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Structure and Function of the Benthos in Estuarine and Coastal Ecosystems in relation to actual and future Anthropogenic Impacts

Final Report

PART I.

SUMMARY

composed by

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Table of Contents

PART I. : Summary (task A - task J)

PART II. : Publications 1-9 pags : 1 - 147

PART III : Publications 10-22 pags : 148 - 396

List of publications :

1. Li Jian & M. Vincx. 1993. The temporal variation of intertidal nematodes in the Westerschelde. The importance of an estuarine gradient. Neth.J. Aquatic Ecol., 27 (2-4), 319-326. 1

2. Li Jian, M. Vincx & P.M.J. Herman. 1996. Carbon flows through meiobenthic nematodes in theWesterschelde estuary. Fundamental and Applied Nematology., in press 9

3. Soetaert, K., M. Vincx, J. Wittoeck, M. Tulkens & D.Van Gansbeke. 1994. Spatial Patterns of Westerschelde meiobenthos. Estuarine, Coastal and Shelf Science, 39, 367-388. 26

4. Soetaert, K., M. Vincx, J. Wittoeck & M. Tulkens. 1995. Meiobenthic distribution and nematode community structure in five European estuaries. Hydrobiologia, 311, 185-206. 48

5. Moens, T., A. Vierstraete, S. Vanhove, M. Verbeke & M. Vincx. 1996. A handy method for measuring meiobenthic respiration. J; Exp. Mar. Biol. Ecol., 197, 177-190 70

6. Moens, T., Vierstraete, A. & M. Vincx. 1996. Life strategies in two bacterivorous marine nematodes : preliminary results. P.S.Z.N.I.:Marine Ecology, 17 (1-3), 509-518. 84

7. Moens, T. & M. Vincx. 1996. Observations on the feeding ecology of estuarine nematodes. J. mar.biol.Ass.U.K., in press. 94

8. Vincx, M., Dewicke, A., Mees, J., Steyaert, M. & Van Gansbeke D. 1996. Benthos of the North Sea : able to recover or desperately lost ? Federal Office for Scientific and Cultural Affairs, Brussels, Proceedings of the symposium held on the occasion of the 10th anniversary of the Belgica, Ostend, October 1994, 33-41. 115

9. Steyaert, M., Van Gansbeke D. & M. Vincx. In prep. Meiobenthos and interstitial processes in eutrophic sediments. 124

10.Mees, J., A. Dewicke & O. Hamerlynck, 1993. Seasonal composition and spatial distribution of hyperbenthic communities along estuarine gradients in the Westerschelde. Netherlands Journal of Aquatic Ecology 27, 359-376. 148

11.Mees, J., N. Fockedey & O. Hamerlynck, 1995. Comparative study of the hyperbenthos of three European estuaries. Hydrobiologia 311, 153-174. 166

(pags 188 - 200 missing in numbering)

12.Cattrijsse, A., J. Mees & H. R. Dankwa, submitted. Nursery function of an estuarine tidal marsh for the brown shrimp *Crangon crangon*.. 201

13.Cattrijsse, A., J. Mees, K. Hostens & E.S. Makwaia, in press. The aquatic fauna of two intertidal salt marsh creeks in the Westerschelde estuary. Belgian Journal of Zoology. 230

14.Mees, J., A. Dewicke, A. Cattrijsse & N. Fockedey, in press. Seasonality in hyperbenthic communities of the Westerschelde estuary. Belgian Journal of Zoology. 250

15.Dewicke, A. & J. Mees, 1996. The hyperbenthic fauna of the Belgian continental shelf: spatial variability in community structure. Progress in Belgian Oceanographic Research 1996: 55-58. 276

16.Dewicke, A. & J. Mees, in preparation. Spatial patterns in the hyperbenthos of the Belgian continental shelf. 280

17.Dewicke, A. & J. Mees, in preparation. Temporal patterns in the hyperbenthos of the Belgian continental shelf. 306

18.Fockedey, N. & Mees, J. (1996). The diet of *Neomysis integer* (Crustacea, Mysidacea) in the maximum turbidity zone of estuaries. Royal Acad. Belgium. Nat. Comm. Oceanol. Progr. Belgian Oceanogr. Res.: 79-82. 329

19.Fockedey, N. & Mees, J. (submitted). Feeding of the hyperbenthic mysid Neomysis integer in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries. J. Mar. Syst. 333

20.Mees, J., Abdulkerim, Z. & Hamerlynck, O. (1994). Life history and production of *Neomysis integer* in the Westerschelde estuary (SW Netherlands). Mar. Ecol. Prog. Ser., 109: 43-57. 361

21.Mees, J., Fockedey, N., Dewicke, A., Janssen, C.R. & Sorbe, J.C. (1995). Aberrant individuals of *Neomysis integer* and other mysidacea: intersexuality and variable telson morphology. Neth. J. Aquat. Ecol., 29: 161-166 376

22.Fockedey, N. & J. Mees. In prep. Laboratory studies on growth and development of the brackish water mysid *Neomysis integer*. 382

Task A

Temporal variablity of the meiobenthos in the Westerschelde

Free-living nematodes are the most abundant meiobenthos in estuaries. Temporal variability in nematode communities shows that abundance peaks of dominant species can be present in any season in the Westerschelde. The nematode community in the brackish zone has a lower biomass and is more variable in time than in the marine zone. The temporal variability in the mesohaline zone has been investigated in great detail in order to develop a time dynamic carbon flow model for the nematode community. The model is calibrated with nematode biomass data (divided over the four feeding types) during three days interval and monthly interval sampling. The driving variables of the model include meiobenthos, macrobenthos, bacteria, chlorophyll-a, salt marsh grass and other environmental factors such as POC, temperature and day length.

In the mesohaline station, 12 nematode species contribute for more than 94% of the community. Average density is 2300 ind.10cm^2 with an average biomass of 0.2 gC/m^2.
The developed model MONIW predicts a standing stock of 249 mgC/m^2 (51% consists of omnivores among the nematodes). The consumption has been calculated as 98 mg C/m^2.day and the production as 22 mgC/m^2.day (53% for the omnivores). The defecation is 73 mgC/m^2.day. The loss in respiration, excretion and natural death is only 3 mgC/m^2.day. These data are consistent with the importance of nematodes in the decomposition of the POC and of nematodes as a pathway from organic detritus to higher trophic levels in the benthic system. It shows that 75% of the nematode carbon production returns to the ecosystem (as faeces) and that 20% of the carbon can be consumed by higher trophic levels.
The yearly P/B equals 32, a value three times higher than the P/B=10 as generally accepted for meiofauna. The model predicts the temporal fluctuations of the biomass of the nematode trophic groups : the biomass of the nematodes is controlled by deposit-feeding predators, mainly macrobenthos. Food availability in the nutrient rich Westerschelde causes less modification in nematodes biomass than predators do. Dominant nematode species in the community area characterized by lower rates in respiration, excretion and natural death. Temperature is important not only in the regulation of the physiological activity of the nematodes but also in the regulation of predatory activities on nematodes.

Articles on the subject produced during the programme :

1. *Li Jian & M. Vincx. 1993. The temporal variation of intertidal nematodes in the Westerschelde. The importance of an estuarine gradient. Neth.J. Aquatic Ecol., 27 (2-4), 319-326.*
2. *Li Jian, M. Vincx & P.M.J. Herman. 1996. Carbon flows through meiobenthic nematodes in theWesterschelde estuary. Fundamental and Applied Nematology., in press*

Task B

Spatial variability of the meiobenthos in the Westerschelde

The meiobenthos was investigated from six transects along the salinity gradient of the Westerschelde, including the intertidal, subtidal and channel area. Meiobenthic densities were higher in the intertidal than in most permanently submersed areas. The subtidal sites below 7 psu salinity were nearly devoid of meiobenthic life. Nematodes were by far the most abundant meiobenthic organisms in the intertidal, but were less dominant in the other areas. Gastrotrichs, turbellarians, copepods and large ciliates were usually more numerous in the subtidal and channels compared to the intertidal, both in relative and absolute terms. Vertical distribution of the meiobenthos within the sediment was rather heterogeneous.
The average meiofauna density is lowest near the mouthof the estuary, is increasing towards Terneuzen, decreases until Ossenisse Valkenisse and increases to a maximal level Bath. Diversity variations along the salinity gradient are more correlated to sediment characteristics than to salinity.
Canonical correspondance analysis showed sediment depth to be as important as water depth, salinity or sedimentary characteristics in the determination of community structure. Intertidal communities exhibited a well-developed community gradient with depth into the sediment, whereas the vertical structure of subtidal and channel stations was different from the intertidal zonation and in some cases showed a distorted pattern. This was probably caused by sediment disturbance dur to higher current velocities and dredging activities in these regions. It is argued that, although at some subtidal sites a characteristic subtidal nematode population may persist, in many cases the sublittoral of the Westerschelde is either too dynamic an environment or food availability is too low to meet requirements for growth and reproduction of the nematodes. The populations are probably not self-sustaining but persist due to continuous replenishment from less harsh areas by means of the estuarine circulation.
The extreme low densities in the intertidal sites near the Maximum Turbidity Zone (upstream of Saefthinghe) can probably be explained by the presence of polluted sediments. Organic and inorganic pollutants result in very low oxygen concentration caused by microbial activities.

Articles on the subject produced during the programme :

3. *Soetaert, K., M. Vincx, J. Wittoeck, M. Tulkens & D.Van Gansbeke. 1994. Spatial Patterns of Westerschelde meiobenthos. Estuarine, Coastal and Shelf Science, 39, 367-388.*
4. *Soetaert, K., M. Vincx, J. Wittoeck & M. Tulkens. 1995. Meiobenthic distribution and nematode community structure in five European estuaries. Hydrobiologia, 311, 185-206.*

Task C

Trophic role of the meiofauna

In marine and estuarine benthic systems, meiofauna are the dominant metazoans in terms of both density and diversity. Nematodes are usually the most abundant meiofaunal organisms, occurring in densities of 10^5 to 10^7 ind.m^{-2}, sometimes even more. Inspite of their remarkable presence, their role in the benthos is still poorly understood. It has been suggested that meiofauna can significantly contribute to nutrient recycling in the benthos. As by far the highest meiofauna densities are found in the upper two cm of the sediment, they may thus have an important role in benthic-pelagic coupling and, in shallow waters, perhaps even influence water column primary productivity. Still, the processes by which meiofauna could influence nutrient recycling have been scarcely documented and have never been properly quantified. A major flaw in our understanding of meiofauna functioning in benthic ecosystems is the overall scarcity of information on meiofauna feeding ecology. This involves both qualitative and quantitative aspects, and is to a large extent due to methodological difficulties involved in the study of meiofaunal feeding. The present study reports on both qualitative and quantitative aspects of the feeding ecology of estuarine and marine nematodes.

Data are presented from an observational study of almost 40 species from the Westerschelde estuary. Our observations are compiled into a dynamic classification in six major feeding guilds. These are the microvores, the deposit feeders, the ciliate feeders, the epigrowth feeders, the predators and the facultative predators. It is stressed that nematodes are often very opportunistic feeders, which may change their feeding behaviour in response to available food, and that on the other hand probably very species-specific nematode-food interactions exist. It is therefore vital not to use this or any other feeding type classification as a static scheme, but to recognize its plasticity, inherent to the diversity of species and functions in a meiobenthic community. A scheme is proposed that illustrates how the presented information can be used to include nematodes in conceptual models of carbon flow pathways in, into and out of the benthos.

Improvements of methodologies currently used to measure meiofauna grazing on bacteria and microalgae are proposed. Special attention has been paid to aspects of meiofauna preservation after incubation and of relevant incubation times of grazing trials. On the basis of our data, it is argued that hitherto published estimates of meiofauna grazing should be used with due caution. Inspite of the proposed improvements, a thoroughly reliable method for grazing experiments on sediment samples is still not in sight. However, a methodology to study fundamental processes in meiofauna feeding, such as selectivity, density-dependence, etc. is now available.

Articles on the subject produced during the programme :

5. *Moens, T., A. Vierstraete, S. Vanhove, M. Verbeke & M. Vincx. 1996. A handy method for measuring meiobenthic respiration. J; Exp. Mar. Biol. Ecol., 197, 177-190*
6. *Moens, T., Vierstraete, A. & M. Vincx. 1996. Life strategies in two bacterivorous marine nematodes : preliminary results. P.S.Z.N.I.:Marine Ecology, 17 (1-3), 509-518.*
7. *Moens, T. & M. Vincx. 1996. Observations on the feeding ecology of estuarine nematodes. J. mar.biol.Ass.U.K., in press.*

Task D

Meiobenthos and interstitial processes in eutrophic marine sediments

The vertical microdistribution of the nematode community, being the dominant meiofauna along the Belgian coastal area, is studied on species level and linked to the biogeochemical environment. Three stations along the Belgian coast and one station in the Westerschelde are seasonally examined to cover the periods of high and low organic input into the sediment. The concentration of important oxidized (nitrate and nitrite) and reduced (ammonia) nitrogen compounds together with redoxpotential values will be used to evaluate the oxidation status of the sediment.

Fine sediments, with an important fraction of mud, are characterised by steep depth gradients (oxygen, redoxpotential, nutrients, ...), which becomes more accentuated in summer (oxygen depleted sediments). *Sabatieria punctata* and *Daptonema tenuispiculum,* both typical species of this type of sediment, show a specific vertical distribution into the sediment; *S.punctata* having a maximum abundance in the subsurface layers (3 to 4 cm depth) and *D.tenuispiculum* being rather restricted to the superficial layers. However, a coupling between this distribution pattern and the redox state, as postulated in earlier studies, is not found, instead a relation is shown with the food sources. The effect of antropogenic influence, in terms of eutrophication, can rather be detected by a detailed vertical scanning of the nematode community on the whole. In the strongly oxidized situation of early spring, diversity of the nematode community seems not to be dependent on the redox chemistry of the sediment. Diversity increases with higher mud accumulations, which can be attributed to diversification possibilities of non-selective deposit feeders. In early summer, after an increased sedimentation of nutrients, the reduced sediment layer is slightly shifting towards the surface layers and is obviously influencing the distribution of the nematode community. The decreasing diversity with increasing depth into the sediment is due to more favourable redox conditions at the superficial layers, enabling more species to coexist.

A coarse sandy sediment with large interstitials implicates a deep oxygen penetration and a relative stable redox chemistry in depth, as well as seasonally. As a consequence, the nematofauna, with *Ixonema sordidum* and *Viscosia langrunensis* as most important species, is highly divers and not affected by the redox processes in its vertical distribution.

In conclusion, antropogenic impacts on the whole community can be detected through species diversity of nematode communities. The information gained out of the distribution profiles of a single nematode species is less valid than the information on diversity profiles of the whole nematode community.

Articles on the subject produced during the programme :

Vincx, M., Dewicke, A., Mees, J., Steyaert, M. & Van Gansbeke D. 1996. Benthos of the North Sea : able to recover or desperately lost ? Federal Office for Scientific and Cultural Affairs, Brussels, Proceedings of the symposium held on the occasion of the 10th anniversary of the Belgica, Ostend, October 1994, 33-41.

Steyaert, M., Van Gansbeke D. & M. Vincx. In prep. Meiobenthos and interstitial processes in eutrophic sediments.

Task E

The hyperbenthos of the Westerschelde estuary: spatial patterns

The hyperbenthos is the association of animals living in the water layer close to the sea bed. It includes all bottom-dependent species and life history stages (mainly crustaceans) which perform, with varying amplitude, intensity and regularity, seasonal or daily vertical migrations above the sea-floor. As functional members of the bottom animal community, they thus constitute the uppermost component of the benthos. The hyperbenthos differs from other ecosystem compartments, both in quantity and quality. In nearly all marine subsystems that have been investigated to date, a general increase in biomass at the benthic boundary layer relative to the water column immediately above it, has been observed. Furthermore, the species composition of the hyperbenthos is distinctly different from that of other benthic and planktonic strata. Besides endemic species that are resident in the near-bottom environment, it also contains elements suggestive of two different origins: (1) species derived from downward extensions, often seasonal in nature, of pelagic planktonic populations, and (2) endo- or epibenthic species emerging into the water column, often in diel cycles. The existence and importance of hyperbenthic communities is now well established, but as hyperbenthic research is still a young discipline, the zone and its fauna remain relatively poorly studied.

Here, spatial patterns in the hyperbenthos of the Westerschelde are described. Samples were taken with a sledge in different seasons at 14 stations along the entire salinity gradient. Mysidacea were shown to dominate the hyperbenthos. Other important species, either permanently (e.g. amphipods and isopods) or temporarily (e.g. fish larvae and decapod larvae) hyperbenthic, belonged to a variety of faunistic groups. Spatial structure was stable through time: the estuary could be divided in the same geographically defined zones in each season. Each zone had a characteristic fauna. Throughout the year, the hyperbenthic community of the mouth region of the estuary was markedly different from that of the upstream brackish area, both in terms of density and species composition. Gradients in salinity, dissolved oxygen and turbidity correlate strongly with the observed variation in community structure. In each season, the upstream (brackish) communities were characterized by few species occurring in very high numbers, whereas the downstream (marine) communities were composed of many species but at lower densities.

Further, the hyperbenthos of the Westerschelde was compared with that of two other, less polluted European estuaries: the Eems (north of the Westerschelde) and the Gironde (south of the Westerschelde). The three estuaries were sampled at regularly spaced stations covering the entire salinity gradient from marine conditions at the mouth to nearly freshwater conditions upstream within a 15 day period in summer. The diversity of the samples and the distribution of the species along the main estuarine gradients were assessed. Again, hyperbenthic communities were identified using different multivariate statistical techniques. The species composition and the density and biomass of the dominant species of each community were compared among communities. Spatial patterns in density, biomass and diversity of the hyperbenthos were similar in the three estuaries: diversity was highest in the marine zone where density and biomass were lowest. Diversity decreased upstream and was lowest in the brackish part where density and biomass reached maximal values. In the Eems and the Gironde there was a slight increase in diversity towards the freshwater zone. Within each estuary two (Westerschelde) or three (Eems and Gironde) communities could be distinguished and their position along the unidirectional salinity-turbidity-temperature gradient was similar: a marine community in the high salinity zone, a brackish water community in the middle reaches and a third community (absent in the Westerschelde) in the stations with the lowest salinities. Qualitative and quantitative differences in the corresponding hyperbenthic communities among estuaries were evident. Some species were restricted to one or two of the estuaries studied, while others, especially the abundant species in the brackish part, were common to all three. Still, these differences were marginal compared to the overriding similarity of the hyperbenthos in the three estuaries. The distribution of single species in the estuaries varied to some extent but the among estuary differences in density and biomass in comparable salinity zones rarely exceeded an order

of magnitude. In the Westerschelde, the low salinity hyperbenthic community was completely absent. Upstream of the 10 psu isohaline the dissolved oxygen concentration dropped to a critical threshold value for hyperbenthic life. The populations of a number of species, which in Gironde and Eems reached highest density and biomass in this zone, seem to have (almost) disappeared from the Westerschelde (e.g. *Gammarus zaddachi* and *Palaemon longirostris*). Other brackish water species did not occur in their "normal" salinity range and their populations have shifted to higher, atypical salinity zones (e.g. *Neomysis integer*, *Mesopodopsis slabberi*, *Pomatoschistus microps* and *Gammarus salinus*).

Articles on the subject produced during the programme :

10. *Mees, J., A. Dewicke & O. Hamerlynck, 1993. Seasonal composition and spatial distribution of hyperbenthic communities along estuarine gradients in the Westerschelde. Netherlands Journal of Aquatic Ecology 27, 359-376.*

11. *Mees, J., N. Fockedey & O. Hamerlynck, 1995. Comparative study of the hyperbenthos of three European estuaries. Hydrobiologia 311, 153-174.*

Task F

The hyperbenthos of the Westerschelde estuary: temporal patterns

Temporal patterns in the hyperbenthos of the Westerschelde estuary are described from year cycles of monthly and forthnightly samples. Throughout the year, the hyperbenthic community of the mouth region of the estuary was markedly different from that of the upstream brackish area, both in terms of density and species composition. In each season, the brackish communities were characterized by few species occurring in high numbers, whereas the marine communities were composed of many species at lower densities. Seasonal patterns in the marine zone were quite pronounced and they were dominated by the recruitment, maximal abundance and subsequent disappearance of temporary hyperbenthic species. In the brackish zone, seasonal patterns were less obvious. Still, spring was characterized by the presence of postlarval flounder and clupeoids and summer by postlarval shrimp, while other seasonal differences seemed to be mainly due to reproduction and natural mortality of endemic species and to migration of marine permanent hyperbenthic species in and out of the area.

The hyperbenthos of two intertidal salt marsh creeks in the brackish basin of the Westerschelde estuary, was also surveyed during eighteen months. Every month a stow net passively sampled the fish and the crustaceans migrating in and out of the marsh creek habitat. A complete description of the fauna of both marshes and their temporal and spatial variability is given. During most seasons the fauna of both marshes were dominated by the mysid shrimps *Neomysis integer* and *Mesopodopsis slabberi*. In spring the communities were typified by high abundances of early juvenile brown shrimp (*Crangon crangon*), and the occurrence of postlarval flounder (*Pleuronectes flesus*) and sole (*Solea solea*). In summer the juveniles of common goby (*Pomatoschistus microps*), seabass (*Dicentrarchus labrax*) and shore crab (*Carcinus maenas*) characterized the communities. Multivariate analyses indicated that the seasonal appearance of these juvenile stages in spring and summer substantially altered the communities. In Saeftinghe, a true brackish marsh, the community appeared to be rather stable and only two different assemblages were identified: one during the winter-spring period (January until May-June) and one during the summer-autumn season (May-June until December). In the more saline marsh of Waarde seasonal changes in species composition were more pronounced, partly due to the appearance of typical marine species. In this marsh the winter-spring community could be divided into a winter-early spring (January-March) and a late spring assemblage (April-June). Despite the difference in the salinity regime between both marshes, their nekton communities remained quite similar. The spatial difference between the two sites converged towards the winter months. Apart form the salinity factor, the position of the marshes along the estuarine channel possibly plays an important role in the species specific accessibility of the marsh habitat, and may accordingly cause a difference in species composition.

Further, the hypothesis is defended that brown shrimp *Crangon crangon* migrate into the brackish part of the Westerschelde estuary shortly after metamorphosis and use the tidal marsh habitat as a nursery until they reach a length of about 10 mm. The importance of the marsh as a nursery was evaluated by estimating foraging activity, predation mortality and residence time. Early postlarval stages of *C. crangon* utilised the creeks of an estuarine tidal marsh from early spring (March-April) until late autumn (October-November). Postlarval shrimp leaving the marsh with the ebb tide always had significantly more food in their stomachs than shrimp entering the marsh with the incoming flood water. The predation pressure on the shrimp population was relatively low during most months, but it increased between August and October when seabass *Dicentrarchus labrax* and common goby *Pomatoschistus microps* occurred with high densities. The marsh creeks thus function both as foraging areas and as predation refugia. Depending on environmental temperature, the postlarval shrimp stayed in the marsh for a period of 2 to 3 weeks. Quantitatively, the value of the marsh as a nursery area changed drastically during a second year of sampling. Recruitment to the subtidal adult population represents an export of animals from the marsh to the estuary. This export is negligible in terms of biomass as compared to the total biomass of the estuarine population, but it may be important in terms of numbers of individuals.

Articles on the subject produced during the programme :

12. *Cattrijsse, A., J. Mees & H. R. Dankwa, submitted. Nursery function of an estuarine tidal marsh for the brown shrimp Crangon crangon..*
13. *Cattrijsse, A., J. Mees, K. Hostens & E.S. Makwaia, in press. The aquatic fauna of two intertidal salt marsh creeks in the Westerschelde estuary. Belgian Journal of Zoology.*
14. *Mees, J., A. Dewicke, A. Cattrijsse & N. Fockedey, in press. Seasonality in hyperbenthic communities of the Westerschelde estuary. Belgian Journal of Zoology.*

Task G

Spatial patterns in the hyperbenthos of the Belgian continental shelf

The hyperbenthos of the Belgian coastal waters, the Westerschelde estuary and part of the Dutch delta was sampled within a 2-week period at 41 locations in the summer of 1993. At each location sampling was done at two depth strata, covering all major sandbanks in the area, and the gullies separating them. Sampling depth ranged from 6 to 40 meter.

The samples were taken with a hyperbenthic sledge equipped with four nets (1 mm and 0.5 mm mesh size), covering two lower strata of the watercolumn: 0 to 0.5 meter and 0.5 to 1 meter above the bottom. Trawling (5 minutes at 1.5 knots: approx. 200 meter per trawl) was always done during daytime and against the tide. The sledge was equipped with an automatic opening-closing device, an odometer and a current meter. Several environmental variables (salinity, temperature,...) were recorded at the beginning of each trawl. In addition, sediment and water samples were taken at each station for grain size distribution, nutrient and pigment analyses.

In the laboratory, all animals were identified to species level, counted and measured. Density (numbers per 100 m^2) was calculated and biomass was derived from length-ashfree-dryweight regressions. Both density and biomass data were subjected to 3 multivariate statistical analyses: a classification (group-average sorting clustering based on the Bray-Curtis dissimilarity index), an ordination (Canonical Correspondance Analysis) and a hybrid technique (Two-way Indicator Species Analysis).

A total of 135 species were recorded in the hyperbenthic fauna of the Belgian continental shelf. The most abundant faunistic taxa included Mysidacea (10 species), Brachyura (20 species), Amphipoda (33 species) and Caridea (14 species). Chaetognatha, Pisces, Anomura, Polychaeta, Isopoda, Copepoda and Cumacea were caught to a lesser extent. The hyperbenthos of the Belgian coastal area could be divided into 6 geographically defined communities, each characterised by a specific species composition, diversity, density and biomass. In the eastern part of the Westerschelde, a typical brackish water community was found (community 'ws'). Two communities were found onshore: community 'onshore east' was located on the east coast (between Oostende and Zeebrugge) and included the marine part of the Westerschelde estuary; community 'onshore west' was found on the west coast (between Oostende and De Panne). Communities 'mid bkp' and 'mid vd' are transitional communities between the two onshore communities and more offshore area. The 'offshore' community was found towards the open sea.

The community structure changed sharply along a gradient perpendicular to the coastline: highest densities and biomasses were recorded onshore. The onshore communities (and the 'ws' community) were characterised by a low diversity and consisted mainly of Mysidacea. Diversity increased in offshore direction and the species composition of the hyperbenthic fauna changed drastically: morefaunistic groups became almost equally represented.

Articles on the subject produced during the programme :

15. Dewicke, A. & J. Mees, 1996. The hyperbenthic fauna of the Belgian continental shelf: spatial variability in community structure. Progress in Belgian Oceanographic Research 1996: 55-58.

16. Dewicke, A. & J. Mees, in preparation. Spatial patterns in the hyperbenthos of the Belgian continental shelf.

Task H

Temporal patterns in the hyperbenthos of the Belgian continental shelf

The hyperbenthic fauna of the Belgian coastal area was sampled monthly at 15 stations from may 1994 through december 1995.

For this report, the hyperbenthos of 4 stations in the Belgian coastal waters was investigated. Three of these were rather situated onshore, spread along the coastline: station 115s on the east coast near De Panne, station 120 near Nieuwpoort and station 140 on the west coast near Zeebrugge. One site, station 330, was located more offshore.

The samples were taken with a hyperbenthic sledge equipped with four nets (1 mm and 0.5 mm mesh size), covering two lower strata of the watercolumn: 0 to 0.5 meter and 0.5 to 1 meter above the bottom. Trawling (5 minutes at 1.5 knots: approx. 200 meter per trawl) was always done during daytime and against the tide. The sledge was equipped with an automatic opening-closing device, an odometer and a current meter. Several environmental variables (salinity, temperature,...) were recorded at the beginning of each trawl. In addition, sediment and water samples were taken at each station for grain size distribution, nutrient and pigment analyses.

In the laboratory, all animals were identified to species level, counted and measured. Density (numbers per 100 m^2) was calculated and biomass was derived from length-ashfree-dryweight regressions. Density data were subjected to 3 multivariate statistical analyses: a classification (group-average sorting clustering based on the Bray-Curtis dissimilarity index), an ordination (Canonical Correspondance Analysis) and a hybrid technique (Two-way Indicator Species Analysis).

A total of 125 species were recorded in the hyperbenthic fauna of the Belgian coastal area. A strong seasonal variation in density was apparent for several species. The species composition of the hyperbenthic fauna was seasonally altered by the recruitment and disappearence of different temporary hyperbenthic species. The most important members for the permanenthyperbenthos were mysids.

Multivariate analyses yielded more insight into community structure. A discontinuity between onshore and offshore samples was obvious. Temporal patterns strongly dominated the onshore communities (stations 115s, 120 and 140). Three communities could be identified: the first one occurred during winter, the second one during spring and the other community lasted from summer until autumn. Offshore, this seasonal trend wass less pronounced: during the whole year the fauna of station 330 resembled the spring fauna of the onshore stations.

Total density was comparable for the different communities. The winter community was the least diverse and consisted mainly of permanent hyperbenthic species (mysids and amphipods) and the already fairly abundant pelagic fish eggs. A high variety of temporary representatives characterised the spring community: polychaete larvae, larval decapods, and postlarval fish appeared in high densities. Mysids were again dominant in the 'summer-autumn' community, supplemented by considerable densities of temporaryhyperbenthic species.

Articles on the subject produced during the programme :

17. Dewicke, A. & J.Mees, in preparation. Temporal patterns in thehyperbenthos of the Belgian continental shelf.

Task I

Population dynamics and feeding of the hyperbenthic mysid Neomysis integer

1. Population dynamics in the field

The population dynamics of the key species of the Westerschelde hyperbenthos, the mysid *Neomysis integer* was investigated from data collected in the field. The population was sampled on a fortnightly basis from November 1990 to December 1991. Density, biomass, population structure and brood size were recorded. The Bhattacharya method was applied to the length-frequency data for the detection and separation of cohorts. Growth is described both by a generalised von Bertalanffy function and by a von Bertalanffy function incorporating seasonal oscillations in growth. Secondary production was estimated for each cohort using four approaches. The seasonal pattern in density and biomass showed three peaks: a relatively small, yet distinct, peak in early March and two main peaks in late spring and in summer. Throughout winter, *Neomysis* density remained low. Three periods of increased reproductive activity and subsequent input of juveniles were found. This suggests that three cohorts were produced per year. The overwintering generation lived from autumn until the following spring. The spring generation was born in early spring and lived for about three months, while the summer generation lived from summer until early winter. The three cohorts showed marked differences in their biology. The overwintering generation showed seasonal growth oscillations, larger brood size, and a larger size at maturity. Individuals belonging to the other two cohorts generally grew faster, produced less young per female, and attained maturity at a smaller size. Within each cohort, both sexes exhibited different growth characteristics: the females generally lived longer, grew faster and consequently became larger than the males. The size-frequency, growth summation and removal summation methods yielded comparable production estimates. The annual production was 0.3 g AFDW m^{-2} yr^{-1} with an annual P/B ratio of 6. The average cohort P/B was 3. The size-frequency method gave similar results only when applied to the three cohorts and to both sexes separately. The spring cohort accounted for almost half of the annual production. Despite the longer life span of the overwintering generation, it generated only a quarter of the annual production. An independent estimate of production using the mortality rate of the different cohorts, resulted in values comparable to those obtained by the other methods for the overwintering cohort, while the production of the other two cohorts was overestimated. We further reported on intersexuality in the mysid *Neomysis integer* collected in the 3 estuaries mentioned above, supplemented with samples from the Elbe (Germany). Individuals which had an irregularly shaped or nearly symmetrically rounded, rather than a typically truncated, telson were also recorded from the four populations studied. A culture experiment with damaged specimens revealed that every type of abnormal telson morphology found in the field can result from regeneration of damaged parts. It is concluded that both intersexuality and aberrant telson morphology are widespread phenomena among estuarine *Neomysis* populations. Both abnormalities were found to be rare in the other dominant mysid species in the study area.

2. Laboratory growth experiments

The life history characteristics of a species depend to a certain extent on the characteristics of its habitat. The combined effects of temperature, salinity, and quality and quantity of available food are likely to influence the growth parameters of brackish water species. The aim of this study was to determine the influence of a broad range of salinity-temperature combinations on growth parameters, moulting frequency and sexual development of the mysid *Neomysis integer*. *N. integer* was cultured in the laboratory and the offspring of the cultured animals was used in 'longterm' growth experiments.

Individual *N. integer* were followed from birth (immediately after hatching from the marsupium) until they reached asymptotic length (maximum duration of the experiments was 4 months). They were kept at several constant temperature-salinity conditions: at 15 °C salinity was 1, 5, 15 and 30 psu; at 8, 20 and 25 °C salinity was 5 psu. They were fed freshly hatched *Artemia* nauplii *ad libidum*. Growth was studied by collecting and measuring the moults. The total length of the exuvia could not be measured (too elastic). Hard appendages (antennal scale, uropod, telson) were measured and converted to standard length with allometric linear regressions. The growth of *Neomysis integer* was described by logistic functions. Within each treatment individual variability was small. Absolute or incremental growth rates ($\Delta L/\Delta t$) fluctuated nonlinearly with size and age, and were maximal around the inflection point (t_0). The developmental time before reaching the inflection point increased with increasing salinity and decreased with increasing temperature.

3. Feeding ecology of *Neomysis integer*

The diet of the mysid *Neomysis integer* in the maximum turbidity zone (MTZ) of three European estuaries (Elbe, Westerschelde and Gironde) was investigated in spring 1993. The quality and quantity of the diet were assessed through measurement of the stomach fullness and microscopical analysis of the stomach content combined with image analyses. *N. integer* was found to be an omnivore which mainly utilizes mesozooplankton and detritus carbon pools. The quality of the diet did not differ between the sexes nor between different developmental stages, although smaller individuals consumed fewer items. In all three estuaries the diet was dominated by Copepoda Calanoida (5-10 *Eurytemora affinis* ind^{-1} for adults; 2-5 ind^{-1} and 2-3 ind^{-1} for subadults and juveniles, respectively) and was supplemented with Rotifera and Cladocera. Phytoplankton and benthic organisms, though present in the stomachs, were negligible. Macrophytal detritus and amorphous material, the latter unidentifiable under the light microscope, were very abundant food items. The amorphous detritus was found to originate from the suspended sediment flocs which are characteristic for the MTZ. These mainly consist of clay minerals, as shown by EDAX-analysis. The energetic value of the flocs for *N. integer* remains unclear.

Articles produced on the subject during the programme :

18. *Fockedey, N. & Mees, J. (1996). The diet of Neomysis integer (Crustacea, Mysidacea) in the maximum turbidity zone of estuaries. Royal Acad. Belgium. Nat. Comm. Oceanol. Progr. Belgian Oceanogr. Res.: 79-82.*
19. *Fockedey, N. & Mees, J. (submitted). Feeding of the hyperbenthic mysid Neomysis integer in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries. J. Mar. Syst.*
20. *Mees, J., Abdulkerim, Z. & Hamerlynck, O. (1994). Life history and production of Neomysis integer in the Westerschelde estuary (SW Netherlands). Mar. Ecol. Prog. Ser., 109: 43-57.*
21. *Mees, J., Fockedey, N., Dewicke, A., Janssen, C.R. & Sorbe, J.C. (1995). Aberrant individuals of Neomysis integer and other mysidacea: intersexuality and variable telson morphology. Neth. J. Aquat. Ecol., 29: 161-166.*
22. *Fockedey, N. & J. Mees. In prep. Laboratory studies on growth and developent of the brackish water mysid Neomysis integer.*

Task J

Anthropogenic influences on the benthos in the Westerschelde and the North Sea

The life in the sediments (i.e. the benthos) in the North Sea and the Westerschelde estuary has not been a large public concern in the past. It was thought that the sea was one big reservoir where all material could be dumped without too much damage because most substances are dispersed and disappear quickly out of the view (and most likely into the bottom).
Within the programme, the meiobenthic and the hyperbenthic components have been studied in detail. Within marine and estuarine sediments, meiobenthos (i.c. nematodes) are the dominant metazoans in terms of both density and diversity. The link with the pelagic is very much accomplished by means of the hyperbenthos (the association of animals living in the water layer close to the sea bed).

Following **NEW INSIGHTS IN BENTHIC ECOLOGY** in the North Sea and Westerschelde have been obtained :

- The **INTERTIDAL AREAS** in the Westerschelde are by far much richer in meiobenthic life than the permanently submersed areas, and therefore more **VULNERABLE**. Diversity of the meiofauna is more related to sedimentological characteristics than to salinity. Extreme low densities in the Maximum Turbidity Zone (upstream Saefthinghe) can probably be explained by the presence of polluted sediments (organic and inorganic pollutants in combination with low oxygen concentration), indicating strong anthropogenic influences.

- The development of a **CARBON MODEL** for nematodes in the Westerschelde predicts that 75% of the nematode carbon production (which is calculated as 22mgC/m².day) in estuarine sediments returns to the ecosystem (as faeces) and that 20% of the carbon can be consumed by higher trophic levels (i.e. macrobenthos and juvenile fish).

- Improvements of methodologies to investigate the **MICROBIAL LOOP** within sediments (by measuring meiofauna grazing on bacteria and microalgae) are proposed (although a throroughly reliable method for grazing experiments on sediment samples is still not in sight). A dynamic classification in six major feeding guilds is proposed for estuarine nematodes. It is stressed that nematodes are often very opportunistic feeders, but that on the other hand species-specific nematode-food interactions exist.

- Following anthropogenic influences on the species diversity of the nematode communities in the coastal sediments of the North Sea can be detected : in early summer, after an increased sedimentation of nutrients in the coarser sediments of the west coast, the reduced sediment is shifted to the surface layers and is obviously influencing the distribution of the nematode community. Bacterial feeders are more linked to the availability of bacteria in the muddy sediments than to the oxygen availability within these sediments. These data are a first step in understanding the role of nematodes in the microbial loop within the sediments. **INDICATOR SPECIES** like *Sabatieria punctata* and *Daptonema tenuispiculum* and indicator values like diversity of the nematode community include important information about the eutrophication level of the sediments and are recommended as **BIOMONITORING** tool.

- The hyperbenthic communities of the **WESTERSCHELDE** are characterized by typical estuarine communities which are stable along the salinity gradient, but with one important feature, compared to e.g. the Gironde and Eems : the **ABSENCE OF THE LOW SALINITY COMMUNITY** (upstream of 10 psu) in the Westerschelde coincides with the drop of dissolved oxygen below a critical treshold. In normal conditions, this would be the most productive area of the estuary. The lack of hyperbenthos within this area put a severe restriction on the distribution of fish in the low salinity part of the Westerschelde (hyperbenthos being a very important food source for fish). The recovery of the Westerschelde in terms of reduction of organic input would lead to an increase in the oxygen level. Comparison with other European estuaries predict that the hyperbenthos and fish will follow a positive evolution in terms of density and diversity (species richness) after a reduction of the organic input. Recent investigations confirm this trend. The population dynamics of a dominant hyperbenthic species, *Neomysis integer*, together with investigations of the diet, indicate the important link with the zooplankton. Hyperbenthos is critical in the **BENTHO-PELAGIC COUPLING**, certainly in esturaine systems.

- The spatial and temporal patterns of the hyperbenthos of the Belgian continental shelf are characterised by increasing diversity in offshore direction with a clear gradient perpendicular to the Belgian coast but with the highest densities and biomasses onshore. In some months, extreme high numbers of eggs and larvae from commercially important fish and shelfish are recorded within the hyperbenthos. The information on the spatial and temporal distribution of these vulnerable young stages is important for the **MANAGEMENTS OF THE FISH STOCKS.** The potential feeding-grounds for adult fish (a lot of demersal fish feed on benthic animals) are indicated and should be monitored with caution within the future.

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Structure and Function of the Benthos in Estuarine and Coastal Ecosystems in relation to actual and future Anthropogenic Impacts

Final Report

PART II.

Publications 1 - 9
Meiobenthos

composed by

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October 1996

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Table of Contents

PART I. : Summary (task A - task J)

PART II. : Publications 1-9 pags : 1 - 147

PART III : Publications 10-22 pags : 148 - 396

List of publications :

1. Li Jian & M. Vincx. 1993. The temporal variation of intertidal nematodes in the Westerschelde. The importance of an estuarine gradient. Neth.J. Aquatic Ecol., 27 (2-4), 319-326. 1

2. Li Jian, M. Vincx & P.M.J. Herman. 1996. Carbon flows through meiobenthic nematodes in theWesterschelde estuary. Fundamental and Applied Nematology., in press 9

3. Soetaert, K., M. Vincx, J. Wittoeck, M. Tulkens & D.Van Gansbeke. 1994. Spatial Patterns of Westerschelde meiobenthos. Estuarine, Coastal and Shelf Science, 39, 367-388. 26

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5. Moens, T., A. Vierstraete, S. Vanhove, M. Verbeke & M. Vincx. 1996. A handy method for measuring meiobenthic respiration. J; Exp. Mar. Biol. Ecol., 197, 177-190 70

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(pags 188 - 200 missing in numbering)

12.Cattrijsse, A., J. Mees & H. R. Dankwa, submitted. Nursery function of an estuarine tidal marsh for the brown shrimp *Crangon crangon*.. 201

13.Cattrijsse, A., J. Mees, K. Hostens & E.S. Makwaia, in press. The aquatic fauna of two intertidal salt marsh creeks in the Westerschelde estuary. Belgian Journal of Zoology. 230

14.Mees, J., A. Dewicke, A. Cattrijsse & N. Fockedey, in press. Seasonality in hyperbenthic communities of the Westerschelde estuary. Belgian Journal of Zoology. 250

15.Dewicke, A. & J. Mees, 1996. The hyperbenthic fauna of the Belgian continental shelf: spatial variability in community structure. Progress in Belgian Oceanographic Research 1996: 55-58. 276

16.Dewicke, A. & J. Mees, in preparation. Spatial patterns in the hyperbenthos of the Belgian continental shelf. 280

17.Dewicke, A. & J. Mees, in preparation. Temporal patterns in the hyperbenthos of the Belgian continental shelf. 306

18.Fockedey, N. & Mees, J. (1996). The diet of *Neomysis integer* (Crustacea, Mysidacea) in the maximum turbidity zone of estuaries. Royal Acad. Belgium. Nat. Comm. Oceanol. Progr. Belgian Oceanogr. Res.: 79-82. 329

19.Fockedey, N. & Mees, J. (submitted). Feeding of the hyperbenthic mysid Neomysis integer in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries. J. Mar. Syst. 333

20.Mees, J., Abdulkerim, Z. & Hamerlynck, O. (1994). Life history and production of *Neomysis integer* in the Westerschelde estuary (SW Netherlands). Mar. Ecol. Prog. Ser., 109: 43-57. 361

21.Mees, J., Fockedey, N., Dewicke, A., Janssen, C.R. & Sorbe, J.C. (1995). Aberrant individuals of *Neomysis integer* and other mysidacea: intersexuality and variable telson morphology. Neth. J. Aquat. Ecol., 29: 161-166 376

22.Fockedey, N. & J. Mees. In prep. Laboratory studies on growth and development of the brackish water mysid *Neomysis integer*. 382

NETHERLANDS JOURNAL OF AQUATIC ECOLOGY 27(2-4) 319-326 (1993)

THE TEMPORAL VARIATION OF INTERTIDAL NEMATODES IN THE WESTERSCHELDE I. THE IMPORTANCE OF AN ESTUARINE GRADIENT

JIAN LI and MAGDA VINCX

KEYWORDS: temporal variation; nematodes; feeding type; estuarine gradient.

ABSTRACT

Biannual meiobenthic sampling (Spring and Autumn) was carried out in 1983-1989 at two fine sandy intertidal stations in the Westerschelde estuary. Both stations are exposed daily for more than one hour and are situated in the polyhaline and the mesohaline zone of the estuary.

Average density data of non-selective deposit-feeders > predators > epigrowth-feeders > selective deposit-feeders for both stations are presented in spite of different nematode species composition. No difference between Spring and Autumn nor trend over 7 years could be detected.

Higher total nematode densities are found at the polyhaline station (average 3200 ind. 10 cm^{-2}) in comparison with the mesohaline station (average 2300 ind. 10 cm^{-2}), a difference mainly due to higher non-selective deposit-feeders and predators densities in the polyhaline station. Each year, heterogeneous variance is found for all feeding types at the mesohaline station, but only for epigrowth-feeders and predators at the polyhaline station. The higher nematode density at the polyhaline station is probably caused by the more stable nematode structure. An unstable nematode temporal pattern at the mesohaline station is suggested to be combined with the detritus food chain system in the mesohaline zone. The unstable estuarine habitats are mainly caused by their upstream effects: the River Schelde, which clearly influences the stability of the nematode communities.

INTRODUCTION

The structural characteristics (density, biomass and species composition) of estuarine and brackish water nematodes in relation to salinity are well documented (for a review see HEIP *et al.*, 1985). Studies which investigate generation time and reproductive potential of nematodes (TIETJEN and LEE, 1972) in relation to salinity are less numerous. Most time series studies of nematodes have a low temporal resolution. Seasonal cycles of nematodes can be very different from site to site according to different local environmental conditions and depending on the species composition (see HEIP *et al.*, 1985 for a review). The abundance peak of dominant species can be present in Spring, Summer, Autumn or Winter. Great variability from year to year was found for nematode abundance (MCINTYRE and MURISON, 1973) and sometimes this variability was even much larger than the seasonal variability (COULL, 1985). Even for separate species, several irregular seasonal patterns can be found (WIESER, 1977; VINCX, 1989). COULL (1985) showed that nematode abundance had a negative correlation with salinity in mud sites and a positive correlation with temperature in sand site (11 years data). However, temporal variability of nematodes for intertidal areas is not well known, especially in relation to estuarine gradients.

The Westerschelde is a completely mixed estuary (HEIP, 1989) with a long water residence time (75 days or 150 tides). The Westerschelde is

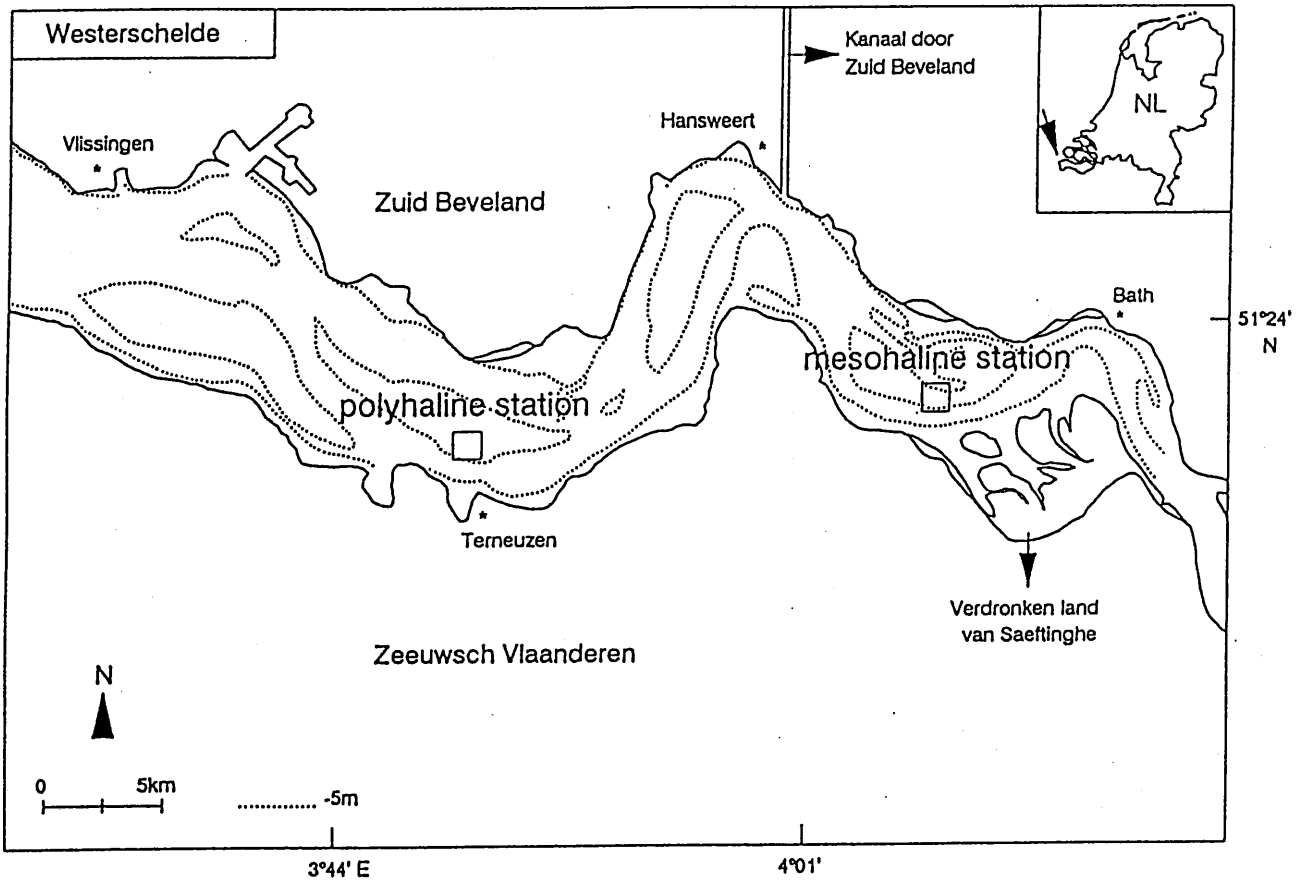

Fig. 1. Location of the sampling stations in the Westerschelde.

Table 1. General characteristics of the two stations in the Westerschelde. Md = median particle size of sediment. Elevation is given in m above mean sea level.

station	mesohaline	polyhaline
position	51°21′42″N 04°05′39″E	51°21′33″N 03°49′12″E
salinity ‰	6.1-21.0	24.3-32.0
Md (1979/1990) µm	127/133	138/167
mud % (1979-1986)	4-7	3-5
O.M. % (1979)	3.2	4.9
TOC (1979) mg l^{-1}	5.1-15.4	6.1-12.0
elevation (1979/1987) m	+1.0/+1.5	+0.5/+0.5

partitioned into 3 zones based on abiotic and biotic gradients (HUMMEL *et al.*, 1988): a marine zone (from mouth to 36 km), a brackish zone (from 36 km to 78 km) and a fresh water tidal zone (after 78 km).

The ecological study of the meiobenthos in the Westerschelde has been mainly restricted to the general spatial distribution (VAN DAMME *et al.*, 1980). Nematodes comprises more than 95% of density and 85% of biomass of the total meiobenthos. Nematode density decreases with decreasing salinity. The knowledge of the temporal distribution of meiobenthos in the Westerschelde is restricted to samples (5 times) from one year (VAN DAMME *et al.*, 1980).

This study investigates how the temporal variability in nematode abundances is influenced by different salinity regimes in both sampling stations. Special attention will be given to the temporal variability of the different feeding types of the nematode communities.

MATERIAL AND METHODS

From 1983 to 1989, biannual meiobenthic samples were taken in March (February in 1988 and April in 1983) and September (October in 1989) in two intertidal stations in the Westerschelde estuary. The two stations (polyhaline – mesohaline) have similar sediments (fine sand and low organic matter) but are situated in different salinity zones (Fig.1, Table 1). Organic matter (O.M.) in the sediment was determined according to WAKEEL and RILEY (1956), organic carbon (TOC) according to PARSONS *et al.* (1984). Mud is defined as the fraction <63 µm.

Sampling occurred at low tide, and within a

circle of < 10 m diameter from the fixed point (except the Sept. 1983 samples which were 100 m away and Sept. 1986 700 m away, in the polyhaline station; the one of Sept. 1984 is 150 m away in the mesohaline station).

Two replicate core samples (diameter = 3.56 cm, sampling depth ≈ 10 cm) were taken at both stations. Fixation, decantation and the Ludox centrifugation-flotation technique (HEIP *et al.*, 1985) were used to extract the nematodes.

The main meiobenthic group, the nematodes, were identified to the genus level and classified into four feeding types (WIESER, 1953): 1A = selective deposit-feeders, 1B = non-selective deposit-feeders, 2A = epigrowth-feeders and 2B = predators/omnivores. Every replicate sample was subsampled to identify at least 10% (or a minimum of 120 individuals) of the total density of nematodes).

The 3-way ANOVA test after p >0.1 for Bartlett's test was used to test for differences in nematode abundances between stations, for each year and between the two seasons.

A simple multiplicative decomposition method (BOWERMAN and RICHARD, 1979) and a similar additive decomposition method were used for calculating linear trends and seasonal fluctuations of the time series. In the multiplicative model, the observed series Y(t) is considered as the multiplicative sum of a linear trend series TR(t), a seasonal series SN(t), a cyclical series CL(t) and an irregular series IR(t): $Y(t) = TR(t)*SN(t)*CL(t)*IR(t)$. After calculating the 1-year moving average of the series Y(t), the seasonal effects were omitted and results in a second series $TR(t)*CL(t)$ (in this study, $TR(1)*CL(1) = (Y(1)+Y(2))/2$, $TR(2)*CL(2) = (Y(2)+Y(3))/2$, ..., $TR(t-1)*CL(t-1) = (Y(t-1)+Y(t))/2$). The slope of the trend comes from the linear regression of the second series. The seasonal fluctuation, therefore, is the average fluctuation of the third series $SN(t)*IR(t) = Y(t)/(TR(t)*CL(t))$. In the additive model, the observed series Y(t) is considered as the additive sum: $Y(t) = TR(t)+SN(t)+CL(t)+IR(t)$. A 1-year moving average of the series Y(t) results in a second series $TR(t)+CL(t)$. The slope of the trend comes from the linear regression of second series. The seasonal fluctuation is the average fluctuation of the third series $SN(t)+IR(t) = Y(t)-(TR(t)+CL(t))$.

The correlogram is constructed from the autocorrelation values at different lags k. The autocorrelation at lag k is the correlation coefficient between the series Y(1), Y(2),...Y(N-k) and the series Y(k), Y(k+1),...Y(N) ($k \geq 0$, $k < N/4$). In this study, lag (k) = 1, 2, 3 means lag (year) = 0.5, 1, 1.5. To avoid the influence of linear trend, TR(t) is extracted from Y(t) in this study.

RESULTS

The meiobenthos of the two stations of the Westerschelde consisted of 96.4% Nematoda, 1.9 % Annelids (Polychaeta and Oligochaeta), 0.9% Arthropods (Copepoda, Amphipoda, Ostracoda, Isopoda and Cumacea) and 0.8% others including Cnidaria (Hydrozoa), Platyhelminthes (Turbellaria), Gastrotricha, Mollusca (Gastropoda and Bivalvia), Priapulida and Tardigrada (Table 2).

Nematode densities

Total nematode density over seven years (Fig. 2) showed differences ($p < 0.001$ between stations by 3-way ANOVA test) between the polyhaline and the mesohaline station; the second station had a lower density (on average about 1000 ind. 10 cm^{-2} less) but a higher coefficient of variation (Table 2). However, no significant diffe-

Table 2. Nematode density (ind. 10 cm^{-2}), coefficient of variation (c.v.) and relative abundance (%) of main meiobenthic groups at two stations in the Westerschelde. Between brackets: min. and max. values.

	mesohaline station	polyhaline station
nematode density	2268 (200-5176)	3240 (1740-5328)
c.v. (%)	670	226
nematodes (%)	94.9 (83.0-99.4)	97.9 (94.3-99.7)
annelids (%)	3.0 (0-9.3)	0.8 (0-4.6)
arthropods (%)	1.4 (0-6.0)	0.5 (0-2.3)

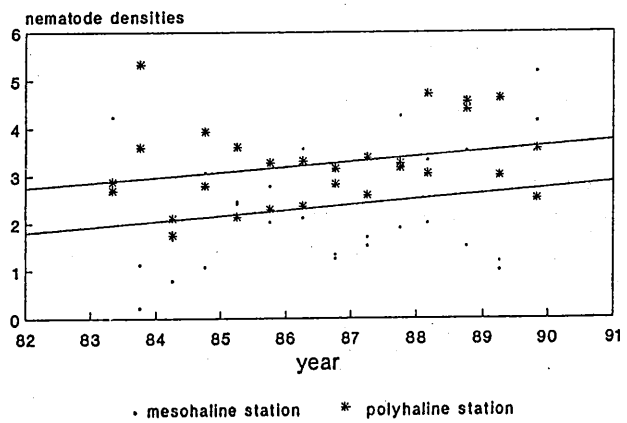

Fig. 2. The temporal variation of nematode densities and their trends over seven years from two stations. (Densities = 1000 ind. 10 cm^{-2}).

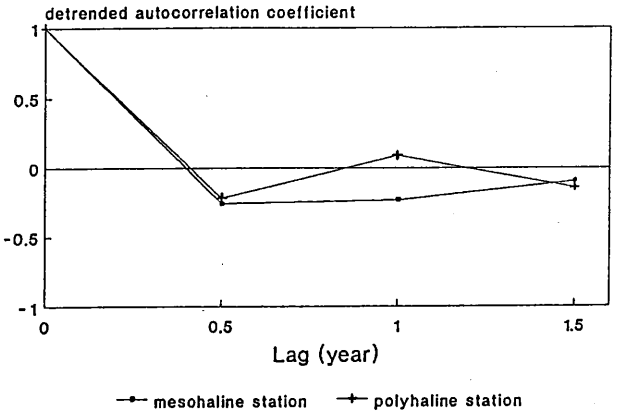

Fig. 3. Autocorrelogram of nematode densities after extraction of linear trends.

Table 3. Slope and seasonal change of the nematode densities (ind. 10 cm^{-2}) from multiplicative and additive model. Percentage seasonal change as in BOWERMAN and RICHARD (1997).

	mesohaline station		polyhaline station	
	multipl. model	additive model	multipl. model	additive model
slope	+ 120	+ 116	+ 106	+ 94
seasonal change	± 0.2%	± 31	± 8%	± 240

Table 4. Average density (min.-max.) (ind. 10 cm^{-2}) average relative abundance of total nematodes (%), and coefficient of variation (c.v.; %) of the nematode feeding types from all the single sample data over seven years.

	mesohaline station			polyhaline station		
	density	%	c.v.	density	%	c.v.
selective deposit-feeders	211 (9-1271)	9	282	187 (22-352)	6	36
non-selective deposit-feeders	804 (23-3439)	36	598	1301 (226-3038)	41	230
epigrowth -feeders	564 (29-2202)	25	641	450 (86-1001)	14	111
predators	681 (119-2893)	30	576	1221 (429-4026)	39	474

Table 5. The probability of homogeneous variances (Bartlett's test) among 7 years for the densities of total nematodes and the four feeding types.

	mesohaline station	polyhaline station
total nematodes	p >0.1	p >0.5
selective deposit-feeders	p <0.005	p >0.5
non-selective deposit-feeders	p <0.05	p > 0.1
epigrowth-feeders	p <0.005	p <0.05
predators	p >0.001	p <0.05

rence was found between the variances at the two stations (p >0.05).

In both stations, a similar nematode density trend (p <0.05 among years by 3-way ANOVA test) was not revealed by Kendall's τ (p >0.05), although the slopes of the trends in both stations are around +100 ind. 10 $cm^{-2}y^{-1}$ (both from the multiplicative and the additive model) (Table 3).

The seasonal fluctuations between Spring and Autumn are not clear (p >0.1 among months by 3-way ANOVA test). Autocorrelation coefficients were negative or very low positive (Fig. 3). The seasonal change calculated from the multiplicative and the additive model (Table 3) indicates a low fluctuation: from the additive model, a density fluctuation of ±31 for the mesohaline station and of ±240 for the polyhaline station is low compared with the total density which is 2300 ind. 10 cm^{-2} for the mesohaline station and 3200 ind. 10 cm^{-2} for the polyhaline station; the fluctuations were thus only 1.4% or 7.5% of the total density; they were near to the results of multiplicative model.

Nematode feeding type

The average densities of the four feeding types followed the same ranking in the two stations: the non-selective deposit-feeders were the most dominant group followed by predators, epigrowth-feeders and selective deposit-feeders (Table 4; see also the average density ratio). However, the fluctuation of the density of the four feeding types differed remarkably between the two stations, also when compared with the total nematode density.

First, there were higher densities of non-selective deposit-feeders (p <0.005) and predators (p <0.001) (about 500 ind. 10 cm^{-2} higher for each) and lower coefficients of variation for all feeding groups in the polyhaline station compared with the mesohaline station (Table 4). But significant differences of variance between the two stations (Bartlett's test) were found for selective deposit-feeders and epigrowth-feeders (both p <0.001) not for non-selective deposit-feeders and predators (both p >0.1).

Secondly, the determination of the homogeneity of variances for the seven years data within each station separately (Bartlett's test) shows (Table 5) significant heterogeneous variances for all four feeding types in the mesohaline stations, but only for the epigrowth-feeders and predators in the polyhaline station.

Finally, the distributions of feeding types (Figs. 4, 5) showed more heterogeneous peaks (>60% of total nematodes average densities) in the mesohaline station compared with the polyhaline

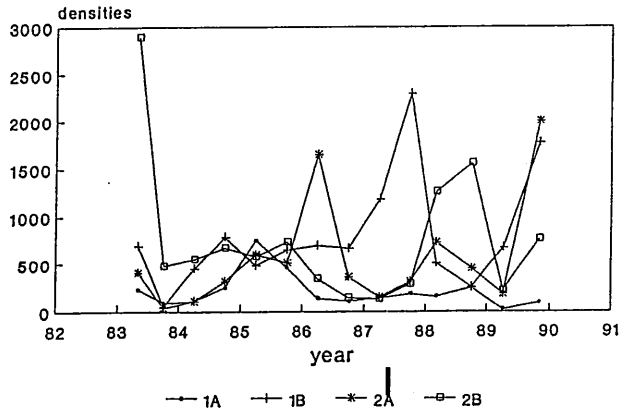

Fig. 4. The temporal density variation of four nematode feeding types over seven years at the mesohaline station (densities = ind. 10 cm^{-2}; 1A = selective deposit-feeders, 1B = non-selective deposit-feeders, 2A = epigrowth-feeders and 2B = predators).

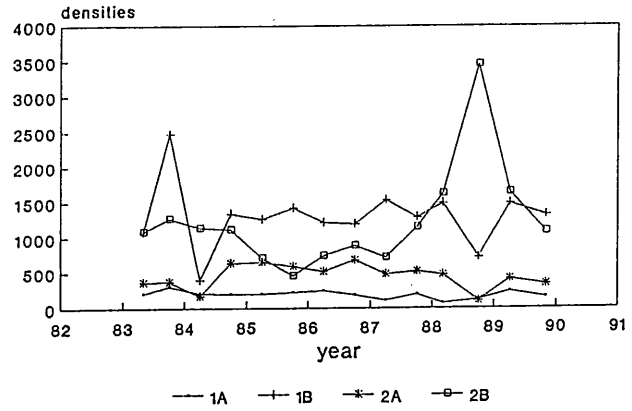

Fig. 5. The temporal density variation of four nematode feeding types over seven years at the polyhaline station. Densities = ind. 10 cm^{-2}.

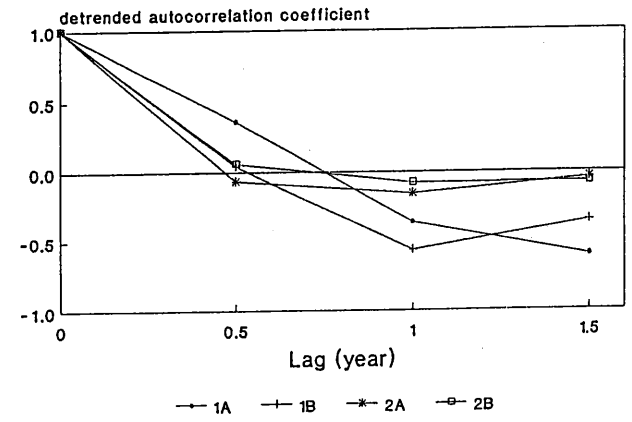

Fig. 6. The autocorrelogram of four feeding types densities at the mesohaline station after extraction of linear trends.

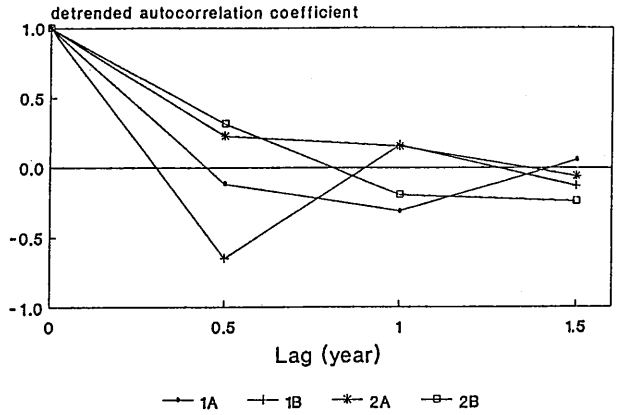

Fig. 7. The autocorrelogram of four feeding types densities at the polyhaline station after extraction of linear trends.

station over the seven years: in the polyhaline station only, one non-selective deposit-feeders peak (1983) and one predators' peak (1988) is present in Autumn; in the mesohaline station, two non-selective deposit-feeders peaks (1987 and 1989) were found in Autumn, two predator peaks (1983 and 1988) in Autumn and Spring and two epigrowth-feeder peaks (1986 and 1989) in Spring and Autumn.

However, no seasonal differences in the abundance of the four feeding types were found in both stations ($p > 0.1$ for each one). Also, the autocorrelograms (Fig. 6 and 7) showed that a positive autocorrelation coefficient at a lag of one year, is only found for selective deposit-feeders and epigrowth-feeders at the polyhaline station. These autocorrelation coefficients were however low and not significant ($p > 0.05$). No positive autocorrelation coefficient was found for the four feeding types at the mesohaline station.

Nematode composition

The number of genera of the nematode community for the two stations was very similar: 32 genera for the polyhaline station and 31 genera for the mesohaline station. But the dominant nematode genera and the composition of feeding types were different (Table 6). In the polyhaline station, there were only 4 genera with a maximal density >20 % in a single sample; in the mesohaline station, 12 such genera were present. Both stations had 3 genera with average density >10%: *Daptonema*, *Theristus* and *Dichromadora* in the mesohaline station and *Daptonema*, *Viscosia* and *Metachromadora* in the polyhaline station.

The genus composition of the different peaks of the abundance patterns of the four feeding types

Table 6. The average and maximal share (%) of nematode genera for all single samples over seven years. The season (S = Spring; A = Autumn) of peak occurrences is given in brackets.

	mesohaline station		polyhaline station	
	average	maximal	average	maximal
selective deposit-feeders:				
Halalaimus	6.02	47.15	0.15	1.27
Terschellingia	1.66	8.25	2.98	15.04
Tubolaimoides	0.18	2.05	0.11	1.83
Cyartonema	0.17	1.53	0.26	2.00
Leptolaimus	0.08	1.04	0.00	0.00
Antomicron	0.10	0.92	0.02	0.42
Trefusia	1.43	7.34	2.56	8.39
Pselionema	0.00	0.00	0.05	0.74
non-selective deposit-feeders:				
Daptonema	13.20	27.52 (A)	19.63	43.29 (A)
Theristus	10.13	46.97 (A)	0.20	1.33
Trichotheristus	6.03	38.21	1.66	10.85
Anoplostoma	5.00	23.43	0.22	3.57
Odontophora	0.12	0.94	8.81	18.32
Ascolaimus	0.30	4.05	4.81	11.76
Axonolaimus	0.20	1.37	1.03	10.71
Eleutherolaimus	0.38	2.63	2.62	6.98
Sabatieria	0.00	0.00	0.10	0.78
Richtersia	0.06	1.20	0.03	0.70
Tripyloides	0.38	5.26	0.65	9.03
Parlinhomoeus	0.03	0.68	2.62	9.04
Bathylaimus	0.35	3.42	0.33	2.29
epigrowth-feeders:				
Praeacanthonchus	3.61	29.48 (A)	0.85	3.10
Dichromadora	11.99	53.51 (S)	4.40	15.29
Ptycholaimellus	0.00	0.00	3.58	12.59
Microlaimus	1.56	12.39	4.72	12.20
Chromadorita	4.77	29.66	0.84	9.03
predators:				
Enoploides	8.53	49.32 (S)	7.26	54.98(A)
Viscosia	7.17	33.54 (A)	16.49	33.12
Metachromadora	0.02	0.58	12.05	36.36 (A)
Calyptronema	7.30	41.35 (A)	0.45	4.86
Oncholaimus	4.55	26.70 (S)	0.11	1.16
Sphaerolaimus	2.00	10.36	0.33	3.17
Enoplus	2.67	8.33	0.07	0.72

were remarkably different for the two stations. At the polyhaline station, the non-selective deposit-feeders' peak was composed of *Daptonema* and the predators' peak was made by *Enoploides*. At the mesohaline station, the non-selective deposit-feeders' peaks were formed by *Theristus* and *Daptonema*; the predators' peaks by *Enoploides* and *Oncholaimus* in Spring and *Viscosia* and *Calyptronema* in Autumn; the epigrowth-feeders' peaks consisted of *Dichromadora* in Spring and *Praeacanthonchus* in Autumn (Figs. 8, 9, 10, 11 and 12).

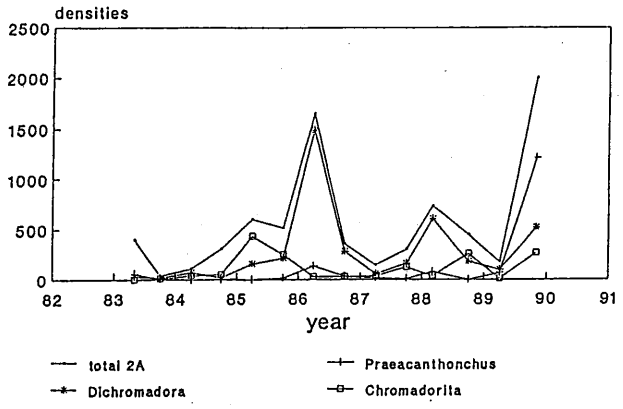

Fig. 8. The temporal density variation of epigrowth-feeders (2A) and its dominant genera at the mesohaline station. Densities = ind. 10 cm^{-2}.

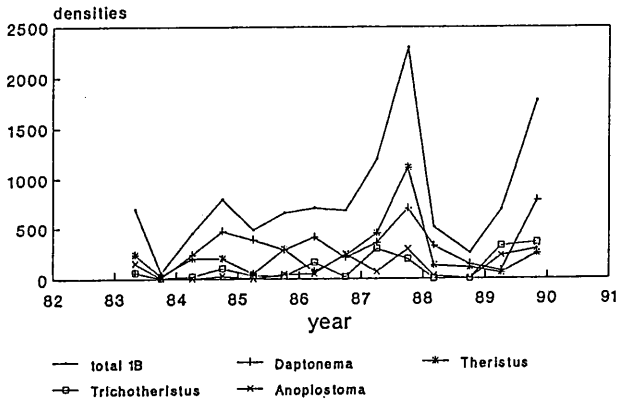

Fig. 9. The temporal density variation of non-selective deposit-feeders (1B) and its dominant genera at the mesohaline station. Densities = ind. 10 cm^{-2}).

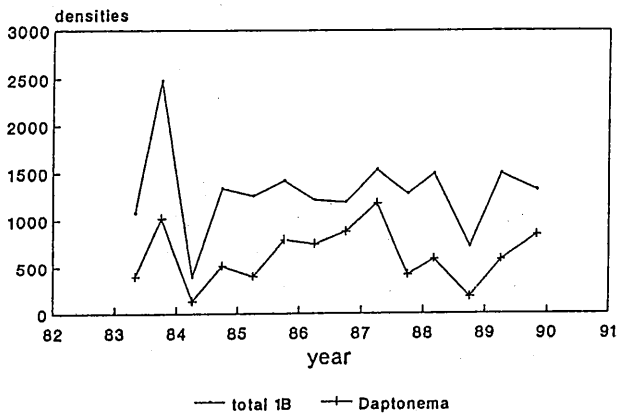

Fig. 10. The temporal density variation of non-selective deposit-feeders (1B) and its dominant genus at the polyhaline station. Densities = ind. 10 cm^{-2}.

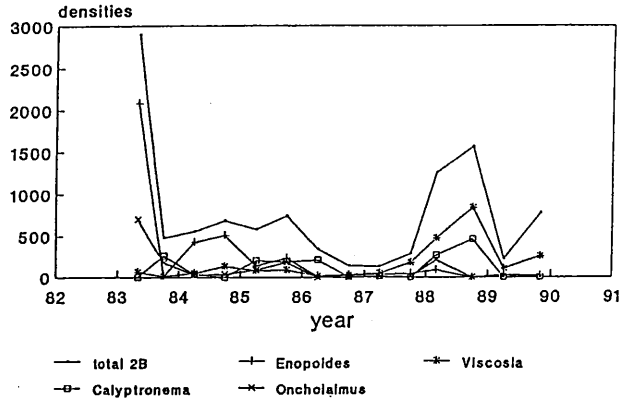

Fig. 11. The temporal density variation of predators (2B) and its dominant genera at the mesohaline station. Densities = ind. 10 cm^{-2}.

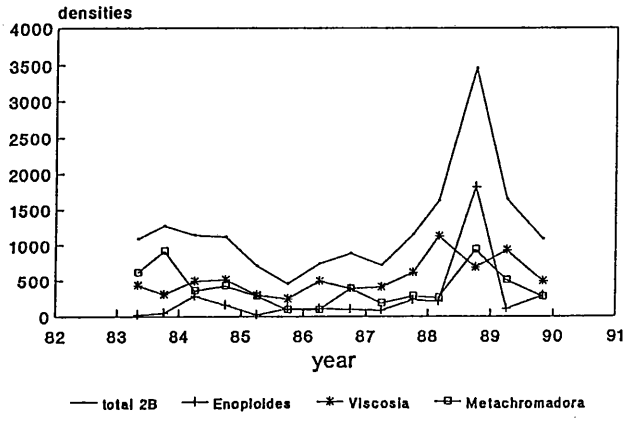

Fig. 12. The temporal density variation of predators (2B) and its dominant genera at the polyhaline station. Densities = ind. 10 cm^{-2}).

DISCUSSION AND CONCLUSION

Despite the 25 km distance between the two stations in the Westerschelde and the different salinity regimes, there were no major differences in the general characteristics of the two nematode communities. No difference between Spring and Autumn could be detected. No statistically significant trend in nematode densities could be found over 7 years. Salinity and salinity fluctuations did not influence the ratio of average feeding type abundances (1B > 2B > 2A > 1A). The variance of the nematode density data was homogeneous over the 7 years at both stations, although the nematode composition (in terms of relative abundances of genera) was different between the two stations.

However, a more detailed analysis in the feeding type structure and the genus composition discovered remarkable differences between the two stations. First, the mesohaline station had a lower total nematode density (especially for non-selective deposit-feeders and predators) than the polyhaline station. Second, all four feeding types had higher density variances per year among the 7 years of investigation at the mesohaline station than at the polyhaline station. Third, the composition of the dominant taxa was more stable in their abundance at the polyhaline station than at the mesohaline station.

Significant trends were found only for the decrease of selective deposit-feeders ($p < 0.05$) in the polyhaline station, when the April sample of 1983 was not included, and the increase of the total nematode density ($p < 0.05$) at the mesohaline station. VAN DAMME *et al.* (1980) showed also that April has the highest density values at the mesohaline station and is therefore clearly different from other months. These authors also found a trend of decreasing density from the mouth of the estuary (2200 ind. 10 cm^{-2}) at Vlissingen to the upstream area (160 ind. 10 cm^{-2}) at Doel. The nematode density for the polyhaline zone was nearly double that for the mesohaline zone (1500 ind. 10 cm^{-2} to 800 ind. 10 cm^{-2}). The ratio of four feeding types is different compared with our results with 1B >2B for the mesohaline zone, but 2B >1B for the polyhaline zone. However, VAN DAMME *et al.* (1980) compared density values in the entire marine or brackish water zone based on the average densities of two intertidal sites, two subtidal sites and one channel site and their interpretation was based on five times sampling during the period 1978-1979.

Since the nutrients are probably never limiting in the Westerschelde (HEIP, 1989) and the studied intertidal stations are covered with water briefly (2-5 hours/tide), we suppose that primary production in the intertidal zone (mainly by diatoms) was not effected much by the water transparency gradient. The more unstable nematode structure in the mesohaline intertidal site, therefore, could be less combined with biotic factors (fluctuation following primary production), but with abiotic factors (fluctuations in submersion, salinity, inorganic and organic pollutants). This explanation is in accordance with the one obtained by HUMMEL *et al.* (1988) who defined a detritus food chain system for the mesohaline zone and a coastal food chain system for the polyhaline zone. In the mesohaline zone, therefore, the flocculated aggregates of detritus and bacteria were a main food source.

The Westerschelde estuary is young and has very unstable habitats (HEIP, 1988). At the polyhaline station, the higher density of the total nematode community can be combined with a more stable

composition of the feeding types (which contains a lower number of dominant genera) and with a stable (homogeneous variance) non-selective deposit-feeders and selective deposit-feeders distribution, which is the result of a non-detritus food chain system. The more unstable nematode structure of the mesohaline station is the result of the detritus food chain system that highly relates to suspended matter input from upstream. The stable/unstable situation in the nematode structure of the polyhaline/mesohaline station, therefore, indicates that unstability of the estuarine habitats may be influenced by the upstream River Schelde.

However, the error in this study could be caused by the different intertidal position of sampling site over the period of investigation (there is a change of tidal range for the same point from 1.0 m in 1979 to 1.5 m in 1987 in the mesohaline station), which influences of course the daily intertidal primary production. Further studies, on smaller time scale, combining biological data with other environmental data (*i.e.* chlorophyll-*a* content) are needed to detect regulating factors for nematode communities.

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Carbon flows through meiobenthic nematodes in the Westerschelde Estuary

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ABSTRACT

A time dynamic model was used to estimate carbon flows through nematodes in an intertidal benthic ecosystem in the Westerschelde Estuary, Belgium. The model calibrated nematode biomass observed from March 1991 to February 1992. The forcing functions of the model include meiobenthic biomass, macrobenthic biomass, bacteria's concentration, chlorophyll-a concentration and other abiotic data, such as, temperature and day length.

We estimate that the nematode population had a low level of standing stock, 249 mg C $\cdot m^{-2}$, but a high level of carbon flow. It consumes 98 mg C $\cdot m^{-2} \cdot d^{-1}$ and produces 22 mg C $\cdot m^{-2} \cdot d^{-1}$. Defecation is 73 mg C $\cdot m^{-2} \cdot d^{-1}$. Losses in respiration, excretion and natural death are only 3 mg C $\cdot m^{-2} \cdot d^{-1}$. Annual P/B is 32 for local nematode community. These data establish the importance of nematodes in the decomposition of POM, and as a pathway from organic detritus to higher trophic levels in the benthic ecosystem.

1. INTRODUCTION

Nematodes are the most abundant meiofauna in benthic system (Coull, 1988), and consist primarily of organic detritus and diatom feeders (Montagna, 1980; Findlay & Tenore, 1982; Alkemade et al., 1993). Annual turnover rate in biomass (P/B) of nematodes has been estimated as high as nine (Gerlach, 1971), or even reach 69 (Vranken & Heip, 1986). Nematodes are consumed by other meiobenthos such as Protohydra (Heip & Smol, 1975; Elmgren, 1976), other large nematodes (Weiser, 1953), or epibenthos such as goby (Hamerlynck & Vanreusel, 1993). Thus, the nematodes may be an important pathway in energetics from the primary production and detritus to higher trophic levels.

Studies on energetics of nematodes in the past have been based on populations of single species (Tietjen, 1980; Warwick, 1981; Schiemer, 1982; Herman & Vranken, 1988). Energetics have been studied in only a few species because of the difficulty in culturing. Most studies concentrate on species feeding on bacteria or diatoms, such as Monhystera disjuncta (Tietjen, 1980; Vranken & Heip, 1986; Herman & Vranken, 1988), Diplolaimelloides bruciei (Warwick, 1981) or Caenorhabditis briggsae (Schiemer et al., 1980; Schiemer, 1982). People often estimate energy flow of a nematode community based on indirect methods, e.g., by estimating respiration rates or assuming an annual P/B of nine (Van Damme et al., 1980; Heip et al., 1984; Heip et al., 1990). A defecation rate was unknown and the variation of P/B was not considered. People often based modeling ecosystems in energetics on a whole marine ecosystem rather than focusing on a single compartment (Warwick et al., 1979; Carrada et al., 1983; Baretta et al., 1988; Steele, 1974; Fransz et al., 1991; Chardy & Dauvin, 1992). All nematodes, all meiobenthos, or all benthos, were set as one state variable. Less information within the compartment comes out of these models. Modeling nematodes on feeding type level may provide significant information different from

modeling single species and modeling whole benthos.

Nematodes in the Westerschelde Estuary comprise >95%, in density, and >85%, in biomass, of the total meiobenthos (Van Damme et al., 1980). Abundance decreases from the sea to a brackish zone. Nematode communities in brackish zones have a higher temporal variation than communities in the mouth area (Li & Vincx, 1993). Predation pressure from the deposit-feeding macroinfauna is the main source of this temporal variation (Li et al., 1996a). A detritus food chain system is suggested for a brackish zone, which is distinguished from a producer food chain system with a higher abundance nematode in the mouth area (Hummel et al., 1988). We will model carbon flows through nematodes in this "predator controlled" and "detritus food chain's" system.

2. MATERIALS AND METHODS

The model consists of several mathematical equations that calculate variation of nematode biomass in response to variation in environmental data. The model was provided with input of observed environmental data, and produced, as output, a simulated nematode biomass. The model used FORTRAN77 and eased by the PC software SENECA (de Hoop et al., 1992). SENECA is designed to work with and develop simulation models of time dependent processes like ecosystem models. It simplifies the model setup and supports techniques of calibrations.

The study site is in the brackish intertidal zone of the Westerschelde Estuary (Fig. 1). Nematodes were sampled from March 1991 to February 1992 and classified into four feeding types (Wieser, 1953), which are selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A) and omnivore/predator (2B). Two replicates were taken at each time to reduce the spatial variation of nematodes within m-scale (Li et at., 1996b). Significant differences ($p<0.05$) among time samples were found for each feeding group. Average data were used in the model. Environmental data include total meiobenthos biomass, macrobenthos abundance and biomass, bacterial density, TOC (Total Organic Carbon) contents and sedimentary chlorophyll-a concentration.

The development of the model included four processes: consumption, assimilation, losses (include respiration, excretion and natural death) and predation (Fig. 2). The food source system was based on the hypothesis of a detritus food chain system for the study site (Hummel et al., 1988). So the POC (Particle Organic Carbon) will be the main food source for nematodes, it can be calculated from TOC. Other food sources include diatom, bacteria and prey nematodes. Predators in the model included four types: Protohydra, turbellarians, polychaetes and epibenthos. The estimating of the model parameters is based on the observed nematode biomass. The simulation of carbon flows through nematode standing stocks was based on repeats the cyclic observed environmental condition. So the out put of model was stable fluctuation. The detailed structure of the model has been described by Li et al. (1996a), and summarized as the list of

equations (Table 1). The validation of the model was according to the three points: (1) The model calibrated by first three months observed nematode biomass can fit the observation of a whole year. (2) The simulations based on one year data predict that the group 2B has the highest stock followed by 1B, 2A, and 1A, which is the same as seven years' observation at a nearby station (Li & Vincx, 1993). (3) The result of the calibration showed that small nematode has a higher respiration rate than a large nematode, which fitted the biological rule.

3. RESULTS

The predicted standing stock of nematodes was 249 mg C $\cdot$ m^{-2} (Table 2, Fig. 3). Nematodes consumed 98 mg C $\cdot$ m^{-2} $\cdot$ d^{-1} and produced 73 mg C $\cdot$ m^{-2} $\cdot$ d^{-1} of decomposed organic matter, which was 74% of what they consumed. The production from this stock was 22 mg C $\cdot$ m^{-2} $\cdot$ d^{-1}, which was 23% of what they consumed and 88% of what they assimilated. Losses including respiration, excretion and natural death were 3 mg C $\cdot$ m^{-2} $\cdot$ d^{-1} that were only 3% of consumption and 12% of assimilation. Annual biomass turnover rate (P/B) is 32 and production efficiency was up to 88%.

Different food sources had different assimilation efficiencies ($p_{(4,i)}$ in Table 2). Feeding on the POC, nematodes assimilated lower carbons (18-36%) than feeding on bacteria (24-64 %), diatoms (63-78 %) or prey nematodes (55-80 %). The average assimilation efficiency for nematodes in the Westerschelde was as low as 24-26%.

4. DISCUSSION

4.1. Standing stock

The predicted nematode standing stock at a brackish zone of the Westerschelde Estuary was 249 mg C $\cdot$ m^{-2}. This is lower than in other areas. For example, standing stock is 120-190 mg C $\cdot$ m^{-2} in the Belgian coastal zone of the North Sea (Vincx, 1989), but 412 mg C $\cdot$ m^{-2} in the mouth of the Westerschelde (Van Damme et al., 1980), and 788 mg C $\cdot$ m^{-2} in the Lynher Estuary (Warwick et al., 1979). Three factors make standing stock lower in a brackish zone than in the marine zone of the Westerschelde (Li & Vincx, 1993). First, detritus dominates food in the brackish zone (Hummel et al., 1988). Thus, food quality and assimilation efficiency in a brackish zone are lower than in the marine zone where the primary production is much higher (Hummel et al., 1988). Second, sediment size in this location (mean diameters: 74-98 μm) is smaller than in the marine zone (mean diameters: 138-167 μm). Sediments with smaller grain size select for smaller nematodes. Animal body size is negatively correlated with respiration rate; thus nematodes within the brackish zone may have a higher respiration rate than in the marine zone because of this smaller size. Higher respiration rates cause lower production efficiencies. Thus, the nematode community in the brackish zone may maintain a low of standing stock through a low of production

efficiency. Finally, the main predator, Heteromastus filiformis (polychaete), decrease from brackish zones to the sea (Meire et al., 1991). Thus, a lower standing stock of the nematode community at a brackish zone could be maintained by a high of predation mortality. So food quality, sedimentary type and predation pressure are the key factors for the level of nematode standing stock in the Westerschelde.

4.2. Productivity

Although biomass is low in the Westerschelde Estuary, production is as high as 8 g C · m^{-2} · y^{-1}, almost twice that reported in previous studies. Production was 4.1 g C · $m^{-2}y^{-1}$ for nematodes in the mouth of the Westerschelde (Van Damme et al., 1980), and 1.5-2.0 g C · m^{-2} · y^{-1} for the total meiobenthos in the Southern Bight of the North Sea (Heip et al., 1984). The estimated production efficiencies were 53-95% for four feeding groups. The total production efficiency was 88% because an omnivore (2B) was dominant. This production efficiency level is high in comparison with studies on individual species in the laboratory (Table 3). These species are different from those studies here, but it is possible that production efficiency of a nematode is higher in field than in the laboratory. Chardy & Dauvin (1992) has simulated the carbon flows in the western English Channel with total meiofauna as one compartment in their system. Although nematodes compose up to 85% of biomass, the total assimilation efficiency is much higher and total production efficiency is much lower than the laboratory observation (Table 3). This shows that nematodes have much higher productivity than other meiofauna and they are varied among different species.

The present model estimates a biomass turnover rate, annual P/B, at 32, three times higher than values from previous studies. Gerlach (1971) estimated an annual P/B of nine for nematodes, and this value has been used to calculate the production in many studies. The annual P/B, is the product of generation number per year multiplied by a life cycle turnover rate ($P/B_{generation}$). A $P/B_{generation}$ of three for nematodes was estimated by Herman & Vranken (1988) but only for the juvenile period. Adult nematodes in the Westerschelde varied greatly in body size. Table 4 show that growth occurs during adult period and that probably has a $P/B_{generation}$ higher than the most nematode species in the Westerschelde Estuary. Maximal $P/B_{generation}$ may be as high as five, postulated by Sanders (1956) for short-lived benthic species and by Waters (1969) for freshwater invertebrates. The average minimal generation time of nematodes is often less than 1/3 year (see review by Heip et al., 1985). Nine of annual P/B underestimate for nematodes. Table 5 lists some examples that annual P/B of nematodes are larger than nine. Heip et al. (1990) suggested that the annual P/B is 10.1 for nematodes in the Southern Bight of North Sea if production efficiency is 40% and would become 35.3 with a production efficiency of 70%. The estimating of nine by Gerlach (1971) in fact is for Monhystera disjuncta (1B) and Chromadorita tenuis (2A). Feeding types 1A, 2A and 1B are predicted to have lower production efficiency than

feeding type 2B (Table 3). A nematode community is composed of all feeding groups and frequently has high species diversity. So the $P/B_{generation}$ and annual P/B are expected to be higher than three and nine respectively for the nematode community in the Westerschelde Estuary. These may be the reasons that present estimating for production is twice that reported by Van Damme et al. (1980).

4.3. Decomposition of organic matter

Daily defecation of a 249 mg C · m^{-2} standing stock of nematodes (about 2,000 ind. · 10 cm^{-2}) is 73 mg C · m^{-2} · d^{-1} or 74% of their consumption. The present assimilation efficiencies, 24-26%, are low in comparison with previous studies, that were from 6% to 52% (Table 3). We may explain this as the high POC composition in the food sources for the detritus food chain system (Hummel et al., 1988). We suggest that average assimilation efficiency be dependent on a food source. We estimated the POC up to 98% of total nematode food sources in the present study site. This may be due to the most dominant species, Viscosia viscosa, is a scavenger rather than a predator defined later (Jensen, 1987). It feeds on large particles. Nematodes, like Viscosia viscosa, are significant players in the decomposition of organic detritus, thus promoting remineralization of bacteria. Findlay & Tenore (1982) found that maximal mineralization rate can be doubled by adding only ten nematodes · 10 cm^{-2}.

The standing stock of organic matter in the Westerschelde Estuary is 10^6 mg C · m^{-2} that dwarfs the decomposition rate of nematodes, estimated at 73 mg C · m^{-2} · d^{-1}. Although decomposition of the POC by nematodes is as important in promoting its mineralization by bacteria, it may not influence overall carbon dynamics. However, nematodes have a high potential to decompose POC and may be more important than shown by this study, because nematodes often have far higher densities than 2,000 ind. · 10 cm^{-2} that we observed.

4.4. Ecological efficiency

The ecological efficiency (production per consumption) was estimated as 23% in the detritus food chain system of a brackish zone of the Westerschelde. It is similar to the producers' food chain system in the Lynher Estuary, which is 22% (Warwick et al., 1979). Simulation of meiofauna in the western English Channel presented much lower ecological efficiency, which is only 11% (Chardy & Dauvin, 1992). Due to the fact that the nematodes compose 65-86% of biomass within the meiofauna compartment there, the meiofauna other than nematodes must have very low ecological efficiency than nematodes. Marchant & Nicholas (1974) found an ecological efficiency of 22.4% for a single freshwater species Pelodera sp. feeding on bacteria, Escherichia coli. The assimilation efficiency for feeding on bacteria has been reported as 59.8% (Marchant & Nicholas, 1974), which is consistent with our prediction (54.8% as a best fit value in Table 2). An ecological efficiency of 22-23% may be characteristic of nematodes in most ecosystems.

4.5. Next development of model

Chardy & Dauvin (1992) predict a 30% respiration rate to consumption (10 g C $m^{-2} \cdot y^{-1}$ respiration from 33.3 g C $m^{-2} \cdot y^{-1}$ consumption), which daily respiration rate to meiofauna biomass is 10%. This predicted that respiration rate is 10 times higher than our prediction for nematodes. However, Chardy & Dauvin's (1992) meiofauna compartment includes 15-35% of other meiofauna, such as microgastropods, copepods, gastrotrichs, halacarids, turbellarians, oligochaetes, polychaetes, amphipods and bivalves. It is possible that nematodes have much lower respiration rates than that meiofauna like fast-moving copepods. The respiration of meiofauna is generally considered very high in marine meiofauna and nematodes are known to excrete ammonium, amino acids and produce mucus trails. A 3% of carbon loss rates for nematodes predicted by present study seams underestimated. However, it is consisted with 2.8-7.4% of respiratory energy losses calculated using Woombs & Laybourn-Parry's (1985) data. A lipid metabolism of a free-living nematode is more important than a carbohydrate metabolism because of higher lipids content than glycogen content when comparing the different functions of the nematodes (Lee & Atkinson, 1977). The current model is based on carbon flows. It will be interesting to combine a model with nitrogen flows to recalibrate the nematode respiration rate in a next study.

This modeling study was based on the hypothesis of a detritus food chain system for the study site (Hummel et al., 1988), where the deposit-feeders compose more than 85% in biomass at the study site. However, setting POC as the main food sources for all four feeding types of nematodes may bring some error in the simulation, especially, for those diatom feeders who may not be fed on POC at all. Further modeling with species level may be required to correct those errors.

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Table 1. The formula used in the mode. Variables: B_j = nematode biomass (j=1-4 for four feeding groups), C_j = consumption, A_j = average assimilation efficiency, D_j = predation death, S_j = total food source, L_j = loss in respiration excretion and natural death,). Forcing function: T = temperature °C, M_k = predator density (k=1-4 for protohydra, turbellarians, polychaets and epibenthos), $F_{(i,j)}$ = single food source (I=1-7 for four nematode feeding groups, bacteria, diatom and particle organic matter). Parameters: $p_{(1)}$ = maximal consuming rate of nematodes, $p_{(2)}$ = temperature effects Q_{10} for nematodes, $p_{(3)}$ = food concentration that nematode reach half of $p_{(1)}$, $p_{(4,i)}$ = assimilation efficiencies for a single food source I, $p_{(5)}$ = loss rates of nematodes include respiration, excretion and natural death, $p_{(6,k)}$ = grazing rates of predator k, $p_{(7,j)}$ = effect of aggregating distribution of nematode j for their predation death, $p_{(8,k)}$ = temperature effects Q_{10} for predator k.

$$\frac{d(B_j)}{d(t)} = C_j \cdot A_j - L_j - D_j \tag{1}$$

$$C_j = \frac{B_j p_{(1)} \cdot S_j p_{(2)}^{\frac{T-20}{10}}}{S_j + p_{(3)}} \tag{2}$$

$$S_j = \sum F_{ij} \tag{3}$$

$$A_j = \sum \frac{p_{(4,i)} \cdot F_{(i,j)}}{S_j} \tag{4}$$

$$L_j = B_j p_{(5)} \cdot p_{(2)}^{\frac{T-20}{10}} \tag{5}$$

$$D_j = \sum M_k p_{(6,k)} \cdot e^{-\frac{p_{(7,j)}}{B_j} \cdot p_{(8,k)}^{\frac{T-20}{10}}} \tag{6}$$

Table 2. The mean values of energetic data for the total nematodes based on the simulation from 1991 to 1995 calculated by the environmental data from 1991 to 1992.

Items	Related toTable 1	Estimations
Standing Stock (mg C · m^{-2})	B	249.2
Available Food Source (mg C · m^{-2})	S	867384
Consumption (mg C · m^{-2} · d^{-1})	C	97.6
Daily Consumption Rate %	$C / B \cdot 100$	39
Defecation (mg C · m^{-2} · d^{-1})	$C - C \cdot A$	72.5
Average Assimilation Efficiency %	A 100	26
Assimilation Efficiency % Feeding on POM	$p_{(4,1)}$ 100	23
Assimilation Efficiency % Feeding on Bacteria	$p_{(4,2)}$ 100	55
Assimilation Efficiency % Feeding on Diatom	$p_{(4,3)}$ 100	72
Assimilation Efficiency % Feeding on Prey Nematodes	$p_{(4,4)}$ 100	73
Loss[a] (mg C · m^{-2} · d^{-1})	L	3
Loss Rate (d^{-1})	L / B	0.012
Production or Predation (mg C · m^{-2} · d^{-1})	$C \quad A - L$ or D	22
Production Efficiency %	$(C \cdot A - L) / (C \cdot A)$	88
Annual P/B (y^{-1})	$C \cdot A / B$	32
Ecological Efficiency %	$D / C \cdot 100$	23

[a]include: respiration, excretion and natural death.

Table 3. The comparison of estimated assimilation efficiency (A. E.) and production efficiency (P. E.) to the previous studies.

Authors	Nematodes	A. E. (%)	P. E. (%)
Present study	Group 1A (Halalaimus gracilis[a])	24	53
Present study	Group 1B (Daptonema setosum[a])	26	86
Present study	Group 2A(Chromadora macrolaima[a])	26	77
Present study	Group 2B (Viscosia viscosa[a])	26	95
Marchant & Nicholas (1974)	Pelodera sp. (1A)	n/a	38
Warwick et al. (1979)	Nematodes[b]	60[c]	38[c]
Tietjen (1980)	Chromadorina germanica (2A)	6	79
Tietjen (1980)	Monhystera disjuncta (1B)	18	80
Tietjen (1980)	Rhabditis marina (1A)	26	97
Schiemer et al. (1980)	Plectus palustris (1A)	12-52	35-87
Warwick (1981)	Diplolaimelloides bruciei (1B)	n/a	71-87
Schiemer (1982)	Caenohabditis briggsae (1B)	n/a	48-63
Woombs & Laybourn-Parry (1985)	Diplogasteritus nudicaptatus (1A)	16-34[c]	54-78[c]
Woombs & Laybourn-Parry (1985)	Paroigolaimella bernensis (1A)	12-28[c]	48-78[c]
Woombs & Laybourn-Parry (1985)	Rhabditis curvicaudata (1A)	9-13[c]	24-49[c]
Herman & Vranken (1988)	Monhystera disjuncta (1B)	18-27	60
Chardy & Dauvin (1992)	Meiofauna (65-86% nematodes[d] in biomass)	46[c]	31[c]

[a]dominant species within the group; [b]all 40 species include: 27% 1A, 27% 1B, 44% 2A and 1% 2B in population; [c]calculated according to the published data.; [d]dominant species include: Richtersia kreisi (1B), Microlaimus conspicuus (2A), Chromaspirina renaudae (2B), Cylindrotheristus divertens (1B) and Prochromadorella ditleveni (2A).

Table 4. The ranges of female body size for dominant nematode species calculated by the observed length and width (Andrassy, 1956).

Dominant nematodes	individual wed weight (μg)
Daptonema setosum (**1B**)	1-15
Anoplostoma viviparum (**1B**)	1-10
Tripyloides gracilis (**1B**)	0.5-2
Viscosia viscosa (**2B**)	1-2
Thalassoalaimus septentrionalis (**1A**)	1.5-2.6
Dichromadora geophila (**2A**)	0.5-5
Dichromadora cephalata (**2A**)	0.4-1.5
Chromadora macrolaima (**2A**)	0.3-2

Table 5. Example that annual P/B of nematode species are larger than nine.

Nematodes	Annual P/B	References
Monhystera disjuncta (**1B**)	69	Vranken & Heip, 1986
Sabatieria punctata (**1B**)	14.1-16.9	Vincx, 1989
Daptonema tenuispiculum (**1B**)	28.5-31.9	Vincx, 1989
Ascolaimus sp. (**1B**)	11.5-14.8	Vincx, 1989

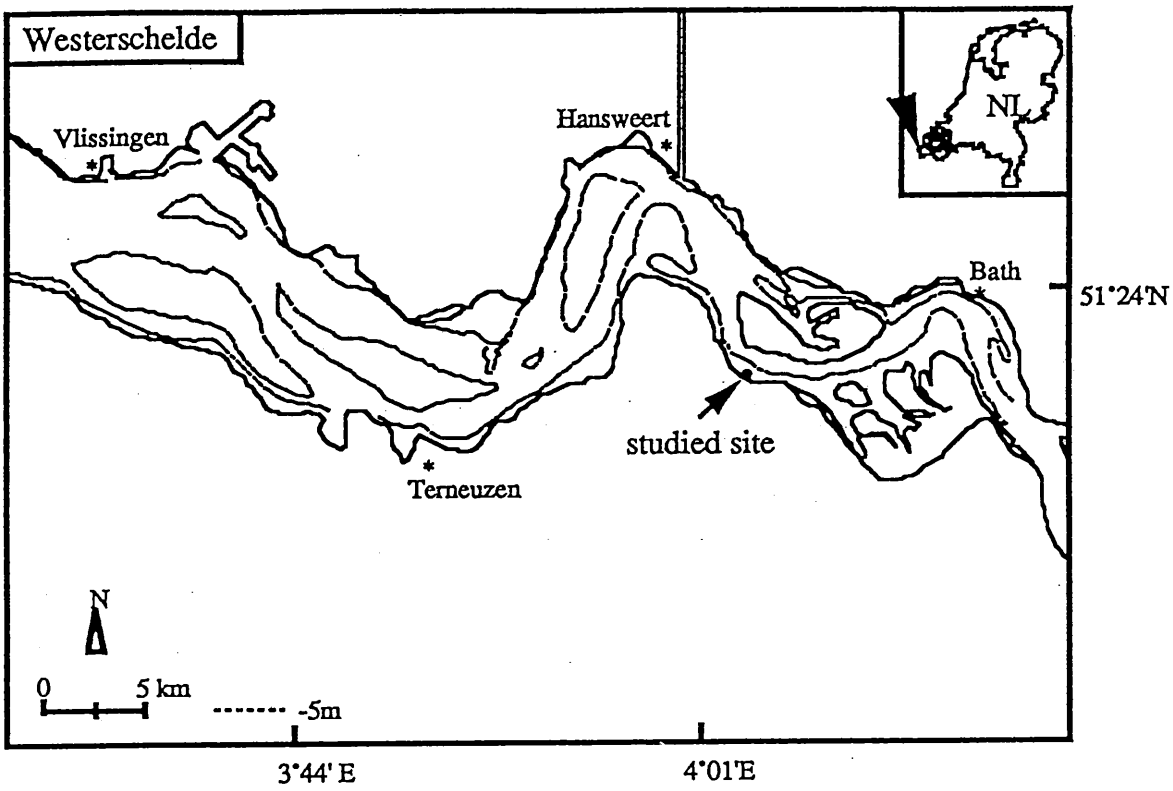

Fig. 1. The location of the studied station at the intertidal brackish zone of the Westerschelde Estuary (NL=The Netherlands).

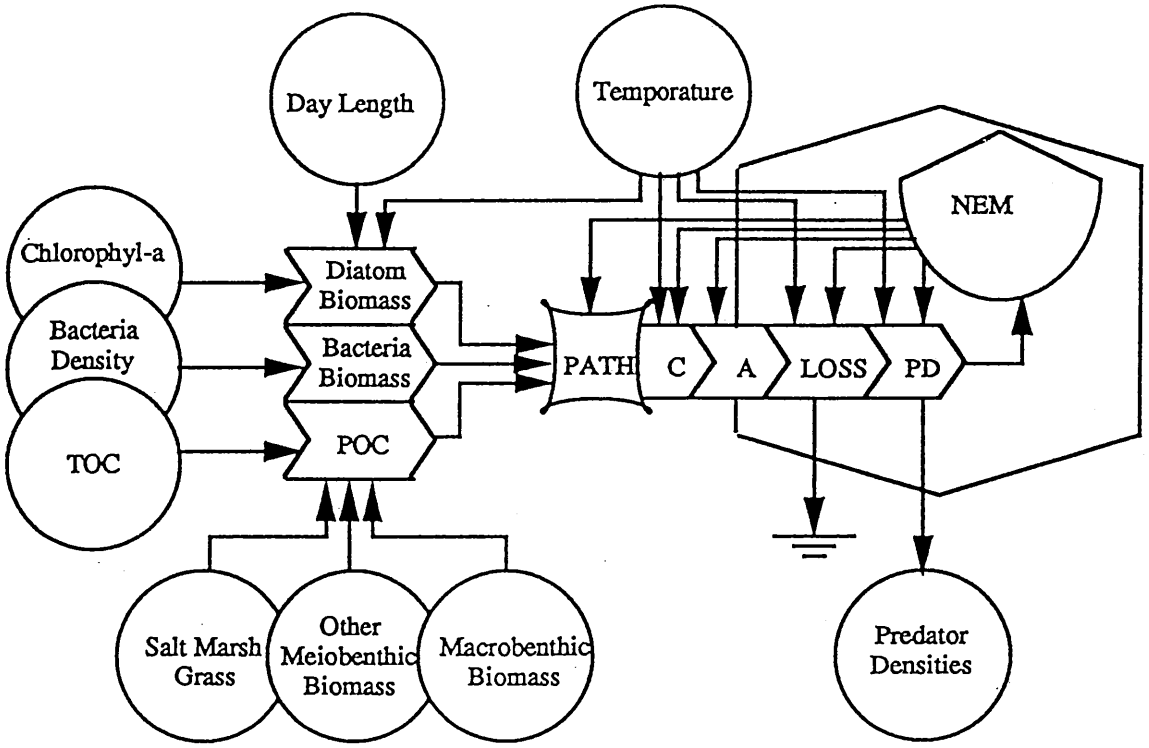

Fig. 2. The model structure with the energy-circuit language (Odum 1972).

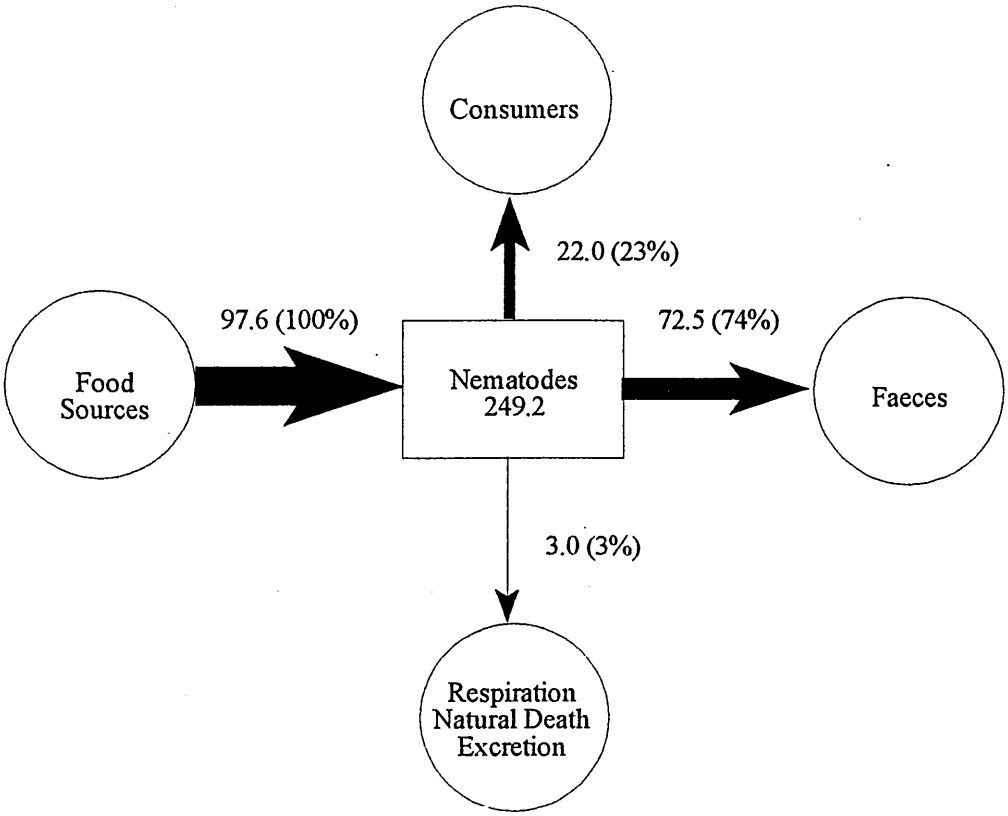

Fig. 3 . The carbon flow (mg C · m^{-2} · d^{-1}) through nematode standing stock (mg C · m^{-2}) according to the simulation (1991-1995) of the model for nematodes in the brackish tidal flat of the Westerschelde.

Spatial Patterns of Westerschelde Meiobenthos

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The meiobenthic fauna of the Westerschelde, a highly polluted and physically disturbed estuary in the south-west Netherlands, was investigated. Samples were taken in spring from six transects, including the intertidal, subtidal and channel area, and located along the salinity gradient. The samples were subdivided into slices to examine the vertical distribution of meiobenthos. Meiobenthos densities were higher in the intertidal than in most permanently submersed areas; the subtidal sites below 7 salinity were nearly devoid of meiobenthic life. Nematodes were by far the most abundant meiobenthic organisms in the intertidal, but were less dominant in the other areas. Gastrotrichs, turbellarians, copepods and large ciliates were usually more numerous in the subtidal and channels compared to the intertidal, both in relative and absolute terms. Vertical distribution of the meiobenthos was rather heterogeneous. Most intertidal stations exhibited a subsurface density peak, whereas in the subtidal and channel area, both subsurface and surface maxima were found. The nematode fauna was examined in more detail and the distributional characteristics of the most important species, with respect to salinity, grain size, water depth and sediment depth were reported. The majority of species had their centre of distribution in the intertidal, although some extended substantially into the subtidal zone. Only a few species had a predominantly subtidal distribution. Most nematode species penetrated relatively deep into the sediment and only some species were real surface dwellers. The nematode diversity per unit of surface reflected more or less the density differences and was higher in the intertidal than in the subtidal sites within a comparable salinity regime. When expressed per common number of individuals, however, there were no differences in diversity in the different areas. Canonical correspondence analysis showed sediment depth to be as important as water depth, salinity or sedimentary characteristics in the determination of community structure. Intertidal communities exhibited a well-developed community gradient with depth into the sediment, whereas the vertical structure of subtidal and channel stations was different from the intertidal zonation and in some cases showed a distorted pattern. This was probably caused by sediment disturbance due to higher current velocities and

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dredging activities in these regions. It is argued that, although at some subtidal sites a characteristic subtidal nematode population may persist, in many cases the sublittoral of the Westerschelde is either too dynamic an environment or food availability is too low to meet requirements for growth and reproduction of the nematodes. The populations are probably not self-sustaining but persist due to continuous replenishment from less harsh areas by means of the estuarine circulation.

Introduction

The Westerschelde is the only remaining true estuary of the Dutch delta and it is subjected to a large input of, mainly untreated, industrial and domestic wastes. It is also the site of intense dredging and dumping activities (Heip, 1989).

Benthic organisms are especially vulnerable to all kinds of disturbances. Many contaminants typically end up in the sediment where they may persist for a long period of time and can be incorporated into the benthic organisms. Physical disturbances of natural (tides, waves, storms) and of man-made origin (dredging) will have an effect on the benthos. Dredging mainly affects benthic animals in the channels, where sediment is extracted, and in the vicinity of the major dump sites, where they could suffer from increased sedimentation. However, the usefulness of the benthos as indicators of disturbances may be hampered by the great spatial and temporal variability that these organisms usually exhibit (Heip *et al.*, 1985; Coull, 1988) and which in part reflects the variability of the habitat.

Estuaries have strongly pronounced gradients of various characteristic substances and typically show a large temporal variability in these gradients. The most obvious is the salinity gradient, and many abiotic factors (nutrients, suspended matter) change in parallel with this. Several geomorphological benthic structures result from the interplay of the topography and differential action of currents and wind-induced waves: intertidal flats which are semi-diurnally flooded are the sites of a more intense sedimentation; the bottom of the subtidal areas and channels is subjected to a resuspension–sedimentation cycle related to tidal currents. These currents are especially strong in the deeper channels, and along their edges most dredging activities take place in the Westerschelde. However, the most pronounced gradients occur vertically into the sediment and the meiofauna species composition and abundance can be substantially different even over a depth of a few centimetres (Joint *et al.*, 1982; Jensen, 1983).

Despite its evident importance, there are remarkably few studies of estuarine meiobenthos that cover both a wide range of salinity regimes and of benthic morphological units (Gerlach, 1953; Riemann, 1966; Warwick, 1971; Van Damme *et al.*, 1980, 1984; Bouwman, 1983; Smol *et al.*, 1994). Some meiobenthic studies were restricted to only a few estuarine sites along the salinity gradient (Capstick, 1959; Warwick & Gee, 1984; Austen & Warwick, 1989). Few of these studies have considered vertical stratification (Bouwman, 1983; Warwick & Gee, 1984).

Studies of Westerschelde macrofauna have indicated that the density as well as the diversity is higher in the more saline part of the estuary, and that the subtidal areas and channels are nearly defaunated in terms of both species and biomass (Vermeulen & Govaere, 1983; Ysebaert & Meire, 1992). The meiobenthic fauna of the Westerschelde was examined by Van Damme *et al.* (1980) and copepods were studied by Van Damme *et al.* (1984). However, these studies (dating back to the period 1977–78) were mainly restricted to the intertidal zone and samples were vertically integrated. Claassen (1991)

examined the meiobenthos in Saaftinge, which is the largest salt marsh of the estuary, situated in the brackish part.

As part of the Joint European Estuaries Programme (JEEP 92) of the EEC, the community structure of the Westerschelde meiobenthos was examined. Samplings were performed along a salinity gradient and included the intertidal, the subtidal and the deep channels. The vertical distribution into the sediment was investigated. Both the general meiobenthic community and more specifically the nematode species assemblages were examined.

Study site

The Westerschelde is a partly mixed estuary in The Netherlands and drains large areas in Belgium and France. Yearly averaged river outflow ranges from 100 to 150 $m^3 s^{-1}$ which is relatively small compared to tidal exchange (Van Eck *et al.*, 1991). Dispersion induced by the tidal currents and geomorphology of the Westerschelde provokes the establishment of salinity and related gradients. The tides and freshwater discharge produce a shift of particulates within the estuary (Postma, 1967), which results in the formation of a zone of maximal turbidity near Antwerp. Here sediment of fluviatile and marine origin is deposited (Van Eck & de Rooij, 1990). The high load or organic matter in this zone induces a high microbial activity which causes a serious depletion of oxygen in the water column in summer (Billen *et al.*, 1988; Heip, 1988). Annual phytoplankton primary production is lowest in the high-turbidity region and increases both upstream and downstream (Kromkamp *et al.*, 1992). The zooplankton shows maximum biomass in a zone downstream from the turbidity maximum in winter and early spring, while in summer highest biomass is observed near the mouth of the estuary (Soetaert & Van Rijswijk, 1993). The macrobenthos in the estuary is well developed on the intertidal flats only (Vermeulen & Govaere, 1983; Meire *et al.*, 1991; Ysebaert & Meire, 1992).

The Westerschelde is a highly polluted estuary due to the injection of large amounts of, mainly untreated, domestic sewage and industrial effluents. Moreover, in order to preserve the shipping lanes of the port of Antwerp, a large amount of sediment (15 million m^3 $year^{-1}$) is removed from the channels and largely dumped back again into the flood channels (Belmans, 1988).

Methods

The meiobenthos was sampled in March 1990 along six transects located at different salinity regimes and comprising three different morphological units: the intertidal, the subtidal area and the channels (Figure 1). Four subtidal samples were taken from the part more upstream.

Samples were taken with a Reineck box-corer (surface 170 cm^2) which was subsequently subsampled with plastic cores of 10-cm^2 surface. Some intertidal stations (of transect numbers 2 and 4) were directly sampled with cores. Some samples were used for chemical and sediment analyses; at least two cores were used for meiobenthic study. They were vertically subdivided into slices, 0–1, 1–2, 2–4 and 4–10 cm deep and fixed with a hot (70 °C) 4% formaldehyde solution. Various abiotic characteristics of the overlying water column at the time of sampling were measured: salinity, oxygen content ($g m^{-3}$), temperature and suspended matter content ($g m^{-3}$).

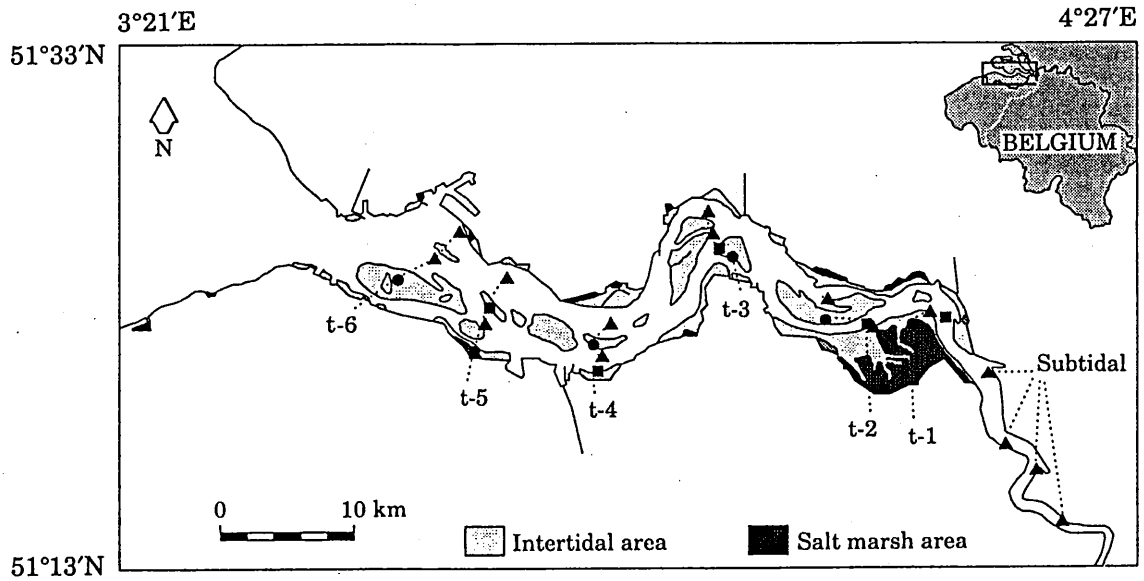

Figure 1. Position of the sampling stations with indication of the transect number (t-1–6) and the four additional subtidal stations. ●, Intertidal; ▲, subtidal; ■, channel.

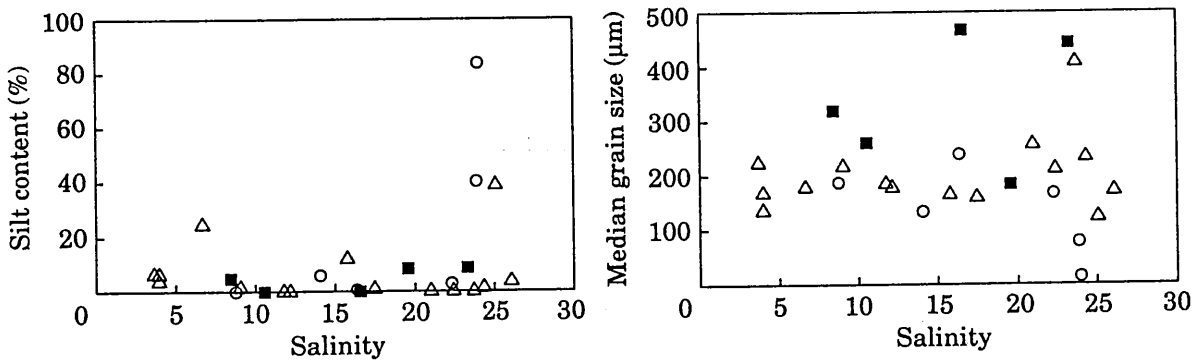

Figure 2. Grain size characteristics of the stations along the salinity gradient. ○, Intertidal; △, subtidal, ■, channel.

A first extraction of the meiobenthos occurred through decantation over a 38 µm sieve. The meiobenthos of fine sedimentary samples was then further extracted using LUDOX TM as described in Heip *et al.* (1985). After colouring with Rose Bengal all meiofaunal taxa were enumerated after which nematodes of one replicate were put onto slides for further identification. The other replicate was processed directly under an inverted microscope LEITZ Diavert.

Sediment particle-size distribution was determined using Coulter LS particle size analysis equipment. Nutrient load (ammonium, nitrate, nitrite, phosphate and dissolved silicate) in the sediment was determined for sediment slices of 0–1, 1–2, 2–4 and 4–6 cm. They were analysed with a Cenco M4 automatic analyser.

At most 100 nematodes per sediment slice (or all if total abundance was less than 100) were determined to species level. The multivariate species-abundance data (ind. $10\ cm^{-3}$) were explained as a function of the major environmental gradients by a canonical correspondence (CANOCO) analysis (Jongman *et al.*, 1987). Diversity was calculated as Hill's diversity indices of various order as recommended in Heip *et al.*

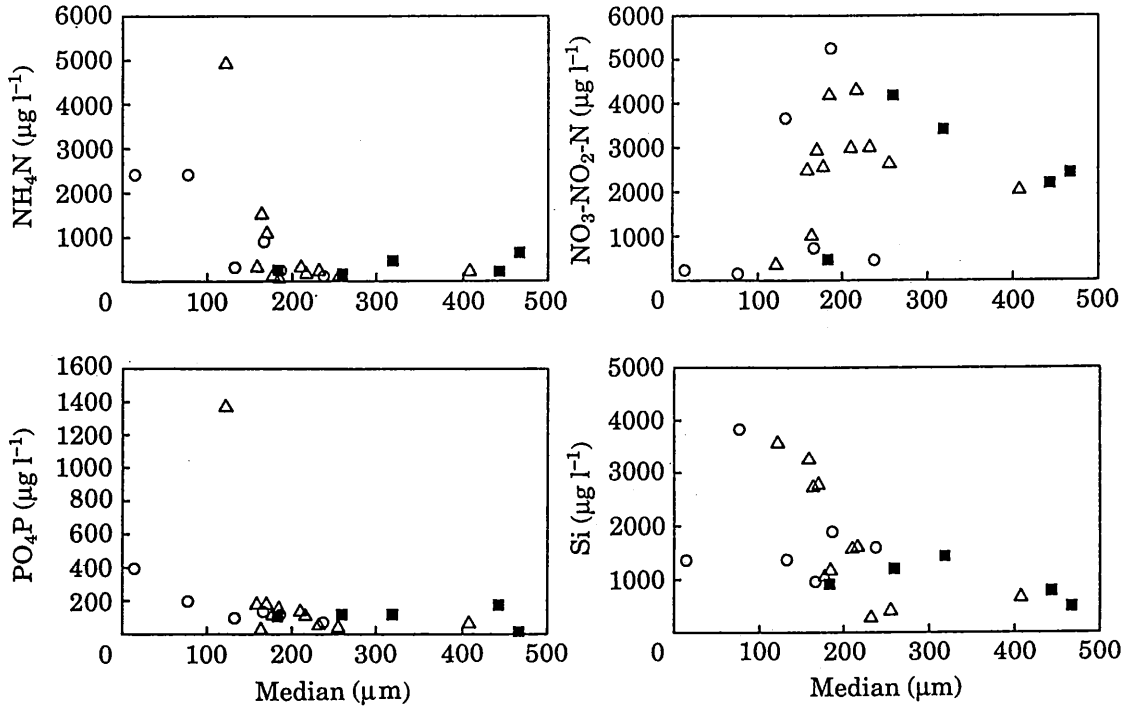

Figure 3. Mean nutrient load of the first 6 cm sediment in relation to median grain size. ○, Intertidal; △, subtidal; ■, channel.

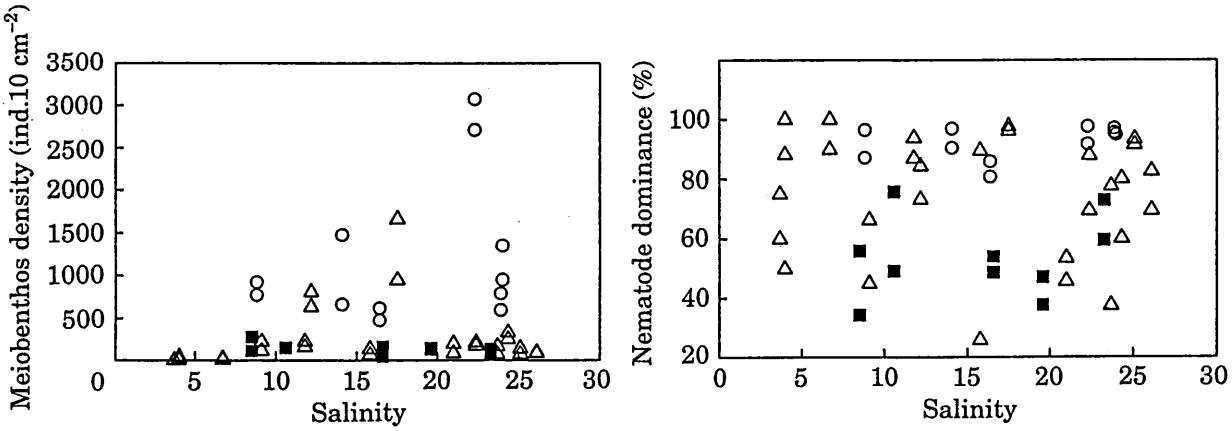

Figure 4. Vertically integrated meiobenthos density and fraction of nematodes along the salinity gradient. ○, Intertidal, △, subtidal; ■, channel.

(1988) and calibrated on a common number of individuals as described in Soetaert and Heip (1990). Areal densities were integrated through the vertical column.

Results

Sediments in the Westerschelde varied from muddy in the marine intertidal and some subtidal stations to very coarse (median grain diameter larger than 400 µm) in the channels. On average the sediments in the intertidal stations (average median grain diameter 136 µm, 22% silt) were finer than in the subtidal stations (average 200 µm, 7% silt) which in turn had finer sediments than most stations situated in the channels (334 µm, 4% silt) (Figure 2). Somewhat related to the grain characteristics was the

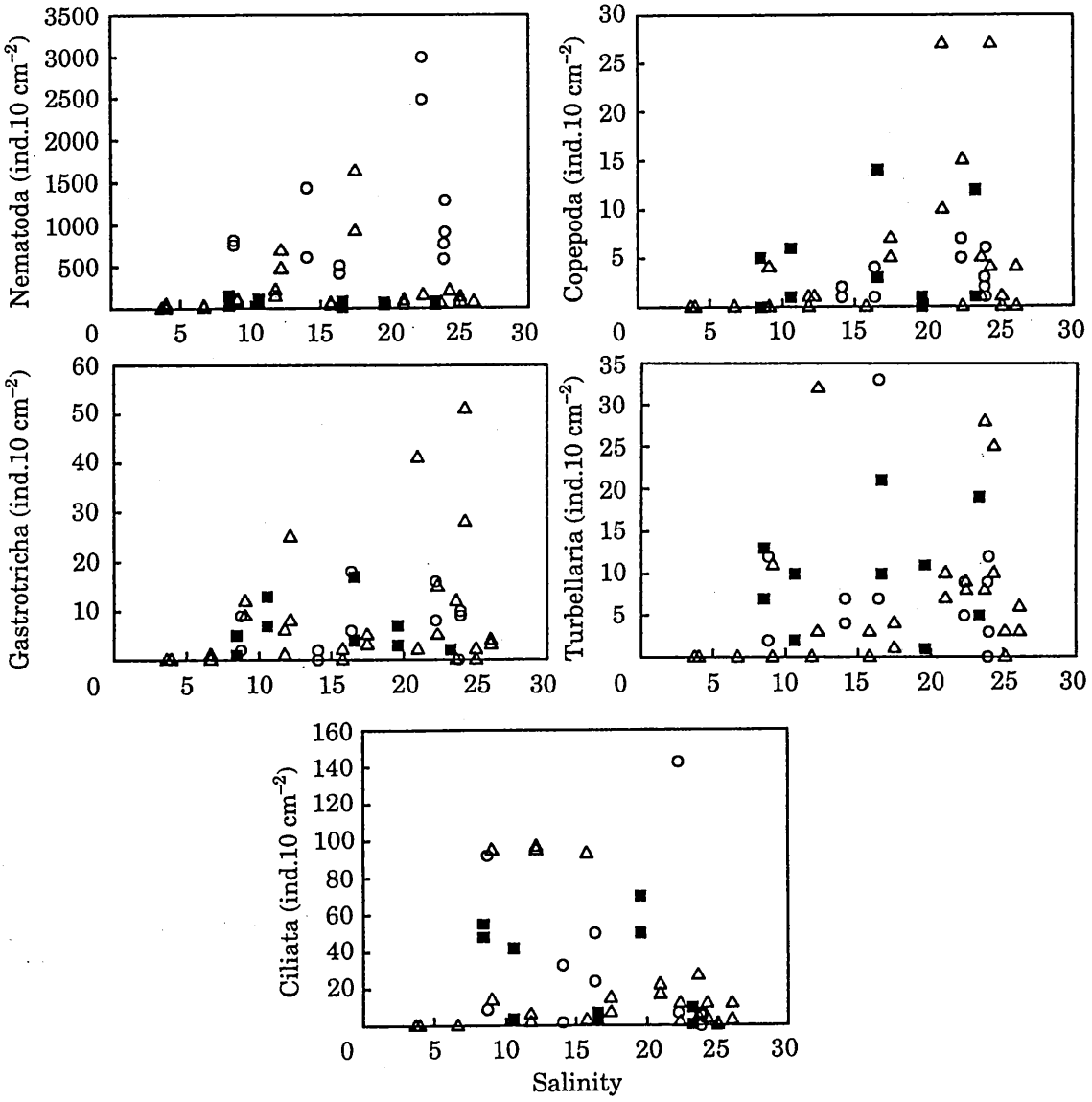

Figure 5. Vertically integrated density of various meiobenthic taxa along the salinity gradient. ○, Intertidal; △, subtidal; ■, channel.

nutrient load of the sediment: relatively high concentrations of ammonium and silicate were observed in sediment with a median grain size less than about 150–200 μm. Nitrate content was low in the most silty sediments and highest in those with intermediate grain sizes (Figure 3). Phosphate concentrations were rather constant.

Meiobenthos densities (Figure 4) were higher in the intertidal zone (482–3076, mean 1204 ind. 10 cm^{-2}) than in most permanently submersed stations. When excluding the channel and the subtidal sites most upstream, sublittoral densities varied from 67 to 1666 (mean 235 ind. 10 cm^{-2}). The channel stations always had very low meiobenthos densities (43–282, mean 137 ind. 10 cm^{-2}). The most upstream subtidal stations were nearly devoid of meiobenthic life (2–46, mean 16 ind. 10 cm^{-2}). There was no significant trend of meiobenthic density with either the sedimentary or salinity gradient, but densities tended to have a broader range with increasing salinity. Nematodes were in most instances the most important member of the meiobenthos, and this dominance was especially well pronounced in the intertidal zone (81–98%, Figure 4). Many subtidal and

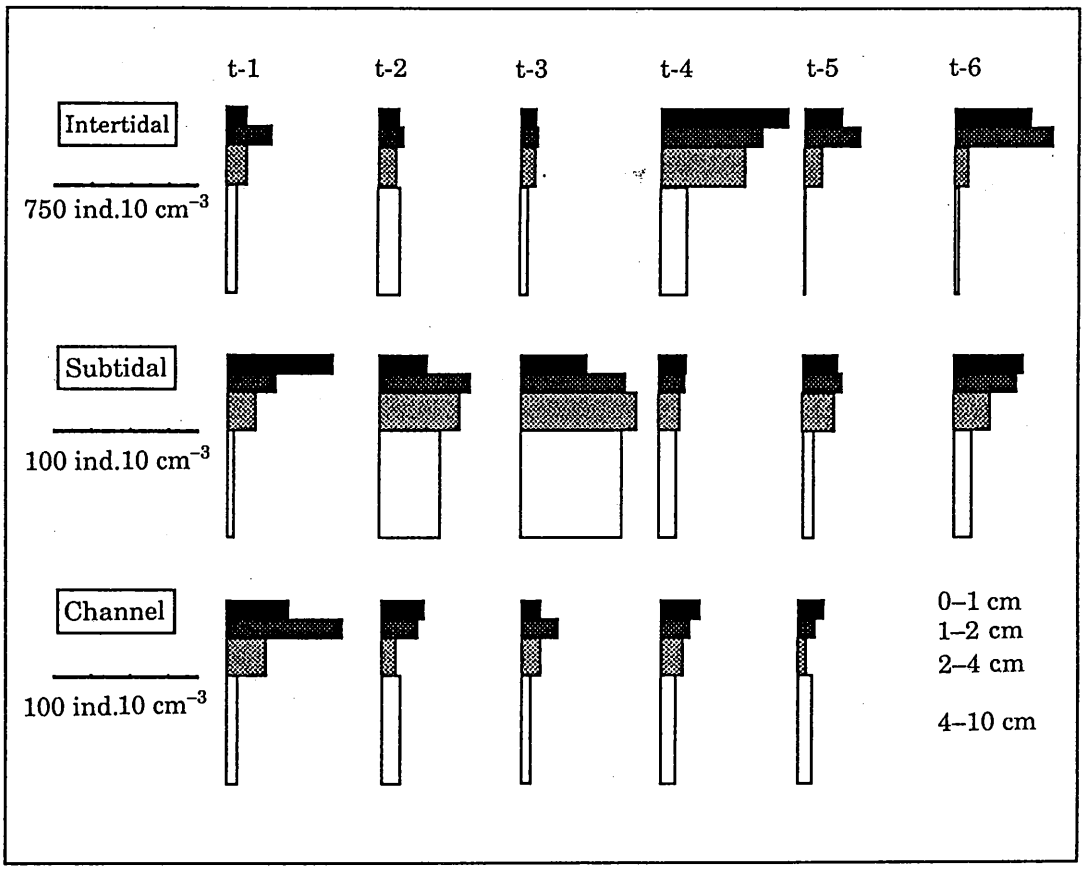

Figure 6. Vertical distribution (0–1, 1–2, 2–4 and 4–10 cm) of total meiobenthic density for intertidal, subtidal and channel stations (six transects).

channel stations on the other hand were characterized by a much higher contribution of other groups: Copepoda, Gastrotricha and, less so, Turbellaria and Ciliata were often both in absolute and relative terms more abundant in the permanently submersed areas (Figure 5). The nematode contribution to total meiofauna density in these areas was in many instances lower than 60% and in some places dropped to less than 30% (Figure 4).

The vertical distribution of total meiobenthos was such that the second centimetre was usually most densely populated in the intertidal area, whereas subtidal and channel stations had a more variable vertical distribution, with densities peaking either in the surface layer or deeper down (Figure 6).

A total of 148 nematode species, belonging to 79 genera, and 37 families were found in this study (see Appendix for a species list).

The distribution characteristics of the 33 most abundant species (defined as making up at least 10% of the total community in at least one station and observed in more than three stations) were calculated in relation to salinity, sediment grain, water depth and sediment depth preferences (Figures 7 and 8). Many species showed a preference for some of these factors, the degree of which can be appreciated by comparing with the distribution of total nematode density.

The majority of the species had a predominantly intertidal distribution, but some penetrated fairly extensively into deeper water (Figure 7): *Theristus blandicor, Ascolaimus elongatus, Viscosia viscosa, Theristus pertenuis, Enoplolaimus propinquus* and *Daptonema setosum*. Few were most commonly observed in the permanently submersed area:

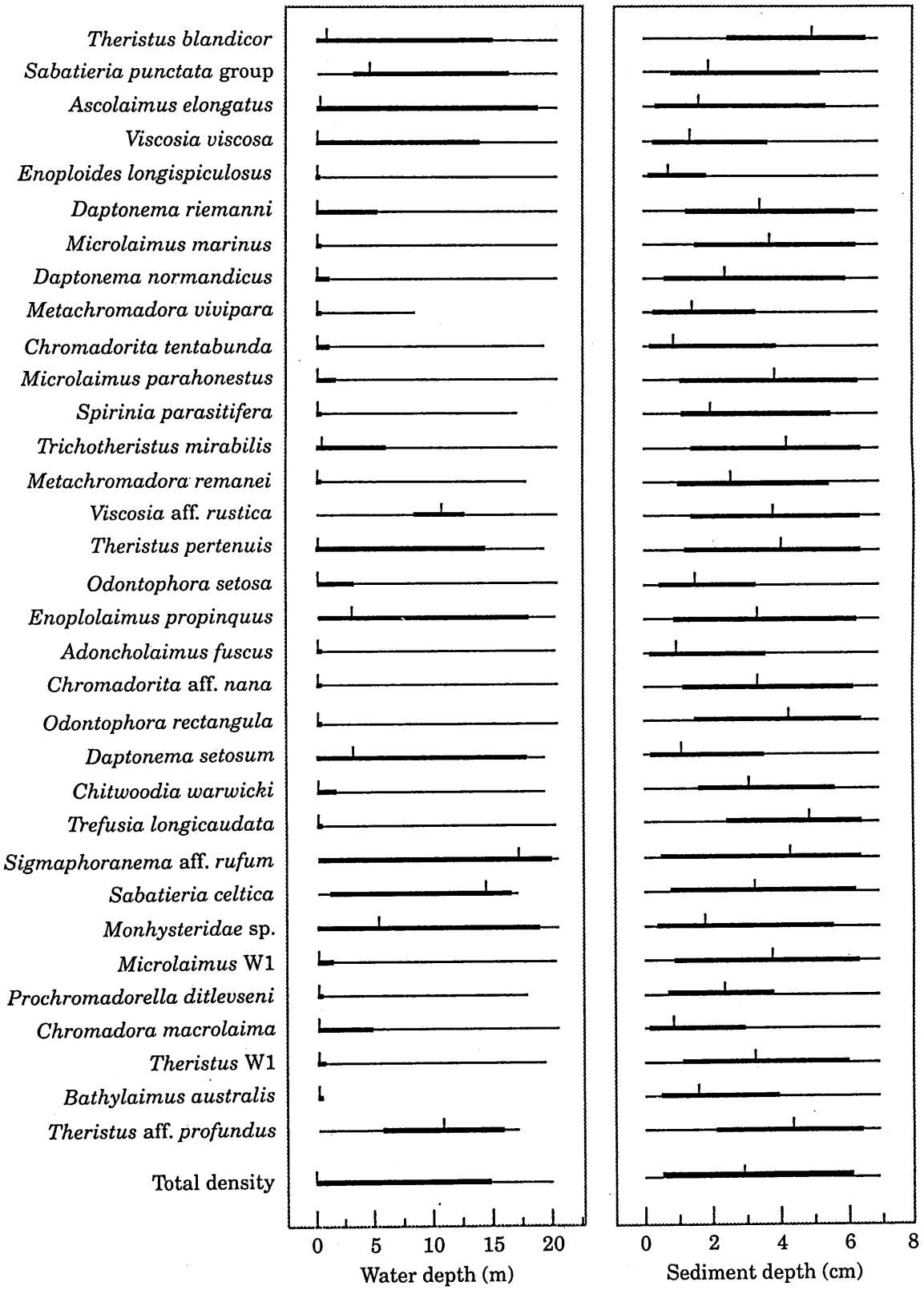

Figure 7. Distribution characteristics of the most abundant nematode species with respect to water depth and sediment depth. Indicated are the total range (horizontal line), the median occurrence (vertical dash) and the 10–90% occurrence (horizontal bar). Species are arranged according to decreasing abundance.

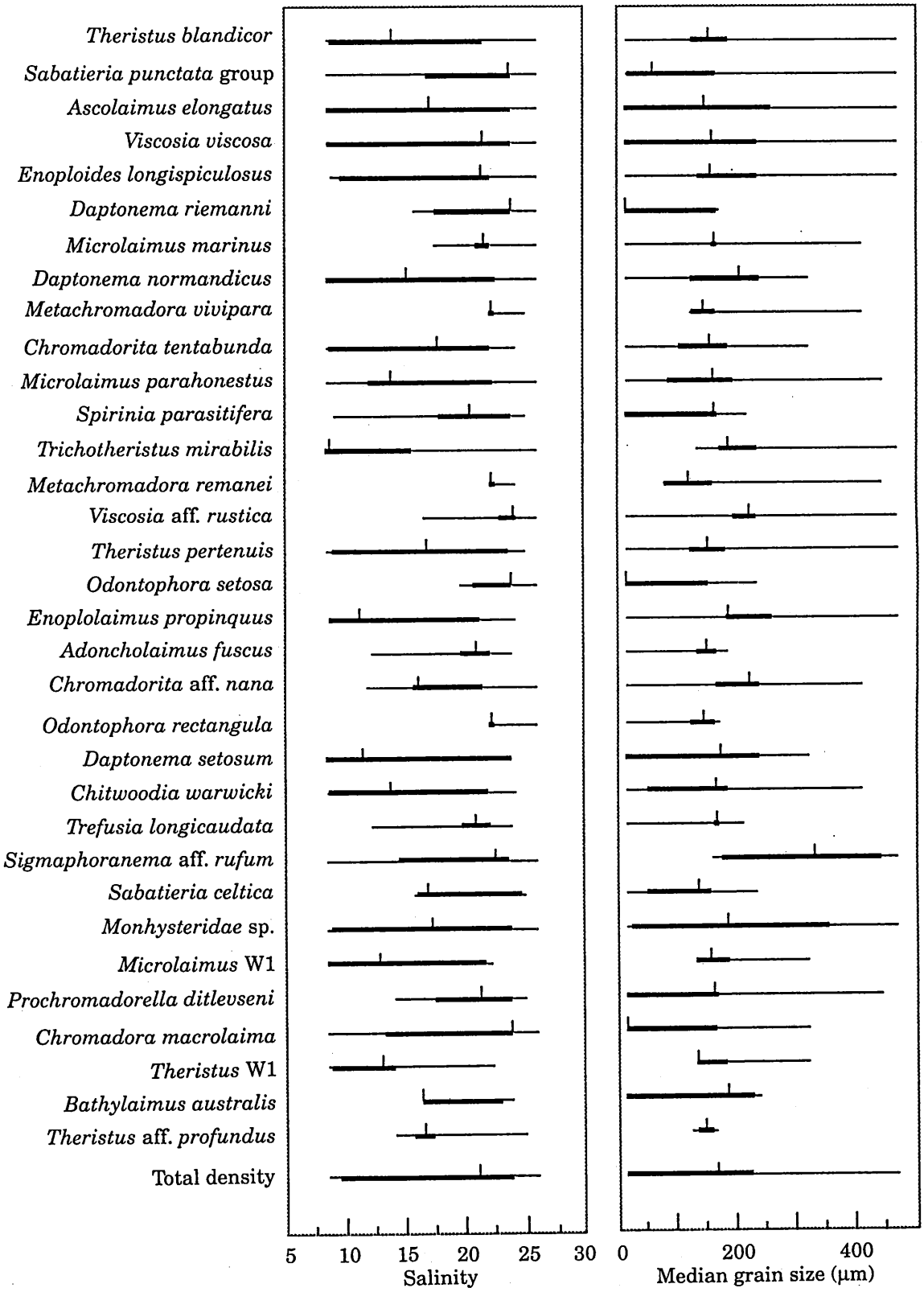

Figure 8. Distribution characteristics of the most abundant nematode species with respect to salinity and median grain size. For further explanation, see Figure 7.

Sabatieria punctata (group) (comprising *S. breviseta* and *S. pulchra*), *Viscosia* aff. *rustica*, *Sigmaphoranema* aff. *rufum*, *Sabatieria celtica*, *Monhysteridae* sp. and *Theristus* aff. *profundus*. For the calculation of sediment depth preferences, it was assumed that

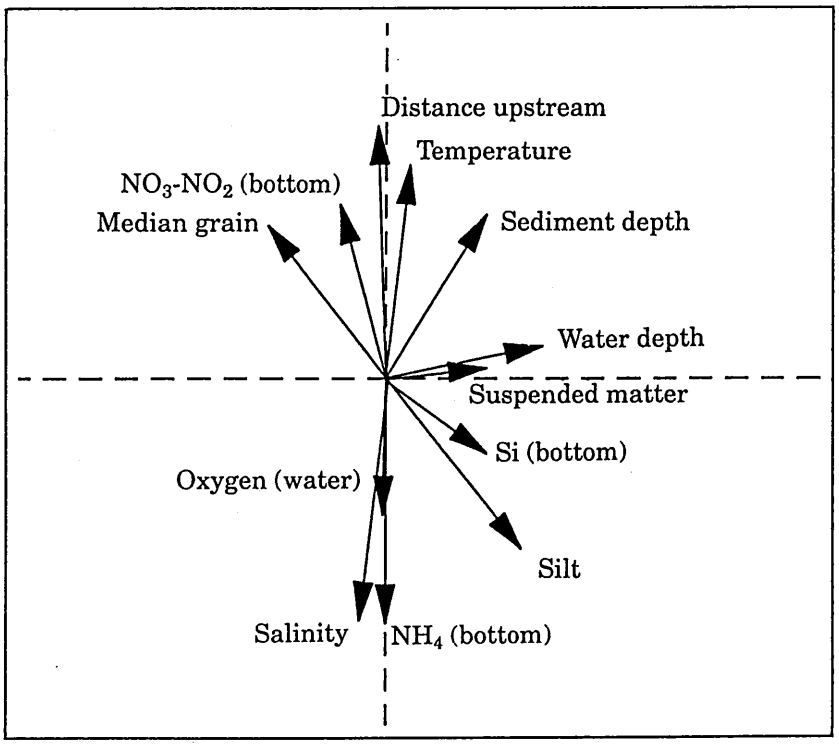

Figure 9. CANOCO plot of environmental variables along the two main ordination axes.

nematodes were evenly distributed in the sediment slices and that nematodes did not penetrate deeper than 7 cm. The median, 10% and 90% occurrence were then calculated as in Sokal and Rohlf (1981). It appeared that the bulk of species extended relatively deep into the sediment and some were real deep-dwelling species, having more than half of the population deeper than 3 cm: *Daptonema riemanni, Microlaimus marinus, Odontophora rectangula* and *Trefusia longicaudata* in the marine part; *Theristus blandicor, Microlaimus parahonestus, Trichotheristus mirabilis, Enoplolaimus propinquus, Chromadorita* aff. *nana, Chitwoodia warwicki* and *Microlaimus* W1 in the brackish part of the estuary (Figures 7 and 8). *Theristus pertenuis* was observed in the deeper sediment layers of both the marine and brackish stations. *Viscosia* aff. *rustica, Sigmaphoranema* aff. *rufum* were observed in the deeper layers of the marine subtidal; *Theristus* aff. *profundus* and *Sabatieria celtica* were brackish subtidal deep-dwelling species. Only few species occurred predominantly in the first centimetre and could be considered as real surface dwellers. They were *Adoncholaimus fuscus* and *Chromadora macrolaima* (marine part), *Daptonema setosum* (brackish area), *Enoploides longispiculosus* and *Chromadorita tentabunda* (all salinity regimes).

A canonical correspondence analysis on nematode species composition revealed upstream distance (with correlated salinity, oxygen and temperature), grain characteristics (median grain, silt content), water depth and sediment depth to be important parameters in the determination of community structure (Figure 9). In Figure 10, the position of samples in two-dimensional CANOCO space is represented with indication of the vertical distribution (arrow). Although the sediment slices of all samples were combined into one analysis, the samples belonging to different transects and morphological units are plotted separately for convenience. There is a pronounced vertical gradient in the intertidal samples: subsequently deeper sediment slices follow a

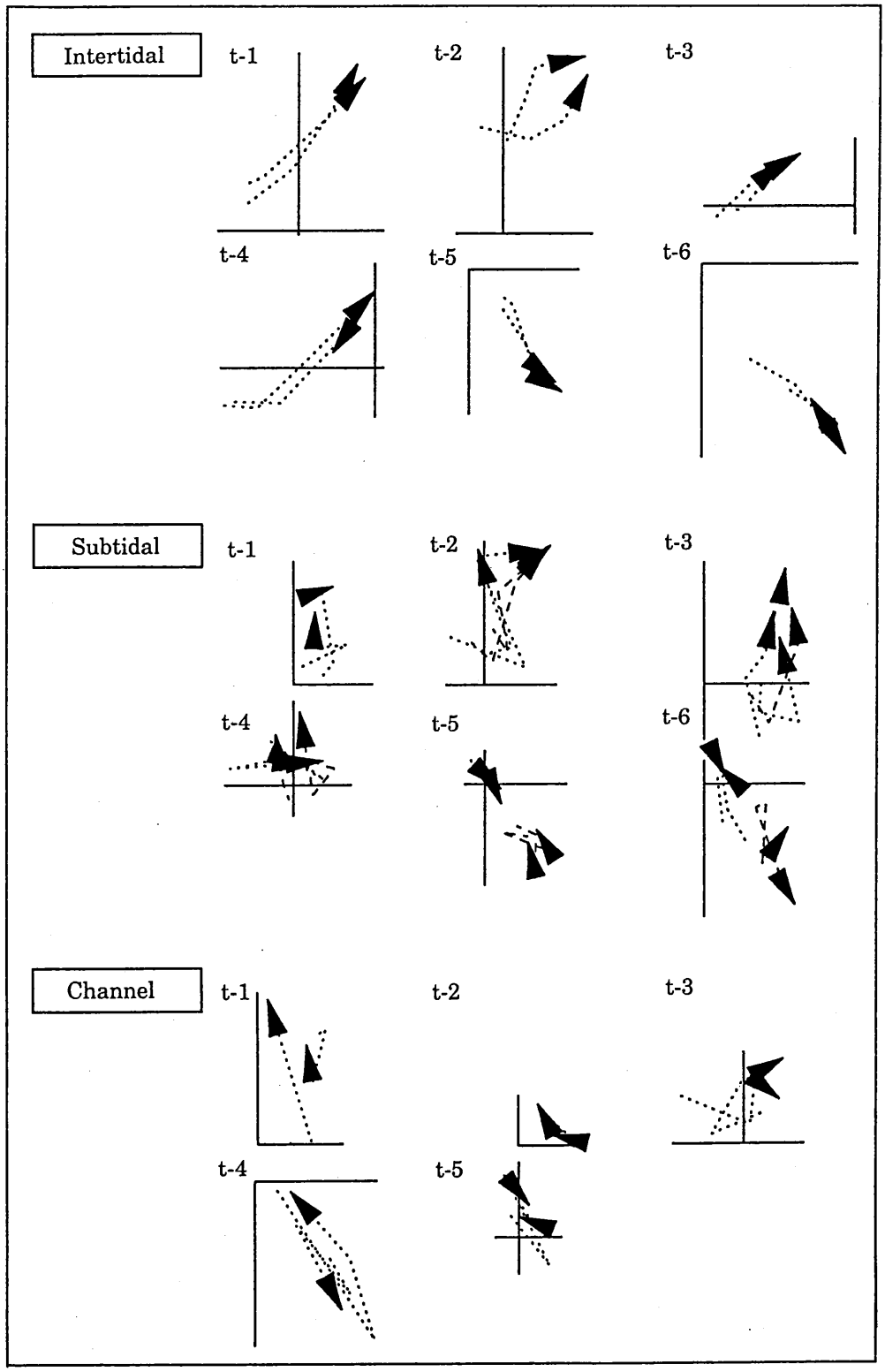

Figure 10. CANOCO plot of the various sediment slices along the same ordination axes as in Figure 9. For clearness' sake, the samples (consisting of four sediment slices and two replicates) of different morphological types and transect numbers (t-1 to t-6) are plotted separately. For each replicate, the arrow represents the vertical gradient. It connects the position of the 0–1-cm slice in CANOCO space with the 1–2-cm slice, the 2–4-cm slice and ends with the position of the 4–10-cm slice.

TABLE 1. Relative abundance (%) of the most dominant nematode species (>10% of total abundance in at least one sediment layer) in the sediment layers of the intertidal samples of transects 5 and 6

Species	Sediment layer (cm)			
	0–1	1–2	2–4	4–10
Transect 5				
Viscosia viscosa	10	7	1	1
Daptonema riemanni	2	6	12	4
Sabatieria punctata group	27	62	70	82
Transect 6				
Ascolaimus elongatus	25	15	3	2
Viscosia viscosa	24	15	2	2
Daptonema riemanni	2	13	57	68
Sabatieria punctata group	2	10	2	2
Metalinhomoeus aff. *biformis*	0	1	2	10
Odontophora setosa	9	15	4	0

more-or-less straight path and there is a strong resemblance of subsamples. Subtidal and channel stations showed a much more distorted pattern and less resemblance among replicates. The same analysis was done excluding the intertidal stations, and now some of the subtidal stations showed a more straight course in two-dimensional CANOCO space, but most were still heavily erratic (not depicted).

Whereas the arrows representing the vertical structure in the intertidal of the transects 1–4 (Figure 10), are parallel to the environmental axis of sediment depth (Figure 9), this is not the case for the intertidal stations of transects 5 and 6. These stations had silty sediments and subsequently deeper sediment layers are stretched along the axis indicating increasing silt content. Yet all these sediment layers have the same granulometric characteristics. This phenomenon can be explained by the fact that nematodes of the superficial layers occur in more sediment types whereas the distribution of nematode species observed in the deeper layers is more restricted to silt. In Table 1 the relative abundance of the most dominant species in the different sediment layers is represented. In the uppermost sediment layers of the intertidal station of transect 6, *Ascolaimus elongatus* and *Viscosia viscosa* were most dominant. Both species attain greatest abundance in more sandy sediments (see Figure 8). With increasing depth, species like *Daptonema riemanni* and *Metalinhomoeus* aff. *biformis* became more important. The first species was also observed in sandy sediments, but was most prominent in silts (Figure 8), the latter species was exclusively found in silts (not depicted). *Odontophora setosa* and *Sabatieria punctata* (group), also silt-colonizing species (Figure 8), were most prominent in the second layer of sediment. In the intertidal station of transect 5, *Viscosia viscosa* (most common in sand) was most prominent in the upper sediment layers, while *Sabatieria punctata* (group) became more dominant with increasing depth into the sediment. Here too *Daptonema riemanni* (silt-colonizing species) was prominent in the deeper layers.

Nematode diversity of whole samples was somewhat higher in the intertidal compared to many permanently submersed areas for a comparable salinity regime [Figure 11(a,b)]. However, as diversity indices are sensitive to the number of individuals on which they were calculated (Soetaert & Heip, 1990), this trend in whole sample diversity partly

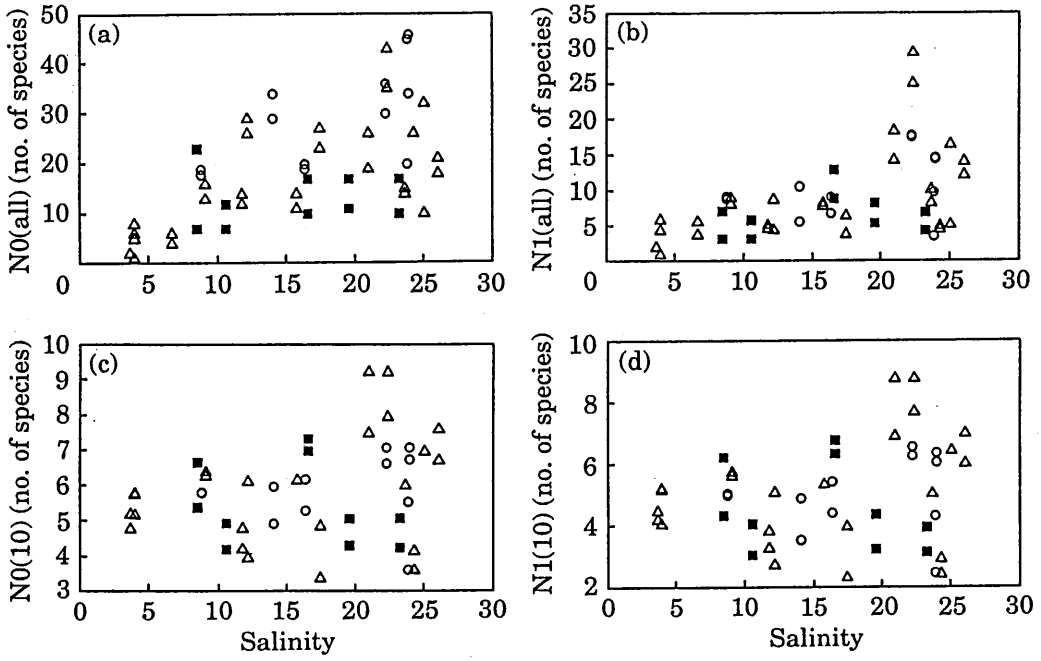

Figure 11. Nematode diversity indices (Hills number of order 0 and 1), based on total abundance (a,b) and on 10 randomly drawn individuals (c,d) along the salinity gradient. N0 is equivalent to the number of species, N1 equals exp (H') where H' is the Shannon–Wiener diversity index. ○, Intertidal; △, subtidal; ■, channel.

reflects density differences. Using this method, one is likely to find more species in an intertidal sample, with about 1000 nematodes, compared to a subtidal site where only 20 nematodes are present. To correct for the density dependence of diversity indices, the diversity of a common number of individuals (i.e. 10) was also calculated [Figure 11(c,d)]. There was no real trend with salinity nor other abiotic parameters, nor could a difference between the gross morphological units be detected. Due to the low nematode densities in many submersed sites and the relatively high diversity there, the mean density per species is very low. When taking into account only stations with a total density of less than 100 ind. 10 cm^{-2} it appears that 50% of the species had an abundance of less than 1 ind. 10 cm^{-2} and 90% of all species had densities of less than 6 ind. 10 cm^{-2}.

Discussion

An estuary such as the Westerschelde is a very dynamic environment. With the incoming tide, sediment is resuspended, carried inward and settles when the current velocities are low. With the outgoing tide the process is repeated, now shifting sediment downstream but typically the current velocities are lower and hence resuspension of the sediment is not so pronounced (Postma, 1967). This results in a net transport of particles towards the tidal flats and upstream (Peters & Sterling, 1976; de Jonge, 1985). In the intertidal zone, currents will rework and sort the sediments during submersion, a process which is amplified by wind-induced waves and storms. The eroded matter is partly redistributed onto the intertidal flat, but some of it is transported to the deeper parts, where it is incorporated in the global estuarine circulation.

Within the estuary, muds will be deposited predominantly in the more sheltered areas, while the high current velocities in the deep channels prevent small particles from

settling there (Oenema *et al.*, 1988). Along with the finer sedimentary material, organic matter is deposited and subsequently degraded, a process which ultimately produces reduced conditions and ammonium. Thus, in the current study one can distinguish between sandy sediments with a low ammonium and silicate load but a higher nitrate content and more fine-grained sediments where the nutrient balance is reversed. The transition between both sedimentary types seemed to lie at 150–200 μm median grain size. The coarse type of sediment was typical for channels and also occurred in some subtidal and intertidal samples; the fine type was observed only in the intertidal and subtidal.

Apart from the larger amount of sedimented organic matter in some intertidal sites, phytobenthos primary production is mainly restricted to these regions and many meiobenthic animals are known to feed on the unicellular algae (Admiraal *et al.*, 1983; Bouwman, 1983; Heip *et al.*, 1985). This at least partly explains the higher meiobenthic densities observed in the intertidal (Bouwman, 1983; Smol *et al.*, 1994; this study). In the Westerschelde, algal feeders comprised about 20% of the total nematode population in the intertidal, while they accounted for less than 10% in the permanently submersed sites.

The greater stability of the sediment environment in the intertidal could provide an additional explanation for the higher densities of nematodes there. Although intertidal sediments are also subjected to a resuspension–sedimentation cycle when flooded, current velocities are somewhat smaller here than in the channels and much of the sediment is redistributed on the tidal flat. Sediments in the sublittoral and especially in the channels experience higher current velocities and are transported over larger (net upstream) distances. Moreover, dredging and (less so) dumping activity mainly affects sublittoral communities. As opposed to nematodes, harpacticoids, gastrotrichs and, less so, turbellarians and large ciliates had higher densities in the permanently submersed areas. This was also the case in the Eastern Scheldt (Smol *et al.*, 1994) and it could indicate that these latter groups are better adapted to sediment disturbances. The predominantly interstitial life-style of, for example, Gastrotricha could further explain why they are so scarce in the more fine-grained intertidal.

Compared to the other meiobenthic groups, nematodes need an intimate contact with the sediments, both for moving (undulatory propulsion) and feeding, and sediments which are in constant turmoil may thus interfere with their basic requirements. This could be the case in many subtidal stations. Moreover, the densities of most nematode species are extremely low at many of these sublittoral sites and this may prohibit their sexual reproduction (for which a minimal specific density is required). It is, thus, very likely that these communities are not self-sustaining but are constantly replenished from less marginal sites. The ability of nematodes to survive in hostile conditions could then allow them to persist at least for a period of time after which they will die. Indicative in this respect was the presence of many badly fixed nematodes in the low-density sublittoral sites, which suggested that many were dead before fixation took place.

The most obvious way of large-scale transport of nematodes is by means of the currents and it has been shown that individual nematodes become suspended in the water column (Palmer & Gust, 1985; Palmer, 1988). Nematodes were also shown to be present in the Westerschelde plankton and were especially abundant near to the turbidity maximum. Here their density (some 10 ind. l^{-1}, i.e. about 100 ind. 10 cm^{-2} in spring 1990), even exceeded the, albeit low, densities in the subtidal bottom beneath (data from

Soetaert & Van Rijswijk, 1993). This involuntary entering of nematodes into the water phase, and their displacement to other sites may be responsible for the continuous repopulation of the subtidal areas.

If the meiobenthic density in many submersed areas was rather low, the four subtidal sites in the most upstream part (salinity <7) were nearly devoid of meiobenthic life: in two stations densities of less than 10 ind. 10 cm^{-2} were found and the maximum density was 46 ind. 10 cm^{-2}. This area is near to the turbidity maximum and it is the site of deposition of polluted sediments (Van Eck & de Rooij, 1990) and consequently lots of dredging takes place (Belmans, 1988). Moreover, the organic and inorganic pollution is very high here. Microbial degradation of these organics results in low oxygen concentrations in the water and near-anoxia is observed during summer months. Finally, pelagic primary productivity is lowest in this region (Kromkamp *et al.*, 1992) which could result in less high-quality food for the benthos. Probably a combination of some of these factors is responsible for the aberrantly low meiobenthic densities here.

Intertidal areas are subjected to cyclic flooding and drying, which will impose stress to the benthos there. This stress is partly due to the sinking groundwater in the sand- and mudflats, but the water table typically does not sink more than 0–2 cm below the surface at low tide (Oenema *et al.*, 1988). Apart from desiccation stress, the absence of the buffering capacities of the overlying water will induce more extreme fluctuations of, for example, temperature and salinity in the upper sediment layers, and this too will affect the benthos. Resuspension and sedimentation processes also act predominantly on the upper layers of the sediment. Hence one observes that the intertidal meiobenthic community in general, and the majority of nematode species in particular, penetrate relatively deep into the sediment, and only few species are real surface dwellers. This is in contrast with the vertical distribution in many subtidal sediments where a high degree of surface accumulation is usually observed (Coull, 1988). In the subtidal area of the Westerschelde, vertical density distributions exhibit either a surface or a subsurface maximum and the nematode community gradients are at least different from gradients in the intertidal areas and in many instances they show a distorted pattern. These atypical vertical distributions are a further indication of the deteriorating effects of human and natural physical disturbances on the subtidal and channel communities.

The distribution of nematode species in the estuary, and consequently the composition of assemblages, is greatly influenced by the sedimentary and geomorphological heterogeneity. Together with salinity and related gradients, these environmental factors explain many of the differences observed in nematode communities. However, apart from this obvious large-scale horizontal heterogeneity, the sharpest gradients in environmental variables such as light, oxygen, temperature, food etc., occur vertically into the sediment and environmental fluctuations are dampened with increasing depth into the sediment. Being small, meiobenthic organisms, and nematodes in particular, strongly experience these small-scale gradients and their distributions are likely to reflect their environmental tolerances. Although on average nematode species had a relatively broad vertical range, a variety of vertical distributions were observed in this study. Moreover, although acting on the scale of centimetres, the effect of sediment depth on community structure proved to be as important as the other abiotic variables which act on the scale of hundreds of metres. Similarly, Joint *et al.* (1982) showed that nematodes and copepods in the intertidal had a typical vertical distribution even on a much finer scale than reported here, though with a broad overlap of species.

Whereas information on the vertical structure is interesting, it is not a prerequisite to include vertical heterogeneity in many studies. Core samples are by definition integrated in the vertical sense and it is much more difficult to deal with horizontal patchiness adequately. However, our ecological understanding can sometimes greatly profit from knowledge on the vertical structure. It has, for instance, been argued that the vertical separation of species will reduce the number of (competitive or predative) interactions, and this could explain the often very high number of species that coexist in a certain small patch (Joint *et al.*, 1982). Species that occur in the surface layers will be more susceptible to epibenthic predation and the vertical distribution will also result in a differential resuspension and transport of nematodes with the sediment. Thus, Warwick and Gee (1984) showed that an abundant surface-dwelling nematode in the Tamar Estuary migrated quickly along with the changing sediment movement, whereas deeper dwelling species did not. Something similar was observed in the intertidal silty sediments of transects 5 and 6: nematode species that were present in the upper layers of these sediments had a more general distribution compared to deeper dwelling species of the same stations. This could be explained by the fact that surface dwellers are more amenable to resuspension and are more easily transported to other areas. It could be interesting to see whether these species also show a more rapid response in time as was the case in the Tamar Estuary (Warwick & Gee, 1984).

The density values found in the intertidal of the Westerschelde in this study are comparable with values from Van Damme *et al.* (1980) from a previous study of the same area, but they are lower than what has been found in an estuary nearby, the Oosterschelde (Smol *et al.*, 1994). Soetaert *et al.* (in press) compared the intertidal densities in the Westerschelde with other estuaries, and concluded that although the abundances in the marine part of the estuary were somewhat lower than in many other estuaries, Westerschelde densities were not aberrant. In the sheltered salt marsh of Saaftinghe, peak densities of 50 000 ind. $10\,cm^{-2}$ were reported by Claassen (1991). Thus, although Saaftinghe is located at a short distance from transects 1 and 2 (this study), it has densities that are almost two orders of magnitude higher than the nearby intertidal sites. Van Damme *et al.* (1980) also observed higher densities and biomasses here and attributed it to the larger amount of organics present and the more sheltered position with respect to extreme environmental conditions and direct pollution.

The intertidal area of the Westerschelde differed from five other estuaries mainly by the near-absence of harpacticoid copepods (Soetaert *et al.*, in press). This feature was also observed by Van Damme *et al.* (1984) who attributed it to chemical pollution effects. In the current study, slightly higher copepod densities were observed in the subtidal of the Westerschelde but these were still much lower than what was observed in the subtidal zone of the Eastern Scheldt (Smol *et al.*, 1994) and in some stations in the Tagus (unpubl. data). Thus, it appears that the trend of low harpacticoid abundance in the Westerschelde is consistent both in the intertidal and in the subtidal zone.

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Appendix

Species list, indicating the maximum density (10 cm^{-2}) of each species in the Westerschelde. *0–4, **4–16, ***16–64, ****64–256, *****256–1024 individuals.

Enoplida	
Enoplidae	
Enoplus sp.	*
Thoracostomopsidae	
Enoploides longispiculosus	*****
Enoplolaimus propinquus	****
Anoplostomatidae	
Anoplostoma W2	*
Anoplostoma viviparum	**
Ironidae	
Syringolaimus sp.	*
Oxystominidae	
Halalaimus gracilis	***
Thalassoalaimus septentrionalis	*

(*Continued*)

Oncholaimidae	
Adoncholaimus fuscus	****
Oncholaimellus calvadosicus	*
Oncholaimellus mediterraneus	**
Oncholaimus oxyuris	*
Viscosia aff. *rustica*	****
Viscosia viscosa	****
Enchelidiidae	
Calyptronema maxweberi	**
Tripyloididae	
Bathylaimus australis	***
Bathylaimus stenolaimus	**
Bathylaimus tenuicaudatus	**
Tripyloides gracilis	**
Trefusiida	
Trefusiidae	
Trefusia W2	*
Trefusia W3	*
Trefusia longicaudata	****
Chromadorida	
Achromadoridae	
Achromadora sp.	*
Chromadoridae	
Atrochromadora microlaima	*
Chromadora axi	*
Chromadora macrolaima	***
Chromadorita aff. *nana*	****
Chromadorita sp.	*
Chromadorita tentabunda	****
Dichromadora cephalata	**
Dichromadora cucullata	*
Dichromadora geophila	***
Euchromadora sp.	*
Hypodontolaimus W1	**
Hypodontolaimus shuurmansstekhoveni	*
Prochromadorella ditlevseni	***
Ptycholaimellus ponticus	**
Spilophorella candida	*
Spilophorella paradoxa	**
Comesomatidae	
Sabatieria celtica	****
Sabatieria punctata (group)	*****
Setosabatieria hilarula	*
Ethmolaimidae	
Neotonchus aff. *cupulatus*	*
Cyatholaimidae	
Cyatholaimidae sp.	*
Paracanthonchus heterodontus	*
Paracanthonchus thaumasius	***
Paracyatholaimoides W1	**
Paracyatholaimus W1	**
Paracyatholaimus pentodon	*
Pomponema sp.	*
Praeacanthonchus punctatus	**

(*Continued*)

Selachinematidae	
Richtersia inaequalis	*
Desmodoridae	
Metachromadora aff. *suecica*	***
Metachromadora remanei	****
Metachromadora vivipara	*****
Molgolaimus cuanensis	*
Molgolaimus turgofrons	**
Onyx sagittarius	*
Sigmaphoranema aff. *rufum*	***
Sprinia parasitifera	****
Southernia zosterae	**
Microlaimidae	
Microlaimus W1	***
Microlaimus W3	*
Microlaimus W4	*
Microlaimus arenicola	**
Microlaimus globiceps	**
Microlaimus marinus	*****
Microlaimus parahonestus	****
Microlaimus robustidens	**
Monoposthiidae	
Monoposthia mirabilis	**
Leptolaimidae	
Camacolaimus tardus	**
Dagda bipapillata	*
Deontolaimus papillatus	*
Leptolaimus acicula	*
Leptolaimus aff. *membranatus*	*
Leptolaimus aff. *minutus*	**
Leptolaimus ampullaceus	**
Leptolaimus elegans	**
Leptolaimus papilliger	*
Leptolaimus sp.	*
Stephanolaimus elegans	*
Haliplectidae	
Haliplectus dorsalis	*
Aegialoalaimidae	
Cyarthonema W1	*
Southernia zosterae	**
Tubolaimoididae	
Chitwoodia warwicki	***
Ceramonematidae	
Dasynemoides albaensis	*
Monhysterida	
Monhysteridae	
Monhysteridae sp.	**
Xyalidae	
Daptonema cfr Bouwman	**
Daptonema W1	**
Daptonema normandicus	****
Daptonema riemanni	****
Daptonema setosum	***
Daptonema sp.	**
Daptonema tenuispiculum	**

(Continued)

Metadesmolaimus 2	*
Metadesmolaimus gaelicus	**
Paramonohystera aff. *albigensis*	**
Paramonohystera sp.	*
Theristus W1	***
Theristus (Penzancia) W1	*
Theristus W5	**
Theristus aff. *profundus*	***
Theristus blandicor	*****
Theristus cfr *subcurvatus*	**
Theristus ensifer	**
Therisuts interstitialis	**
Theristus longus	**
Theristus pertenuis	***
Theristus sp.	*
Trichoteristus W3	*
Trichotheristus mirabilis	****
Xyala striata	*
Sphaerolaimidae	
Sphaerolaimus sp.	**
Siphonolaimidae	
Siphonolaimus sp.	*
Linhomoeidae	
Eleutherolaimus iniquisetosus	*
Eleutherolaimus stenosoma	***
Eumorpholaimus sp.	*
Linhomoeidae W1	*
Linhomoeidae W2	**
Linhomoeidae W4	*
Linhomoeidae W5	**
Linhomoeidae sp	**
Megadesmolaimus W1	***
Metalinhomoeus aff. *biformis*	***
Metalinhomoeus biformis	***
Terschellingia communis	*
Axonolaimidae	
Ascolaimus elongatus	****
Axonolaimus paraspinosus	**
Odontophora W4	***
Odontophora aff. *paravilloti*	*
Odontophora rectangula	****
Odontophora setosa	****
Diplopeltidae	
Diplopeltula W1	*
Diplopeltula asetosa	*
Diplopeltula belgica	*
Pararaeolaimus nudus	**
Rhabditida	
Cephalobidae	
Cephalobus sp.	**
Diploscapteridae	
Diploscapter sp.	*
Odontopharyngidae	
Odontopharynx sp.	**

(*Continued*)

Tylenchida	
Aphelenchoididae	
Aphelenchoididae sp.	*
Tylenchida	
Tylenchida W1	*
Dorylaimida	
Dorylaimida	
Dorylaimida W1	*
Dorylaimida W2	*
Dorylaimida W3	*
Indeterminata	
Indeterminata	
Indeterminata sp.	***

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Meiobenthic distribution and nematode community structure in five European estuaries

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Key words: Meiobenthos, nematoda, estuary

Abstract

Meiofauna from the intertidal zone of five European estuaries (Ems, Westerschelde, Somme, Gironde, Tagus) was investigated. Samples represented a cross section of various benthic habitats from near-freshwater to marine, from pure silts to fine-sandy bottoms. The meiobenthic community comprised everywhere a fauna strongly dominated by nematodes, with meiobenthic density increasing with increasing salinity. The Ems differed from the other estuaries due to the presence of a well developed community of Copepods, Gastrotrichs, large Ciliates and/or soft-shelled Foraminiferans in some sites. The Westerschelde stood out due to the near-absence of harpacticoid copepods and, as in the Tagus, the lower meiobenthic densities in the marine part of the estuary. For nematode community analysis, we also included data from the Tamar which were obtained from the literature (Warwick & Gee, 1984). This resulted in the enumeration of 220 species, belonging to 102 genera, each with a characteristic distribution along the salinity, sedimentary and latitudinal gradients. Using the multivariate technique CANOCO, a zonation along these different physicochemical determinants was observed as well although salinity and sediment characteristics (scale of hundreds of meters to kilometers) proved to be more important in explaining community structure than latitudinal differences (scale of hundreds of kilometers). Nematode diversity was nearly entirely determined on the genus level and was positively related to salinity. Deviations from this general trend in the Gironde and the Tamar were attributed to sedimentary characteristics or to low macrobenthic predation. The presence of a typical opportunistic colonizing nematode species *Pareurodiplogaster pararmatus* in the low-salinity region of the Gironde could indicate (organic?) pollution or disturbance of the intertidal mud-flats.

Introduction

Meiobenthic communities in European estuaries have only been studied in the U.K. (Capstick, 1959; Warwick, 1971; Warwick & Price, 1979; Warwick & Gee, 1984; Moore, 1987; Austen & Warwick, 1989), Germany (Gerlach, 1953; Riemann, 1966; Skoolmun & Gerlach, 1971), the Netherlands (Van Damme *et al.*, 1980; Bouwman, 1983; Smol *et al.*, 1994) and northern France (Gourbault, 1981). No data exist on more southern estuaries. Moreover, intercomparison between estuaries is complicated by taxonomic difficulties. Especially in the past, the chaotic taxonomy of *e.g.* the nematodes made this taxon only accessible to the specialist (Gerlach, 1980). Thanks to the publication of pictorial keys (Platt & Warwick, 1983; 1988) nematode identification is now much easier – at least to the genus level. However, the identity of many species remains problematic. If different estuaries are investigated by the same researcher, as in this study, it becomes more easy to distinguish within-species variability from between-species variability and, although determinations may not always be exact, this will introduce more consistency into the results.

This study is part of a general research program, which aims at the understanding of major biological processes in European tidal estuaries (MAST CEC project, JEEP92). As part of this programme, a base-

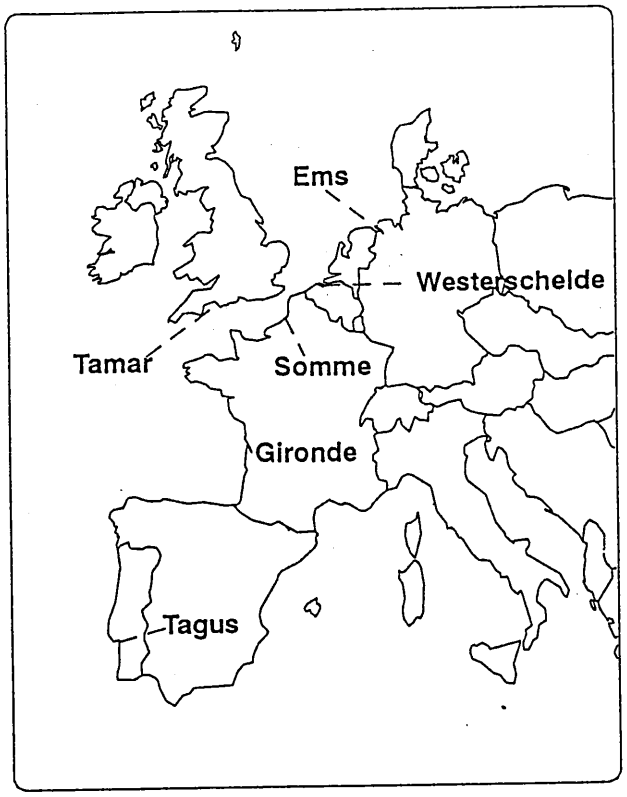

Fig. 1. Location of the various estuaries under study.

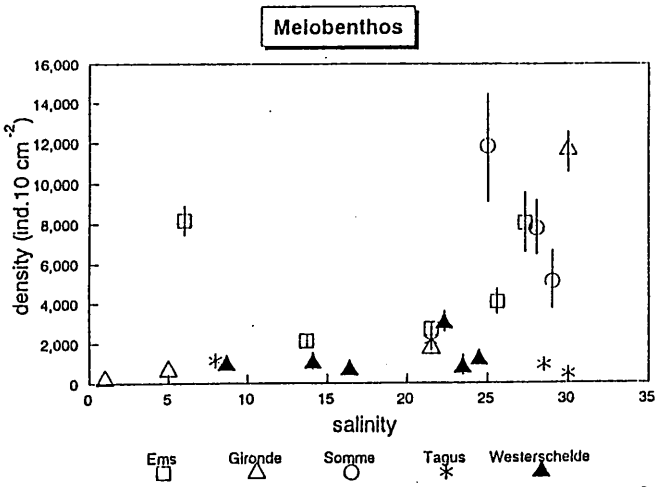

Fig. 2. Mean meiobenthos density and total range (ind 10 cm^{-2}) for the five European estuaries under study.

line study of the meiobenthos in five European estuaries was made, with emphasis on the nematode species composition. For this purpose, data on the Tamar estuary, as provided in Warwick & Gee (1984) were included. The distribution of the most important species and the influence of large-scale spatial structure on the community level are reported.

Material and methods

Samples were taken from intertidal areas of the Ems, the Tagus, the Gironde, the Somme (April 1992), and the Westerschelde (April 1990) along a salinity gradient (Fig. 1). A total of twenty five sites were sampled, and at least two replicates (10 cm^2) per station were taken with plastic cores for meiofauna and an additional one for sediment analysis. Samples from the Gironde were taken from the mean tidal level and subdivided into 0–1 and 1–5 cm slices. Samples from the Westerschelde were also vertically subdivided. However, information on the vertical distribution was not retained in this study. Vertical distribution of nematodes in the Westerschelde is discussed in Soetaert *et al.* (1994).

Salinity was measured in the water column. All sediment and meiofauna samples were treated in the same laboratory (Ghent). Sediment particle size distribution was determined using Coulter LS particle size analysis equipment.

The meiofauna was extracted using LUDOX TM as described in Heip *et al.* (1985). After colouring with Rose Bengal the meiofaunal taxa (i.e. those animals retained on a 38 μm sieve) of two replicates were enumerated after which at least 200 randomly chosen nematodes per replicate were mounted into slides for further identification. If total nematode density was less than 200 individuals, all were processed. Identification to genus – and in some cases species – level was done using the pictorial keys in Platt & Warwick (1983, 1988) and Bongers (1988). For many species further identification required consultation of the relevant literature.

The species-abundance matrix was analysed using the multivariate technique CANOCO (CANOnical COrrespondence analysis; Jongman *et al.*, 1987; Ter Braak, 1989). This method was used to relate the observed trends to the major environmental gradients. Diversity was measured as Hill's diversity numbers of order 0 (i.e. N0, the number of species present), 1 (i.e. N1, the exponential of the Shannon Wiener index), 2 and ∞ (i.e. N∞, the reciprocal of the dominance of the most common species) as suggested by Heip *et al.* (1988). This spectrum of diversity indices gives a clear representation of the influence of rare (lower order indices) and more common (higher order indices) species on diversity. In order to correct for the size-dependency of diversity indices (Soetaert &

Heip, 1990) and as a means of comparing with other studies, the indices were calibrated on 100 randomly chosen individuals as described in Soetaert & Heip (1990). The life-history traits of the communities were summarized into the 'maturity index' as described in Bongers (1990) and Bongers *et al.* (1991). In short, a higher value of this index indicates a higher degree of persistence, a lower value a more opportunistic community.

The different estuarine environments

The estuaries under investigation are situated along a north-south gradient (Fig. 1). The climate changes from temperate (mild winters, relatively cool summers) in the North of the Netherlands, with a well pronounced variation in temperature and a relatively smooth precipitation curve, to warmer in Portugal, where precipitation is very variable.

The Ems estuary (the Netherlands, Germany) connects the north-eastern part of the Netherlands and the north-western part of Germany with the North Sea. It is an important shipping route with three harbours along its side. Sediments are dredged from the estuarine channels and dumped in other parts (de Jonge, 1992). The major freshwater input to the Ems estuary, the river Ems and the Westerwoldsche AA are also important sources of nutrient enrichment (de Jonge & Essink, 1992). In its upper reaches, the Ems estuary has a vast intertidal mudflat: the Dollard. The total average annual discharge varies from 80 to 180 $m^3\ s^{-1}$ with seasonal variation; tidal currents are moderate.

The Westerschelde estuary (the Netherlands) provides the link from the harbour of Antwerp towards the North Sea. It has busy shipping lanes and concurrent dredging and dumping activities of polluted sediments. Large cities on the river banks, and the high degree of industrialization stand for a high input of organics, inorganics and nutrient wastes. The high load of organic matter in the turbidity maximum zone causes strong anoxic conditions in summer (Heip, 1989). The average freshwater flow is about 100 $m^3\ s^{-1}$ and it fluctuates in a seasonal manner.

The Somme (North of France) is an embayment of the Somme and Maye river system and opens to the west in the English Channel; the northern half of the bay is subjected to strong waves from the Channel (Ducrotoy & Sylvand, 1989). The Somme is characterized by a low freshwater output (30 $m^3\ s^{-1}$) and is thus primarily under marine influence. It consists of large intertidal areas with high biomasses of cockles (Rybarczyk *et al.*, 1992).

The Gironde estuary (South of France) is fed by the rivers Garonne and Dordogne and has the city of Bordeaux along its banks. The mean river discharge is high, varying from 600 to 1000 $m^3\ s^{-1}$ and is subjected to seasonal variations. It is a highly turbid estuary but with a distinct turbidity maximum (Castel & Feurtet, 1989).

The Tagus (Portugal, Spain) is the largest river of the Iberic peninsula and is characterized by an extensive intertidal area (>30%) (Moreira *et al.*, 1992). It is flanked by the city of Lisbon which disposes its untreated wastes into the estuary. The port of Lisbon is also an important source of industrial waste in the marine part of the estuary (Gaudencio *et al.*, 1991). Another centre of industrialization causes heavy pollution along a narrow channel in the brackish part (R. Neves, pers. comm.). The freshwater outflow of the Tagus is very variable, both annually and interannually; it varies from 30 to 18 000 $m^3\ s^{-1}$ and the salinity varies concurrently.

The Tamar estuary is situated in South-West England and is characterized by extensive tidal flats and mud banks. River discharge varies in a seasonal way from about 2 to 150 $m^3\ s^{-1}$ (Morris *et al.*, 1985). A turbidity maximum in the upper estuary acts as a trap for particles of marine, estuarine and fluviatile origin (Bale *et al.*, 1985).

Results

Sediments

The majority of intertidal stations had fine-grained sediments although some stations in the three most northerly estuaries were more coarse. Grain characteristics, salinity and name of the stations are presented in Table 1.

Meiobenthos

Meiobenthic taxa observed included Nematoda, Copepoda, Gastrotricha, Plathelminthes, soft-shelled Foraminifera, Ciliata, Polychaeta, Oligochaeta, Ostracoda, Halacarida, Cnidaria, Priapulida and Tardigrada.

Meiobenthos densities (Fig. 2) varied from 130 to 14 500 ind 10 cm^{-2} and were highest in the Somme and the marine part of the Gironde and Ems. Low meioben-

Table 1. Salinity, silt content and median grain size of the intertidal samples.

Estuary	Station	Salinity (‰)	Silt (%)	Median (μm)
Ems	E 6	6	78	18
Ems	E 5	14	16	124
Ems	E 4	22	23	107
Ems	E 2	26	45	73
Ems	E 1	27	6	136
Westerschelde	WS 1	9	0	187
Westerschelde	WS 22	14	6	133
Westerschelde	WS 32	16	1	238
Westerschelde	WS 42	22	3	167
Westerschelde	WS 61	28	40	77
Westerschelde	WS 53	29	84	15
Somme	HH	25	17	165
Somme	LC	28	17	169
Somme	LM	29	15	181
Tamar	Clifton (*)	9	94	11
Tamar	Neal point (*)	23	94	15
Tamar	West mud (*)	31	87	34
Gironde	Lamarque	2	96	9
Gironde	St Estephe	5	85	23
Gironde	Richard	22	58	60
Gironde	Le Verdon	30	92	9
Tagus	Cala do Norte	8	95	9
Tagus	Banco do Ladeiro	22	36	148
Tagus	Banco dos Cavalos	30	77	11
Tagus	Banco do Destroi	29	72	13

(*) = data from Warwick and Gee (1984).

thic densities were observed all along the transect in the Westerschelde and the Tagus and in the brackish part of the Gironde. In general, meiobenthic density increased with increasing salinity (Fig. 2).

Nematodes were always the most abundant taxon (Fig. 3) and their dominance was in the order of 81 to 99%, except for the Ems. The Ems seemed to be the only estuary where other groups were of some significance: the most upstream station had fairly large numbers of soft-shelled Foraminiferans and Copepods, the most marine station had a large Gastrotrich community (30% of all meiobenthic animals), while Turbellarians, Ciliates and soft-shelled Foraminiferans were numerous in the two most marine stations (Fig. 3).

Apart from the Ems, turbellarians and large ciliates were also present in low quantities (at most 20, resp. 75 ind 10 cm^{-2}) in the Westerschelde, while virtually absent in other estuaries. Gastrotrichs were observed in high densities in the Ems and the Somme only, while soft-shelled Foraminiferans were also numerous in the most marine station of the Gironde. Harpacticoids were present in low quantities in most estuaries; in the Westerschelde (maximum 6 ind 10 cm^{-2}) and the Somme (7 ind 10 cm^{-2}) harpacticoid densities were very low (Fig. 3).

Nematodes

A total of 220 species, belonging to 102 genera and 35 families, were recorded in the intertidal of the investigated estuaries. The majority of these species were confined to only one estuary, some were found in two or three estuaries (Appendix). Only three species were common to all estuaries: *Dichromadora cephalata*, *Halalaimus gracilis* and *Viscosia viscosa*. Another thirteen species were observed in all but one estuary: *Daptonema normandicum*, *Chromadorita tentabunda*, *Anoplostoma viviparum*, *Calyptronema maxweberi*, *Metalinhomoeus aff biformis*, *Daptonema setosa*, *Sabatieria punctata group*, *Dichromadora geophila*, *Ptycholaimellus ponticus*, *Praeacanthonchus punctatus*, *Axonolaimus paraspinosus*, *Metachromadora remanei* and *Chromadora macrolaima*.

As salinity and sediment characteristics were fairly evenly distributed (Table 1), the distribution characteristics of the most important species and genera (defined as making up at least 15% of the total community in at least one station and observed in more than two stations) were computed with respect to their salinity and sediment grain size preferences. The median distribution (50%) and the 10% and 90% occurrences as well as the total range along which the species were observed were calculated and represented as box-whisker plots (Figs 4–5). By comparing the specific distribution with the repartition of all nematodes combined (total DENSITY), the degree of selectivity for any one parameter can be evaluated. Some species exhibit broad ecological tolerances to both factors and have a distribution along the sedimentary and salinity axis that is not noteworthy different from the total nematode density: *Viscosia viscosa* and *Dichromadora cephalata*. These two species were also observed in all estuaries (see above). Most species or genera have more clear preferences.

A Canonical correspondence analysis based on species, genera or families yielded – after permutation of the X-axis in the genus and family level – very similar results. The CANOCO plot of species (or genera) and environmental variables (Fig. 6) yields

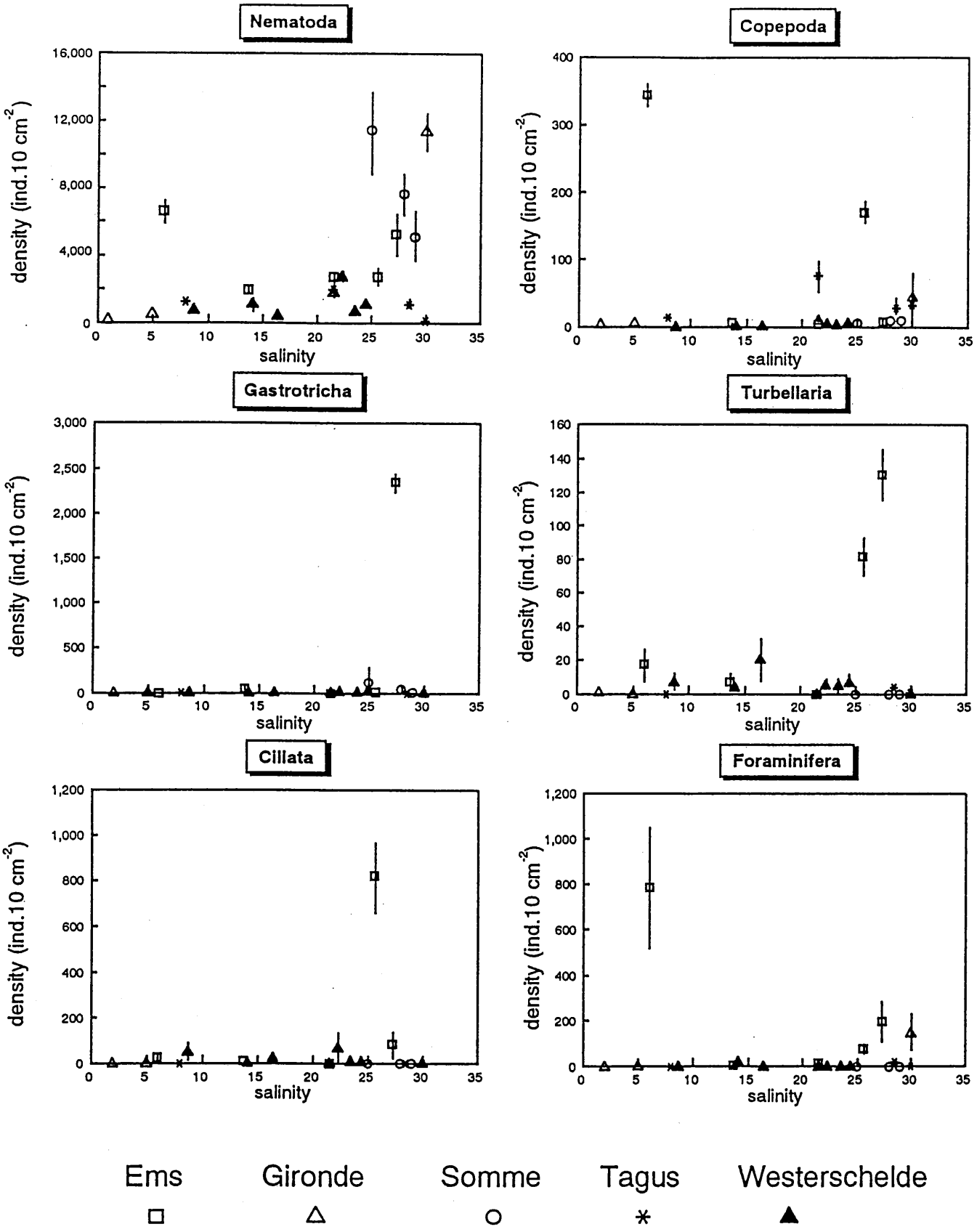

Fig. 3. Mean densities and total range (ind 10 cm^{-2}) of nematodes, copepods, gastrotrichs, turbellarians, ciliates and softshelled foraminiferans in the five European estuaries under study.

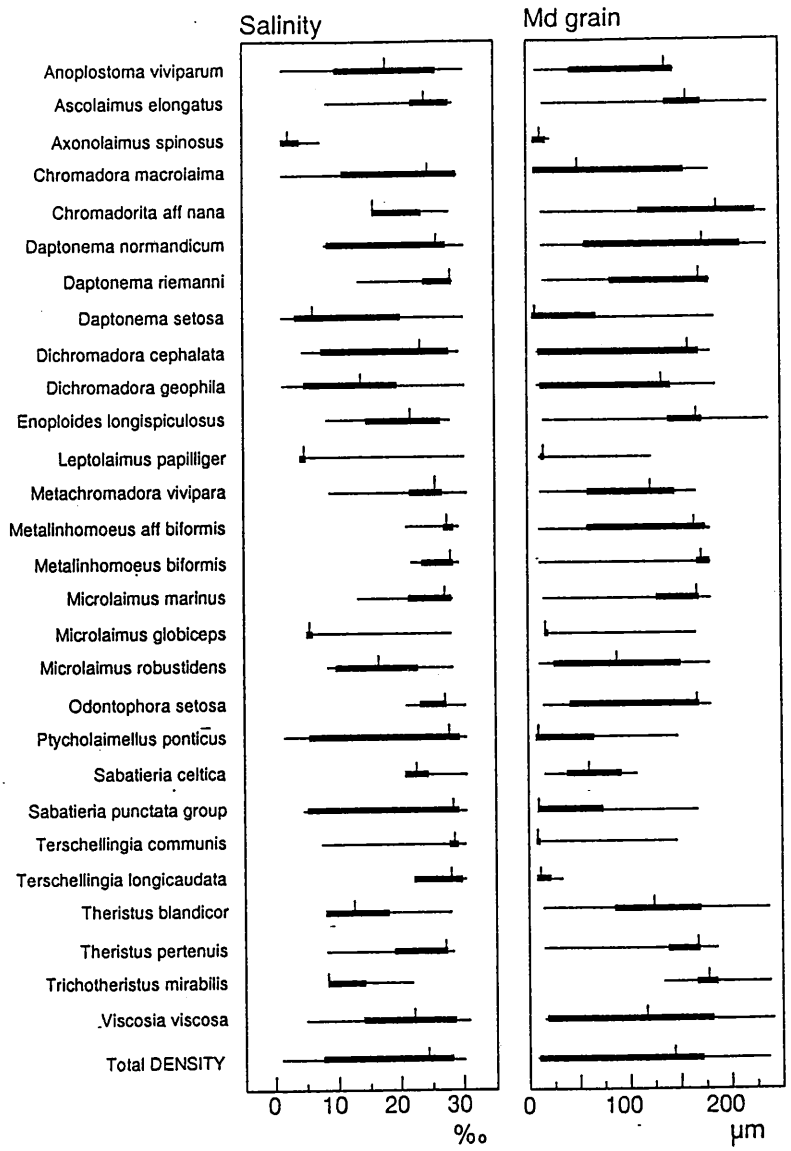

Fig. 4. Distributional characteristics of the most important species along the salinity and sediment grain gradient (all studied estuaries combined). Indicated are the total range (horizontal line), the median occurrence (vertical dash) and the 10 to 90% occurrence (horizontal bar).

similar information with respect to the figures (4–5) and (appendix): specific preferences for any variable can be 'appreciated' by orthogonally projecting the species position on the environmental axis. The positions of the species (Fig. 6) can aid in the interpretation of the station position in the same graph as they are weighted averages of the species positions. The length of the environmental arrows indicates their relative importance in explaining community structure. The relative position of the arrows reflects the relationship of the environmental variables, with orthogonality indicating no correlation, paralellism indicating positive (same direction) or negative (opposite) correlation. Thus salinity and grain characteristics (order of kilometres) were about equally important and independent factors, while latitudinal differences (correlated with grain size), although on the scale of hundreds to thousands of kilometres were much less pronounced.

The positions of the stations in the CANOCO plot with the same axes are represented in Fig. 7 for the species level. Results were very similar for genus or even family level (not depicted). There was a great overlap between stations of different estuaries. Within estuaries, the community gradually changed along the salinity gradient (arrows in Fig. 7) rather than along the sediment gradient (not depicted). This could indicate that the predictive ability of the type of sediment was overemphasized by the analysis, due to its relationship with latitude.

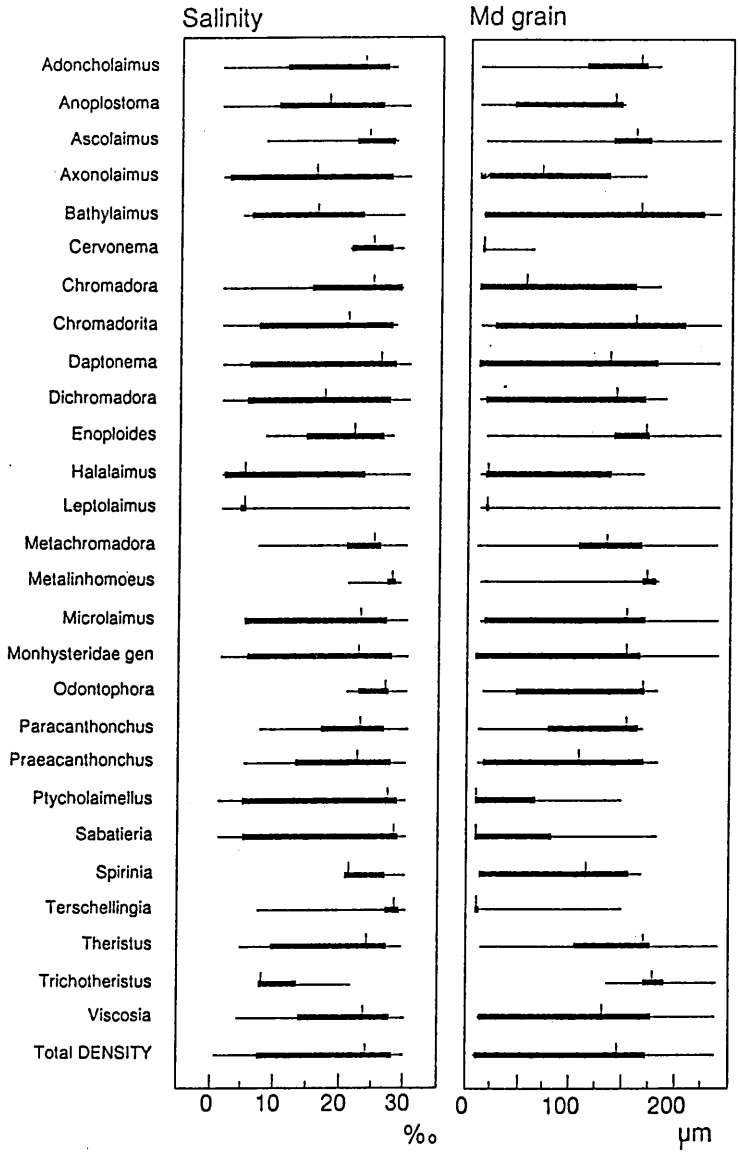

Fig. 5. Distributional characteristics of the most important genera along the salinity and sediment grain gradient. For an explanation, we refer to Fig. 4.

Nematode distributions (Figs 4–6, appendix)

The most important nematode families in terms of total density were the Xyalidae, the Axonolaimidae, the Comesomatidae, the Microlaimidae, the Linhomoeidae and the Chromadoridae.

The Comesomatidae were predominantly observed in silty sediments and preferred to some extent a more saline environment. There are two important members of this group. Species which have been described in the literature as *Sabatieria punctata*, *S. pulchra* and *S. breviseta* were present in this study both in their typical morphology as well as in all kinds of intermediate forms. This makes their systematic identity rather problematic and they were consequently grouped as one species which we called the '*Sabatieria punctata group*'. They were more strongly bound to silty sediments than the other member of the genus, *S. celtica*. Whereas the genus *Sabatieria* seemed to be more indifferent with respect to salinity and latitude, the genus *Cervonema* is a clear representative of the more saline and silty bottoms of estuaries at the lower latitudes (Gironde, Tagus).

The Xyalidae were a very abundant family encompassing three important genera: *Daptonema*, *Theristus* and *Trichotheristus*. *Trichotheristus mirabilis* and *Theristus blandicor* were only present in the most northerly estuaries (WS, resp. Ems and WS) and both prefer brackish waters. *Daptonema setosa* was found in the brackish part of all but one estuaries in silty sed-

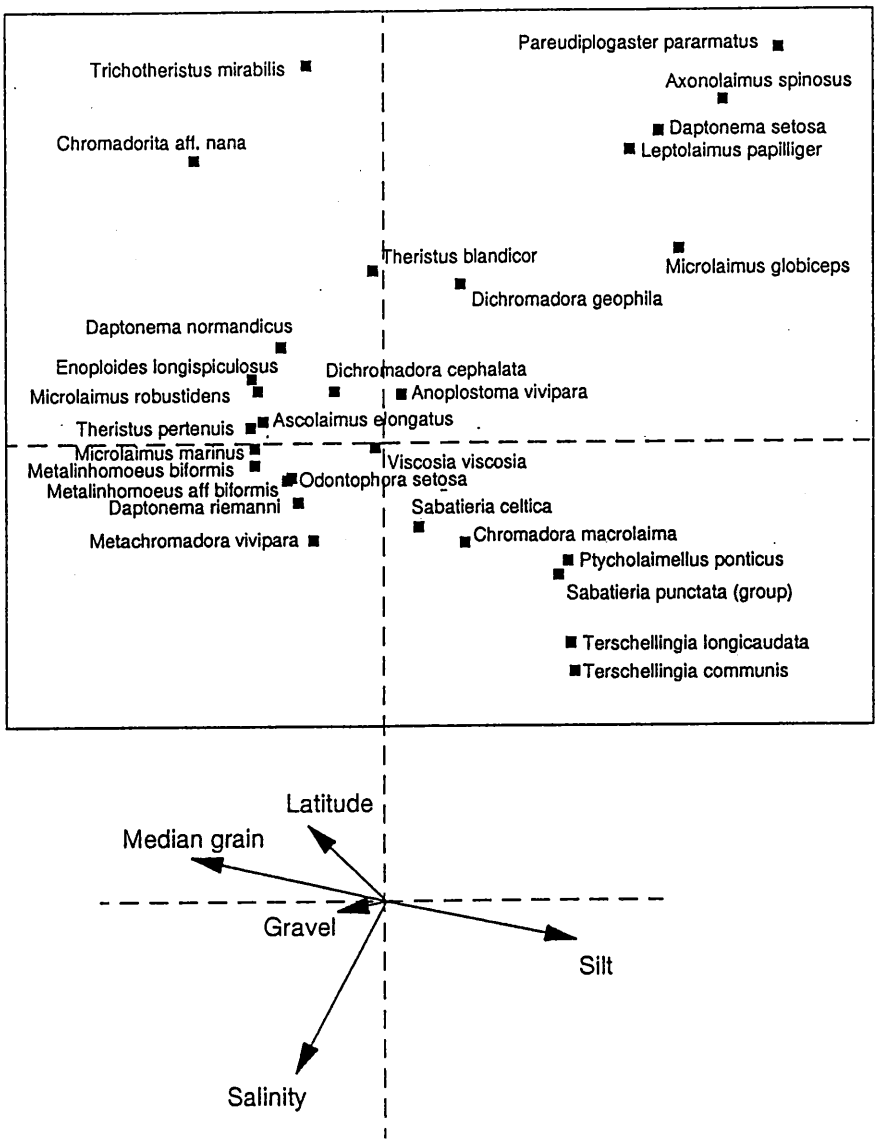

Fig. 6. Upper part: position of the most important species in two-dimensional CANOCO space (first two axes). Lower part: environmental arrows in the same CANOCO space.

iments (in the Somme the brackish area was not sampled). *Daptonema riemanni* was a common species in more marine and sandy bottoms of the northern estuaries. *Daptonema normandicum* and *Theristus pertenuis* are more northerly species with an affinity for coarser-grained and saline sediments.

The Linhomoeidae were represented by *Terschellingia longicaudata* and *Terschellingia communus* which co-occur in the silty sediments of the marine part in the Tagus, the Gironde and the Tamar and by two closely related species of the genus *Metalinhomoeus* (called *Metalinhomoeus typicus* and *M. biformis* in Bouwman, 1981, but referred to as *Metalinhomoeus biformis* resp. *M. aff biformis* in this study), which had greater preference for sandy sediments in the marine part.

The Chromadoridae were a very diverse group with important species as *Chromadora macrolaima* and *Ptycholaimellus ponticus* which are more common in silty sediments and the genus *Chromadorita* (*C. aff nana*, *C. tentabunda*) which was more frequently observed in coarser sediments and/or higher latitudes. Within the genus *Dichromadora*, we note the predominance of *D. geophila* in brackish water and the more neutral position of *D. cephalata*.

The Axonolaimidae encompassed amongst others *Ascolaimus elongatus* and *Odontophora setosa*, two northerly species which had an affinity for somewhat coarser sediments in the marine area. The

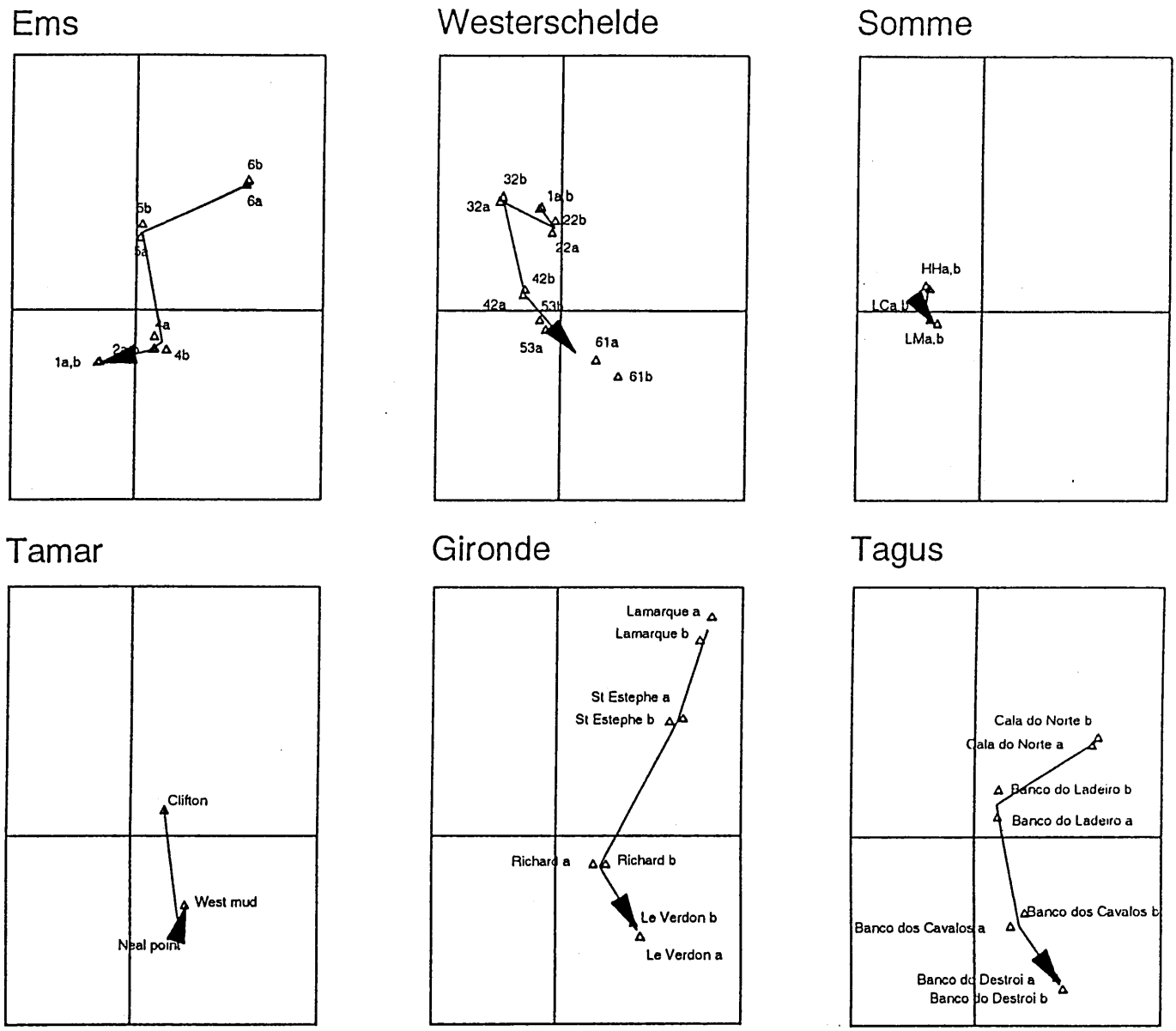

Fig. 7. Station plot of the various European estuaries in two- dimensional CANOCO space (first two axes). For convenience, the stations of one estuary were depicted separately. All plots have the same scale. The salinity gradient is depicted by the arrows.

genus *Axonolaimus* was represented by *A. spinosus*, a southern species in silty brackish-water bottoms, and *A. paraspinosus* having a broader distribution.

The Desmodoridae had as most common representatives *Metachromadora vivipara* and *Spirinia parasitifera*, which are marine species.

Amongst the Microlaimidae *Microlaimus marinus* and *M. robustidens* were more common in coarser sediment, while *M. globiceps* peaked clearly in the more freshwater part of the estuaries.

The Neodiplogasteridae – with their only representative *Pareudiplogaster pararmatus* – were only observed in a freshwater station of the Gironde, together with *Tobrilus diversipapillatus*.

Amongst the important species we also note *Anoplostoma viviparum* and *Viscosia viscosa* which had a very broad distributional range.

Nematode diversity and life-history traits

When randomly drawing 100 nematodes from the samples, a total of 8 to 29 species was found (N0, Fig. 8). Hill's diversity number of the first order (N1) varied from 3 to 22 equivalent species (Fig. 8), while N2 varied from 2 to 21, N∞ from 1 to 9.

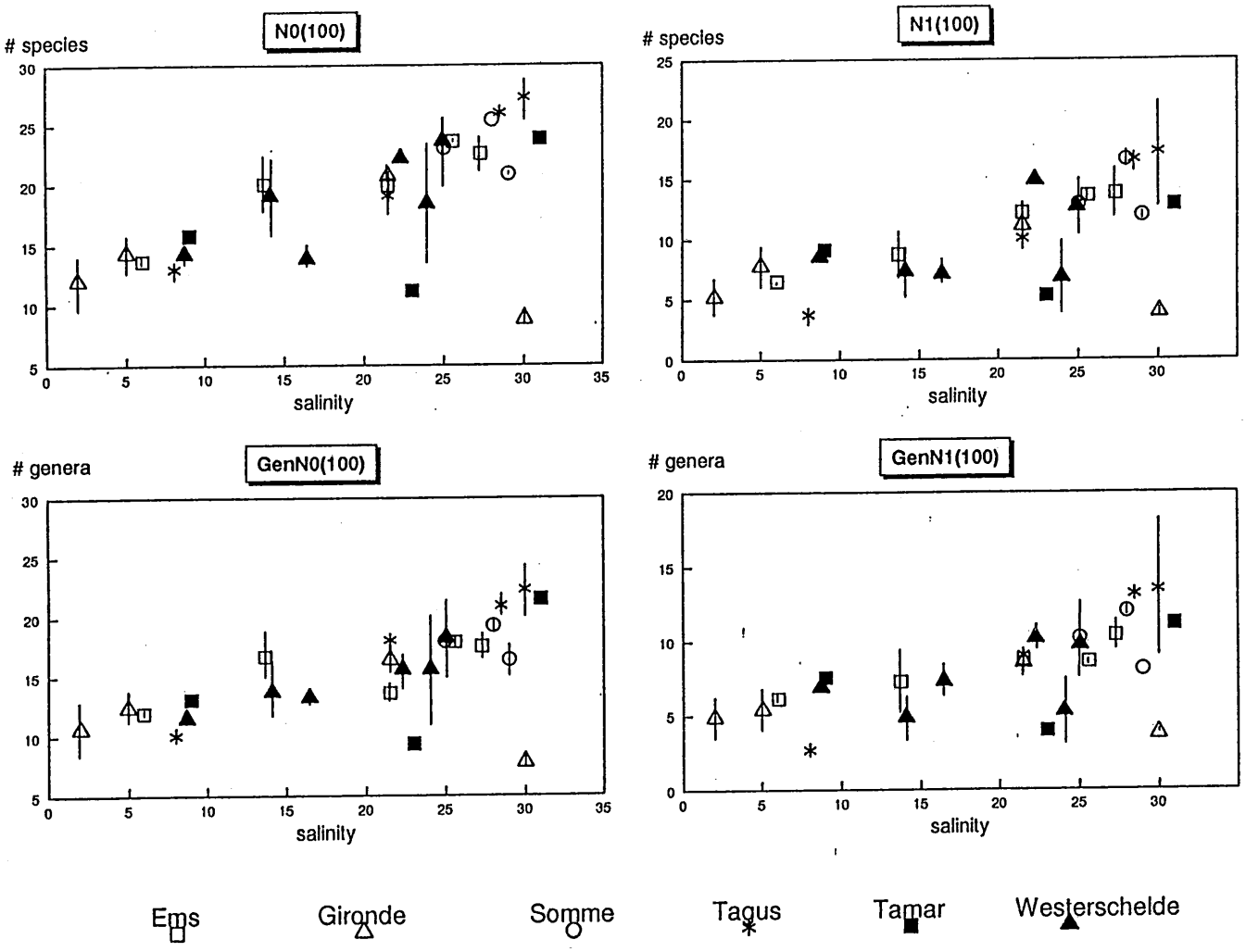

Fig. 8. Hill's diversity numbers of order 1 and 2, calibrated on 100 randomly picked nematodes for the six European estuaries, along the salinity gradient. Upper half: diversity on the species level, lower half: diversity on the genus level.

All estuaries combined, diversity increased with increasing salinity and there was no relationship with granulometry nor with latitude. No estuary could be clearly separated from the others on the basis of diversity although the Gironde and the Tamar had one station that scored relatively low.

Nematode diversity was nearly entirely expressed at the generic level: the diversity at the genus level was about 80 percent of total diversity (all Hill's numbers) and also increased with increasing salinity. On average only 1.2 species per genus were present in any one station and the vast majority were monospecific genera, even on the scale of the entire estuary. Considering the entire study area, many genera were represented by very few species (Addendum). Genera which are successful, both in number of species and density, are *Dichromadora* (5 species), *Microlaimus* (7 species), *Theristus* (9 species) and *Daptonema* (14 species).

The maturity index revealed no real trend (Fig. 9), but the fresh-water station in the Gironde had a much lower index than the other stations, which should indicate a low quality environment. This low value is caused by the relatively high concentrations of *Pareudiplogaster pararmatus* in this station, a species which has a great colonizing ability (Bongers, 1990).

Discussion

The estuarine environment shows large fluctuations of e.g. salinity, temperature and oxygen over different time scales, from a tidal cycle to a year (and longer). Meiobenthic organisms, being bound to the sediment, have to adapt to a range of these conditions and their present occurrence is based on a past set of environ-

mental conditions. How far back one should consider the environmental history for explaining a specific distribution and abundance depends on the life-history characteristics of the species: fast-reproducing and fast-growing species will show a quick response to favourable conditions, while slow-growing and slow-reproducing nematodes have experienced much larger fluctuations. How to translate this into the values of abiotic factors (i.c. salinity) is uncertain, particularly since extreme events (ice cover, storm, dessication) could possibly be more important. We therefore chose to relate meiobenthic distributions to the abiotic parameters measured at the time of sampling.

Whereas the use of the *in situ* salinity as environmental determinant can cause concern, one could also be sceptical about the scale of this study, encompassing five (resp. 6) estuaries separated up to a thousand kilometres one from another. Moreover, only a few sites in each estuary were sampled. It has for instance been shown that the nematode and copepod fauna can be significantly different at sites as close as several metres to kilometres apart (Eskin & Coull, 1984, Phillips & Fleeger, 1985) and nematodes generally have aggregated distributions on the scale of centimetres (Heip *et al.*, 1985). Moreover, in the intertidal area some zonation along the tidal elevation gradient exists (Warwick, 1971) and for this study, samples were taken at a random spot along this gradient. Even when discarding the temporal variation, it is clear that in this study, in which we had to compromize between spatial coverage and labour intensity, a large degree of intertidal variability within any one of the estuaries has been missed. In view of all these restraints it is remarkable to find a high consistency in the nematode structure along the different estuaries. Mesoscale variability (in the order of kilometres) due to salinity changes or grain-size differences are more important than 'huge'-scale variability (hundreds of kilometres) among estuaries. Microscale variability (centimetres) seemed negligible in view of the great resemblance between subsamples. Of course the importance of such factors as salinity or grain size characteristics on nematode community structure is well documented (e.g. Ward, 1975; Warwick & Gee, 1984; Austen & Warwick, 1989; Vanreusel, 1990; Vincx *et al.*, 1990) but as this is the first study to consistently include among-estuarine variability, the similarity of these gradients in the various estuaries has been clearly demonstrated. Likewise there is nothing innovating in the salinity and sedimentary preferences reported for the species in this study, as they greatly confirm what has been observed in other areas. However, the species distributions form the basis for sophisticated multivariate techniques like CANOCO and as such they may contribute to our appreciation of and help in understanding the results from these multivariate techniques.

A striking feature of nematode assemblages is the large number of species present in any one habitat – usually an order of magnitude higher than for any other taxon (Heip *et al.*, 1985). The highest known species diversity values for nematode communities were reported from the deep sea (Soetaert *et al.*, 1991), while the lowest nematode diversity was observed in the polluted subtidal muds off the Belgian East coast (Vincx, 1990) where at some sites only one species was present. The diversity values reported here fall well in between these extremes. Diversity in the marine part of the estuaries can be compared to the ones observed in the sublittoral coastal North Sea as reported by Vincx (1990) and Vanreusel (1990).

According to Bouwman (1983) and Heip *et al.* (1985), the estuarine environment was invaded by marine species which have adapted to reduced salinities in varying degrees and these species vanish with decreasing salinity. On its upstream boundary, penetration of freshwater species (up to a salinity of about 10‰) or even of species of terrestrial origin (Bouwman, 1983) add up to those of marine origin. Hence nematode diversity usually increases from about 5‰ salinity towards both the marine and the freshwater zone (Heip *et al.*, 1985). This general trend has been confirmed in this study except for the freshwater part that was not sampled. If such a clear trend can be demonstrated, deviations from this pattern become interesting. Why was the diversity in the most marine station of the Gironde and in the mid-saline station of the Tamar (Neal point) lower than one would expect? Diversity patterns in the Tamar were discussed by Warwick & Gee (1984) and by Austen & Warwick (1989). They argue – in agreement with Huston's dynamic equilibrium hypothesis – that the lower diversity at mid-salinity could be related to the lower degree of disturbance by macrofauna, which is far less numerous here. The nematode community in the marine station of the Gironde (Le Verdon) was very similar to the low-diversity site of the Tamar: it was largely dominated by *Sabatieria punctata (group)* and *Terschellingia communis*, whereas *T. communis*, *T. longicaudata*, *Metachromadora vivipara* and *S. punctata (group)* were co-dominant in Neal point. All these species are conservative with low respiration rates and long generation times. They are typical for tidal mud

flats with rather anoxic sediments (Vincx *et al.*, 1990) and this might also be the case for the sediments at Le Verdon (Castel, pers. comm.). Although the low diversity in these stations could be due to a lower degree of disturbance, as invoked by the dynamic equilibrium hypothesis, the macrofauna cannot be the cause of this at Le Verdon where macrofaunal biomass is highest (Castel, 1992) and, remarkably enough, the macrofaunal diversity is also reasonably high in this station. It may be that the low oxygen concentrations in some of the mud flats do not allow the establishment of the higher-diversity assemblage one could expect according to salinity, as only a few species have physiological tolerances suited for persisting in such a harsh environment.

Nematodes have been shown to be possible indicators of pollution or other kinds of disturbances (Heip *et al.*, 1985) and especially the influence of perturbations on diversity has been well documented (Lambshead *et al.*, 1983; Platt *et al.*, 1984) and debated (Hodda & Nicholas, 1986). Amongst the estuaries studied, organic and inorganic pollution have the highest levels in the Westerschelde, and one would expect to find significant differences in diversity in this estuary, as compared to the other estuaries. This was shown to be the case for intertidal copepods (Van Damme *et al.*, 1984), when compared to the Ems. However, the deviation of nematode diversity from the general trend in the Westerschelde is only suggestive at most and not consistent enough to establish a possible effect of pollution on nematode diversity. As for the study of copepods in Van Damme *et al.* (1984), the diversity differences between the Ems and the Westerschelde could be reflections of the density differences, because of the large dependence of diversity indices on the number of individuals (Soetaert & Heip, 1990).

Perhaps the most striking result of this study lies in the distribution of higher meiofaunal groups in the various estuaries. Whereas nematodes were overall the most abundant organisms in the intertidal zone, their dominance was much lower in the Ems compared to the other estuaries. Only in the Ems have we observed important populations of harpacticoids, turbellarians, ciliates and gastrotrichs in some stations. Although the occurrence of these high densities could very well be short-term events, this does not explain their absence in other estuaries and the causes of this are uncertain.

The paucity of harpacticoid copepods in the Westerschelde, when compared to the Ems, was already noted by Van Damme *et al.* (1984) and ascribed to pollution effects in the Westerschelde. From our study, the Westerschelde indeed appeared to be nearly devoid of intertidal populations of harpacticoids, whereas the other estuaries had, albeit weakly developed, populations in at least some of their stations. However, harpacticoids were shown to be more abundant in the subtidal area of the Westerschelde (max. 27 ind 10 cm^{-2}, Soetaert *et al.*, 1994) compared to the intertidal and it would be interesting to see whether these subtidal abundances are also consistently lower when compared to the other estuaries. Subtidal samples analyzed from the Tagus indeed suggest that Westerschelde abundances are lower since mean densities of more than 1500 ind 10 cm^{-2} were observed in one Tagus station (own unpublished data).

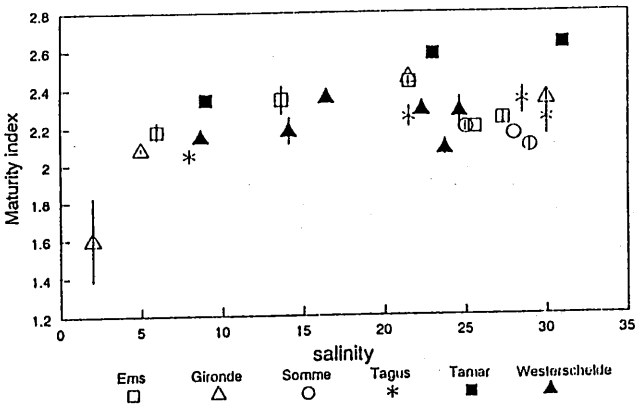

Fig. 9. Maturity index of the various stations along the salinity gradient.

Total meiobenthic densities tended to increase exponentially with increasing salinities but were low in the most saline range of the Tagus and to a smaller extent of the Westerschelde. The lack of low-salinity tolerance of species of marine origin is usually invoked to explain the decreasing densities in the low salinity ranges of an estuary (e.g. Coull, 1988; Montagna & Kalke, 1992). However, whereas this could very well explain the presence or absence of species (and hence diversity), it is unclear why species that have adapted to brackish situations cannot attain large densities. Moreover, large densities were observed in the salt marshes of the brackish area of the Westerschelde (Van Damme *et al.*, 1980) and this seems to contradict the thesis of salinity tolerance.

Other explanations could be linked with food availability and both the supply and reactivity of the organic matter in the bottom is important. In the turbid regions of the estuaries primary production in the water is lowest (Kromkamp *et al.*, 1992; Soetaert *et al.*, 1994b) and this may result in a lower food supply for the benthos. Bacterial mineralization in this turbid area

is high (Goosen *et al.*, 1992; Soetaert & Herman, 1995) and hence the residual organic matter of fluviatile descent, downstream from this region, will be more refractory (Soetaert & Herman, 1995) and thus less suitable as a food source. On the other hand primary pelagic productivity increases downstream as turbidity decreases (Soetaert *et al.*, 1994b). The closer to the marine zone, the more important the input of organics from marine origin will become (Soetaert & Herman, 1995) and this higher quality food source could allow the benthos to increase in abundance. Also of possible importance in explaining meiobenthic density is macrofaunal predation (e.g. Warwick & Gee, 1984). However, the magnitude of macrofaunal interaction with the meiofauna along the salinity gradient is difficult to assess. In the Westerschelde for instance, macrofaunal biomass increases with increasing salinities but the trophic structure of the macrofauna changes concurrently from detritus-feeding (and thus meiofauna consuming) in the brackish part to filter feeding in the marine part (Meire *et al.*, 1991). Van Damme *et al.* (1980) even invoked pollution effects to explain the lower densities of the Westerschelde meiobenthos compared to the Ems. However, as may be apparent from this study, meiobenthic densities of the Westerschelde are not consistently lower, except perhaps in the marine part. Moreover, the Ems was not so well chosen as a reference estuary as from our study it appears to be the only estuary where large densities in the brackish part were observed. Nevertheless, in many instances pollution decreases in parallel with increasing salinity and thus cannot be ruled out as a possible cause of benthic depauperation. Another factor which could be of possible importance is the degree of environmental fluctuation, with brackish areas that are usually more unpredictable (varying current velocities, steeper gradients of several solutes and particulates), while the human impact (dredging) usually is more intense in this region.

Pollution is expected to be high in the Tagus, which receives high amounts of untreated waste from the city of Lisbon, and many industrial effluents are pumped into the estuary. This estuary has low numbers of meiobenthic animals all along its banks, and one could be tempted to conclude that this is a pollution effect. But seasonal environmental fluctuations in the Tagus are much more pronounced than in the other estuaries and this too could cause low densities. However, too little is known about the abiotic and biotic factors from this area to be conclusive about the causes of meiobenthic distributions.

Another way of looking for anomalies among stations is by comparing the life-history traits of nematodes: are they predominantly *r* or *K* strategists? Very recently, an index has been proposed that summarizes this information into one number: the maturity index (Bongers, 1990; Bongers *et al.*, 1991). On the whole, not much information could be extracted from this measure and it appeared to be unable to reveal gradients. However, it did point to a spuriously low 'maturity' of the station located in the most freshwater part of the Gironde (Lamarque). This muddy intertidal flat was also very poor in macrobenthos, which was dominated by oligochaetes (Castel, 1992) and it is situated at about 30–40 km from the city of Bordeaux.

The low maturity of the nematode community could nearly entirely be accounted for by the dominance of the freshwater nematode *Pareudiplogaster pararmatus*. This nematode is known to be successful in waste-water exposed intertidal mud flats in the low-salinity range of estuaries (Romeyn *et al.*, 1983) and belongs to a family which has high colonization capabilities (Bongers, 1990). Being ovoviviparous, it can survive under extreme conditions and become dominant due to the disappearance of less resistant competitors. Its dominance at Lamarque could thus be indicative for some kind of organic enrichment in this area although the total densities were relatively low. Another possibility is that we have observed a pioneer community after some kind of 'catastrophic event' (e.g. storm) has taken place.

In 1980 *Pareudiplogaster pararmatus* was the most important member of the nematode fauna in an area exposed to organic pollution in the Ems (Bouwman *et al.*, 1983). The fact that it was not observed in the Ems during this study could indicate the improved conditions in this part of the estuary as was shown from the macrobenthos (Esselink *et al.*, 1989).

Although ecological interpretations are difficult to make without an extensive background environmental data set, this study has provided some insight in the structure of estuarine meiobenthic communities. The results of this work could serve as a base-line for a more intensive study where smaller spatial scales and temporal variation should be taken into account and a more extensive environmental data set should be procured. If one wants to spot pollution or produce conclusive evidence for the causes of benthic distributions, a global research project as this one is not appropriate. Yet it can provide a reference frame against which other results can be put into perspective.

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Appendix. Species list, indicating the maximum density (10 cm^{-2}) of each species in the five estuaries of the current study and the Tamar (data derived from Warwick & Gee, 1984).

	Ems	Wester-schelde	Somme	Tamar	Gironde	Tagus
Enoplida						
Enoplidae						
Enoplus brevis	–	–	**	–	–	–
Enoplus sp	*	*	–	–	–	–
Thoracostomopsidae						
Enoploides longispiculosus	**	***	***	–	–	–
Enoplolaimus litoralis	–	–	**	–	–	–
Enoplolaimus propinquus	–	**	–	–	–	–
Anoplostomatidae						
Anoplostoma viviparum	***	**	–	**	**	***
Chaetonema riemanni	**	–	–	–	–	–
Ironidae						
Syringolaimus sp	–	*	–	–	*	*
Leptosomatidae						
Leptosomatidae T1	–	–	–	–	–	**
Oxystominidae						
Halalaimus gracilis	***	**	**	*	**	**
Halalaimus sp	–	–	–	*	**	–
Halalaimus T1	–	–	–	–	–	**
Halalaimus T2	–	–	–	–	–	**
Halalaimus T3	–	–	–	–	–	*
Nemanema cylindricaudatum	**	–	–	*	–	–
Oxystomina elongata	–	–	**	*	–	–
Oxystomina sp	**	–	–	–	–	–
Thalassoalaimus septentrionalis	**	*	–	–	–	**
Oncholaimidae						
Adoncholaimus fuscus	***	***	***	–	–	–
Adoncholaimus thalassophygas	–	–	–	–	*	–
Oncholaimellus mediterraneus	–	*	–	–	–	–
Oncholaimidae sp	–	–	–	–	–	**
Oncholaimus oxyuris	–	*	***	–	–	–
Oncholaimus sp	**	–	–	–	*	–
Viscosia abyssorum	–	–	–	*	–	–
Viscosia aff rustica	***	**	–	–	–	–
Viscosia glabra	**	–	–	–	–	–
Viscosia viscosa	***	***	***	**	***	***
Enchelidiidae						
Calyptronema maxweberi	**	**	***	*	**	–
Eurystomina sp	–	–	–	–	–	**

Appendix (Continued).

	Ems	Wester-schelde	Somme	Tamar	Gironde	Tagus
Tripyloididae						
Bathylaimus australis	–	**	–	–	–	–
Bathylaimus sp	–	–	–	–	*	**
Bathylaimus stenolaimus	–	*	–	–	–	–
Tripyloides gracilis	–	**	***	*	**	–
Tobrilidae						
Tobrilus diversipapillatus	–	–	–	–	*	–
Trefusiida						
Trefusiidae						
Trefusia Longicaudata	–	***	–	–	–	–
Trefusia multipapillatus	***	–	–	–	–	–
Trefusia S1	–	–	***	–	–	–
Trefusia S2	–	–	**	–	–	–
Trefusia W2	–	*	–	–	–	–
Chromadorida						
Chromadoridae						
Atrochromadora microlaima	*	*	–	**	–	–
Chromadora aff nudicapitata	–	–	–	–	–	**
Chromadora axi	–	*	–	–	–	**
Chromadora macrolaima	***	**	***	–	****	***
Chromadorida sp	**	–	–	–	*	**
Chromadorina germanica	–	–	–	–	*	–
Chromadorita aff nana	**	***	–	–	–	–
Chromadorita tentabunda	**	***	***	–	**	**
Dichromadora cephalata	**	*	***	**	**	**
Dichromadora cucullata	–	*	–	–	–	–
Dichromadora geophila	***	**	–	**	**	***
Dichromadora hyalocheile	–	–	***	–	–	–
Dichromadora sp	–	–	**	–	–	**
Dichromadora T3	–	–	–	–	–	*
Hypodontolaimus balticus	–	–	–	*	–	–
Hypodontolaimus W1	–	**	–	–	–	–
Neochromadora sp	–	–	–	*	–	–
Prochromadorella ditlevseni	–	**	***	–	–	–
Ptycholaimellus ponticus	***	*	–	**	****	**
Spilophorella candida	–	*	–	–	–	*
Spilophorella paradoxa	–	*	**	*	–	*

Appendix (Continued).

	Ems	Wester-schelde	Somme	Tamar	Gironde	Tagus
Comesomatidae						
Cervonema G1	–	–	–	–	**	–
Cervonema T1	–	–	–	–	–	***
Paracomesoma sp	–	–	–	*	–	–
Sabatieria celtica	***	*	–	*	–	–
Sabatieria longisetosa	–	–	–	–	–	**
Sabatieria longispinosa	***	–	–	–	–	–
Sabatieria punctata group	****	***	–	**	****	***
Sabatieria sp	**	–	**	–	*	**
Setosabatieria hilarula	–	–	–	*	–	**
Ethmolaimidae						
Neotonchus aff cupulatus	–	*	–	–	–	–
Neotonchus sp	–	–	–	*	–	–
Cyatholaimidae						
Cyatholaimidae sp	–	–	***	–	–	–
Paracanthonchus aff caecus	–	–	–	*	–	–
Paracanthonchus aff heterodontus	–	–	–	–	–	***
Paracanthonchus aff thaumasius	–	–	****	–	–	–
Paracanthonchus caecus	**	–	–	–	–	–
Paracanthonchus heterodontus	***	–	–	–	–	**
Paracanthonchus thaumasius	–	**	–	–	–	–
Paracyatholaimoides W1	–	*	–	–	–	–
Paracyatholaimus W1	–	**	–	–	–	–
Praeacanthonchus punctatus	***	*	***	*	**	–
Selachinematidae						
Halichoanolaimus robustus	**	–	–	–	–	*
Desmodoridae						
Desmodora A	–	–	–	*	–	–
Desmodora B	–	–	–	*	–	–
Leptonemella sp	–	–	**	–	–	–
Metachromadora aff suecica	***	**	–	–	–	–
Metachromadora remanei	**	***	***	–	**	**
Metachromadora sp	–	–	**	–	–	–
Metachromadora vivipara	****	***	–	***	–	–
Molgolaimus cuanensis	***	–	–	–	–	–
Molgolaimus S1	–	–	***	–	–	–
Molgolaimus S2	–	–	**	–	–	–
Molgolaimus sp	–	–	***	–	–	*
Molgolaimus tenuispiculum	–	–	–	*	–	–
Molgolaimus turgofrons	–	*	–	–	–	–
Onyx sagittarius	–	*	–	–	–	–
Sigmaphoranema aff rufus	–	**	–	–	–	–
Spirinia parasitifera	**	***	–	*	–	–

Appendix (Continued).

	Ems	Wester-schelde	Somme	Tamar	Gironde	Tagus
Microlaimidae						
Aponema torosa	–	–	–	*	–	–
Calomicrolaimus S1	–	–	****	–	–	–
Calomicrolaimus S3	–	–	****	–	–	–
Microlaimus arenicola	–	**	***	–	–	–
Microlaimus globiceps	****	**	***	–	–	–
Microlaimus marinus	***	***	****	–	–	–
Microlaimus parahonestus	***	***	–	**	–	–
Microlaimus robustidens	–	–	****	*	–	–
Microlaimus sp	**	–	–	–	–	–
Microlaimus W1	–	**	–	–	–	–
Microlaimus W4	–	*	–	–	–	–
Monoposthiidae						
Monoposthia mirabilis	–	**	–	–	–	–
Monoposthia sp	**	–	–	–	–	–
Nudora bipapillata	–	–	–	**	–	–
Leptolaimidae						
Antomicron elegans	**	–	**	–	*	*
Camacolaimus tardus	–	**	***	–	–	–
Dagda bipapillata	–	*	–	–	–	–
Deontolaimus papillatus	–	*	–	–	–	–
Leptolaimus acicula	–	*	–	–	–	–
Leptolaimus ampullaceus	–	*	–	–	–	–
Leptolaimus elegans	–	**	–	–	–	–
Leptolaimus luridus	–	–	–	–	–	*
Leptolaimus papilliger	****	–	–	*	**	–
Leptolaimus S1	–	–	***	–	–	–
Leptolaimus sp	–	*	–	–	*	*
Stephanolaimus aff spartinae	–	–	***	–	–	–
Haliplectidae						
Haliplectus wheeleri	–	–	–	–	–	**
Aegialoalaimidae						
Aegialoalaimus aff tenuicaudatus	–	–	–	–	–	**
Aegialoalaimus elegans	**	–	–	–	**	*
Cyarthonema E1	**	–	–	–	–	–
Cyarthonema germanica	***	–	–	–	**	–
Cyarthonema W1	–	*	–	–	–	–
Southernia zosterae	**	**	–	–	–	–

Appendix (Continued).

	Ems	Wester-schelde	Somme	Tamar	Gironde	Tagus
Tubolaimoididae						
Chitwoodia warwicki	–	**	–	–	–	–
Meyliidae						
Meyliidae T1	–	–	–	–	–	**
Desmoscolecidae						
Calligyrus sp	–	–	–	*	–	–
Desmoscolex falcatus	–	–	–	**	–	–
Tricoma sp	–	–	–	*	–	–
Monhysterida						
Monhysteridae						
Diplolaimella sp	–	–	***	–	*	*
Monhysteridae sp	**	**	***	*	**	**
Monhysteridae T1	–	–	–	–	–	**
Xyalidae						
Daptonema acc Bouwman	***	*	***	–	–	–
Daptonema cfr biggi	–	–	–	–	**	–
Daptonema G1	–	–	–	–	***	–
Daptonema kornoense	–	–	–	–	–	**
Daptonema normandicum	**	***	***	*	**	–
Daptonema oxycerca	***	–	–	*	***	*
Daptonema procera	–	–	–	*	–	–
Daptonema riemanni	****	***	****	–	–	–
Daptonema setosa	***	**	–	*	***	***
Daptonema sp	**	**	***	–	***	***
Daptonema T1	–	–	–	–	–	**
Daptonema T2	–	–	–	–	–	*
Daptonema tenuispiculum	–	**	–	–	–	–
Daptonema W1	–	**	–	–	–	–
Daptonema xyaliforme	**	–	–	–	**	**
Metadesmolaimus 2	–	*	–	–	–	–
Metadesmolaimus E1	**	–	–	–	–	–
Metadesmolaimus gaelicus	–	*	–	–	–	–
Paramonohystera E1	***	–	–	–	**	**
Paramonohystera sp	–	*	–	–	–	–
Pseudotheristus furcatus	–	–	–	–	–	*
Theristus 1	–	**	–	–	–	–
Theristus acer	**	–	***	*	–	**
Theristus aff profundus	–	*	–	–	–	–
Theristus blandicor	****	***	–	–	–	–
Theristus cfr subcurvatus	**	**	–	–	–	–

Appendix (Continued).

	Ems	Wester-schelde	Somme	Tamar	Gironde	Tagus
Theristus ensifer	–	*	***	–	–	–
Theristus G1	–	–	–	–	*	–
Theristus longus	***	**	***	–	–	–
Theristus pertenuis	***	**	****	–	–	–
Theristus sp	**	–	***	–	–	–
Trichotheristus mirabilis	–	***	–	–	–	–
Xyala striata	–	*	–	–	–	–
Sphaerolaimidae						
Sphaerolaimus balticus	–	–	–	*	–	–
Sphaerolaimus gracilis	–	–	–	*	**	**
Sphaerolaimus hirsutus	**	–	–	*	**	*
Sphaerolaimus sp	–	**	–	–	–	–
Siphonolaimidae						
Siphonolaimus sp	–	*	–	–	–	–
Linhomoeidae						
Desmolaimus S1	–	–	***	–	–	–
Desmolaimus T1	–	–	–	–	–	*
Desmolaimus zeelandicus	***	–	–	**	–	–
Eleutherolaimus aff stenosoma	–	–	–	–	–	**
Eleutherolaimus amasi	**	–	–	–	–	–
Eleutherolaimus sp	**	–	–	–	–	–
Eleutherolaimus stenosoma	**	**	–	*	–	–
Linhomoeidae T1	–	–	–	–	–	**
Linhomoeidae sp	**	*	***	–	**	*
Linhomoeidae W1	–	*	–	–	–	–
Linhomoeidae W2	–	*	–	–	–	–
Linhomoeidae W4	–	*	–	–	–	–
Linhomoeidae W5	–	**	–	–	–	–
Linhomoeus S1	–	–	***	–	–	–
Megadesmolaimus W1	–	**	–	–	–	–
Metalinhomoeus aff biformis	**	**	****	–	**	***
Metalinhomoeus biformis	–	**	****	–	–	**
Paralinhomoeus ilensis	**	–	–	–	–	–
Paralinhomoeus sp	**	–	–	–	–	**
Paralinhomoeus T1	–	–	–	–	–	**
Terschellingia communis	–	*	–	**	****	**
Terschellingia longicaudata	–	–	–	***	***	**

Appendix (Continued).

	Ems	Wester-schelde	Somme	Tamar	Gironde	Tagus
Axonolaimidae						
Ascolaimus elongatus	***	***	****	–	–	–
Axonolaimus cfr orus	–	–	–	–	–	**
Axonolaimus paraspinosus	***	*	–	**	**	**
Axonolaimus spinosus	–	–	–	–	**	*
Odontophora aff. paravilloti	–	*	–	–	–	–
Odontophora rectangula	***	***	–	–	–	–
Odontophora setosa	***	***	****	*	–	–
Odontophora sp	**	–	***	–	–	–
Odontophora W4	–	**	–	–	–	–
Pseudolella granulifera	–	–	–	–	***	***
Diplopeltidae						
Campylaimus gerlachi	–	–	–	–	–	**
Diplopeltis incisus	–	–	–	*	–	–
Diplopeltula asetosa	**	–	–	–	–	–
Rhabditida						
Diploscapteridae						
Diploscapter sp	**	*	–	–	–	–
Neodiplogasteridae						
Pareudiplogaster pararmatus	–	–	–	–	***	–
Dorylaimida						
Dorylaimida						
Dorylaimida W2	–	*	–	–	–	–

(–) absent, (*) 0–10 ind, (**), 10–100, (***) 100–1000, (****) >1.000

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A handy method for measuring meiobenthic respiration

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Abstract

Our current understanding of meiofaunal respiration rates, and especially of the way they are influenced by changing abiotic factors, is still far from complete. Meiofaunal respiration is traditionally measured using Cartesian divers or related manometric techniques, but these are extremely time-consuming and labour-consuming. We have evaluated the use of the Strathkelvin polarographic electrode model 1302 and the O_2 monitor model 781 in determining the O_2 consumption of meiofaunal animals. Respiration rates obtained in this way of the terrestrial nematode *Caenorhabditis elegans* compared well with results obtained from Cartesian diver respirometry. Experiments with 3 estuarine nematode species show that 5% accuracy levels are obtained with respiration rates down to 200 nl O_2 h^{-1}. This involves the use of a few tens to a few hundred individuals, depending on the size and the respiratory activity of the animals. Several practical problems that relate to accurate determinations of O_2 consumption are discussed. It is concluded that short-term measurements and fairly easy procedures make polarographic O_2 electrodes an interesting and reliable tool for routine measurements of meiofaunal community respiration and of the influence of abiotic factors on meiofaunal aerobic metabolism.

Keywords: Respiration; Electrode; Method; Meiobenthos; Nematodes

1. Introduction

The role of meiofauna in benthic energy flow processes is still a controversial matter. Calculations of production (e.g. Faubel et al., 1983; Heip et al., 1984; Witte and Zijlstra, 1984) are commonly based on an annual *P/B*-ratio of 9 (Gerlach, 1971). Warwick and Price (1979) re-evaluated this *P/B*-ratio using an empirical relationship between

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respiration and production (McNeil and Lawton, 1970), while Vranken and Heip (1986) recalculated annual *P/B* for marine nematodes from data obtained from laboratory experiments. Nematodes are by far the dominant component in marine and estuarine meiobenthic communities, but energy budgets have been established for only four marine species (Tietjen, 1980; Warwick, 1981), and annual production has but been estimated for one (Herman and Vranken, 1988; Vranken et al., 1988). Relatively more data are available on fresh-water nematodes (e.g. Duncan et al., 1974; Marchant and Nicholas, 1974; Schiemer et al., 1980; Schiemer, 1983, 1987; Woombs and Laybourn-Parry, 1985). Thus there remains a wide gap in our knowledge of meiofaunal respiration and energetics, and of the way these are influenced by varying abiotic factors.

The paucity of papers on meiofauna respiration is evidence of the difficulties involved in its study. Nematode respiration has hitherto been measured using Cartesian divers or related techniques (Linderstrom-Lang, 1937, 1943; reviews in Lasserre, 1976; Heip et al., 1985). Although the fairly high sensitivity of this method allows respiration measurements with few (less than 10) individuals, it is less suited for studies on community respiration; moreover, Cartesian diver respirometry is extremely time-consuming and labour-consuming, and it is therefore an unlikely tool for routine use or for respiration studies under a range of abiotic conditions (temperature, salinity, pO_2, etc.). O_2 electrodes have only rarely been used to study nematode respiration, mainly because of methodological problems (for a discussion of technical problems involved in electrode-based respiration studies of small aquatic invertebrates, see, for example Gnaiger, 1983a). Respiration of large enoplid nematodes has, however, been measured on individual animals (Atkinson and Smith, 1973; Atkinson, 1973a,b). The present study evaluates the use of the Strathkelvin polarographic electrode model 1302 and the oxygen monitor model 781 in determining meiofaunal respiration rates. Results so obtained of experiments on nematode respiration are verified on the basis of previously published data. Finally, the applicability of the described method to other than nematode research is stressed.

2. Materials and methods

2.1. Description of the oxygen monitor and the respiration vials

Essentially, the Strathkelvin respirometer consists of a polarographic Clark-electrode (Clark, 1956), contained in a polystyrene-based coat. The electrode can be inserted into different types of respiration vials. The polypropylene membrane spanning the electrode tip is kept taut by a neoprene '0'-ring. When it is in measurement position, the membrane forms part of the respiration chamber (Fig. 1). O_2 concentration is displayed on a decimal meter, which is connected to a potentiometric pen recorder for continuous registration of O_2 consumption. The temperature during measurements is regulated by connecting the water mantle of the respiration vial to a thermostatic water bath; a Haake KT 38 was used in our experiments. All presently reported tests were performed at 20°C, unless stated otherwise. This paper discusses the use of the RC 200 and the RC 300 respiration vials, which have respiration chambers with adaptable volumes of 50–180 μl

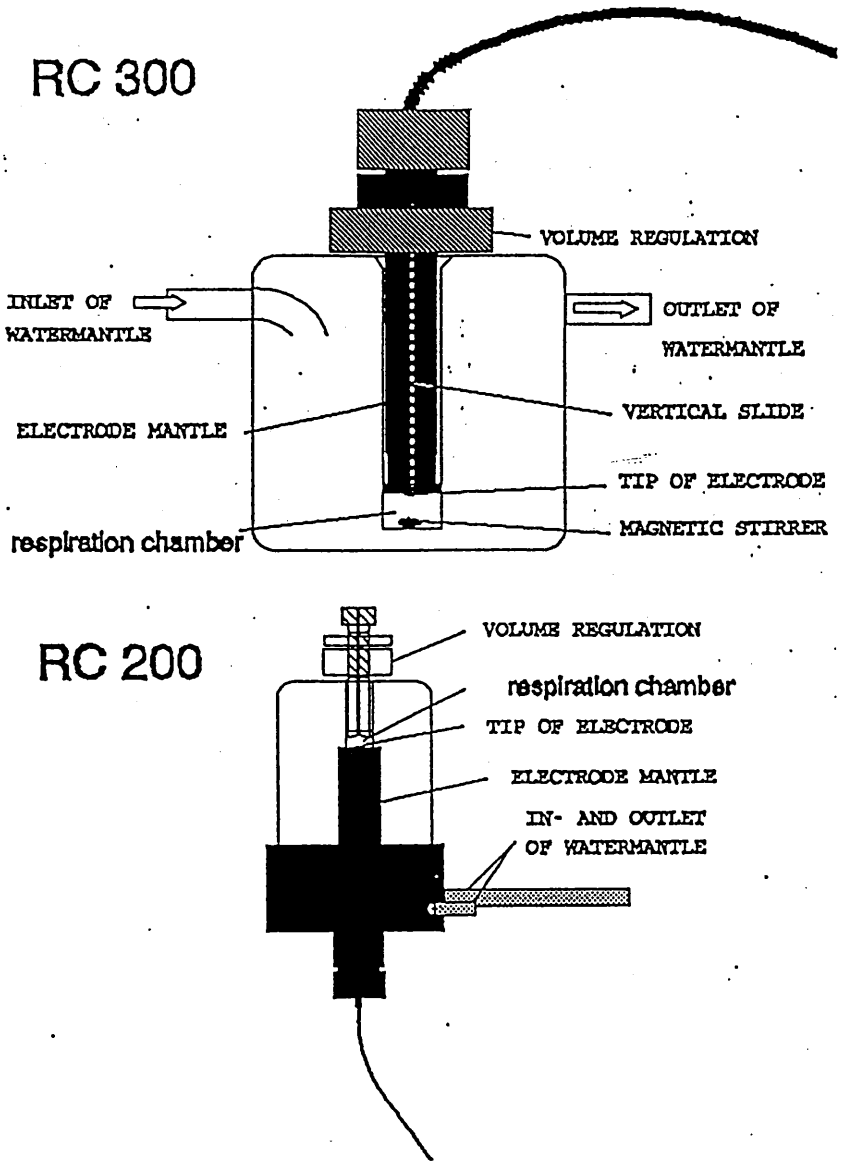

Fig. 1. The RC 300 and the RC 200 respiration vials. The drawings are not to relative size. Both respiration chambers have a diameter of 1.5 cm; the diameter×height dimensions are 5×5.6 cm and 3.7×7.7 cm for the RC 300 and the RC 200 vials, respectively.

and of 300–1000 μl, respectively (Fig. 1). The reason for testing both vial types was to check on methodological aspects, such as reproducibility, sensitivity and O_2 consumption of the electrode, etc. in the RC 300, and then to further improve the sensitivity of the procedure—and hence reduce the number of animals needed per experiment—by decreasing the sample volume in the RC 200.

2.2. Calibration of the electrode

Zero O_2 was calibrated in a 3% solution of sodiumsulfide or sodiumdithionite. 100% saturation was determined using presaturated distilled water. The accuracy of the monitor was then tested by comparing O_2 measurements of a set of tap water samples of

different concentrations obtained with the electrode with a parallel series of measurements following the Winkler method (as described in Strickland and Parsons, 1972). These measurements were performed at 15.7°C.

To determine O_2 consumption by the electrode itself, the respiration chamber of the RC 300 vial was filled with 1 ml of a presaturated 1% formaldehyde solution, and O_2 concentration was registered for 2 h. The influence of temperature and salinity on the O_2 consumption by the electrode was assessed in the following way: O_2 consumption of the electrode was measured at 2, 5, 10, 15, 20, 25 and 30°C during 20 min incubations of 600 μl samples of 0.22 μm Millipore-filtered water from the Westerschelde estuary (southwest Netherlands), with a salinity of 20‰. A parallel test was run on a series of 600 μl samples of water of different salinities as prepared from 35‰ of artificial seawater (Dietrich and Kalle, 1957) through the addition of distilled water. Distilled water served as the 0‰ control.

2.3. Experimental procedures

The respiration in different sample volumes and of different nematode species was compared. Measurements were performed with the brackish-water nematodes *Diplolaimelloides meyli* (Timm, 1966), *Pellioditis marina* (Bastian, 1865) and *Panagrolaimus* (Fuchs, 1930) sp. and with the terrestrial *Caenorhabditis elegans* (Maupas, 1900). Adult sizes of these species are approximately 0.75–1.25, 1.4–2.4, 0.6–1.2, and 1.15–1.75 mm, respectively. The latter species was cultured either monoxenically on *E. coli* or axenically (Vanfleteren et al., 1990). The other species were cultured on agar plates, consisting of a mixture of bacteriological and nutrient agar in a 4/1 or a 10/1 ratio, synxenically with unidentified bacteria from the natural habitat as a food source.

For all experiments described, nematodes were harvested from the stock cultures, either by washing them from the agar surface with small volumes of sterile water, or, if only low numbers were required, by hand-sorting. Nematodes were aseptically washed prior to measurements, and antibiotics were added to the sample medium in order to block microbial development. Applicability of different aseptic washing techniques was compared by determining the antibiotic efficiency and the recovery rate of nematodes (defined as the actual number of nematodes that were still present and alive after treatment). Antibiotic efficiency was qualitatively assessed by plating 10 μl subsamples on nutrient-enriched agar plates, and observing bacterial growth after 3, 5, and 10 days of incubation at 22°C in the dark. Aseptic washing techniques were modified after Koenning and Barker (1985); washing with sterile habitat water, or through serial changes of antibiotic solutions (basically 10 mg/ml streptomycin and 10 000–20 000 units/ml penicillin), and preincubation with the same antibiotics, were performed. After treatment, nematodes were collected by centrifugation (5 min, 3000 rpm). *C. elegans* was freed from contaminants by washing with sucrose (Sulston and Brenner, 1974).

All measurements with nematodes were accompanied by blanks, in which the O_2 consumption of the 0.22 μm Millipore-filtered sample medium was determined. All results presented below have been corrected for this 'background-respiration'. 100% saturation was calibrated whenever the temperature or the sample medium was changed.

The reproducibility of the respiration measurements was determined using bacteria

(unidentified species) or nematodes (*C. elegans*). Stocks were adequately mixed to ensure subsample homogeneity. A magnetic stirrer was added in all of the following experiments with the RC 300 vial to ensure optimal O_2 diffusion. The reproducibility of the measurements as a function of the sample volume (1000, 600 and 500 μl in the RC 300; 150, 100 and 50 μl in the RC 200) was also determined with bacteria and nematodes (*C. elegans*). Both respiration vials were compared on the basis of measurements of O_2 consumption of *C. elegans*.

The respiration of a dense sample of monoxenically cultured *C. elegans* was compared with that of subsamples of declining nematode numbers. The respiration rates of monoxenically cultured and axenically cultured *C. elegans* were compared with values from the literature to independently assess the accuracy of our measurements. To determine the minimum number of nematodes necessary for reproducible measurements of O_2 consumption, small numbers (in between 20 and 500) were hand-picked from laboratory cultures, aseptically washed and the respiration measured and calculated per individual, following the equation:

$$R = \frac{(a-b)\cdot v\cdot 60}{1000\cdot t\cdot n}$$

with:

a and b	the oxygen concentration at the beginning and at the end of a measurement, respectively
v	sample volume (ml)
t	time in minutes (we usually measured for 20–40 min)
n	number of individuals
R	respiration rate in mg O_2 ind^{-1} h^{-1}

or, if respiration was measured as a percentage of the O_2 used from the initial concentration:

$$R = \frac{x\cdot v\cdot z\cdot 60}{1000\cdot y\cdot t\cdot n}$$

with:

x	difference in oxygen concentration between the beginning and the end of a measurement
z	maximal concentration of oxygen dissolved in the sample medium at the temperature of measurement
y	% of oxygen at the beginning of the experiment

3. Results

3.1. Calibration of the electrode

The measurements of the O_2 concentration with the Strathkelvin monitor and according to the Winkler method, respectively, differed from 0.18 to 0.20 mg O_2 l^{-1}

Table 1
Comparison of O_2 measurements with the Strathkelvin 1302 electrode and the Winkler method

Electrode measurement	Winkler measurement
9.92	9.73
9.92	9.74
8.24	8.05
7.00	6.80
5.56	5.38
4.48	4.29

Experiments were performed at 15.7°C.

(Table 1). The observed discrepancy can be accounted for by a small calibration error of the 100% saturation value, which was due to a loss of O_2 from the saturated sample upon transfer to the respirometer. As a consequence, the 100% sample was only 97.5% saturated. The sensor output is linear with O_2 concentration, and so the discrepancy with the Winkler method is fairly constant over the entire concentration scale (Table 1). Hence, calculations of O_2 consumption, which use the difference between O_2 content at the beginning and at the end of a measurement, are not affected.

O_2 consumption by electrodes depends on the cathode surface area and on the O_2 partial pressure of the buffer (Haller et al., 1994). O_2 consumption by the Strathkelvin electrode ranged from 0 to 1% (mostly from 0.2 to 0.6%) of a saturated water sample over a 20 min. period at 20°C; this percentage did not differ between water samples of different salinity, so O_2 consumption was indeed proportional to O_2 partial pressure. Equally, O_2 consumption by the electrode, expressed as a percent of O_2 used, should not be influenced by temperature. At a room temperature of about 21°C, this was true for sample temperatures from 15 to 25°C. At 2–10°C and at 30°C, however, we found, in terms of percentage, a higher (1 to 2%) and lower (0 to −0.6%) consumption, respectively. This was overcome by using longer equilibration times at the more extreme temperatures; this problem is probably due to the signal being transferred between the highly different temperatures of the sample/electrode and the monitor.

Next to O_2 consumption by the electrode, O_2 diffusion into the respiration chamber via the vertical slit in the electrode jacket is a potential problem in respiration measurements (Hinkle and Yu, 1979; Haller et al., 1994). This rate of O_2 diffusion is proportional to the pO_2 gradient between the respiration chamber and the surrounding air. Haller et al. (1994) found the diffusion rate in their setup to be typically in between +1 and +3 pmol O_2 s^{-1}, far less than values reported for several other respirometers. In their study, the gateway for O_2 diffusion from outside was an injection cannula of 100 mm length and 1.2 mm diameter, which compares well with a slit of 65 mm length but only 1 mm diameter in the protective coat of the Strathkelvin electrode. At low sample pO_2, however, this aspect deserves closer attention.

3.2. Experiments

Table 2 presents data on the recovery rate and the antibiotic efficiency of four aseptic treatments. Aseptic washing was most efficient through serial changes of antibiotics.

Table 2
The applicability of different aseptic washing techniques for experimental nematodes

Treatment	% nematodes recovered after treatment±SD		Infected spots (x/15 inocula)
	A (without agar)	B (with agar)	
a	53.74±4.78	81.69±2.62	13
b	45.93±5.31	73.82±2.87	2
c	44.27±2.62	77.95±3.40	3
d	32.97±3.68	69.12±4.64	0

Treatments: (a) rinsing with sterile habitat water; (b and c) overnight preincubation at 22 and 6°C, respectively; (d) washing through serial changes of an antibiotic solution. Nematodes were harvested after treatment by centrifugation. More than 98% of the recovered nematodes were alive, without significant differences between treatments. A and B refer to treatment without and with addition of 0.1% agar, respectively. Both series (A and B) are averages of 3 replicate treatments. The number of infected spots was determined after a 5-day incubation.

Loss of nematodes from the samples during centrifugation was strongly reduced through addition of a small volume of 0.1% agar. This improved pellet formation during centrifugation but did not interfere with antibiotic efficiency. For most respiration measurements, however, a simple preincubation with antibiotics will sufficiently reduce bacterial contamination, even at treatments as short as 1 h. The combination of antibiotics did not significantly affect the respiration rates of the four nematode species used in our experiments. However, if toxicity of the antibiotics is a problem, transferring the experimental animals through a sterile medium will also strongly reduce the number of microbial contaminants, except when there is a prominent microbial epiflora coating the body surface of the animals.

Determinations of bacterial or nematode respiration using the RC 300 were not significantly influenced by the sample volume (500, 600 or 1000 μl; $P>>0.05$, post hoc test). Measurements with bacteria gave low variances as percentages of the mean (Fig. 2). Values obtained with dense nematode samples, however, were extremely variable during early experiments. This could be attributed to inefficient O_2 diffusion upon use of the small stirrer provided with the RC 300 vial. A second set of measurements with a rectangular magnetic stirrer (0.8×0.3×0.2 cm), gave highly reproducible results (Fig. 2). Survival of the nematodes was checked after a series of measurements using three different types of stirrer at different stirring speeds (10–200 rpm). Only occasional mortality (usually less than 1%) of nematodes was observed upon use of the above-mentioned rectangular stirrer at a speed of up to 30 rpm; occasional increases in mortality during measurements were due to 'irregular' stirring, either at too high a speed or with contact between the stirrer and the vial wall, causing physical damage to the nematodes (see also Marks and Sörensen, 1971). Larger stirrers at a speed of well above 60 rpm caused highly variable mortality rates, sometimes exceeding 50%.

Measurements of O_2 consumption by homogeneous bacterial samples, using the RC 200 vial, were highly reproducible. Values obtained with dense nematode cultures, however, showed large variation. Furthermore, respiration was significantly influenced by the sample volume (50, 100 and 150 μl; $P<0.005$, post hoc test). The high variance

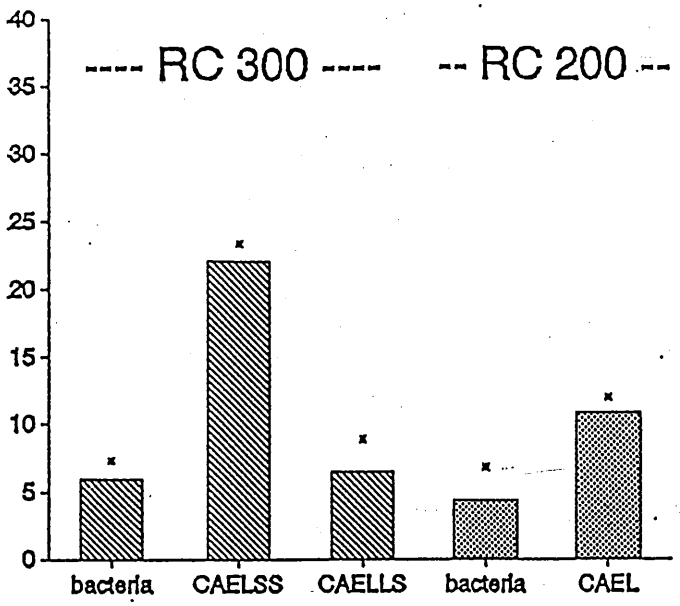

Fig. 2. The variance levels of the respiration measurements with bacterial and nematode samples, using the RC 300 and RC 200 vials. The average variance and the STD are shown for 3 series of 3 replicate measurements each. CAEL, CAELSS and CAELLS represent data on the nematode *C. elegans*, without stirring, with a small stirrer and with a larger stirrer, respectively.

on measurements with the RC 200 can be accounted for by the position of the electrode, which is at the bottom of the RC 200. Nematodes will precipitate during measurements and cover the electrode, thus causing local O_2 depletion. This explanation was verified by changing the position of the respiration vial during measurements (Fig. 3). Although reproducibility of the measurements was greatly enhanced by altering the position of the

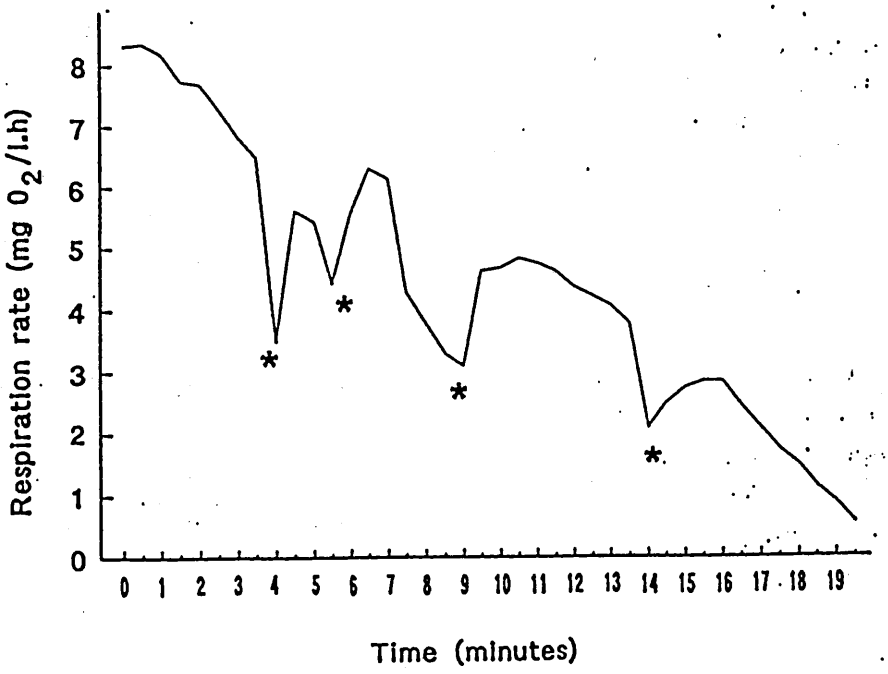

Fig. 3. The respiration profile as a function of the vial position. The measurements were taken with the RC 200 vial. * marks changes caused by an 80° rotation of the vial.

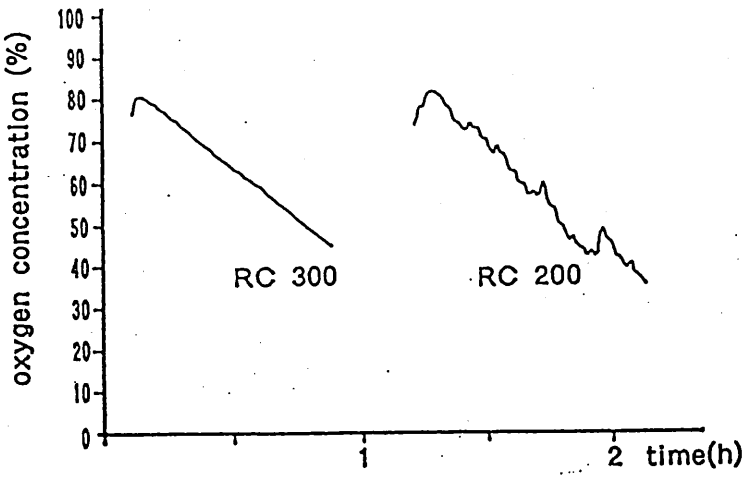

Fig. 4. The respiration profiles obtained for a single nematode sample with the RC 300 (left) and the RC 200 (right), respectively.

vial, the respiration pattern obtained with the RC 200 was usually more irregular and deviant from a linear slope than that obtained when using the RC 300 (Fig. 4). We compared data sets on nematode respiration obtained using the RC 200 and RC 300, and found that respiration rates obtained with the RC 300 were, on average, significantly higher ($P<0.05$, post hoc test). A likely explanation for this phenomenon is the absence of stirring in the RC 200; movement of the nematodes does not suffice for an adequate O_2 diffusion. It is possible to provide a stirring facility in the RC 200, by positioning the vial top-down over a magnetic plate. However, even then, respiration rates determined with the RC 200 were dependent upon the sample volume, and the recorder signal remained much more irregular than when the RC 300 was used. As a consequence, all subsequently reported measurements were performed using the RC 300 respiration vial.

Fig. 5 shows an inverse linear relationship between the number of nematodes (*C. elegans*) in a sample and the sample respiration. The minimum numbers necessary for accurate and highly reproducible measurements differed from species to species (Fig. 6), primarily as a consequence of the species-specific respiratory activity, but also in relation to the physiological homogeneity of animals within one sample. The relatively large variation in *P. marina* respiration rates, even at higher numbers of experimental animals, is probably due to the fact that *P. marina* from our cultures reproduces partly ovoviviparously; the presence or the absence of a few juvenile-containing females (there may be tens of juveniles in a gravid female) may cause significant differences in respiration between subsamples.

Data from our experiments and from Cartesian diver respirometry were highly comparable (Table 3). Moreover, the respiration of axenic *C. elegans* as determined in our experiments was 63% of that of monoxenic nematodes, which corresponds well with an activity ratio of 5:3 for monoxenic to axenic *C. elegans* (Johnson, 1985).

4. Discussion

Respiration measurements with polarographic electrodes face bias mainly in two

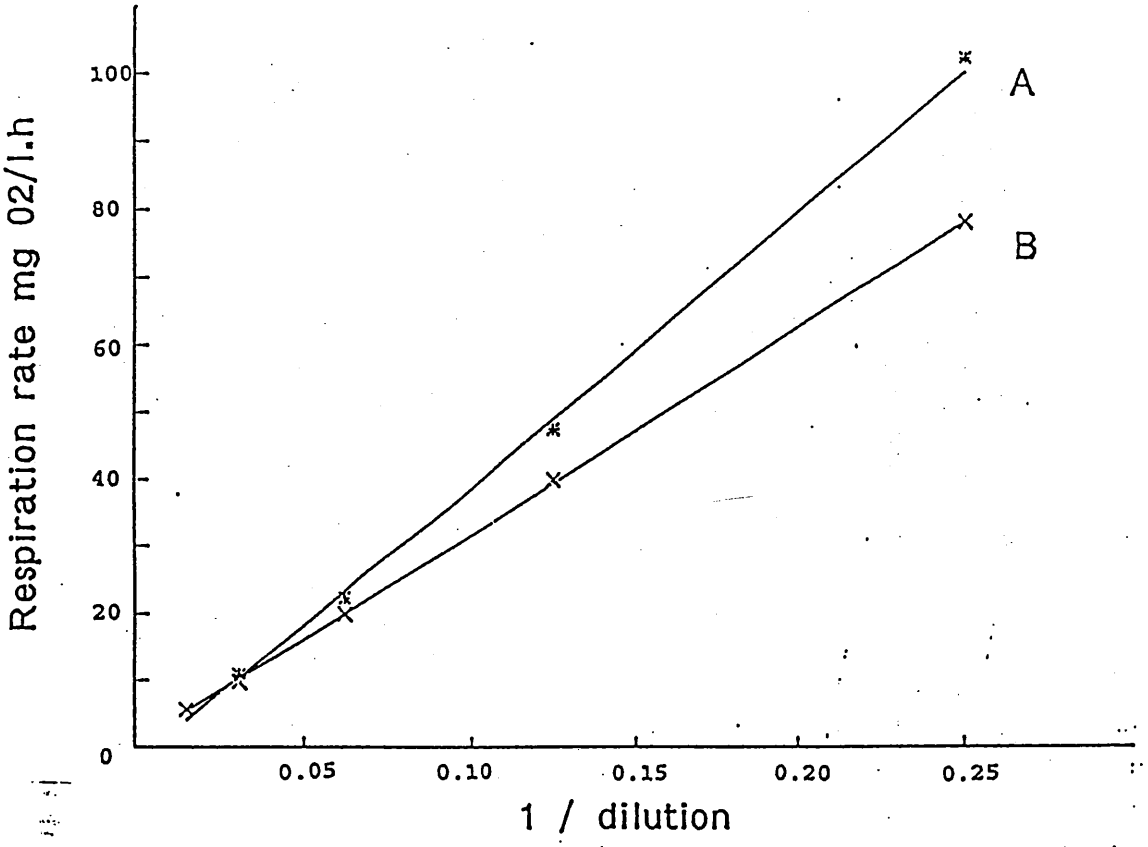

Fig. 5. The respiration as a function of the nematode numbers. A and B represent samples with adults only and a mixture of adults and J4-juveniles, respectively.

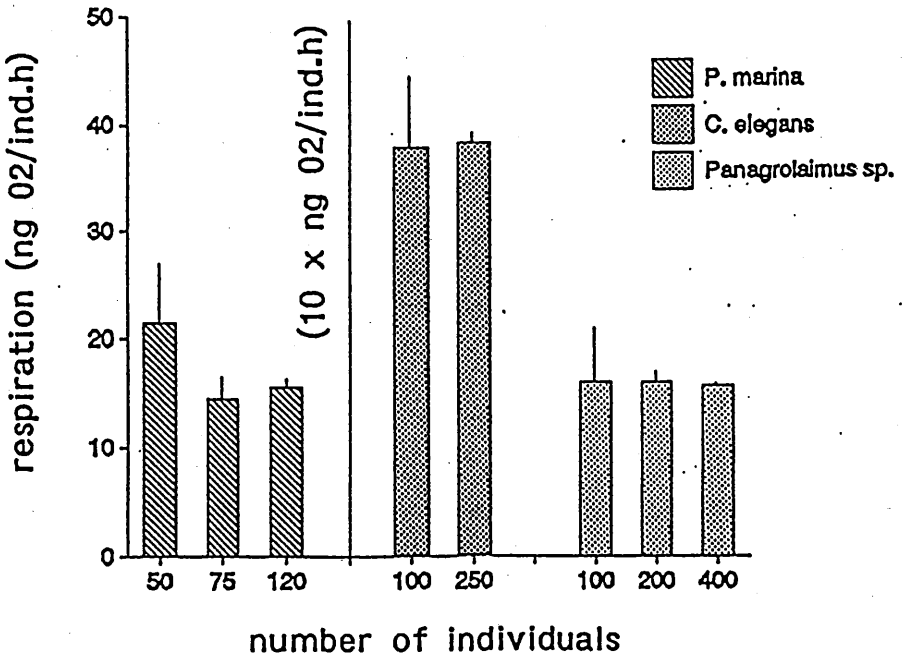

Fig. 6. The respiration as a function of the nematode numbers in 3 species of rhabditid nematodes. The average and the maximum of 2 replicate values are shown.

Table 3
Respiration of axenically and monoxenically cultured *C. elegans*: comparison between data from electrode measurements (our experiments) and from Cartesian diver respirometry

Culture	Age	Respiration (ng oxygen/h per ind)	
		Electrode	Cartesian Diver
monoxenic	adult	5.74±0.56	5.64±0.69[a]
axenic	adult	3.65±0.35	3.39±0.41[b]
axenic	J4/adult	2.35±0.33	2.30±0.32[b]

Shown are data±SD.
[a] The value for monoxenic respiration was calculated from De Cuyper and Vanfleteren (1982), taking into account the ratio of respiration of monoxenically to axenically cultured *C. elegans*, as proposed by Johnson (1985). The second axenic value was calculated, assuming a 50/50 ratio of J4 to adults. Our measurements were performed with 250 nematodes per replica.
[b] Data from De Cuyper and Vanfleteren (1982).

ways: through O_2 consumption by the electrode and through O_2 diffusion into the respiration chamber. O_2 consumption by the Strathkelvin electrode is proportional to O_2 concentration, and averages about 0.5% of full scale in 20 min, with a range from 0–1%. O_2 diffusion into the respiration chamber is probably negligible for most applications proposed in this paper. However, at low pO_2 this aspect should be carefully considered.

The Strathkelvin respirometer provides conditions ideally suited for measurements of respiration under varying environmental parameters, such as temperature, salinity and O_2 tension.

The sensitivity of the presently described method can be determined using data shown for *Panagrolaimus* sp. (Fig. 6). A 5% accuracy level can be reached with O_2 consumption rates down to 200 nl O_2 h^{-1}. This matches the conclusions of Holter and Zeuthen (1966) that a consumption of 100 nl h^{-1} is a minimum value for reaching this accuracy level. This inevitably puts some constraints on measurements with smaller juveniles or species, since a high number of individuals is then still needed for sufficiently accurate experiments. The lower limit for detection of O_2 consumption may in part be set by the ability of the materials in contact with the sample to absorb and release small volumes of O_2 (Atkinson and Smith, 1973; Haller et al., 1994). As such, using Viton instead of neoprene in the 'O'-ring may further improve the sensitivity of the presently described equipment.

Clearly, the amount of O_2 consumed is not the only parameter influencing variance. Physiological status (similar body volume, age, sex, etc.) will importantly influence the minimum number of individuals necessary for adequately reproducible measurements. Prolonging the duration of the measurements may further reduce nematode numbers needed, but we prefer short experimental incubation times, since longer measurements are likely to increase stress to the animals. Furthermore, when working in closed respiration chambers, prolonged measurements imply intrapolation of respiration rate over a timescale during which incubation conditions (e.g., O_2 tension) may significantly change (Gnaiger, 1983b; Moens et al., unpublished data).

The relevance of the respiration rates obtained under laboratory circumstances may be questioned in view of the stress conditions resulting from differences with the in situ

conditions of the animals. Overestimations or underestimations could be a consequence of metabolic adaptations to experimental stress, i.e., stirring and floating of benthic animals in water, without contact with a substrate. However, any mechanical damage caused by stirring can simply be assessed by determining mortality after measurements. An important influence of stirring is unlikely in view of the very similar respiration rates obtained with Cartesian diver respirometry (no stirring) and with the presently described procedure. Furthermore, our observations show that nematodes may change their behaviour in reaction to floating, either by remaining quite immobile (e.g., *Viscosia, Daptonema*), or in contrast by increased activity (vigorous body shaking or active swimming, the latter behaviour especially in monhysterids). The impact of motility rather than metabolic activity on experimentally obtained respiration rates still remains uncertain. However, experiments with a non-motile mutant of the terrestrial *C. elegans* indicate that metabolism largely dominates motility in respiration (J. Vanfleteren, personal communication), and even extreme movement would probably cause less than a doubling of O_2 consumption. This is further supported by studies cited in Schiemer (1987).

At present, experiments are in progress to determine the degree to which several meiobenthic representatives, and in particular dominant nematode genera, partake in total sediment respiration. From the results presented in this paper, it is clear that the respiratory activity of at least the dominant nematode genera can be derived from determinations of numbers and biomass on the one hand, and from laboratory measurements of respiration with batches of animals, collected from sediment samples, on the other. If sufficient numbers, i.e., from some tens to a few hundred, in contrast with the "as few as 5000 individuals" (for nematodes, Marks and Sörensen, 1971) — are available, similar experiments can be performed with other representatives of the meiobenthos, like harpacticoid copepods and oligochaetes. Preliminary experiments on harpacticoids and individual amphipods, as well as many previous reports of electrode measurements of respiration in a variety of small invertebrate organisms, indicate the present and the related electrode-based methods to be applicable in much wider a field than nematology. The aim of this paper, therefore, is not to hint at any superiority of the presently used equipment over similar devices from other companies, but rather to enhance the use of O_2 electrodes in unravelling meiobenthic respiration rates.

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Life Strategies in Two Bacterivorous Marine Nematodes: Preliminary Results

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With 8 figures and 1 table

Key words: Nematodes, feeding, respiration, life strategies.

Abstract. Temperature dependence of sex ratio and maximal densities of the estuarine, deposit-feeding nematodes *Pellioditis marina* (*Rhabditidae*) and *Diplolaimelloides meyli* (*Monhysteridae*) were investigated *in vitro*. Both species are characteristic for organically enriched habitats. Data from competition culture experiments with both species are integrated with information from respiration measurements at different temperatures, and from observations on the influence of temperature and bacterial density on uptake rates.

Sex ratio was significantly influenced by temperature in both species, with the highest relative numbers of males at the highest temperatures. Total numbers attained fluctuated only moderately in the 10 to 20 °C interval, but increased and decreased highly significantly at 25 °C for *D. meyli* and *P. marina*, respectively.

Respiration at temperatures from 5 °C to 30 °C was measured with nematodes from monospecific cultures using a modified Clark electrode procedure. Respiration was dependent upon temperature in the entire range for *P. marina*, but not for *D. meyli*, where only at 25 °C a clear respiratory acceleration was observed.

Feeding experiments with *P. marina* showed a dominant influence of bacterial density on uptake rates, with a lower but still significant temperature effect.
These data are discussed in relation to the overall life strategies of both species.

Problem

Several studies have suggested a role for nematodes in the mineralization of organic matter and nutrient turnover (review in SCHIEMER, 1987; recent data in FINDLAY & TENORE, 1982; RIEPER-KIRCHNER, 1989; TIETJEN & ALONGI, 1990; ALKEMADE *et al.*, 1992 a, b). It is generally believed that nematodes exert their influence through stimulation of bacterial growth by one or a combination of the following processes: (a) direct grazing, whereby bacterial populations could be kept in a prolonged log phase of growth, (b) increasing available surface for microbial degradation processes through mucus secretion (RIEMANN & SCHRAGE, 1978) and/or tube building (NEHRING *et al.*, 1990; NEHRING, 1991), and (c) increasing O_2-diffusion by bioturbation (CULLEN, 1973; ALKEMADE *et al.*, 1992 b).

Pellioditis marina (BASTIAN, 1865) ANDRASSY, 1983 and *Diplolaimelloides meyli* TIMM, 1966 are brackish-water nematodes characteristic for organically enriched habitats. Both species are generally considered to be deposit feeders and to

primarily benefit from bacterial carbon as a food source (WIESER, 1953; JENSEN, 1987). Both species also co-occur on decaying plant material in the Westerschelde estuary (Southwest Netherlands), but in spite of its much shorter generation times and comparable reproductive potential and abiotic tolerance ranges (review in HEIP *et al.*, 1985; MOENS, unpubl.), *P. marina* apparently is never dominant. Moreover, *P. marina* and *D. meyli* have preferences for somewhat different types of detritus, with the former species being most commonly found on decaying *Fucus* thalli, the latter on detritus of variable plant origin.

The present study illustrates differences in life strategy in relation to changing abiotic variables, and in trophic position of both nematode species. Data from competition culture experiments are integrated with information obtained from respiration measurements at different temperatures, and from observations on the influence of temperature and bacterial density on uptake rates. It is suggested that integrating data from laboratory cultures on the one hand and from respiration and uptake experiments on the other, will lead to a better understanding of the mechanisms involved in the apparent coexistence of *P. marina* and *D. meyli*; it would also improve our insight into the role of both species in detritus mineralization.

Material and Methods

1. Culturing of the nematodes

Nematode cultures were initiated from spotplates, where pieces of plant litter are inoculated on a 0.5 to 1% sloppy bacto-agar layer, enriched with modified KILLIAN nutrient medium (VON THUN, 1966). Monospecific cultures were established by transferring adults or J4-juveniles of either species to nutrient-rich agar plates (a mixture of bacto and nutrient agar in a 10/1 or a 4/1 ratio, with the addition of the above-mentioned KILLIAN medium). Contaminants other than accompanying bacteria were gradually removed during early subculturing by one or a combination of the following treatments: a) nystatin treatment to remove fungi, b) repeated rinsing of nematode inocula with autoclaved habitat water to remove contaminating ciliates, algae, and diatoms, and c) prolonged dark incubations, in combination with b), to suppress further algal development.

Monospecific cultures were grown in 9-cm-diameter petri dishes at 20 °C in the dark and subcultured every 10 and 20 days for *P. marina* and *D. meyli*, respectively. Occasionally, newly sampled, rinsed, and antibiotics-treated nematodes were added in order to increase population variability in our stock cultures.

2. Culturing experiments

For the two-species-culturing experiments, 50 adults and/or J4-juveniles of *D. meyli* and 20 adult and/or J4 *P. marina* were randomly picked from monospecific cultures and transferred to bacto/nutrient (4/1 ratio) agar plates. Nematode inocula were not standardized for male/female ratios in order to recognize standard population development patterns from sex ratio-induced variation. Bacterial food was not preincubated on the plates but was derived from the microflora associated with both nematode inocula. Three replicate plates were incubated in the dark at each of the following temperatures: 10, 16, 20, and 25 °C ± 1 °C, and counted every 2 or 3 days during the first 10 days of the experiment, then regularly up to a population platform for each species. Density patterns and sex ratio were determined. Maximal densities as a primary indication of the nematodes' ability to successfully colonize the agar plates were used preferentially to growth rate r or intrinsic rate of increase r_m, because the latter assume density-independent population growth – in the case of r_m starting from an inoculum with a stable age distribution (HEIP *et al.*, 1985). Since both nematode species used in our experiments are supposedly bacterivorous, interspecific competition and hence limited food supply could not be ruled out; more-

over, nematode inocula contained adults and J4 only. Our observations showed a stable age distribution during exponential population growth. Only after reaching (close to) maximal densities, accumulation of non-maturing juveniles was noted.

3. Respiration measurements

Measurements of O_2-consumption were performed with a Strathkelvin 781 O_2-monitor, which basically consists of a CLARK electrode (CLARK, 1956), inserted into a respiration vial with adaptable volume (RC 300 vial, volume of 0.3 to 1 ml). The RC 300 is surrounded by a water mantle, with a connection to a thermostatic water bath.

Nematodes were collected from monospecific plates and treated with an antibiotic mixture (10 to 20 mg · ml^{-1} streptomycin sulphate and 10,000 to 20,000 units benzylpenicillin) prior to respiration measurements. Measurements with nematodes were accompanied by blanks, in which the O_2-consumption of a 0.22 µm millipore-filtered sample medium was determined. All data presented below have been corrected for this background respiration. It is important to note that acclimation to temperatures different from 20 °C was attained gradually but for short periods (30 min to 1 h per 5 °C). Respiration measurements with *P. marina* used adults and J4 only; those with *D. meyli* used culture samples containing all life stages, although here too, adults and older juveniles (J3 and J4) were dominant. One hundred and about two hundred individuals were incubated per measurement for *P. marina* and *D. meyli*, respectively.

4. Feeding experiments

The influence of variable food supply and temperature on the uptake rates of *P. marina* was checked by counting the number of oesophageal contractions (WOOMBS & LAYBOURN-PARRY, 1984) under a Leitz Dialux inverted microscope at 200 x magnification. For this purpose, unidentified bacteria from the habitat were grown for 24 to 48 h at 25 °C on DIFCO nutrient broth (1.25 mg/100 ml), harvested by centrifugation, resuspended in autoclaved habitat water and diluted to the desired concentration. Pulsation rates of *P. marina* were determined at 5, 10, 15, 20, and 25 °C, and at food concentrations from 10^6 to 10^{11} bacteria · ml^{-1} (which on average was the maximal density obtained in nutrient broth cultures). Nematodes were preincubated overnight before observations of feeding activity at the conditions specific for each treatment. A similar procedure, however, was not useful for *D. meyli*, since this species lacks a pronounced oesophageal bulb with distinctly visible valves. Additional feeding experiments using ^3H-thymidine-labelled bacteria were performed, but failed to give consistent results.

Results

1. Competition culture experiments

Figure 1 displays a typical colonization model of the agar plates by both nematode species. In spite of the smaller *P. marina* inoculum, this rhabditid first colonized the plates at all temperatures investigated. Its population increased exponentially towards a maximum, whereafter numbers almost immediately started to decline. The slope of increase and decrease of this growth curve was strongly dependent on temperature (data not shown). At 25 °C, only the first generation progeny reared on the plates matured normally, whereas from subsequent generations only few individuals reached adulthood and started reproduction.

The population increase for *D. meyli* at 16 °C and 20 °C was predictable from generation times of this and other monhysterid nematodes from monospecific cultures. Significant increases of *D. meyli* followed sharp decreases in *P. marina*, so it is unlikely that *D. meyli* actually outcompeted *P. marina*. At 10 °C, a slow

decrease in *P. marina* correlated with a prolonged lag before the expected increase of *D. meyli*. At 25 °C, however, the monhysterid rapidly reached high population densities, this significant population increase coinciding with an early decrease of the rhabditid. At all temperatures investigated, the population platform was maintained for prolonged periods, contrary to *P. marina*.

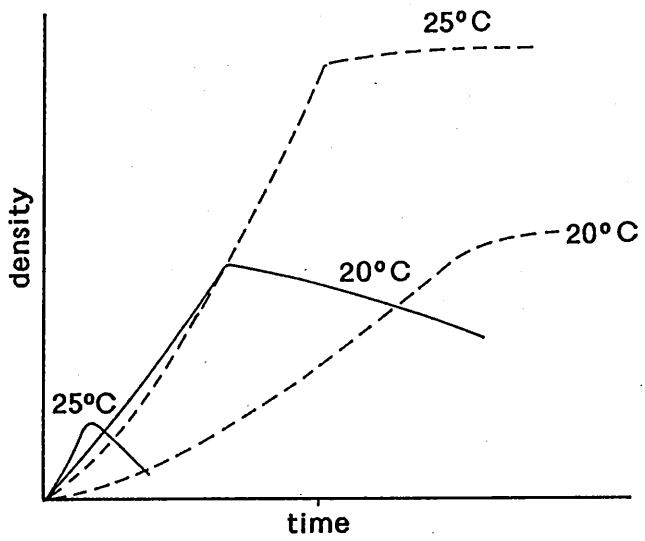


Fig. 1. General model of population development of *P. marina* (full lines) and *D. meyli* (dashed lines) in mixed laboratory culture. Relative development rates at 20 °C are similar to those at 15 and 10 °C.

Maximal *P. marina* numbers reached were not significantly influenced by temperature in the 10 °C to 20 °C interval, but were five to ten times lower at 25 °C (Figs 2 and 3, Table 1). Maximal *D. meyli* densities were affected by temperature over the entire range investigated, but highly significantly only at 25 °C, where numbers were two to three times higher than at the other temperatures.

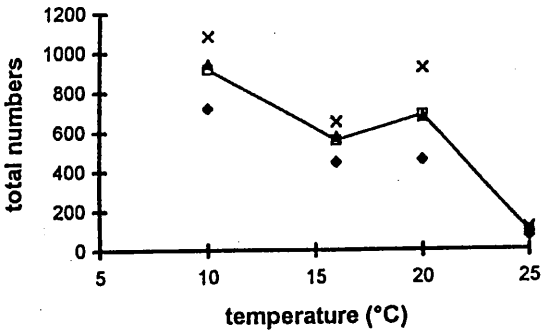


Fig. 2. Influence of temperature on maximal numbers of *P. marina* reared on agar plates. Three replicate and average values are depicted.

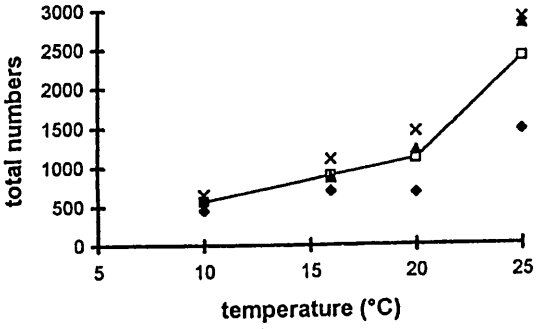
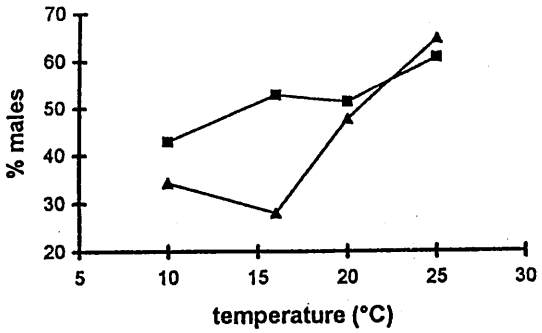


Fig. 3. Influence of temperature on maximal numbers of *D. meyli* reared on agar plates. Three replicate and average values are depicted.

Table 1. Time elapsed before reaching maximal densities at four different temperatures. Average of three replicate plates and SD are given.

temperature (°C)	time before reaching maximal densities (days ± SD)	
	P. marina	*D. meyli*
10	14 ± 2	49 ± 7
16	10 ± 2	32 ± 4
20	7 ± 1	22 ± 2
25	5 ± 1	12 ± 2

Fig. 4. Influence of temperature on the sex ratio of *P. marina* (■) and *D. meyli* (▲) in laboratory culture. Data shown are averages of three replicate treatments.



The sex ratio was significantly influenced by temperature in both species (Fig. 4), but this effect was clearly more pronounced in *D. meyli* than in *P. marina*. In the latter species, the percentage of males varied between 42% at 10 °C and 60% at 25 °C, with an almost equal sex ratio at the intermediate temperatures. In the monhysterid, however, this percentage ranged from almost 65% at 25 °C to only slightly over 25% at 16 °C. At 20 °C and 10 °C, males averaged about 50% and 33%, respectively. Sex ratios were fairly constant over time; one strongly deviant value upon maturation of the second generation of adults in *D. meyli* was probably due to slightly different generation times for males and females.

2. Respiration

Respiration of adults and J4 of *P. marina* was strongly dependent upon temperature in the entire range investigated (Fig. 5). Q_{10} values averaged slightly over 1.5 and slightly over 2 in the 5 °C to 15 °C and the 10 °C to 25 °C interval, respectively. Q_{10} values were also influenced by age of the adults, but the overall pattern was hereby not affected. At temperatures above 25 °C, nematode respiration decreased. This phenomenon is commonly observed in other nematodes as well, although in some species respiratory activity can still increase up to 30 °C (WIESER & SCHIEMER, 1977). The respiration-vs-temperature pattern in *D. meyli* is quite different (Fig. 6). In the 5 °C to 20 °C interval, almost no temperature dependence could be found, with a maximal Q_{10} of about 1.25 between 15 °C and 20 °C. Towards 25 °C,

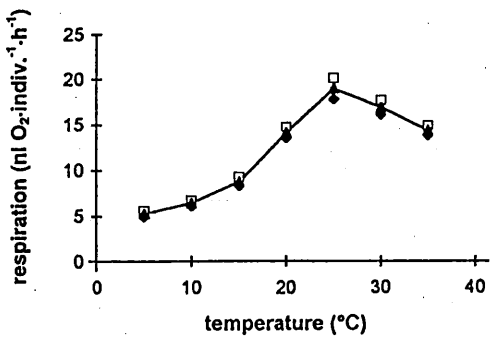


Fig. 5. Influence of temperature on the respiration rate of adult *P. marina*. Two replicate and average values are shown.

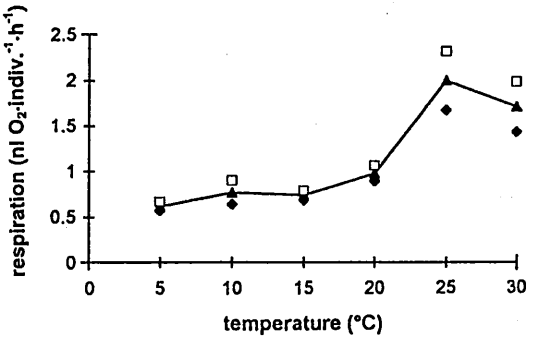


Fig. 6. Influence of temperature on the respiration rate of adult and juvenile *D. meyli*. Two replicate and average values are shown.

however, a spectacular increase in respiration was found, with an average Q_{10} of slightly over 4. At still higher temperatures, respiratory activity declined, as in *P. marina*.

3. Feeding experiments

Oesophageal contraction rates were only observed in adult *P. marina* females. The data presented in Figs 7 and 8 clearly show that both food density and temperature significantly influenced ingestion rates. The optimal food density for *P. marina* was almost 10^{11} bacteria · ml^{-1}, a concentration at which adult females on average showed more than 200 oesophageal contractions per minute at 25 °C. At a food density one order of magnitude lower, this pulsation rate was less than half the above value. A further decline, although not highly significant, was observed towards 10^9 particles · ml^{-1}. Ingestion rates at densities down to 10^7 · ml^{-1} were similar to those at 10^9 · ml^{-1}. At 10^6 bacteria · ml^{-1}, nematodes actively moved their heads and still pumped at low rates (about 20 pulsations per minute), but this probably represents a probing rather than an actual feeding behaviour.

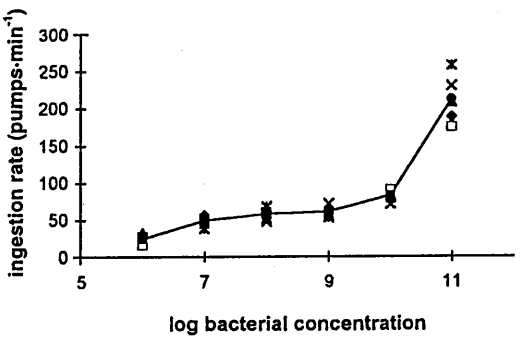


Fig. 7. Influence of food concentration on the ingestion rate of adult *P. marina* females. Data on 5 adult females and averages are shown.

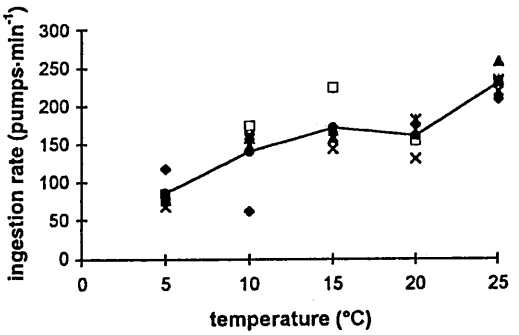


Fig. 8. Influence of temperature on the ingestion rate of adult *P. marina* females. Data on 5 females and averages are shown.

The effect of temperature on ingestion rates was only significant at the extreme temperatures investigated, with clearly over 200 contractions · min^{-1} at 25 °C and less than 100 contractions · min^{-1} at 5 °C. The overall effect of temperature on feeding activity, therefore, is much less pronounced than that of food density, since ingestion at 5 °C with 10^{11} bacteria · ml^{-1} still equalled ingestion at 25 °C at a food density only one order of magnitude lower. The pulsation rates in *P. marina* were in the same order of magnitude as those reported for other rhabditid nematodes (MAPES, 1965; DUNCAN *et al.*, 1974; WOOMBS & LAYBOURN-PARRY, 1984). Again, it must be stressed that our data stem from adult females only. Ingestion rates in juveniles, although not studied in detail, were clearly higher, as reported previously for other rhabditid species (WOOMBS & LAYBOURN-PARRY, 1984). Different uptake rates in males versus females are also likely to occur (WOOMBS & LAYBOURN-PARRY, 1984).

Discussion

The importance of bacteria in the diet of rhabditid and monhysterid nematodes has been demonstrated both directly from grazing experiments (e.g., TIETJEN *et al.*, 1970; HERMAN & VRANKEN, 1988) and indirectly from culturing experiments (*e.g.*, VRANKEN *et al.*, 1981, 1984; VRANKEN & HEIP, 1985; MOENS, unpubl. observ.). At first glance, this compares reasonably with their preferred natural habitat, which is usually densely crowded with bacteria. However, no clear-cut scheme exists on how important bacterial carbon is for nematode survival, activity, and reproduction, since few qualitative and virtually no quantitative data are available on the role of dissolved and small particulate organic matter in any marine nematode's diet.

Our data for *P. marina* are consistent with a mode of feeding primarily directed at bacteria. Clearly, this nematode prefers extremely high bacterial densities (Fig. 7). A *P. marina* population at its highest density will probably consume most of the bacteria present on the limited agar plate environment, thereby bringing bacteria below an optimal density. This could explain the pattern of growth and decline of our *P. marina* cultures. Moreover, the prolonged abundance of *P. marina* in cultures kept at 10°C and lower coincides with the observed lower uptake rates at these temperatures; this might prolong food abundance. The remarkable population development pattern of *P. marina* at 25°C is, however, difficult to explain and contradicts previous data (HOPPER *et al.*, 1973). Since bacterial food was not standardized in our experiments, the bacteria developing at 25°C may have differed from those at the other temperatures and been less favourable to *P. marina*. Furthermore, although non-selective with respect to ingestion, nematodes like *P. marina* can enforce their grazing effect on bacteria through their digestive specificity. However, repeating of the experiment with different bacterial isolates from the habitat of both nematodes always yielded a similar pattern.

The population dynamics of *D. meyli* at different temperatures permit two conclusions on energy use. On the population level, the highest relative amount of energy directed towards reproduction is at 16°C, where females make up almost three quarters of the adult population. On the individual level, however, this occurs at 25 °C, according to the coincidence of higher nematode densities with lower percentages of females. This extra reproductive effort may be reflected in the extremely high respiration rates per individual at this temperature. The predominance of males should not automatically be considered an overall energy waste for the population, since excess sperm may be utilized as an energy source by reproductive females (JENNINGS & DEUTSCH, 1975).

A relationship between food source and respiration-vs-temperature has been proposed previously (PRICE & WARWICK, 1980). Meiobenthos with a stable food source (other meiobenthos or organic matter) would have a low Q_{10} value of about 1, whereas species with food sources which vary with temperature (bacteria and algae) would have Q_{10} values typically around 2. *P. marina* fits well into this scheme, since it is bacterivorous and its respiratory activity is temperature-dependent over the entire temperature range investigated. Q_{10} values are close to 2 on average. The preference for bacterial densities as high as 10^{11} ml^{-1} reasonably reflects the *Fucus* thalli habitat: this and several other brown algae are often covered with a slimy exudate layer (*e.g.*, SIEBURTH, 1969; PREGNALL, 1983), offering both

a suitable substrate for the nematodes and high numbers of bacteria (BOUWMAN *et al.*, 1984).

The relation between food source and respiration-vs-temperature pattern (PRICE & WARWICK, 1980) suggests a stable food source for *D. meyli*. In our opinion, this makes sense if *D. meyli* is attracted to the easily assimilable dissolved or small particulate organic matter of decaying plant material. Bacteria, although also ingested and partly digested, might merely be an additional food source. This hypothesis awaits direct information on the uptake of dissolved and/or small particulate organic matter from radiotracer experiments; it remains intriguing that the use of dissolved organic matter in nematodes has hitherto been so poorly documented compared with less abundant marine invertebrates (CHIA & WARWICK, 1969; TIETJEN & LEE, 1975; LOPEZ *et al.*, 1979; RIEMANN *et al.*, 1990).

The respiration-vs-temperature pattern obtained here for *D. meyli* does not fit the data on the closely related *D. bruciei* (WARWICK, 1981). *D. bruciei* exhibited Q_{10} values of almost 4 over a temperature range from 5 °C to 25 °C. Highly significant interspecific differences in temperature dependence of developmental patterns, for example, have been observed within the monhysterids (VRANKEN & HEIP, 1985). Other explanations for the discrepancies between WARWICK'S and our study concern the experimental setup, although neither the shorter acclimation times used here nor the longer incubation times in the conflicting study are likely to be responsible (MOENS, unpubl. data). The absence of a bacteriocidal treatment in the study on *D. bruciei* may have increased the risk of microbial contamination, although this would have further accelerated respiration above 25°C, which was not the case. New and replicate measurements with both species, cultured in similar media, are necessary to substantiate the temperature dependence of respiration.

The present data permit no final conclusions on competition effects between *P. marina* and *D. meyli*. The competition cultures, however, clearly substantiate a different colonization ability. *P. marina* has a tremendous potential to react to increased food supply, mainly because of its extremely short generation times, short reproductive and prereproductive periods, and high reproductive capacity (VRANKEN & HEIP, 1983; HEIP *et al.*, 1985). On the other hand, it probably has a very high food threshold, with optimal densities around 10^{11} bacteria · ml^{-1}. An increased food supply for *P. marina* apparently means high bacterial densities, as attracted by decaying plant material. Some adaptations to lower food densities are apparent from reduced feeding rates at lower particle concentrations, but only to a limited extent. The very high production and assimilation efficiencies of *P. marina* (TIETJEN, 1980) – if only at high food supply, which was not stipulated – are consistent with a species adapted to environments with constantly high food supply (SCHIEMER, 1983). Since we consistently failed to reestablish *P. marina* cultures from old culture plates, contrary to *D. meyli*, it is plausible that the former species has no true stress-resistant life stages. This may explain why *P. marina* is rather rare on small bits of stranded plant material, which also offer a high food supply. *D. meyli* and other monhysterids, however, are much more frequent in such microhabitats (MOENS, pers. observ.). The long survival periods of *D. meyli* juveniles on old, food-depleted agar plates indicate a better adaptation to food stress. Together with its short generation times and high reproductive capacity, this enables it to readily exploit new organic enrichments.

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Observations on the feeding ecology of estuarine nematodes
and
Quantification of meiofauna grazing rates on bacteria and microalgae: an evaluation of methodology.

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A summary of task C.

General introduction

Meiofauna are the numerically dominant metazoans in marine and estuarine benthic ecosystems, but their feeding biology and role in the benthos are still rather poorly understood. Meiofauna may feed on a variety of sources, including particulate detritus, bacteria, diatoms and other small microalgae, cyanophytes, ciliates, other meiofauna and dissolved organic carbon. What species feed on what sources remains, however, largely unknown.

For a long time, the meiobenthos has been considered as a sort of black box, receiving energetic inputs from the lower trophic levels (primary producers and microheterotrophs), but otherwise not participating in benthic energy flow (McIntyre, 1969, McIntyre *et al.*, 1973). In more recent years, meiofauna have been demonstrated to play a potentially substantial role in the energy flows to the higher trophic levels, both directly, since meiofauna can be significant prey to macrofauna (e.g. Gerlach & Schrage, 1969, Bell & Coull, 1978, Gee, 1989, Coull, 1990, Feller & Coull, 1995), and indirectly, as they may contribute to nutrient recycling processes. It has been suggested that meiofauna stimulate heterotrophic breakdown of organic matter in sediments (e.g. Findlay & Tenore, 1982, Tietjen, 1980, Tietjen & Alongi, 1990, Schiemer, 1987, Alkemade *et al.*, 1992a, Rieper-Kirchner, 1989) through their grazing activity on bacteria (e.g. Montagna, 1995), through mucus production (Riemann & Schrage, 1978), and through bioturbation of the sediment, increasing both the penetration depth of oxygen into the sediment (Alkemade *et al.*, 1992b, Cullen, 1973) and the available space for heterotrophic processes (Nehring, 1991, Nehring *et al.*, 1990, Alkemade *et al.*, 1992a).

Nematodes are the most abundant meiofaunal component in marine and estuarine soft sediments, with densities up to several million individuals m^{-2}, representing an average biomass of 0.2 - 0.5 g C m^{-2} (Heip *et al.*, 1985). In organically polluted sites with a predominance of large nematodes, biomass values of up to 50 g wet weight m^{-2} have been reported (Bett & Moore, 1988). In terms of ATP, nematodes may comprise up to 92 % of living carbon in intertidal sediments, a contribution over ten times more important than that of bacteria (Sikora *et al.*, 1977). Bouwman (1983) attributed nematode dominance in estuarine sediments to three main factors: 1. their burrowing capacity, in combination with their small and slender shape, allowing the occupation of interstitial spaces in coarse grained sediments as well as the invasion of soft sediments; 2. their tolerance, as a taxon, of a variety of

environmental stresses; 3. the diversification in buccal structures, enabling nematodes to exploit a broad range of food items present in the benthos.

C.1. Observations on the feeding ecology of estuarine nematodes

Introduction

Wieser (1953) linked buccal morphology of free-living aquatic nematodes to feeding ecology in a feeding type classification discriminating between 1A: selective deposit feeders and 1B: non-selective deposit feeders, both without a buccal armature, and between 2A: epistrate feeders and 2B: predators or omnivores, both with a buccal armature. Wieser's feeding type classification (Fig. 1) has been widely used since, primarily because it enables the putative assignment of any nematode specimen to a trophic guild. However, the reduction of a huge species diversity into four feeding types, suggesting but a limited functional diversity, is likely to underestimate the true functional complexity of nematode communities. The same may hold true for the four feeding groups recognized among harpacticoid copepods (B.M. Marcotte, cited in Hicks & Coull, 1983), often the secondmost dominant meiofaunal taxon.

Wieser's scheme, and the modifications subsequently proposed to it, are primarily lacking in factual data obtained from observations of living nematodes in the presence of different candidate food particles. As part of task C, we performed an observational study on the feeding behaviour of nematodes from an intertidal mudflat at Walsoorden, in the Westerschelde estuary (Fig. 2), with additional data on species from the margin of a *Spartina* marsh near Terneuzen (the Paulinaschor). A total of almost 40 species has been observed during feeding, and the most relevant data from this study have been compiled in a publication, presently in press in the *Journal of the marine biological Association of the UK*, and proposing significant modifications to the feeding type classification of Wieser (1953). The discussion of this paper is reproduced here in a slightly modified version to highlight the main points which emerged from our observations.

Results

Contrary to Jensen (1987a) but in agreement with Wieser (1953) we suggest a subdivision within the deposit feeders for the following reasons. The difference in maturity index (Bongers *et al.*, 1991) between selective and non-selective deposit feeders, as well as their relative proportion in organically enriched as opposed to unenriched sediments (Vincx, 1989, Smol *et al.*, 1991), suggest significant ecological differences. Moreover, in WO22 selective and non-selective deposit feeders have clearly different seasonal abundance patterns (Fig. 3). An ecologically relevant subdivision between Wieser's groups 1A and 1B is futher supported by the analysis of functional groups in deep-sea nematode communities (Thistle *et al.*, 1995). To avoid confusion with previously used terminology (Wieser, 1953, Jensen, 1987a), we propose the terms **microvores** and **deposit feeders *sensu lato*** as opposed to selective and non-selective deposit feeders, respectively.

In view of their small buccal cavities, **microvores** are automatically restricted to small particulate food or dissolved organic matter. For these nematodes, picking out bacteria on an individual basis may prove to be energetically favourable compared to non-selective oesophageal pumping, but it has to be stressed that processes involved in food selection in these and other free-living aquatic nematodes remain largely unknown.

Based on observations of food ingestion, selectivity in **deposit feeders *sensu lato*** would appear to be mainly a function of particle size. Obtainable food should, however, not be narrowed down on the mere basis of a nematode's mouth size. *Theristus* sp. (mouth size 10 mm) was reported ingesting diatoms with a diameter of 24 mm and a length up to 220 mm (Boucher, 1973). The nematodes' ability to widen their mouth during feeding clearly broadens their food range.

Within the deposit feeders *sensu lato*, some species have a food selection mechanism which is not mainly based on particle size; diatoms and algae, which would fit into their buccal cavity, are not

ingested, while similarly sized protozoa are. As yet, our observations on *Tripyloides gracilis* and *T. marinus* and those of von Thun (1968) on *Anoplostoma viviparum* are the only ones implementing marine nematodes in significant predation on ciliates. In view of their relative abundance - they comprise up to 25 % of nematodes in WO22 (Li, 1993) - and because of the different selection mechanism involved, the phenomenon is significant enough to erect another category within the traditional deposit feeders. Protozoa are a food source the importance of which for nematodes might, up to now, have seriously been underestimated. Bacteria probably make up part of **ciliate feeders'** diets as well, their importance possibly being greater in juvenile stages.

Diatoms are an important food source for several representatives of the **deposit feeders** ***sensu stricto***. These nematodes have no buccal armature; diatoms are ingested entirely and (partly) digested during passage through the intestine (Nehring, 1992b). **Epigrowth feeders**, however, are characterized by the presence of a buccal armature, supposedly used to either scrape off particles from a substrate, or to damage and open food items before emptying them. Diatoms and other microalgae are an important food for many representatives of this group. There are two strategies in which diatoms are preyed upon: cracking and piercing (Nehring, 1992a). The qualitative feeding pattern of epigrowth feeders is found throughout all life stages, from freshly hatched juveniles to adults. Obviously, the range of diatoms available as prey will at least in part depend on their size, thus confining smaller juveniles to a more limited diet.

Diatoms are clearly an important food for epigrowth feeders. However, the high abundances of representatives of this feeding type at several deep-sea locations (e.g. Vincx *et al.*, 1994, Thistle *et al.*, 1995) are but one indication for the involvement of other components in their diets. Although it has been suggested that epigrowth feeders do not significantly feed on bacteria (Tietjen & Lee, 1973, Jennings & Deutsch, 1975, Deutsch, 1978), their teeth may be used to scrape off microbiota from solid surfaces or from mucus threads (cf. Boucher, 1973, Jensen, 1982). Alongi & Tietjen (1980) cultured *Chromadorina germanica* on a diet of bacteria, provided that enough bacteria were attached to a solid substrate. Riemann & Schrage (1978) argue that non-particle-induced oesophageal pumping is a mechanism to feed on all sorts of small particles entrapped in the nematodes' mucus threads. The buccal apparatus would thereby act as a filter to prevent the oesophagus from becoming clogged, rather than as an actual scraping device.

The mechanisms involved in discriminating between edible and non-edible particles, and among the edible ones, in preferring some to others, are at present unknown. Particle size (Tietjen & Lee, 1973) and rigidity (Romeyn *et al.*, 1983) may play a role in food selection. Orientation of a diatom may also be important, since many collisions of several chromadorid species with suitable food items did not result in any feeding response at all.

Nematodes belonging to Wieser's group 2B (1953) have diverse feeding habits and candidate food sources. Wieser (1960) therefore used the name omnivores rather than predators, but this too is misleading in that it suggests additional food for species which indeed are strictly or mainly predatory. In WO22, *Sphaerolaimus gracilis* and *Enoploides spiculohamatus* feed predominantly or exclusively via predation on nematodes and nematodes, oligochaetes and perhaps still other metazoans, respectively.

The remainder of Wieser's group 2B are commonly considered as omnivorous. The nature of this 'omnivory' is, however, poorly understood. Oncholaimids and enchelidiids scavenge on dead animals, as illustrated by several observations. Jensen (1987a) therefore used the name scavengers, but other feeding strategies may be equally or predominantly important. *Metoncholaimus scissus* forages by random ingestion of fine sediment and detrital material (Meyers *et al.*, 1970), the presence of mat-forming organisms being an important factor to render a substrate attractive (Meyers *et al.*, 1970, Meyers & Hopper, 1966, Hopper & Meyers, 1966). This mode of feeding was also observed by us in *Viscosia viscosa* in mats of epipelic diatoms. Significant label uptake by *Adoncholaimus fuscus* in ^{3}H-adenine-impregnated sediment devoid of living nematode prey and bacteria indicates that sediment particles are also ingested directly (Verbeeck & Moens, unpubl. results). Microbiota often pass through the gut of *Adoncholaimus thalassophygas* undigested and label from ^{14}C-glucose-impregnated bacteria was not incorporated by this nematode (Lopez *et al.*, 1979). Label from dissolved glucose, however,

was readily incorporated, and it was therefore concluded that juvenile *A. thalassophygas* benefit primarily from dissolved organic carbon (DOC), a food source still obtainable for older juveniles and adults, which further feed by predation and scavenging (Lopez *et al.*, 1979). A shift from juvenile omnivory to adult carnivory has also been documented for an enoplid nematode (Hellwig-Armonies *et al.*, 1991).

The only character common to all scavengers *sensu* Jensen (1987a) observed by us, is the ability to forage on living nematode prey. Prey is ingested, not pierced, and in *Adoncholaimus fuscus* and *Oncholaimus oxyuris* predation can be commonly observed in juveniles, from J1 onwards, as well as in adults. The quantitative importance of predation in these nematodes is unclear, but *A. fuscus* adults and J4 were able to significantly reduce numbers of *Diplolaimelloides meyli* on agar plates deficient in other food items, though to a lesser extent than did *Enoploides longispiculosus*. In organically enriched medium, however, *A. fuscus* was never observed ingesting prey, whereas *E. longispiculosus* actively preyed on oligochaetes and a variety of nematodes (Verbeeck & Moens, unpubl. observ.). It seems plausible that oncholaimid nematodes are very opportunistic feeders, and that predation is merely a facultative mechanism to obtain extra food. We therefore propose the term **facultative predators** instead of omnivores (Wieser, 1953) or scavengers (Jensen, 1987a).

Our observations on the predatory behaviour of *Calyptronema maxweberi* demonstrate that any enchelidiid or oncholaimid nematode cannot automatically be considered a facultative predator, and suggest the involvement of some "chemical warfare" in prey capture by this nematode. To our knowledge, no other reports on the use of paralysing or lethal substances in predation of aquatic nematodes have hitherto been published. Enoplid nematodes, on the other hand, should not automatically be considered strictly predatory. *Enoplus brevis* not only ingests other nematodes and oligochaetes, but also cyanophytes, diatoms, rotatoria, and detritus (Hellwig-Armonies *et al.*, 1991). In fact, although its buccal morphology would classify it as a predator, its feeding behaviour apparently is much like that of a facultative predator.

Our observations illustrate major relations between (groups of) nematode species and particular food sources, which offer a more direct basis for assigning the six feeding guilds proposed in Fig. 4 than mere morphological characters, which give information on a nematode's ability to handle food rather than on any actual feeding preference. However, quoting Yeates *et al.* (1993), it is clear from the opportunistic feeding behaviour of many nematodes observed in the present study that "...Ideally the feeding habits of each nematode species should be determined in each particular ecological setting". Because of major difficulties in long-term maintenance of most marine species, no such detailed account of nematode feeding can at present be given. However, as the role of nematodes in sediments is still poorly understood, ecologists and modellers have a dire need of schemes which illustrate the possible pathways of carbon flow through this component of the benthos, and the present data may allow a more refined interpretation of nematode feeding ecology than previous schemes. A tentative scheme of carbon flow pathways through the nematode component of the benthos is proposed in Fig. 5.

A major flaw in our understanding of the trophic position of nematodes in marine sediments is the virtually complete lack of information on the role of dissolved organic carbon (DOC) in their nutrition. Jensen (1986, 1987a,b) demonstrated significantly lower body volume to body surface ratios in thiobiotic nematodes as opposed to oxybiotic species, and hinted at the possible involvement of transepidermal uptake of DOC in their survival strategy. Experimental evidence for uptake of DOC by a meiofaunal community with nematode predominance was given by Montagna (1984a). DOC uptake was demonstrated for two oncholaimid and one comesomatid nematode species by Chia & Warwick (1969), Lopez *et al.* (1979), and Riemann *et al.* (1990), but found to be of lesser importance for the rhabditid *P. marina* (Tietjen & Lee, 1975). Riemann & Schrage (1988) showed attraction of *A. thalassophygas* to CO_2, suggesting that motile oxybiotic species might react to the release of fermentation products from anaerobic sediment layers. Uptake of DOC is mainly through the intestine (Chia & Warwick, 1969). Hence, non-particle-induced oesophageal action might be a strategy for obtaining dissolved organic compounds, released by microbial activity. Microbial and microphytobenthal exopolymer secretions (EPS), which by theirself can offer an easily assimilable organic food source for

meiofauna (Decho & Moriarty, 1990) are known to trap DOC (Decho , 1990, Decho & Lopez, 1992), so any EPS-covered particle may be a strongly nutrient-enriched food source.

C.2. Quantification of meiofauna grazing rates on bacteria and microalgae: an evaluation of methodology

Introduction

Most experiments to date investigating rates of meiofauna grazing on bacteria or microalgae have used radioactive tracer techniques, the label either being added directly *in situ* (i.e. to sediment samples) (Daro, 1978, Montagna, 1983, 1984b, 1993) or indirectly (as prelabelled food particles added *in situ* or under laboratory conditions) (Haney, 1971).

Published estimates of meiofauna grazing on benthic bacteria and microalgae on average approximate 0.01 h^{-1} (Montagna, 1995). Put differently: meiofauna consume about 1 % of bacterial and microalgal standing stock per hour, which suggests a tight coupling of benthic meiofauna to benthic microbiota (Montagna, 1995). There are, however, serious flaws in the presently used techniques to measure meiofaunal microbivory, and questions pertaining to relevant experimental incubation times, sample preservation procedure, periodicity in meiofauna feeding activity etc. have so far received too little attention. As part of task C, we started with field grazing experiments along a salinity gradient in the Schelde up to the North Sea (Mees *et al.*, 1993), but, though employing protocol recommended in the literature (Montagna, 1983, 1984, 1993, Montagna & Bauer, 1988), immediately faced methodological problems which urged a shift towards the assessment of key aspects of methodology. Some of these aspects have already received ample attention, and are discussed below on the basis of an overview of the pertinent literature, while others have been acknowledged but not investigated. As part of task C, we focused on sample fixation procedure and experimental incubation time.

Most experiments to date have been performed to determine meiofauna community grazing rates on bacteria or microalgae, and have for this purpose used direct addition of radioactive label ($NaH^{14}CO_3$ for microalgae and [methyl-^{3}H]-thymidine for bacteria) to sediment samples. This is the so-called three-compartment model, where grazing rates can be calculated from the equation

$$G = 2M/m.t$$

- where G = grazing rate, M = the amount of label entering grazers via feeding on bacteria or microalgae , m = the amount of label in the bacteria or microalgae, and t = incubation time (Daro, 1978, Montagna, 1984b, Montagna & Bauer, 1988). For this equation to yield accurate grazing rates, two essential assumptions should be met: 1. label uptake in the grazed compartment should be linear, and 2. label uptake in the grazer compartment should be hyperbolic with time (as the grazers are feeding on increasingly more labelled food). It is further assumed that added label is not depleted and that grazer label recycling is zero during the experimental time course. In this case, G is expressed in units of t^{-1} , mostly h^{-1} (Daro, 1978, Montagna & Bauer, 1988, Montagna, 1984b, 1993).

A first methodological difficulty in trials with sediments is how to administer the label in such a way that it is rapidly and evenly distributed, at the same time, however, minimizing disturbance to sediment microbial and meiofaunal organisms. Frequent use has been made of slurries, which cause severe disruption of the sediment, but ensure rapid and homogeneous distribution of the label. Alternatives to this are the pore water replacement method, which also yields a good homogeneous label distribution, but may still adversely affect the benthos, and horizontal injection of label into the sediment, which does not significantly disrupt the sediment, but results in a poorer label homogenization (Carman *et al.*, 1989, Montagna, 1983, 1984b).

Once a tracer has been added to a sediment, controls have to be adopted for label adsorption to grazers' body surfaces, and for the ingestion of free label (i.e. not assimilated by the grazed compartment) (Montagna, 1983, 1993, Montagna & Bauer, 1988). Poisoned controls, typically using formaldehyde-preserved samples, account for the former aspect, while the latter can be balanced by running parallel dark (in the case of grazing on microalgae) or inhibitor-poisoned (in the case of grazing on bacteria) incubations, where label uptake by the grazed compartment is considered non-existent or minimal (in the latter case strongly influenced by the efficacy of the prokaryote inhibitor used) (Montagna, 1983, 1993, Montagna & Bauer, 1988). Whereas ingestion of inorganic free label ($NaH^{14}CO_3$) by meiofauna is apparently all together not very high, and the use of parallel dark incubations remains pretty straightforward, uptake of free organic label in, e.g., the deposit-feeding fraction of the meiobenthos, together with surface adsorption, may account for more than 80 % on average of label entering grazers (Montagna & Bauer, 1988), and the use of prokaryotic inhibitors requires time-consuming efficiency screening and may adversely affect meiofauna activity. It is therefore not surprising that fairly well reproducible grazing rates on microalgae have been obtained in some studies, while rates of bacterivory remain prone to unacceptably high variance.

Even in a two-compartment model with grazers and prelabelled food, some significant methodological problems exist. Recycling of label from the food compartment during the test incubation period should be low, since free label may enter grazers via non-grazing activity (Montagna & Bauer, 1988), which in this setup is not corrected for. It also tends to yield higher control values for adsorption of label to the grazers.

More important yet is the fixation procedure used to stop an experimental incubation. Formaldehyde has commonly been used in trials with meiofauna (Montagna, 1995), but apart from permeabilizing their cuticle to some extent, it may also induce egestion or defaecation of (parts of) the grazers' gut contents. Other chemical preservatives are likely to cause similar bias, though probably to a variable extent (Montagna, 1995). We have compared bacterial grazing rates in meiofauna preserved chemically (formaldehyde in several concentrations, glutaraldehyde, ethanol,...) or physically (freezing or heating) or with a combined chemical and physical approach, with rates obtained from non-preserved meiofauna.

Another important question is how long a grazing trial should be incubated in order to yield values representative of grazing and not of assimilation. Most studies have hitherto used incubations of at least two hours (Montagna, 1995), a period during which actively feeding rhabditid nematodes, e.g., can fill and subsequently empty their guts as often as eight times (Mapes, 1965, pers. observ.).

Materials and Methods

We previously reported on a methodology for laboratory grazing experiments with monhysterid and rhabditid nematodes (Dewicke *et al.*, 1995) without , however, special focus on aspects of sample preservation and incubation time.

For the experiments reported here, bacteria were isolated from a culture of the nematode *Pellioditis marina* and grown in liquid nutrient broth medium on a rotary shaker at room temperature. 250 µl of ^{3}H-adenine was added to 30 ml of bacterial culture. Bacteria were harvested by centrifugation and washed a minimum of 5 times in sterile artificial seawater to remove non-assimilated label (Dewicke *et al.*, 1995). Bacteria were enumerated via serial dilution on nutrient agar plates, or spectrophotometrically after calibration of an optical density curve by comparison with numbers obtained by serial dilution.

35 adult and J4 *P. marina* were hand-picked from a laboratory culture on agar and transferred into a 300 µl drop of sterile artificial seawater (ASW) with a salinity of 25 psu. 300 µl of washed bacteria were then added to the nematodes, and the total 600 µl spotted in 3.5 cm diameter plastic petridishes. This created a thin water layer on a solid substrate, where the nematodes showed an improved feeding action over nematodes floating in water (Dewicke *et al.*, 1995). Grazers were then allowed to feed for 1 h at room temperature (approximately 20°C). Experiments were stopped by chemical or physical preservation or by a combination of both. **Chemical preservation** was done 1) by

addition of 600 µl of 8, 4 and 2 % formaldehyde, yielding final concentrations of 4, 2 and 1 %, respectively; 2) by addition of 600 µl 2 % glutaraldehyde and 3) of reagent grade ethanol. The effect of storage time in formaldehyde was tested in the following way: immediate label losses were quantified by comparison of grazing rates obtained without preservation of the grazers (in this case, the living nematodes were hand-picked from the plates, transferred twice in sterile artificial seawater to remove adhering label, and then directly into tissue solubilizer) with rates obtained from animals which were formaldehyde-preserved but then immediately (after 15 min) rinsed and transferred into tissue solubilizer. These rates were further compared with rates obtained from nematodes which remained in formaldehyde for 1 day and 1 week, respectively, and from nematodes which remained in formaldehyde for 1 day and then in sterile artificial seawater for one more week.

Physical preservation was done 1) by sample heating to 80 °C, and 2) by transfer of the samples to liquid N_2. The following **combinations of chemical and physical preservation** were tested: 1) liquid N_2 with postfixation in 2 % (final concentration) formaldehyde; 2) addition of 600 µl heated (80 °C) 4 % formaldehyde; and 3) cooling the samples on ice, followed by the addition of 600 µl cold (4 °C) 4 % formaldehyde.

Each treatment was done in triplicate. Parallel controls for label adsorption to the nematodes' body surfaces were run by incubating and preserving equal numbers of formaldehyde-preserved nematodes in exactly the same way as in the trials with living nematodes. After termination of an experiment, nematodes were kept in the preservative for 24 h (except in the above-mentioned time series with formaldehyde), and subsequently transferred manually to tissue solubilizer. Before that, they were rinsed twice (also by manual transfer) in ASW to remove a majority of adhering label. We found this procedure to be superior to others and all together not too time-consuming.

Activity in the nematodes was determined through liquid scintillation counting in a Beckmann LS6000. Nematodes were first solubilized in 1 ml Lumasolve (LUMAC) for 24 h at room temperature, followed by the addition of 10 ml of scintillation cocktail (Lumasafe +, LUMAC), Each sample was run twice and counted twice per run, each time for 10 minutes.

The influence of **incubation time** on calculated grazing rates was assessed using experimental incubations on two different time scales. In a first test, nematodes were incubated as described above, but for 1, 2, 3, 4, 6, 8, and 24 h, respectively. In a second trial, incubation times of 15, 30, 45, and 60 min were used. There were three replicates for each incubation time. Experiments were stopped through addition of 600 µl of 4 % formaldehyde. Samples were then further treated as described above.

Results and Discussion

Grazing rates obtained after sample preservation with formaldehyde were roughly the same for the different concentrations of fixative used, but were all significantly lower than rates obtained with other chemical preservatives. Glutaraldehyde yielded rates twice as high as an equal concentration of formaldehyde, while ethanol yielded rates more than tenfold those obtained with formaldehyde and approximately 70 % of rates from non-preserved specimens. Preservation in formaldehyde, followed by immediate rinsing in water, gave rates slightly over 60 % of those from non-preserved specimens. A 24h stay in formaldehyde reduced label in the nematodes by more than 90 %, while a one week incubation in water after preservation in formaldehyde yielded a further reduction of 75 % (Fig. 6).

Using heated or cold formaldehyde as a preservative approximately doubled and tripled, respectively, rates obtained from samples preserved in formaldehyde at room temperature. The use of liquid N_2 gave results comparable to those after preservation with hot formaldehyde (Fig. 6).

If we assume that upon manual transfer of living nematodes label losses via egestion or defaecation are negligible, than the patterns of label loss upon preservation with formaldehyde probably illustrate two main ways in which label is lost after chemical or physical termination of a grazing experiment. If nematodes are immediately rinsed and transferred following a 15 min. treatment with formaldehyde, grazing rates average slightly over 60 % of those from non-preserved samples. It is hypothesized that this discrepancy is mainly due to egestion or defaecation of ingested particles. It should be born in mind here that death is not immediate upon preservation in formaldehyde, and that the

addition of the chemical induces an abrupt stress reaction, causing, e.g., enhanced egg deposition. Label loss during prolonged incubation in formaldehyde is probably due to label leakage at the cellular level, and is more than substantial: activities measured in nematodes one day and one week after preservation with formaldehyde average only 5 and 1.25 %, respectively, of activities in non-preserved nematodes.

The differences in rates obtained after preservation in cold or hot formaldehyde as compared to the same chemical at room temperature again reflect the aspect of egestion/defaecation. Cooling down the samples before preservation reduces nematode activity and thus tempers egestion/defaecation upon preservation, while hot formaldehyde kills the nematodes more rapidly than at room temperature.

Liquid N_2 should minimize label egestion or defaecation, because fixation is immediate, but significant leakage of low molecular weight proteins upon thawing can be a problem. In trials with ^{14}C-glucose as a tracer, Nicholas *et al.* (1973) showed that ^{14}C taken up by nematodes was not evenly distributed in the nematodes' bodies, but was strongly concentrated in a pool of low molecular weight components. The relatively poor values obtained after fixation in liquid N_2 probably reflect leakage of this material upon thawing. Furthermore, material preserved in liquid N_2 is usually rather "sloppy" and therefore difficult to handle, as specimens easily fall apart when picked out with a needle.

An early experiment with incubations of one or several hours showed label uptake to increase as a linear function of incubation time up to 8 h (Fig. 7). However, *P. marina* is a very active feeder, relatives of which may fill and subsequently empty their guts up to eight times per hour (Mapes, 1965). It is therefore not surprising that on a shorter time scale, this linearity is not found. In two separate experiments, we found grazing rates calculated after a 1 h incubation to underestimate 15 min rates by a factor of 2 to 4 (Fig. 8).. The results of these and other parallel experiments were not always consistent, and are more diffcult to interprete because a formaldehyde preservation, causing egestion of particles, was used. Nevertheless, it is fairly obvious that for this nematode a 1 h incubation gives rise to a grazing rate which is biased as it will integrate only one "gut equivalent" with an amount of assimilated label. The linear curve in Fig. 7 likely shows an equilibrium between assimilation and respiration, rather than grazing. In order to discriminate between ingestion and assimilation, time scales should be as short as possible, and should definitely not exceed half an hour.

It is clear from the present results that virtually all hitherto published meiofauna grazing rates on bacteria are strongly biased as a result of basic methodological problems. While the need for adequate controls for non-grazing label uptake has been recognized from the start (Montagna, 1983, Montagna & Bauer, 1988), the possibility of problems involved in sample preservation and incubation time has been mentioned (Montagna, 1993) but disregarded in practice. As a consequence of these methodological shortcomings, it can be argued that meiofauna grazing rates sofar obtained have consistently been over- or underestimated. There are arguments on both sides, and it is yet to be established whether they are in balance or tend to either direction.

Possible causes for underestimated grazing rates are function of 1) tracer administration to the sediment, of 2) sample preservation, and of 3) experimental incubation time.

The use of slurries may disrupt patches of microalgae and bacteria, and our unpublished observations demonstrate that nematodes are attracted to and feed more efficiently on patchily rather than homogeneously distributed food. We also demonstrate that, since grazing experiments on sediment samples, involving a substantial number of replicates and control treatments, are rather time-consuming and thus on average take at least one or more days just for the sorting of the meiofauna, and since formaldehyde has almost routinely been used to terminate experimental incubations, the rates reported will be but a fraction of true rates considering the aspect of label leakage out of grazers. As in most trials sofar incubation times of several hours have been used, the possibility of nematodes clearing their guts several times within this interval is a further reason to suggest that reported rates have strongly underestimated real values.

The latter aspect, however, can also be held as an argument to suggest that grazing rates have sofar been overestimated. The key question here is whether or not rates obtained from relatively short incubations can be extrapolated to 24h rates. In an intertidal environment, e.g., it seems plausible that there would be a significant tidal impact on feeding, as meiofauna migrate vertically in the sediment in response to a tidal cycle, and as a significant part of their suspected microalgal food (e.g. epipelic

diatoms) is tidally resuspended and therefore only available during ebb tide. In subtidal environments, hydrodynamics and perhaps other abiotic factors may similarly influence feeding activity.

Overestimation of grazing rates could also result from leakage of label into the grazers. We found activity in nematodes to increase during prolonged stays in formaldehyde and in the presence of free label (data not shown). These results still need confirmation, but suggest that in trials where meiofauna were sorted several days after termination of the experiment rates may rather reflect the amount of free label present in the sediment samples, and as label adsorbs to sediment particles, differences obtained between several locations might reflect differences in sediment chemistry and texture rather than in meiofauna feeding activity.

Either way, it is clear that attention has to be focused on improving methodology. Reagent grade ethanol appears to minimize problems of both particle egestion/defaecation and of label leakage, yielding rates as high as 70 % of those from non-preserved samples. It remains to be tested whether the patterns of label loss observed in the present study can be generalized for other meiofauna (cuticular structure, e.g., is quite variable and might influence label loss upon chemical preservation) and for other types of food or label (will, e.g., ^{14}C-labelled microalgal substances be stored, respired and leaked upon preservation in the same way as ^{3}H-labelled bacterial products?).

Recently, a shift from radioactive to fluorescent tracers in grazing experiments has evolved. Fluorescent tracer techniques have become fairly common practice in protist grazing research (Borsheim, 1984, Sherr *et al.*, 1987, Sherr & Sherr, 1993, Starink *et al.*, 1994). A comparison between rates obtained from radioactive and fluorescent tracer trials could yield new insights. As similar preservation procedures are used, our results on egestion/defaecation are relevant for this type of experiments too. A major problem in the early use of fluorescent tracers in nematode feeding experiments is the autofluorescence of the nematodes (pers. observ., Dr. R. David, pers. comm., Dr. K. Carman, pers. comm.), which can be prominent in some species and almost negligible (rarely) in others. This aspect, together with egestion/defaecation, may explain the low ingestion rates of nematodes reported by Epstein & Shiaris (1992). The only other published report sofar on aquatic nematode grazing based on fluorescent tracer techniques is by Borchardt & Bott (1995).

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Fig. 1. The feeding type classification of Wieser, 1953.

Fig. 2. Location of the sampling station WO22 in the Westerschelde estuary.

Fig. 3. Monthly abundance data of selective (1A) and non-selective deposit feeders *sensu* Wieser (1953) at station WO22 during March 1991-January 1992. 1B+ denotes deposit feeders *sensu* Moens & Vincx (present study) together with ciliate feeders, 1B- denotes deposit feeders only.

Fig. 4. Estuarine nematode feeding guilds and their particulate food sources, as derived from observations on representatives from an intertidal mudflat in the Westerschelde estuary (SW Netherlands).

Fig. 5. A tentative scheme of patterns of carbon flow into and through different nematode feeding guilds.

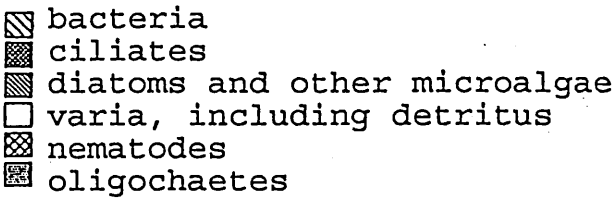


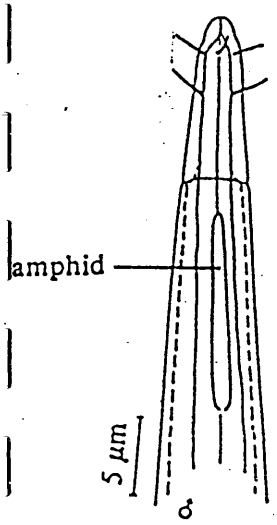
Fig. 6. Influence of sample preservation procedure on grazing rate determination in *Pellioditis marina*. All data shown are means of three replicates. SD are given.

Fig. 7. Influence of incubation time (per hour) on grazing rates obtained with *Pellioditis marina*. Data points are means of three replicates.

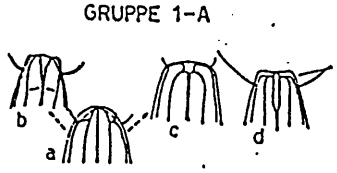
Fig. 8. Influence of incubation time (per 15 minutes) on grazing rates obtained with *Pellioditis marina*. Data points shown are means of three replicates.

WIESER (1953)

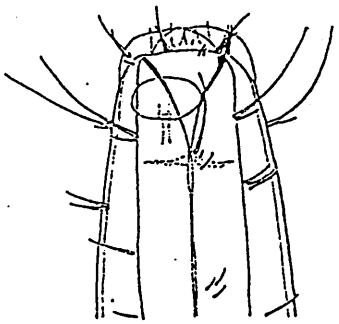
4 feeding categories:

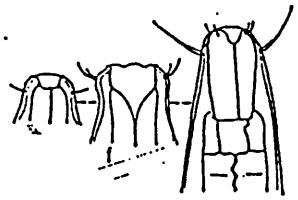


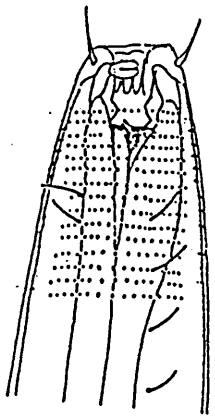
* 1A: selective deposit feeders
  - no buccal armature
  - minute buccal cavity
  - mainly bacterial feeders



* 1B: non-selective deposit feeders
  - no buccal armature
  - bacteria, diatoms, algae, macromolecules...

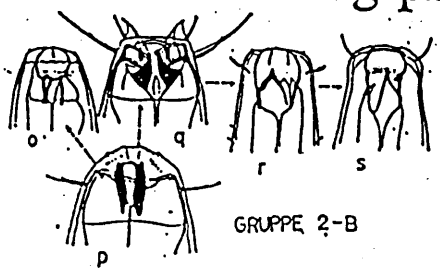






* 2A: epistrate feeders
  - buccal armature
  - especially diatoms and algae

* 2B: omnivores or predators
  - strong buccal armature
  - variable feeding strategies, including predation



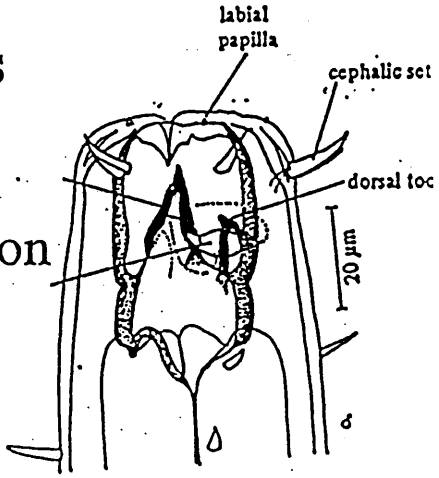


FIGURE 2.

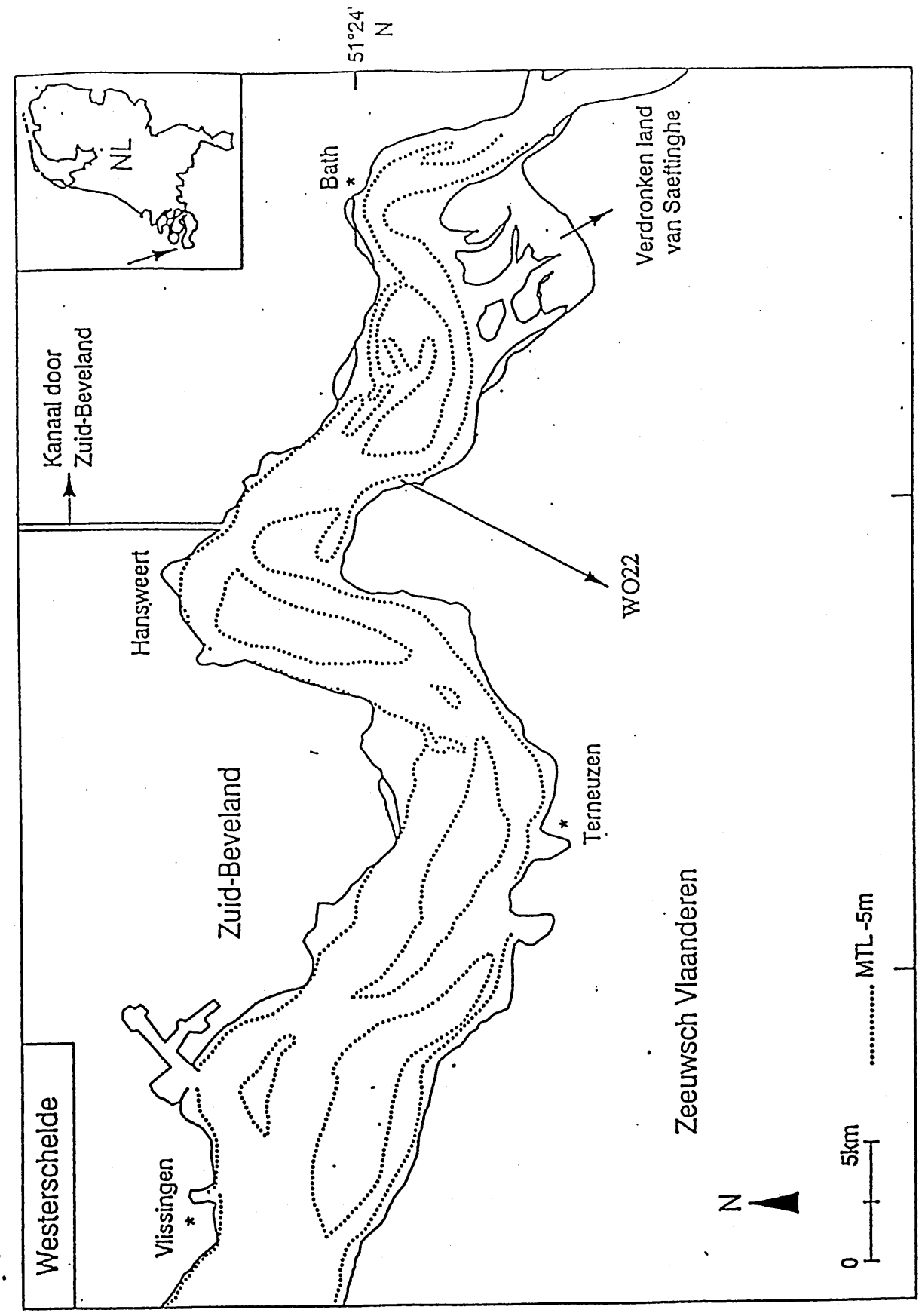
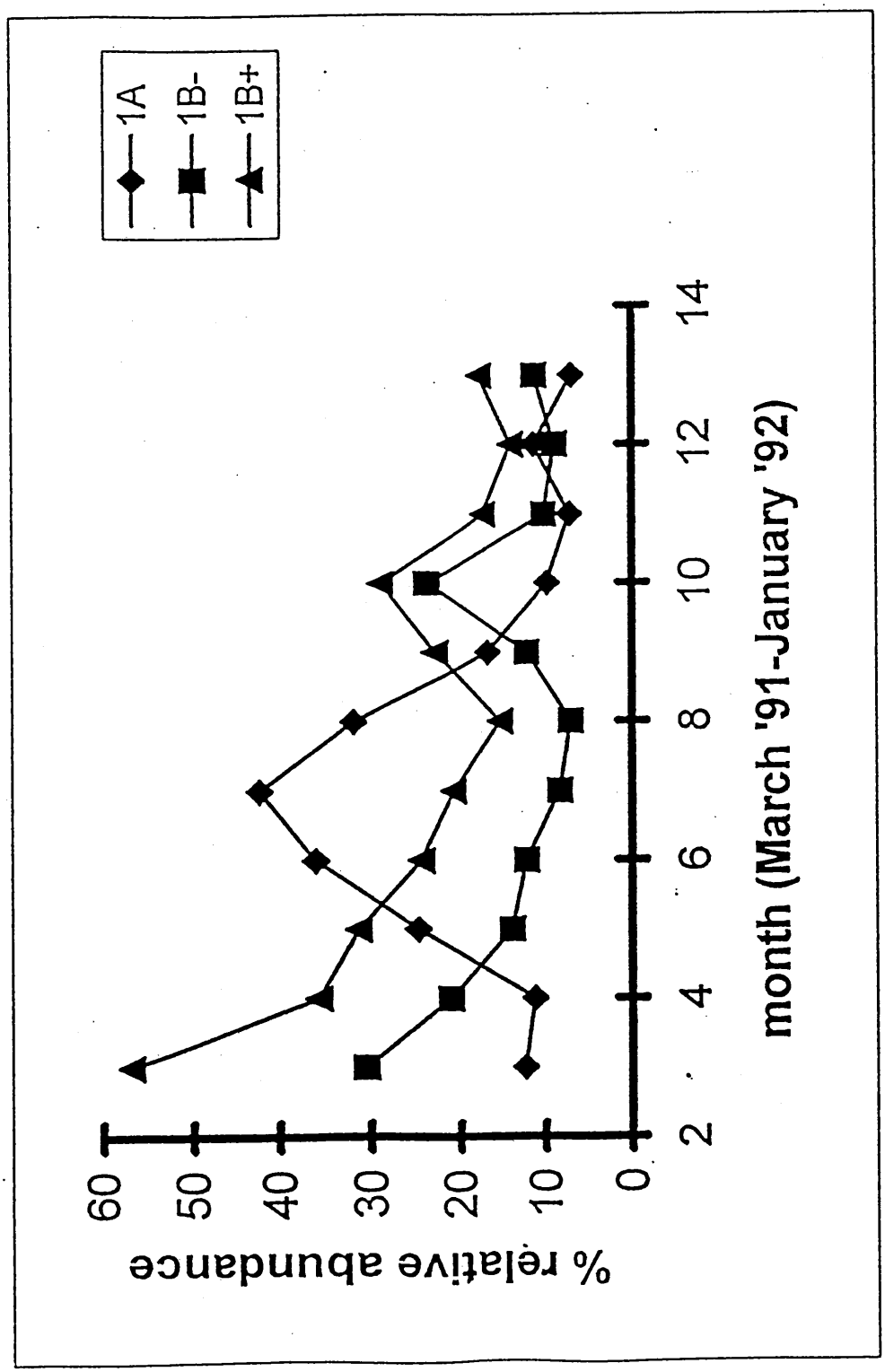

51°24' N
NL
Bath
Verdronken land van Saeftinghe
Kanaal door Zuid-Beveland
Hansweert
WO22
4°01'
Zuid-Beveland
Terneuzen
Westerschelde
Vlissingen
Zeeuwsch Vlaanderen
3°44' E
N
0
5km
MTL-5m

FIGURE 3.


1A
1B-
1B+
% relative abundance
0
10
20
30
40
50
60
2
4
6
8
10
12
14
month (March '91-January '92)

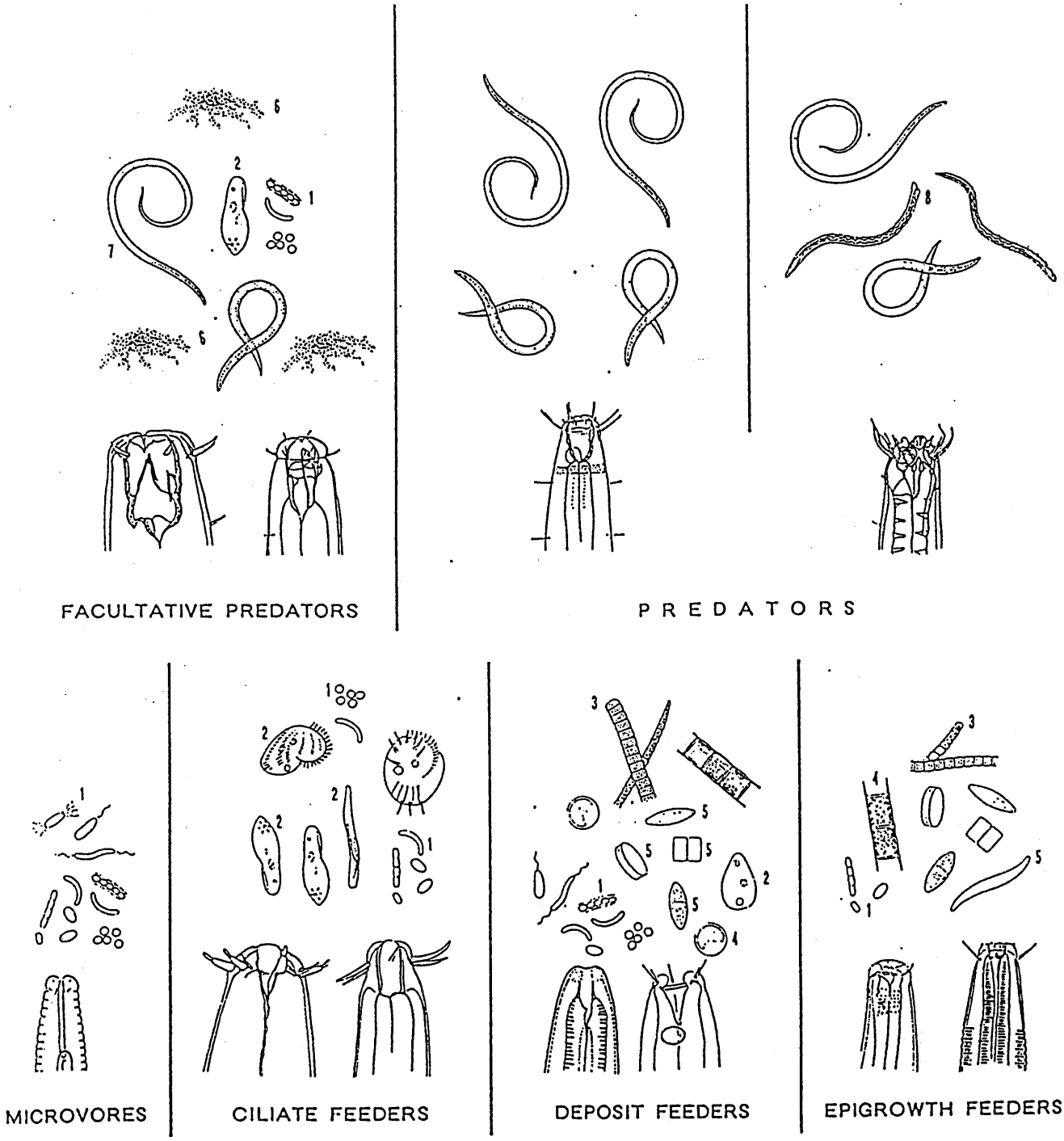

FACULTATIVE PREDATORS
PREDATORS
MICROVORES
CILIATE FEEDERS
DEPOSIT FEEDERS
EPIGROWTH FEEDERS
1. bacteria
2. ciliates
3. cyanophytes
4. green algae
5. diatoms
6. detritus
7. nematodes
8. oligochaetes

FIGURE 5.

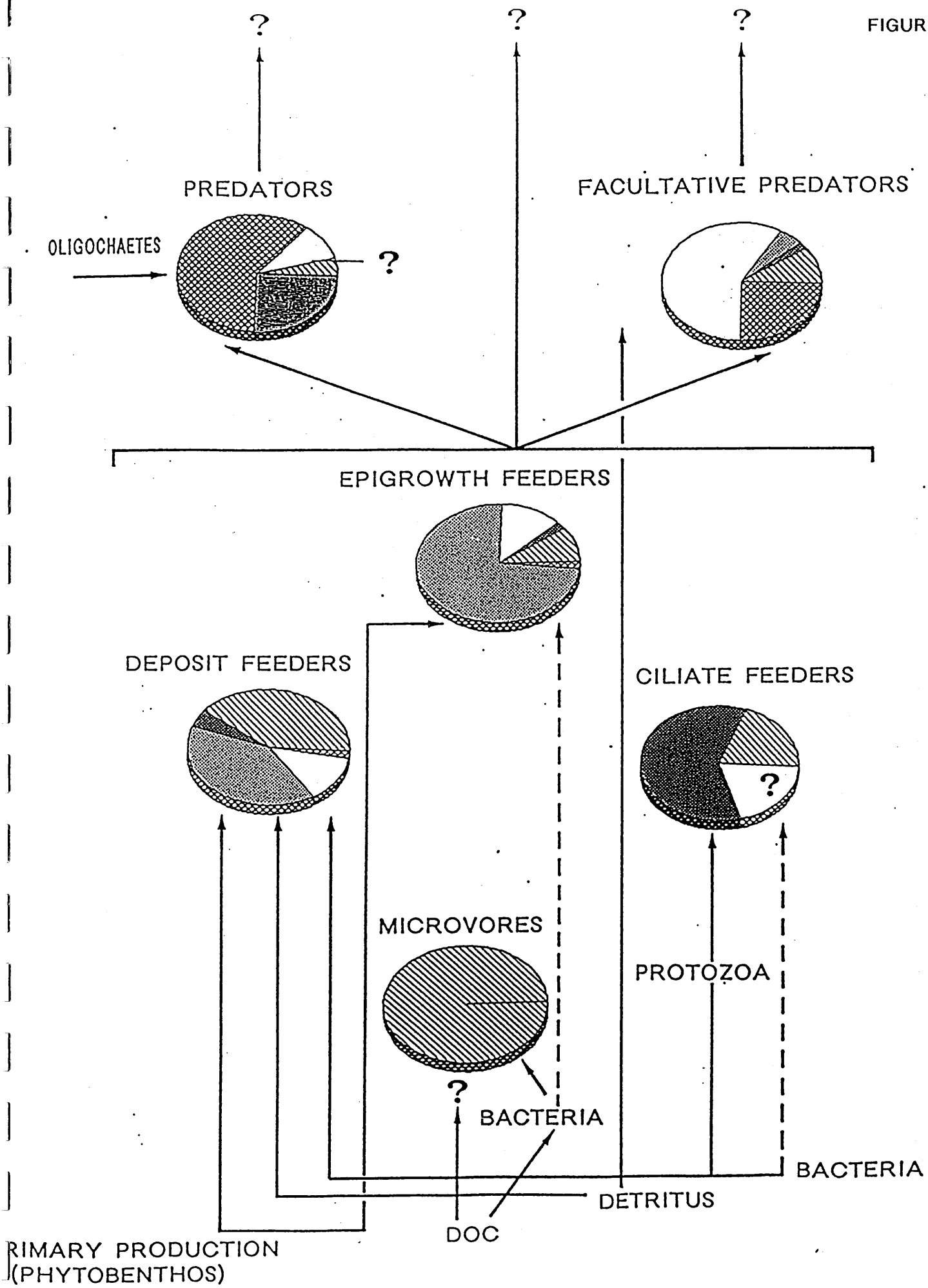

?
?
?
PREDATORS
FACULTATIVE PREDATORS
OLIGOCHAETES
?
EPIGROWTH FEEDERS
DEPOSIT FEEDERS
CILIATE FEEDERS
?
MICROVORES
PROTOZOA
?
BACTERIA
BACTERIA
DETRITUS
DOC
RIMARY PRODUCTION
(PHYTOBENTHOS)

FIGURE 6.

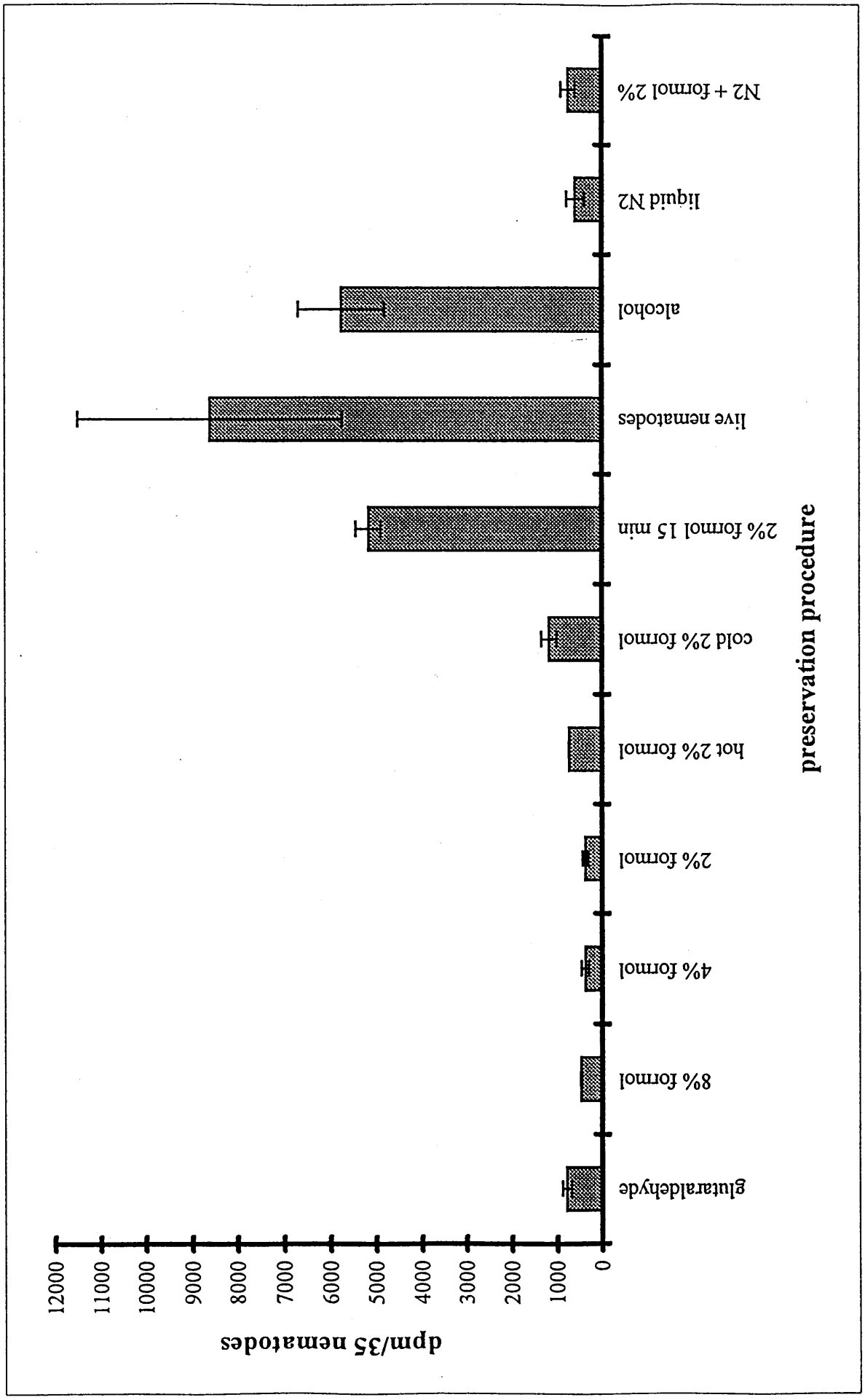

dpm/35 nematodes
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
11000
12000
preservation procedure
glutaraldehyde
8% formol
4% formol
2% formol
hot 2% formol
cold 2% formol
2% formol 15 min
live nematodes
alcohol
liquid N2
N2 + formol 2%

FIGURE 7.

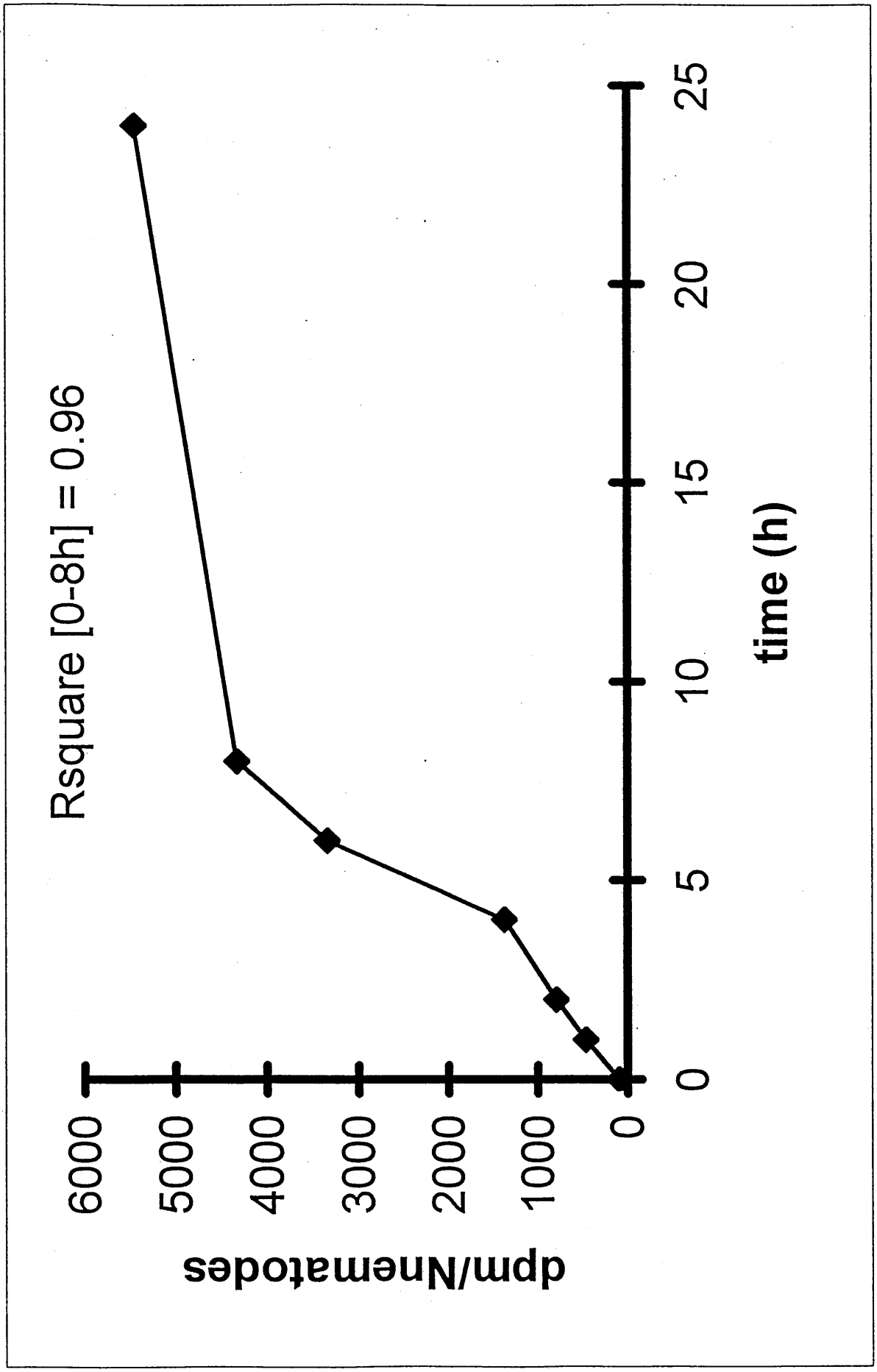
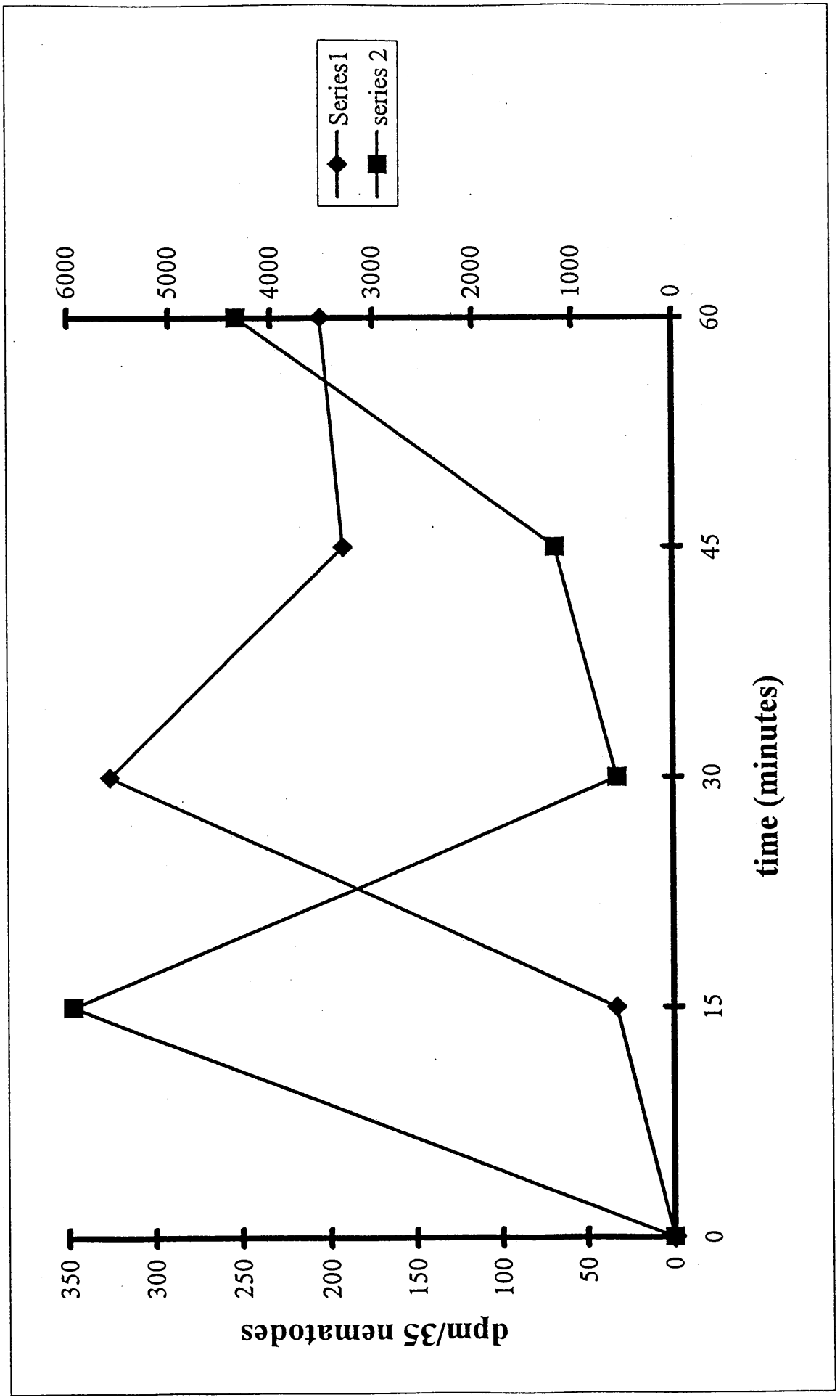

Rsquare [0-8h] = 0.96
dpm/Nematodes
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
time (h)

FIGURE 8.


Series1
series 2
6000
5000
4000
3000
2000
1000
0
0
15
30
45
60
time (minutes)
350
300
250
200
150
100
50
0
dpm/35 nematodes

BENTHOS OF THE NORTH SEA: ABLE TO RECOVER OR DESPERATELY LOST?

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1. Introduction

The living creatures of the sediments (*i.e.* benthos) in the North Sea have not been a large public concern in the past. It was thought that the sea was one big reservoir where all material could be dumped without too much damage because most substances are dispersed and quickly disappear from view (most likely into the bottom). However, it has been shown, e.g. in the Quality Status Report of the North Sea (North Sea Task Force, 1993), that life in the sea bottom is especially influenced by human activities in the coastal zone, and that this in turn influences all levels of the ecosystem. Indeed the benthos, the plankton and the nekton (e.g. fish) are in continuous interaction with each other and with the abiotic environment.

The flux of energy and organic matter through ecosystems is the most important driving force of these systems. Benthic organisms, especially in coastal waters, play a crucial role in the functioning of the ecosystem, for example in the control of phytoplankton and macroalgal blooms, and in the exchange of materials between the sediments and the water column. Within the plankton, primary production needs sufficient nutrients (nitrogen, phosphorus), which are formed within the sediments through mineralisation of the sedimented organic material. The benthos has no primary production of its own (except in intertidal areas and in coral reefs) and completely depends for its organic matter on sedimentation from the water column.

The structural characteristics of the benthic communities have been relatively well documented in the last 20 years. The state of the art is summarized in the Quality Status Report of the North Sea, Subregion 4 (North Sea Task Force, 1993).

The coastal zone is the most vulnerable since environmental impacts of human activities (e.g. fishing, industrial operations, dredging etc.) and reductions in benthic biodiversity are most likely to occur there.

However, our knowledge of patterns of secondary production of the benthos is limited because of the tedious species-by-species methods of estimating production (in contrast with photosynthesis for primary production). Effects of e.g. eutrophication on primary production are well documented whereas the impact on production of higher trophic levels are far less understood.

2. Belgian coastal subtidal zone

The Belgian coastal zone used to be subdivided in the early eighties in a polluted eastern zone with muddy sediments near the mouth of the Westerschelde and a less polluted western zone near the French border (Vincx and Herman, 1989). The mud content in the eastern zone shows significant seasonal fluctuations; this fluctuation has no effect on the structure of the meiobenthic communities. Despite the strong relationship between the characteristics of the meiobenthic communities and the sediment structure, the influence of the Westerschelde is reflected in a clear decrease in trophic, family and species diversity of meiobenthic communities.

Benthic communities are very well suited for monitoring purposes because they do not migrate very much, are abundant throughout the year and show an integrated picture of long-term pollution. In Figure 1a, a typical North Sea benthic community is illustrated and the arrows indicate the important trophic interactions. In Figure 1b, the impoverished situation of parts of the Belgian coastal zone is characterized by benthic communities which consist mainly of only a few species of deposit feeders. The richer situation is found in the sandier sediments where the redox potential becomes negative

only at a sediment depth of a few centimetres. Here the zoobenthic community consists of a high number of individuals, species, and trophic levels from the different size classes of the faunal communities within the sediments. The poorer situation is typical of the muddy substrates off the Belgian coast where dissolved oxygen is depleted within the top few millimetres (high turbidity of the water column, low diversity and no complex higher trophic levels).

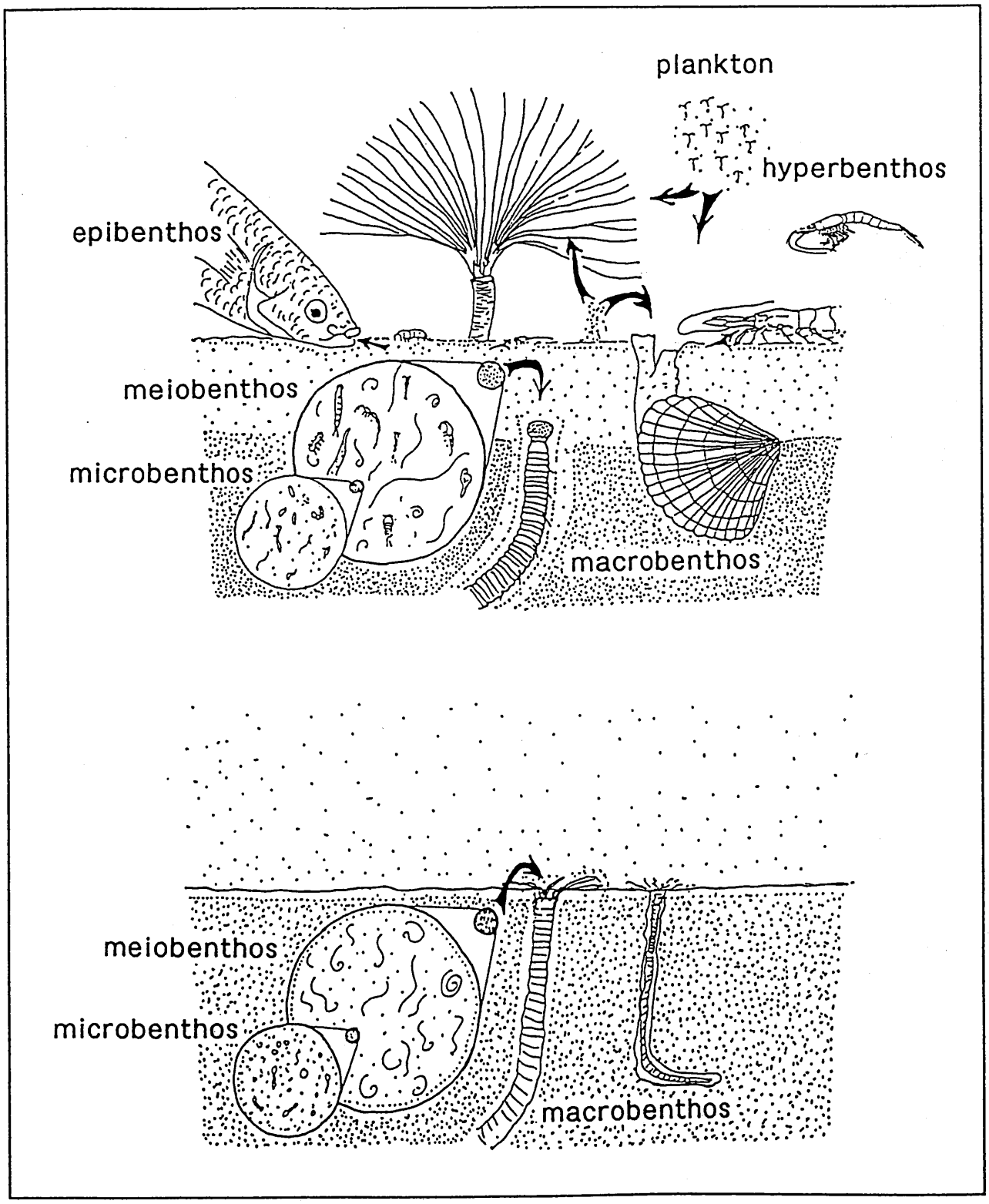

Fig. 1a - (top) *Typical benthic foodweb for sandy sediments in the coastal subtidal zone of the North Sea (adapted after Platt* et al., *1983).*

Fig. 1b - (bottom) *Impoverished benthic community for silty (polluted) sediments in the coastal subtidal zone of the North Sea.*

In recent years this typical division between east and west coast has become less obvious since impoverished communities are found along the whole coast, depending on the siltation of the sediments. However, comparison of data on interstitial fauna of 1977-1985 with data from 1993 indicates that its recovery is possible as long as non-polluted areas for recruitment are sufficiently close. Nevertheless, the huge amount of nutrients in the coastal waters cause severe stress on the benthic communities since dissolved oxygen is quickly removed from the sediments.

The following example shows that recovery of the benthic communities is possible as long as the oxygen concentrations are enhanced and when the sediment conditions become less 'toxic'.

Example 1: MEIOBENTHOS

Meiobenthos consists of microscopic animals which live in the bottom of the sea and which are shown to be good indicators of pollution studies (Vincx and Heip, 1987). Within the meiobenthos, nematodes are always dominant (>90%).

In general, the composition, distribution and biomass of the North Sea meiobenthos is well known (*cf.* Heip *et al.*, 1990; Huys *et al.*, 1992) but the role of the meiobenthos within the food web is not well documented. The numerical abundance of the meiofauna indicates that this size category of organisms (<1 mm) is important in the total energy flow of marine benthic ecosystems. However, their contribution as a food source is poorly understood. There is a general agreement that their main importance lies in an enhancement of decomposition processes by stimulating microbial activity as a result of grazing, excretion and bioturbation. The bacterivores and deposit feeders especially may alter the abundance, metabolic activities and composition of the microbial community, which in turn affects sediment biogeochemistry.

It is known that grain size, oxygen and chemical substances determined by the redox reactions, pH, salinity, temperature and pollution have a strong structuring impact on the meiobenthos. The abiotic environment is built up by the interaction of these factors. The synergistic impact of some factors stresses the complex character of the system. The reaction of the meiofauna with each of the components of the abiotic environment is difficult to estimate and can only be tested by experiments. Nevertheless, as the meiofaunal assemblage lives in, and is adapted to, the abiotic, interstitial environment, a detailed knowledge of the interaction of the meiofauna with its abiotic environment as a whole is needed.

This study is part of the project the Belgian Impulse Programme *Marine sciences* of the Federal Office for Scientific, Technical and Cultural Affairs.

Two stations along the Belgian Coast were selected in order to test extreme habitat conditions (Figure 2). Station 702 is situated near the mouth of the Westerschelde and station 115 is located on the western part of the Belgian coast, near the French border. Original data from both stations will be presented from June 1993 (Steyaert *et al.*, unpublished) and compared with data from the period 1977-1985 (Vincx and Herman, 1989).

Samples are taken by subsampling a box-corer on board the R.V. *Belgica*. Subsequently, the cores are vertically subdivided into 1 cm slices and used for the determination of meiofauna density, bacterial density, sediment composition, redox potential, organic carbon, pigment and nutrient concentrations (Steyaert *et al.*, unpublished).

Station 115 has extremely high numbers of meiobenthos (2898 individuals/10 cm^2) in comparison with station 702 (791 individuals/10 cm^2) (Figure 2). This difference in density is the result of the complex interaction of biological, physical and chemical factors. Grain size has a direct impact on structural and spatial conditions and an indirect influence on the chemical and the physical environment (Heip *et al.*, 1985). Sandy sediments (median 200 μm) are in general believed to be the most attractive habitats. In these sediments the interstitial spaces - the habitat for most meiobenthos - are most conspicuous in comparison with finer or coarser sediments (Marcotte, 1986).

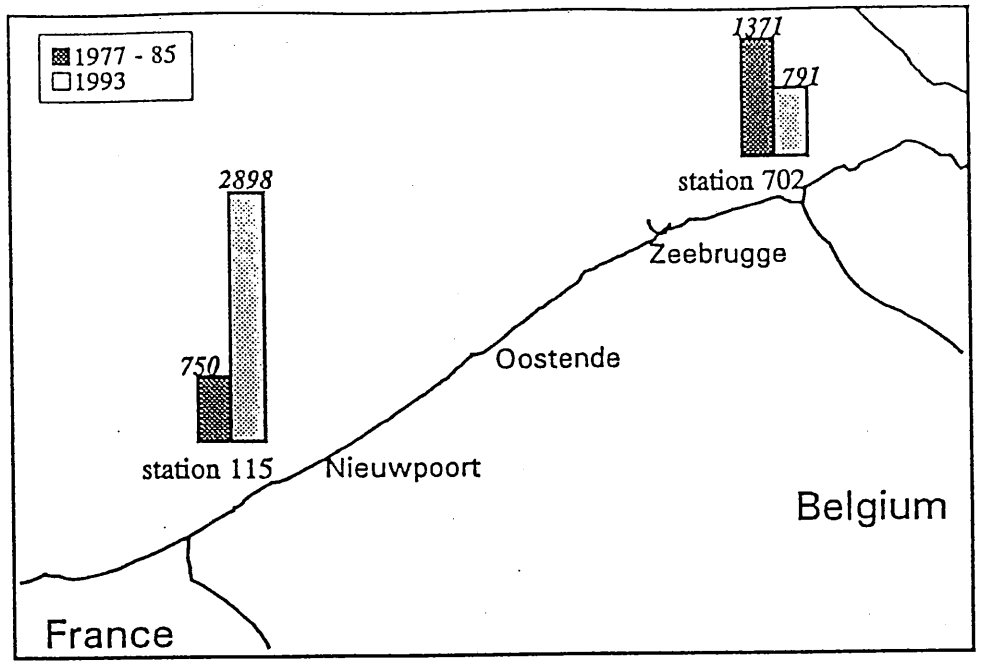

Fig. 2 - *Densities (individuals/10 cm²) of meiobenthos in two coastal stations along the Belgian coast.*

The sediment in station 702 consisted in 1993 mainly of fine sand with a median diameter of the total sediment fraction of 188 μm; the silt and clay fraction is less than 10%. In contrast station 115, with a fivefold higher meiobenthic density, has a rather silty fine sandy sediment (median of the total sediment fraction is 47 μm; silt and clay fraction is 97%).

A second strong structuring parameter is the oxygen supply, as most meiobenthic animals have very high oxygen demands. As a consequence they are restricted to the uppermost oxic layers of the sediment where free oxygen is available. Apart from these oxygen reducers, there exist organisms which can generate energy from the reduction of other chemical species. This will result in a typical species assemblage around the oxic, the oxidized, the RPD (Redox Potential Discontinuity) transition zone and the reduced layer. The overall reducing and oxidizing capacity of the sediment is given by the redox potential.

Nematode density, bacterial density, redox potential, pigment concentration - as a quantitative indicator for algae -, nutrients and organic carbon are measured in a vertical profile at both stations (Figures 3 and 4).

In station 702, the RPD layer is situated at a depth of approximately 3 cm. The oxygenated layer has higher numbers of individuals in the nematode, bacteria and algae communities. The organic carbon concentration, which gives an indication of the overall present energy, shows maximum values at 3 cm depth. The downward and upward gradients of nitrate, nitrite, ammonia, phosphate and silicate are directly coupled to, and the result of, the geochemical cycles. At station 115 the redox potential is negative over the whole sediment depth, which does not seem to have a negative influence on the numbers of nematodes and bacteria. The question of whether the species present are especially adapted to these anoxic conditions is still under investigation.

Comparison of recent data with earlier studies

Figure 5 demonstrates positive evolution to an enriched meiobenthic community (station 702) around the mouth of the Westerschelde, as evidenced by both enhanced diversities and abundances. On the other hand the meiofauna of the western part of the Belgian coast (station 115) shows a clear decreasing trend, probably related to the finer sediments. Lower densities as well as a lower diversity have been demonstrated.

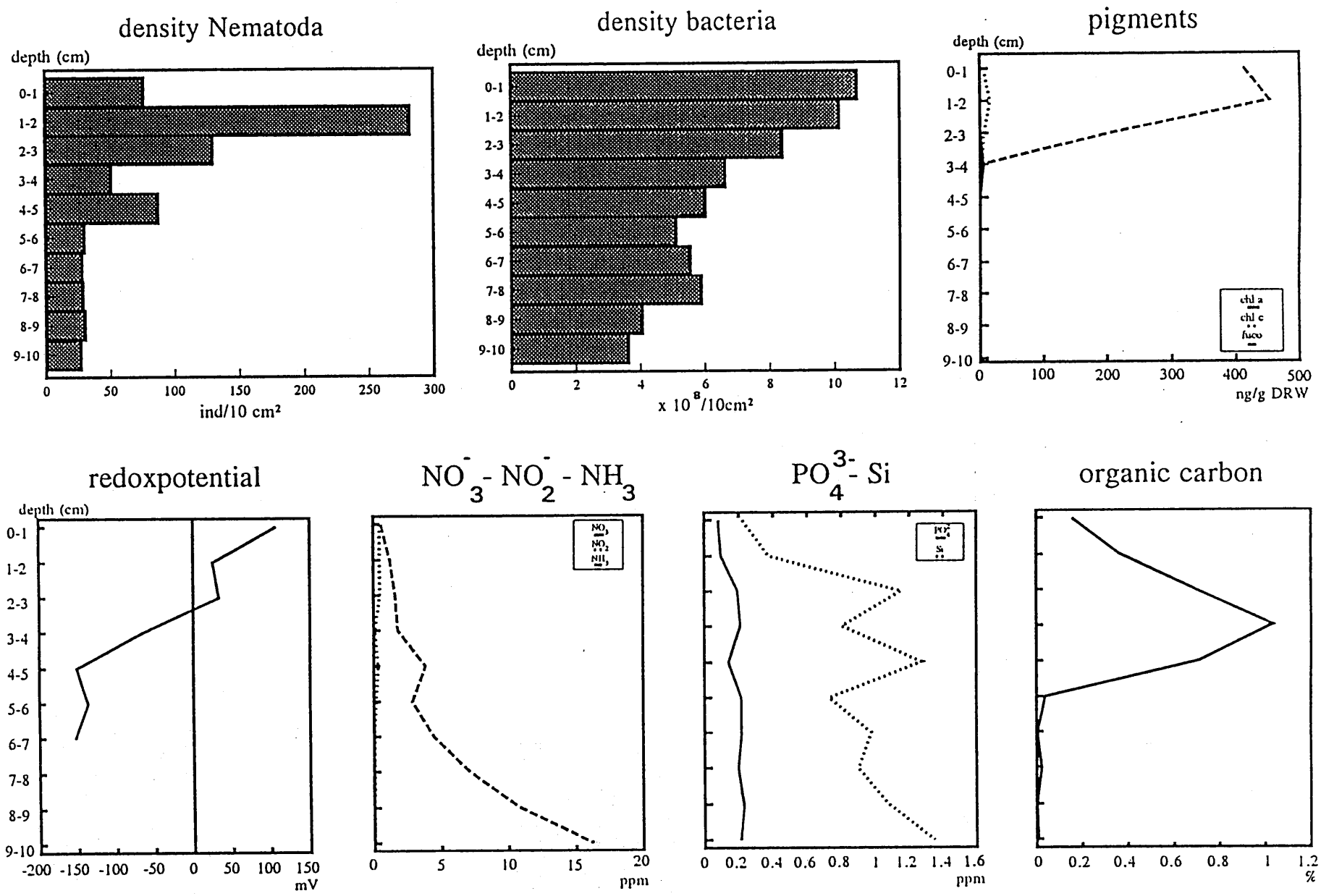

Fig. 3 - *Vertical profiles within the sediment of densities of nematodes and bacteria versus environmental factors (pigments, redox potential, N-nutrients, phosphates, silicates and organic carbon) at station 702 (June, 1993) (Steyaert* et al., *unpublished).*

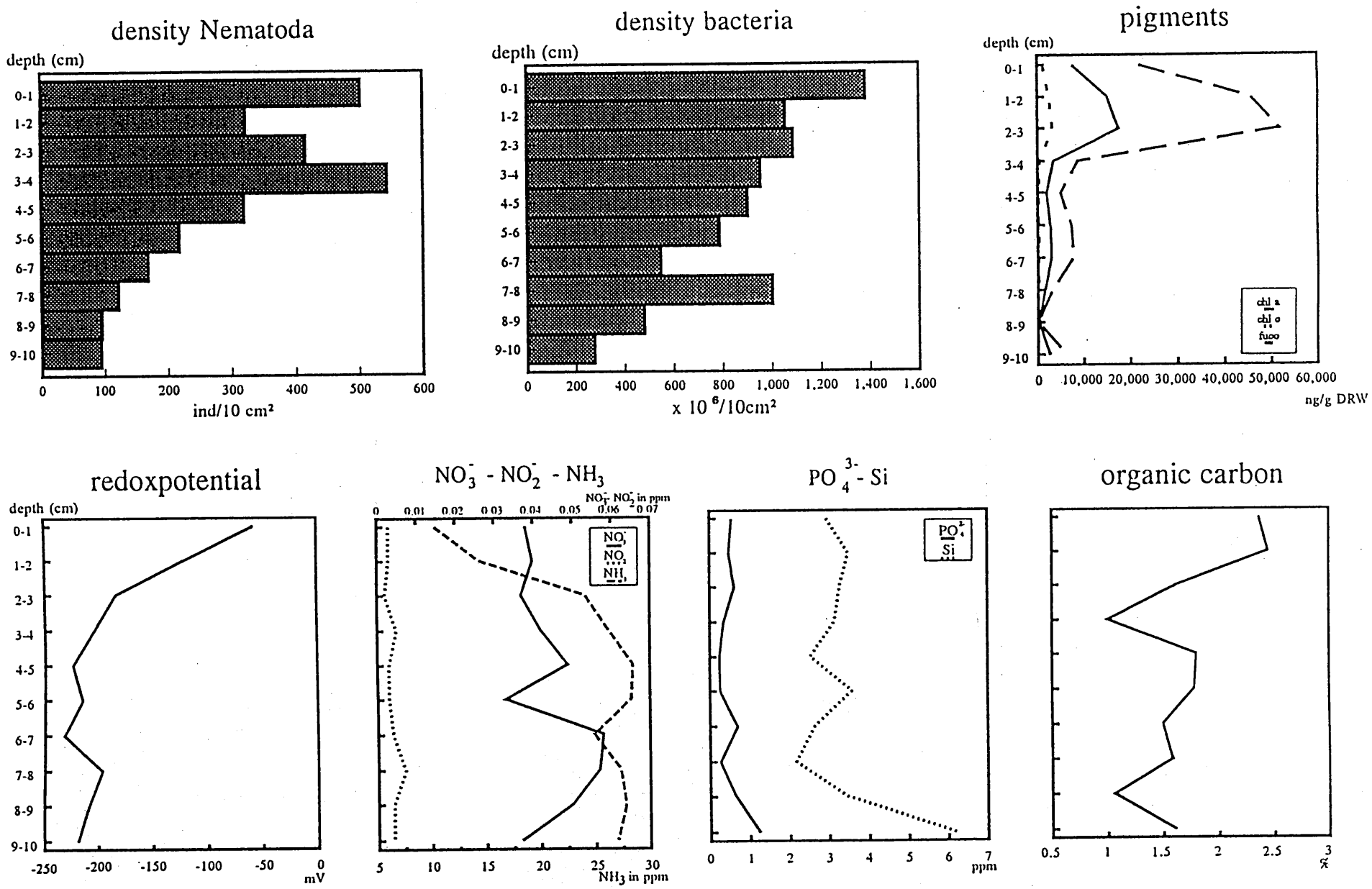

Fig. 4 - *Vertical profiles within the sediment of densities of nematodes and bacteria versus environmental factors (pigments, redox potential, N-nutrients, phosphates, silicates and organic carbon) at station 115 (June, 1993) (Steyaert* et al., *unpublished).*

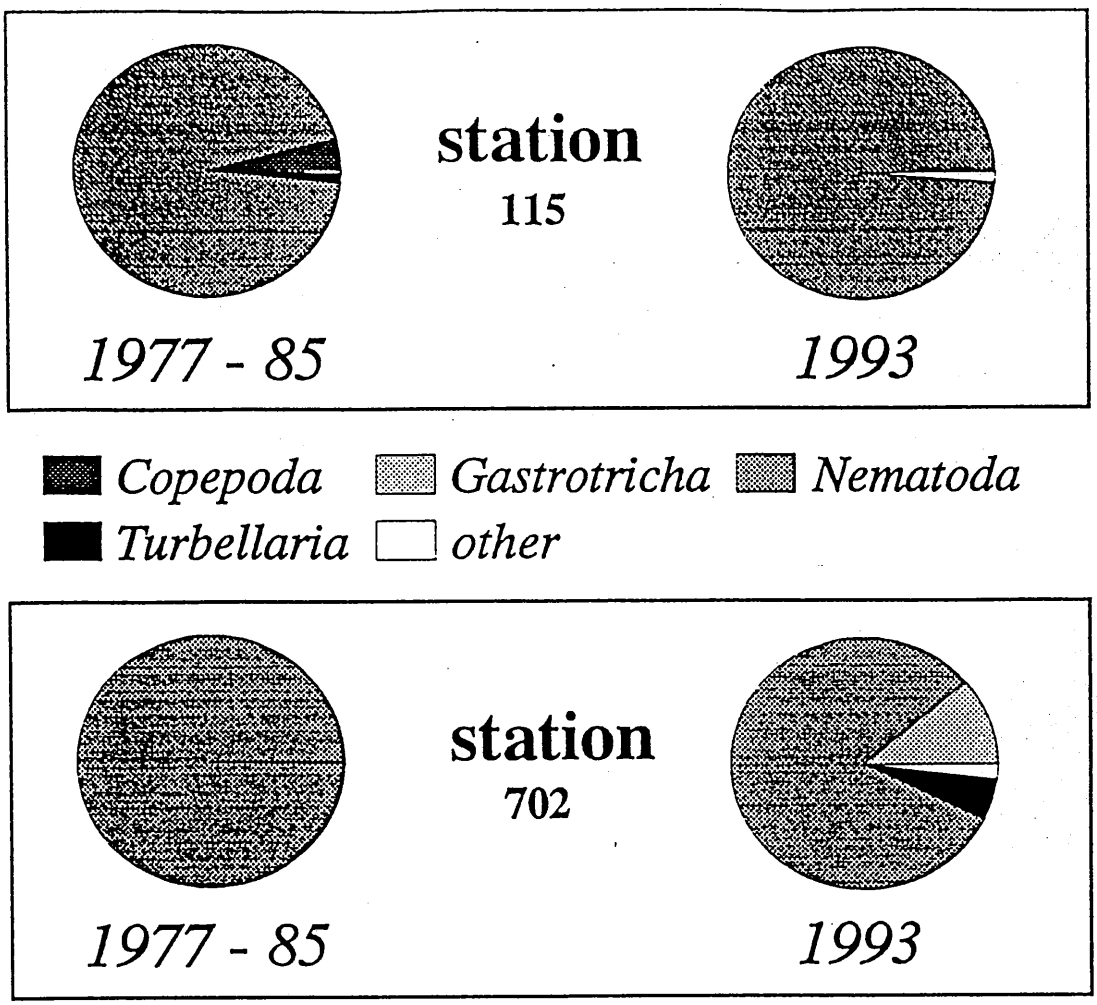

Fig. 5 - *Comparison of meiobenthic composition in station 115 and station 702 of the periods 1977-1985 and 1993 (Steyaert* et al., *unpublished).*

The extreme changes along the Belgian coastal zone are probably the result of the high sediment transports (Anonymous, 1993). This stresses the fact that recovery of the interstitial fauna is possible as long as non-polluted areas for recruitment are sufficiently close and water quality becomes better.

Example 2: HYPERBENTHOS

The hyperbenthos is the association of animals living in the water layer close to the seabed. It includes all bottom-dependent species and life history stages (mainly crustaceans and fish) which perform, with varying amplitude, intensity and regularity, seasonal or daily vertical migrations above the sea floor. Since it is not possible to sample hyperbenthic animals quantitatively with conventional techniques used in zooplankton or macrobenthos research, the study of the hyperbenthos is often neglected even in comprehensive ecological studies. Still, mobile hyperbenthic animals are an important component of the biomass of coastal regions. They contribute substantially to the diet of fish. They can be significant predators, structuring zooplankton populations, and can have an important role as grazers of organic matter and in the coupling of benthic and pelagic food webs (*cf.* Figure 1a).

A hyperbenthic sledge which consists of a heavy metal frame carrying two pairs of nets (3 m in length, mesh sizes of 0.5 mm on the right side and 1 mm on the left side) samples the hyperbenthos from 0 to 50 cm and from 50 to 100 cm above the bottom, respectively. The sledge is equipped with an automatic opening-closing mechanism, current meters and an odometer.

Under the Impulse Programme *Marine Sciences* an intensive sampling campaign has been organised in the North Sea and the first results along the Belgian coast (Dewicke and Mees, unpublished) show that a typical east-west gradient along the coast is present, as was found for the meiobenthic communities. Along the west coast the numbers of hyperbenthos reach the very high numbers found in the maximum turbidity zone of estuaries (Mees *et al.*, 1993) (Figure 6). Their temporal fluctuations and possible recovery has been under investigation since the summer of 1994.

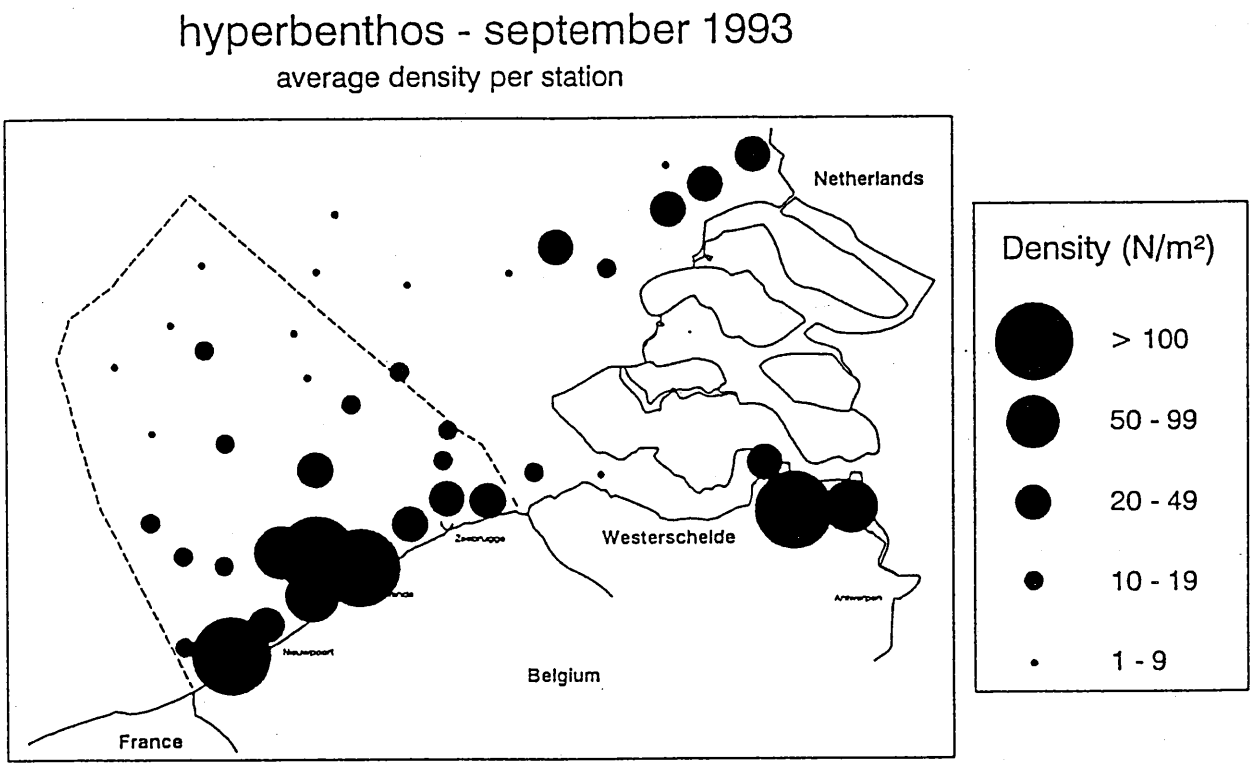

Fig. 6 - *Average density of hyperbenthos in the Southern Bight of the North Sea and the Westerschelde (Dewicke and Mees, unpublished).*

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Meiobenthos and interstitial processes in eutrophic marine sediments

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INTRODUCTION

Meiobenthos often showed an aggregated spatial occurrence within the sediment, both in the horizontal and in the vertical distribution. The causes of this patchy occurrence may be complex and involve a variety of biological, physical and chemical variables. Important structuring factors determining variation in meiobenthic distribution are granulometry, salinity, oxygen tension, food availability and chemical compounds in the pore water (Montagna, 1983; Heip *et al.*, 1985; Vincx, 1989; Vanreusel, 1990; Giere, 1993; Jian, 1993; Ndaro, 1995 and others). Understanding the processes that generate and maintain the vertical distribution pattern in different localities is an important subject for contemporary ecological research. The description of a sulphide 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). Nevertheless, oxygen and hydrogen sulphide are thought to be of prime importance for nematode distribution (Fenchel and Riedl, 1970; Ott, 1972; Platt, 1977; Nicholas et al., 1991; Rosenberg, 1991; Jensen, 1992; Giere, 1993; Hendelberg, 1993; Wetzel, 1995). Moreover, the former two ecofactors are both indirect or direct determining factors for all other biogeochemical factors.

In the present investigation, the vertical microdistribution of the nematode community, being the dominant meiofauna along the Belgian coastal area (Heip *et al.*, 1985; Vincx, 1989; Heip *et al.*, 1990), is studied on species level and linked to the biogeochemical environment.

MATERIAL & METHODS

The Southern Bight of the North Sea is strikingly free of muddy sediments, with the exception of the river mouths. Earlier studies revealed that, in front of the eastern Belgian coast, a muddy sedimentation is developing to a considerable extent due to local affluents of the Ijzer, but mostly to suspension material dragged out of the Schelde estuary. The presence of the Schelde estuary seems to induce a gyre in front of the Belgian coast, where the freshwater from the Schelde resides for some times (Nihoul and Ronday, 1975).

To illustrate the interactions between antropogenic factors and the impact on the nematode community, three subtidal stations along the Belgian coast and one intertidal station, located in the mesohaline zone of the Westerschelde, are seasonally sampled on board of the R.V. Belgica (Fig. 1). Station 115 is situated in the western part of the Belgian coast, near the mouth of the Ijzer, station 790 in the central part and station 702 is located in the eastern part, near the Westerschelde mouth. Each station was sampled in March, June and August to cover the periods of high and low organic input into the sediment.

The influence of the biogeochemistry on the vertical distribution of the nematodes will be treated in sedimentologically different subtidal systems. Three subtidal North Sea stations with different sedimentological characteristics are seasonally analysed

in terms of species diversity and abundance patterns. The diversity is expressed as the N1 diversity index (Hill, 1973) which is a normalisation of the Shannon Wiener index, H. The distribution pattern of the most abundant species is followed during the field study.
The concentration of important oxidized (nitrate and nitrite) and reduced (ammonia) nitrogen compounds together with redoxpotential values will be used to evaluate the oxidation status of the sediment.
In the second part of the study, the biogeochemical impact on the vertical distribution of the nematode community will be focused in an intertidal station in the Westerschelde (WO22), with similar sedimentological characteristics as station 702 (mouth of the Westerschelde) and station 115 (west coast).

All samples were taken by pushing perspex cores (diameter 3.6 cm) into the sediment. Subsequently the samples were divided into ten slices of one centimetre (Fig. 2). For each slice, a subsample of 120 nematodes was identified onto species level. The following environmental variables were measured: bacteria densities, pigment concentrations (chlorophyl a, chlorophyl c and fucoxanthine), redox potential, nutrient concentrations (nitrate/nitrite, ammonia, silicate and phosphate), amount of organic carbon and grain size composition. For a detailed description of the used materials and methods is referred in two former reports of the Federal Programme Impuls Sea (October 1994 and October 1995).

RESULTS

1. Description of the abiotic environment

Station 115 is characterised by fine sediments with a relative high percentage of mud. In table 1 the averaged grain size and mud content are showed for each month. Out of the profiles of the redoxpotential and the nitrogen compounds it can be concluded that the sediment in winter or early spring is oxidized in the top 5 cms (Fig. 3). The sediment is nearly anoxic from the top onwards in June and returned back to a somewhat less reduced situation in August.

The sediment of **station 790** consists of fine to coarse sand, almost devoid of mud and is characterised by large interstitials (Table 1; Fig. 4). As a consequence of the high aeration, oxygen can penetrate deep into the sediment. Only positive redoxpotential values were measured. Concerning the nitrogen compounds, a relatively stable and constant depth pattern was found over the periods.

Station 702 consists mainly of fine sand with a lower mud content (Table 1; Fig. 5). It has a fully oxidized sediment in March with nitrate concentrations ranging not lower than 0.021 mmol/l and very low ammonia concentrations (< 0.078 mmol/l). An intermediate situation with only the upper three centimetres oxidized is found in June, whereas in August the sediment turned out to be completely reduced. No positive Eh values were measured and the ammonia concentrations are extremely high (up to 4.09 mmol/l).

The intertidal **station (WO22)** has a sandy sediment with a high percentage of fine materials (Table 1; Fig.6). In both periods, the nitrogen compounds and redox potential values (not higher than - 53 mV in March 1994 and - 92 mV in June 1993) stresses the highly reduced character of the sediment.

For further analysis, the four stations are treated as four specific systems.
Station 115 and station 702 have similar sedimentological characteristics. In terms of eutrophication pressure, the following order can be hypothesised going from low to high eutrophication impact:
station 115: March, August, June
station 702: March, June, August
In general, station 115 is less reduced than station 702 and the situation of June in station 115 is comparable with the situation of August in station 702.
Station 790 has a coarser sediment with larger interstitials which has a direct effect on the geochemical conditions and on the nematode assemblage. The muddy to fine sandy sediment of station WO22 is considered taking into account the unstable, estuarine origin of the sediment.

2. North Sea stations

a. On nematode community level

The average relative abundance of the most important species are shown in table 2 for the three stations. In station 115 and station 702, a superdominance of non-selective deposit feeders is obvious, whereas in station 790 the epistratum feeders is the most important trophic group.

In both **muddy to fine sandy** stations (115 and 702), the highest species diversity is found in March and the lowest in August (Fig.7).
At station 115 in March, diversity is decreasing with depth, except from 8 cm onwards from where an abrupt increment to a level even higher than the top centimetre occurred. This higher diversity is attributed to five species, *Daptonema normandicum*, *Leptolaimus* spec., *Metalinhomoeus* spec., *Microlaimus conothelis* and *Molgolaimus cuanensis*, which became suddenly important in these deep sediment layers. A positive correlation between the species diversity and the mud content by a Spearman rank test suggests a direct or indirect stimulation of the nematode species diversity trough sedimentation of fine particles (< 62 µm) (Fig. 8).
Further analysis revealed that species diversity within non-selective deposit feeders is also correlated to the amount of mud in the sediment. In June and August, diversity decreases with depth, showing a sharp contrast between the first centimetre and the remainder of the sediment column, however only in August, the less reduced situation, a correlation was found with the nitrate/nitrite concentration in the sediment (Fig. 9).
In the highly oxidized sediment of March at station 702, diversity decreases slowly and here again is correlated with the mud fraction of the sediment (Fig.10), however no further correlations could be assessed for species diversity within trophic groups.
In June, a similar diversity pattern is found as for June and August in station 115, although here species diversity is positively correlated with the mud content and negatively correlated with the ammonia concentration in the sediment (Fig. 11).
Species diversity within non-selective deposit feeders, the dominant trophic group of station 702, is correlated with the redox state of the sediment (redox potential, nitrate/nitrite and ammonia). In the most reduced situation of August at station 702, diversity showed over all depth layers relatively low and nearly constant values. No correlation with any measured abiotic factor is established.

Concerning diversity in station 790, characterised by the **coarse sandy** sediment, differences are found between the former two fine sandy stations by the overall higher diversity levels and by an increase in average diversity over the three periods (Fig.12). In March, the species diversity remains relatively low and nearly constant over depth and is not correlated with any abiotic factor. The species diversity pattern is showing a slowly decreasing trend in June, however is not coupled onto any measured abiotic factor. An extraordinary increase of species diversity with depth is showed in August, which is also reflected in the species diversity of the different trophic groups, however here only a relation between food sources is suggested (Impuls Sea report, 1995).

Out of these data, four models, according to the redox state of silty sediments, can be put forward to stress the antropogenic impact on the nematode species composition:
1. in strongly oxidized sediments (March of station 115 and 702), there exists an obvious positive correlation between the mud content of the sediment and diversity of nematodes. This positive correlation could also be assessed for species diversity within non-selective deposit feeders (station 115)
2. in oxidized sediments, with the redox potential discontinuity layer (RPD layer) situated somewhat higher into the sediment column (June of station 702), diversity is coupled onto both the mud content and ammonia concentrations. Here again, a relation is found between non-selective deposit feeders and the redox state of the sediment
3. in rather reduced sediments with an oxidized layer of only about one centimetre (August of station 115), the amount of mud shows no impact on the species diversity of the community. Instead, a positive trend is shown between oxidized pore water and diversity
4. in strongly reduced sediments with an oxidized layer of a few millimetres (June of station 115 and August of station 702) the diversity is always low and not influenced by the sediment grain size nor redox values.

b. On species level

Sabatieria punctata has the highest abundances in both fine sandy, subtidal stations (115 and 702), followed by *Daptonema tenuispiculum*, whereas in the coarse sandy station (790), both *Ixonema sordidum* and *Viscosia langrunensis* are the most important species.
Highest abundances - over the total sediment column - for *Daptonema tenuispiculum* and *Sabatieria punctata* are found in the most reduced periods; June in station 115 and August in station 702 (Fig. 13). Both species have extreme low abundances in the most oxidized situation (March, station 702). Besides these differences in total abundances, similar trends in depth distribution can be distinguished at each period, in both stations, apart from the only aberrant situation for March at station 702. The maximum density for *D. tenuispiculum* is in all cases measured in the surface layers. From three to four centimetres onwards, densities are halved or can even reach zero levels. Hereby it is proved that *D.tenuispiculum* is a typical surface dweller and is not clearly affected by the redox state. *S.punctata* appears to penetrate deeper down into the sediment, although highest densities are

found in the subsurface (three to four centimetres depth). Neither *S.punctata* seems to be influenced by the redox status of the sediment. Spearman rank correlations between both species and the amount of bacteria suggests that the depth distribution of *D.tenuispiculum* and *S.punctata* is rather related to the available food resources.

Compared to *D.tenuispiculum* and *S.punctata* in fine sediments, *Ixonema sordidum* and *Viscosia langrunensis,* the two dominant species of the coarse sandy station (790) seems to be less important in terms of total abundance. The density of *I.sordidum* is low and almost constant over depth in March, however, a six-fold higher density is found in the upper sediment layers in June and twenty-fold higher density at four to five centimetres depth in August (Fig. 14). An opposite situation is found for *V.langrunensis;* over the total depth the density is low and rather constant, except in the upper layers of March where it reaches six-fold higher densities.
Regardless of the oxidation state in the sediment of station 790, which doesn't exert significant differences in depth, nor in periods, there exists an apparent periodicity in the depth distribution of both species which is not attributed to any single abiotic factor, measured in this study.

2. Westerschelde
Station WO22 is an intertidal station with a typical nematode community, build up by mainly Xyalidae (Table 3). Compared with the genus diversity of the former subtidal stations, WO22 has in both periods a relatively high genus diversity relative to the strongly reduced sediment (Fig. 15). Although no obvious differences of the environmental characteristics were found in both periods, the nematode community shifted from a deposit feeder dominated community in March to an epistratum feeder/omnivore-predator community in June, which stresses the highly unstable origin of the Westerschelde station. Furthermore diversity was nearly constant over depth in March, whereas in June, it was decreasing from 8.6 in the surface of the sediment to 1.8 in the deeper layers. This steep decreasing diversity is coupled onto increasing ammonia concentrations and secondly onto a decreasing algae amount in the sediment.

DISCUSSION

Distribution profiles of dominant nematode species

Literature of the autoecological characteristics of *Daptonema tenuispiculum, Ixonema sordidum, Sabatieria punctata, Viscosia langrunensis* and in general on most of the freeliving nematode species are very scarce. However, *D.tenuispiculum* and *S.punctata,* both non-selective deposit feeders, are typical for silty sediments. They are known as eurytopic species, which can tolerate unstable, highly polluted environments (Vincx, 1989). *S.pulchra* is also found to be very abundant in disturbed environments, independent of the sediment composition. It is suggested by several authors that *S.pulchra* is extremely physiologically adapted to stressed life conditions (Heip and Decraemer, 1974; Tietjen, 1980 and Heip *et al.*, 1984). This species even survives as a facultatively anaerobic species in deoxygenated sediments (Jensen, 1984). Hendelberg *et al.* (1993) postulated that it is incorrect to state that *Sabatieria* species in general exhibit tolerance to long periods of anoxia. Based on morphological characteristics and microhabitat preferences a distinction

was made between a *S.pulchra* group and a *S.ornata* group. The *S.pulchra* group consists of *S.pulchra, S.punctata*, *S.granulosa* and *S.claviculata,* and lives in reduced sediments, while the *S.ornata* group consists of *S.ornata, S. proabyssalis* and *S.abyssalis*, and is limited to oxidized sediments. Furthermore, some authors found that *S.pulchra* and closely related *Sabatieria* species in muddy sediments are known to have their population maximum at the RPD-layer (Jensen, 1981; Bouwman *et al.,* 1984; Platt, 1985; Jensen *et al.,* 1992). Out of this study, *S.punctata* appears to penetrate deep down into the sediment, having the highest density at three to four centimetres depth in reduced sediments as well as oxidized sediments. *S.punctata* seems to be rather food dependent for the vertical distribution into the sediment. By this the need for identification to higher taxonomic levels is once more proved and generalisations of *Sabatieria* species to one genus need to be done with caution when considering ecological tolerance facilities. It is suggested to make a further distinction of *Sabatieria* into three groups, *S.pulchra*, *S.punctata* and *S.ornata,* according to the morphological characteristics and habitat preferences.

Diversity versus sediment composition

Although relations between diversity and sediment grain size have been suggested many times in literature, some controversy about the subject exists. In the seventies, Heip and Decraemer (1974) found that nematode species diversity was positively correlated with median grain size and negatively correlated with silt-clay content of the sediment. They ascribed this to the wider range of microhabitats available for meiofauna in sandy sediments compared to muddy sediments. This finding was confirmed by several other authors and hereby the need to compare only areas of similar substrate was stressed, when studying the consequences of the disposal of effluents. However, this study suggests a positive relation between species diversity and the mud content of the sediment i.e. an increasing species diversity with increasing mud content in oxidized sediments. These controversial results may arise from the different scale which is viewed. Earlier studies considered diversity fluctuations within different localities, while the actual study concentrates on variations within one type of sediment. Furthermore, it was stated by Etter *et al.* (1992), that the nature of the sediments should be important in structuring deep sea communities because deposit feeders rely on the sediment for nutrition and comprise most of the organisms in the deep sea. If deposit feeders in the deep sea partition the sediments with respect to size, species diversity may in part be a function of sediment particle size diversity. Also, sediment particle size diversity may reflect habitat complexity because the organisms live on or within the sediments. In this respect, an accumulation of fine particles in these fine sandy sediment may lead to diversification possibilities of feeding guilds, which has indeed been proved by a positive correlation between species diversity of the non-selective deposit feeders and the amount of mud.

The antropogenic impact on the nematode community

Earlier research revealed that the nematode communities of silty sediments along the Belgian coast are remarkably poor in species diversity compared to other areas of the same sediment type in the North Sea and in Europe. This impoverishment was attributed to the higher level of pollution. The Belgian coastal area is used to be

subdivided into a muddy eastern zone and a more sandy western part during the seventies/eighties, mainly originating from the current pattern in the eastern part and the precipitation of finer polluted materials out of the Westerschelde (Vincx *et al.,* 1989). This was reflected in the nematode communities along the Belgian coast which showed a decreasing species diversity towards the Westerschelde mouth. Out of the data of this investigation, a positive evolution to an enriched nematode community near the Westerschelde mouth could be stressed over a period of almost 20 years, visualised by enhanced species diversity values. On the other hand the nematofauna of the western part of the Belgian coast (station 115) shows a clear decreasing trend, probably related to the finer sediments. Lower species diversity have been demonstrated. The extreme changes along the Belgian coastal zone is probably the result of the high sediment transports (Anonymous, Study IHE-BMM, 1993). A refinement of the sediment along the Belgian coast is stated during a period of 1979 - 1993 and can probably be assessed onto dredging activities (Vyncke, 1994).
The effect of antropogenic influence, in terms of eutrophication, can be detected locally on silty sediments of both the eastern and western Belgian coast by detailed vertical scanning of the nematode community. Taking in consideration a constant seasonal input of antropogenic pollutants, the effect of the antropogenic influence is lowest in early spring, before the onset of the springbloom. In this strongly oxidized situation, diversity of the nematode community seems not to be dependent on the redox chemistry of the sediment. However in early summer, after an increased sedimentation of nutrients, the reduced sediment layer is slightly shifting towards the surface layers and is obviously influencing the distribution of nematodes, through a decrease of species diversity. A tight coupling of the vertical distribution of a nematode assemblage to seasonal hypoxic bottom water has been proved by Hendelberg *et al.* (1993). As a consequence of oxygen deficiency and sulphide poisoning induced by hypoxic bottom waters during late summer, a mortality of the surface fauna and an upward migration by the subsurface fauna was observed.

Diversity in the strongly reduced silty sediments was low and was not related to any of the redox components of the pore water. A plausible explanation is that the variations in biochemical composition of the pore water can change over relatively short periods and the meiofauna is not in phase with these changes.

The coarse sandy sediment of station 790 has a minute fraction of mud which implicates large interstitials, a deep oxygen penetration and a relative stable redox chemistry in depth as well as seasonally. As a consequence, the nematofauna of this type of sediment is highly divers and not affected by the redox processes in its vertical distribution. However, periodicity in the vertical species distribution and species composition is detected, and rather related, directly or indirectly, by the distribution of food sources (Impuls Sea report, 1995).

In conclusion, antropogenic impact on the whole community can be detected through species diversity of nematode communities. In early spring, before the algae bloom, the pore water of the sediments is strongly oxidized which implies high species diversity deep down into the sediment. In these situations, diversity increases with higher mud accumulations, which can be attributed to diversification possibilities of non-selective deposit feeders. In reduced sediments, the decreasing

diversity with increasing depth into the sediment is due to more favourable redox conditions at the superficial layers, enabling more species to coexist. Furthermore, information gained out of distribution profiles of a single nematode species is much less valid than the information on diversity profiles of the whole nematode community. The value of identifying indicator species or ecological indicators for sustainable management with nematodes still needs to be interpreted with much caution.

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Mud fraction (< 62 μm) of the sediment (in %)				
Station	115	702	790	W022
March	36.1	9.5	0.0	37.7
June	83.6	5.9	0.3	45.1
August	65.9	53.3	0.3	
Median grain size of the sediment (μm)				
Station	115	702	790	W022
March	143.8	198.1	461.5	67.0
June	47.2	187.5	402.5	71.0
August	47.8	76.5	487.1	

Table 1

Relative abundance of the most important species (> 1 %)				
	Trophic group	115	702	790
Ascolaimus elongatus	1b	2.1	7.7	
Chromadorina demani	2a			2.4
Cyartonema elegans	1a			3.0
Daptonema spec.	1b	5.3		1.1
Daptonema normandicus	1b	2.3		
Daptonema tenuispiculum	1b	6.5	11.2	
Daptonema trichinius	1b		1.6	
Desmodora spec.	2a			1.3
Desmolaimus zeelandicus	1b		1.1	
Enoploides spec.	2b			2.9
Ixonema sordidum	2a			24.7
Metalinhomoeus spec.	1b	2.3		
Microlaimus spec.	2a			1.5
Microlaimus conothelis	2a			2.2
Neochromadora hyalocheile	2a			7.1
Odontophora phalarata	1b		2.2	
Onyx perfectus	2b			1.6
Paracanthonchus spec.	2a		1.0	1.8
Rhynchonema quemer	1b			8.1
Richtersia spec.	1b		1.5	
Richtersia inaequalis	1b		7.2	
Sabatieria punctata	1b	73.3	51.4	2.4
Sigmophoranema rufum	2b		1.6	1.7
Spirinia parasitifera	2a	1.3		
Syringolaimus spec.	2b		1.8	1.5
Syringolaimus caspedum	2b			2.8
Tubolaimoides spec	1a			4.1
Viscosia langrunensis	2b		4.4	7.9
Xyala imparis	1b			1.9
Total		93.1	92.6	80.1

Table 2

Composition of the nematode community in WO22 on genus level		
Relative abundances of most important genera (>1)		
	Trophic group	Relative abundancy
Daptonema	1b	39.3
Viscosia	2b	10.0
Halalaimus	1a	9.9
Sabatieria	1b	7.7
Dichromadora	2a	6.7
Prooncholaimus	2b	5.9
Neochromadora	2a	5.5
Gammarinema	1b	2.5
Hypodontolaimus	2b	2.1
Ptycholaimellus	2b	1.8
Calyptronema	2b	1.6
Sphaerolaimus	2b	1.4
Leptolaimus	1a	1.0
Anoplostoma	1b	1.0
Total		96.3

Table 3

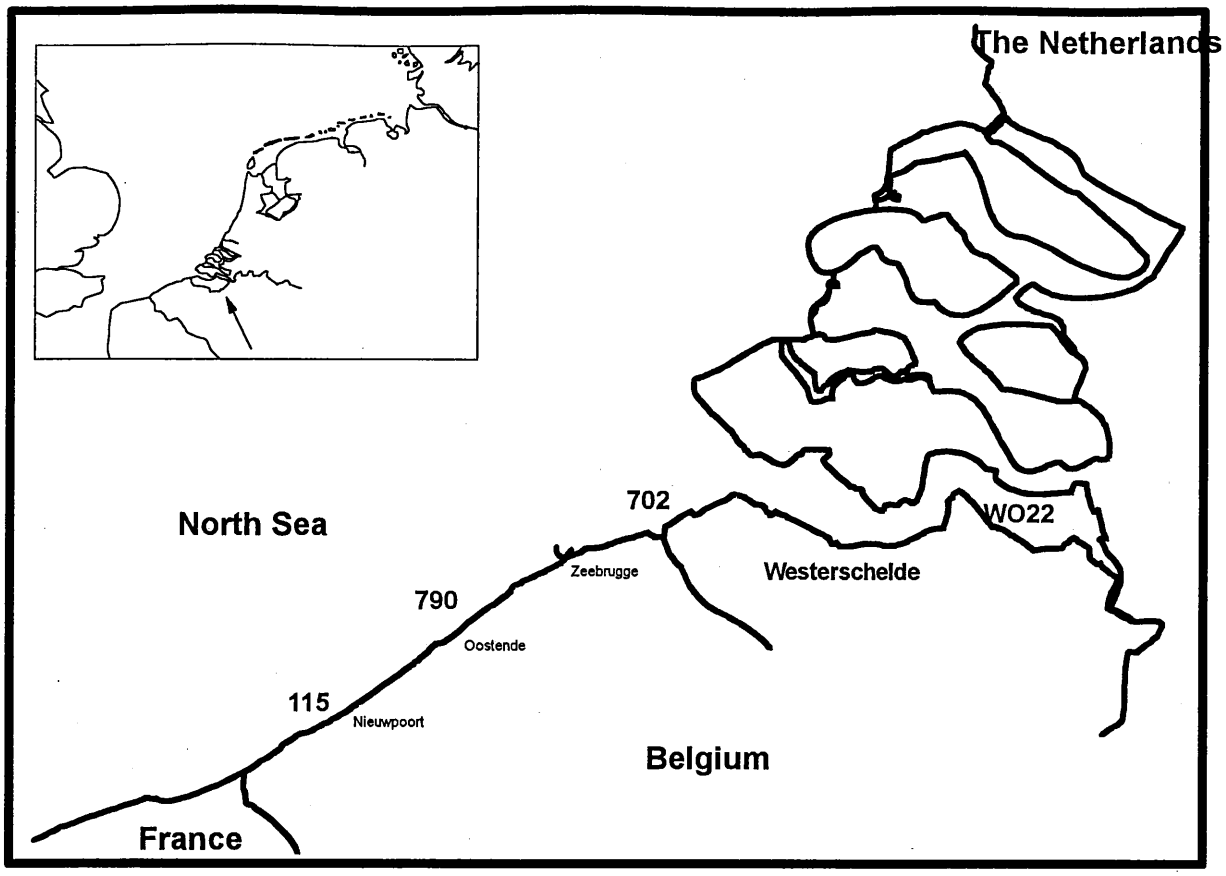


Figure 1. Location of the sampling stations

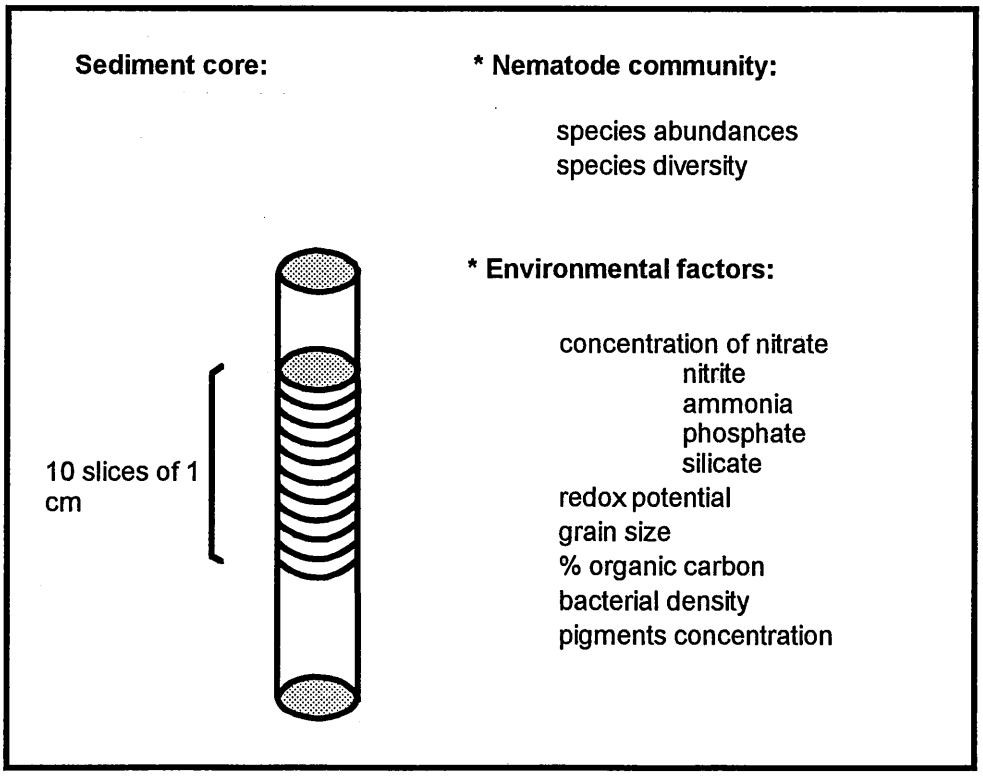


Figure 2.

Station 115

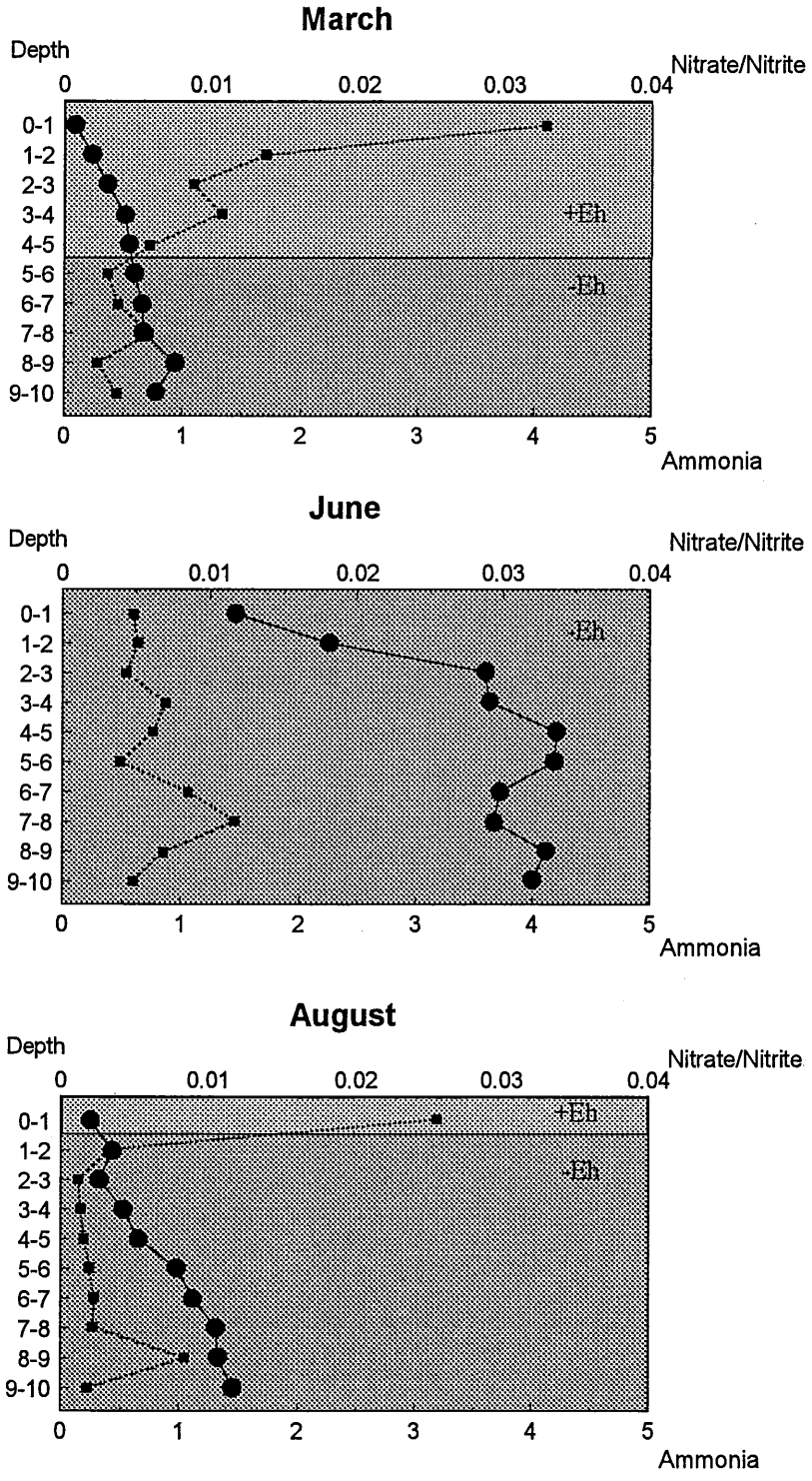


Figure 3. Environmental characteristics in station 115

Station 702

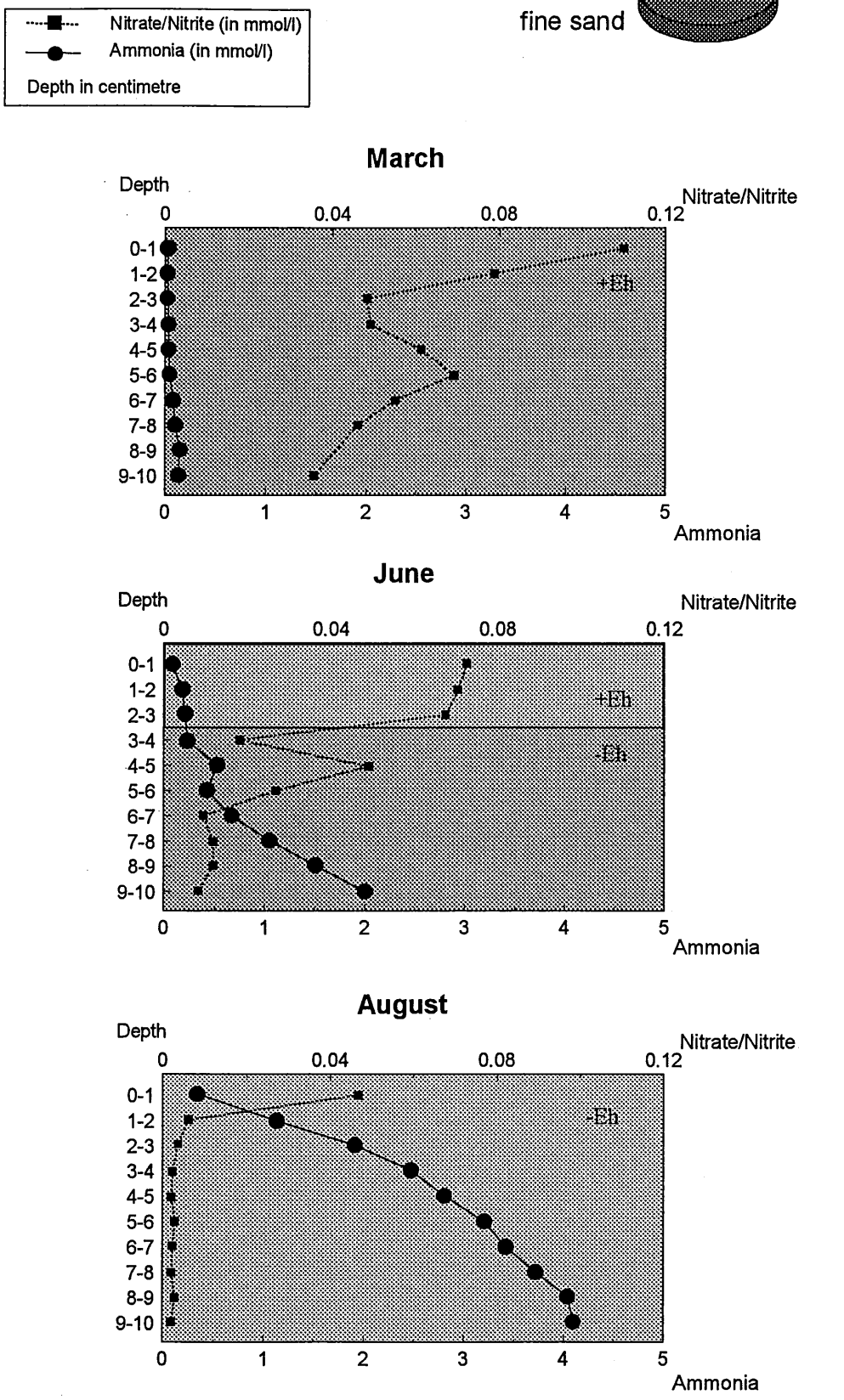
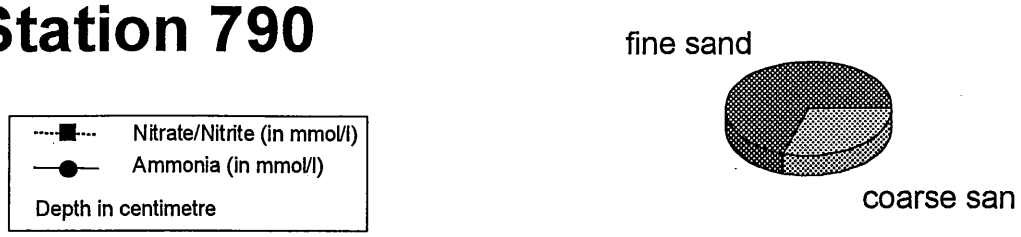


Figure 4. Environmental characteristics in station 702

Station 790



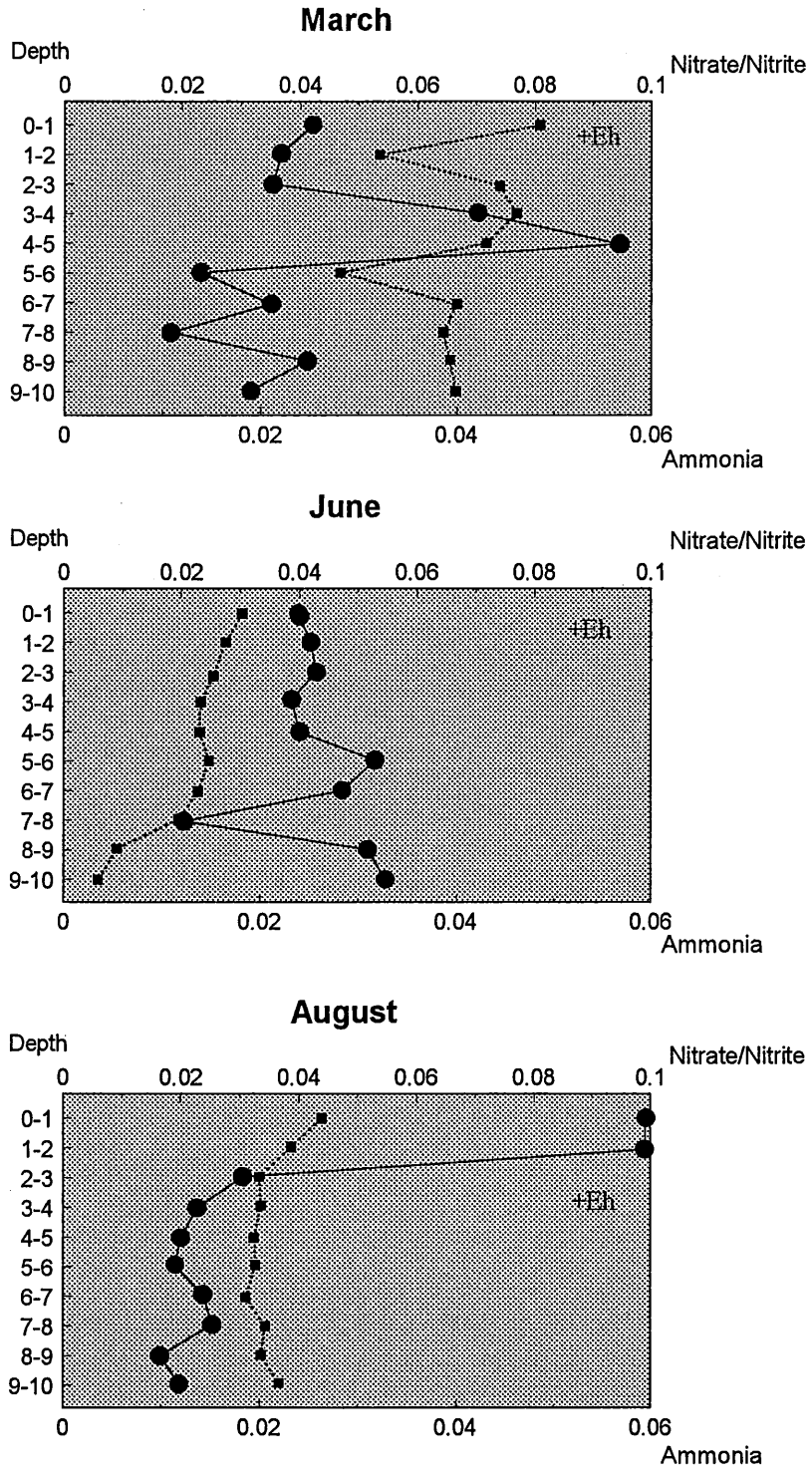
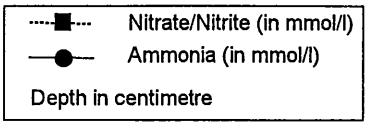
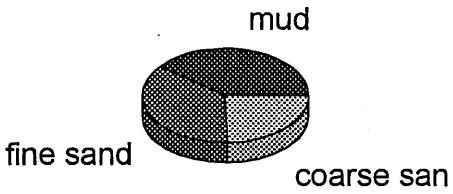
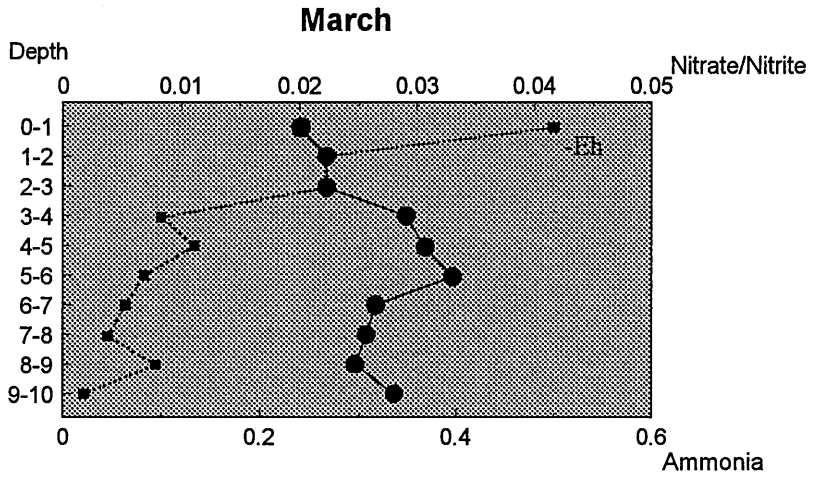


Figure 5. Environmental characteristics in station 790

Station WO22







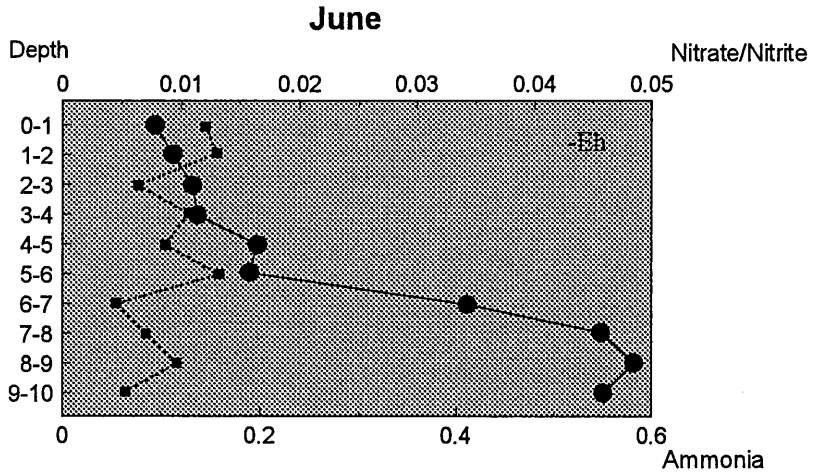
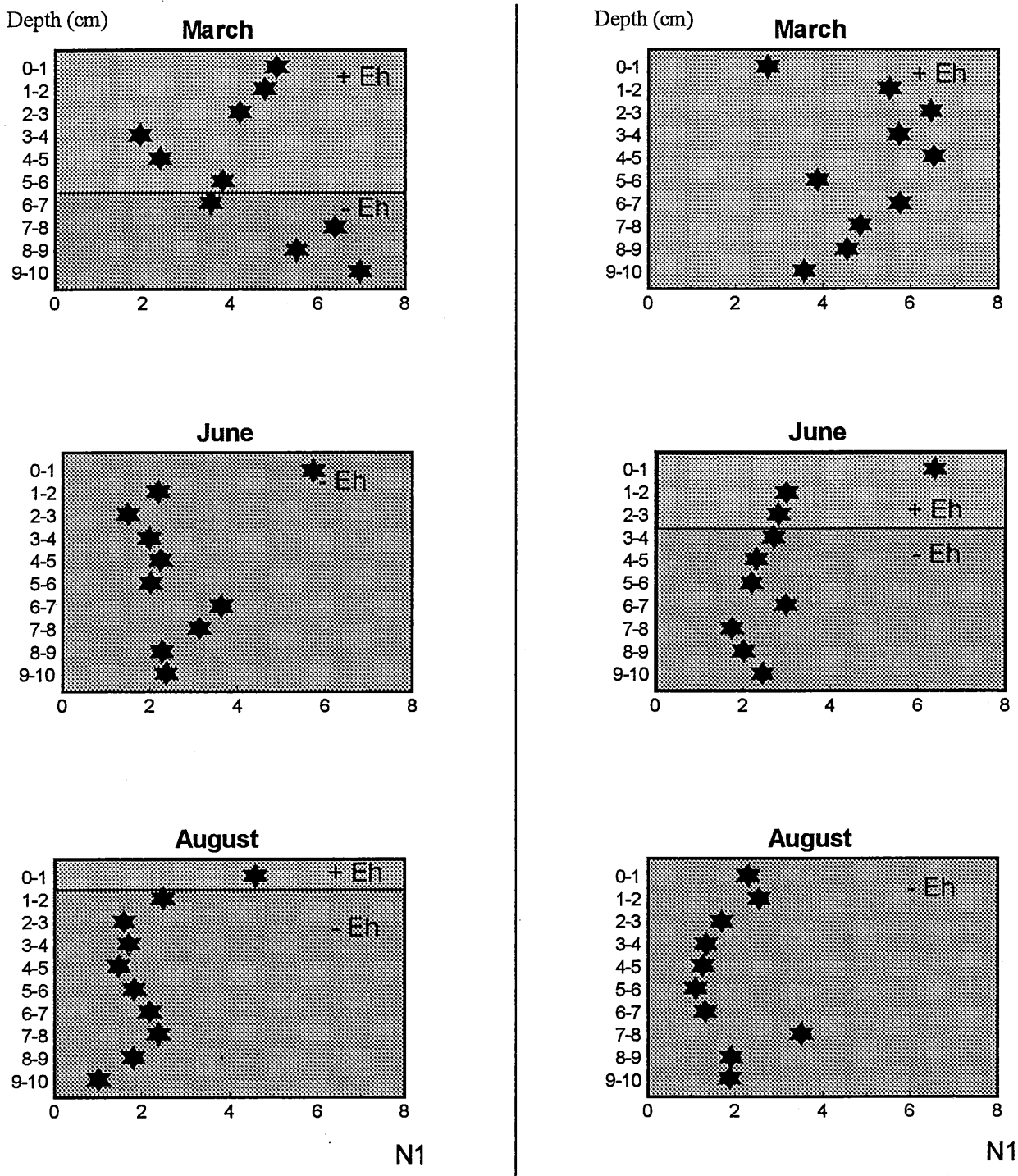


Figure 6. Environmental characteristics in station WO22



Figuur 7.Variability in depth of N1 species diversity

Station 115 - March

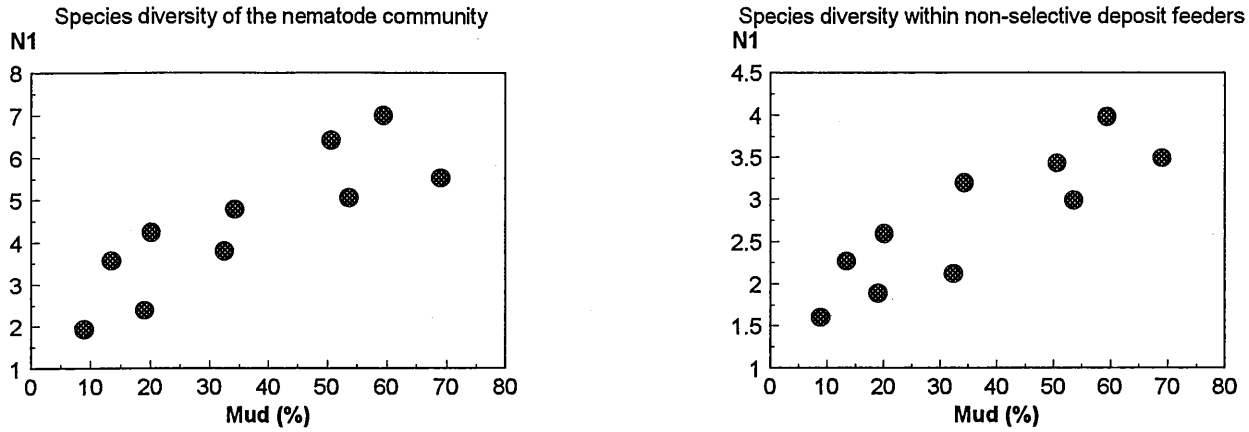


Figure 8. Spearman rank correlations for station 115 in March

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Station 115 - August

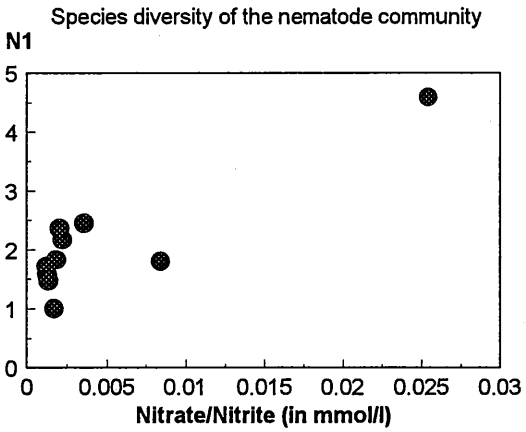


Figure9. Spearman rank correlations for station 115 in August

Station 702 - March

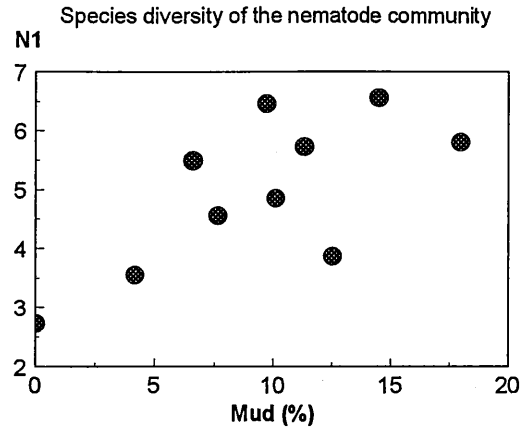
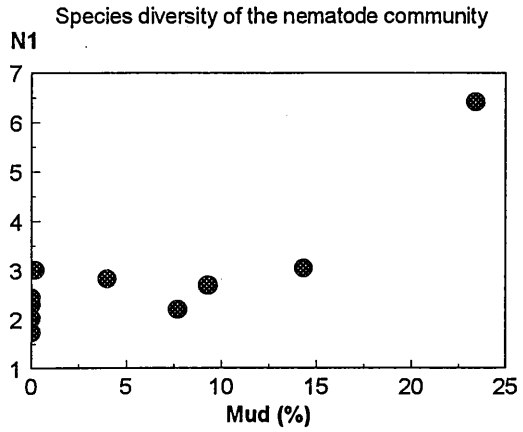
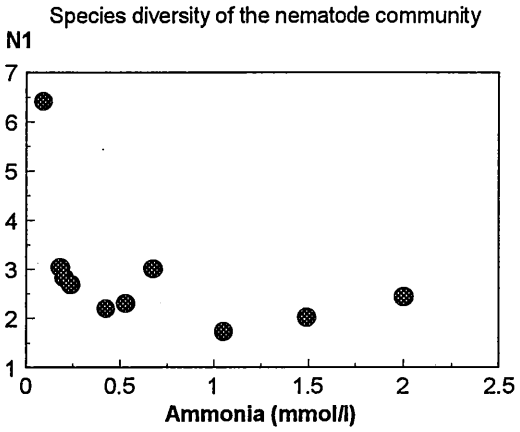
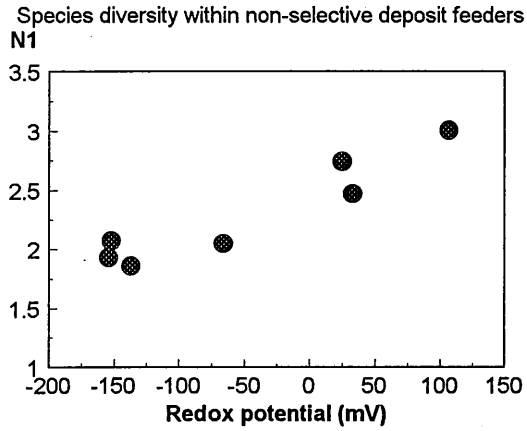


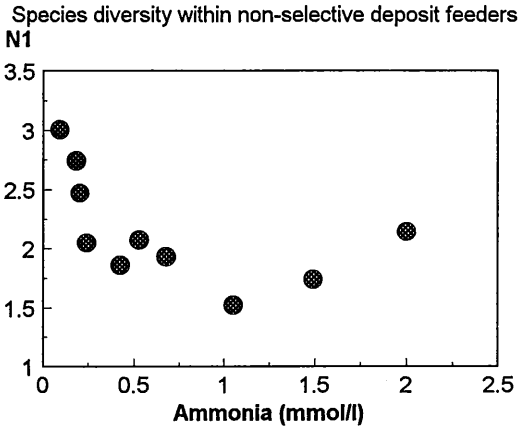
Figure 10. Spearman rank correlations for station 115 in March

Station 702 - June









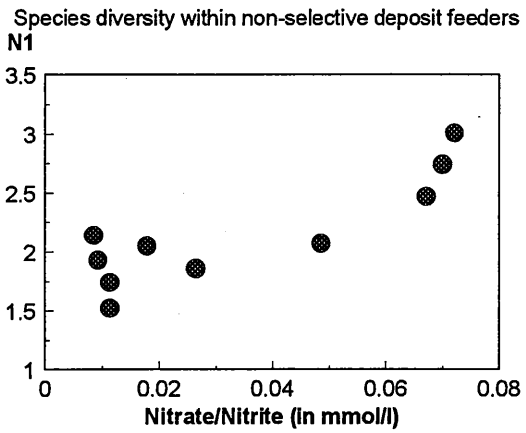
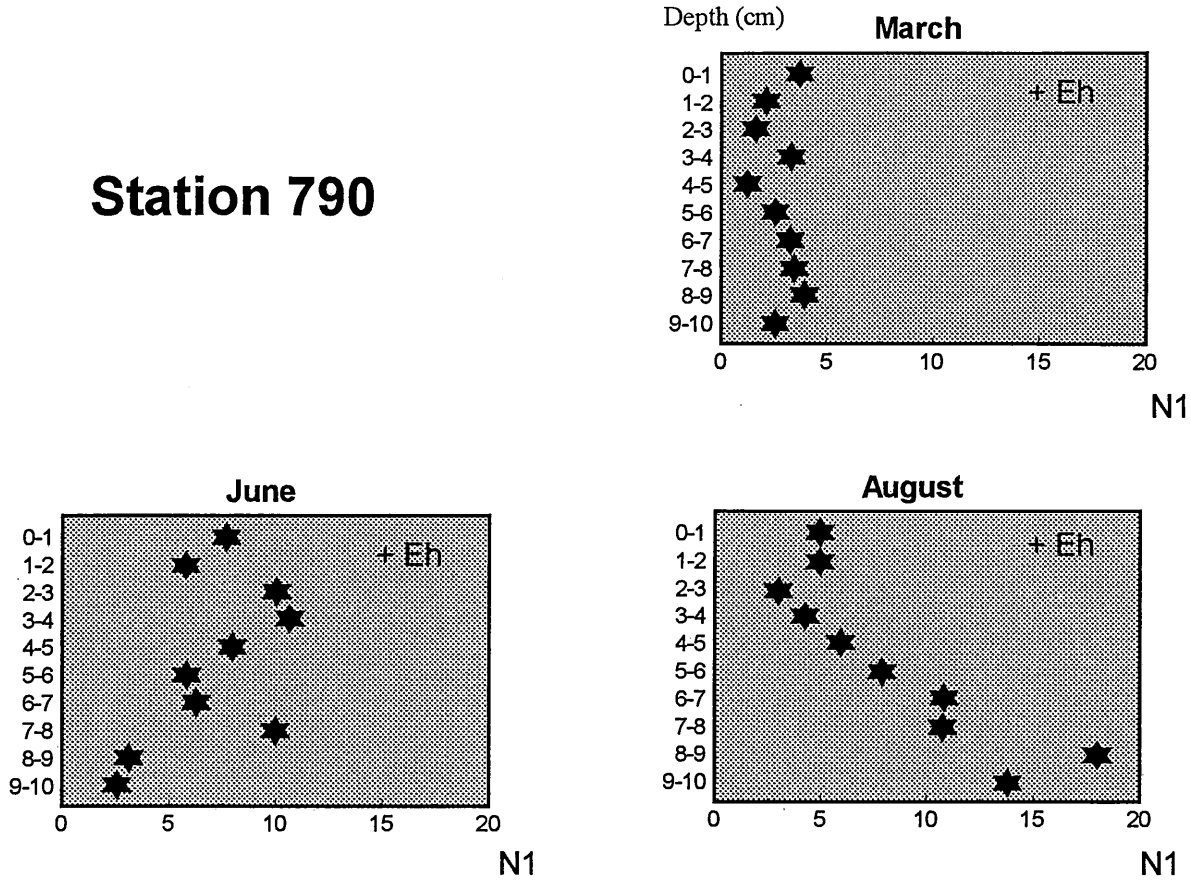


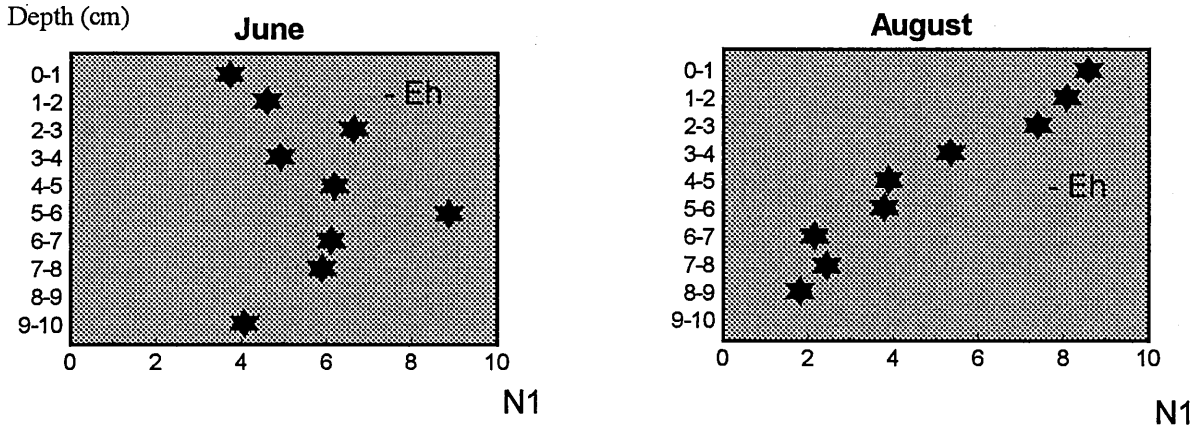
Figure 11. Spearman rank correlations for station 702 in June

Station 790

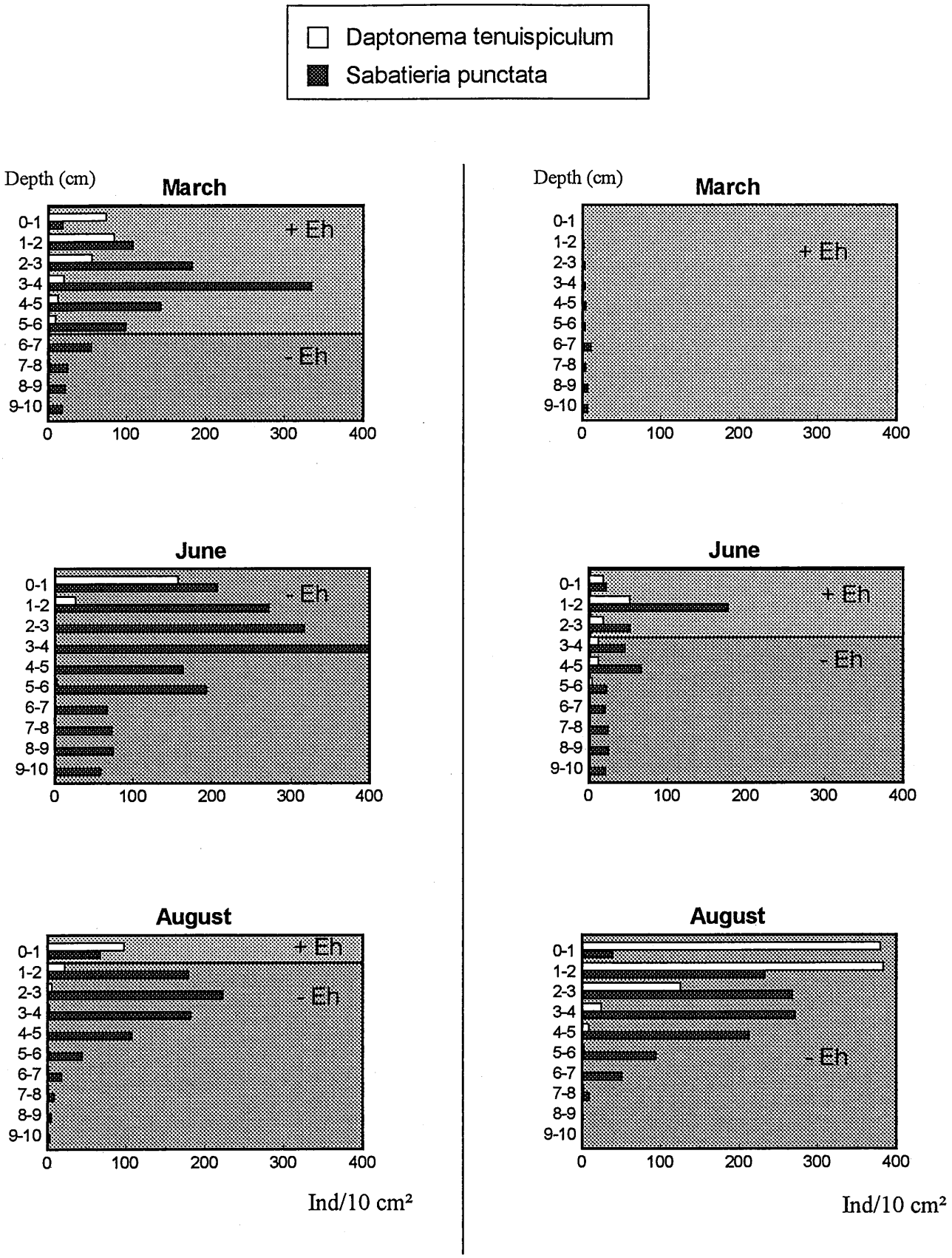


Figuur 12.Variability in depth of N1 species diversity

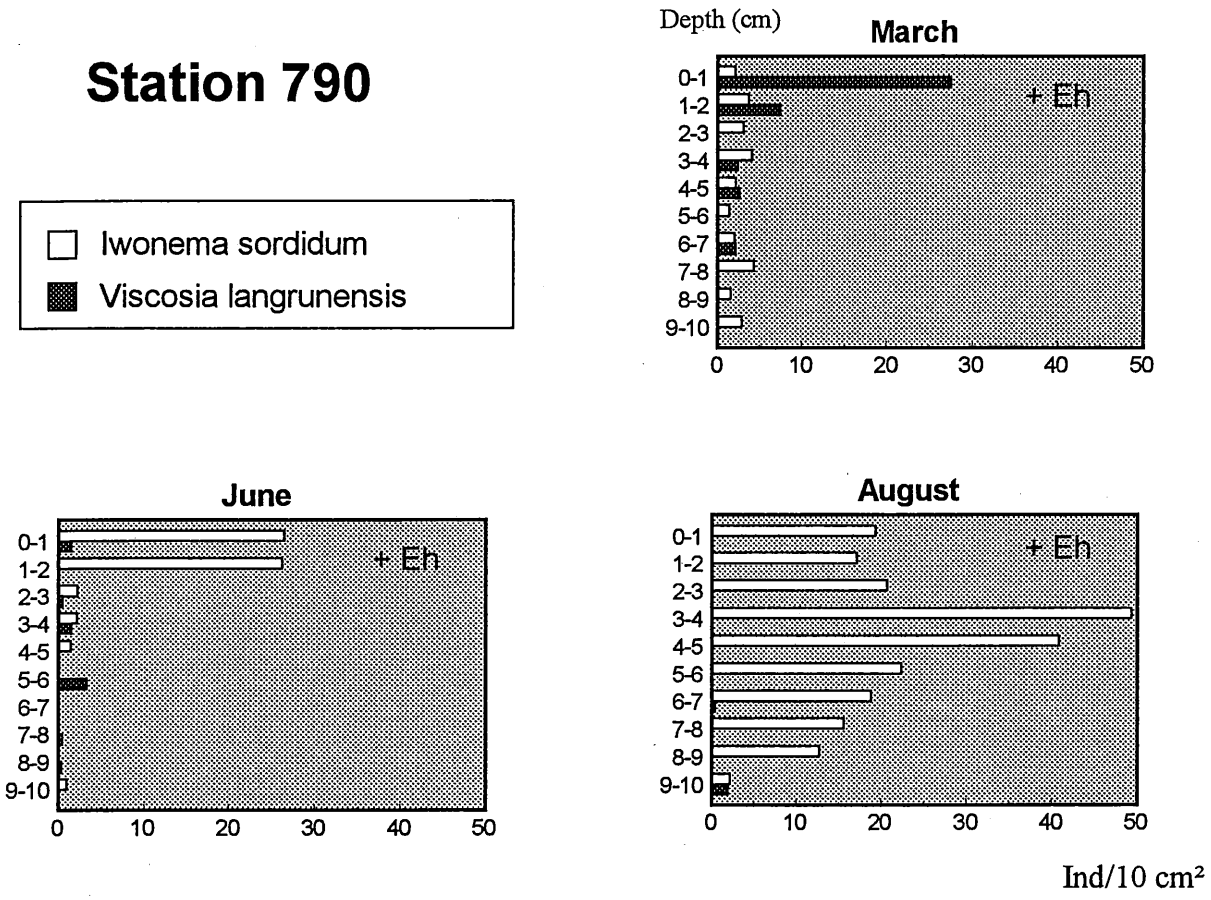
Station WO22



Figuur 15.Variability in depth of N1genra diversity



Figuur 13. Depth distribution of D.tenuispiculum and S.punctata



Figuur 14. Depth distribution of I.sordidum and V.langrunensis