ON THE POTENTIAL OF BASING AN ECOLOGICAL TYPOLOGY OF AQUATIC SEDIMENTS ON THE NEMATODE FAUNA: AN EXAMPLE FROM THE RIVER RHINE

TOM BONGERS and JAN VAN DE HAAR

KEYWORDS: freeliving nematodes, Rhine sediments, community ecology, environmental, assessment anaerobic conditions, ammonia.

ABSTRACT

In Dutch river sediments, nematodes can occur in high numbers; azoic sediments are as yet unknown. In a series of samples taken from the river Rhine and its estuaries, nematode density varied from 5.000 to 1.7 million per square metre, and about 50 genera were identified. Anoxic conditions appear to have less influence on the nematode abundance than the presence of ammonia. Based on nematode genera, an ecological typology of aquatic soils would appear to be feasible. The use of nematodes in environmental studies is discussed.

INTRODUCTION

Nematodes have hitherto received relatively little attention in environmental studies. Traditionally, nematologists interested in environmental studies have mainly focussed on marine nematodes whilst terrestrial nematodes have been studied by phytopathologists. However, interest in nematodes as a tool in river sediment and soil quality assessment has increased recently, since papers of ZULLINI (1976, 1976a), ZULLINI and RICCI (1980) and PREJS (1977).

The reasons for this increasing interest are primarily the high density and diversity of the nematofauna in aquatic, temporarily submersed and terrestrial systems leading to more robust data sets than can be achieved by most other organisms.

Nematodes are aquatic organisms. Even those that live in unsaturated habitats are dependent on the water film around particles. In aquatic systems, nematodes also live in the deeper sediment layers, as far as the ground water. With their permeable cuticle they are in direct contact with xenobiotics in the interstitial water. They are permanent members of the benthos and therefore are unable to escape from

any contamination by pollutants. Especially in cases where the xenobiotic load in water and sediment is not evenly balanced, as is often the case, nematodes offer a potential means of assessing sediment quality, either directly or by a bio-assay method such as that described by TIETJEN and LEE (1984). Certain nematodes are relatively easy to establish and maintain in laboratory cultures. They require little space, do not require running water, can be grown on simple foods and are easy to manipulate and to transfer. Their small size makes them especially useful in conducting experiments on the effects of toxicants on populations, rather than single individuals (TIETjEN and LEE, 1984). For the routine analysis of soil samples efficient techniques have been developed. After a soil or sediment sample arrives in the laboratory, it is possible to produce a nematode analysis consisting of diversity estimate and species composition within two hours because time consuming handpicking is superfluous.

In the Netherlands, about 800 terrestrial and freshwater nematode species are known to occur (BONGERS, 1988) and about 400 marine nematode species. They occur in all types of surface water

sediments including heavily polluted harbours, paper sludge and heavily eutrophicated or polluted aquatic systems where macrofaunal elements have already disappeared.

Nematodes form a heterogeneous group of organisms. Some species are relatively sensitive to pollutants, other can withstand most extreme pollutions or anaerobic conditions.

Within the benthic network of organisms, nematodes absorb dissolved organic compounds (LO-PEZ *et al.*, 1979), consume fungi and other organisms, parasitize higher plants, regenerate nutrients (GERLACH, 1978), influence sediment texture by their mucus secretion (RIEMANN and SCHRAGE, 1978; WAR-WICK, 1981), improve gas diffusion and serve as food for other organisms including predators belonging to their own taxon. Some species show a generation time of some days, other reach ages of one year or more.

Based on their life strategy, nematodes can be scaled from colonisers (r-strategists s.l.) to persisters (K-strategists s.l.). The weighted mean of these values gives an indication of the stability of the ecosystem from which the sample originates (80N-GERS, 1990). Nematodes appear to be appropriate organisms for the study of colonisation and ecosystem development in newly reclaimed land, harbour sludge dumped on land and in newly formed lakes,

Within the soil ecology programme of the National Institute for Public Health and Environmental Protection, an identification key (BONGERS, 1988) was written to facilitate nematode identification by non-specialists. Also within the same programme, a study of the development of an ecological typology of unsaturated soils was carried out (BONGERS et al, 1989), being a prerequisite for the development of a biological soil assessment system.

Focussing on this typology in the unsaturated zone, a number of season-independent nematode assemblages could be distinguished that appeared to be correlated with physical/chemical and species assemblage parameters such as density, diversity and evenness. The next step is to include (temporarily) submersed soils in that typology.

Although some preliminary studies have been undertaken on the nematode fauna of Dutch freshwater habitats (STRAATSMA, 1977; TAMIS, 1986; JA-COBS, 1987b; DE WINTER, 1988b), the sediment of the river Rhine had not hitherto been studied. The density of the nematode fauna, the depth distribution and relation to physical/chemical parameters, the base of the typology, were all unknown and are the subject of this paper.

MATERIAL AND METHODS

At 16 sites (Fig. 1; Table 1), samples were taken by SCUBA-diving using a perspex corer with an inner diameter of 60 mm and length of 25 cm. The sediment cores were subsequently sliced in layers of 0.5, 0.5,1.0, 3.0, 5.0 and 5.0 cm thickness; the upper layer is labelled as layer 1, the layer between 10 and 15 cm as layer 6. In total three cores were combined per sampling point. For detailed site descriptions and physical/chemical characteristics see DE JONG *eta/.* (1988).

The sediment samples were fixed in the field by adding formalin to produce a final concentration of 5%. Nematodes were extracted using the Oostenbrink elutriator; the extract was subsequently centrifuged with MgSO4-solution (density 1.28). After counting, the total nematode fauna was transferred to glycerin, a subsample mounted on mass-slides (BONGERS *et al.*, 1989) and, where possible, 100 specimens identified to genus. Numbers are expressed in percentages. The nematode assemblages in the two upper layers and in the total cores were analysed with TWlNSPAN. In the sediments studied here, the nematode fauna was composed not only of aquatic nematodes but also contained nematodes introduced by rain-water: the Tylenchida specimens (excluding *Hirschmannie//a)* were of terrestrial origin. The position of Aphelenchidae, Cephalobidae and some dorylaimids is uncertain; they are frequently found in aquatic habitats.

Table 1. Code, compartment and locality, depth in metres and texture of the upper centimetre of the sampled sites.

	Code Compartment and locality	Depth	Texture
1	Haringvliet, Stellendam (1)	-4.6	silty sand
2	Haringvliet, Slijkplaat	-2.1	silty sand
3	Haringvliet, Slijkplaat	-5.9	silty sand
4	Haringvliet, Ventiagersplaat	-11.6	silty sand
5	Haringvliet, Ventjagersplaat (2)	-0.5	silty sand
6	Hollandsch Diep, Willemstad	-7.2	silty sand
7	Hollandsch Diep, Willemstad	-2.2	silty sand
8	Hollandsch Diep, Sassenplaat	-7.8	silt
9	Hollandsch Diep, Sassenplaat	-3.0	silt
10	Noorder Gat, Anna Jacominaplaat	-4.4	silty sand
11	Nieuwe Merwede, Lage Zwaluwe	-4.3	silt
12	Nieuwe Merwede, Kop van 't Land	-3.0	sand
13	Nieuwe Merwede, Ottersluis	-1.8	silty sand
14	Nieuwe Merwede, Werkendam	-4.5	silt
15	Waal, Sliik Ewijk	-2.0	sand
16	Waal, Sliik Ewiik	-5.0	sand

(1) Marine sediment

(2) High benthic primary production

Fig. 1. Sampling stations in the Rhine estuary (adapted from OE JONG *et a/,.* 1988)

RESULTS

The number of nematodes ranged from 5.000 to $1.770.000$ m -2 . About 50 nematode taxa were identified (see appendix).

In table 2, the TWlNSPAN ordination from two upper sediment layers is given together with the number of nematodes m^{-2} and maturity index. Because only a few terrestrial plant parasitic nematodes were present, the ordination is essentially the same whether they are included or not. The maturity index is based on values as given by BONGERS (1990). For the marine taxa, the following values were used: 2 for the pollution-resistant genera Axonolaimus and *Sabatieria, 3 for Ascolaimus and Odontophora and 4* for *Ca/yptronema* and *Viscosia.*

If the TWINSPAN analysis is based on the whole core, sample 5 is combined with sample 2 and 6, and sample 10 and 13 constitute a separate group. The remaining samples of that group (11 and 14) are combined with 12 , 15 and 16 .

Tabel 2. Site number, nematode density (number m^{-2}), Shannon-Weaver index (H), Evenness (J') and Maturity Index (MI) per sample, based on the whole core, ordinated according to the TWINSPAN analysis of the upper two layers. MI (2) gives.the MI for the upper two layers.

In general, the number of nematodes decreased with increasing depth; only in samples 8 and 9 was maximum below the upper centimetre. On average, 50.8% of the total number is found in layer 1, 27.6% in layer 2, 10.9% in layer 3, 4.0% in layer 4 and 3.3% in layer 5 and 6. The depth distribution per layer, expressed in number of nematodes per 100 ml sediment, is presented in table 3.

DISCUSSION

The lowest number of nematodes was found at locality 10: about 5.000 specimens per square metre. This site is characterized by a high sedimentation rate. The nematodes found here belong to the stress-tolerant family Monhysteridae, which are rstrategists or colonizers. Below 2 cm depth nematodes were absent. In the density of nematodes, this sample resembles the polluted samples from the Vossemeer (JACOBS, 1987b) and harbour basins of Vlaardingen (TAMiS, 1986). However, the maturity index is lower. It is possible that the fauna at this site is subject to a constant process of extinction and recolonisation (VAN URK, pers. comm.).

Many nematode species are able to survive periods of anaerobic conditions. Following a mass mortality of demersal fish in Kiel Fjord due to anoxic conditions, PREIN (1988) described patches of 2- 3 m in diameter in which the layer of *Pontonema vulgare* was several millimetres thick, forming white fluffy carpets of nematodes. Within the framework of a DBW/RIZA project (Nature Developments Volkerakmeer/Zoommeer), DE WINTER (1988a) described a bloom of *Terschellingia communis* below the 'Volkeraksluizen' where the macrofauna had disappeared as a result of anaerobic conditions. *Sabatieria,* which feeds on sulphur reducing bacteria (BOUWMAN, 1978), can also withstand these extreme conditions.

In freshwater sediments the genera *Monhystera* and *Tobrilus* occur in oxygen-stressed conditions (JACOBS, 1987a; NUSS, 1984) and the contractile parts of their muscle cells are often filled with crystalloids. Sulphur was found to constitute a significant component of the crystalloid inclusions of *Tobri/us.* According to NUSS and TRIMKOWSKI (1984), these crystal-Ioids are the product of a detoxification mechanism for survival of H₂S-rich conditions. SCHIEMER and DUNCAN (1974) concluded that the metabolism of *Tobri/us graci/is* is partially anaerobic, even when oxygen is available.

From our results, it seems that the presence of ammonia has a greater influence on nematode abundance than absence of oxygen. The shallow

sample from the Ventjagersplaat (5), a site with a high benthic primary production where oxygen penetrates the upper 4 mm of the sediment, exceeds all other samples in number of nematodes. Even in the anaerobic layer 5, at a depth of 5 - 10 cm, more than 350.000 nematodes $m⁻²$ occur. It is remarkable that the ammonia concentration at this site does not exceed 50 μ M, at the other sites it varied between 1000 and 5000 μ M (DE JONG *et al.*, 1988). Ammonia is toxic to nematodes and has been used as a nematicide (RODRIGUEZ-KABANA, 1986).

The first division of the TWINSPAN analysis separated the marine station from the freshwater samples due to the presence of the marine general *Odontophora, Sabatieria, Ascolaimus* and members of the Linhomoeidae. Sample 1 is the only one in which the common freshwater genus *Eumonhystera* is absent. Marine sediments differ in their nematode assemblage at the highest level: a general trend is also observable in other organisms (BILIO, 1966) and is also reflected in biophysical sediment processes as redox profile and hydrogen sulphide content (DE JONG *et al.,* **1988).**

At the second level, the sandy river eroding sites (12, 15 and 16) together with the production site (5) are split off from the silty sites; the eroding sandy sites being characterized by *Chromadorita.* At the first three sites, sedimentation hardly occurs; the sandy soils had a high oxygen content, high permeability and little accummulation of pigments. Sample 5 however contained more silt but the oxygen concentration was rather high as a result of photosynthesis by the algae covering the sediment. The division into coarse and fine textured sediments is comparable to the main division in clayey and sandy soils in the terrestrial system (BONGERS *et al.,* 1989) and also in marine sediments. In finer marine sediments, the nematode density is higher but the diversity is higher in coarser sediments (JUARIO, 1975; HElP *et al.,* 1985).

The remaining group of the second level is composed of silty river sites and sites in the basins of Haringvliet and Hollandsch Diep where sedimentation occurs. These sediments are characterized by a high content of chlorophyll, dissolved organic material, nitrogen and a low oxygen level. A characteristic genus of this sediment type is *Monhystera* which usually occurs in oxygen stressed conditions (JA-COBS, 1987a).

The silt rich samples are divided into two groups: river samples with *Chromadorita* and relatively high numbers of *Monhystrel/a,* and basin samples characterized by *Paraphano/aimus.*

The two remaining samples 2 and 6 are silt-

sandy basin samples which show low numbers of *Monhystera* and *Paraphanolaimus* but high numbers of *Monhystrella, Eumonhystera* and *Tobrilus.*

An enlarged sample series of the Dutch surface waters is necessary to confirm the relation between nematode species and the depth in which they occur and to indicate which species are aquatic and wich are of terrestrial origin.

The bottom types, a result of sedimentation, can be distinguished based on nematode genera and appear to be characterized also by ecosystem processes as production and denitrification. Also a geographical influence is perceptible. OE JONG *et aL* (1988) distinguished four compartments: Haringvliet, Hollandsch Diep, Nieuwe Merwede and Waal. Based on their fig. 3.4.3 however, it appears that these compartments are also characterized by physical/chemical properties.

Considering the upper sediment layers of this series the following six types of bottoms can be distinguished.

- A: The marine locality 1, with *Odontophora, Aphelenchoides cf. bicaudatus* and several *Daptonema* species in the upper layer and *Sabatieria* in the deeper anaerobic layers. From the abundance of sulphate and the shape of the redoxprofile a high H₂S concentration can be assumed (DE JONG, pers. comm.).
- B: The shallow site 5 with its high benthic primary production. The 02 production is high, ammonia is lacking. The presence of *Asco/aimus* and *Axono/aimus* indicates its marine origin. This type is characterized by a relatively high density of *Microlaimus* and *Paracyathdlaimus.*
- C: The sandy river samples 12, 15 and 16, characterized by the absence of sedimentation and a high oxygen penetration. *Chromadorita* occurs in high concentrations and *Theristus* occurs in the upper sediment layer.
- D: The silty river samples 10, 11, 13 and 14, with a high sedimentation rate and a low number of nematodes in the deeper layers, contain high numbers of *Monhystrella,* the typical silt inhabitant *Monhystera* (also in E) and *Chromadorita* is. still present. Sample 13 deviates from the other samples in this series by the absence of Chromadoridae (eutrophication?).

E: Silty basin samples 3, 4, 7, 8 and 9 with

Paraphanolaimus as the characteristic genus, *Monhystera* which is shared with type D and an absence of *Chromadorita.* Sample 3 deviates from the other in this series by the reduced number of nematodes in the deeper layers, by the presence of *Sabatieria* and *Calyptronema* and absence of Chromadoridae and *Tobri/us.* Sample 7 deviates by the absence of *Monhystrella,* a low chlorophyll-a level and absence of nitrogen.

F: Silt sandy basin samples 2 and 6, characterized by a low chlorophyll-a content, low level of nitrogen and organic material compared to type E. Type F contains high densities of nematodes. *Chromadorita* is still present but *Monhystera* is almost absent. Probably these two samples represent two types: sample 6 contains a higher level of N and P than sample 2. In sample 6 the pollution resistant genus *Tobrilus* is dominant, *Daptonema* in sample 2.

According to VAN URK *etaL* (1985), based on the macrofauna, (river) sediments in The Netherlands can be separated into three pollution classes: azoic sediments in some harbour basins, sediments with only Tubificidae and sediments with at least four tubificid species, together with chironomids and molluscs. For the assessment of water quality, tubificids are considered to be relatively tolerant to pollutants; according to VAN URK *et aL* (1985), they only have an indicator value in extreme cases of pollution. The nematode fauna has the potential to act as an additional instrument to develop an overall typology and assessment system of sediments and terrestrial soils.

ACKNOWLEDGEMENTS

This study is a part of a project that was carried out by the Delta Institute for Hydrobiological Research (DIHO) on request of the National institute of Public Health and Environmental Protection (RIVM). The authors wish to express their gratitude to Wim Admiraal, Peter Hofman, Loes de Jong and Adri Sandee for the opportunity to participate in this project and for taking sediment samples; Ron de Goede for helpful suggestions and Howard Platt of the British Museum (Natural History) for scrutinizing the English text.

42 BONGERS and VAN DE HAAR

APPENDIX 1. Nematode percentage abundancy for samples of which more than 10 specimens were identified.

Nematodes of Rhine sediments **43**

APPENDIX 1. (Continued)

44 BONGERS and VAN DE HAAR

APPENDIX 1. (continued)

Other genera found in omitted samples with a low nematode density are: Pleurotylenchus (10-5), Nagelus (11-4, 13-6) and Leptolaimus (13-3).

REFERENCES

- BILIO, M., 1966. Die aquatischen Bodenfauna van Saizwiesen der Nord- und Ostsee. II. Okologische Faunenanalyse: Hydrozoa, Nematoda, Rotatoria, Gastrotricha, Nemertini, Polychaeta, Oligochaeta, Halacaridae, Ostracoda, Copepoda, Intern. Rev. Ges. Hydrobiol, und Hydmgraph., 51: 147-195.
- BONGERS, T,, 1988. De nemstoden van Nederland, Pirola Schoorl. Natuurhist. Bibiioth. KNNV nr. 48. 406 p.
- BONGERS. T., R.G.M. DE GOEDE. F.I. KAPPERS and R. MANGER, 1989. Ecologische typologie van de Nederlandse bodem op basis van de vrijlevende nematodenfauna. RIVM-rapportnr. 718602002.
- BONGERS, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia: in press.

BOUWMAN, L.A., 1978. Investigations on nematodes in the Ems-Dollart estuary. Annales Soc. r. Zool. Belg., 108: 103-105.

- DE JONG, S.A., P.A.G. HOFMAN and A.J.J. SANDEE, 1988. Gradiënten van fysisch-chemische en biologische parameters in de Rijnbodem. Rapport DIHO. 55 p.
- DE WINTER, W., 1988a. Veraoderingen in de nematodeofauna. In: E.H. van Nes and H. Smit. Natuurontwikkelingen Volkerakmeer/Zoommeer. Voortgangsrapport over de pefiode oktober 1987 t/m februari 1988. DBW/RIZA notitie 88.029x. p. 26-30.

DE WINTER, W., 1988b. Nematodenfauna van hat Volkerakmeer/Zoommeer. Rapp. Vg. Nematologie LUW - DBW/RIZA Dordrecht. 22 p.

- GERLACH, S.A., 1978. Food-chain relationships in subtidal silty sand marine sediments and the role of meiofauna in stimulating bacterial productivity. Oecologia, 33: 55-69.
- HELP, C. and W. DECRAEMER, 1974. The diversity of nematode communities in the southern North Sea. Journal of the Marine Biological Association of the United Kingdom, 54: 251-255.

HELP, C., M. VINCX and G. VRANKEN, 1985. The ecology of marine nematodes. Oceanogr. Mar. Biol. Ann. Rev. 23: 399-489.

JACOBS, L.J., 1987a. A checklist of the Monhysteridae (Nematoda, Monhysterida). Publ. Randse Afrik. Univ. C 46 Johannesburg. 186 p. JACOBS, L.J., 1987b. Inleiding tot de biologische kwaliteitsbeoordeling van onderwaterbodems in Nederland door middel van de

Nematofauna. Vg. Nematologie LUW - DBW/RIZA. JUARIO, J.V., 1975. Nematode species composition and seasonal fluctuation of a sublittoral meiofauna community in the German Bight.

- Veröff. Inst. Meeresforsch. Bremerh., 15: 283-337.
- LOPEZ, G., F. RIEMANN and M. SCHRAGE, 1979. Feeding biology of the brackisch water oncholaimid nematode *Adoncholaimus tha/assophygas.* Mar. Biol., 54:311-318.

NUSS, B., 1984. Ultrastructurelle und ökophysiologische Untersuchungen an kristalloiden Einschlüssen der Muskeln eines suffidtoleranten limnischen Nematoden (Tobrilus gracilis). Veröff. Inst. Meeresforsch. Bremerh., 20: 3-15.

NUSS, B. and V. TRIMKOWSKI, 1984. Physikalische Mikroanalysen an kristalloiden Einschlüssen bei *Tobrilus gracilis* (Nematoda, Enoplida). Ver6ff. Inst. Meeresforsch. Bremerh., 20: 17-27.

PREIN, M., 1988. Evidence for a scavening lifestyle in the freeliving Nematode *Pontonema vu/gare* (Enoplida, Oncholaimidee). Kieler Mearesforsch. Sonderh., 6: 389-394.

PREJS, K., 1977. The littoral and profundal benthic nematodes of lakes with different trophy. Ekologia Polska, 25 (1): 21-30.

RIEMANN, F. and M. SCHRAGE, 1978. The mucus-trap hypothesis on feeding nematodes and implications for biodegradation and sediment texture. Oecologia, 34: 75-88.

RODRIGUEZ-KABANA, R., 1986. Organic and inorganic nitrogen amendments to soil as nematode suppressants. J. Nematol., 18:120.

SCHIEMER, F. and A. DUNCAN, 1974. The oxygen consumption of a fresh-water benthic nematode, *Tobri/us gracilis* (8astian). Oecologia, 15: 121-126.

STRAATSMA, G., 1977. Oriënterend onderzoek naar de bruikbaarheid van Nematoden bij de beoordeling van de waterkwaliteit van laaglandbeken. Verslag afd. Natuurbeheer (LUW) no. 432: 25 p.

TAMiS, W.L.M., 1986. Nematoden in Vleardings Havenstib en in Papiersiurrie in het Apeldoorns Kanaal. Hydr. Adv. Bur. Klink. Rapp. & Meded., $22.9p.$

TIETJEN, J.H. and J.J. LEE, 1934. The use of free-living nematodes as a bioassay for estuarine sediments. Marine Environmental Research, 11: 233-251.

URK, G. VAN, F.C.M. KERKUM and S.M. WIERSMA, 1985. Bodemfauna in verontreinigde onderwaterbodems. H20, 18 (24): 509-513.

WARWICK, R.M., 1981. Survival strategies of meiotauna. From: Jones, N.V. and W.J. Wolff: Feeding and survival strategies of estuafine organisms. Mar. Sci., 15: 39-51.

ZIJLLINI, A., 1976. Nematodes as indicators of river pollution. Nemst. Medit. 4: 13-22.

ZULLINI, A., 1976a. I Nematodi come bioindicatori. I. Studio di una successione. Accademia Nazionale dei Lincei. Serie VIII, LXI (5): 503-507.

ZULLINI, A. and C. RICCI, 1980. Bdelloid mtifers and nematodes ih a small Italian stream. Freshwater Biology 10: 67-72.

Address of the **authors:**

Nematology Department, Wageningen Agricultural University, PB 8123 6700 ES Wageninoen, The Netherlands.