



## Responses of Baltic benthic invertebrates to hypoxic events

Helene Modig\*, Emil Ólafsson

*Dept. of Zoology, University of Stockholm, S-106 91 Stockholm, Sweden*

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### Abstract

In the Baltic Sea, oxygen-depleted areas are widespread and have increased in a relatively short period of time. In a laboratory experiment, microcosms with natural soft-bottom communities were exposed to (a) hypoxia (0.2 mg O<sub>2</sub>/l) (b) hypoxia (0.2 mg O<sub>2</sub>/l)/normoxia (11–12 mg O<sub>2</sub>/l) altered on a weekly basis and (c) normoxia for two months. Multidimensional scaling ordination separated all treatments distinctly with communities in normoxia very similar to field controls but different from hypoxic treatments. On the basis of this experiment, we group the species into ‘very sensitive’: the amphipod *Monoporeia affinis*, harpacticoids *Microarthridion littorale* and *Pseudobrydia* sp. were totally wiped out in pulses of hypoxia, ‘sensitive’: the surface dwelling nematode species *Axonolaimus spinosus* and the meiofauna groups Oligochaeta and Turbellaria were found in reduced abundance in the hypoxic treatments, ‘less sensitive’: *Macoma balthica*, the ostracod species *Paracyprideis fennica* and the nematode species *Calomicrolaimus honestus*, *Paracanthochus* spp., survived temporary hypoxia, but not permanent hypoxia, and ‘tolerant’: the ostracod species *Heterocyprideis sorbyana*, *Candona neglecta*, the group Kinorhyncha, and the nematode species *Leptolaimus elegans*, *L. papilliger*, *Sabatieria pulchra* were all tolerant to hypoxic conditions. The different responses of the major species and taxa to long- and short-term hypoxic events, may be used as a tool in estimating the duration of naturally occurring hypoxia. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Baltic Sea.; Soft-bottom; Hypoxia; Meiofauna; Macrofauna; Laboratory experiment

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### 1. Introduction

Oxygen is one of the most important environmental factors structuring benthic communities. In a short period of time, areas with hypoxic and anoxic bottom water have increased and many marine ecosystems in the world are now under severe stress due to low oxygen concentrations (Diaz and Rosenberg, 1995).

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\*Corresponding author. Tel.: + 46 8-164009; fax: + 46 9-167715; e-mail: helene.modig@zoologi.su.se

The effects of low oxygen concentrations can be drastic and entire macrobenthic communities may disappear as a result of hypoxic conditions (Josefson and Widbom, 1988). Under low-oxygen conditions in marine systems, sulphide may accumulate as a result of the activity of sulphate-reducing bacteria. Sulphide is toxic to many aerobic organisms and several tolerance studies show that the negative effects of low oxygen concentrations are aggravated by sulphide exposure (see Ref. Bagarinao, 1992; Nilsson and Rosenberg, 1994).

Many invertebrates are however naturally exposed to oxygen deficiency and high sulphide concentrations during periods of their life and they have different strategies to endure such conditions. Intertidal species can switch to anaerobic metabolism during low tide (Schöttler et al., 1984; Grieshaber et al., 1992), many burrowing species such as *Saduria entomon* are tolerant to low oxygen concentrations and sulphide (Hagerman and Szaniawska, 1988, 1990; Hagerman and Vismann, 1993) and have a mechanism to detoxify sulphide (Vismann, 1991). The responses of marine benthic invertebrates to low oxygen concentration and the presence of sulphide have been documented in a number of investigations (Theede et al., 1969; Rosenberg et al., 1991; Vismann, 1990, 1991, 1996; Oeschger and Vetter, 1992; Hagerman and Vismann, 1995; Abele-Oeschger and Oeschger, 1995; Völkel et al., 1995; Diaz and Rosenberg, 1995; Gamenick et al., 1996; Sobral and Widdows, 1997).

Several meiofaunal species inhabit sulphidic zones which has been regarded as an ecosystem of its own (e.g. Fenchel and Riedl, 1970) termed thiobios (Boaden and Platt, 1971). Reise and Ax (1979) argued that the deep-dwelling meiofauna did not live in the sulphidic zone but in a microoxic environment near faunal tubes or burrows. With microelectrode measurements, Meyers et al. (1987) found a continuum of oxic and sulphidic microhabitats around burrows attracting both oxybios and thiobios. Similarly Wetzel et al. (1995) found enhanced oxygen concentrations around the burrows of the large polychaete *Arenicola marina*. Smaller burrowing invertebrates can also cause a mosaic of chemically different microniches beneath the sediment surface (Fenchel, 1996). Further, it has recently been suggested that thiobiotic meiofauna are able to utilise extremely low oxygen concentrations, undetectable with current measuring techniques (Raffaelli and Hawkins, 1996).

Generally, nematodes are considered to be the most resistant meiofauna taxa to low oxygen concentrations and sulphide exposure, and copepods to be more sensitive (Murrell and Fleeger, 1989; Hendelberg and Jensen, 1993). However this seems to be species specific as the diversity of nematodes may decrease after a hypoxic event (Austen and Widbom, 1991) and some copepod species appear to be tolerant to low oxygen concentrations and sulphide exposure (Vopel et al., 1996).

In the Baltic, due to the lack of water mixing and the rare intrusions of more saline oxygenated water from the North Sea, large areas below the halocline (around 60–80 m) are characterised by low oxygen concentrations (Andersin et al., 1978). Shallower areas may also become depleted of oxygen, typically after spring bloom events when the sedimentation rate is high and the break-down of organic matter is intense (Larsson pers. comm). Not only phytoplankton spring bloom material, but also drifting macroalgal mats may induce hypoxia when accumulating at the bottom (Bonsdorff, 1992; Norkko and Bonsdorff, 1996a; Bonsdorff, 1992). Hypoxic events in shallow areas are often short

since they may be interrupted by mixing of the water column, induced by stormy weather.

The main aim of this study was to identify the responses of a common infaunal (macrofauna and meiofauna) benthic community in the Baltic proper to hypoxic events. Our hypothesis is that the faunal response is different after two months of continuous hypoxia compared with two months of hypoxic conditions interrupted by pulses of normoxia. We postulate that some species are adapted to short-term hypoxic events and will therefore tolerate such events. We do not intend to separate the confounding effects of low oxygen concentrations and sulphide exposure on the benthic community.

## **2. Materials and methods**

We define hypoxia as dissolved oxygen concentrations between 0 mg/l (the point of anoxia) and 2.8 mg/l (2 ml/l) (Diaz and Rosenberg, 1995).

Microcosms were established at the Department of Zoology, Stockholm University in May 1995. Each microcosm consisted of a 1.5-l plastic jar of internal diameter 12 cm and 13 cm height and a plastic lid. Sediment was sampled with a Kajak-corer equipped with polycarbonate liners (50 cm<sup>2</sup>) from a 33-m muddy station ca. 3 km south of Askö Laboratory in the north western Baltic proper (58°49'N, 17°38'E). The top 7 cm of the sediment cores were carefully placed in the microcosms, and immediately brought back to the laboratory. A total of 21 microcosms were established to permit 3 treatments of 7 replicates each. Further, five cores were fixed in 4% formaldehyde immediately upon sampling to be used as field controls. The microcosms were partly sealed with plastic lids placed in a dark thermoconstant room, temperature and salinity were kept at ambient levels i.e. 4–5°C and 6.7–7.2‰, and the overlying water was oxygenated with airstones. Water was not replaced during the course of the experiment. After 12 days, 7 microcosms were randomly assigned to the three treatments; normoxic with air supply during the whole experiment (A1–A7), hypoxia/normoxia altering air and N<sub>2</sub> supply on a weekly basis (B1–B7) and hypoxia with only N<sub>2</sub> (C1–C7).

Dissolved-oxygen concentrations were measured daily in the near bottom water with an oxygen electrode (WTW Oxi 191). After each measuring occasion, nitrogen gas was bubbled for twenty minutes into the hypoxic microcosms to keep the oxygen concentrations low. The oxygen concentrations were always well below the limits of hypoxia (avg. 0.5 mg O<sub>2</sub>/l, min. 0.1 and max. 0.8 mg O<sub>2</sub>/l). Microcosms kept in a normoxic environment (A1–A7 and every second week B1–B7), were bubbled with air and had oxygen levels ranging between 11 and 12 mg O<sub>2</sub>/l. Salinity was measured regularly in the microcosms with a conductivity meter. At the end of the experiment, after two months, the pH in the water was measured with a pH-meter and the sulphide concentration in the water column was measured using a colorimetric method (determination of sulphide content of pure water and nonpolluted natural water, SIS). Oxygen penetration into the sediment was measured with a 50-μm tip electrode (Diamond General #737) at the end of the experiment. Hypoxic/normoxic microcosms (B1–B7) (hypoxic at this moment) and hypoxic microcosms (C1–C7) were connected to air bubbling. This was done in order to compare oxygen penetration into the sediment

among the treatments. Oxygen profiles were measured after 6 and 28 h of air supply to the hypoxic microcosms. Oxygen profiles in the normoxic microcosms were also measured twice with a 20-h interval. Finally the microcosms contents were fixed in 4% formaldehyde.

The sediment was sieved through a 500- $\mu\text{m}$  and 40- $\mu\text{m}$  mesh sieve. The 40- $\mu\text{m}$  fraction samples were then transferred to Ludox (colloidal silica polymer) with a specific gravity of 1.15. After stirring, the samples were left for 45 min and then the supernatant liquid decanted through a 40- $\mu\text{m}$  sieve. This procedure was repeated then once more. Macro- and meiofauna retained on the 500  $\mu\text{m}$  and 40  $\mu\text{m}$  sieves were enumerated and identified to major taxa or species in a petri dish under a stereodissecting microscope. Nematodes from the 40- $\mu\text{m}$  fraction were impregnated with anhydrous glycerine (Platt and Warwick, 1983) and mounted on slides for identification under a high-power microscope.

One-way analysis of variance, ANOVA, was used to investigate differences among treatments, in total abundance of major taxa, species, dissolved-oxygen concentration in the sediment and pH and the Tukey a posteriori test used for pairwise comparisons. To check the assumption of homoscedasticity Cochran's *C* test was applied and when necessary, data were  $\log_{10}(x + 1)$  transformed. Patterns in macrofauna species, major meiofauna taxa and nematode-species assemblage structure were examined with nonmetric multidimensional scaling ordination (MDS) using the PRIMER v4.0 statistical package from the Plymouth Marine Laboratory. Before analysing the macrofauna species and major meiofauna taxa pattern, data were double square root-transformed. This was done in order to reduce the dominance of major meiofauna taxa abundance. The Bray–Curtis similarity index was used in all cases. One-way ANOSIM (analysis of similarities) was used to test whether there was significant difference between treatments in assemblage structure (Warwick et al., 1990a,b). The SIMPER computer program was then used to identify those species contributing to differences observed in the ordination analysis (Warwick et al., 1990a,b).

### 3. Results

#### 3.1. Physical parameters

The pH was, on average, 7.5 at the end of the experiment and did not differ among treatments ( $P > 0.05$ , ANOVA). In the normoxic microcosms, dissolved oxygen was measurable in the sediment down to a maximum depth of 20 mm. The dissolved oxygen concentration (%) in normoxic, normoxic/hypoxic and hypoxic microcosms were compared at the sediment surface, and four-depth layers below the sediment surface (Table 1). The hypoxic/normoxic microcosms had a shallower oxygen penetration than normoxic microcosms, 6 h after the air pumps had been switched on and significantly less dissolved oxygen was measured in depth layers 6 and 7 mm below the sediment surface ( $P < 0.001$ , ANOVA, Table 1, Fig. 1). However, at the second measuring occasion when air pumps had been on for 28 h, normoxic and hypoxic/normoxic microcosms did not differ at any of the depth layers compared ( $P > 0.05$ , ANOVA,

Table 1  
Average values (Avg) and standard error (SE) of dissolved oxygen concentrations in different depth layers

Depth layer (mm)	A1–0 h		A2–20 h		B1–6 h		B2–28 h		C1–6 h		C2–28 h		ANOVA p-value	Tukey test
	Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE		
Water column	93	2	87	4	90	2	88	1	85	1	85	1		
Sediment surface	68	5	68	2	66	3	72	2	14	4	40	4	$P < 0.001$	A1, A2, B1, B2 > C1, C2 *** C2 > C1 ***
Sediment	66	6	65	4	64	2	69	2	8	3	345	3	$P < 0.001$	A1, A2, B1, B2 > C1, C2 *** C2 > C1 ***
	60	6	60	3	56	3	65	2	2	1	255	1		
	55	6	56	3	49	3	60	3	0	0	164	0		
	52	7	54	4	43	3	55	3	0	0	93	0		
	48	7	51	5	36	2	52	3	0	0	52	0		
	45	8	49	6	30	2	47	3	0	0	21	0		
	41	8	45	6	24	2	45	4	0	0	1	0	$P < 0.001$	A1, A2, B1, B2 > C1, C2 *** A2 > B1 * B2 > B1 * C2 > C1 *
	38	9	43	7	20	2	41	4	0	0	0	0		
	35	9	41	7	14	2	39	4	0	0	0	0		
	32	10	39	8	10	2	35	4	0	0	0	0		
	30	10	37	8	7	2	32	4	0	0	0	0		
	28	11	35	8	5	2	30	5	0	0	0	0	$P < 0.001$	A1, A2, B1, B2 > C1, C2 *** A1, A2, B2 > B1 ***
	24	11	33	9	4	1	26	5	0	0	0	0		
	22	11	31	9	3	1	25	5	0	0	0	0	$P < 0.001$	A1, A2, B2 > B1, C1, C2 **
	20	11	30	10	2	1	23	5	0	0	0	0		
	18	11	28	10	1	1	21	5	0	0	0	0		
	17	11	27	10	2	1	19	4	0	0	0	0		
	15	11	26	10	2	1	18	4	0	0	0	0		
	14	11	25	10	1	0	16	4	0	0	0	0		
	12	11	25	10	0	0	15	4	0	0	0	0		
	11	10	23	10	12	3	12	3						
	9	9	21	10	10	3	10	3						
	20	9	20	9	8	3	8	3						
	19	9	19	9	6	2	6	2						
	17	8	17	8	5	2	5	2						
	16	8	16	8	4	2	4	2						
	15	7	15	7	3	1	3	1						
	15	7	15	7	2	1	2	1						
	13	7	13	7	1	1	1	1						
	12	6	12	6	1	1	1	1						

In control microcosms (A), oxygen profiles A1 (N = 5) were measured at the point of experiment interruption and A2 (N = 5) 20 h after the first measurement. In hypoxic (C) and hypoxic/normoxic microcosms (B) oxygen profiles were measured after 6 h with air supply B1 (N = 7), C1 (N = 7) and after 28 h with air supply B2 (N = 7), C2 (N = 7). Results from ANOVA and Tukey test comparing dissolved oxygen concentrations (%) are also presented. Positive depth values indicate above sediment surface and negative values are measurements done under.

\*  $P < 0.005$ , \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

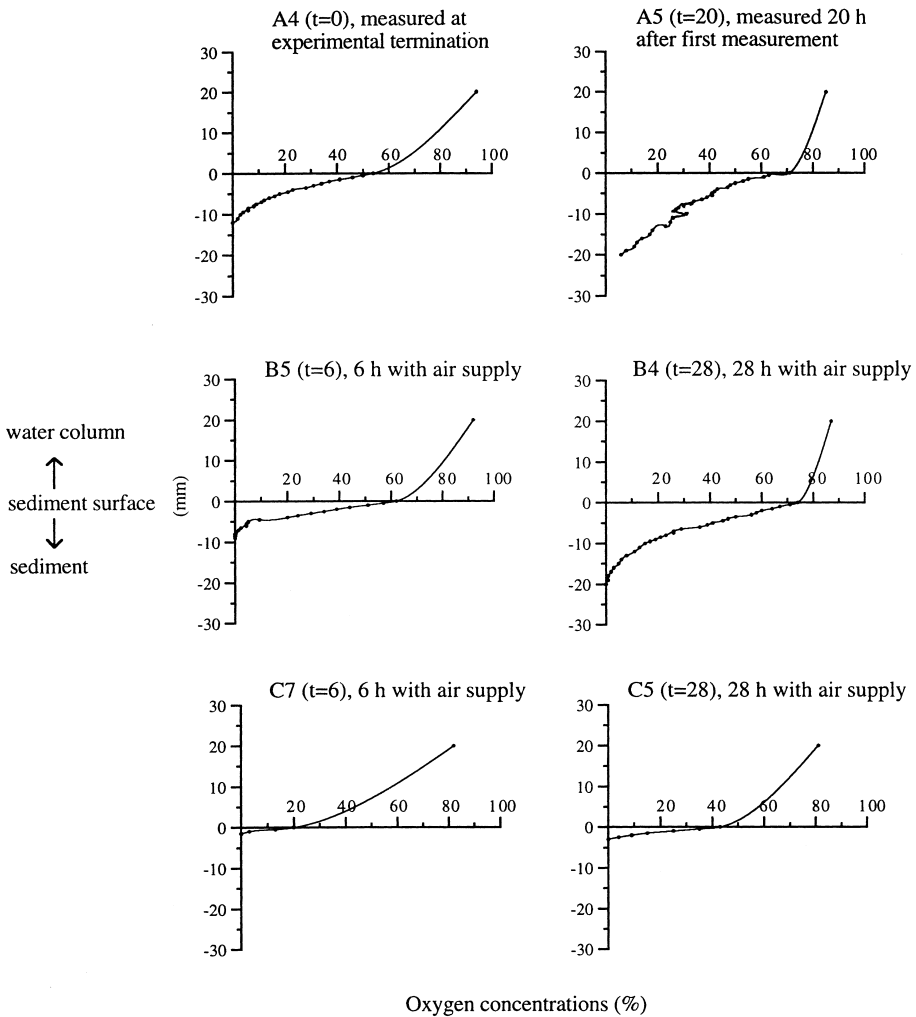


Fig. 1. Oxygen profiles in microcosms A (control), B (hypoxia alternated with normoxia on a weekly basis) and C (2 months of hypoxia). Profiles were measured at two occasions for control and treated microcosms. Positive depth values indicate above sediment surface and negative are measurements done under the sediment surface. Profile A5 was chosen to show an oxygen profile indicating animal activity while all other profiles were chosen with the purpose of showing representative profiles for each occasion.

Table 1, Fig. 1). The profiles measured in hypoxic microcosms differed from both normoxic and hypoxic/normoxic microcosms at both measuring occasions. The oxygen penetration was shallower and significantly less dissolved oxygen was measured in the hypoxic microcosms at the sediment surface and depth layers 0.5, 3.5 mm at both occasions ( $P < 0.001$ , ANOVA, Table 1, Fig. 1).

At the end of the experiment, the sulphide concentration in the water above the sediment surface was close to the detection limit of the method or  $1.9 \mu\text{mole l}^{-1}$  in four

of the hypoxic microcosms. In the other microcosms, no sulphide was measured in the water.

### 3.2. The fauna

Altogether, three macrofauna species and seven meiofauna taxa were recorded in the field control and normoxic microcosms. Two macrofauna species, *Macoma balthica* and *Halicryptus spinulosus* were to a large extent part of the meiofauna size fraction and should therefore be considered as temporary meiofauna. There was no significant difference between field control and normoxic microcosms in the abundance of meiofauna or macrofauna ( $P > 0.05$ , ANOVA). Nematoda were the most abundant taxa while Harpacticoida and Ostracoda were the second and third most abundant groups. Kinorhyncha, Turbellaria, Halacaridae and Oligochaeta were other meiofauna taxa registered in the microcosms.

In hypoxic microcosms, the macrofauna species, *Monoporeia affinis* and *Macoma balthica* died. *Halicryptus spinulosus*, appearing mainly in the meiobenthic size range (80% of all animals found passed the 0.5-mm sieve), survived two months of hypoxia though in significantly reduced numbers ( $P < 0.01$ , ANOVA) compared with the normoxic microcosms. Most meiofauna taxa were found in much lower abundance than in normoxic microcosms with a total disappearance of the numerically important taxon Harpacticoida, (*Microarthridion littorale* and *Pseudobryadia* sp.) (Fig. 2). Ostracoda as a group was significantly reduced in hypoxic microcosms (ANOVA,  $P < 0.05$ ). The numerically dominant ostracod species, *Paracyprideis fennica*, was in significantly lower numbers (ANOVA,  $P < 0.05$ ) in hypoxic microcosms while no significant difference was found in numbers of the two other species *Heterocyprideis sorbyana* and *Candona neglecta*. *C. neglecta* was found in low abundance in all treatments. Nematoda was found in significantly lower numbers in the hypoxic microcosms compared to the normoxic (ANOVA,  $P < 0.001$ ). Oligochaeta and Turbellaria were in lower abundance in the hypoxic microcosms (ANOVA,  $P < 0.001$ ). Further, kinorhynchs tended to decrease in hypoxic microcosms compared with normoxic but there was no significant difference between treatments (ANOVA,  $P > 0.05$ ) (Fig. 2).

Weekly pulses of hypoxia also resulted in a total wipe out of *Monoporeia affinis*, *Microarthridion littorale* and *Pseudobryadia* sp. Turbellarians and oligochaetes were also heavily reduced in the microcosms compared to the normoxic microcosms (ANOVA,  $P < 0.05$ ,  $P < 0.001$ ). *Halicryptus spinulosus* (75% of meiobenthic size) was in lower abundance (ANOVA,  $P < 0.05$ ) while the abundance of *Macoma balthica* (59% of meiobenthic size), ostracods, kinorhynchs and nematodes were not numerically affected by pulses of hypoxia (Fig. 2).

Weekly hypoxic and hypoxic microcosms did not differ concerning the number of the macrofauna species *Monoporeia affinis* (wiped out in both hypoxic treatments) and *Halicryptus spinulosus* (Fig. 2). However the third macrofauna species, *Macoma balthica*, was in significantly reduced number in hypoxic microcosms compared with hypoxic/normoxic microcosms. Ostracods as a group and the dominant ostracod species, *Paracyprideis fennica*, were in significantly lower numbers in hypoxic microcosms compared with hypoxic/normoxic microcosms (ANOVA,  $P < 0.05$ ). There was however

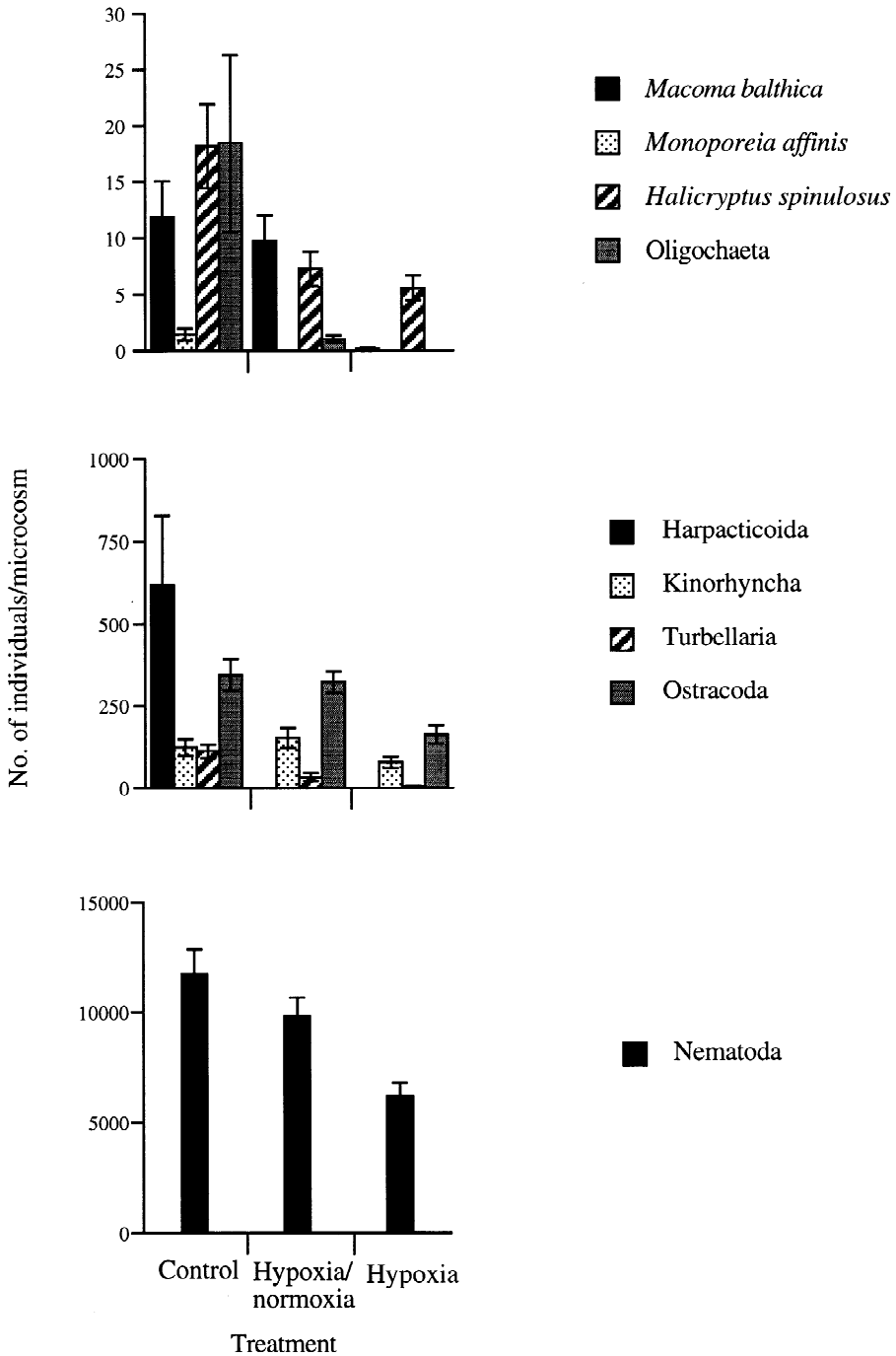
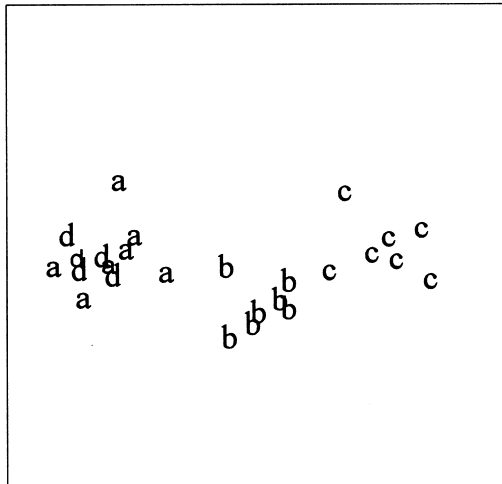
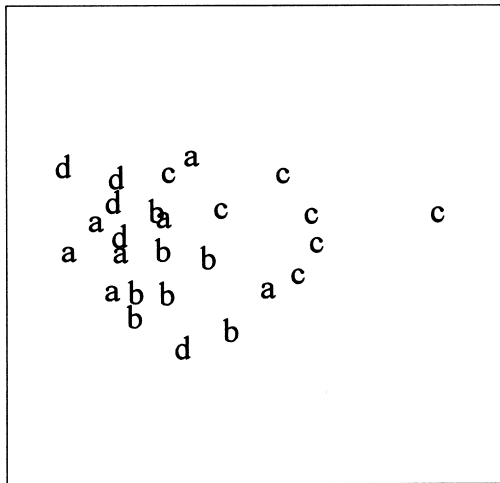


Fig. 2. Number of individuals per microcosm of macrofauna species and major meiofauna taxa in all treatments. Error bars represent standard error.

no difference in numbers of the two other ostracod species, neither of *Oligochaeta* nor *Harpacticoida* (totally wiped out in both hypoxic treatments). Two other meiofauna taxa differed in abundance between the two hypoxic treatments; *Nematoda* was in significantly lower numbers in hypoxic microcosms compared with hypoxic/normoxic microcosms (ANOVA,  $P < 0.05$ ) as well as *Turbellaria* (ANOVA,  $P < 0.01$ ).



a)



b)

Fig. 3. (a) Nonmetric MDS (multidimensional scaling ordination) of macrofauna species and major meiofauna taxa abundance data. a: Normoxic microcosms, b: hypoxic/normoxic microcosms, c: hypoxic microcosms and d: field control. Stress value = 0.07. (b) Nonmetric MDS (multidimensional scaling ordination) of nematode species data. Stress value = 0.15.

Table 2

ANOSIM, pairwise test comparing assemblage structure among treatments and field control for macrofauna species and major meiofauna taxa (global  $R = 0.818$ ) and nematode species (global  $R = 0.317$ ) a = normoxia, b = hypoxia/normoxia, c = hypoxia, d = field control.  $R^*$ -values and significance levels are presented

	Macrofauna species and major meiofauna taxa		Nematode species	
	Significance		Significance	
	$R$ -value	Level	$R$ -value	Level
(a, b)	0.963	0.10%	0.07	19.20%
(a, c)	1	0.10%	0.497	0.40%
(a, d)	0.061	28.50%	-0.038	59.00%
(b, c)	0.842	0.10%	0.522	0.10%
(b, d)	1	0.10%	0.224	7.10%
(c, d)	1	0.10%	0.581	0.50%

\*  $R = 1$  if all replicates within one treatment are more similar to each other than any replicates from different treatments.  $R$  is approximately zero if the similarities between and within treatments are the same on average.

The MDS (multidimensional scaling ordination) for the macrofauna species and major meiofauna taxa clearly separated normoxic, hypoxic and weekly hypoxic microcosms from each other (Fig. 3a). They were all significantly different from each other (ANOSIM,  $P < 0.001$ , Table 2), while the field control did not differ from normoxic microcosms (Table 2, Fig. 3a).

### 3.3. Nematode assemblage structure

Altogether 40 species/genera belonging to 17 families were identified in the microcosms. In the normoxic and field controls, more than 70% of all nematodes belonged to five species: *Calomicrolaimus honestus* (29%), *Leptolaimus elegans* (18%), *L. papilliger* (11%), *Paracanthochus* spp. (9%) and *Microlaimus globiceps* (7%).

*Calomicrolaimus honestus* and *Paracanthochus* spp. were heavily reduced in hypoxic microcosms (Table 3). *Axonolaimus spinosus* was reduced, not only in hypoxic but also weekly hypoxic microcosms (Table 3). There was also a significant difference in the numbers of *Desmolaimus zeelandicus* ( $P < 0.05$ , ANOVA, Table 3) being in ca. 3 times lower numbers in the hypoxic treatments than in normoxic ones. *Microlaimus globiceps*, the fifth most common species, tended to decrease in hypoxic microcosms but the difference was not statistically significant (Table 3). The dominant species *Leptolaimus elegans* and *L. papilliger* were found in similar numbers among treatments ( $P > 0.05$ , ANOVA, Table 3). *Sabatieria pulchra* tended to increase in the hypoxic microcosms but there was no significant difference in abundance of *S. pulchra* among treatments (Table 3).

The nematode assemblage structure in normoxic and hypoxic microcosms differed from each other ( $P < 0.01$ , ANOSIM, Table 2, Fig. 3b). The numerically dominant species *Calomicrolaimus honestus* contributed 23% to the dissimilarities (SIMPER). Weekly hypoxic and constantly hypoxic treatments also differed concerning the nematode assemblage structure (ANOSIM  $P < 0.001$ , Table 2, Fig. 3b) and again

Table 3

Average number (avg) per microcosm and standard error (SE) of the eleven most abundant nematode species. Results from ANOVA and Tukey test are also presented

	Hypoxia/						ANOVA	Tukey test
	Normoxia (A)		Normoxia (B)		Hypoxia (C)			
	Avg	SE	Avg	SE	Avg	SE		
<i>Calomicrolaimus honestus</i>	2926	517	2681	244	1184	282	0.007**	A,B > C
<i>Leptolaimus elegans</i>	2228	376	1274	162	1724	246	0.075	
<i>Leptolaimus papilliger</i>	1275	183	1467	234	999	163	0.261	
<i>Paracanthochus</i> spp.	1119	275	1233	306	162	57	0.010**	A,B > C
<i>Microloaimus globiceps</i>	884	164	667	54	492	59	0.052	
<i>Daptonema</i> sp.	797	173	641	148	280	95	0.053	
<i>Campylaimus</i> sp.	278	81	203	22	99	27	0.069	
<i>Desmolaimus zeelandicus</i>	335	75	122	49	110	66	0.040*	n.s
<i>Sabatieria pulchra</i>	76	51	154	87	337	138	0.188	
<i>Halalaimus</i> sp. 3	182	65	221	75	161	60	0.818	
<i>Axonolaimus spinosus</i>	381	96	143	51	18	12	0.002**	A > B,C

\*  $P < 0.05$ ; \*\*  $P < 0.01$ .

*Calomicrolaimus honestus* contributed more than 20% (24%) to the dissimilarities (SIMPER). There was no significant difference between normoxic and weekly hypoxic microcosms concerning the nematode assemblage structure nor between the field and experimental normoxic microcosms (ANOSIM  $P > 0.05$ , Table 2, Fig. 3b).

#### 4. Discussion

Depending on the faunal responses to constant and alternated hypoxic events we can divide some of the Baltic meiofauna and macrofauna into four groups. These four groups are not an absolute categorisation of these animals, rather an attempt to summarise the results from the experiment. The responses of different species to low oxygen concentrations may, for example, differ depending on the life stage (Wang and Widdows, 1991; Breitburg, 1992). The first group, 'very sensitive species', include the crustaceans, *Monoporeia affinis* (Amphipoda) and the harpacticoid copepods *Microarthridion littorale* and *Pseudobryadia* sp. neither of which survived weekly nor constant hypoxia. According to Johansson (1997), *M. affinis* may be reduced to 50% after 24 h in hypoxic water (0.2 mg O<sub>2</sub>/l). Generally, crustaceans as a group are not found in hypoxic-stressed areas and they are less tolerant to low oxygen concentrations than polychaetes and molluscs (Diaz and Rosenberg, 1995). There are, however, exceptions among crustaceans with individual species showing high tolerance to low oxygen concentrations and sulphide concentrations, such as *Saduria entomon* (Hagerman and Szaniawska, 1988, 1990; Hagerman and Vismann, 1993; Vismann, 1991), the harpacticoid species *Cletocampus confluens* (Vopel et al., 1996), and the ostracod *Cyprideis torosa* (Jahn et al., 1996). The ostracod species in our experiment also represented

another exception from the general assumption that crustaceans do not tolerate low oxygen concentrations.

'Sensitive' species included the surface-dwelling nematode species *Axonolaimus spinosus* and the groups Turbellaria and Oligochaeta which were all heavily reduced in both weekly and constant hypoxia. Fox and Powell (1986) showed that a surface-living, oxybiotic turbellarian species was negatively affected by sulphide exposure regardless of the presence or absence of oxygen. They concluded that H<sub>2</sub>S was the limiting factor for the vertical distribution of this species into the sediment. Three other subsurface thiobiotic species (two turbellarians and one gastrotrich) showed different degrees of tolerance to sulphide depending on the presence or absence of oxygen. These species seemed to be adapted to different microhabitats with varying concentrations of oxygen and sulphide (Fox and Powell, 1986). In our experiment, the sediment in hypoxic microcosms was black at the end of the experiment indicating high concentrations of iron sulphide. In four of the seven microcosms, sulphide was measurable in the overlying water column which indicates that the sulphidic zone had reached the surface. Black spots were visible in the hypoxic/normoxic microcosms indicating a patchy distribution of iron sulphide in the sediment. The presence of sulphide in the sediment in combination with low oxygen concentrations may have a stronger negative effect on the benthic fauna (Bagarinao, 1992; Nilsson and Rosenberg, 1994) and most likely the limiting factor for the benthic fauna in this experiment was the low oxygen concentration in combination with exposure to sulphide.

The third group, 'less sensitive' species such as *Macoma balthica*, the ostracod species *Paracyprideis fennica*, and the surface-dwelling nematode species *Paracanthochus* spp. and *Calomicrolaimus honestus* were reduced in hypoxic conditions but not in weekly hypoxic microcosms. *M. balthica* is known to be tolerant to low oxygen conditions and Henriksson (1969) showed, in an experiment with natural sediment, that 50% of the bivalves survived 18 days in oxygen deficiency. Further, adult *M. balthica* are able to efficiently detoxify sulphide (Jahn et al., 1997). Some bivalve species close their shells and reduce their metabolism as a strategy to survive in an oxygen depleted environment (Theede, 1973). Possibly, *M. balthica* survived the weekly hypoxic periods through shell closure and a lower metabolic rate. In the pulses of oxygenated water, the bivalve seemed to be able to recover which may be due to its protection mechanism against sulphide toxification which implies oxidation of sulphide to nontoxic compounds (Jahn et al., 1997). However, two months with low oxygen conditions was fatal, possibly due to starvation as feeding may cease in hypoxic environments (Diaz and Rosenberg, 1995) and/or due to the accumulation of sulphide in the tissues. Finally, the fourth group of 'tolerant' species, not significantly reduced after two months in any of the hypoxic treatments, include the two ostracod species, *Heterocyprideis sorbyana*, *Candona neglecta*, kinorhynchs, and the numerically important nematode species, *Leptolaimus elegans*, *L. papilliger*. In another study (Jahn et al., 1996), the ostracod species, *Cyprideis torosa*, common in the shallow waters of the Baltic Sea, showed the ability to survive in sulphidic habitats mainly through long-term anaerobiosis. *C. torosa* also showed the ability to detoxify sulphide but not very effectively due to its small size (Jahn et al., 1996). It is possible that the two tolerant ostracod species in this study have

the capability to endure a long period in an oxygen depleted milieu mainly through anaerobic metabolism. The large deep-dwelling nematode species, *Sabatieria pulchra*, also showed high tolerance and even tended to increase in hypoxic microcosms. *Sabatieria pulchra* is commonly found in high densities at depths near the RPD (redox potential discontinuity) layer, and has shown a tolerance to long periods of oxygen deficiency (Wieser and Kanwisher, 1961; Jensen, 1981, 1983; Warwick and Gee, 1984; Ólafsson, 1992; Hendelberg and Jensen, 1993). Finally, *Halicryptus spinulosus* (Priapulida) did not respond as expected but was significantly reduced in numbers in the hypoxic/normoxic treatment compared with normoxic microcosms: this species has, before, shown high tolerance to hypoxic conditions (Weigelt and Rumohr, 1986) through a multiple strategy of preventing some of the sulphide from entering the body wall through an external barrier of iron-sulphide formation, sulphide detoxification and passive anaerobic metabolism (Oeschger and Vetter, 1992). In this experiment, most of *H. spinulosus* appeared in meiobenthic size and possibly the larvae are not as tolerant to hypoxic conditions as adults. This might explain the significantly lower numbers in hypoxic/normoxic microcosms compared with normoxic microcosms.

Some of the oxygen profiles in the sediment were irregular (Fig. 1., A5) in normoxic microcosms. This was probably due to higher faunal activity in the normoxic microcosms. Bioturbation may enhance dissolved oxygen concentrations in sediment layers below the oxic layer (Reise and Ax, 1979; Wetzel et al., 1995) and thus cause an uneven distribution of oxygen in the sediment. Oxygen penetration in hypoxic/normoxic microcosms did not differ from normoxic microcosms after being exposed to 28 h of air bubbling. In contrast, in hypoxic microcosms, oxygen penetration was still very shallow after the 28-h air supply. The highly reduced fauna in hypoxic microcosms with a subsequent reduction of bioturbation may be an explanation for the narrow oxic layer in the microcosms remaining even after 28-h air bubbling. Another possibility is that oxygen may have been consumed through microbial activity connected to the S-cycle. The existence of sulphide in the water column of hypoxic microcosms at the termination of the experiment indicated that the oxic zone where sulphide is oxidised, had moved upwards and was now near or at the sediment surface. This was also clearly seen from the O<sub>2</sub> profile (Fig. 1, C7). This may partly explain the slower O<sub>2</sub> penetration into the sediment in hypoxic microcosms upon 28-h air bubbling (Fig. 1, C5).

Long- and short-term hypoxic events are common in the Baltic Sea and oxygen-depleted areas have increased during the last decades partly due to eutrophication (Elmgren, 1989). It is estimated that macrofauna have disappeared from 100 000 km<sup>2</sup> of deep Baltic bottoms due to oxygen deficiency (Elmgren, 1989). Low oxygen concentrations and subsequently sulphide exposure may be among the most important structuring factors in the sub thermocline soft-bottom communities. Changes in a zoobenthic community under hypoxic conditions may evolve rapidly (Norkko and Bonsdorff, 1996a) not only as a consequence of different faunal responses to low oxygen concentrations per se but also due to altered benthic predator–prey interactions (Norkko and Bonsdorff, 1996b). Furthermore, the different responses of the major species and taxa to long- and short-term hypoxic events, may be used as a tool in estimating the duration of naturally occurring hypoxia. In benthic communities, where larger organisms

(macrofauna) may disappear as a response to low oxygen conditions, the whole spectrum of invertebrates should be examined to obtain an idea of the severity and duration of hypoxic events.

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