

Responses of intertidal nematodes to short-term anoxic events

M. Steyaert^{a,*}, L. Moodley^b, T. Nadong^c, T. Moens^a, K. Soetaert^b, M. Vincx^a

^a Ghent University, Biology Department, Marine Biology Section, Krijgslaan 281/S8, B-9000 Gent, Belgium

^b Centre for Estuarine and Marine Ecology (NIOO-KNAW), Korrिंगaweg 7, 4401 NT Yerseke, The Netherlands

^c Biological Sciences Department, College of Arts and Sciences, University of Southern Mindanao, Kabacan, Cotabato, Philippines

Received 9 June 2006; received in revised form 29 January 2007; accepted 1 March 2007

Abstract

We investigated tolerance to, and activity of, nematodes in hypoxic and anoxic/partially sulphidic conditions and their ability for recovery after reoxygenation of anoxic sediment. To this end, sediments from an intertidal flat were incubated under oxic, suboxic and anoxic/partially sulphidic conditions for a 14-day period and the final density of nematodes, as a group, and of the most abundant species were assessed. In one treatment, oxygen was restored after anoxic incubation. The incorporation of ¹³C, originating from labeled algae added on top of the sediment, was taken as an indication of nematode activity.

Short-term suboxic and anoxic/partially sulphidic conditions had similar structuring impact on the nematode community, reducing total densities by about one third. Survival in suboxic and anoxic/partially sulphidic conditions was species-specific. *Daptonema setosum*, *D. tenuispiculum* and *Chromadora macrolaima*, dominant in the oxic incubation, disappeared when the oxygen level was reduced. The density of the other dominant species was slightly reduced (*Sabatieria pulchra*), similar (*Terschellingia communis*) or even increased in the suboxic and anoxic conditions (*Metachromadora vivipara*). The activity level of these three species was, however, reduced under oxygen limitation. Our results are discussed in terms of the life-history strategies of these species.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Estuarine sediments; Experiment; Isotopes; Nematodes; Oxygen deficiency; Sulphide

1. Introduction

The oxygenation status of sediments is a well studied determinant for the distribution of marine benthic organisms. Conditions where oxygen is limiting have a great impact on the benthic communities including meiofauna (Diaz and Rosenberg, 1995; Gamenick et al., 1996; Moodley et al., 1997; Modig and Ólafsson, 1998; Rosenberg et al., 2002). Tolerance of benthic organisms

to oxygen stress is probably related to exposure history, phylogenetic constraints and life history. Within a given habitat, specific species of foraminiferans and nematodes are typically most tolerant to hypoxia/anoxia, and members of these groups are found in sediments where suboxic or anoxic conditions prevail (Moodley et al., 1997; Modig and Ólafsson, 1998). In addition, nematodes and foraminiferans may reach high abundances in marine Oxygen Minimum Zones (OMZ), where they presumably benefit from the abundant food supply and the release from predation (Neira et al., 2001; Levin, 2003). The nematode, and more generally the meio-benthic communities of these habitats have received

* Corresponding author. Tel.: +32 09 264 85 16; fax: +32 09 264 85 98.

E-mail address: maaik.steyaert@UGent.be (M. Steyaert).

substantial attention (e.g. Fenchel and Riedl, 1970; Bernhard et al., 2000; Thiermann et al., 2000; Ott et al., 1991, 2004; Van Gaever et al., 2006).

Free-living nematodes are an important component of marine suboxic, anoxic and sulphidic benthic habitats. Sulphidic habitats occur whenever an excess of organic material leads to exhaustion of the available electron acceptors O_2 , nitrate, iron oxides and manganese oxides by bacterial respiration. In these environments, not only is oxygen lacking, but the resident higher organisms have to avoid the toxic effects of hydrogen sulphide. Switching to an anaerobic metabolism, excluding sulphide from sensitive tissues, or oxidizing sulphide to more benign forms are physiological adaptations that organisms have to cope with the presence of sulphide (Somero et al., 1989). In marine nematodes, the formation of oily viscous inclusions in the epidermis consisting of elemental sulphur has been demonstrated for *Oncholaimus campylocercooides* when exposed to sulphidic conditions (Thiermann et al., 2000).

Field studies (Coull, 1969; Josefson and Widbom, 1988; Murell and Fleeger, 1989; Hendelberg and Jensen, 1993) following hypoxic episodes typically demonstrate a reduction in overall meiofaunal abundance and in species and higher taxon diversity, as well as an increase in the relative abundance of nematodes. All meiofaunal organisms are sensitive to permanent anoxia, but some may tolerate extended periods of low-oxygen or anoxic conditions that range from hours to several months. In addition, migration into the water column until normoxic conditions are re-established is recognized as a strategy to survive hypoxic and/or sulphidic events (Wetzel et al., 2001). Depending on the local conditions, re-colonisation of sediments disturbed by various processes, including hypoxia/anoxia, may be a fast (Sherman and Coull, 1980; Colangelo and Ceccherelli, 1994) or a slow process (Austen and Wibdom, 1991; Wetzel et al., 2002). Recovery of a

nematode community after re-establishment of normal oxic conditions can happen either through immigration and/or through reproduction of surviving individuals, but it is difficult to assess the importance of these two processes under field conditions.

The aims of this study were to identify experimentally the response of intertidal nematodes (at species level) to (1) oxygen conditions ranging from saturation to deficiency, and (2) (traces of) sulphide, and to assess (3) their ability to recover after reoxygenation of anoxic sediments. We studied changes in the nematode community composition after relatively short (2 weeks) incubations.

2. Materials and methods

Surface (0–2 cm) sediment was collected from an intertidal muddy site (51°32.0' N; 03°52.5' E) in the Oosterschelde (The Netherlands) on June 03, 2003 during low tide. The sediment was homogenized and wet sieved; the fraction passing through a 500 μm mesh but retained on a 38 μm mesh was used for the experiment. 1 cm layers of sediment were incubated in perspex jars (i.d. 10 cm; height 10 cm). All jars were directly filled with fresh Oosterschelde water and sealed tightly (Fig. 1). The following treatments were established, each in duplicate: T_0 , control, suboxic, anoxic and anoxic+reoxygenated. The T_0 were preserved immediately after sieving and homogenization. In all other treatments, a specific oxygen regime was established by flushing the overlying water with air (control) or gas mixtures. The controls were flushed with air throughout the incubation. Suboxic conditions were established through a connection to a gas cylinder filled with a gas mixture containing 3% oxygen. In the anoxic treatment, jars were flushed with N_2 gas until 0 $\mu\text{mol/l}$ oxygen was measured; hereafter the lids were closed tightly. Control, suboxic and anoxic treatments were incubated for 14 days. Finally, two jars were made

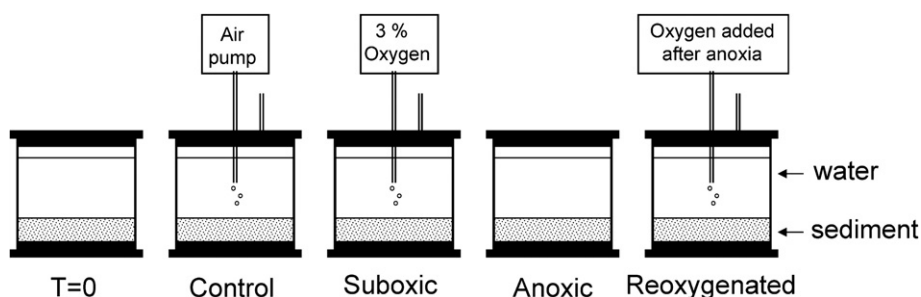


Fig. 1. Schematic drawing of the experimental set up.

Table 1

P-levels for the one-way ANOVA tests (post-hoc Tukey HSD) for total nematode abundance and the six nematode species (*df*=4)

	Treatments compared						
	T ₀ –control	Control–suboxic	Control–anoxic	Control–reoxygenated	Anoxic–suboxic	Anoxic–reoxygenated	Suboxic–reoxygenated
Total nematode abundance	0.023	0.000	0.000	0.000	0.417	0.511	0.996
<i>Chromadora macrolaima</i>	0.294	0.000	0.000	0.000	1.000	1.000	1.000
<i>Daptonema tenuispiculum</i>	0.195	0.012	0.011	0.011	0.997	1.000	0.997
<i>Daptonema setosum</i>	0.633	0.000	0.000	0.000	1.000	1.000	1.000
<i>Metachromadora vivipara</i>	0.179	0.112	0.008	0.111	0.064	0.064	1.000
<i>Sabatieria pulchra</i>	0.966	0.057	0.035	0.479	0.914	0.127	0.233
<i>Terschellingia communis</i>	0.784	0.415	0.963	0.992	0.266	0.877	0.533

Figures in bold indicate significant ($P \leq 0.05$) different *P*-levels.

anoxic for 8 days and subsequently reoxygenated with air, as in the control treatment, for 11 days. All jars were kept in the dark at 16 °C.

The oxygen concentration at the end of the incubation was measured with a trace oxygen dripping probe. An oxygen electrode was built into the lid of the jars and connected to a dissolved oxygen meter (dissolved oxygen accuracy $\pm 0.1 \text{ mg l}^{-1}$) (Moodley et al., 1997). Dissolved oxygen was $0 \text{ } \mu\text{mol l}^{-1}$ in the anoxic treatment; $32\text{--}77 \text{ } \mu\text{mol l}^{-1}$ (or $1\text{--}2 \text{ mg l}^{-1}$) in the suboxic treatment; and $179\text{--}249 \text{ } \mu\text{mol l}^{-1}$ (or $6\text{--}8 \text{ mg l}^{-1}$) in the control and reoxygenated treatments. Sulphide concentration was measured spectrophotometrically with the methylene blue method as described by Trüper and Schlegel (1964). Sulphide concentrations at the end of the 14-day incubation ranged from $13\text{--}31 \text{ } \mu\text{mol l}^{-1}$ (or $0.41\text{--}0.97 \text{ mg l}^{-1}$) in the anoxic treatments and were $0 \text{ } \mu\text{mol l}^{-1}$ in all other treatments, including the reoxygenated one. We have, however, no measurements on sulphide concentrations in this last treatment at the end of the 8-day anoxic incubation.

Prior to preservation, samples were examined for living nematodes under an inverted microscope. This is a qualitative verification of the staining method as dead nematodes will also stain with Rose Bengal; dead nematodes were excluded from further analysis. After the live-check was performed, samples for nematode identification and enumeration were preserved in 4% formaldehyde. Nematodes were stained with Rose Bengal and extracted from the sediment by centrifugation with Ludox (Heip et al., 1985). All nematodes were enumerated and a subsample (150 ind.) was mounted on Cobb slides for identification.

In order to establish whether nematodes remained active (feeding) during the experimental incubation, ^{13}C -labeled diatoms ($\sim 2 \text{ mg C}$) were added on top of the sediment in all jars after one week of incubation. Frozen diatoms were thawed and injected through the lids of the

microcosms. Details on the identity, cultivation, labeling and harvesting of the diatoms are described by Moodley et al. (2002). After fixation (4% formalin), juvenile and adult nematodes were hand-picked in duplicate samples for isotope analysis. Uptake of diatom carbon would be reflected in ^{13}C -enrichment in nematodes. Nematode carbon isotopic ratios were measured according to Moens et al. (2002) and are reported in the δ notation relative to the conventional Vienna Pee Dee Belemnite standard. Results are reported as specific uptake ($\Delta\delta^{13}\text{C}$), i.e. the difference in $\delta^{13}\text{C}$ of nematodes before and after enrichment.

Absolute abundances of nematodes and of six abundant nematode species were compared using a one-way ANOVA. Since variances were usually heterogeneous (tested with Cochran's *C*-test, Sokal and Rohlf, 1995), they were square root transformed. The Tukey HSD post-hoc test was used in pairwise comparisons of controls and treatments. Univariate analyses were performed using the software package Statistica 6.0.

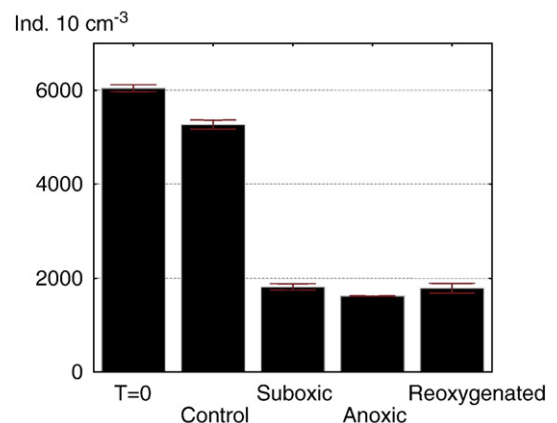


Fig. 2. Total nematode abundance for the T₀, the control and the three treatments (mean values \pm s.e.).

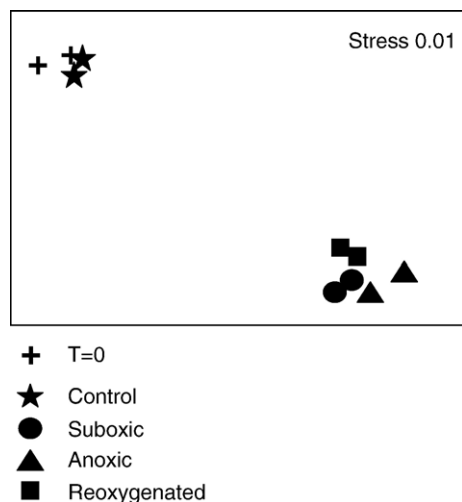


Fig. 3. MDS plot for the T₀, the control, the suboxic, the anoxic and the reoxygenated treatment.

Non-parametric multidimensional scaling (MDS) ordination with the Bray–Curtis similarity measure was performed (PRIMER version 5.2.9., Clarke and Warwick, 1994) on non-transformed species abundance data to determine the response of nematode assemblages to the different oxygen conditions. In order to determine the contribution of individual species to the average Bray–Curtis dissimilarity between samples, the similarity percentages program (SIMPER) was applied using the same software.

3. Results

Nematodes were the most abundant (>67%) metazoan meiofauna taxon in all treatments. Total nematode abundances in the T₀ and the control treatment were of similar magnitude, but were nevertheless significantly different (Table 1). Nematode abundances in the suboxic, the anoxic and the reoxygenated treatments were comparable to each other but much lower than in the T₀ and the control treatment (Fig. 2, Table 1).

In total 44 nematode species were identified. The total number of species in the T₀ and the control treatment ranged from 23 to 27, whereas it dropped to 12, 9 and 11 in the suboxic, anoxic and reoxygenated treatments, respectively. The MDS analysis revealed the largest distinction in community composition between the T₀ and the control treatment on the one hand and the suboxic, the anoxic and the reoxygenated treatments on the other (Fig. 3). The results from SIMPER analysis revealed that differences between the control and the three low-oxygen treatments resulted from changes in the abundances of dominant species including *Chromadora macrolaima*, *Daptonema setosum*, *D. tenuispiculum*, *Metachromadora vivipara*, *Sabatieria pulchra* and *Paracanthonus heterodontus*. These species occurred in markedly higher numbers in the control compared to the low-oxygen treatments (Table 2).

The response was further evaluated for 6 species, accounting for 59 to 90% of the total nematode com-

Table 2
Summary of SIMPER results

Species	Control	Suboxic	Contribution %	Control	Anoxic	Contribution %	Control	Reoxygenated	Contribution %
Average dissimilarity %	60	60		65	65		56	56	
<i>Daptonema setosus</i>	691±32	0±0	16	691±32	0±0	15	691±32	0±0	17
<i>Chromadora macrolaima</i>	629±11	0±0	15	629±11	0±0	14	629±11	0±0	16
<i>Paracanthonus heterodontus</i>	433±21	0±0	10	433±21	0±0	10	433±21	0±0	11
<i>Sabatieria pulchra</i>	536±102	195±42	8	536±102	140±54	9	536±102	399±1	3
<i>Daptonema tenuispiculum</i>	371±82	12±12	8	371±82	0±0	8	371±82	0±0	9
<i>Metachromadora vivipara</i>	124±41	262±1	3	124±41	429±41	7	124±41	262±23	4
<i>Anoplostoma viviparum</i>	248±62	0±0	6	248±62	0±0	5	248±62	0±0	6
<i>Hypodontolaimus balticus</i>	227±62	0±0	5	227±62	0±0	5	227±62	0±0	6
<i>Daptonema normandicus</i>	175±31	0±0	4	175±31	0±0	4	175±31	0±0	4
<i>Desmolaimus zeelandicus</i>	155±93	11±11	3	155±93	0±0	3	155±93	14±14	4
<i>Viscosia viscosa</i>	124±62	0±0	3	124±62	0±0	3	124±62	0±0	3
<i>Ptycholaimellus ponticus</i>	134±72	11±11	3	134±72	21±21	3	134±72	0±0	3
<i>Terschellingia communis</i>	815±53	956±7	3	815±53	778±94	2	815±53	838±38	1
<i>Terschellingia</i> sp.1	62±0	79±8		62±0	75±75	2	62±0	42±15	
<i>Prooncholaimus</i> sp.1	62±0	0±0		62±0	0±0	1	62±0	0±0	2
<i>Spirinia parasitifera</i>	72±10	137±6	2	72±10	67±18		72±10	55±2	

Average abundance of nematode species contribution to Bray–Curtis dissimilarities (%) for the control–anoxic, the control–suboxic and the control–reoxygenated treatment. A cut-off of 90% was used.

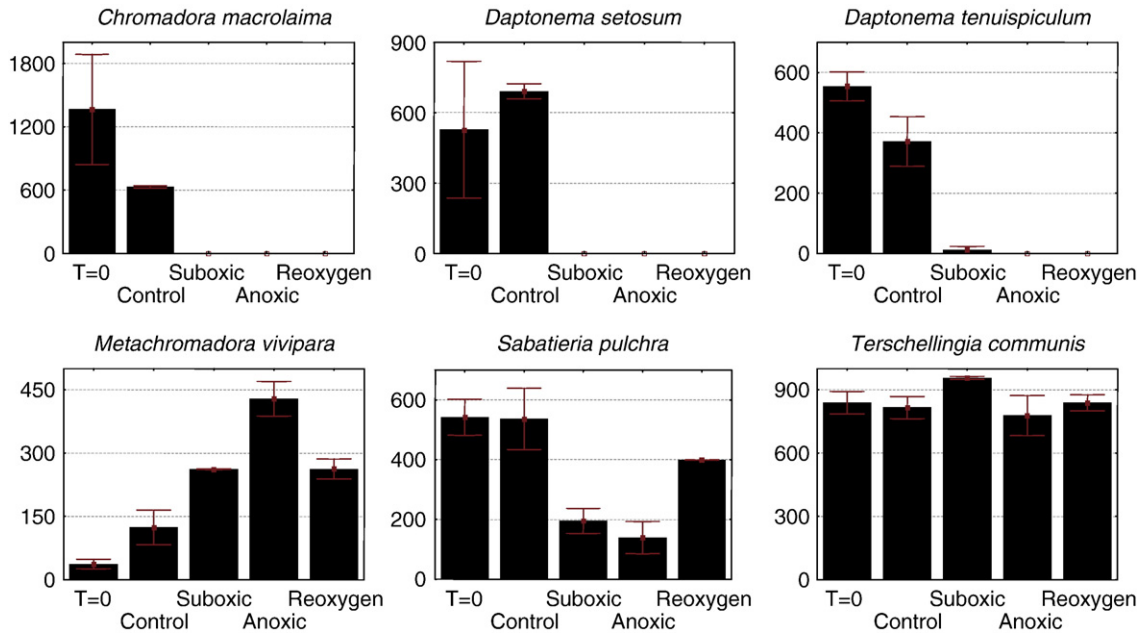


Fig. 4. Nematode species abundance (ind. 10 cm⁻³) for the T₀, the control and the three treatments (mean values ± s.e.).

munity: *C. macrolaima*, *D. setosum*, *D. tenuispiculum*, *M. vivipara*, *S. pulchra* and *Terschellingia communis*. The three most abundant species in all treatments always belonged to this selection of 6 species, with *T. communis* being dominant in all treatments.

In general, the variance in density of individual species was much larger than the variance in total nematode densities, so that, in contrast to total nematode abundances, the abundances of the individual species were not significantly different between the T₀ and the control treatment (Table 1). Density of *T. communis* was overall high and did not differ significantly among treatments (Fig. 4). In contrast, *C. macrolaima*, *D. setosum* and *D. tenuispiculum* were (nearly) absent in the suboxic, the anoxic and the reoxygenated treatment (Fig. 4). Consequently, their high abundance in the control treatment was significantly different from that in the other treatments (Table 1). The abundance of *S. pulchra* was high in the control treatment, lower in the suboxic and anoxic treatment, and intermediate in the reoxygenated treatment (Fig. 4). Abundances were significantly different only between the control and the anoxic treatment (Table 1). In contrast to the other species, *M. vivipara* showed lowest abundances in the T₀ and in the control treatment, highest in the anoxic treatment and intermediate in the suboxic and the reoxygenated treatment (Fig. 4). Only the abundances in the control and anoxic treatment were significantly different (Table 1).

Concerning juvenile/adult ratio, no clear difference between control and oxygen limited treatments was found for *S. pulchra* (Table 3). A higher juvenile/adult ratio (×4) in the anoxic treatment was found for *M. vivipara* compared to the control treatment; while for *T. communis* a lowered ratio was found in the oxygen limited treatments (Table 3).

All species showed uptake of labeled diatoms (Fig. 5). The uptake of the three species that survived the anoxic treatment, *M. vivipara*, *S. pulchra* and *T. communis*, was on average higher in the control compared to the suboxic and the anoxic treatments, but none of these differences proved statistically significant. Natural (*i.e.* unenriched) δ¹³C for all species

Table 3
Average juvenile/adult ratio for the six nematode species

Treatments	T ₀	Control	Suboxic	Anoxic	Reoxygenated
<i>Chromadora macrolaima</i>	2.08	1.61			
<i>Daptonema setosum</i>	16.99	14.06			
<i>Daptonema tenuispiculum</i>	39.23	41.88			
<i>Metachromadora vivipara</i>	4.00	5.55	7.00	22.50	16.50
<i>Sabatieria pulchra</i>	27.48	22.05	15.41	20.03	16.58
<i>Terschellingia communis</i>	16.08	10.33	6.02	5.01	6.69

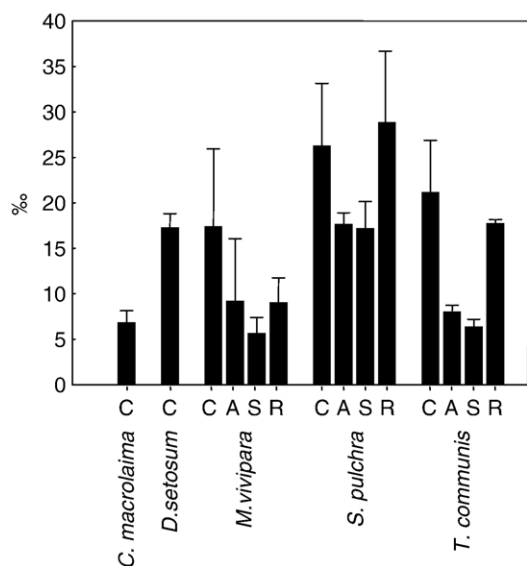


Fig. 5. ^{13}C -incorporation (specific uptake, $\Delta\delta^{13}\text{C}$, in ‰) in the control (C) and the three treatments (suboxic (S), anoxic (A), reoxygenated (R)) for *Chromadora macrolaima*, *Daptonema setosum*, *Metachromadora vivipara*, *Sabatieria pulchra* and *Terschellingia communis*.

ranged between -12.8 and -17.9 ‰ for *C. macrolaima*, *M. vivipara* and *D. setosum*, and between -31.8 and -34.0 ‰ for *S. pulchra* and *T. communis*.

4. Discussion

Before discussing the results of our experiment in detail, we draw attention to some implications of the experimental design. The first pertains to the pretreatment of sediments, *i.e.* mixing and wet sieving. It is likely that this procedure affected the nematode communities inside our microcosms compared to the natural field situation, which we did not analyse. However, the community and its dominant species present at T_0 (*i.e.* after wet sieving and mixing) were highly representative for mud-dwelling nematode communities in a wide range of marine and estuarine areas and habitats. Genera like *Daptonema*, *Sabatieria*, *Terschellingia*, *Metachromadora*, *Chromadora*, *Paracanthochus* and others are all very widespread and often highly abundant (e.g. Heip et al., 1985), hence our experimental community provides a highly relevant case study.

A second issue of the experimental design concerns the addition of diatoms halfway through the experimental incubation, which could potentially have interfered with the effects of the different oxygen treatments by selectively favouring some species over others, particularly those that can feed directly on diatoms over those

that cannot. However, we have good reasons to believe that the addition of the diatoms did not selectively affect nematode population development or survival. First, all dominant nematode genera incorporated diatom carbon to very similar extents relative to their body mass. This implies that both nematodes capable of grazing on diatoms (*C. macrolaima*, *M. vivipara* and *D. setosum*) and nematodes incapable of doing so (but probably capable of utilizing bacteria that were assimilating the diatom carbon) utilized and incorporated the added source to a very similar extent. Second, $\Delta\delta^{13}\text{C}$ of nematodes were consistently low, showing that uptake of the added diatom carbon was very low and could have contributed only very little to nematode growth or activity. This is probably because the amount (~ 2 mg C) of diatom carbon added to the sediment was small compared to what was already present in the sediment, and/or because the diatom species used here was too large for most nematodes to directly graze upon it.

Nematode densities in the control treatment were about 10% lower than at T_0 . This suggests that the experimental incubation negatively affected the nematode community, albeit to a limited extent, irrespective of oxygen treatment. However, the effect of establishing suboxic and anoxic conditions was much more spectacular than that of ‘incubation’ per se, with densities dropping to less than half to about one third of those found in oxic conditions. This confirms that nematodes as a taxon may tolerate oxygen deficiency, although their densities are impaired, as previously reported in field surveys across several habitats as well as in experimental work (e.g. Murell and Fleeger, 1989; Hendelberg and Jensen, 1993; Moodley et al., 1997; Cook et al., 2000; Gooday et al., 2000; Neira et al., 2001; Levin, 2003).

In addition, the species composition changed drastically when nematodes were exposed to suboxic or anoxic conditions. The lowering of oxygen in the three oxygen limited treatments resulted in an altered dominance pattern and favoured species as *S. pulchra* and *T. communis*, both typical inhabitants of the deeper sediment horizons (Hendelberg and Jensen, 1993; Modig and Ólafsson, 1998; Boyd et al., 2000; Wetzel et al., 2002). Moreover *S. pulchra*, and also its congener *S. punctata*, is often the dominant species in species-poor assemblages of (very) disturbed environments (Steyaert et al., 1999; Boyd et al., 2000). Only about half the number of species remained in the oxygen limited treatments, and multivariate analysis clearly separated the control and T_0 from the other treatments. Noteworthy are the similarity of nematode density and assemblage composition in the suboxic and anoxic/sulphidic treatment. This was not expected, as for

macrobenthic invertebrates it is generally assumed that the presence of (traces of) sulphide reinforces the harmful effects of oxygen limitation (Diaz and Rosenberg, 1995; Gamenick et al., 1996).

The dominant nematode species reacted differently to deteriorated oxygen conditions. Our results suggest that *D. setosum*, *D. tenuispiculum* and *C. macrolaima* are very sensitive to oxygen depletion as they totally disappeared during the two weeks of suboxic and anoxic exposure. These three species are surface-dwellers, typically found in intertidal or subtidal mud and sands (e.g. Vincx, 1987; Platt and Warwick, 1988; Warwick et al., 1998; Schratzberger et al., 2000a, 2002; Commito and Tita, 2002; Steyaert et al., 2005). Surface-living species are often very sensitive to hypoxia or anoxia, and they may cope with episodic oxygen limitation by migrating to nearby aerated patches in the sediment or by emerging into the water column (Wetzel et al., 2002). Earlier research has demonstrated that both *D. setosum* and *D. tenuispiculum* successfully migrate in upper sediment layers (Schratzberger et al., 2000a; Steyaert et al., 2001). Moreover, several *Daptonema* species as well as *Chromadorita tenuis*, a species that is morphologically closely related to *C. macrolaima*, can emerge into the water column and can colonise sediment after disturbance (e.g. anoxia) through active swimming (Jensen, 1981; Wetzel et al., 2002).

The other dominant species either demonstrated moderate tolerance (*S. pulchra*), or no response (*T. communis*), or even seemed to profit from the suboxic and anoxic conditions (*M. vivipara*).

S. pulchra is a typical mesohaline species which inhabits all types of sediment but with highest densities in mud. It lives from the very shallow down to deeper waters. In anoxic and strongly reduced sediment it is often the only remaining species. It dwells deep into the sediment, and is often found in high densities near the RPD layer (Jensen, 1984; Modig and Ólafsson, 1998; Boyd et al., 2000; Wetzel et al., 2002; Steyaert et al., 2003; Ullberg and Ólafsson, 2003; Steyaert et al., 2005). The very depleted natural $\delta^{13}\text{C}$ of *S. pulchra* in our study indeed suggests a contribution of thiobiotic bacteria to their diet (Spiro et al., 1986; Ott et al., 2004). In our experiments, survival rate of *S. pulchra* was less than 50% in the oxygen limited treatments, confirming some – albeit limited – degree of tolerance, at least for the 14-day duration of the experiment. The similar survival rates encountered in the anoxic, sulphidic treatment suggest that mortality can mainly be ascribed to oxygen limitation rather than to any toxicity of sulphide. Detoxifying mechanisms for

hydrogen sulphide have already been suggested for nematodes, e.g. *O. campylocercoides*, which have oily to viscous inclusions in the epidermis (Thiermann et al., 2000). Such intracellular inclusions have been reported for *S. pulchra* as well (Vincx, 1987; Smol unpubl.). Remarkably, *S. pulchra* was the only species for which the data indicate that abundance was, at least partly, restored within 11 days after an anoxic event of 8 days, since average abundances in the reoxygenated treatment were twice those in the anoxic treatment. However, this difference was not statistically significant. Such a restored abundance could not result from an enhanced reproduction as this would suggest an extremely short life cycle, which is at odds with life cycle estimates based on field studies (Juario, 1975; Vincx, 1987). Moreover, no difference in adult/juvenile ratio was found (Table 3). It could, however, be explained by the comparatively shorter period of anoxia in the reoxygenated treatment (8 days compared to 14 days in the anoxic treatment), suggesting that the duration of anoxia is a crucial factor determining the survival potential of *S. pulchra*. We conclude that *S. pulchra* requires oxygen for its metabolism, but has developed adaptation mechanisms that allow this species to survive episodes of anoxia.

T. communis, the most abundant nematode species in the community, survived the reduced oxygen conditions and presence of sulphide very well as no significant differences in densities between treatments were observed. This species is a typical inhabitant of estuarine, intertidal and subtidal areas with highest abundances in muddy sediments (Warwick and Gee, 1984; Austen and Widdicombe, 1998; Warwick et al., 1998; Schratzberger et al., 2000b; Steyaert et al., 2005). Like *S. pulchra* it has a long and slender body form, well adapted to its deep-dwelling life style. In general, *T. communis* occurs vertically within the sediment down to 10 cm, and it is typically more dominant in deeper sediment layers than at the surface (Steyaert unpubl.). Like in *S. pulchra*, the stable carbon isotopic signatures of *T. communis* were significantly more depleted than those of the other nematode species. The $\delta^{13}\text{C}$ of *C. macrolaima*, *D. setosum* and *M. vivipara* matched closely with that of microphytobenthos (Moens et al., 2002), in line with observations and/or mouth-morphology based predictions on their diatom-feeding behaviour (Moens and Vincx, 1997). The strongly negative $\delta^{13}\text{C}$ values of *T. communis* and *S. pulchra*, however, fall outside the range of typical end member carbon isotopic ratios in this area (Middelburg and Nieuwenhuize, 1998; Moens et al., 2002; Moens unpubl.), and resemble the isotopic composition of many thiobiotic micro-organisms (Spiro

et al., 1986; Ott et al., 2004), suggesting at least a partial dependence of these nematodes on thiobiotic organisms, either through grazing or through some symbiotic relationship. We have not found any evidence of ectosymbiosis. Records of endosymbiosis in marine nematodes are so far restricted to 5 mouthless species which belong to the family Siphonolaimidae (Ott et al., 2004).

M. vivipara is an ovoviviparous nematode, common in estuarine intertidal muddy and sandy environments (Platt and Warwick, 1988; Schratzberger and Warwick, 1999; Schratzberger et al., 2000a). While it is typically most abundant in surface sediment layers (0–2 cm; pers. obs.), it also occurs deeper down (down to 5 cm or deeper). In contrast to the other nematode species in this study, *M. vivipara* reached highest densities in the anoxic/sulphidic treatment. The higher juvenile/adult ratio ($\times 4$) compared to the control treatment clearly demonstrated reproduction in the experimental microcosms (Table 3). The ovoviviparous reproductive strategy of *M. vivipara* can be considered as an advantage of life in reduced environments (Allg n, 1953) in the sense that parents secure the survival and development of their brood in the anoxic/sulphidic environment (Van Gaever et al., 2006). Keeping the juvenile stages in the adult body may guarantee a better oxygen supply if adult *M. vivipara* migrate in between oxic and sulphidic layers. The enhanced hatching of *M. vivipara* juveniles under anoxic/sulphidic conditions may be linked to a potentially higher resistance to anoxia in juveniles, as demonstrated for *Theristus anoxybioticus* (Jensen, 1995).

5. Conclusion

We have shown that different nematode species are differentially adapted to living and/or surviving in low-oxygen environments. Some of our findings were expected. For a number of species, the sensitivity to oxygen limitation is consistent with the habitat in which they are found: this is so for surface-dwelling *C. macrolaima*, *D. setosum* and *D. tenuispiculum*, which did not survive anoxia, while deep-dwelling organisms such as *S. pulchra* and *T. communis*, were moderately to very tolerant to episodic oxygen limitation. Other findings were unexpected. First of all, nematodes as a group as well as the different species responded similarly to the suboxic and sulphidic treatment, suggesting that the presence of sulphide did not have a discernable effect over that of low-oxygen concentration. Secondly, the ovoviviparous nematode *M. vivipara* showed an increased reproduction in the anoxic

treatment. This finding was especially surprising as this species is usually associated with oxic conditions. Apparently, conditions that negatively affect other nematode species trigger the hatching of juveniles of *M. vivipara*. Finally, the two species that are common in suboxic and anoxic sediments showed strongly depleted natural carbon isotopic signatures, and therefore clearly rely on other food sources than *M. vivipara* and the surface-dwelling *C. macrolaima* and *D. setosum*. These depleted signatures point to a dependence on thiobiotic organisms, either via grazing or symbiosis.

Acknowledgements

Thanks to Lennart van Ijzerloo and Bart Beuselinck for their assistance during the processing of the samples. Financial support was obtained from the Flemish and Dutch Science Foundation (VLANEZO project # 3GO43702) and the Flemish Fund for Scientific Research (FWO) through project 31521704. T.M. is a postdoctoral fellow with the FWO. This is publication Nr. 4019 of the NIOO-CEME. [RH]

References

- Allg n, C., 1953. Revision der freilebenden marinen Nematoden aus der Umgebung der Staatlichen Zoologischen Station Kristineberg an der Westk ste Schwedens. Zool. Jahrb. (Syst.) 81, 548–603.
- Austen, M.C., Wibdom, B., 1991. Changes in and slow recovery of a meiobenthic nematode assemblage following a hypoxic period in the Gullmar Fjord basin, Sweden. Mar. Biol. 111, 139–145.
- Austen, M.C., Widdicombe, S., 1998. Experimental evidence of effects of the heart urchin *Brissopsis lyrifera* on associated subtidal meiobenthic nematode communities. J. Exp. Mar. Biol. Ecol. 222, 219–238.
- Bernhard, J.M., Buck, K.R., Farmer, M.A., Bowser, S.S., 2000. The Santa Barbara basin is a symbiosis oasis. Nature 403, 77–80.
- Boyd, S.E., Rees, H.L., Richardson, C.A., 2000. Nematodes as sensitive indicators of change at dredged material disposal sites. Estuar. Coast. Shelf Sci. 51, 805–819.
- Clarke, K.R., Warwick, R.W., 1994. Change in Marine Communities: an Approach to Statistical Analysis and Interpretation. Plymouth Marine Laboratory, Plymouth, England.
- Colangelo, M.A., Ceccherelli, V.U., 1994. Meiofaunal recolonization of azoic sediment in a Po Delta lagoon (Sacca di Goro). Bull. Zool. 61, 335–342.
- Commito, J.A., Tita, G., 2002. Differential dispersal rates in an intertidal meiofauna assemblage. J. Exp. Mar. Biol. Ecol. 268, 237–256.
- Cook, A.A., Lamshead, P.J.D., Hawkins, L.E., Mitchell, N., Levin, L.A., 2000. Nematode abundance at the oxygen minimum zone in the Arabian Sea. Deep-Sea Res., Part II 47, 75–85.
- Coull, B.C., 1969. Hydrographic control of meiobenthos in Bermuda. Limnol. Oceanogr. 14, 953–957.
- Diaz, R., Rosenberg, R., 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioral responses of benthic macrofauna. Oceanogr. Mar. Biol. Annu. Rev. 33, 245–303.

- Fenchel, T., Riedl, J., 1970. The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. *Mar. Biol.* 7, 255–268.
- Gamenick, I., Jahn, A., Vopel, K., Giere, O., 1996. Hypoxia and sulphide as structuring factors in a macrozoobenthic community on the Baltic Sea shore: colonisation studies and tolerance experiments. *Mar. Ecol. Prog. Ser.* 144, 73–85.
- Gooday, A.J., Bernhard, J.M., Levin, L.A., Suhr, S.B., 2000. Foraminifera in the Arabian Sea oxygen minimum zone and other oxygen-deficient settings: taxonomic composition, diversity, and relation to metazoan faunas. *Deep-Sea Res., Part II* 47 (1–2), 25–54.
- Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. *Oceanogr. Mar. Biol. Ann. Rev.* 23, 399–489.
- Hendelberg, M., Jensen, P., 1993. Vertical distribution of the nematode fauna in a coastal sediment influenced by seasonal hypoxia in the bottom water. *Ophelia* 37, 83–94.
- Jensen, P., 1981. Phyto-chemical sensitivity and swimming behaviour of the free-living marine nematode *Chromadorita tenuis*. *Mar. Ecol. Prog. Ser.* 4, 203–206.
- Jensen, P., 1984. Ecology of benthic and epiphytic nematodes in brackish waters. *Hydrobiologia* 108, 201–217.
- Jensen, P., 1995. Life history of the nematode *Theristus anoxybioticus* from sublittoral muddy sediment at methane seepages in the northern Kattegat, Denmark. *Mar. Biol.* 123, 131–136.
- Josefson, A.B., Widbom, B., 1988. Differential response of benthic macrofauna and meiofauna to hypoxia in the Gullmar Fjord basin. *Mar. Biol.* 100, 31–40.
- Juário, J.V., 1975. Nematode species composition and seasonal fluctuation of a sublittoral meiofauna community in the German Bight. *Veröff. Inst. Meeresforsch. Bremerhaven* 15, 283–337.
- Levin, L.A., 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanogr. Mar. Biol.* 41, 1–45.
- Middelburg, J.J., Nieuwenhuize, J., 1998. Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. *Mar. Chem.* 60, 217–225.
- Modig, H., Ólafsson, E., 1998. Responses of Baltic benthic invertebrates to hypoxic events. *J. Exp. Mar. Biol. Ecol.* 229, 133–148.
- Moens, T., Vincx, M., 1997. Observations on the feeding ecology of estuarine nematodes. *J. Mar. Biol. Assoc. UK* 77, 211–227.
- Moens, T., Luyten, C., Middelburg, J.J., Herman, P.M.J., Vincx, M., 2002. Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. *Mar. Ecol. Prog. Ser.* 234, 127–137.
- Moodley, L., van der Zwaan, G.J., Herman, P.M.J., Kempers, L., van Breugel, P., 1997. Differential response of benthic meiofauna to anoxia with special reference to Foraminifera (Protista: Sarcodina). *Mar. Ecol. Prog. Ser.* 158, 151–163.
- Moodley, L., Middelburg, J.J., Boschker, H.T.S., Duineveld, G.C.A., Pel, R., Herman, P.M.J., Heip, C.H.R., 2002. Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus. *Mar. Ecol. Prog. Ser.* 236, 23–29.
- Murell, M.C., Fleeger, J.W., 1989. Meiofauna abundance on the Gulf of Mexico continental shelf affected by hypoxia. *Cont. Shelf Res.* 9, 1049–1062.
- Neira, C., Sellanes, J., Levin, L.A., Armtz, W.E., 2001. Meiofaunal distributions on the Peru margin: relationships to oxygen and organic matter availability. *Deep-Sea Res., Part I* 48, 2453–2472.
- Ott, J.A., Novak, R., Schiemer, F., Hentschel, U., Nebelsick, M., Polz, M., 1991. Tackling the sulphide gradient — a novel strategy involving marine nematodes and chemoautotrophic ectosymbionts. *P.S.Z.N. I, Mar. Ecol.* 12, 261–279.
- Ott, J., Bright, M., Bulgheresi, S., 2004. Symbioses between marine nematodes and sulfur-oxidizing chemoautotrophic bacteria. *Symbiosis* 36, 103–126.
- Platt, M.H., Warwick, M.R., 1988. Free living marine nematodes. Part 2: British Chromadorids. *Synopsis of the British Fauna (N.S.)* 38. The Linnean Society of London and The Estuarine and Brackish-water Sciences Association, London.
- Rosenberg, R., Agrenius, S., Hellman, B., Nilsson, H.C., Norling, K., 2002. Recovery of marine benthic habitats and fauna in a Swedish fjord following improved oxygen conditions. *Mar. Ecol. Prog. Ser.* 234, 43–53.
- Schratzberger, M., Warwick, R.M., 1999. Impact of predation and sediment disturbance by *Carcinus maenas* (L.) on free-living nematode community structure. *J. Exp. Mar. Biol. Ecol.* 235, 255–271.
- Schratzberger, M., Rees, H.L., Boyd, 2000a. Effects of simulated deposition of dredged material on structure of nematode assemblages — the role of burial. *Mar. Biol.* 136, 519–530.
- Schratzberger, M., Gee, J.M., Rees, H.L., Boyd, S.E., Wall, C.M., 2000b. The structure and taxonomic composition of sublittoral meiofauna assemblages as an indicator of the status of marine environments. *J. Mar. Biol. Assoc. UK* 80, 969–980.
- Schratzberger, M., Dinmore, T.A., Jennings, S., 2002. Impacts of trawling on the diversity, biomass and structure of meiofauna assemblages. *Mar. Biol.* 140, 83–93.
- Sherman, K.M., Coull, B.C., 1980. The response of meiofauna to sediment disturbance. *J. Exp. Mar. Biol. Ecol.* 46, 59–71.
- Sokal, R.R., Rohlf, F.J., 1995. *Biometry: the Principles and Practice of Statistics in Biological Research*, 3rd edition. W. H. Freeman and Co., New York. 887 pp.
- Somero, G.N., Childress, J.J., Anderson, A.E., 1989. Transport, metabolism, and detoxification of hydrogen sulfide in animals from sulfide-rich marine environments. *Crit. Rev. Aquat. Sci.* 1, 591–614.
- Spiro, B., Greenwood, P.B., Southward, A.J., Dando, P.R., 1986. $^{13}\text{C}/^{12}\text{C}$ ratios in marine invertebrates from reducing sediments: confirmation of nutritional importance of chemoautotrophic endosymbiotic bacteria. *Mar. Ecol. Prog. Ser.* 28, 233–240.
- Steyaert, M., Garner, N., Van Gansbeke, D., Vincx, M., 1999. Nematode communities from the North Sea: environmental controls on species diversity and vertical distribution within the sediment. *J. Mar. Biol. Assoc. U.K.* 79, 253–264.
- Steyaert, M., Herman, P.M.J., Moens, T., Widdows, J., Vincx, M., 2001. Tidal migration of nematodes on an estuarine tidal flat (the Molenplaat, Schelde Estuary SW, Netherlands). *Mar. Ecol. Prog. Ser.* 224, 299–304.
- Steyaert, M., Vanaverbeke, J., Vanreusel, A., Barranguet, C., Lucas, C., Vincx, M., 2003. The importance of fine-scale, vertical profiles in characterising nematode community structure. *Estuar. Coast. Shelf Sci.* 58, 353–366.
- Steyaert, M., Moodley, L., Vanaverbeke, J., Vandewiele, S., Vincx, M., 2005. Laboratory experiments on the infaunal activity of intertidal nematodes. *Hydrobiologia* 540, 217–223.
- Thiermann, F., Vismann, B., Giere, O., 2000. Sulphide tolerance of the marine nematode *Oncholaimus campylocercoides* — a result of internal sulphur formation. *Mar. Ecol. Prog. Ser.* 193, 251–259.
- Trüper, H.G., Schlegel, H.G., 1964. Sulfur metabolism in Thiorhodaceae. I. Quantitative measurements on growing cells of *Chromatium okenii*. *Antonie Van Leeuwenhoek* 30, 225–238.
- Ullberg, J., Ólafsson, E., 2003. Effects of biological disturbance by *Monoporeia affinis* (Amphipoda) on small-scale migration of

- marine nematodes in low-energy soft sediments. *Mar. Biol.* 143, 867–874.
- Van Gaever, S., Moodley, L., de Beer, D., Vanreusel, A., 2006. Meiobenthos at the Arctic Håkon Mosby Mud Volcano with a parental caring nematode thriving in sulphide-rich sediments. *Mar. Ecol. Prog. Ser.* 321, 143–155.
- Vincx, M., 1987. Free-living marine nematodes from the Southern Bight of the North Sea. Ph.D. Thesis, Ghent University.
- Warwick, R.M., Gee, J.M., 1984. Community structure of estuarine meiobenthos. *Mar. Ecol. Prog. Ser.* 18, 97–111.
- Warwick, M.R., Platt, M.H., Somerfield, J.P. 1998. Free living marine nematodes. Part 3: Monhysterids. *Synopses of the British Fauna* (N.S.) 53. The Linnean Society of London and The Estuarine and Coastal Sciences Association, London.
- Wetzel, M.A., Fleeger, J.W., Powers, S.P., 2001. Effects of hypoxia and anoxia on meiofauna: a review with new data from the Gulf of Mexico. In: Rabalais, N.N., Turner, R.E. (Eds.), *Coastal Hypoxia: Consequences for Living Resources and Ecosystems*. Coastal and Estuarine Studies, vol. 58. American Geophysical Union, Washington, pp. 165–184.
- Wetzel, M.A., Weber, A., Giere, O., 2002. Re-colonization of anoxic/sulfidic sediments by marine nematodes after experimental removal of macroalgal cover. *Mar. Biol.* 141, 679–689.