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Oxygen/sulfide regime and nematode fauna associated with *Arenicola marina* burrows: new insights in the thiobios case

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Abstract Profiles of oxygen and sulfide around the burrows of the lugworm, *Arenicola marina*, from a North Sea tidal flat were examined with microelectrodes, and the steep gradients were related to the microdistribution of nematodes. Around the tail shaft free oxygen penetrated only 2 mm into the burrow wall, coinciding with a bright zone sharply limited by the ambient black sediment. Contrastingly, in normal bottoms of the tidal flat ("controls") only the surface of the bright zone was supplied with free oxygen. Here, the dark colouration coincided with the presence of free hydrogen sulfide. Around the tail shaft the nearest free hydrogen sulfide was detected 6 mm from the burrow wall leaving several millimetres of black sediment without measurable free sulfide. We discuss how these divergencies may relate to the stability of the oxygen/sulfide gradients and the course of time involved in their formation. A total of 54 nematode species were identified. Based on non-metric Multidimensional Scaling Ordination, four nematode assemblages corresponded to four microhabitats of the *A. marina* burrow: the funnel, the feeding pocket, the tail shaft and the feacal cast. The tail shaft assemblage (oxic plus partly anoxic zones) was similar to that of the anoxic zone of the control sediment. It was dominated by the most abundant nematode in the present study, *Metalinhomoeus biformis* (mean abundance in tail shaft 202 ind $\times$ 10 cm^{-3}). Adults of common nematode species from sulfidic microhabitats had a significantly higher length/diameter ratio than those inhabiting the oxic zone of the control sediment ($p < 0.001$). The chemical recordings and metric analysis indicate that these slender nematodes around the *A. marina* tail shaft and in the reduced horizons of the reference sites represent thiobiotic assemblages, as compared to the shorter and stouter oxybiotic species characterising the assemblages from the surface zone and (partly) the funnel.

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Introduction

The description of a sulfide system as a specific biotope by Fenchel and Riedl (1970) has led to a number of controversial studies on the fauna of this habitat, termed "thiobios" by Boaden and Platt (1971). From the very beginning, the steep gradient system around the burrows of the common lugworm, *Arenicola marina* (Polychaeta), played a key role in the discussion about the presence or absence of a thiobios (Reise and Ax 1979; Boaden 1980; Powell and Bright 1981; Scherer 1985; Reise 1987). Jensen (1986, 1987) showed that thiobiotic nematode species are more slender than oxybiotic ones. However, none of the early papers on oxybios and thiobios were based on actual measurements of the two crucial ecofactors, oxygen and sulfide. Instead, the position of the redox potential discontinuity layer (RPD-layer) was usually considered a valid criterion discriminating an oxic from a reduced/sulfidic milieu. Moreover, none of the debated studies on the meiofauna around animal tubes focussed on the most abundant group at the oxic/sulfidic interface, the nematodes. Since it is known that oxygen and hydrogen sulfide are of prime importance for nematode distribution (Ott 1972; Nicholas et al. 1991), a detailed analysis of their microdistribution in relation to the gradient fields of these ecofactors along the *A. marina* burrow would seem of relevance. The present investigation needle-electrometrically analysed oxygen and hydrogen sulfide as interacting factors, and linked them for the first time to the microdistribution and

morphometrics of nematode species around the tube structure of this characteristic macrobenthic worm. Interpretation of the present results sheds light on previous discussions on the existence of a thiobios.

Material and methods

The field study was carried out in September 1992 on an intertidal flat in the outer part of the shallow bay of Königshafen on the island of Sylt, Germany (Fig. 1). For detailed descriptions of the area see Wohlenberg (1937) and Reise (1985). During ebb tide sediment was sampled from four burrows of about equal size made by *Arenicola marina* and from four adjacent "control" sites without *A. marina* burrows. The study site was approximately 100 m below the high water line. The average tidal flat sediment consists of three differently coloured layers: an uppermost brownish horizon, followed vertically by a black layer and, deeper down, by a grey layer (see Reise 1981).

The temperatures of the air, the overlying water and of various sediment horizons were measured in situ by insertion of a thin thermistor probe. Salinity of pore water and surface water was recorded with a hand refractometer. In order to collect pore water from the deeper sediment horizons (5 and 10 cm depth), a suction pore-water sampler (modified after Howes and Wakeham 1985) was used. Water content and grain size composition were determined in 2-cm steps down to 12 cm. The uppermost fraction was further subdivided into two 1-cm layers. Four cores each were taken with a perspex corer (internal diameter 5 cm, length 30 cm), and the respective fractions were pooled prior to analysis. For grain size analysis sediment collected from five feeding pockets and approximately 20 tail shafts (for designation of the various burrow compartments see Fig. 2) was pooled and wet sieved (for methodological details see Giere et al. 1988).

Sulfide and dissolved oxygen were measured with a combined microelectrode (Visscher et al. 1991). Since the use of needle-electrodes for field-ecological purposes is still an uncommon and rather sophisticated procedure the methods involved are described here in some detail. In the sediment of the control cores taken into the laboratory, free sulfide and dissolved oxygen were measured using combined needle-electrodes with the 100-µm thin sensors placed closed to each other in a stainless steel needle of 1 mm diameter (Visscher et al. 1991). Conventional calomel electrodes served as references. Elapsed time between sampling and start of measurements was not more than 30 min. In the laboratory the sediment surface was covered with sea water, and sulfide and oxygen profiles were measured under non-stirred conditions. In the sediment around the tail shaft made by *Arenicola marina* the steep microgradients of oxygen and sulfide were assessed with a different method. A spate was pushed into the sediment approximately 10 cm away from a faecal cast and the sediment forced to break open along the *A. marina* burrow. After careful removal of the overlying sediment, the lower half of the burrow wall with its adjacent sediment, lay open as in a longitudinal section, could be sampled with minimal disturbance by inserting a perspex corer (internal diameter 5 cm) parallel to the sediment surface.

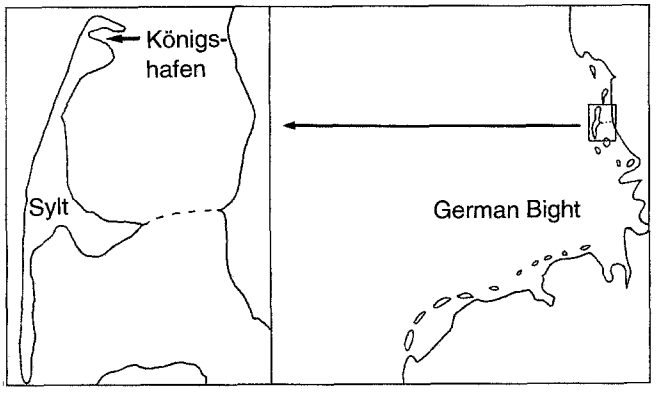


Fig. 1 Sampling area in Königshafen Bay on the island of Sylt, North Sea coast of Germany

The sediment in the corer was immediately covered with argon to prevent oxidation of sulfide, and the stoppered core sampler was transported to the nearby laboratory. Here, the cores were covered with sea water, and sulfide and oxygen measured as follows: the microelectrode was mounted to a motor-driven micromanipulator. A depth gauge with a resolution of 10 µm provided readings of electrode depths relative to the sediment surface. Oxygen and sulfide profiles were obtained only during vertical insertion of the needle-electrode. Since the degree of dissociation of hydrogen sulfide is strongly dependent on the hydrogen ion activity, pore water pH was measured in intervals of 1 cm with a thin needle electrode at the same locations as, and immediately following, the readings of sulfide and oxygen. Values at intermediate depths were calculated by interpolation.

Prior to measurement, the sulfide and oxygen sensors were calibrated. Sulfide calibration was carried out in oxygen-free phosphate buffer (flushed with nitrogen) to which sulfide was added stepwise and the resulting electrode reading plotted against the given sulfide concentration. During calibration the liquid was continuously stirred using a magnetic stirrer and covered with argon gas to prevent sulfide from being oxidised by air oxygen. Calibration curves in the form of logarithmic regressions were made for the pH values of 7.5, 8.0 and 8.5. Their parallel course allowed for graphical design of intermediate curves referring to other pH-values. The sulfide stock solution was calibrated by titration against a silver nitrate solution of 0.1 *M*.

The oxygen sensor was two-point calibrated by measuring in 0 and 100% air-saturated sea water under non-stirred conditions at in situ salinity and temperature (Revsbech 1983). For conversion of relative oxygen values into concentration, the tables by Boutilier et al. (1984) were used. For technical details of the measuring instruments and measuring process see Visscher et al. (1991).

Sediment samples from four burrows and four control sites were collected in order to examine nematode distribution. Sampling meiofauna of the fine-layered sediments around an *Arenicola marina* burrow could only be performed to a limited degree of precision due to the complicated structural pattern involved. We differentiated between the funnel, the wide feeding pocket, the narrow tail shaft and the surficial faecal cast (Fig. 2; see Reise 1981; Scherer 1985). The usual perspex corers (internal diameter 5 cm, length 30 cm) could only be applied for samples from the funnel and the control sites. Both were subdivided into horizons 1 cm of length from 0 to 2 cm depth and 2 cm of length from 2 to 12 cm depth. To sample sediment from the feeding pocket a wide corer (11 cm internal diameter) was pushed into the appropriate depth, dug out, and 10 cm^3 of the sediment in the pocket was collected with a spatula. Similarly, for the feeding pocket controls, 10 cm^3 sediment was sampled from the adjacent grey sediment not influenced by the feeding activity of the lugworm. Sediment lining the tail shaft was sampled out of a large sediment block containing a tail shaft and subsequently breaking up the sediment block alongside the burrow. The fairly stable wall of the tail shaft allowed meticulous removal of the differentiated lateral sediment structures with a spatula, i.e. the thin bright zone ("halo") and the black adjacent layer. From both compartments, 6 cm^3 sediment was sampled. Faecal cast samples (6 cm^3) were obtained with a spatula. All the samples were transported to the nearby laboratory in a portable cooler, fixed in buffered formalin (10% final concentration) and extracted following the elutriation method described in Pfannkuche and Thiel (1988). Elutrates containing high nematode numbers were subdivided using a sample splitter (Jensen 1982). The nematodes were mounted in glycerol on slides according to Riemann (1988) and identified under a Zeiss microscope equipped with interference contrast. Body length (L, excluding tail) and diameter (D) of adults (average value for 7 to 10 ind) of the most abundant species were drawn with a camera

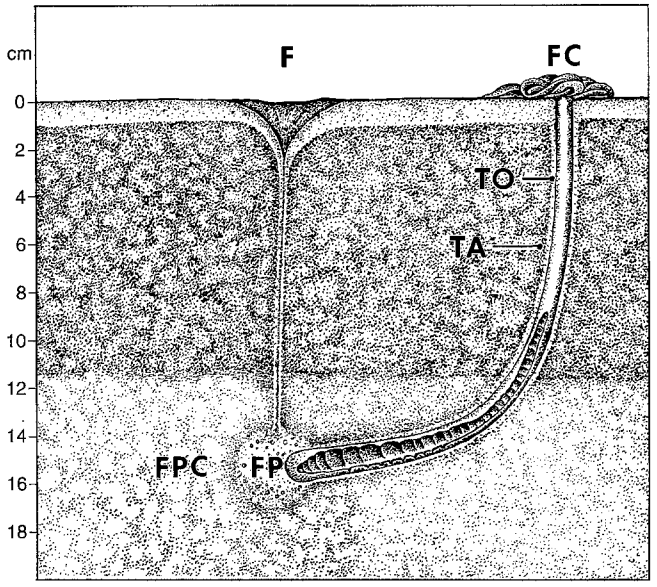


Fig. 2 Schematical structure of an *Arenicola marina* burrow indicating its various compartments. (*F* funnel; *FC* faecal cast; *FP* feeding pocket; *FPC* "controls" of feeding pocket; *TA* tail shaft, anoxic; *TO* tail shaft, oxic)

lucida. An analysis of covariance (Sokal and Rohlf 1981) was applied to the L/D data of the most abundant species found in the tail shaft, the funnel and the control sediment (adult specimens) in order to test for significant differences in body shape between relevant species.

Abundance data for nematode species were analysed using the non-metric multivariate statistical methodologies reviewed by Clarke (1993). For each separate burrow system the Bray–Curtis similarity coefficient was calculated between all pairs of samples, based on square-root transformed species abundances. This coefficient was then subjected to non-metric Multidimensional Scaling Ordination (MDS). A similar analysis was conducted for the samples from all four burrow systems combined. For the latter, species abundances for the control and funnel samples from individual depth horizons were averaged to provide a single depth-integrated sample (values suffered as a result of repeated calculations, and are not strictly independent of each other). The significance of differences in assemblage structure between treatments (i.e. locations within the burrow system) were tested using the randomisation/permutation test ANOSIM, regarding corresponding samples from each of the four burrow systems as replicates. The contribution of each species to the Bray–Curtis similarity coefficient within treatments, ranked in order of importance, was determined using the computer program SIMPER. This defines those species which contribute most to similarities and dissimilarities, respectively, between particular locations in the burrow systems. For further details on this method and its application see Clarke (1993) and Warwick (1993).

Results

Hydrographical and sedimentological parameters

Neither temperature nor salinity showed any unusual range or large gradient relevant for the present study, hence the pertinent data are given here as brief background information only. Temperature varied between 13 and 20 °C, greatest temperature difference was 2.9 °C between 0 and 10 cm depth. Salinity, usually around 33 to 35‰, had a maximal difference of 2‰ between 0 and 10 cm depth. Water content varied from 16.1 to 19.2% without any recognisable gradient.

Table 1 Characteristic granulometric indices for various sediment layers and compartments in the *Arenicola marina* burrow. (*Md* median; *QDI* sorting ceofficient)

Sediment	Md(μm)	Sand type	QDI	Sorting
0–1 cm	483	medium	1.03	poor
1–2 cm	569	medium	0.95	moderate
2–4 cm	500	medium	1.04	poor
4–6 cm	579	medium	0.97	moderate
6–8 cm	716	medium	0.94	moderate
8–10 cm	748	medium	0.94	moderate
10–12 cm	852	medium	0.87	moderate
12–14 cm	948	medium	0.76	moderate
feeding pocket	1377	coarse	1.30	poor
tail shaft (oxic halo)	365	medium	1.30	poor

The granulometric pattern (Table 1) was more differentiated. The sediment became coarser with depth, i.e. the median grain size increased from Md = 483 μm at the surface to Md = 948 μm at 12 to 14 cm depth. All sediment horizons, except the feeding pocket and the oxic halo around the tail shaft, had medium sand, most of them moderately sorted according to the Wentworth-scale (see Giere 1993). Both the bright zone around the tail shaft of *Arenicola marina* with its rather fine sand and the feeding pocket with coarse sand consisted of poorly sorted sediment. These results are in accord with Cadée (1976) and Baumfalk (1979).

Oxygen and sulfide profiles

In the sediment of the controls dissolved oxygen was only recorded down to 2–3 mm depth (Fig. 3), but, as indicated by the bright sediment colour, oxidised compounds extended down to 10 mm depth (in one measuring series even down to 20 mm). This deeper, oxidised sediment horizon was anoxic, i.e. with neither free oxygen nor free hydrogen sulfide. In the underlying black sediment layer, here termed "reduced", free dissolved sulfide was measured with concentrations in the range of 125 to 1150 $\mu mol\,l^{-1}$, increasing with depth. Its presence coincided well with the black colouration of the sediment (Fig. 3). Along the tail shaft the bright oxic zone was sharply limited by black sediment already at 2 mm distance (Fig. 4). The ambient zone between 2 mm and 6 to 15 mm was black due to iron sulfide, but no free hydrogen sulfide was detectable. Hydrogen sulfide was recorded only in the peripheral black layer at a distance of about 6 to 15 mm from the tail shaft (variation in distance relates to different sampling series).

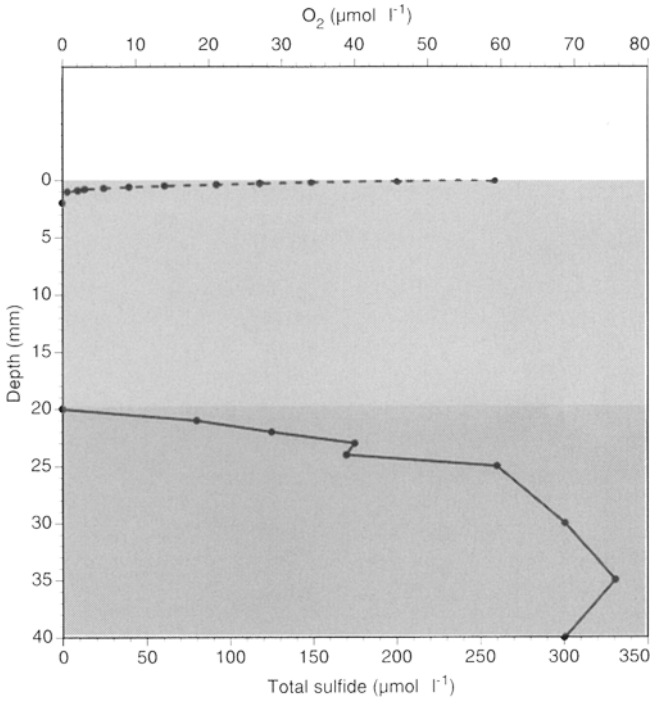


Fig. 3 Typical vertical profiles of oxygen and sulfide in "control" sediment, measured on 20 September 1992 (*Dashed line* oxygen profile; *continuous line* total sulfide profile; *lightly shaded area* oxygenated sediment; *darkly shaded area* black sediment)

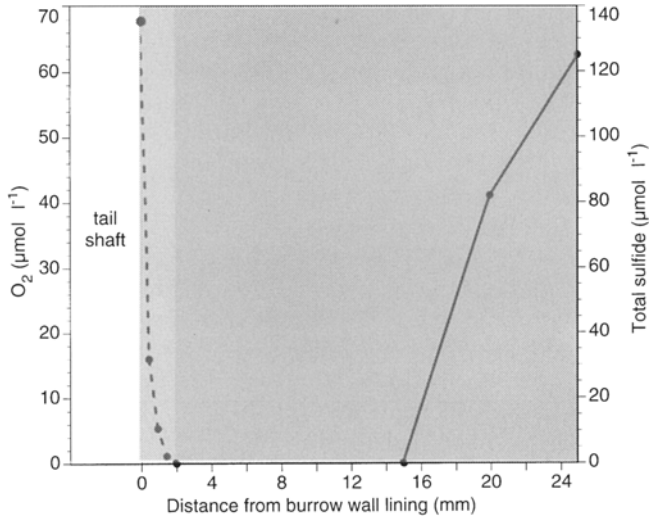


Fig. 4 Lateral profiles of oxygen and sulfide in burrow wall of tail shaft made by *Arenicola marina* (16 September 1992) (*Dashed line* oxygen; *continuous line* total sulfide; *lightly shaded area* oxygenated sediment; *darkly shaded area* black sediment)

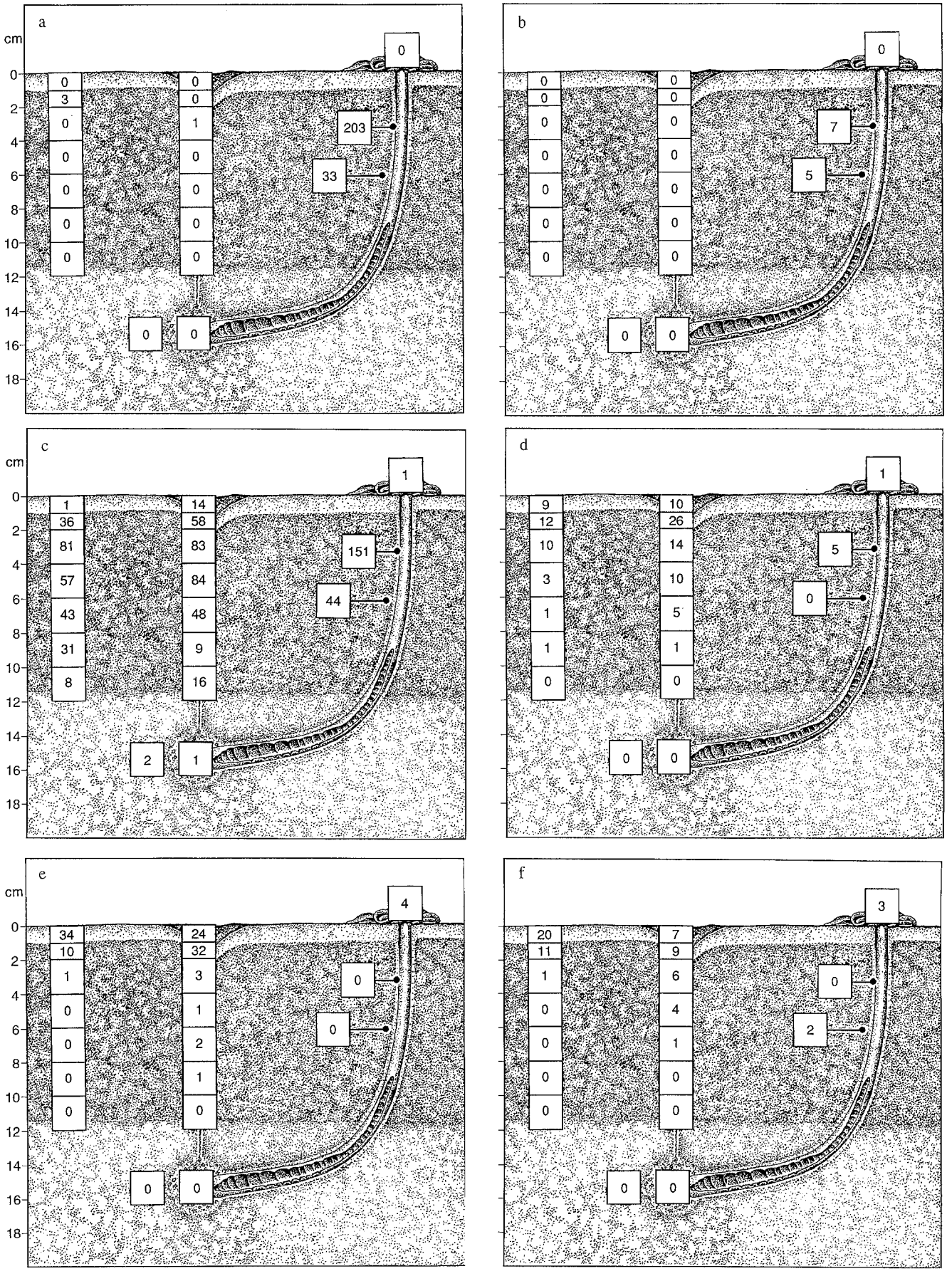
Nematode species distribution

Altogether 54 nematode species belonging to 40 genera could be distinguished in all the samples. Combining oxic and anoxic layers, 50 species occurred in the control sediments, but a more detailed analysis reveals a rather heterogeneous picture and suggests discrimination between various nematode assemblages related to different microhabitats (Table 2). The most apparent feature in Table 2 is the fairly homogeneous occurrence of nematode species in both sublayers of the tail shaft (oxic and anoxic) and in the anoxic layer of the control. This overlapping distribution, regardless of their assignment to oxic or anoxic microhabitats, is typical for almost all the species listed in the upper part of Table 2. The best example of this pattern is *Calomicrolaimus marinus* (Fig. 5c), with its main occurrence in the anoxic control sediment (maximum 81 ind × 10 cm^{-3}, equivalent to 64% of all the anoxic inhabitants). In the tail shaft it occurred less frequently, but was encountered in both oxic and anoxic layers, with 15 ind × 10 cm^{-3} and 44 ind × 10 cm^{-3}, respectively. The eurytopic nature of this species was underlined by its further occurrence in the sediment of the funnel consisting of sediment of surficial origin (not indicated in Table 2). *Metalinhomoeus biformis*, the most abundant of all species, inhabited predominantly the oxic zone of the tail shaft, with up to 202 ind × 10 cm^{-3}, but was also found in the surrounding anoxic zone and, in low numbers, also in the anoxic, non-sulfidic zone of the control

Table 2 Relative abundance of the more frequent (>1%) nematode species (adults and juveniles) in the *Arenicola marina* tail shaft and the "control" sediment (% for each of four microhabitats calculated separately) (+ present; – absent)

Species	Tail shaft		Control	
	oxic	anoxic	oxic	anoxic
Metalinhomoeus biformis	30	18	–	+
Calomicrolaimus marinus	22	24	1	64
Rhadinema sp.	9	–	–	+
Euchromadora vulgaris	7	6	5	+
Linhomoeus sp.	5	8	–	2
Microlaimus conothelis	2	5	–	6
Spirinia sp.	3	4	+	6
Viscosia viscosa	2	2	20	4
Choniolaimus papillatus	1	3	–	+
Dagda bipapillata	1	3	–	–
Odontophora sp.	–	–	–	2
Spirinia laevis	+	1	+	4
Spirinia parasitifera	+	–	+	5
Metachromadora suecica	–	–	26	+
Daptonema vicinum	–	1	15	+
Enoplolaimus litoralis	–	–	7	–
Dichromadora cephalata	+	1	3	+
Enoploides longispiculosus	–	–	3	–
Monoposthia mirabilis	–	2	3	+
Axonolaimus sp.	–	–	2	+
Paracanthonchus heterodontus	–	–	2	–

Fig. 5 Distribution of most abundant nematode species around the *Arenicola marina* burrow. Numbers in rectangles indicate ind × 10 cm^{-3}. **a** *Metalinhomoeus biformis*; **b** *Dagda bipapillata*; **c** *Calomicrolaimus marinus*; **d** *Spirinia parasitifera*; **e** *Metachromadora suecica*; **f** *Daptonema vicinum*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(33 ind × 10 cm^{-3}, Fig. 5a). A very even distribution pattern was also recorded for *Spirinia* sp. which populated all habitats except for the oxic control. A clear distributional concentration in the tail shaft was observed in species such as *Dagda bipapillata* and *Rhadinema* sp. (Fig. 5b). In contrast *S. laevis* and *S. parasitifera* had a well defined population centre in the anoxic control sediment (Table 2, Fig. 5d). No nematode species had a population maximum in the anoxic layer of the tail shaft.

The distinct character of the oxic layer in the control sediment is underlined by the presence of *Metachromadora suecica*, followed by *Choniolaimus papillatus* and *Daptonema vicinum* (Table 2, Fig. 5e, f), but they were rare at the anoxic depth and absent from the oxic halo of the tail shaft. Comparing only the two oxic microhabitats, their nematode populations are very different, with only *Euchromadora vulgaris* occurring in common (at least more regularly). Although both microhabitats are oxic, they have apparently little ecological coherence.

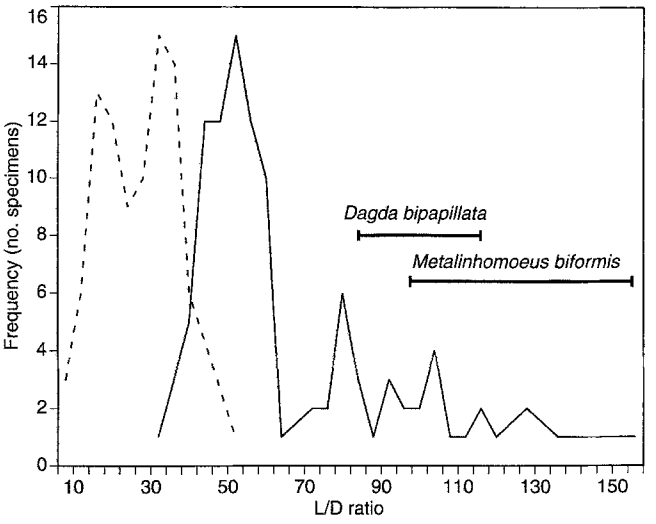


Fig. 6 Length/diameter (*L/D*) ratios of the most frequent nematode species listed in Table 3 (adult specimens) plotted against their frequency. L/D range of two prominent tail shaft species, *D. bipapillata* and *M. biformis*, indicated. (*Dashed line* oxybiotic species; *continuous line* thiobiotic species)

Body elongation

In order to further analyse the character of the nematodes inhabiting the *Arenicola marina* burrow, body elongation was assessed by calculating the L/D ratio of adults (Table 3). The "size spectrum" is shown in Fig. 6. Three types of body shapes can be discriminated, i.e. one group with a predominantly stout body characterised by an L/D range between 10 and 40 and extending to 52 (dashed curve). A second group is composed of predominantly slender species with a range in L/D ratios between 32 and about 80, but mostly with values of 40 to 65 (left part of continuous line). The third group consists of very slender species with L/D ratios of about 80 to 150 (right part of continuous line). It is best represented by *Dagda bipapillata* and *Metalinhomoeus biformis*, both occurring mainly in the tail shaft. These three types of body shapes of the observed species correlate to habitat characteristics: stout, oxybiotic species in the oxic sediment horizons, and more slender, thiobiotic species

Table 3 Calculation of length/diameter (*L/D*) ratios in common nematodes (adult specimens) from different compartments (sequence of species arranged according to L/D ratio) and assignment to characteristic compartments (*TO* tail shaft, oxic; *TA* tail shaft, anoxic; *S* sulfidic sediment; *O* oxic sediment)

Species	Body length (µm)	Body diameter (µm)	L/D ratio	Compartment
Metalinhomoeus biformis	2006	17	122	TO, TA
Dagda bipapillata	3879	38	103	TO, TA
Odontophora sp.	4227	47	91	S
Spirinia laevis	3000	44	68	S
Spirinia sp.	3275	58	58	S, TO, TA
Microlaimus conothelis	1917	34	56	TO, TA, S
Daptonema proprium	1060	19	55	S
Calomicrolaimus marinus	1395	26	55	TO, S, TA
Spirinia parasitifera	2588	51	51	S
Neochromadora sp.	1362	28	50	TA, TO
Viscosia viscosa	1902	46	42	O, S, TO, TA
Enoplolaimus litoralis	2124	55	39	O
Dicromadora cephalata	1252	55	37	O, TO
Monoposthia mirabilis	1454	40	37	O
Daptonema vicinum	1272	43	31	O
Paracanthonchus longus	2364	77	31	TO, TA, O, S
Metachromadora suecica	1247	48	26	O
Chromadora nudicapitata	731	30	24	O
Daptonema setosum	1562	78	20	O
Chromadorita tentabunda	619	41	15	O

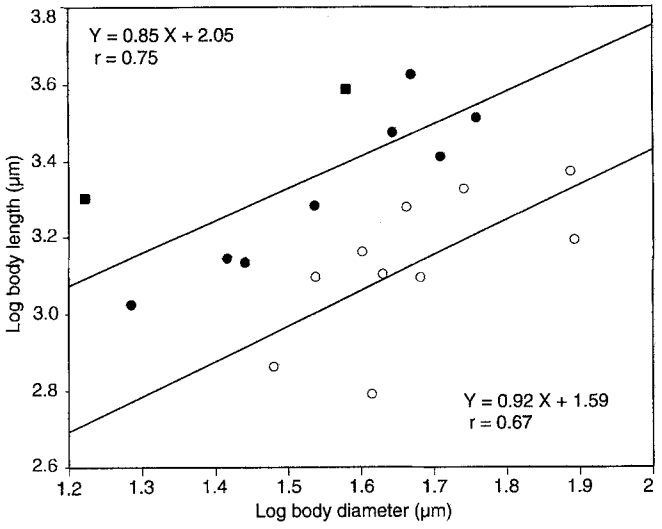
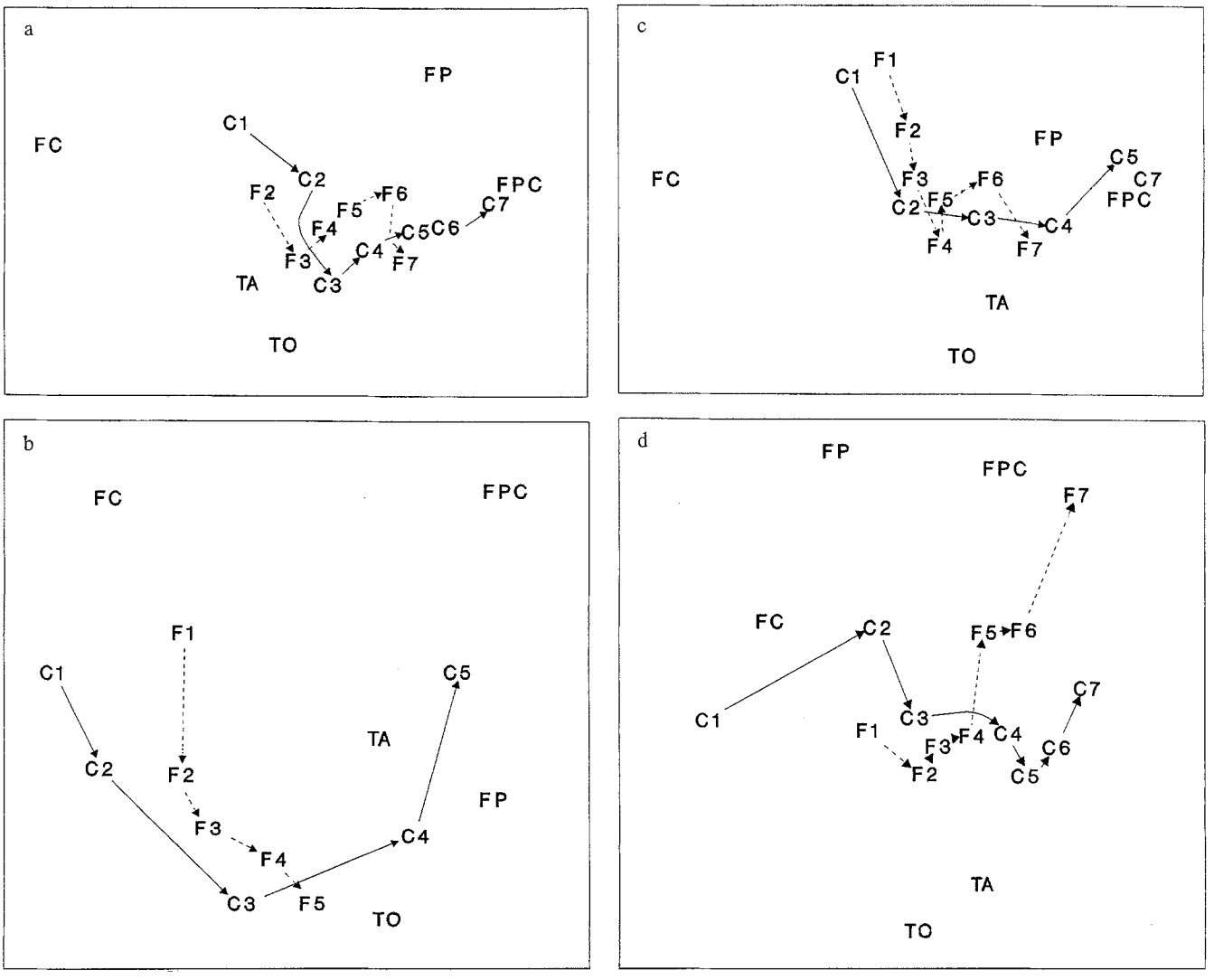


Fig. 7 Regression of body elongation on log-transformed scales of most abundant nematode species (adult specimens) inhabiting the tail shaft of *Arenicola marina* (*squares*), the anoxic and oxic horizons of "control" sediment (*circles*). *Filled symbols* thiobiotic species; *open circles* oxybiotic species

represented by two other groups of species mainly in the anoxic horizons and in the tail shaft of *A. marina* burrows.

In order to test whether these differences in body shape are reliable indicators of oxybiotic and thiobiotic species, the mean adult body length was plotted against the mean body diameter of the most abundant species (adults of ten species per group) with a population maximum either in oxic horizons (i.e. oxybiotic species) or in anoxic/sulfidic horizons (i.e. thiobiotic species). The two groups of plots are described by the equations of the regression lines: $Y = 0.92X + 1.59$ and $Y = 0.85X + 2.05$, with corresponding correlation coefficients of $r = 0.67$ and 0.75, respectively (Fig. 7). The

Fig. 8 Two dimensional multidimensional scaling ordinations of nematode species **a–d** for each of four *Arenicola marina* burrow systems investigated. This method shows degree of similarity between nematode assemblages of different compartments of burrow system. (*C* "control"; *F* funnel; *FC* faecal cast; *FP* feeding pocket; *FPC* "controls" of feeding pocket; *TA* tail shaft, anoxic; *TO* tail shaft, oxic). For clarity, lines connect species occurring in same compartment. *Numbers* refer to sediment depth in centimeter



two groups of plots are significantly different from each other (analysis of covariance, $p < 0.001$) confirming that they represent different nematode assemblages, with the two most slender species, *Metalinhomoeus biformis* and *Dagda bipapillata*, on the one (thiobiotic) side and the stoutest nematode, *Metachromadora suecica*, at the other (oxybiotic) extreme.

Allocation of nematode assemblages

Two-dimensional MDS ordinations for each burrow system enabled discrimination between nematode assemblages in the various compartments of the *Arenicola marina* burrow, and further statistical treatment allowed assessment of their degree of similarity. The figures emerging from the MDS ordination (Fig. 8a–d) indicated for the control and funnel samples a similar trend: with increasing depth (from left to right in each case) the composition of nematode assemblages changed gradually. The faecal cast samples were most similar in species composition to the surface layers of the control and funnel (both at the left side of the ordinations). The feeding pocket and those samples of the controls corresponding in depth to the feeding pocket were most similar to the deeper sections of the control and funnel, i.e. to the right of the ordinations. The tail-shaft samples (anoxic and oxic) were intermediate in composition between these two. The combined MDS ordination for all burrows (Fig. 9) indicated four separate clusters of samples: a central, tight group comprising all the control and funnel samples, the four faecal cast samples to the left, the feeding pocket and feeding pocket control samples to the upper right and the samples from both the oxic and anoxic layers of the tail shaft to the lower right.

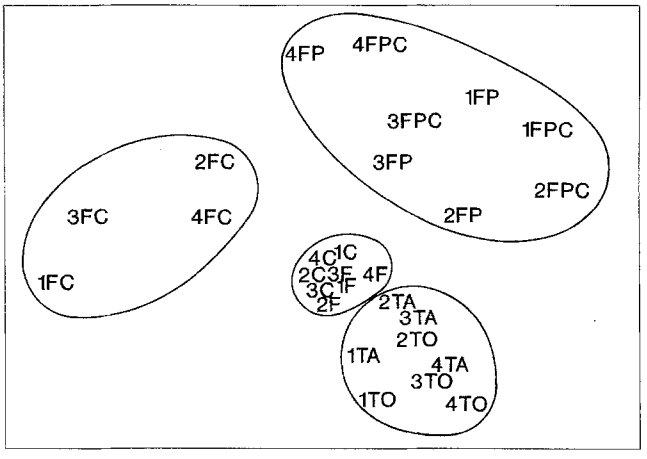


Fig. 9 Two dimensional multidimensional scaling ordinations of nematode species combined for all four *Arenicola marina* burrow systems investigated. Clustering of "nematode assemblages" assigned to different compartments of burrow system becomes evident. For abbreviations see Fig. 8

A similar analysis in which the abundance of each species, taken as a percentage of the total in each sample, was standardised showed an essentially similar pattern. This proves that the clusters represent genuine differences in species composition and not simply differences in overall abundance. The ANOSIM test showed high R values and significant differences between different compartments of the *Arenicola marina* burrow (significance level $< 5\%$) in most pairwise comparisons. But there were low R values and no significant differences between the control and funnel samples, the oxic and anoxic sections of the tail shaft, or the feeding pocket and feeding-pocket control samples. This underlined the conformity of these compartments indicated already by MDS ordination. For the purpose of SIMPER analysis, these treatments have not, therefore, been considered separately; i.e. there are four coherent assemblages corresponding to the four clusters in Fig. 9.

Discussion

The results of this investigation will be discussed under two aspects: (1) the biogeochemical aspect, considering the small-scale pattern and dynamics of oxygen and hydrogen sulfide around the *Arenicola marina* burrow, and (2) the meiobenthic aspect, considering the occurrence and variations of nematode assemblages. These two aspects will be considered together in relationship to the existence and habitat conditions of a possible thiobios.

The application of needle-electrodes measuring oxygen and sulfide concentrations revealed the absence of a correlation between sediment colouration and the presence of oxygen and sulfide (compare Revsbech et al. 1980; Sikora and Sikora 1982; Meyers et al. 1987). Around the chemocline, sediment horizons with a bright colour (and positive redox potentials) may just indicate the presence of oxidised compounds, but not the presence of free oxygen (see Fig. 3). Conversely, a black stratum indicates the presence of iron sulfides and anoxia, but not necessarily that of free hydrogen sulfide (see considerations on tail shaft region of *Arenicola marina* burrows in "Discussion").

Regarding the availability of oxygen for meiofauna, the recording conditions applied here may not truely reflect field conditions. In our cores the water was stagnant resulting probably in lower oxygen concentrations and penetration depths than are to be expected in the field, where bioturbation and flow velocity play important roles (e.g. Revsbech et al. 1980; Booij et al. 1991). Zühlke and Reise (1994) showed that even at sheltered sites, the upper 1 to 2 cm of sediment may drift during submergence, allowing the oxygen gradient to penetrate much deeper into the sediment.

Another discrepancy between the factual presence of (low) oxygen and its registration in our measurements may be due to the detection limit of electrodes (1 to 2 μM). This could, at least temporarily, suffice as an oxygen supply for animals with small body diameters like the species found in the present study (Powell 1989; Dubilier et al. 1995).

Around the lugworm burrow we attribute the discrepancy between sediment colouration and sulfide gradient to the time course of chemical processes, e.g. to the persistence of the chemical regime influenced by the bioturbative and irrigational activity of the burrowing macrofauna (Anderson and Meadows 1978; Yingst and Rhoads 1980).

The variable biogenic or merely physical (no irrigational activity during low tide) oxygen fluxes through the sediment layers cause an unstable chemical system. Oxic sediment exposed to reducing conditions will soon lose its free oxygen content, leaving temporarily an anoxic sediment of still bright colouration. Then the diffusion of hydrogen sulfide from the deeper, reduced strata results in the precipitation of iron sulfides which stain the sediment black. During the initial phase of anoxia in this black layer, free hydrogen sulfide is not yet recordable. Animals living here will, in this phase, have to overcome anoxia, but not additionally the toxic sulfide. Only after a continuing diffusive intrusion of hydrogen sulfide and after complete reduction and precipitation of the dissolved oxic iron compounds as iron sulfides will the excess hydrogen sulfide create a coincidence between black sediment colouration and presence of toxic hydrogen sulfide. This demonstrates why the presence or absence of free oxygen and free hydrogen sulfide in the narrow gradient system around the chemocline drastically depends on its persistence, or the time course of its formation. This, in turn, can be altered by the intensity of ventilation exerted by endobenthic animals. Intermittent pauses of irrigational activity quickly result in anoxia and diffusion of hydrogen sulfide into the burrow system (Meyers et al. 1988; Watling 1991; Völkel and Grieshaber 1992). Hence, the narrow halo of bright sediment around the tail shaft is only an ephemeral oxic environment momentarily spot-recorded by the thin point of the electrode. Considering the rapid diffusion of hydrogen sulfide from the ambient sediment whenever *Arenicola marina* ceases to flush its burrow, the meiobenthic animals living here are at ebb-tide, in fact, repeatedly exposed to hydrogen sulfide.

On the other hand, Wells (1945) observed that lugworms kept in experimental tubes responded to low water with peristaltic movements of their hindbody transporting air bubbles to their gills. This behaviour could prevent the burrow water from sulfide diffusion during low tides. We performed continuous sulfide measurements in *Arenicola marina* tail shafts for longer ebb-tide periods. These data (not presented here) revealed oscillations of sulfide concentrations in the range of 0 to 115 $\mu mol\,l^{-1}$ with an average frequency of 40 min. This may indicate a periodic influx of sulfide into the tail shaft, alternating with phases of sulfide oxidation. When the sediment is flooded again, the oxygen gradient is re-established due to the pumping activity of the worm, and the sulfide gradient is forced back into the sediment. Sulfide in the oxic zone is oxidised by Fe^{3+} and in the anoxic sediment surrounding the oxic zone the sulfide is precipitated as black ironsulfide. The variable flux of dissolved iron into the burrow system and its lining may also cause fluctuating periods of presence or absence of free hydrogen sulfide (Aller and Yingst 1978).

This conception of the compartment "tail shaft" not only underlines its nature as an environment under continuous change, with rapid fluxes of oxygen and hydrogen sulfide and steep but transient chemical gradients. It also implies that the narrow bright halo and its black environment represent one ecological unit with a narrow chemical threshold, repeatedly crossed by chemical fluxes and the mobile meiofauna. Supplied by mucus, excretion products and an enhanced supply of organic substances derived from the activity of *Arenicola marina*, this is the favourable microhabitat for a particularly rich stock of bacteria metabolising sulfur compounds (Aller and Aller 1986; Meyers et al. 1988; Reichardt 1988; Thomsen 1989; Hüttel 1990).

It is exactly this variability of the oxic and sulfidic micro-gradients to which the close similarity of the nematode spectrum in the oxic and anoxic parts of the tail shaft can be ascribed (Table 2). Indicated both by the ordination method and L/D ranking, it is evident that only one assemblage of thiobiotic, slender nematodes inhabited both micro-habitats. Moreover, adult body length of some of the abundant nematodes inhabiting the tail shaft sediment (e.g. *Metalinhomoeus biformis*, *Spirinia laevis*, *Dagda bipapillata*) is 2 to 4 mm, i.e. beyond the thickness of the oxic layer. This suggests that parts of their body are easily subjected to anoxia. Therefore, the two different layers, although momentarily of a different chemical character, belong together ecologically, because they are both frequently dominated by hydrogen sulfide. This ecological factor is a link also to the deeper layers of the control. Consequently, the oxic halo of the tail shaft and the oxic surface layer of the control sediment cannot simply be equated. In fact, their major ecological divergence, discussed above, is indicated by the difference in nematode assemblage.

Reise (1981, 1984, 1987) showed a marked increase in diversity and abundance of many turbellarian orders and of gnathostomulids around the burrow system of *Arenicola marina*. He ascribed to this biogenic habitat structure a prominent influence on the heterogeneity of distributional patterns. In the overall structure of a lugworm burrow he discriminated various distinct subhabitats with particular, although seasonally varying and dynamic, turbellarian assemblages. Knowledge of

this salient ecological role of *A. marina* burrows is (partly) confirmed and supplemented here by a detailed analysis of nematode microdistribution. Hence, two dominant meiofauna taxa seem to respond similarly to the differentiated factorial system established in lugworm biogenic structures.

The existence of four separate nematode assemblages tightly coupled to four microenvironments could be demonstrated for the first time by mathematical treatment (MDS ordination analysis). Among these microenvironments, the funnel, with its sediment sliding down from the surface, had a nematode assemblage structure best comparable to the surrounding control sediment (Fig. 9), but it also had similarities to the surface sediment of the control. This intermediate nature resulted also from the L/D calculation, where species collected from funnel samples were considered either representatives of the oxic or anoxic layer of the controls.

The assemblage inhabiting the oxic and anoxic sediment around the tail shaft showed a close relationship to the control samples (Fig. 9). It was characterised by the most slender nematode species found (Fig. 5a, b; Fig. 6; Fig. 7). We ascribe this body elongation to the low oxygen concentration available in the tail shaft minimising diffusion limitation (cf. Powell 1989).

The assemblage from the feeding pocket was, in species composition and abundance, similar to the control adjacent to the pocket sand. This is in contrast to the findings of Reise (1984, 1987) for Turbellaria. He suggested a particular set of abundant species in the pocket sand of lugworm burrows at a site close to our sampling area. However, a direct comparison with samples from the adjacent sediment was not made. Hylleberg (1975) found in the feeding pocket made by *Abarenicola pacifica* a significantly higher nematode number compared to the neighbouring control sediment.

The nematode assemblage found in the faecal casts represents a unique cluster in the MDS ordinations (Fig. 9). However, some of the faecal cast inhabitants were abundant also in the uppermost oxidised control sediment (Fig. 5d, e, f). This similarity could be an effect of the tidal currents continuously dispersing the casts and their inhabitants over the adjacent surface sediment. This would contrast to the situation studied by Jensen (1987), where the casts were deposited on a rather stable sea bottom at 16 m water depth, possibly persisting for several days (cf. Fig. 1 in Jensen 1981).

Conclusions

In tidal flat sediments, the occurrence and structure of the meiofauna seems to a major extent dominated by the oxygen/hydrogen sulfide profiles, particularly steep and changing around biogenic structures. This was assumed already by Reise and Ax (1979), discussed in more detail by Scherer (1985) and confirmed on the basis of microelectrode data by Meyers et al. (1987). The present investigation relates the nematode microdistribution to different microhabitats alongside the *Arenicola marina* burrow characterised by variables of oxygen and sulfide. The main conclusions can be summarised in four points:

1. The tail shaft of *Arenicola marina* burrows and the control sediment were inhabited by different assemblages, but a closer comparison required differentiation between the oxic, surface layer and the anoxic, deeper horizon of the control.
2. There was a relatively high species overlap between anoxic control inhabitants and those in the tail shaft. The most abundant species, *Metalinhomoeus biformis*, inhabited mainly the tail shaft, but a few specimens were also present in the anoxic control. In contrast, the oxic control sediment and the tail shaft had only few species in common.
3. The specific conditions at the tail shaft created a genuine environment to which some representatives apparently are restricted: two species, *Dagda bipapillata* and *Rhinema* sp., were exclusively found here.
4. The tail shaft and the anoxic control sediment were both characterised by the influx of hydrogen sulfide. Representative inhabitants of both microhabitats were significantly more slender than those inhabiting the oxic control sediment, a result which corroborates former calculations from other habitats (Jensen 1986, 1987) and justifies the discrimination between thiobiotic and oxybiotic nematode species from sandy habitats on the basis of their L/D-ratio.

Distinct microsites around lugworm tubes, assessed here for nematodes, have been found to exist also for turbellarians (Reise 1984, 1987). For both meiofauna groups the tail shaft compartment played a prominent ecological role.

The detailed layering of the chemical microgradients, recordable only with needle-electrodes and the continuous interaction of biotic factors, have altered the earlier more static conception of a two-layered chemocline around the *Arenicola marina* burrows. Today we conceive of this system as permanently fluctuating, with drastic variations in the mm-range meeting ideally the requirements of "sulfur bacteria". The distinct nematode fauna living preferably around the tail shaft is alternatingly exposed to irregular changes in concentrations of sulfide, sulfide-free anoxia and oxygen. The seeming contradiction that thiobiotic species can be encountered in oxic microenvironments (compare Giere et al. 1991; Ott et al. 1991) is easily resolved by a modern conception of the thiobiome which takes into account the continuous fluctuations of oxygen and sulfide around the chemocline (Meyers et al. 1988), rendering clear-cut delineations of oxic and sulfidic

layers around the burrow walls of *Arenicola marina* problematical. A fauna typically encountered in, and sometimes even restricted to, this biotope dominated by hypoxia and hydrogen sulfide has an ecological configuration defined as "thiobios" (Powell et al. 1983; Giere 1992).

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