased oxygen availability strongly affects benthic communities and may also indirectly affect the availability of contaminants. Decreased oxygen content in bottom waters may also result from low rates of water exchange such as that found in many Norwegian fjords.

Effects from eutrophication alone may modify how contaminants affect pelagic and benthic organisms in several ways. One of the characteristics of eutrophic systems compared to oligotrophic systems is the increased biomass in the former. Thus, a given contaminant load could conceivably be "diluted" into more individuals and more biomass in an eutrophic system than in an oligotrophic system, resulting in lower contaminant level in individual organisms (Olsson & Jenssen, 1975). The concentrations of dissolved and particulate organic material in the water column will furthermore be higher in more eutrophic systems, causing a decrease in the water-soluble fraction of contaminants (Baker et al., 1985; Evans, 1988). Finally, sedimentation rates are generally higher in more eutrophic systems, resulting in shorter residence times for particle-bound contaminants in the water column (Pavoni et al., 1990; Jonsson, 1992; Millard et al., 1993). The entire question of what interacts with what may also be turned the other way around, as the presence of contaminants may also affect the biological response to increased eutrophication. Increased food availability such as that found in more eutrophic systems would be expected to result in increased growth and reproduction (Dauvin & Gentil, 1989; Josefson & Jensen, 1992; Sköld & Gunnarsson, 1996). Such responses may however be decreased or abolished in the presence of contaminants above toxic thresholds (Herman et al., 1991; Weisse, 1991).

The third factor referred to above, decreased oxygen availability, may affect the bioavailability of contaminants (e.g. Krantzberg, 1994), decrease growth and affect reproduction (Nilsson & Sköld, 1996) and have deleterious effects on benthic organisms (Reish, 1974; Rosenberg et al., 1991; Diaz & Rosenberg, 1995).

Sediments are complex habitats. The exposure routes of contaminants to benthic organisms depend on factors such as physico-chemical composition of the sediments (grain size, organic carbon content, redox conditions) (Di Toro et al., 1991; Rule & Alden III, 1996) and biological factors (behaviour, feeding strategy, life cycle strategies) (Landrum & Faust, 1994; Lee II, 1992). Hence, it is difficult to predict by *in situ* studies the environmental implications of contaminated sediments due to the complexity of factors that may or may not be essential. Sediments may act both as a sink (Jonsson, 1992; Millard et al., 1993; Salomons et al., 1987) and as a potential source (Di Toro & Horzempa, 1982; Wildish et al., 1980; Wood et al., 1987) for contaminants and organic matter (Graf, 1992). Some obvious environmental effects of eutrophication are found in sediments, including excessive organic enrichment, oxygen deficiency, increased biomass of some species and/or the abolition of other species (Pearson & Rosenberg, 1978; Rosenberg & Loo, 1988; Gray, 1989). Decreased oxygen availability, caused by eutrophication-related processes, low rates of water exchange or a combination of the two, is also typical for coastal sediments. Furthermore, most contaminants accumulate in sediments up to several orders of magnitude above the levels found in water. Coastal sediments are therefore highly relevant as model systems to study how eutrophication, oxygen deficiency and the presence of contaminants may affect marine organisms. This project aimed to address interactions

between eutrophication and contaminants in marine sediment microcosms. We selected the following three factors for this study:

(1) organic enrichment;
(2) oxygen availability;
(3) the presence of contaminants.

One, two or all three of the above are found in most enclosed coastal systems. As indicated above, (1) and (2) are processes clearly related to eutrophication, although (2) can also be affected by rates of exchange of bottom waters in fjord-type environments. Both (1) and (2) may affect the bioavailability of contaminants, either directly through association between contaminant and organic material (1) or through modification of redox conditions in the sediment (both (1) and (2)). In general, increased content of organic material would be expected to decrease the bioavailability of organic contaminants. Although less important than for organic contaminants, organic enrichment would also be expected to decrease metal availability, especially for metals such as Hg and Cu. As mentioned above, both organic enrichment and reduced oxygen availability would be expected to reduce the redox values (Eh) in sediments. The redox value gives an approximation of the integrated charge of all chemical components in sediment and is low under reducing conditions (low oxygen, high sulphide) and high under oxygenated conditions. Low redox values will be associated with elevated concentrations of sulphide, thereby reducing the bioavailability of metals due to the low solubility of metal sulphides. This property has been suggested as a general method to assess the bioavailable fraction of metals (acid volatile sulphide, AVS; Di Toro et al., 1990).

To approach this problem we designed a marine sediment microcosm experiment with three experimental factors: organic enrichment, oxygen availability and the presence of contaminants. The factors were included singly and in combination, resulting in 8 treatments. Identical numbers of three sediment-dwelling invertebrates were added to all aquaria. In addition, blue mussels were kept in aquaria downstream each sediment aquarium. The microcosms were kept for 93 days, following which samples were taken of sediment, pore water and organisms. Samples were analysed for concentration of contaminants and the effects on sediment-dwelling organisms assessed. Effects on the experimental organisms were evaluated by growth, arm-regeneration and biomarkers (metallothionein-like proteins, glutathione reductase, glutathione *S*-transferase) at the end of a 93-days experimental period. The biomarkers were selected to reflect expected exposure to Cd and Hg (metallothionein-like proteins), variations in oxygen availability and oxidative stress (glutathione reductase) and exposure to benzo(a)pyrene (glutathione *S*-transferase).

1.2 Objectives

The project aimed to answer the following questions:

1. Will differences in organic enrichment affect the partitioning of environmental contaminants between sediment, pore water and sediment-dwelling organisms?

2. Will differences in the redox-conditions of the sediment (as caused by different levels of oxygen and/or organic enrichment) affect the partitioning of environmental contaminants between sediment, pore water and sediment-dwelling organisms?

3. Will organic enrichment, oxygen variability, and/or the presence of environmental contaminants affect growth or biomarkers in sediment-dwelling invertebrates?

4. Will the amount of contaminants released from the sediment differ between sediments receiving different levels of organic loading and/or deoxygenated/normal oxygen levels?

Management questions that may be approached through the project are:

1. To what extent need decisions considering improvements in the eutrophication status of an area be related to the level of environmental contaminants in that area?

2. To what extent need decisions considering improvement in the water exchange of an area be related to the level of contaminants in and/or eutrophication status of that area?

3. To what extent may eutrophication or oxygen depletion affect the role of contaminated sediment as a source to contaminants?

2. Materials and methods

2.1 An overview

The design consisted of two levels of each of three factors (organic enrichment, oxygen availability, contamination) and three replicate aquaria for each treatment (**Table 1**). One factor, the presence or absence of contaminants (only one concentration of each contaminant), was introduced through mixing into sediment before start of the experiment. A second factor, oxygen availability, was introduced through reducing oxygen in the water supplied to half the aquaria. The third factor, organic enrichment, was introduced in two steps. Organic material was mixed in with the sediment before start (at the same time as contaminants), but an additional batch given through water to the same ("enriched") aquaria on day 50.

Table 1. Overview of treatments. All aquaria contained 4 cm "base" sediment with 2 cm treated sediment layered on top. The code indicates the oxygen availability (D-deoxygenated, S-saturated), the presence or not of contaminants cadmium (Cd), mercury (Hg), benzo(a)pyrene (BaP) (C-contaminated, R-reference), the addition of organic material or not (O-organic material added, N-normal, no material added). "Trophic status" indicates where the treatment could be positioned in a oligotrophic – eutrophic gradient (most oligotrophic – HL: high oxygen, low organic; most eutrophic – LH: low oxygen, high organic).

Code (aquarium no) – replicates			"trophic status"	Sediment		Seawater	
				Cd, Hg, BaP	organic material	Flow (l/h)	Oxygen (mg/l)
DCO (3)	DCO (9)	DCO (12)	LH	+	+	1-2	deoxygenated
SCO (17)	SCO (18)	SCO (21)	HH	+	+	1-2	saturated
DCN (1)	DCN (5)	DCN (7)	LL	+	-	1-2	deoxygenated
SCN (14)	SCN (19)	SCN (22)	HL	+	-	1-2	saturated
DRO (4)	DRO (6)	DRO (11)	LH	-	+	1-2	deoxygenated
SRO (15)	SRO (20)	SRO (24)	HH	-	+	1-2	saturated
DRN (2)	DRN (8)	DRN (10)	LL	-	-	1-2	deoxygenated
SRN (13)	SRN (16)	SRN (23)	HL	-	-	1-2	saturated

Whole-glass aquaria were used and the total sediment-depth in the aquaria was approximately 6 cm. The bottom 4 cm was a marine sediment without contaminants or organic material added (identical for all aquaria). The top 2 cm contained contaminants and/or organic material (concentrated planktonic algae, *Skeletonema costatum*) or no addition. 12 aquaria received deoxygenated water. The remaining 12 aquaria received untreated seawater.

The contaminants used, cadmium (Cd), mercury (Hg) and benzo(a)pyrene (BaP), were added as pure compounds mixed with radioactive tracer (^{109}Cd, ^{203}Hg, ^{14}C-BaP, respectively).

Twenty polychaetes (*Nereis diversicolor),* 10 bivalves (*Abra alba)* and 10 brittle stars (*Amphiura filiformis*) were added to each aquarium. All animals were added on day 0. The outflowing water from each aquarium was aerated and passed through smaller aquaria with 10 blue mussels (*Mytilus edulis)* in each.

The end-points in the experiment were: (1) contaminant concentrations in sediment, pore water and organisms; (2) changes in sediment biogeochemistry (pH, redox, sulphide, organic content); (3) effects on growth, arm regeneration or biomarker responses in sediment-dwelling invertebrates.

2.2 Sediment and organisms

2.2.1 Sediment

A "base" sediment was prepared as a mixture of 70% subtidal and 30% intertidal defaunated sediment. The sediment contained 80-85% clay/silt (passed through a 63 µm-sieve) and 1.1 % total carbon. This sediment was used for the bottom 4 cm in all aquaria and as matrix for the addition of contaminants and organic material in the upper 2 cm. This sediment contained a large proportion of "fines", commonly found in bottom sediment at depths greater than 50 m. The concentration of total carbon is low compared to natural sediments.

2.2.2 Organisms

For the experiment we wanted a set of sediment-dwelling invertebrates that would fulfil the following requirements:

1. Represent important faunal groups in marine sediments;
2. Available in the Oslofjord/Skagerrak and known to survive experimental situations;
3. Represent different modes of feeding;
4. Known or expected not to interfere with other species in the system;
5. Their biology should be reasonably well documented

On the basis of the above, we selected one intertidal species, the polychaete *Nereis (Hediste) diversicolor*, and two subtidal species, the brittle star *Amphiura filiformis* and the bivalve *Abra alba*. Polychaetes, brittle stars and bivalves are dominant components in soft-bottom fauna and the project group had experience with all three species. The three species are ecological key-species, in intertidal (*N. diversicolor*; Möller et al.,1985) or subtidal (*A. alba* Rainer, 1985, *A. filiformis*; Sköld et al.,1994) soft-bottom communities.

The polychaete *N. diversicolor* is among the most-used marine invertebrates both in field studies and for experimental work. This species may use different modes of feeding, but the most important is filter-feeding and foraging on the sediment surface for organic material and detritus. *N. diversicolor* will construct U-shaped burrows lined with mucus, produce a mucus net and pump water through the burrow. This behaviour is to some extent regulated by the concentration of algae in the overlying water, although it is probable that *N. diversicolor* to a large extent irrigates is burrow with surface water even in the absence of algae.

The brittle star *A. filiformis* is a filter-feeder. Under oxic conditions the disc will be buried in sediment with only the arms showing. The arms have bristles that will collect material in the water immediately over the sediment surface and transport it to the mouth on the ventral side of the disc. Much of the oxygen-transport within this species will probably be by the water-channel system found in all echinoderms. Thus, contaminants may enter *A. filiformis* both through food and through tube feet (water channel system). This species will be more exposed to pore water than *A. alba*, but less than a sediment-dwelling polychaete such as *N. diversicolor*.

The bivalve *A. alba* is a surface deposit feeder that will select organic material on the surface with a long siphon. This species would probably only to little extent be exposed to pore water as water for respiration would also be taken from the surface. *A. alba* is common in areas with high organic loads, it is a short-lived bivalve that grows quickly when food (=organic carbon) is available.

In addition to the three sediment-dwelling species, blue mussel (*Mytilus edulis*) were kept in aquaria downstream of the sediment-containing aquaria. The intention of including blue mussels was to quantify the loss of contaminants from the sediments during the experimental period and under the different treatments.

All four species to be used in the experiment were analysed for Cd, Hg, Cu and Zn prior to starting the experiment (**Table 2**).

Table 2. Concentrations of the metals Cd, Hg, Cu and Zn in the four species used in the experiment (n=3).

species	Cd-ng/g dw (mean; range)	Hg-ng/g dw (mean; range)	Cu-µg/g dw (mean; range)	Zn-µg/g dw (mean; range)
Nereis diversicolor	13; 12-14	10; 8-11	2.5; 2.3-2.7	17.4; 16.8-18.5
Amphiura filiformis	95; 84-111	43; 26-60	2.9; 2.4-3.6	54.9; 41.5-65.4
Abra alba	107; 93-134	76; 60-103	5.7; 5.0-6.5	66.2; 57.0-76.9
Mytilus edulis	294; 281-310	15; 15	1.2; 1.0-1.3	30.4; 20.6-37.7

2.3 The test-system

2.3.1 General description

Twenty-four glass aquaria (285 x 465 x 230 mm) were used and the total sediment-depth in the aquaria was 6 cm. The bottom 4 cm was base sediment without contaminants or organic material (**Figure 1**). The top 2 cm contained contaminants and/or organic material or no addition. 12 aquaria received deoxygenated water (**Figure 2**). The remaining 12 aquaria received oxygen-rich seawater.

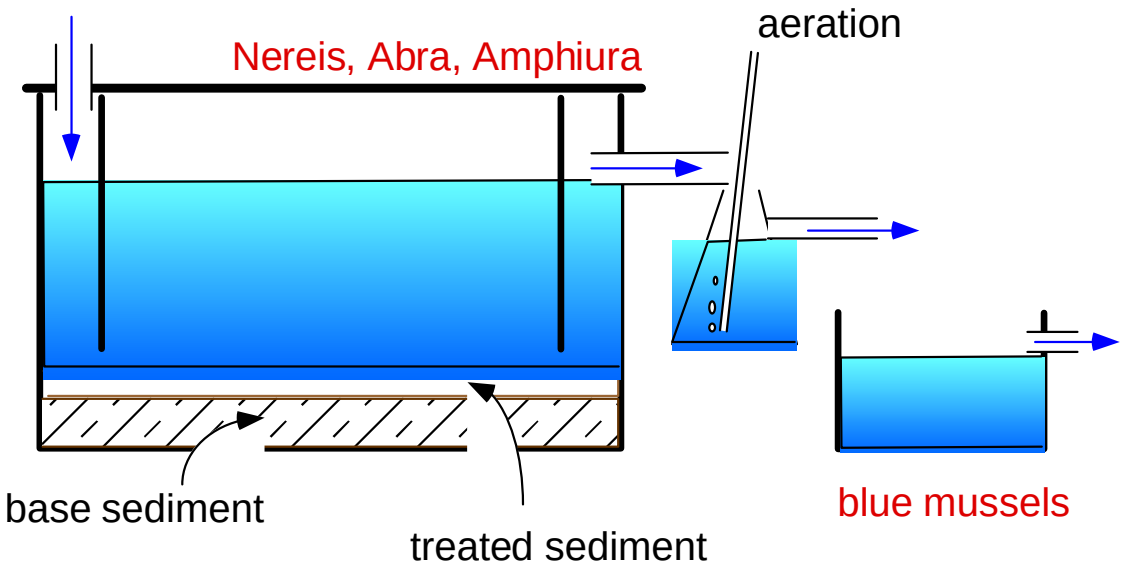

Figure 1. Individual aquaria: 2 cm sediment treated with organic material and/or contaminants overlying 4 cm of base sediment. Water supplied from 60-m depth, filtered through a series of 25-µm filters. Outflowing water from the large aquarium was led through an aeration flask before being fed to smaller aquaria with blue mussels.

2.3.2 Supply of seawater

Marine Research Station Solbergstrand (MRSS) has supply of different qualities of both seawater and freshwater. In this study we used water from 60-m depth outside MRSS. The quality (salinity, temperature) of this water varies little throughout the year. This 60-m water was filtered through a series of 25-µm filters before use. A flow-through system with separate header-tanks was used for both deoxygenated and oxygenated seawater. Seawater of the same quality was used to stabilise the

temperature in the aquaria (water-bath). Before being released to the sea the wastewater draining from each water-bath was passed through a filter of active carbon.

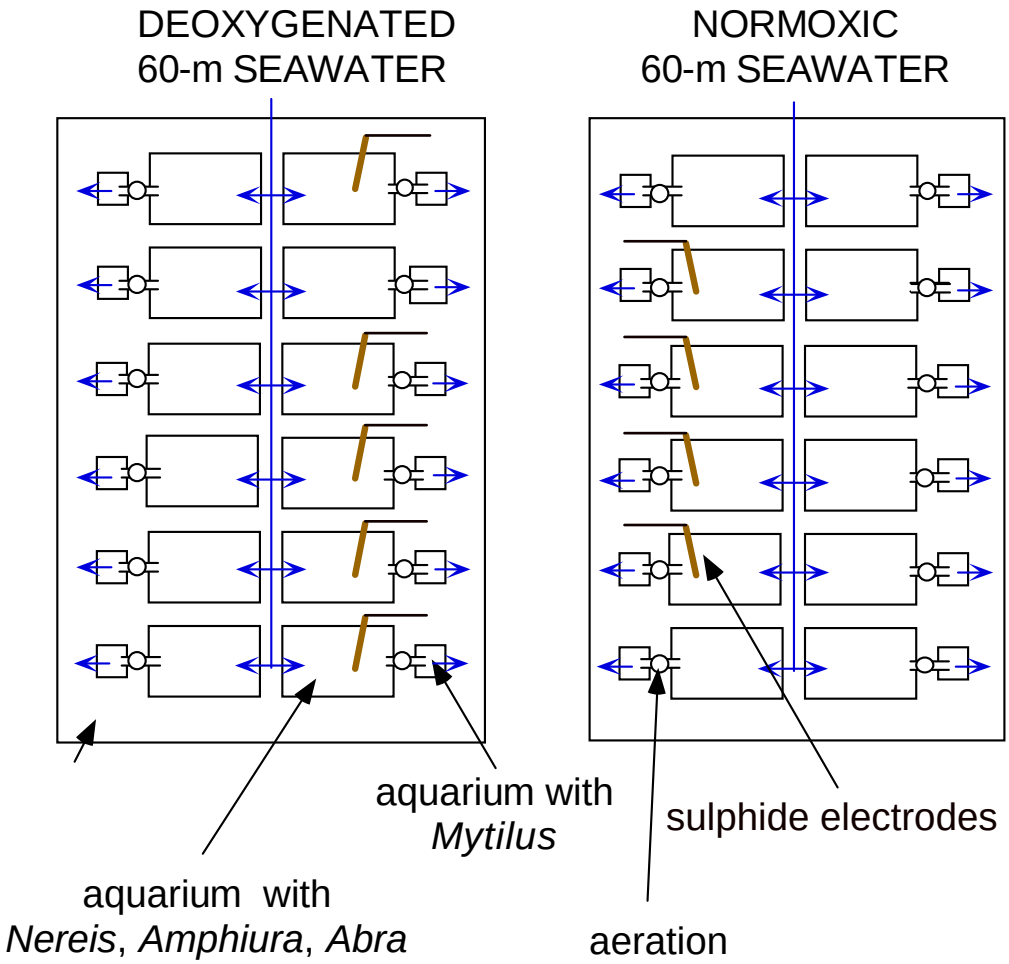

Figure 2. The set-up in two large water-baths with separate feeder tanks. Sediment and sediment-dwelling organisms were added to the large aquaria (285 mm x 465 mm i.m.), whereas the smaller aquaria (150 mm x 20 mm i.m.) were used to hold blue mussels.

2.3.3 Addition of contaminants and organic material to sediment

The stock solution for metals was prepared by dissolving the respective chloride salts and appropriate amounts of tracer (^{109}Cd and ^{203}Hg) in seawater. 390 ml of this stock solution was added directly to each of the two contaminant stock sediment flasks (see below). Benzo(a)pyrene (BaP) was dissolved in acetone, to which appropriate amounts of ^{14}C-BaP in toluene were added. 140 ml of this stock solution was also added to the same two flasks. To each of the two flasks with contaminated sediment was added 206 mg Cd (18.3 MBq), 253 mg Hg (50.4 MBq) and 11 mg BaP (9.25 MBq). The two sediment flasks with no contaminants received seawater and acetone only. The specific activities of the three tracers were therefore: 88.83 kBq/mg Cd, 199.21 kBq/mg Hg, 36.56 kBq/mg BaP.

Organic matter was prepared by concentrating frozen algal cultures containing predominantly diatoms (*Skeletonema costatum*). The cultures were allowed to settle, after which as much water as possible was siphoned off. This procedure was repeated until the maximum possible volume for addition to two

treatments was reached. The organic material added was estimated to yield approximately 2 mg C/g dry sediment in the final test-sediment (eqivalent to 20 g C/m^2).

Stock sediment for each of the four treatments was prepared by initially preparing a sediment slurry from 2 l of the base sediment. Equal amounts of this slurry were transferred to each of four 5-l erlenmeyer flasks with magnetic stirrers. Contaminants (SCO, DCO, SCR and DCR) or acetone/seawater carrier (SRO, DRO, SRN and DRN) and organic matter (SCO, DCO, SRO and DRO) or seawater (SCN, DCN, SRN and DRN) were added to make a total volume of 4.5 l (see **Table 1** for explanation of codes). All four stock sediments were covered and allowed to mix under stirring for 48 hrs.

In addition to the treatment of sediment, additional organic material (approximately 20 g/m^2) was added to all aquaria with organic enrichment (SCO, DCO, SRO and DRO) on day 60. The total amount carbon added is similar to the sedimentation in an unpolluted Norwegian fjord through one year (approx. 50 g C /m^2).

2.3.4 Addition of organisms

Abra alba and *A. filiformis* were transferred to MRSS some days prior to day 0 and were kept in 60-m water awaiting transfer to experimental aquaria. *Nereis diversicolor* was collected one week before day 0 and was kept in clean sediment awaiting transfer. *Mytilus edulis* was collected the week before day 0 and kept in 60-m water until commencement of the experiment. On day 0, 10 individuals of *A. alba*, 10 individuals of *A. filiformis* and 20 individuals of *N. diversicolor* were transferred to each aquarium. Similarly, 10 individuals of *M. edulis* were transferred to small aquaria, one for each sediment-containing aquarium.

2.3.5 Schedule for the experiment

The experiment was started on December 2nd, 1994 and was terminated on March 3rd - 4th, 1995 (**Table 3**).

Table 3. Schedule for the experiment.

days (with respect to 2.12.94)	action
-14	adding 4 cm sediment in each aquarium
-9	start mixing of stock sediment
-7	mixing stock sediment with standard sediment, distributing to aquaria
-6	starting water flow through aquaria
-4 - -1	adjusting deoxygenation
-4 - -1	inserting S-electrodes, connecting logger
-2 – 0	preparing addition of organisms and sampling
0	sampling organisms; sampling sediment, preparing pore-water; sampling water; marking and adding organisms
60	addition of more organic material
93-94	sampling organisms; sampling sediment, preparing pore-water; weighing or video-filming organisms; biochemical procedures

2.4 Measurement of oxygen, salinity and temperature

Salinity and temperatures were continuously recorded on electrodes permanently positioned in the source water before entering the deoxygenation tower. Oxygen was measured daily in deoxygenated aquaria and every second day in aquaria receiving normal seawater. Oxygen was measured in each aquarium by inserting a standard oxygen electrode to a fixed depth (15 cm) in the outflowing water (between the end-wall of the aquarium and the plexiglass-separator (see **Figure 1**).

2.5 Sulphide ion activity, pH and redox potential

Sulphide ion activies in selected aquaria were continuously recorded on Ag|AgS electrodes at different depths in the sediments and overlying water. In each aquarium, six sensors (2 x 10 mm) were mounted on a multielectrode at a distance of cc20 mm. The multielectrodes were inserted into the sediment so that the second sensor from top was aligned with the sediment-water interface. Thus, initial recordings were performed in the overlying water 1 cm above the interface (sensor 1), at the interface (sensor 0), and in the sediment 1, 2, 3 and 4 cm below the interface (sensors -1, -2, -3 and -4). During the course of the experiment, sediment compaction and various disturbance altered sensor positions relative to the sediment-water interface. Thus, whereas sensor 1 was always recording in the overlying water and sensors -2, -3 and -4 were always recording in the sediments, sensors 0 and -1 may have recorded at, below or above the sediment-water interface.

Available logger capacity and cable lengths limited sulphide monitoring to 54 sensors distributed in aquaria nos. 7, 9, 10, 11, 12, 14, 15, 16 and 17. Thus, at least one replicate of each treatment was monitored. The monitored aquaria were interconnected by stainless steel conductors. A common Ag|AgCl reference electrode was placed in aquarium no. 10.

The potential on the Ag|AgS electrode is proportional to the activity of the S^{2-}-ion. If the pH is known, the total concentration of hydrogen sulphide:

(Eq. 1) $$pS = -\log([H_2S] + [HS^-] + [S^{2-}])$$

can be calculated from the equation:

(Eq. 2) $$pS = -pK_2 + pH + (E_{obs} + E_{ref} + E_o')/0.0295 - \log(10^{(pK1-pH)} + 1)$$

which is valid at pH≤10.

K_1 and K_2 = first and second dissociation constant for H_2S
E_{obs} = recorded cell potential
E_{ref} = half-cell potential of the applied reference electrode
E_o' = standard potential of Ag|AgS electrode

Constant values of $pK_1 = 13.9$, $pK_2 = 7.0$, $E_{ref} = 0.234$ V, $E_0' = 0.6689$ V and pH = 7.8 was used throughout the calculations.

Reliability of pS-measurements

In a previous experiment, the standard deviation of E_o' found by calibration of 58 Ag|AgS-sensors was found to be 0.0009 V. This correspond to 0.03 pS-units. pH variations at similar depths are unlikely to exceed ±0.2 pH-units. Thus, comparing pS at similar depths, the random error should be <0.2 pS-units. The pH may vary from 8.1 in the overlying water to 7.5 at depths in the sediment.

herefore, comparing between different depths, the corresponding error may be up to 0.6 pS-units. Large absolute errors may result from uncertainties in thermodynamic constants and variations in temperature and ionic strength of the seawater. Different calibrations have resulted in values of E_o' between 0.65 and 0.68 V, which correspond to a difference of 1.0 pS-units.

The absence of colour-changes in the sediments and any smell of hydrogen sulphide throughout this experiment confirmed that the levels of hydrogen sulphide were below the detection limits of 10^{-7} M (pS = 7) of conventional methods.

2.6 Sampling of sediment and preparation of pore water

Sediment core samples were collected using a modified 13 mm (ID) PVC-syringe. Five cores were drawn from each chamber. One-cm sections were pushed into a sectioning chamber, cut off and transferred to 18-ml centrifuge tubes. Thus, each sample represented a pool of five samples drawn at different locations within each aquarium. The pore water was extracted by centrifugation at 20 000 g for 15 minutes and carefully transferred to 5 ml scintillation vials. The sediment was freeze-dried within the centrifuge tubes. Centrifugation was completed less than 12 hours after the cores had been removed from the aquaria. Thus prepared, the samples in the scintillation vials and centrifuge tubes were sent to IFE for analyses (gamma-emission, Cd and Hg).

Initial samples were drawn on day 0. Pooled samples for pore water and sediment analyses were taken from the 0-1cm depth interval, only. A sixth core was drawn from each aquarium and 0-1 cm sections were transferred to vials for counting of total sediment.

2.7 Sampling of organisms

The aquaria were processed in random order within each of the groups containing contaminants and those not containing contaminants (due to waste treatment). Organisms were extracted from the sediment by turning each aquarium on its side and removing sediment by gentle hosing with seawater. The organisms were transferred to beakers with seawater as they became visible and special care was taken not to damage *A. filiformis*. All sediment was passed through a 1-mm sieve to retrieve all individuals escaping visual inspection. *A. alba* from each aquarium was immediately transferred to a smaller aquarium with clean sediment, wherein they were left for 24 hrs before further processing.

2.8 Growth and arm regeneration

Nereis diversicolor
Before being added to the aquaria each individual polychaete was inspected for damage, blotted dry and weighed (in a small volume of water) before being transferred to a larger beaker. On termination the same procedure was used. On both occasions, the polychaetes were kept for at least 6 hours in clean water to void their intestinal contents.

Amphiura filiformis
Before being added to the aquaria one arm was severed between the 7th and 8th arm segment and the individual video-filmed. On terminating the experiment all individuals were video-filmed and the outline of the regenerated arm traced on-screen to quantify the length and area of the regenerated arm.

Abra alba
Each individual *A. alba* added to an aquarium was first marked with a pen, video-filmed and traced. Ten individuals were added to each aquarium. At the end of the exposure all individuals were again video-filmed and traced on-screen. The traces were used to quantify changes in shell area, i.e. growth.

2.9 Processing of organisms for tracer and biochemical analyses

Following other measurements, appropriate pools of individuals from each aquarium of each species (4-7 pools of *N. diversicolor*, 3 pools of *A. filiformis*, 1 pool of *A. alba*) were homogenised in ice-cold 0.1 M potassium phosphate buffer, pH 7.8, containing 1 mM glutathione, 0.1 mM PMSF, 0.15 M KCl and 5% glycerol. A 1.5-ml aliquot of each homogenate was transferred to an eppendorf-tube which was centrifuged at 10 000 x *g* at 4°C for 30 mins. The supernatant (containing cytosol and microsomes, termed PMS, post-mitochondrial supernatant, below) was immediately transferred to a cryo-tube and frozen in liquid nitrogen. The remainder of each homogenate was frozen at -20°C in scintillation vials and stored for tracer analyses.

2.10 Tracer analyses and extraction of benzo(a)pyrene

All analyses for ^{109}Cd and ^{203}Hg were done at Institute for Energy and Technology (IFE), using a Ge(Li) gamma spectrometer for the sediment samples, low level HPGe-gamma spectrometer for the biological samples or a Quantulus low level liquid scintillation spectrometer for the pore water and some of the biological samples. All results were corrected to day zero activities according to the half-lives of 46.6 d for ^{203}Hg and 453 d for ^{109}Cd. Analyses for ^{14}C-BaP in pore water was done at IFE on toluene-extracted samples using Qantulus, whereas all other extractions and analyses of ^{14}C-BaP were done at KMF. Due to interference from gamma-emitters, BaP had to be extracted prior to analysis by scintillation counting. Approximately 1.5 mg DW sediment were taken from each sample, weighed and placed in a teflon vial of the MAE (Microwave assisted extraction) system. Thirty ml of Acetone /Hexane 1:1 were added. MAE extraction was performed for 30 minutes (Pressure: 100 PSI, Power: 100%, T: 120 c, Ramp time: 8 min., Extraction time: 15 minutes). Extracts were transferred to a glass vial of 250 ml with a narrow neck and 235 ml of filtered seawater was added. Glass vials were manually shaken (upside-down), 20 times. Bubbles were vortexed out. A clear phase separation was obtained with the organic fraction (15 ml Hexane) on top and the water phase (235 ml water + 15 ml acetone) below. Ten ml of scintillation cocktail Ultima gold were added to each of the organic extracts. Each scintillation vial was kept for 48 hours at room temperature to remove chemoluminescence and then counted for 10 minutes with a Beckman LSC, using automatic quenching correction based on own quench curves determination.

In order to verify the efficiency of the phase separation, samples were also controlled at IFE for energy spectra and eventual gamma activity. No gamma emitters could be detected in most of the organic samples, whereas most of the water samples showed the presence of gamma activity, indicating a successful phase separation. A few organic samples contained some gamma activity and were omitted from the results.

2.11 Biomarker analyses

Analyses for total protein in the PMS (post-mitochondrial supernatant) were done according to Bradford (1976). Heat-stable sulphydryl-rich proteins (hereafter referred to as metallothionein [MT] - like proteins) in PMS was quantified by differential pulse polarography on heat-denatured samples (95°C for 3 min) according to Olafson *et al.* (1979) and Olafson & Olsson (1991). Glutathione *S*-transferase was analysed in PMS using CDNB (1-chloro-2,4-dinitrobenzene) as substrate as described by Habig *et al.* (1974). Glutathione reductase activity was analysed in PMS using a plate-reader following a minor modification of the method described by Livingstone (1990).

2.12 Statistical analyses

The study was designed for a three-way analysis of variance with the factors **organic enrichment** (normal - enriched), **oxygen** (deoxygenated - normoxic) and **contamination** (contaminated – not contaminated). There were three replicate aquaria for each of the resulting eight treatments. All three treatments and all interactions were included as factors in a nested three-way ANOVA under H_0: no difference between treatments (Sokal & Rohlf, 1981; Underwood, 1981). The level for the rejection of H_0 was set to 0.05. All growth-related variables and biomarkers were analysed using nested three-way ANOVA except biomass change in *N. diversicolor* and biomarkers in *A. alba*, for which there was only one observation for each aquarium (no nesting). In addition, biomarker responses were analysed using multiple regression (Draper & Smith, 1981) with mean wet weight and tissue contaminant levels (Cd and benzo(a)pyrene) as factors. Dependent variables were checked according to Levene (1960) and transformed where necessary to obtain normally distributed residuals and fulfil the criterion of homogeneity of variances.

3. Results

3.1 The experimental environment

3.1.1 Salinity, temperature and light

The temperature in the 60-m seawater used in the experiment dropped from 10.1°C at the start until 6.1°C at the end. During the same period the salinity varied between 33.6 and 34.6. The conditions are close to natural for *A. filiformis* and *A. alba*, but more stable (and warmer) than what *N. diversicolor* would experience in nature (in the intertidal). The system was kept at 12:12 day:night light regime, but with weak lighting as daylight.

3.1.2 Flow and oxygen

Initially, the deoxygenation system did not work well and levels fluctuated in the aquaria that were supposed to receive low levels of oxygen (**Figure 3**). This situation was corrected within a few weeks however and over the entire experimental period the aquaria receiving deoxygenated water had mean oxygen levels of 2.4-3.6 mg/l (**Table 4**). The flow through individual aquaria varied between 52-192 ml/min during the experiment, with aquarium means of 114-166 ml/min.

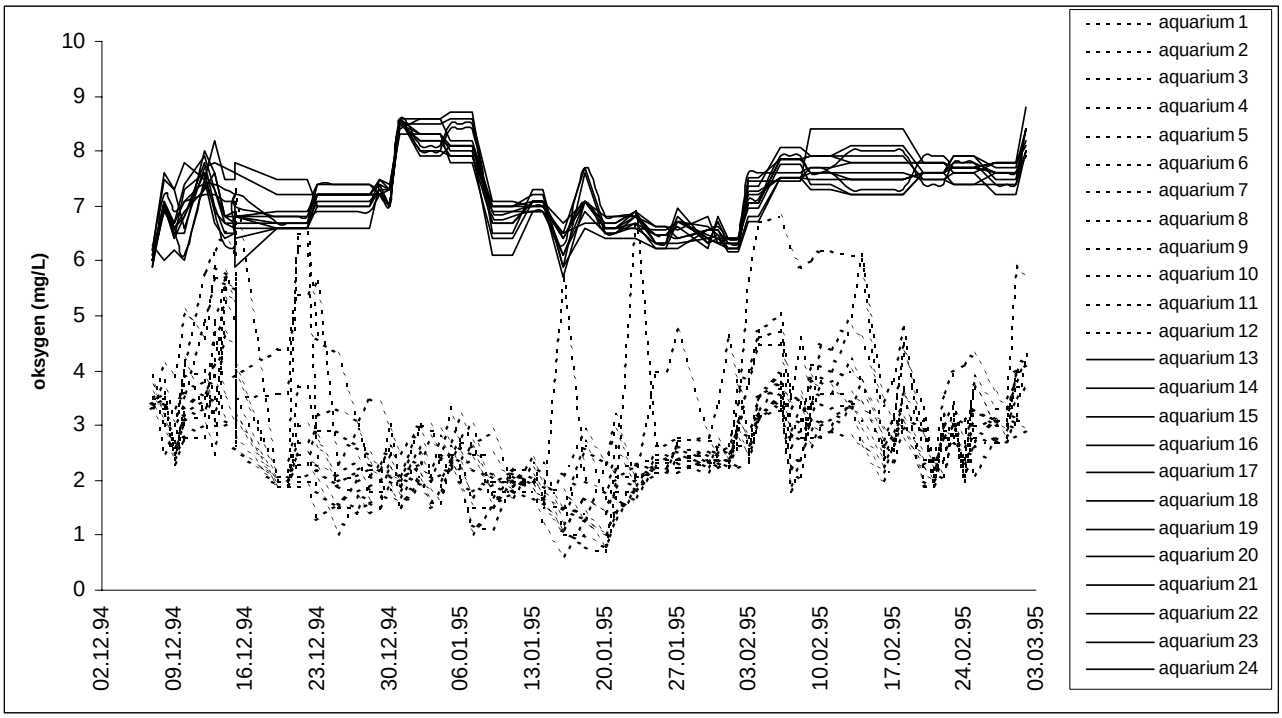

Figure 3. Oxygen in the outflowing water from individual aquaria. Aquaria 1-12 are indicated by grey, dotted lines (deoxygenated), aquarium 13-24 are indicated by black, continuous lines (normoxic). Measurements in the aquaria receiving untreated seawater (13-24) were done at 2-d intervals, whereas measurements in the deoxygenated aquaria were done daily.

Table 4. Oxygen and flow in each aquarium during the experimental period. Aquaria 1-12 received deoxygenated water, whereas aquaria 13-24 received untreated seawater ("normoxic").

	Oxygen (mg/l)				flow (ml/min)			
Aquarium	mean	Std dev	Min	Max	Mean	Std dev	Min	Max
1	2.8	0.9	1.0	5.8	114	17	80	144
2	2.5	0.8	1.0	5.3	117	17	76	152
3	3.5	1.5	1.5	6.8	119	34	72	164
4	2.6	1.0	0.8	4.7	126	19	60	160
5	2.4	1.0	0.6	5.6	152	18	88	188
6	2.6	1.2	0.7	5.9	166	32	64	192
7	3.0	1.1	1.5	6.3	132	24	56	184
8	2.9	1.1	1.1	5.7	133	28	68	184
9	3.6	1.5	1.3	7.3	119	25	52	156
10	2.9	0.9	1.2	4.8	129	17	88	168
11	2.6	1.0	1.1	5.5	140	15	92	184
12	3.0	1.3	1.6	6.9	132	19	64	156
13	7.0	1.2	2.5	8.5	122	19	72	160
14	7.1	0.7	5.7	8.6	123	16	96	156
15	7.0	0.7	5.9	8.5	136	17	96	180
16	7.1	0.7	5.9	8.6	138	21	96	182
17	7.0	0.7	6.0	8.6	133	13	96	160
18	7.0	0.7	6.0	8.5	134	18	84	168
19	7.3	0.8	5.8	8.7	146	16	96	168
20	7.0	0.7	5.9	8.6	121	23	72	164
21	7.1	0.7	5.8	8.5	138	20	80	168
22	7.1	0.8	6.0	8.8	122	19	88	168
23	7.3	0.7	6.2	8.6	136	13	108	164
24	6.9	0.8	5.9	8.6	129	11	104	168

3.1.3 Redox, carbon/nitrogen and sulphide ion activity

Redox-potentials recorded at 1 cm depth on day 46 ranged between 180 and 352 mV. On day 55, loss on ignition (5 hours, 450°C) in sediment from organically enriched aquaria and aquaria with no organic material added were 45.9±5.2 and 43.4±6.2 mg/g, respectively. This lack of difference between the enriched and non-enriched treatments became clear during the experiment and led to the addition of the second dose of algae. Analyses performed at the termination of the experiment confirmed that the effect of the initial algae addition on the levels of carbon and nitrogen was small compared to the effect of the second dose (**Figure 4**). According to the classification system used for Norwegian coastal sediments (Rygg & Thelin, 1993), only the final concentrations of organic C and N in the C-enriched treatments exceeded the upper limit set for a non-polluted environment. Thus, with regard to organic enrichment, the experimental environment could be classified as non-polluted to moderately polluted. Final concentrations of carbon and nitrogen in the sediment had not been affected by different levels of oxygen.

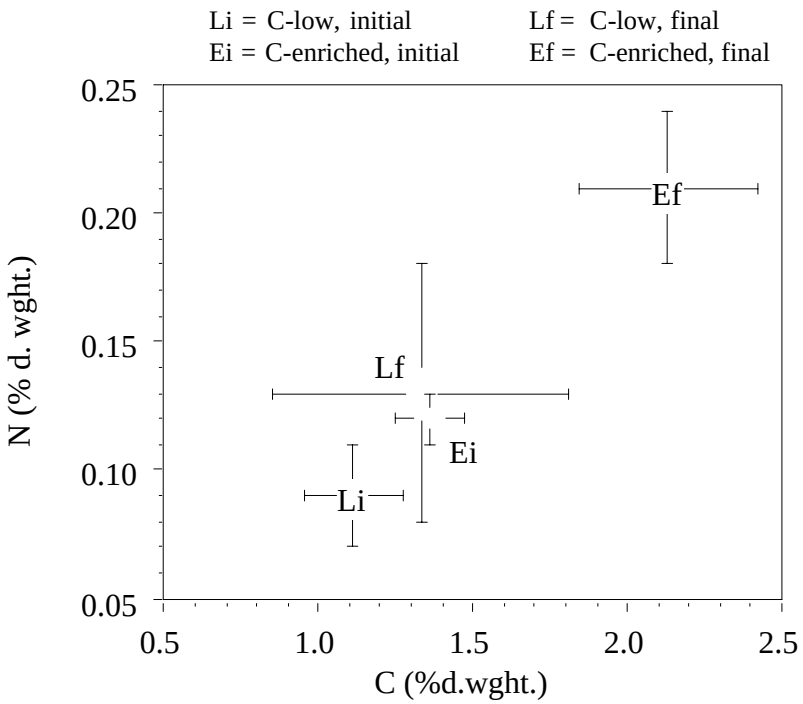

Figure 4. Initial (i) and final (f) concentrations of carbon versus nitrogen in 0-1 cm depth interval in organically enriched (E) and non-enriched (L) aquaria. Mean ± one std. deviation.

Due to long stabilisation times for the electrode potentials and some initial delay, data for the first 12 days of the experimental period has been omitted. For the remaining 12-93 days period, potentials on each sensor were recorded continuously and integrated values were stored on the logger every 12 h. The maximum concentration of hydrogen sulphide ($\Sigma[H_2S]$) was $10^{-8.54}$M (pS = 8.54). This maximum was recorded on day 36, in one of the oligotrophic (low-C, high-O_2) aquaria, at a sensor located below the spiked layer (4-cm depth). The variation on each electrode ($0.98 \leq$ std.dev. ≤ 1.66 at 2 cm depth) was large compared to the variation between treatments. Thus, with regard to maximum $[H_2S]$, no significant difference was observed between treatments. However, the mean values were different and revealed several expected features such as the decrease of pS at 2 cm depth from 13.37 in the most oligotrophic treatment to 10.29 in the most eutrophic treatment and lower pS within the carbon-enriched layers than at corresponding depths in the aquaria with no organic material added (**Figure 5**). Furthermore, reduced penetration of oxygen may explain the displacement of the pS-cline towards the sediment-surface in the deoxygenated aquaria (**Figure 5**).

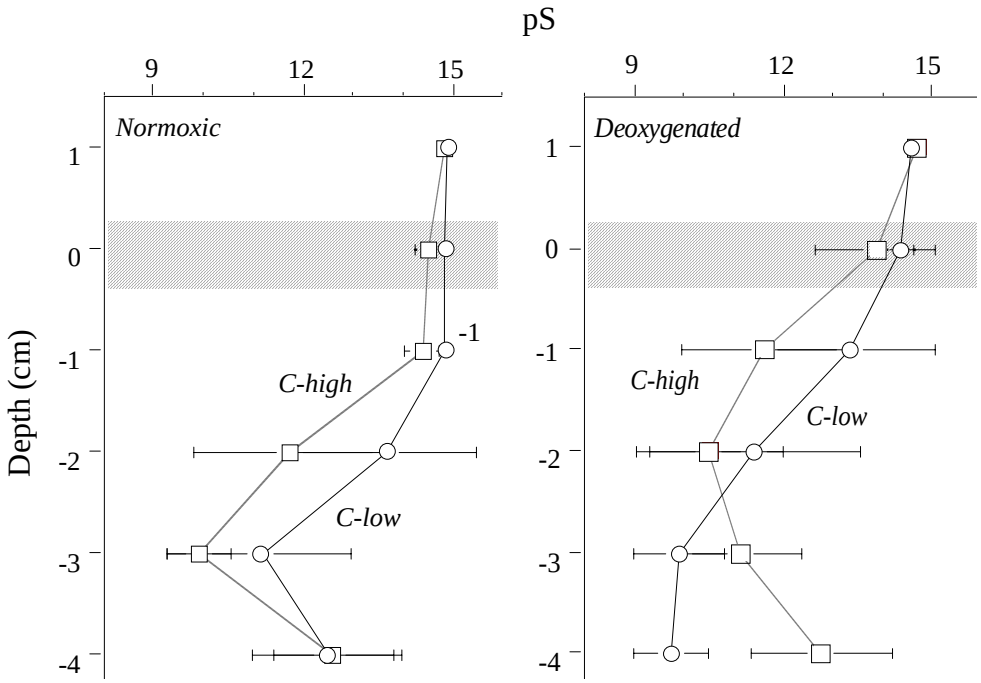

Figure 5. Profiles of pS across the sediment-water interface during the 12-93 days period (continuously recorded on Ag|AgS electrodes, error bars = ± 1 std. dev. (n=360 12h integrated readings).

The low (nanomolar and less) level of H_2S was also confirmed by the high redox potentials measured at one cm depth on day 46 ($180mV \leq E_h \leq 352mV$, duplicate measurements in all aquaria). Thus, it could be concluded that severe sulphide events did not occur in any of the aquaria and that the electrodes appeared suitable for monitoring pS at concentration levels in the sediments which on the basis of smell and colour, would be referred to as oxic or non-sulphidic.

3.2 Sediment and pore water concentrations of Hg and Cd

In all samples of the pore water, the concentration of Hg was below the limit of detection. Sediment concentrations of Hg decreased from maximum values in the spiked surface layer to low levels in the non-contaminated layer below 2 cm depth (**Figure 6**c). The initial concentration of Hg of 158 ± 11 Bq/g dry sediment was not significantly different from the final concentration of 170 ± 23 Bq/g, and no significant difference was found between treatments (**Figure 6**c, **Table 6**). Thus, the data gave no evidence for the presence of any processes acting to redistribute the initially retained Hg. The mean concentration of Hg of 164 Bq/g (0-1cm, all treatments, initial and final) corresponds to 229 ng Hg/g (dry weight) which has been classified as “moderately polluted” (Rygg & Thelin, 1995).

At the start of the experiment the sediment concentration of Cd of 1026±67 Bq/g in the organically enriched aquaria was significantly higher than the concentration of Cd of 874±45 Bq/g in the treatments with no organic material added (**Figure 6**a, **Table 5**). During the experimental period, the concentration of Cd in sediment decreased in all treatments, but in the final samples no significant ($p>0.35$) differences were found between any of the four treatments. Thus, the loss of Cd had been larger in the enriched aquaria than in the aquaria with no organic material added. The mean Cd-concentration of 824±154 Bq/g (0-1 cm, all treatments, initial and final) corresponded to 4.6 µg Cd/g sediment (dry weight), classified as a “markedly polluted” coastal sediment (Rygg & Thelin, 1995).

In the pore water, Cd-concentration ranged between 12.1 and 17.2 Bq/g in the oligotrophic treatment, which was high compared to 2.9-6.8 Bq/g in the other aquaria (**Figure 6**b). In the pore water, both deoxygenation ($p<0.0001$) and algae addition ($p=0.0009$) had significant effects in lowering Cd-concentration.

At depths below 1 cm, neither Cd nor Hg showed any significant difference between treatments and the pore water concentration of Cd was close to the detection limit. The linear profiles of sediment Hg (**Figure 6**c), indicated that the final samples from the 1-2 cm depth interval represented a mixture of 50% of the spiked surface layer and 50% of the non-contaminated base sediment. The fact that the final sediment concentration of Cd at 1-2 cm depth corresponded to 50% of the initially retained Cd-concentration in the surface layer (**Figure 6**a) indicated that loss of Cd was restricted to the 0-1 cm layer. The vertical profiles of Cd in pore water (**Figure 6**b) did not deviate from the concavity expected from a source within the 0-1 cm layer. The profile in the most eutrophic treatment was slightly different and may have resulted from increased precipitation of $CdS_{(s)}$ within the 0-1cm layer after the second addition of algae. Thus, neither sediment nor pore water samples gave any evidence for mobilisation of Cd from depths exceeding 1 cm in any treatment.

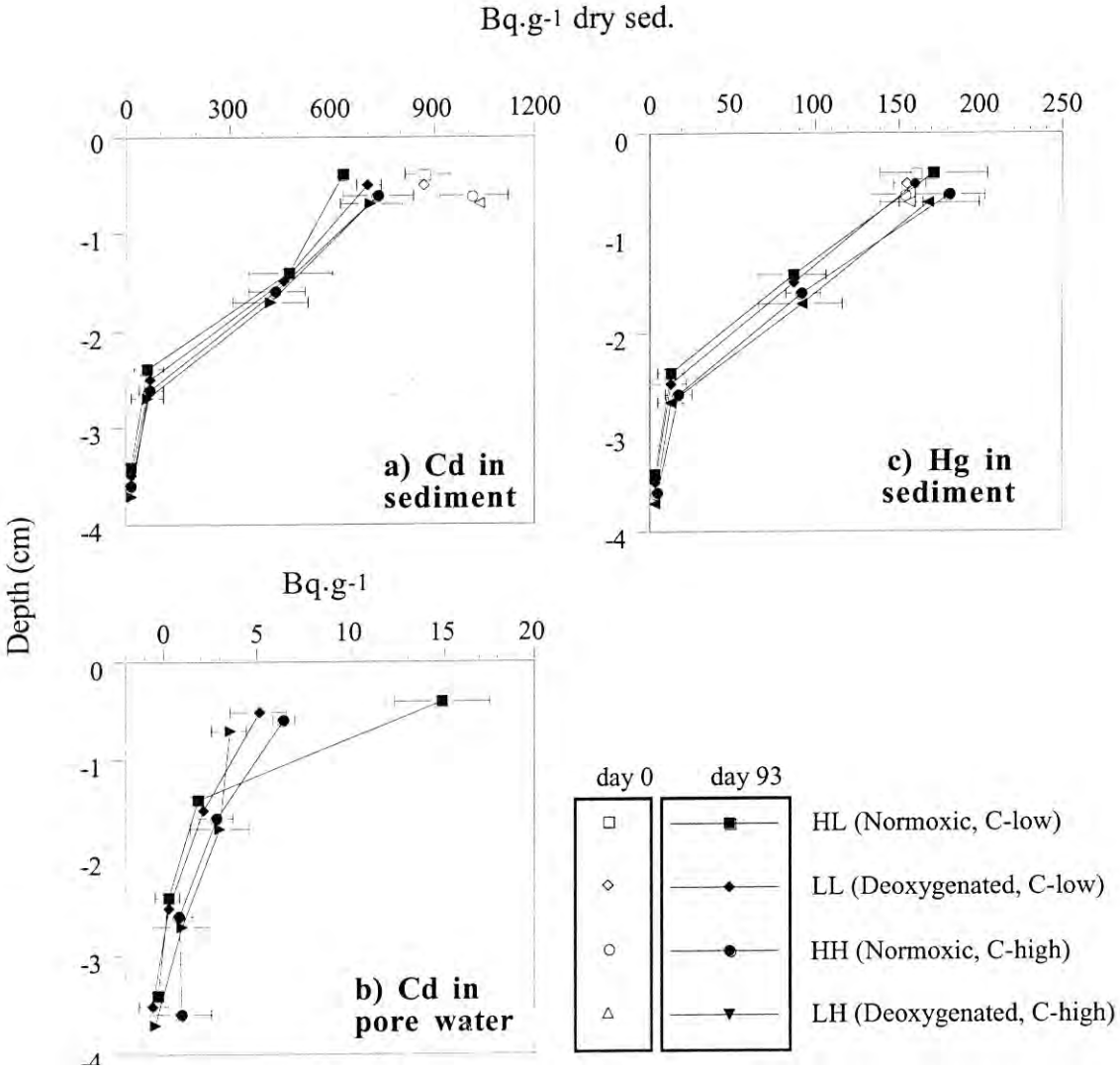

Figure 6. Vertical profiles of mean activities of ^{109}Cd and ^{203}Hg in sediments and pore water on day 0 (0-1cm, open symbols) and day 93 (profiles, filled symbols). Error bars = ± 1 std. dev. (n=3 replicate aquaria). Note: vertical separation of symbols does not affect shape of profiles.

Table 5. Analysis of variance (ANOVA) on the effects of oxygen level, organic addition and oxygen x organic interactions on the activities of Cd in sediment. R^2 = fraction of total variance explained by the model, n = number of analyses and p = probability.

	R^2	n	p		
			oxygen	organic	interactions
Sediment, initial	0.69	12	0.92	0.003	0.72
Sediment, final	0.32	12	0.54	0.20	0.26
Pore water	0.92	12	<0.0001	0.001	0.08

Table 6. Analysis of variance (ANOVA) on the effects of oxygen level, organic addition and oxygen x organic interactions on the activities of Hg in sediment. R^2 = fraction of total variance explained by the model, n = number of analyses and p = probability

	R^2	n	p		
			oxygen	organic	interactions
Sediment, initial	0.06	12	0.75	0.82	0.56
Sediment, final	0.12	12	0.43	0.55	0.99

3.3 Concentrations of benzo(a)pyrene in sediment

There was no detectable benzo(a)pyrene in any pore water-sample. In sediment samples there were clear gradients from the surface downwards (**Figure 7**). In the upper centimeter there were obvious differences in BaP-levels between treatments, but this effect was not apparent from 1 cm downwards. The concentration of BaP in the upper centimeter of the sediment at the end of the experiment was affected by both organic enrichment and oxygen availability (**Table 7**). Addition of organic material resulted in higher BaP-concentrations in the upper centimeter and lowered oxygen availability in lower concentrations of BaP. The effect of organic material is not surprising as BaP has high affinity for organic material, which could serve as a trap for this contaminant (organic material and BaP were both added to the upper two cm). There reasons for the observed differences between normal and deoxygenated aquaria are less obvious.

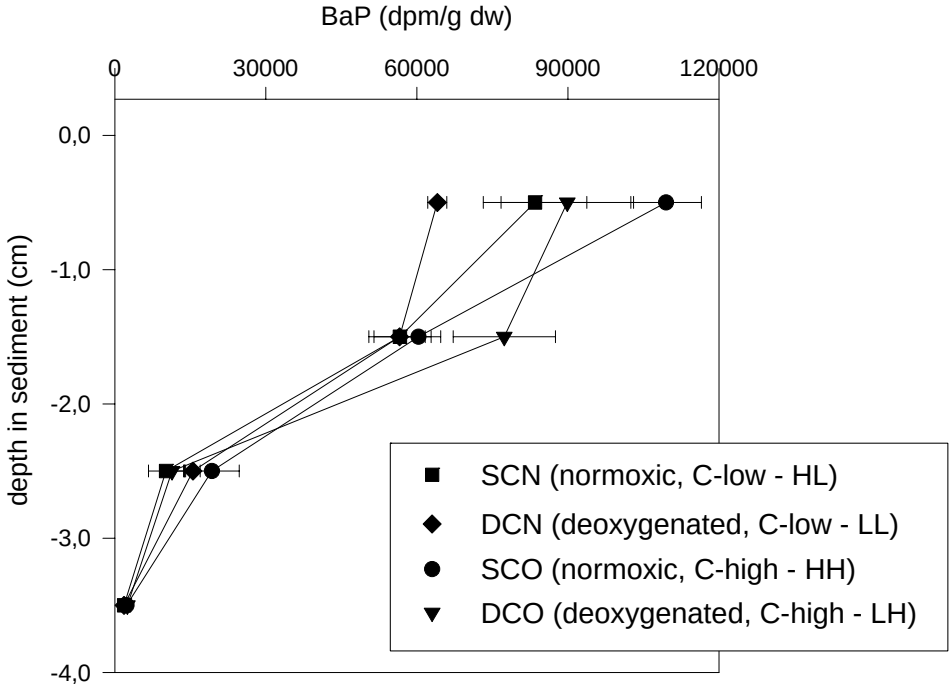

Figure 7. Concentrations of BaP in sediment samples at four depths in the sediment at day 93 (dpm/g dry sediment); mean ± standard error, n=3.

Table 7. Two-way ANOVA with concentration of BaP in sediment as dependent variable and oxygen availability and organic enrichment as factors, n=12. The whole model R^2 was 0.53 (p = 0.014).

effect	degrees of freedom	F ratio	probability
oxygen	1	4.59	0.064
organic	1	**8.11**	**0.021**
oxygen x organic	1	0	0.99

3.4 Bioaccumulation of Cd, Hg and benzo(a)pyrene

3.4.1 Nereis diversicolor

There were significant differences in the accumulation of Cd between groups exposed to contaminants (**Figure 8**). The groups in aquaria with organic enrichment accumulated significantly more than groups kept in aquaria without enrichment (**Table 8**). This effect of organic enrichment was more obvious for Hg than for Cd, as *N. diversicolor* in the two aquaria receiving organic enrichment had significantly higher concentrations of Hg than worms in the non-organic aquaria (**Figure 8**). There were no differences between any groups as regarded the accumulation of BaP (**Figure 8**). There were low levels of all three tracers in the groups not being exposed to contaminants.

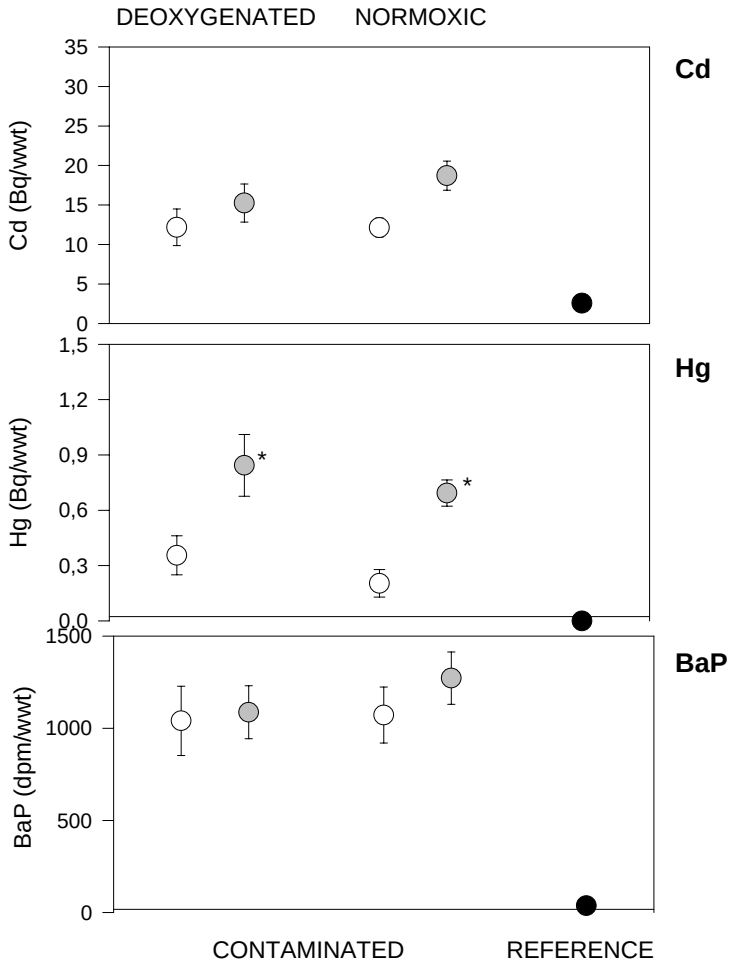

Figure 8. Concentrations of Cd, Hg and BaP in *N. diversicolor* exposed to deoxygenated or normoxic seawater, with (grey symbols) or without (white symbols) organic material added. REFERENCE - no tracer added to the sediment (background). Mean with standard error, n = 4-7. * significantly different from groups without * (0.05≥p). See **Table 8** for further statistical analyses.

The accumulation of BaP was not significantly affected by any of the factors (**Table 8**), although there was a tendency towards more accumulation in the worms kept in the organically enriched aquaria with normal oxygen levels.

3.4.2 Amphiura filiformis

The brittle star *A. filiformis* is a filter-feeder that lives submerged in the sediment. The accumulation of all thre contaminants in *A. filiformis* were of similar order of magnitude as that found for *N. diversicolor*. Although the accumulation of both Cd and Hg appeared to be somewhat higher in the groups receiving deoxygenated water, there were no significant differences between groups for either of the metals (**Figure 9**). In a two-way analysis of variance neither organic enrichment nor different oxygen levels appeared to explain any of the variability (**Table 8**). For the accumulation of BaP there appeared to be effects of both organic enrichment and deoxygenation, giving higher levels in *A. filiformis* kept in an organically enriched, deoxygenated environment (**Figure 9**). This observation was borne out in the two-way analysis of variance in that both factors were found to contribute significantly in explaining the variability in the model (**Table 8**). However, there were unexplainably high levels of both Cd and Hg tracers in groups not exposed to contaminants.

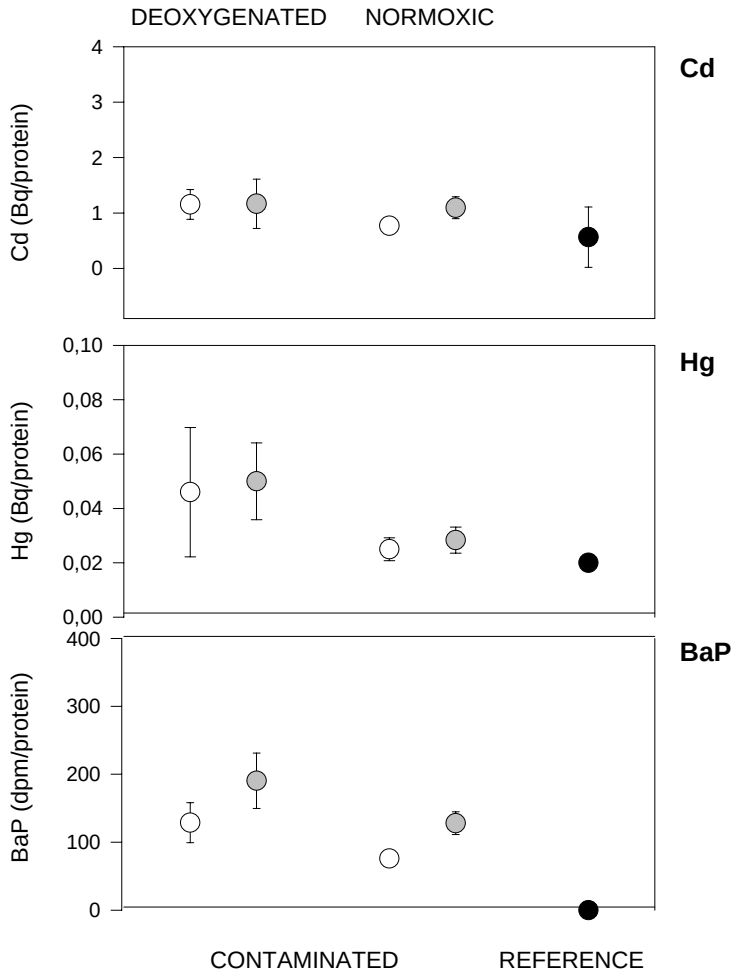

Figure 9. Concentrations of Cd, Hg and BaP in *A. filiformis* exposed to deoxygenated or normoxic sea-water, with (grey symbols) or without (white symbols) organic material added. REFERENCE - no tracer added to the sediment (background). Mean with standard error, n = 9. See **Table 8** for statistical analyses.

3.4.3 Abra alba

In the bivalve *A. alba* there was a dramatically elevated accumulation of both metals Cd and Hg in the groups kept in aquaria with organic enrichment, although this effect was not apparent for BaP (**Figure 10**). As for *N. diversicolor*, there were low levels of all three tracers in groups not being exposed to contaminants. The accumulation of contaminants could have two sources - either food or respired seawater. It is improbable that the food *A. alba* consumes should contain relatively less BaP than the two metals. The opposite would be more probable since BaP is strongly lipophilic and will be tightly bound to organic material. Results for pore water show that there were not detectable levels of BaP in pore water.

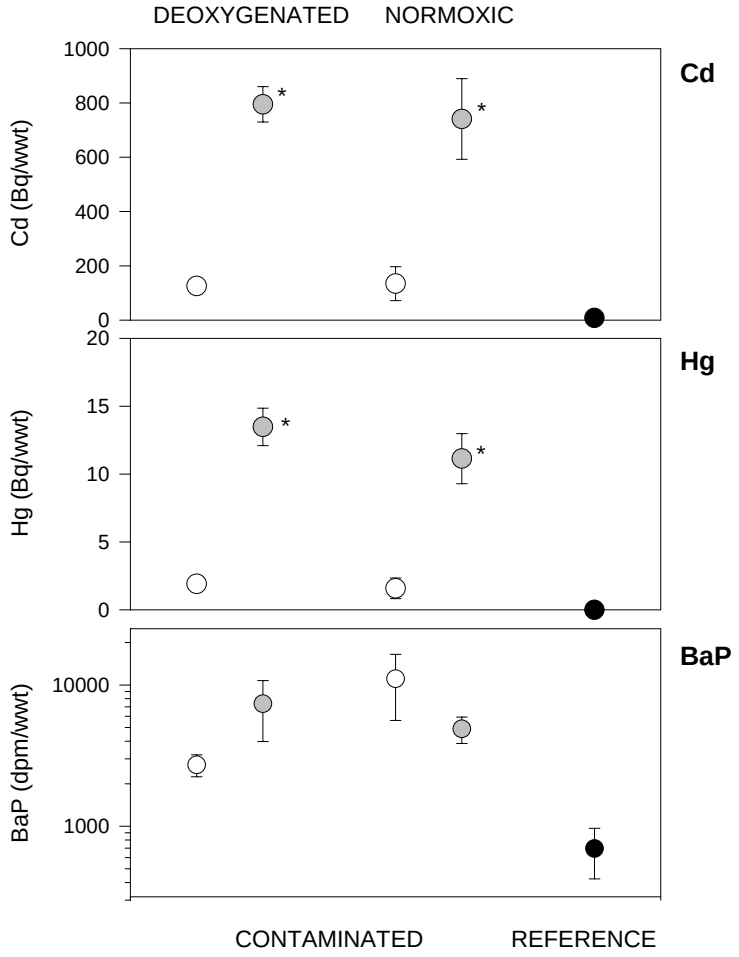

Figure 10. Concentrations of Cd, Hg and BaP in *A. alba* exposed to deoxygenated or normoxic sea-water, with (grey symbols) or without (white symbols) organic material added. REFERENCE - no tracer added to the sediment. Mean with standard error; n = 3. Note log-axis for BaP. * significantly different from groups without * (0.05≥p). See **Table 8** for further statistical analyses.

3.4.4 Mytilus edulis

The blue mussel, *Mytilus edulis*, were kept in aquaria that received outflowing water from each sediment-containing aquarium. There appeared to be an increase in accumulation of Cd in blue mussels downstream aquaria receiving organic enrichment, but the difference was not significant (**Figure 11**, **Table 8**). Similarly, BaP appeared to accumulate more readily in blue mussels kept downstream aquaria with organic enrichment. Levels of Hg were close to the detection limit for the analysis. There was unfortunately only one sample analysed from non-contaminated groups, but this sample could not be used (due to problems during work-up and analysis). As a result, there were no results for blue mussels not being exposed to contaminants.

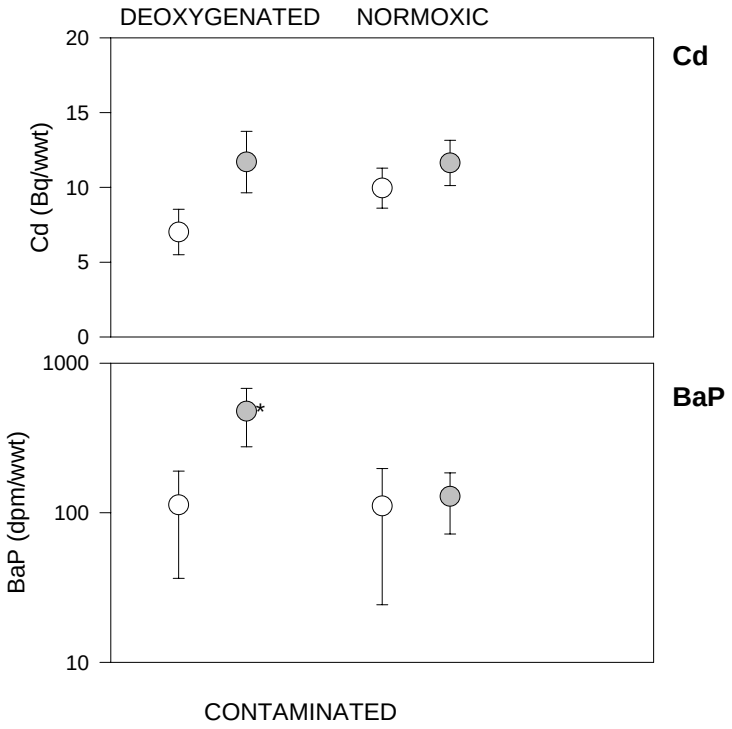

Figure 11. Concentrations of Cd and BaP in *Mytilus edulis* exposed to deoxygenated or normoxic sea-water, with (grey symbols) or without (white symbols) organic material added. There was no data for the reference group Mean with standard error; n = 4. Note log-axis for BaP. * significantly different from groups without * (0.05≥p).

Table 8. Two-way analysis of variance with contaminant concentration in test organisms as dependent variables. Oxygen availability ("oxygen"), organic enrichment ("organic") and the interaction between the two were used as factors. Significant results in bold. Nested analyses were used where applicable.

Species	contaminant	n	model R^2 (adj)*	factors contributing to the model	p-value
Nereis diversicolor	Cd	36	0.02	oxygen	0.20
				organic	**0.05**
				oxygen x organic	0.20
	Hg	36	0.14	oxygen	0.53
				organic	**0.003**
				oxygen x organic	0.33
	BaP	24	0.29	oxygen	0.72
				organic	0.022
				oxygen x organic	0.41
Amphiura filiformis	Cd	35	0.20	**oxygen**	**0.05**
				organic	0.18
				oxygen x organic	**0.01**
	Hg	35	0.24	oxygen	0.95
				organic	0.06
				oxygen x organic	0.14
	BaP	24	0.29	oxygen	0.45
				organic	**0.005**
				oxygen x organic	0.82
Abra alba	Cd	12	0.81	oxygen	0.95
				organic	**0.0001**
				oxygen x organic	0.66
	Hg	12	0.87	oxygen	0.47
				organic	**<0.0001**
				oxygen x organic	0.52
	BaP	9	0	oxygen	0.26
				organic	0.81
				oxygen x organic	0.24
Mytilus edulis	Cd	37	0.13	**oxygen**	**0.01**
				organic	0.10
				oxygen x organic	**0.003**
	BaP	24	0.14	oxygen	0.09
				organic	**0.02**
				oxygen x organic	0.35

3.5 Effects on sediment-dwelling organisms

3.5.1 Survival

There was a high survival (and recovery) of all three experimental species following the 3-month experimental period (**Table 9**). The only exception was high mortality of *Abra* in two aquaria receiving deoxygenated seawater, where only two individuals survived (of 10 added).

Table 9. Numbers of each species added at the start and recovered at the end of the experimental period.

Species	number added (min-max)	number recovered (mean; min-max)	mean recovery (%)
N. diversicolor	20	17; 12-20	85
A. alba	10	8.6; 2-10	86
A. filiformis	10	7.7; 5-9	77
M. edulis	10	10	100

3.5.2 Growth and arm regeneration

Nereis diversicolor

The biomass of *N. diversicolor* populations increased in aquaria with organic material added compared to aquaria without organic material (**Figure 12**). In addition, the biomass of this species was negatively affected by decreased oxygen availability. There was also a trend towards decreasing biomass with the presence of contaminants, but this effect was not statistically significant (**Figure 12**).

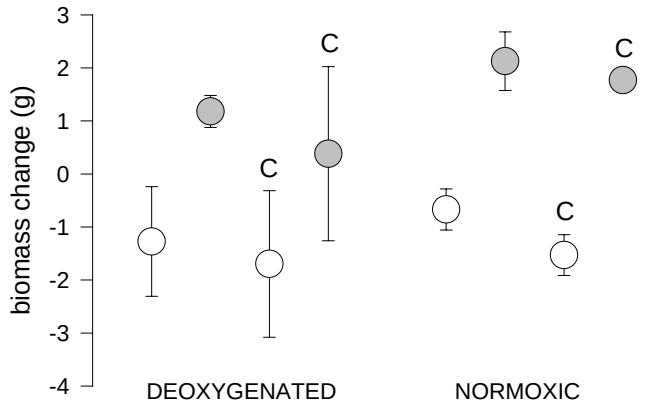

Figure 12. Effects of oxygen availability, organic enrichment and the presence of contaminants on biomass change in the polychaete *Nereis diversicolor*; mean ± standard deviation (n=3). Organic enrichment: gray; no addition: white. Contaminated groups are marked "C", uncontaminated groups are unmarked. See **Table 10** for statistical tests.

There were no statistically significant interactions between the three factors.

Amphiura filiformis

Growth (disk diameter) in the brittle star *A. filiformis* was increased in the organically enriched treatment, but there were no significant effects from oxygen availability, the presence of contaminants or interactions between the three factors (**Figure 13**A, **Table 10**). Arm regeneration in *A. filiformis* increased signficantly in aquaria that received organic enrichment and oxygen availability also affected this growth variable (**Figure 13**B, **Table 10**). There were no significant interactions between pairs of factors, but a weak interaction effect between all three ($F_{1,184}$=3.16, p=0.09). This interaction appeared to be due to decreased arm regeneration under non-enriched, normoxic conditions in the presence of contaminants compared to increased arm regeneration under non-enriched, contaminated, hypoxic conditions. The opposite pattern was seen for arm regeneration in *A. filiformis* in enriched aquaria.

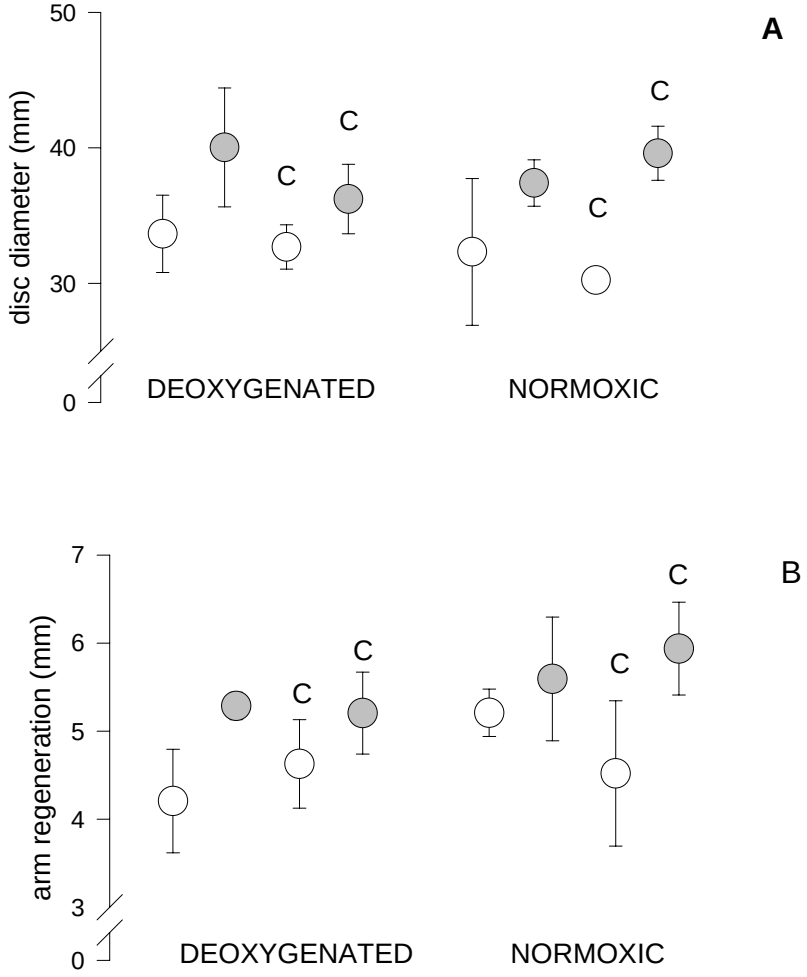

Figure 13. Effects of oxygen availability, organic enrichment and the presence of contaminants on disk diameter (A) and arm regeneration (B) in the brittle star *Amphiura filiformis*;. mean ± standard deviation (n=10). Organic enrichment: gray; no addition: white. Contaminated groups are marked "C", uncontaminated groups are unmarked. See **Table 10** for statistical tests.

Abra alba

The bivalve *A. alba* grew more in aquaria with organic material added (**Figure 14**). In addition, there was a significant interaction between organic enrichment and oxygen availability in their effects on growth of *A. alba* (**Table 10**). The interaction between organic enrichment and oxygen availability was due to a smaller increase in growth in *A. alba* from enriched compared to non-enriched aquaria in the deoxygenated treatment relative to the normoxic treatment. There was no effect of contamination on the growth of *A. alba*.

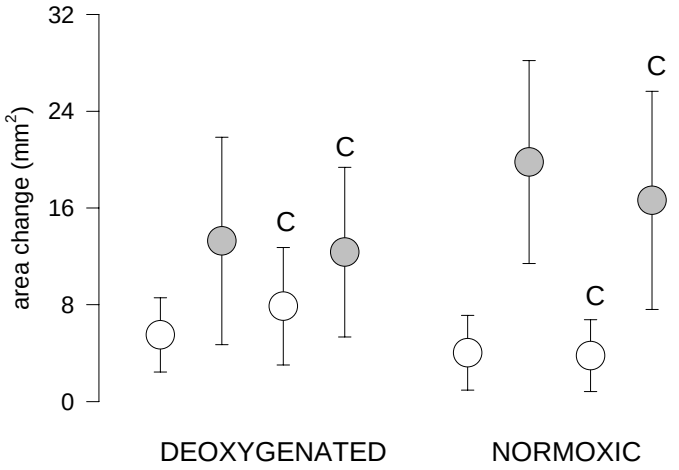

Figure 14. Effects of oxygen availability, organic enrichment and the presence of contaminants on growth (area change of shell) of the bivalve *Abra alba*; mean ± standard deviation (n=2-10). Organic enrichment: gray; no addition: white. Contaminated groups are marked "C", uncontaminated groups are unmarked. See **Table 10** for statistical tests.

Table 10. Three-way nested analyses of variance (ANOVA) with biomass change, growth or arm-regeneration as dependent variables. Oxygen availability ("oxygen"), organic enrichment ("organic"), the presence of contaminants ("contaminants") and all interactions between the three were used as factors. Effects of the primary factors are indicated for all parameters, whereas interactions are only shown where they contributed in the model (i.e. p<0.1).

Species	variable	n	model R^2 (adj)*	within-aquarium effect (p-value)	factors contributing to the model	p-value
Nereis diversicolor	biomass change	24	0.70	n/a	oxygen	0.05
					organic	< 0.0001
					contaminants	0.11
Amphiura filiformis	disk diameter	185	0.28	0.07	oxygen	0.33
					organic	< 0.0001
					contaminants	0.44
	arm regeneration	185	0.13	0.24	oxygen	0.05
					organic	0.002
					contaminants	0.97
					oxygen x organic x contaminants	0.09
Abra alba	shell area change	190	0.51	<0.0001	oxygen	0.94
					organic	< 0.0001
					contaminants	0.99
					organic x oxygen	0.05

* for model including all interactions, n/a - not applicable, ** root-transformed

3.5.3 Biomarkers

Metallothionein-like (MT-like) proteins were measured in samples from all three sediment-dwelling species, glutathione reductase in *N. diversicolor* and *A. filiformis*, whereas glutathione *S*-transferase was measured in *N. diversicolor* and *A. alba*.

The level of MT-like proteins and glutathione reductase activity was higher in *N. diversicolor* kept in aquaria with organic material added than in aquaria with low levels of organic carbon ($F_{1,127}$=19.8, p=0.0003 and $F_{1,92}$=4.16, p=0.05, respectively). Similarly, glutathione *S*-transferase in this polychaete was affected by organic enrichment ($F_{1,126}$=9.76, p=0.006), but there was also a significant interaction between contaminants and organic enrichment for this biomarker ($F_{1,126}$=5.14, p=0.04). None of the three factors (mean weight, benzo(a)pyrene in tissues, Cd in tissues) contributed significantly in a multiple regression analysis on MT-like proteins in *N. diversicolor*, although there was some indication of possible effects from benzo(a)pyrene (**Table 11**). Both the concentration of Cd in tissues and mean wet weight had significant effects in multiple regression on glutathione reductase in *N. diversicolor*. Of the three factors, only mean wet weight contributed significantly in a multiple regression model on glutathione *S*-transferase activity in *N. diversicolor*.

Table 11. Multiple regression analyses on biomarker responses in *Nereis diversicolor* with mean wet weight, tissue benzo(a)pyrene and Cd as regressors (both contaminants standardised to protein).

Biomarker	Number of observations	model R^2 (adjusted)	factors contributing to the model	p-value individual factors
MT-like protein (log-transformed)	35	0.34	mean wet weight	0.70
			benzo(a)pyrene	0.10
			Cd	0.24
Glutathione reductase (log-transformed)	24	0.47	mean wet weight	0.09
			benzo(a)pyrene	0.71
			Cd	0.001
Glutathione *S*-transferase (log-transformed)	35	0.13	mean wet weight	0.01
			benzo(a)pyrene	0.95
			Cd	0.46

Oxygen availability and the presence of contaminants appeared to have weak effects, although non-significant, on MT-like proteins in *A. filiformis* ($F_{1,66}$=3,75, p=0.07 and $F_{1,66}$=4.22, p=0.06). None of the treatments had significant effects on glutathione reductase in this brittle star. Both the level of MT-like proteins and activity of glutathione reductase in *A. filiformis* related to tissue levels of benzo(a)pyrene in multiple regression analyses (**Table 12**).

Table 12. Multiple regression analyses on biomarker responses in *Amphiura filiformis* with mean wet weight, tissue benzo(a)pyrene and Cd as regressors (both contaminants standardised to protein). Glutathione *S*-transferase was not measured in *A. filiformis* due to lack of material.

Biomarker	Number of observations	model R^2 (adjusted)	factors contributing to the model	p-value individual factors
MT-like protein (log-transformed)	28	0.42	mean wet weight	0.89
			benzo(a)pyrene	0.004
			Cd	0.24
Glutathione reductase (log-transformed)	24	0.67	mean wet weight	0.80
			benzo(a)pyrene	0.05
			Cd	0.17

There was a significant contribution from the interaction between oxygen availability and organic enrichment towards explaining the variability in MT-like proteins in *A. alba* ($F_{1,15}$=6.97, p=0.02). Glutathione *S*-transferase activity in this bivalve was not affected by any treatment. None of the factors in multiple regression contributed in the models on MT-like proteins or glutathione *S*-transferase in *A. alba* (**Table 13**).

Table 13. Multiple regression analyses on biomarker responses in *Abra alba* with mean wet weight, tissue benzo(a)pyrene and Cd in tissues as regressors (both contaminants standardised to protein). Glutathione reductase was not measured in *A. alba* due to lack of material.

Biomarker	Number of observations	model R^2 (adjusted)	factors contributing to the model	p-value individual factors
MT-like protein	16	0	mean wet weight	0.99
			benzo(a)pyrene	0.32
			Cd	0.35
Glutathione *S*-transferase (log-transformed)	15	0	mean wet weight	0.26
			benzo(a)pyrene	0.29
			Cd	0.99

4. Discussion

4.1 Experimental system

Experimental work in any scale is subject to artefacts and the results should always be considered with care. The use of meso- or microcosms as models for the "real world" is necessary to obtain realistic results related to interactions in marine sediments. The objective of the experiment described in this report was to study processes that influence the behaviour of contaminants in sediments. This requires manipulations with one factor at a time, which is largely impossible to carry out in the field.

One of the main advantages of the experimental set-up at Solbergstrand is the access to good quality seawater where salinity and temperature may be maintained stable and where the proximity to the sea allows continuous flow of water in the experiments. The facility also has a convenient location for sampling of sediments and organisms to be used in experiments and which are typical for fjord-type environments.

Using deoxygenated water may be a practical and to some extent an economical problem. Purging by nitrogen gas through the source water to maintain oxygen levels at 2-3 mg/l require much nitrogen and such a system will need a period of "tuning" to maintain constant oxygen levels during the experiment.

Spiking sediments with radioactive tracers, instead of using natural contaminated sediments, was done to allow measurements of contaminants in sediments, pore water and organisms at low levels. This was also economically favourable. It may, however, be argued that the speciation of the contaminants in the sediments control the levels in pore water and sediment-dwelling organisms and that equilibrium between sediment and pore water chemistry will not be established during the short time the experiment lasted. In this experiment we allowed two days constant mixing for the initial association of contaminants and organic material to sediment, followed by one week stabilisation in the aquaria under constant flow of water.

It should be emphasised that there is a great demand for knowledge about the environmental implications of contaminated sediments. Decisions are being made concerning discharge of contaminants and organic matter to the marine environment as well as handling of contaminated sediments (dredging, capping etc.). Experimental work will give insight into mechanisms and interactions that are much needed for environmental managers.

4.2 Partitioning and bioaccumulation of Hg and Cd

Contaminant levels in the sediments were within the range of concentrations frequently found in moderately polluted areas, and the concentration of H_2S remained low throughout the experimental period in all treatments.

The observed retention of excess concentration of Cd in sediments treated with *Skeletonema costatum*, as well as the preferential uptake of Cd (relative to Hg) in all of the sediment-dwelling species, was consistent with the frequently reported affinity of Cd to both dead and living organic material (Collier and Edmond, 1983, 1984, Golimowski *et al.*, 1990). *Abra alba* is known to feed selectively on food items, which is picked up by rapid and precise movements of the siphon. In the present experiment, *A. alba* was found to grow more rapidly than the other species, and efficient feeding on the Cd-contaminated algae appeared to be the primary pathway explaining the 100-fold higher Cd-concentration in this organism as compared to the other species. The results in Collier and Edmond (1983) indicate that the Cd associated with planktonic matter from sediment traps was easily desorbed. Consistently, the excess phase of Cd present on day zero disappeared during the experimental period.

Thus, the present experiment indicated that Cd adsorbed on the dead algae represented a highly available source of Cd for organisms feeding on the detritus.

The absence of detectable amounts of Hg both in pore water and *M. edulis* indicated that uptake of Hg via the pore water pathway was not important. The retention of Hg in sediments treated with algae was not different from the retention in the other sediments. Nevertheless, the concentration of Hg had increased in all sediment dwelling species exposed to algae-enriched sediments. Again the effect was most pronounced in *A. alba*, which had ten times higher concentration of Hg than the other species. It appears that feeding behaviour had affected metal uptake even when the metal was not associated with the principal food item. If, however, increased feeding in the algae-enriched sediments had been accompanied by increased ingestion of Hg-contaminated sediment, and some of this Hg was retained in the organisms, bioaccumulation should be stronger in the enriched than in the low-carbon sediments, as observed.

The oxygen factor had no significant effect on bioaccumulation of Hg, but affected the bioaccumulation of Cd. In *M. edulis* exposed to Cd in dissolved and particulate phases flowing through the downstream aquaria, the highest concentration in the oligotrophic treatment was consistent with the high concentration of Cd in the pore water of the corresponding treatment. This showed that leakage via the pore water might have accounted for a significant fraction of the total loss of Cd from the sediments. Significant interactions between the oxygen and organic factor, had however, resulted in high Cd-concentration in the most eutrophic treatment in spite of low Cd-concentration in the pore water. Mechanisms such as increased resuspension due to bioturbation or secretions from more actively feeding organisms, may have been involved to account for the relatively high Cd-concentration and significant interaction effects in the mussels located downstream the C-enriched sediment.

The two sediment-dwelling species *N. diversicolor* and *A. filiformis* might be more dependent on pore water quality than *A. alba* which has a protective shell and can feed and respire through its siphon on the sediment surface. This was confirmed by the ANOVA-analyses which showed lower p-values for interaction effects in *N. diversicolor* and *A. filiformis* ($p=0.2$) than in *A. alba* ($p=0.66$). Significant interactions were, however, only found for *A. filiformis* ($p=0.006$). *Nereis diversicolor* is known to be tolerant to hypoxia (Schöttler, 1979, Vismann, 1990) and in several experiments performed at MRSS, *N. diversicolor* has been observed to survive severe sulphide events in the pore water without leaving their burrows within the sediment. *Amphiura filiformis*, is known to be more sensitive to hypoxia and may respond to low oxygen by escaping from their normal location inside the sediment (Rosenberg *et al.*, 1991, Diaz and Rosenberg, 1995, Nilsson and Sköld, 1996). In the present experiment, sulphide concentrations were not severe and both species remained buried in the sediments. However, only *A. filiformis* showed a significant effect from the oxygen factor. If the accumulation of Cd in *A. filiformis* in the normoxic aquaria was stimulated by the higher Cd-concentration in the pore water, it appears that *A. filiformis* is more exposed to the pore water environment than *N. diversicolor*. Thus, the observed bioaccumulation patterns in the two species were consistent with the different behaviour shown by the two species when exposed to hypoxia.

Low level sulphide solubility control?

Because the Ag|AgS electrode selectively measures the activity of S^{2-} ions, the potentials recorded on such electrodes should be ideal for sulphide solubility calculations. Within the sediment surface, characteristic pS-values ranged between 10 and 14 (Table 1) corresponding to recorded potentials (E_{obs}) between -296 and -415 mV. Solving Eq.1 for this range of potentials gave activities of S^{2-} between $10^{-16.5}$ and $10^{-20.5}$ M. The mean activities of ^{109}Cd in the pore water of the four treatments ranged between 3.5-14.9 Bq/g. Using the specific activity of 0.0056 $\mu gCd \cdot Bq^{-1}$, an activity coefficient of 0.2 for the divalent ion and disregarding Cd-complex formation, activities of Cd^{2+} of $10^{-6.8}$-$10^{-7.5}$ M were calculated. By multiplication of the highest activities of Cd^{2+} with the lowest activities of S^{2-} and vice versa, the CdS ion activity product ($\{Cd^{2+}\} \cdot \{S^{2-}\}$) within the surface layer ranged $10^{-24.0}$-$10^{-27.3}$ which was in reasonably good agreement with the solubility product of $10^{-27.0}$ (Stumm and Morgan,

1981). This gave credibility to the potentials recorded, as well as it did indicate that Cd in the pore water was controlled by hydrogen sulphide, even at the very low concentrations present in this sediment. If sulphide solubility also controlled Hg-concentration, the solubility product of $10^{-52.7}$ for HgS would predict non-detectable Hg-concentration in the pore water, as observed.

It appears that low level sulphide solubility control could explain the complete absence of significant effects of oxygen on bioaccumulation of Hg. The solubility of $HgS_{(s)}$ is so low that even in the most oligotrophic treatment, Hg was maintained below detection limits. If sulphide solubility also was the primary factor controlling Cd-concentration in pore water, the activity of sulphate reducing bacteria yielding low levels of hydrogen sulphide in the apparently non-sulphidic environment, appeared to be responsible for the observed oxygen-organic interaction effects affecting bioaccumulation patterns in *M.edulis, A. filiformis* and, to a lesser extent, in *N. diversicolor*. Only in *A. alba* , bioaccumulation of Cd appeared to be independent on the sulphide ion activities.

4.3 Partitioning and bioaccumulation of benzo(a)pyrene

In all three sediment-dwelling species there was a trend towards increased accumulation of benzo(a)pyrene in organisms kept in aquaria with organic enrichment compared to organisms kept in aquaria with no organic addition. The increased accumulation of B(a)P in the benthic organisms may be either due to an increased uptake of contaminated food particles or caused by a stimulation of feeding and general metabolism triggered by the presence of food (organic material). Landrum et al. (1992) found decreased accumulation of B(a)P with increased feeding activity of the crustacean *Mysis relicta*. The results found in this project indicates the opposite - that benzo(a)pyrene is readily accumulated through feeding on highly available phytoplankton. The results found here and elsewhere (e.g. Wyman & Connors, 1980; Thomann et al., 1986) contrasts with the equilibrium partitioning (EqP) approach, where benthic organisms are regarded as passive lipid “droplets” that are in equilibrium with external concentrations as a function of sediment organic carbon (TOC). Such an ideal equilibrium may be reached in the long term. If bioaccumulation is to any extent affected by selective uptake of contaminated food particles (as the results found here suggest), other processes may be more important in short-term accumulation such as that found during spring blooms. Several studies have suggested that major sedimentation events may temporarily “clear” the water column of contaminants (especially organic contaminants), thereby strongly enhancing bioaccumulation in sediment-dwelling organisms.

Blue mussels kept in aquaria downstream of aquaria receiving organic addition also contained higher concentrations of benzo(a)pyrene than mussels kept downstream aquaria without such addition. This result indicated that the release of benzo(a)pyrene from sediments increased with increased organic loading. The most probable reason was the increased activity of the sediment-dwelling organisms in the aquaria receiving organic material, thereby increasing resuspension and transport of particle-associated contaminants (as found for Cd) as well as more dissolved organic matter (DOM) than sediments with less organic material. Released DOM would be a carrier for organic contaminants (and metals) being released from the sediment.

4.4 Effects on sediment-dwelling organisms

All three sediment-dwelling species, *N. diversicolor, A. filiformis* and *A. alba,* grew better in organically enriched aquaria compared to non-enriched aquaria. The results indicate that food availability was the major limiting factor for macrobenthic production in this system, and also that the source of carbon used, predominantly the diatom *Skeletonema costatum*, was a readily available food source for all three species. All three species are known as facultative suspension feeders and are able to use both sediment-associated carbon and organic material present in the water column (Buchanan, 1964; Martoja *et al.*,1988; Amouroux *et al.*,1989; Esselink *et al.*,1989; Riisgård, 1991; Rosenberg, 1993; Loo *et al.*,1996).

Although organic enrichment had by far the strongest influence on growth in *N. diversicolor*, reduced oxygen availability also affected growth. *Nereis diversicolor* is known to be tolerant to low oxygen levels (Vismann, 1990; Miron & Kristensen,1993a; Miron & Kristensen,1993b; Abele-Oeschger, 1996) and was able to use available organic material for somatic growth even under hypoxia. In experimental studies with the detritus-feeding polychaete *Capitella capitata*, Forbes & Lopez (1990) found interactions between oxygen level and food availability in two out of three experiments with different food sources. The observed growth in *C. capitata* was limited at oxygen levels similar to those used in the present study, even in the presence of food. Thus, the effects of organic enrichment and oxygen availability were additive in their effects on growth in *N. diversicolor*, whereas the same two factors each limited growth in *C. capitata*. In that study, there were some differences between large and small individuals, but no such effects were found here (results not shown). The presence of contaminants did not affect growth in *N. diversicolor* in this study, although exposure to similar concentrations of benzo(a)pyrene in sediment has been found to decrease growth in *N. diversicolor* previously (Hylland, unpublished). In that study, growth reduction was stronger after three weeks than at six weeks, however, a result which suggests some sort of acclimation to this contaminant. Such acclimation would explain the lack of effects following three months' exposure found here.

Both disk size and arm regeneration in *A. filiformis* increased under enriched conditions. Similar results were found by Sköld & Gunnarsson (1996) in their study of *A. filiformis* and *A. chiajei*. Whereas disk size was not affected by other treatments in the present study, hypoxia negatively affected arm regeneration, in accordance with previous observations (Nilsson & Sköld, 1996). The only indication that the presence of contaminants may have affected arm regeneration in *A. filiformis* was by a weak (non-significant) interaction between all three factors, apparently due to a contaminant-related decrease in arm regeneration which was only detectable when there was sufficient oxygen but little food. In two experiments on arm regeneration in the brittle star *Microphiopholis gracillima*, D'Andrea *et al.* (1996) found only minor effects of Cd exposure, at concentrations above those used in the present study. In one study done in early autumn there was an increase in arm regeneration in brittle stars kept in sediment with 7.71 mg/kg Cd compared to brittle stars kept in 1.75 mg/kg Cd. In a second study using the same conditions two months later there were no differences in arm regeneration of brittle stars kept in sediments with Cd concentrations up to 13.75 mg/kg. In contrast to the results for *N. diversicolor* and *A. filiformis*, there was an interaction between organic enrichment and oxygen availability in their effects on growth in *A. alba*. It is not surprising that decreased oxygen availability also affects growth since both regulating oxygen uptake and anaerobic respiration leads to higher energy costs (de Zwaan & Putzer, 1985). Thus, the production of all three species, even the tolerant *N. diversicolor*, was negatively affected by low oxygen availability.

Whereas the effects of organic enrichment and oxygen availability on growth in *N. diversicolor* and *A. filiformis* appeared to reflect a physiological adjustment to low food or low oxygen levels, the results reflect an inability of *A. alba* to utilise the available organic material under hypoxic conditions. In contrast to *N. diversicolor*, both *A. alba* and *A. filiformis* are sensitive to low oxygen levels (Rosenberg & Loo,1988; Rosenberg *et al.*,1991).

Effects of hypoxia on the energy budget of benthic fauna vary considerably between species and may interact with food availability. To a large extent these effects depend on the capability of taking up oxygen even at low levels, i.e. if a species is a regulator or a conformer, and its capacity of anaerobic metabolism. Internal tissues of echinoderms rely to some extent on anaerobic metabolism even under aerobic conditions (Shick, 1983). Anaerobic respiration leading to higher energy costs is a likely explanation to the negtive effects of the hypoxic treatment (2.4-3.5 mg/l) in this study. This is supported by (Forster et a*l.*,1995) who suggested that a shift from aerobic to anaerobic respiration occur in benthic communities during prolonged exposure to 1.8-2.7 mg O_2/l. As will be apparent from the above discussion, it is not possible to generalise and to some species such as *A. alba* oxygen concentrations below 3 mg O_2/l may severely decrease growth and even decrease survival. *Abra alba* is typically found in organically enriched sediments where it may be present in high densities (Dauvin & Gentil,1989). Such areas are also prone to oxygen deficiency and the growth and survival of such

dense *A. alba* populations will therefore be a continuous balance between high energy input (food availability) and high metabolic costs due to periodic anaerobic metabolism and regulation of oxygen uptake.

The presence of contaminants had minor effects on the growth of these three sediment-dwelling invertebrates. As mentioned above, there was a trend towards reduced growth in *N. diversicolor* exposed to contaminants and a possible reduction in arm regeneration in *A. filiformis* under non-enriched, normoxic conditions. The concentrations of the relevant contaminants, especially of Cd (4.8 mg/kg dry wt) and benzo(a)pyrene (1.32 mg/kg dry wt), are well into the range where effects on sediment-dwelling organisms would be expected (Oslo and Paris Commissions, 1994; Long *et al.*,1995). It is not clear why there was so little influence of sediment-bound contaminants on the growth of sediment-dwelling invertebrates in the present study, as effects on growth or similar sublethal parameters in other marine organisms have been observed at far lower concentrations than those used here (Forbes & Depledge,1992). On the other hand, growth or arm regeneration may not be the attributes of sediment-dwelling invertebrates that are most sensitive to contaminants. In most previous studies where contaminant exposure has led to decreased growth or reproductive output, exposure concentrations have been higher than those used here, the exposure has been shorter and organisms have been exposed via water and not via sediment (e.g. Røed, 1980; Jenkins & Sanders,1985; den Besten *et al.*,1991).

In addition to biomass change and growth, the effects of sediment-bound contaminants on the experimental organisms were assessed using subcellular effect measures, commonly referred to as biomarkers (cf. McCarthy & Shugart, 1990). There is little knowledge of how biomarkers in sediment-dwelling invertebrates respond to contaminants or other environmental factors. The biomarkers used here are known to respond to specific contaminants or sets of contaminants in vertebrates, but their response in invertebrates or the influence of other factors, e.g. organic enrichment or oxygen availability, is largely unknown. Metallothionein-like proteins, a biomarker for metals such as Cd, Zn and Cu (Roesijadi, 1992), appeared to primarily respond to growth or growth-related processes and organic enrichment for *N. diversicolor* and *A. alba*, but to contaminants (benzo(a)pyrene) in tissues for *A. filiformis*. Ruffin *et al.* (1994) found increased synthesis of various non-MT proteins, including putative heat-shock proteins, in *N. diversicolor* following exposure to Cd. None of the groups in the present study differed with regard to protein concentration, however, so changes in the rates of total cytosolic/microsomal protein synthesis was not affected by any of the treatments used here (results not shown). Moreover, there has been some discussion as to whether metallothionein or MT-like proteins are at all present in *N. diversicolor*, and other Cd-binding proteins have been identified in this species (Nejmeddine *et al.*,1988; Demuynck *et al.*,1991; Demuynck & Dhainaut Courtois, 1994). This question can not be resolved here as the measured MT-like protein was not characterised biochemically, but *N. diversicolor* was exposed to much lower concentrations and through a longer period in the present compared to previous studies. A strong correlation was found between benzo(a)pyrene concentration in tissues and MT-like proteins in *A. filiformis*, whereas Cd concentration did not appear to affect this biomarker. Since the two contaminants co-occurred in the sediments it is not possible to separate their effects, however, and the concentration of MT-like proteins correlated with both contaminants in isolated simple regression analyses. Metallothionein in both mammals and invertebrates is induced by free-radical generating agents such as benzo(a)pyrene (Sato, 1991; Marsh *et al.*,1992; Martinez *et al.*,1995) and a similar induction of MT-like proteins has been observed in tunicates exposed to PAH from an aluminium smelter (Næs *et al.*, unpublished). A metal-inducible MT has been characterised in some echinoderms (Nemer *et al.*,1984; Harlow *et al.*,1989) and indicated in others (den Besten *et al.*,1990; Aspholm & Hylland, unpublished), but the response of this protein to free radicals is unknown. As for *N. diversicolor*, there has also been some discussion as to whether MT or MT-like proteins are relevant to trace metal metabolism in sediment-dwelling bivalves, e.g. *Macoma balthica* (Johansson *et al.*,1986; Langston & Zhou,1987). In the present study we found no relationship between MT-like proteins in *A. alba* and the presence of contaminants, even at Cd sediment concentrations of 4.8 mg/kg dry wt. Other mechanisms of metal sequestration and detoxification may be more active in sediment-dwelling bivalves than low-molecular-weight proteins such as MT.

It is not clear why glutathione reductase activity in *N. diversicolor* increased in response to tissue Cd. Glutathione reductase is involved in free radical metabolism through its reduction of oxidised glutathione, an important cellular scavenger of radicals. The activity of this enzyme may therefore to some extent reflect cellular levels of either oxyradicals or chemically generated radicals (Halliwell & Gutteridge, 1989). Cd may affect glutathione reductase activity indirectly by depleting intracellular glutathione (cf Cartaña *et al.*,1992). Tissue levels of Cd and benzo(a)pyrene in *N. diversicolor* correlated strongly, however, and effects from individual contaminants could not be separated in the present study. Increased activity of glutathione reductase a response to benzo(a)pyrene is not unexpected as exposure to this contaminant is known to induce antioxidant enzymes in other invertebrates (Livingstone *et al.*,1990; Ribera *et al.*,1991; Schlenk *et al.*,1991). Moreover, increased glutathione reductase activity also related to tissue benzo(a)pyrene in *A. filiformis*. Ideally, other components of the cellular defense against radicals should be investigated simultaneously (cf Regoli & Orlando, 1995), but these results do indicate a possible involvement of antioxidant enzymes in contaminant responses of important sediment-dwelling invertebrates such as polychaetes and echinoderms. The third biomarker, glutathione *S*-transferase, encompasses a family of enzymes involved in conjugation reactions with both endogenous and exogenous lipophilic compounds (Clark, 1989). This biomarker has previously shown some promise as a marker for organic contaminants in invertebrates (Lee *et al.*,1988; Egaas *et al.*,1993). Although not relating directly to tissue levels of contaminants, glutathione *S*-transferase in *N. diversicolor* appeared to be weakly affected by contaminant exposure. There were however some indications that the response in glutathione *S*-transferase in *N. diversicolor* to sediment-bound contaminants was decreased in the presence of organic enrichment.

The major effects on biomarker responses in this experiment was expected to be driven by the presence or absence of contaminants in sediments. Such differences in biomarker responses were weak to non-existent. Nevertheless, some of the biomarkers did relate to tissue levels of contaminants. Since bioaccumulation of contaminants was obviously greater in sediments with contaminants added to them than in uncontaminated sediment and always exceeded 4-5 times the initial tissue concentration of the relevant contaminants, one would expect correspondence between sediment contaminants and biomarker responses. This lack of agreement can not be resolved here, but requires a study specifically focusing on the effects of bioavailability, bioaccumulation and duration of exposure on biomarker responses in sediment-dwelling organisms.

An addition of diatom organic material, equivalent to approximately 40 g C/m^2 and resulting in an increase in sediment organic carbon from 1.21% to 1.92%, caused increased growth in the three sediment-dwelling species *N. diversicolor, A. filiformis* and *A. alba*. Hypoxia (2.4-3.5 mg O_2/l) decreased growth all three species, but the interactive effects between organic enrichment and hypoxia differed between the three species. Whereas *N. diversicolor* and *A. filiformis* appeared to adjust their metabolism in response to low oxygen levels, the growth decrease in *A. alba* indicated that this species was inefficient in its use of the available organic material under hypoxia. The presence of contaminants had minor effects on growth in these organisms, although there was an indication of complex interactions between all three factors on arm regeneration in *A. filiformis*. Biomarker responses in *A. filiformis* and to some extent *N. diversicolor* were affected by tissue contaminant levels, whereas there was no such responses in *A. alba*. There were only weak direct interactions between eutrophication-related processes and sediment-bound contaminants in their effects on growth in the sediment-dwelling invertebrates studied here. To some extent, biomarker responses in these species, especially *A. filiformis*, reflected interactions between eutrophication and contaminants through their response to bioaccumulated contaminants.

5. Conclusions and relevance to environmental management

5.1 Evaluation of the experimental system and design

Although there were initial problems in keeping the oxygen levels sufficiently low in the deoxygenated aquaria, it appears from the results that exposure-levels of all three factors (oxygen, contamination, organic material) were within acceptable limits. There were no visible changes in the sediments and low mortality of sediment-dwelling organisms, but obvious effects on both biogeochemical and biological parameters.

The experimental conditions were presumably close to what is found in moderately contaminated sediments. Notable differences between the experiment and natural conditions is the lack of other macrofauna, possibly reduced diversity of meiofauna and lack of biogeochemical "ageing" and layering of the sediment.

5.2 Addition of organic material - influence on carbon and nitrogen in sediments

Both additions of organic material had moderate effect on sediment levels of carbon and nitrogen. It is worth noting however that even minor concentration differences in total carbon - 1.3% in normal compared to 2.1% in the enriched groups - affected both the bioaccumulation of contaminants and growth in sediment-dwelling organisms. The organic material added is similar to what will be a large proportion of the material in a normal spring bloom in temperate waters (diatoms) and the experiment should in that sense be similar to a natural situation.

5.3 Partitioning and bioaccumulation of contaminants

In this study the contaminants included both trace metals (Hg and Cd) and an organic contaminant (benzo(a)pyrene, BaP). A high and a low organic regime in the sediments was established to study the influence of organic matter on the partitioning of these contaminants. The results showed that the availability to sediment-dwelling organisms increased primarily as a result of enrichment by organic material. Similarly, there was a weak increase of bioaccumulation of BaP when the organic matter in the sediments increased. There were some obvious differences in the patterns of bioaccumulation found for the three contaminants which merit further study into the mechanisms for uptake. The results indicate that the presence of nutritive food sources (e.g. phytoplankton) strongly influences the bioavailability of contaminants in marine sediments. Furthermore, the quality of organic material is presumably important in the interpretation of contaminant bioaccumulation in benthic organisms (Maloney, 1996). All three contaminants were predominantly present in the upper two centimetres of the sediment at the end of the experiment, indicating negligible bioturbation by the three sediment-dwelling species added to the system. Both decreased oxygen availability and organic enrichment affected the distribution of Cd; there was least retention of Cd in sediment (and highest expected bioavailability, at least from water) in the absence of both these eutrophication-related factors. Benzo(a)pyrene and Hg were not detectable in pore water-samples.

Despite obvious species differences and differences between contaminants, there was clearly a strong effect of organic enrichment on the bioaccumulation of trace metals and, to a lesser extent, BaP. *A. filiformis* differed from the other two sediment-dwelling species in that there were only minor differences between treatments with regard to accumulation of trace metals, but that there was a difference with regard to BaP. *Nereis diversicolor* and *A. alba* were similar in that both accumulated

more trace metals under situations with organic enrichment, but that none of the treatments appeared to strongly affect the bioaccumulation of BaP.

The results found here suggest that removal of nutrients or other remedial action to decrease eutrophication in an area could also decrease the contaminant load in sediment-dwelling invertebrates (and conceivably their predators) without changes in the contaminant load. It must be emphasised that this observation relates to oxic sediments, a limited number of species and a selection of contaminants.

5.4 The influence of decreased oxygen availability

The oxygen availability in the sediments is primarily controlled by the supply of oxygen at the sediment-water interface (deep-water renewal) and the quantity of oxygen-consuming substances in the sediments. Furthermore, the availability of oxygen appeared to control the concentrations of Hg and Cd in the pore water due to formation of metal sulphides. Due to the low solubility of mercury sulphide (HgS) the bioaccumulation of Hg is independent of the pore water pathway. The bioaccumulation of Cd in the brittle-star *A. filiformis*, but not in the polychaete *N. diversicolor* or the bivalve *A. alba*, was reduced under a low oxygen regime, presumably due to higher sensitivity towards pore water composition. Hypoxia had no apparent effect on the bioaccumulation of BaP in any of the organisms.

The results found here indicate that increased oxygen availability (through improved water exchange) may increase the bioaccumulation of some metals in sediment-dwelling organisms (and conceivably their predators). There was no indication of increased bioaccumulation of the organic contaminant used (benzo(a)pyrene).

5.5 Mobilization of contaminants in the sediments

Release of contaminants from the sediment to the overlying water during the 3-month experiment was monitored by blue mussels in downstream aquaria. It was assumed that the mussels would accumulate both metals and benzo(a)pyrene in dissolved or particulate form originating from the sediment aquaria (diffusive transport and resuspension due to bioturbation). Accumulation of Cd in the mussels indicated release from the sediments to a larger extent in the low carbon-normoxic regime in which the pore water concentrations were higher. Mercury, however, was tightly bound in the sediments in all treatments. Benzo(a)pyrene was released from the sediments and accumulated in the mussels, particularly when the input of organic matter to the bottom sediments increased.

Thus, decreased eutrophication in a given area (with low current velocities) could cause decreased transfer of contaminants from the sediment to overlying water, probably through decreased activity of sediment-dwelling organisms and/or increased liberation of dissolved organic material (DOM) carrying contaminants.

5.6 Effects on sediment-dwelling organisms

Growth and biomarker responses were determined in the sediment-dwelling organisms to quantify the biological effects of organic enrichment, redox regimes and the presence of contaminants. The results show that organic enrichment stimulated growth (biomass change, shell growth, arm regeneration) of sediment-dwelling organisms. Hypoxia, on the other hand, had a negative effect on growth. Although there was a response in some biomarkers to bioaccumulated contaminants, the effects of sediment-bound contaminants on these sediment-dwelling organisms were minor.

5.7 Answers to initial questions

- *Will differences in organic enrichment affect the partitioning of environmental contaminants between sediment, pore water and sediment-dwelling organisms?*

The results from the project indicate clearly that changes in organic enrichment affect the partitioning of contaminants. Contrary to what may have been expected, increased organic enrichment generally caused increased bioaccumulation of contaminants.

- *Will differences in the redox-conditions of the sediment (as caused by different levels of oxygen and/or organic enrichment) affect the partitioning of environmental contaminants between sediment, pore water and sediment-dwelling organisms?*

Yes, small changes in sulphide levels (below detection limits for conventional methods) clearly affected the solubility (and hence bioavailability and bioaccumulation) of Cd and Hg. Redox conditions were affected by both organic enrichment and oxygen availability.

- *Will organic enrichment, oxygen variability, and/or the presence of environmental contaminants affect growth or biomarkers in sediment-dwelling invertebrates?*

Organic enrichment clearly affected growth in all three sediment-dwelling species. Oxygen had some effect, whereas the presence of contaminants had little effect. There was some response in biomarkers in two of the sediment-dwelling species to bioaccumulated contaminants. It is important to note that the levels of all three factors (organic enrichment, oxygen availability, presence of contaminants) were within ranges found in coastal sediments with a moderate input of pollutants.

- *Will the amount of contaminants released from the sediment differ between sediments receiving different levels of organic loading and/or deoxygenated/normal oxygen levels?*

More Cd and benzo(a)pyrene was released from organically enriched sediment than from sediment not receiving organic enrichment.

- *To what extent need decisions considering improvements in the eutrophication status of an area be related to the level of environmental contaminants in that area?*

The results found here indicate that any improvement, be it decreased eutrophication or decreased input of contamintants, may contribute to decreased concentrations of contaminants in sediment-dwelling biota. The current project did not address processes in the water, for which the answer may be different.

- *To what extent need decisions considering improvement in the water exchange of an area be related to the level of contaminants in and/or eutrophication status of that area?*

Increased oxygen levels in bottom waters, be it from increased rates of water exchange or decreased sedimentation of organic material, may increase the bioaccumulation of some metals. Increased oxygen levels in bottom water may be expected to change trophic pathways and the results found here indicate that some macrobenthic species (e.g. *Abra alba*) will be able to make better use of available organic material under high oxygen levels.

- *To what extent may eutrophication or oxygen depletion affect the role of contaminated sediment as a source of contaminants?*

Organic enrichment caused increased mobilisation of both cadmium and benzo(a)pyrene from the sediment (as evidenced through increased accumulation in blue mussels receiving water from the aquaria). Low oxygen levels decreased the mobilisation of Cd, but did not affect benzo(a)pyrene.

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Appendix A.

Abbreviation/term	Full wording
ANOVA	Analysis of variance
B(a)P, BaP	Benzo(a)pyrene
Cd	Cadmium
C	Carbon
DOM	dissolved organic carbon
EqP	Equilibrium partitioning
IFE	Institute for Energy Technology, Kjeller, Norway
Hg	Mercury
ID	Internal diameter
i.m.	Internal measure
KMF	Kristineberg Marine Research Station, Fiskebäckskil, Sweden
N	Nitrogen
NIVA	Norwegian Institute for Water Research1
TOC	total organic carbon