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Nematode communities of small farmland ponds

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Abstract The nematofauna of 14 farmland ponds, selected according to a gradient of surrounding agricultural land-use intensity, from five regions in North-West of Belgium were studied. The total nematode density (9–411 ind./10 cm^2 per pond), and especially the number of species (4–12 species per pond) was especially low in these ponds. In total, 17 genera of free-living benthic nematodes, belonging to 15 families, are identified. *Tobrilus gracilis* and *Eumonhystera filiformis* were the most common species and were found in 13 and 12 of the 14 sampled ponds, respectively. The genera *Tobrilus* and *Eumonhystera* jointly comprise 77% of the total nematofauna. Consequently, the investigated water bodies were dominated by deposit feeding Monhysteridae and/or by chewing Tobrilidae. Diplogasteridae and Rhabditidae, normally related with eutrophic habitats, were almost absent. In order to explain the variation of total density, diversity, feeding-types composition and the individual density of the six most important species within ponds as well, sets of environmental variables were statistically selected. It was demonstrated that morphologically very similar species can show highly different ecological properties. The presence of a substantial mud layer and of an overall high level of eutrophication as well as the presence of possibly associated anaerobic conditions are put forward as the main factors explaining the observed low density and diversity. Total phosphate concentration and sediment characteristics seem to be the most important variables to explain the nematode community structure. However, a clear pattern of environmental variables, agricultural land use and nematode assemblages was not observed.

Keywords Diversity · Freshwater · Meiofauna · Nematoda · Pool · Water body

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Introduction

Nematodes occur in all substratum and sediment types and are of considerable ecological importance (Giere, 1993; Neher, 2001), they regularly dominate the meiofaunal communities of lentic habitats (e.g., Heip et al., 1985, Traunspurger, 1996a, b). The abundance of limnetic nematodes usually ranges from 60 to ca 1000 ind./10 cm^2, containing about 30–70 different species (Traunspurger, 1996a, 2002). Nematodes are theoretically an excellent taxon to be used as ecological indicators for benthic environments. As they obtain very high abundances, a small sediment sample contains enough animals to produce scientifically sound statements. Moreover, they have an ubiquitous distribution, a high diversity (i.e., from tolerant to very sensitive species) and a short generation time. Additionally, in reproductive potential nematodes range from explosive opportunists to conservative survivalists (Bongers, 1990; Bongers & Ferris, 1999; Ritz & Trudgill, 1999; Höss & Traunspurger, 2003). Freshwater-nematodes proved to be indicators of aquatic pollution or eutrophication, both on the community (e.g., Beier & Traunspurger, 2001; Callahan et al., 1979; Ocaña & Picazo, 1991; Prejs, 1977; Preys, 1983; Zullini, 1976, 1988; Zullini & Ricci, 1980), and on the individual level (Arthington et al., 1986; Eyualem et al., 2001). Despite their significant role in benthic processes and biomonitoring potentials, relatively few studies have been carried out on freshwater-nematodes compared with other benthic invertebrate groups (Traunspurger, 2002) and knowledge of the taxonomy and ecology of freshwater-nematodes is still very limited (Pennak, 1988; Traunspurger, 2000; Beier & Traunspurger, 2003b).

This study is part of a multidisciplinary research project into the integrated management tools for water bodies in agricultural landscapes (MANSCAPE), which is described in detail by Declerck et al. (2006). In spite of their small surface area farmland ponds can significantly contribute to the regional biodiversity, supporting heterogeneous communities of aquatic organisms and often containing rare or unique species (Williams et al., 2003). Ponds and pools also substantially contribute to the ecological functioning and biodiversity levels of both natural and agricultural terrestrial ecosystems, as many organisms rely on such small water bodies for drinking, as foraging sites or as a nursery habitat for several life stages. Small aquatic systems proved to be good model systems for tackling general questions in ecology and evolutionary biology (De Meester et al., 2005). Their sensitivity to anthropogenic impacts makes them excellent sentinel systems for actual or pending changes in ecosystem health. In addition, small aquatic ecosystems also have an economic impact, especially in the agricultural sector. For these reasons, small water bodies deserve special attention of managers and decision-makers dealing with sustainable management of biodiversity. Nevertheless, small water bodies are largely ignored in major ecological surveys and little or no data are available, especially information on the distribution of nematodes in these habitats is, to our knowledge, completely lacking. In this paper, we present the first data concerning the nematode composition of temperate lentic water bodies, and more specifically of the type ‘‘cattle drinking pond’’, thus providing basic information for further investigations. Nematode assemblages were also analysed in combination with environmental variables in order to address the following three questions. (1) First of all, what are the potential environmental variables determining the nematode community structure and the individual density of the most important species? (2) Second, what are the differences in nematode diversity, Maturity Index (MI) values and feeding type composition between the studied ponds and what are the most important environmental variables explaining these patterns? (3) And thirdly, is the nematode community structure of small pools related to the intensity of agricultural land use?

Materials and methods

Study area, sampling and extraction

A total of 14 ponds from five regions (Blankaart, Zottegem, Temse, Geraardsbergen and Knokke) in North-West of Belgium were studied (Fig. 1). Regions were chosen such that each region contained several ponds that were located within a circular area of approximately 20 km^2 and covered a broad gradient in land use. Three

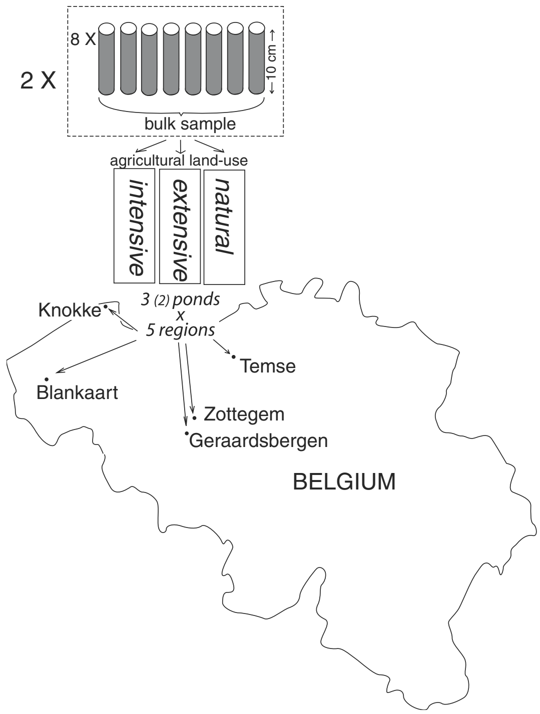

Fig. 1 Map of Belgium with the location of sampled pond clusters. Each cluster contained three small farmland ponds that were located within a circular area of approximately 20 km^2. The ponds within clusters were selected along a gradient of land use intensity, ranging from relatively natural areas to intensive agricultural activities (natural, extensive, intensive). An overview of the sampling strategy is schematically presented

ponds within each region were selected along a gradient of land use intensity, ranging from relatively natural areas (protected areas, nature reserves, pastures with low agricultural intensity) over intermediate (extensive) use to areas with intensive agricultural activities (crop lands, intensive cattle breeding). The three selected ponds were named ''natural'', ''extensive'' and ''intensive'' according to the agricultural land-use intensity. Special care was taken that only land use was applied as selection criterion, and not the aspects of the ponds themselves (e.g., macrophyte cover, water transparency). The extensive pond of Temse could not be sampled since it was completely dried out at the moment of sampling. More detailed information about the study site selection can be found in Declerck et al. (2006).

Sampling was done in August and September 2003. Within each pond, the top 10 cm of the sediment was sampled on eight randomly points using a Perspex core (inner diameter 5.7 cm); the samples were mixed and, out of a total volume of about 2 l, a subsample of 150 ml was taken. This sampling procedure was duplicated. We opted for sub-sampling well mixed bulk samples instead of taking individual samples because meiobenthos in freshwater habitats is known to show an especially high patchiness (Pennak, 1988; Traunspurger, 1996a), for a comprehensive overview of the implications of subsampling bulk samples, see Been & Schomaker (2006).

Summaries of results of physical/chemical water analysis and sediment analysis are listed in Table 1. The pH and conductivity of the water bodies was measured with standard electrodes. Water transparency was not determined with a Secchi disk but with a Snell tube because pond depths were shallow. Water depth and the thickness of the mud layer on the bottom sediments were measured with a graduated stick, along two perpendicular transects at distance intervals of 1 m. Sulphates, chlorides, alkalinity and hardness were measured following standard methods according to the Hach Water Analysis Handbook (Hach, 1992). Nitrate concentration in GF/F filtered water samples was determined with a Technicon autoanalyser III. Total phosphate concentration was measured with the ascorbic acid method after perchlorate digestion (Murphy & Riley, 1962). Chlorophyll a (Chl-*a*) concentrations were spectrophotometrically determined following the protocol of Talling & Driver (1963). Measured oxygen concentrations had to be excluded from further analysis because their unreliability due to frequent technical problems; yet, to approximate oxygen values, measurements were redone 2 years later in the same season. For more details on sampling strategy see Declerck et al. (2006).

Nematodes were extracted by the centrifugal-flotation technique (Caveness & Jensen, 1955); the sediment was centrifuged two times with Ludox AS40 (non-toxic, specific density 1.18 mg/ml; DuPont Chemicals, Wilmington, USA) and kaoline for 5 min at 3,500 rotations/min and the supernatant was rinsed over a 38 μm sieve. Nematodes were counted, 100 individuals were picked out randomly using a stereomicroscope

Table 1 Environmental variables measured in 14 farmland ponds in North-West of Belgium

Variable	Unit	Blankaart			Knokke			Geraardsbergen			Zottegem			Temse	
		Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Int.
Snell depth	cm	4.5	7	1.5	8	9	2	36.0	8	27	16	13	14	5	9
Temperature	°C	18.6	22.9	30.4	21	14	25.3	14.7	15.3	14.7	17.8	18.5	22.1	14	17
Conductivity	μS/cm	304	842	969	685	458	1224	637	526	687	954.0	663.0	513.0	722	510
pH		8.4	9.2	9.2	7.7	7.2	9.8	7.6	7.0	7.6	7.2	8.1	7.4	7.4	8.0
Alkalinity	meq/l	105	281	240.0	300	220	168	301	173	90	373	178.0	177	82	66
Tot P	mg/l	10.9	11.9	11.5	4.3	1.4	2.6	0.6	0.8	0.2	0.5	1.6	5.9	0.9	4.9
NO_3	mg/l	6.2	1	8	1	3.7	1.4	0.7	0.6	0.6	0.5	15.2	2.4	18.8	2.2
Hardness	mg/l	63.0	106	286	235	151.0	270	360	246	369	247.0	234	221	430	217
Ca^{++}	mg/l	57	103	215	116	98	153	286	123	200	190.0	116	135	250	138
SO_4^-	mg/l	27.5	55		33	15	160	28.0	102	115	130.0	116	7.5	124	130
Cl^-	mg/l	21.5	75.3	209.1	59.6	27.5	172	57.0	36.5	45	50.4	68.5	74.2	34	23.5
Chl-*a*	μg/l	136.0	75.1	1880	5.3	3.7	1000	2.2	2.2	5.7	36.7	343.9	4.2	205	19.4
Slib layer	cm	80	4	50	90	15	100	25	10	5	15	20	70	50	50
Surface area	m^2	420	911	234	134.0	56.5	294	163.0	50	70.7	84.8	208	1100	88	336
Volume	m^3	94.6	483.5	23.5	28.7	39.2	30.8	119.8	20.1	23.2	56.5	87.1	224.6	13.8	19.6
Max depth	m	0.4	1.0	0.2	0.5	1.0	0.2	1.1	0.4	0.3	1.0	0.8	0.4	0.3	0.1
Clay	0/1							1	1	1	1	1	1		
Silt	0/1		1												1
Sand	0/1	1		1	1	1	1							1	
Oxygen[a]	mg/l	18.2	2.1	1.2	1.03	5.36	5.76	0.45	0.75	–	0.84	11.0	–	8.35	3.09
Sediment colour[a]		Grey	Dark grey	Dark grey	Dark grey	Dark grey	Dark grey	Black	Grey	–	Black	Grey	–	Dark grey	Dark grey

[a] Measured 2 years later, in same season; average from three replicates

– pond was dried out at the moment of sampling

Snell depth: water transparency (deepest point under water (in cm) at which a mini Secchi disk, lowered in tube filled with water, can be seen); Tot P: total phosphate concentration; NO3: nitrate concentration; Ca^{++}: calcium concentration; SO_4^-: sulphate concentration; Cl^-: chloride concentration; chl-*a*: chl-*a* concentration: Slib layer: amount of mud on bottom of the pond; Clay, Silt and Sand: dominant grain size of sediment

(50× magnification); however, in case of very low densities less than 100 nematodes were collected (see Table 3). Formalin (4% with 1% glycerol) was heated to 70°C and 4–5 ml were quickly added to the specimens to fix and kill the nematodes in one process (Seinhorst, 1966). The fixed nematodes were processed in anhydrous glycerol following the glycerol–ethanol method (Seinhorst, 1959) and mounted on aluminium slides with double coverslips (Cobb, 1917).

Data analysis

Nematodes were identified to species level, whenever possible. Measurements were prepared from camera lucida line drawings using an Olympus BX 51 DIC microscope. The morphology of some important species was also recorded in video clips that mimic multifocal observation through a light microscope following the Video Capture and Editing procedures developed by De Ley & Bert (2002). The resulting virtual specimens are available on the web at: http://www.nematology.ugent.be/VCE.htm. The abundance (individuals/10 cm^2) of each species was determined as well as feeding-type (following Traunspurger, 1997a: deposit-feeder, epistrate-feeder, chewer, suction-feeder). The MI was calculated to provide an index that indicates the condition of an ecosystem based on the composition of the nematode community (Bongers, 1990). Nematode genera were assigned to the 1–5 ''coloniser-persister (cp) scale'' according to their r and K characteristics following Bongers (1990) and Bongers (1999). The MI was calculated both by including cp-1 nematodes (MI) and excluding cp-1 nematodes (MI 2–5); calculations were done without the Infraorder Tylenchomorpha (=predominantly plant-parasitic nematodes). To provide an index of diversity, the Shannon-Wiener index, with log base 2, was calculated.

Before the relationship between environmental factors and nematode communities was investigated, as well as the relation between individual species abundance and the aforementioned indices, the correlation structure among the environmental variables was explored by means of correlation graphs, linear correlation coefficients and principal component analysis (PCA). The nematode community structure was analysed through non-metric Multi-Dimensional Scaling applying the Bray-Curtis similarity measure (MDS) on square-root-transformed nematode species data. Further, the BIO-ENV procedure (Clarke & Ainsworth, 1993) was used to define suites of variables best explaining the nematode assemblage structure. MDS and BIO-ENV analyses were performed with the primer v5.2.9 software package (Clarke & Gorley, 2001). To examine the relationship between total nematode pond density, pond diversity, feeding type composition and individual density of the most important species vis-à-vis environmental variables, a model selection approach was used (Johnson & Omland, 2004). A stepwise forward variable selection was used to select the set of variables that best explained the variation in the diversity estimates (Neter et al., 1996). In this stepwise variable selection procedure, a significance level of $\alpha = 0.05$ was used as criterion to retain or drop a variable into the model (Neter et al., 1996). Analyses were performed in Proc REG in SAS v. 9.1.3.

Results

The environmental variables

As this study is of an observational type, a thorough inspection of the correlation structure among the explanatory variables is a prerequisite for understanding the observed relationships. Correlations among the variables and their significance level are presented in Table 2. One group of inter-correlated variables is associated with the ion-content of the pond, such as conductivity, water hardness, pH, and the concentration of calcium-, chloride- and sulfate-ions. Highly significant correlations include chloride concentration with pH and conductivity, and hardness with calcium concentration. Temperature is highly positively correlated with several environmental variables: pH, total phosphate-, chloride- and chl-*a* concentration. The concentration of phytoplankton chl-*a* appears to be highly positively correlated with conductivity, pH and chloride concentration. Ordination by PCA of

Table 2 Pearson product moment correlation coefficients (rP) among continues environmental variables measured in 14 farmland ponds

	Snell depth	Temperature	Conductivity	pH	Alkalinity	Tot P	NO3	Hardness	Ca^{++}	SO_{4-}	Cl^{-}	Chl*a*	Slib layer	Surface area	Volume
Snell depth															
Temperature	–0.50														
Conductivity	–0.17	0.57*													
PH	–0.45	0.78**	0.57*												
Alkalinity	0.21	0.24	0.37	–0.03											
Tot P	–0.49	0.69**	–0.02	0.63	0.02										
NO_3	–0.30	–0.06	–0.05	0.03	–0.38	–0.02									
Hardness	0.41	–0.22	0.34	–0.23	–0.12	–0.60	0.27								
Ca^{++}	0.51	–0.11	0.38	–0.13	0.13	–0.39	0.15	0.88**							
SO_4^-	–0.18	0.01	0.60*	0.22	–0.29	–0.41	0.21	0.43	0.27						
Cl^-	–0.32	0.88**	0.75**	0.74**	0.22	0.36	0.02	0.14	0.23	0.31					
Chl*a*	–0.45	0.79**	0.60**	0.69**	0.03	0.40	0.25	0.14	0.22	0.53	0.92**				
Slib layer	–0.45	0.46	0.12	0.39	–0.19	0.27	0.06	–0.08	–0.17	–0.07	0.33	0.30			
Surface area	–0.14	0.43	–0.08	0.32	0.00	0.61*	–0.16	–0.45	–0.33	–0.39	0.13	–0.06	0.19		
Volume	0.04	0.24	0.02	0.30	0.29	0.53	–0.19	–0.46	–0.25	–0.39	0.00	–0.20	–0.23	0.80**	
Max. depth	0.46	–0.34	–0.09	–0.29	0.67*	–0.21	–0.15	–0.23	0.01	–0.40	–0.32	–0.42	–0.56*	–0.03	0.41

(* p<0.05; ** $p < 0.01$; *** $p < 0.001$); For abbreviations legend, see Table 1

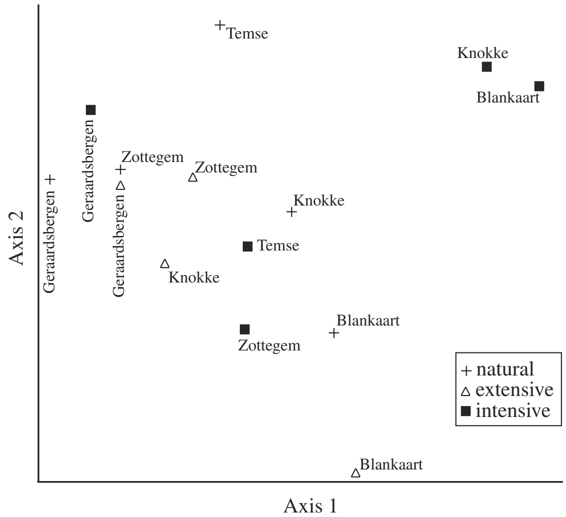

Fig. 2 Two-dimensional correlation-based principal-component analysis (PCA) of squareroot-transformed environmental data from 14 farmland ponds, which are selected along a gradient of land use intensity (natural, extensive, intensive) in five different regions

environmental data revealed an inconsistent trend of ordering natural, extensive and intensive ponds *per* region (Fig. 2). However, the overall picture did not show a clear pattern related to land use, natural, extensive and intensive samples are not aggregated in three separated successive zones on the PCA plot. The first two components (eigenvalues 5.7 and 4.0) explained for 51.5% of the total variance of the original variables. The PC1 axis (30%) is mainly associated with variables that could correspond to increasing intensity of land use and represents increasing values of temperature, pH, total phosphate-, chloride- and chl-*a* concentration (all highly correlated), turbidity and grain size. On the other hand, the PC2 axis (21.5%) is mainly associated with the ion-content and represents an increasing hardness and conductivity and increasing concentrations of calcium-, chloride- and sulfate-ions (all inter-correlated), but a decreasing surface and volume.

Species assemblages, density and diversity

Seventeen genera of free-living benthic nematodes, belonging to 15 families, were identified from the 14 ponds sampled (Table 3). These ponds are characterised by very few species. Only the genera *Tobrilus* Andrassy, 1959; *Monhystera* Bastian, 1865 and *Eumonhystera* Andrássy, 1981 were represented by more than one species. *Tobrilus gracilis* (Bastian, 1865) and *Eumonhystera filiformis* (Bastian, 1865) were the most common species; they were found in 13 and 12 of the 14 ponds, respectively. These two species jointly constitute 58% of the total nematofauna, and the genera *Tobrilus* and *Eumonhystera* comprise 77% of the total nematofauna. One pond (Zottegem-natural) was dominated by a plant-parasitic nematode, the migratory endoparasitic *Hirschmanniella loofi* (Sher, 1968) represents up to 58% of the total nematofauna in this pond. Other significant free-living nematodes in this study were *Monhystera stagnalis* Bastian, 1865, *M. riemanni* Jacobs & Heyns, 1987, *Mononchus aquaticus* Coetzee, 1968 and *Dorylaimus stagnalis* Dujardin, 1845 (Table 3). The species *Tobrilus stefanskii* (Micoletzky, 1925), *Mononchus tunbridgensis* Bastian 1865 and *Aglenchus agricola* (de Man, 1884) were recorded in three or four localities but only in low densities. The other recorded nematode species were recovered in not more than a single pond and in relatively low densities. Two replicas of Knokke-natural contained also a few individuals of unknown juvenile parasitic nematode, apparently free-living stages of Filarioidea (for details, see Bert et al., 2006).

Initial examination of the nematode communities by a MDS analysis did not show a clear pattern related to land use, as natural, extensive and intensive samples are irregularly grouped on the MDS plot (Fig. 3). Pond Zottegem-natural, dominated by plant-parasitic nematodes, and Geraardsbergen-extensive characterised by the single dominance of *T. gracilis*, revealed the highest dissimilarity to the remaining ponds. BIO-ENV analyses showed that the nematode communities can be explained best by the total phosphate concentration, the presence of sand or a combination of total phosphate and sand. These environmental variables alone or in combination display nearly equally, relatively low, Spearman rank correlation coefficients of 0.32, 0.31 and 0.30, respectively. Combinations of total phosphate, nitrate, pH and sand; and total phosphate and pH display slightly lower correlation coefficients of 0.28 and 0.27, respectively.

Table 3 Species list with abundance (ind./10 cm^2; first replicate up, second replicate down) per investigated pond and percent contribution to total nematofauna

	c-p Value	Blankaart			Knokke			Geraardsbergen			Zottegem			Temse		% Σ total abundance
		Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Int.	
Tobrilus gracilis	3	0.5	0	7.7	14.5	16.4	29	22	240	3.6		0.6	3.2	29	34	15.4
		0	0.9	0.9	3	43.6	28	64	182	1.8		0.6	2.7	50	43	
T. stefanskii	3	0								1.8					6.4	0.5
		0.5				0.9				9.1					0	
T. diversipapilatus	3											0.6				0.1
												1.8				
Tobrilus sp.	3	7.7	0	16	9.7	15	46	46	225	5.5	0	4.2	1.8	72	67	23.7
(juveniles)		2.7	1.8	3.6	4.8	25	56	26	131	4.5	0.5	0	7.7	103	110	
Tripyla glomerans	3										0					0.3
											0.9					
Dorylaimus	4	0.5	0.5	0.5	0.6	2.7			3.6			0.6		1.8	13.6	1
stagnalis		0.5	1.4	0	0	0			5.5			0		2.7	0	
Dorylaimus sp.	4	0.5	4.5	3.2	2.4	1.8			24	0.9				1.8	2.7	2.2
		0	7.3	9	1.2	1.8			7	4.5				3.6	0	
Mesodorylaimus sp.	4														29	0.5
															0	
Mononchus	4		0.5			1	2.7		0			4.8	0			0.9
aquaticus			0.5			0	3.6		3.6			6.1	0.9			
M. tunbridgensis	4						1.9					3			4.5	0.3
							1.8					0.6			2.7	
Monhystera	2				0.6		2.7							12		0.4
stagnalis					0		0							3.6		
M. riemanni	2		1.4			15.5		13		68	4.1	9.7			10	7.3
			0.5			28		35		33	0.9	9.1			4.5	
Monhystera sp.	2		1		0.6	53	1.7	1.8		24	0.5	1.2		0	0	3.8
(juveniles)			1.4		0	13	0.6	18		20	0	0		0.9	11	
Eumonhystera	2	2.3	16	18	70	14	94	0		137		75	13	17	57	34.2
filiformis		8.2	47	23	10	23	53	17.3		91		37	0.9	16	24	
E. vulgaris	2				1.8											0.1
					0.6											
Eumonhystera sp.	2	0	2.3			5.5	0	0		0		3	0.5		0.9	2.7
(juveniles)		0.5	7.3			0	2.4	12		5.5		1.2	4.5		0.9	
Protorhabditis	1											10				0.4
oxyuroides												0				
Rhabditis sp.	1					0.9					0		0			0.3
						0					0.5		0.5			
Aglenchus agricola										0.8	0	2.4				0.3
										0	0.5	0				
Amplimerlinius										0.9						0
icarus										0.9						
Geocenamus sp.												3				0.2
												1.1				
Psilenchus sp.			0													0
			0.2													
Hirschmanniella											2.7	2.4				4.3
loofi											8.2	0				
Helicotylenchus sp.												1.8				0.1
												0				
Trichodorus sp.							1.3									0
							0									
Acrobeloides cfr.	2			9.5												0.8
buetschlii.				0												
unknown filaroid					1.1											0.1
					0.7											

Table 3 continued

	c-p Value	Blankaart			Knokke			Geraardsbergen			Zottegem			Temse		% Σ total abundance
		Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Int.	
Individuals collected, if not 100		*31* *33*	*72* *100*	*100* *78*							*20* *31*		*51* *47*			
Total mean abundance per pond		11.8	47	42	60.3	130.5	162.7	127.3	410.9	206.4	9.3	90.3	18	156.8	210.9	

Juveniles of *Tobrilus, Eumonhystera Monhystera* could not be determined to species level and are listed separately. Results based on 100 individuals that were picked out randomly, unless stated differently

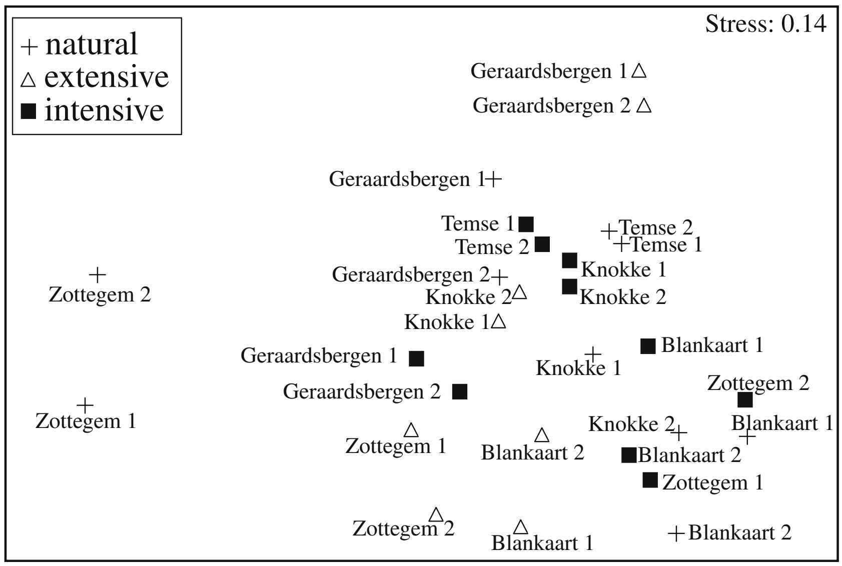

Fig. 3 Output of non-metric Multi-Dimensional Scaling (MDS) on square-root-transformed species abundances from 14 farmland ponds (two replicates), which are selected along a gradient of land use intensity (natural, extensive, intensive) in five different regions (Stress: 0.14)

A set of environmental variables was also statistically selected that best explained the among pond variation in individual density of the six most significant nematode species. Alkalinity, pH, water transparency, hardness, chloride concentration, absence of clay and total phosphate seem to have the highest influence on the density of *E. filiformis*, *M. riemanni*, *M. stagnalis* and *T. gracilis*. The recorded set of environmental variables could not significantly explain variations in density of *M. aquaticus* and *D. stagnalis*. Density of *E. filiformis* decreases with increasing alkalinity, and increases with increasing pH (Alkalinity: $t = -2.32^*$; pH: $t = 2.95^{**}$). *M. riemani* density increases with increasing water transparency (Snell depth: $t = 5.09^{***}$), whereas the density of *M. stagnalis* decreases with increasing water transparency (Snell depth: $t = -5.02^{***}$); both opposite reactions being highly significant. Density of *M. stagnalis* also decreases with increasing chl-*a* concentration, but increases with increasing hardness (chl-*a*: $t = -2.77^*$; hardness: $t = 6.02^{***}$). *T. gracilis* density decreases with increasing total phosphate concentration and significant more *T. gracilis* individuals are found when clay is absent. (total P: $t = -3.53^{**}$; absence of clay: $t = 2.27^*$).

The highest number of species was recorded from Zottegem-extensive (12 species) while the lowest number was recorded from Geraardsbergen-natural and -extensive (three species). This low species richness and dominance of few species leads to very low species diversity, as expressed by the Shannon-Wiener index. (Table 4). Knokke-extensive, Zottegem-extensive and Temse-intensive were characterised by the highest diversity, but the average Shannon-Wiener index was still below 2. The lowest

Table 4 Nematode diversity expressed as Shannon-Wiener index (H′ log base 2), nematode feeding-types (suction-feeders, deposit-feeders, chewers) densities (ind./10 cm^2), and Maturity Index (MI)

Replicate			Blankaart			Knokke			Geraardsbergen			Zottegem			Temse	
			Nat	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Ext.	Int.	Nat.	Int.
Shannon-Wiener index		1	1.1[a]	0.9[a]	1.6	1	2	1.1	0.9[a]	0.1	1.2	1.0[a]	1.9	0.7	1.6	2.4
		2	0.6[a]	0.5	0.2[a]	1.3	1.6	1.3	1.4[a]	0.3	1.3	1.3[a]	1.6	1.7	1.2	1.4
Feeding type	Deposit-feeder	1	2[a]	20[a]	28	72	35	97	13[a]	0	205	4[a]	98	14	29	68
		2	9[a]	55	23[a]	10	51	56	65[a]	0	129	1[a]	47	6	20	29
	Chewer	1	8[a]	1[a]	24	25	85	81	70[a]	465	35	0[a]	15	5	101	113
		2	3[a]	5	5[a]	8	83	90	107[a]	316	35	1[a]	9	11	154	166
	Suction-feeder	1	1[a]	5[a]	4	3	5	0	0[a]	27	3	3[a]	10	0	4	45
		2	0[a]	9	1[a]	1	2	1	0[a]	13	5	9[a]	1	0	6	0
MI		1	2.9[a]	2.4[a]	2.6	2.3	2.3	2.5	2.8[a]	3.1	2.1	2.0[a]	2.1	2.3	2.8	2.9
		2	2.3[a]	2.3	2.2[a]	2.5	2.5	2.6	2.5[a]	3	2.1	2.3[a]	2.3	2.7	2.9	2.8

Comparison between 14 farmland ponds (two replicates); ponds selected along a gradient of land use intensity (natural, extensive, intensive) in five different regions ([a]calculations based on less than 100 individuals, see also Table 3)

diversity value (average Shannon-Wiener index: 0.2) were recorded in Geraardsbergen-extensive. Variations in diversity could not be explained by the observed environmental variables, since no relation with environmental variables met the 0.05 significance level.

The mean nematode density ranged from only nine individuals/10 cm^2 (Zottegem-natural) to 411 individuals/10 cm^2 (Geraardsbergen-extensive), and the overall mean density is 119 individuals/10 cm^2. A model with alkalinity and total phosphate was statistically selected as the combination of variables that best explained density. Nematode density significantly decreases with increasing alkalinity and increasing total phosphate concentration (Alkalinity: $t = -2.72$**; total P: $t = -3.04$**).

Feeding types and Maturity Index

The composition of nematode feeding-types showed a clear dominance of deposit-feeders (50%) and chewers (42%), jointly comprising 92% of all investigated nematodes (Fig. 4). Blankaart-extensive (81%), Geraardsbergen-intensive (92%) and Zottegem-extensive (83%) are distinctly dominated by deposit-feeders; while Geraardsbergen-extensive (95%), Temse-natural (81%) and Temse-intensive (68%), are clearly dominated by chewers (Fig. 4). Suction-feeders dominated only Zottegem-natural, primarily belonging to the same species, the plant-parasitic nematode *H. loofi*. Epistrate-feeders were completely absent. Models with pond volume, conductivity and pH were statistically selected as the combination of variables that best explained the proportion of chewers and deposit feeders. Chewer proportion significantly decreases with increasing volume and conductivity (Volume: $t = -2.50$*; Conductivity: $t = -2.12$*). Deposit feeders density significantly increases with increasing pH (pH: $t = 2.47$*). The proportion of suction-feeders was not significantly related with one of the environmental variables. When feeding type density was considered instead of feeding type proportion, only the significant relation between deposit feeders and pH (pH: $t = 2.65$*) was confirmed.

The MI (Table 4) is noticeably associated with either *Tobrilus* or *Eumonhystera* being the subdominant genus. The MI shows that the community of Geraardsbergen-extensive (dominated by the species *T. gracilis*) had the highest MI value (average 3.1). The communities of Geraardsbergen-intensive (dominated by *E. filiformis*) had the lowest MI value (average 2.1). Variations in MI were not significantly related with one of the environmental variables.

Discussion

Species assemblages, density & diversity

With the only exception of Zottegem-natural, all ponds were dominated by Tobrilidae and/or

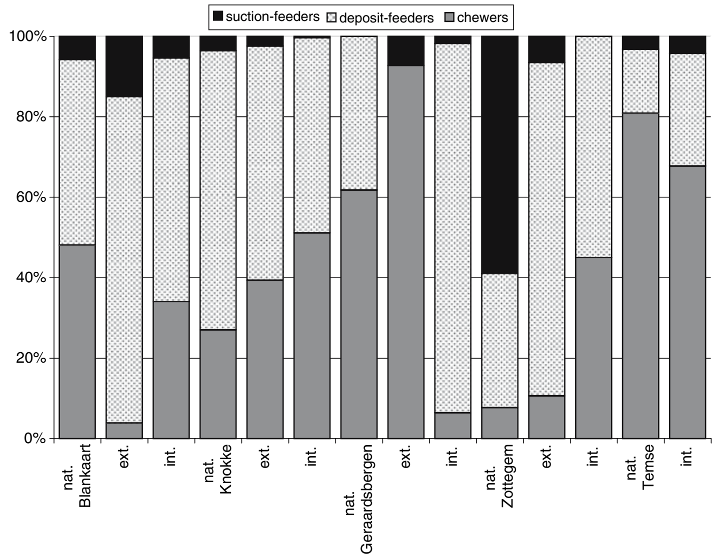

Fig. 4 Relative abundance (means of two replicates) of nematode feeding-types (suction feeders, deposit-feeders, chewers) in 14 farmland ponds, which are selected along a gradient of land use intensity (natural, extensive, intensive) in five different regions

Monhysteridae, mainly the species *T. gracilis* and *E. filiformis*. Their joint contribution to the total nematofauna (58%) versus the contribution to the total nematofauna of the genera *Tobrilus* and *Eumonhystera* (77%) is mainly attributed due to juveniles, which could not be determined on species level (see Table 3). Extrapolated, (assuming that species within one genus have a similar age-class distribution) *T. gracilis* and *E. filiformis*, respectively, constitute 38% and 37% of the total nematofauna. Tobrilidae display a high density in freshwater ecosystems all over the world (Gerlach & Riemann, 1973/74). They are large nematodes with long generation times (Traunspurger, 1997b) and with a great diversity of specific ecological requirements (Beier & Traunspurger, 2003a). *T. gracilis* is a very common freshwater nematode found in lentic as well as in lotic habitats (Traunspurger, 1997b). Some species of *Tobrilus* are considered typical for eutrophic lakes (Traunspurger, 2002), which corresponds to the importance of tobrilids in those hypereutrophic pools (trophy level based on phosphorus and nitrogen values as described by Wetzel, 1983). Since Tobrilidae are tolerant of short anoxic conditions (Prejs, 1977) and since the metabolism of *Tobrilus gracilis* is partly anaerobic, even when oxygen is available (Schiemer & Duncan, 1974), some tobrilids can have a competitive advantage in anaerobic situations related with intense eutrophication. Our actual result that the density of *T. gracilis* is negatively correlated with increasing phosphate concentration does not preclude that tobrilids become proportional more important in eutrophic situations.

The monhysterid *E. filiformis*, which was recorded in all but two ponds, is a common species in freshwater systems (Beier & Traunspurger, 2003a). Monhysteridae are typical opportunists with a small body size and a short generation time (Vranken et al., 1988) and many species have the ability to reproduce parthenogenetically (Beier & Traunspurger, 2003a). *E. filiformis* was found negatively related with NO_2^-, NO_3^-, NH^{4+}, PO_{3-4} and ammonium (Bongers & Van de Haar, 1990; Ocaña & Picazo 1991; Beier & Traunspurger, 2003b), and Prejs (1977), who analysed the nematofauna of 17 lakes with a different trophy, only found this species in oligotrotrophic lakes. The dominance of *E. filiformis* in hypereutropic ponds is in contrast with these literature data, but in agreement with Ocaña & Picazo (1991) who considered *E. filiformis* and *E. vulgaris* as being indifferent to diverse grades of water pollution, since they have found these species in considerable densities in unpolluted, as well as polluted parts of the Monachil river. *Monhystera*, a quantitatively lesser important monhysterid in this study, was represented by two species with apparently different ecological traits. *M. stagnalis* and *M. riemanni* are, respectively, negatively and positively related with water transparency, both relations being highly significant ($p < 0.0001$). The importance of this result is demonstrating once more that morphologically very similar species can have different ecological properties. As several ecological divergent species within the Monhysteridae are hardly morphologically discernible, a review of the Monhysteridae to clarify species' delimitations is highly warranted, at least for those few species that are frequently found in freshwater (A. Coomans, personal communication).

Only the pond Zottegem-natural was not dominated by Tobrilidae and/or Monhysteridae. The plant-parasitic nematode genus *Hirschmanniella* (associated with *Carex acuta* and *Poa trivialis*) is subdominant in this pond, merely because of the very low densities of free-living nematodes (jointly only 4 ind/10 cm^2). The genus *Hirschmanniella* is unique among plant-parasitic nematodes in inhabiting soil, freshwater and marine habitats; some species are of economic significance because of their impact on rice production. This is only the second record of this genus in Belgium (Bert et al., 2003). The species observed in this study, *Hirschmanniella loofi*, is only recorded in The Netherlands and Belgium from reed, canal banks or moist wetland.

On nematode community level, total phosphate concentration and sediment characteristics seem to be the most important variables to explain the nematode assemblages. However, resulting correlation coefficients of about 0.3 are rather low and indicates that important variables are missing. Moreover, combinations of variables did not substantially increase the correlation coefficient suggesting that several environmental variables are correlated, which makes the identification of the determining variables that contribute to the community structure intractable. As revealed by MDS ordination, nematode assemblages per pond are not structured by the assumed intensity of agricultural land use. However, we have to refrain ourselves to describe conclusive relationships between the observed nematode communities and agricultural land use, since environmental PCA analysis did not show a straightforward pattern related to land use, i.e., natural, extensive and intensive samples partially irregularly scattered on the PCA plot. Without a consistent pattern in environmental data, any land use related conclusion is highly speculative. According to Declerck et al. (2006), analyses of the non-nematode parameters on a larger scale (126 ponds covering Belgium), showed a more comprehensive land use pattern, since in their study non-nematode parameters of extensive ponds form intermediates between natural and intensive data. Hence, the situation that was assumed a priori. Possibly, the majority of ponds in current investigation are to a certain extend indirectly influenced by anthropogenic activity so that land-use gradients that are observed in the field are partially erased; e.g., all ponds, including the more natural ones, are hypereutrophic.

The total nematode density and especially the number of species was very low in this study. In general, nematode densities and number of species in freshwater systems tend to be actually lower than in terrestrial and marine environments (Traunspurger, 2002). Nonetheless, the abundance and species richness of limnetic nematodes usually ranges from 60 to *ca* 1,000 ind./10 cm^2, containing about 30–70 different species (Traunspurger, 1996a, 2002). Low diversity and dominance by single species, thus equivalent to what is observed in current study, was found in highly eutropic and dystrophic lakes (Prejs, 1977). These lakes where characterised by only a maximum of four species and the population was also dominated by either tobrilid or monhysterid nematodes. However, even lower species numbers are known from hypersaline lakes with very high conductivity or acidity (summarised in Eyualem, 2004).

Our results indicate negative relations between density and total phosphate concentration. Also, the hypereutrophic status of all ponds can be a major cause for the very low nematode density. This is in agreement with Prejs (1977) who reported the abundance in eutrophic lakes to be much lower than in oligotrophic lakes. However, reports on relationship between abundance and eutrophication remain contradictory (Eyualem, 2004). On the other hand, sediment is a wellrecognised factor that acts on nematode abundance as well as nematode diversity (Eyualem, 2004). Nematode density and diversity in limnetic and marine habitats were shown to be negatively related with the proportion of mud in the sediment (Eyualem et al., 2001; Tietjen, 1977). A muddy environment is homogenous and has fewer microhabitats for nematodes; consequently, the population is more easily dominated by few species. The presence of a mud layer in all water bodies studied, which was nearly always thicker than 10 cm (depth of the cores), could be one of the major influencing factors related to low species density and diversity. Since a mud layer was present in all studied ponds, mud was likely not demonstrably important to explain density and diversity variations among the individual ponds. The availability of oxygen, though its effect on nematode may be less than other meiofauna, is most probable another important factor negatively influencing nematode density and diversity (e.g., Prejs, 1977; Tudorancea & Zullini, 1989; Eyualem et al., 2001; Vidaković & Bogut, 2004). Oxygen concentrations could not be safely included in our statistical analysis. Yet, repeated observations of anoxic-like sediments below oxygen-poor bottom water (Table 1) indicate that the oxygen concentration is especially low in this type of ponds, which is a common condition in hypereutrophic lentic habitats. Hence, it cannot be excluded that oxygen is a critical factor related to the observed low nematode diversities and densities.

Feeding types

The investigated water bodies were dominated by deposit feeding Monhysteridae and/or chewing Tobrilidae. Deposit feeders posses no teeth and feed mainly on bacteria and unicellular eukaryotes, which are swallowed as a whole; chewers are equipped with a voluminous, sclerotised mouth cavity and their feeding-habits seems relatively complex (Beier & Traunspurger, 2003c). A singular dominance of a monhysterid or dominance in combination with a tobrilid has been reported for freshwater lakes repeatedly (e.g., Biro, 1968; Traunspurger, 1996b). Unexpectedly, volume, conductivity and pH were in current study statistically selected as the variables that best explained the proportion and density of chewers and deposit feeders. But, chewers and deposit feeders jointly dominate the total nematode composition so that their, respectively, feeding type proportions are virtually each other's complement. This necessitate density-based test to purify proportional trends, i.e., to know if environmental variables effectively act on one feeding type and not influences the reverse reaction on the complementary feeding type. Ultimately, the subset of proportional and density models only supports the positive relation of deposit feeders and pH. However, variation in pH is expected to have no direct effect on nematode abundance; but, according to Yeates (1981) indirect effects include influence on soil biota, plant growth and solubility of ions. Possibly, a more direct effect on the abundance of deposit feeders could have been caused by chl-*a*-, chloride- or sulphate concentration, given that they were shown to be positively correlated with pH.

The nearly absence of deposit feeding Diplogasteridae and Rhabditidae in this study is remarkable. In general, Diplogasteridae and Rhabditidae are common families associated with eutrophic (Bongers and Bongers, 1998) and lymnetic habitats. Several studies have indicated that deposit-feeding individuals of Monhysteridae in clean rivers are replaced by deposit-feeding Diplogasteridae and Rhabditidae in polluted systems (Zullini, 1976, 1988; Beier & Traunspurger, 2003b for an overview). However, in this case, we have no indication that the limited presence of Diplogasteridae and Rhabditidae is associated with high sediment quality of the analysed pools. Moreover, since no reference studies in these kinds of habitats are available, absence could be rather related to the type of this habitat than to the quality status of the analysed water bodies. Diplogasteridae and Rhabditidae are predominately terrestrial nematodes likely not fully adapted to non-aerated, hypereutrophic, lentic habitats.

Conclusions

This is the first attempt to delineate nematode associations in small ponds in an agricultural landscape, and to describe the potential factors influencing these nematode communities. Small farmland ponds appear to show a relatively low nematode density and an exceptionally low nematode diversity. Only some of these typical freshwater species such as tobrilids and monhysterids are able to reach substantial densities in these small aquatic spots in a terrestrial environment. The knowledge that nematode composition is herein dominated by only a few species is crucial in any further study on small water bodies.

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References

Arthington, A. H., G. W. Yeates & D. L. Conrick, 1986. Nematodes, including a new record of *Tobrilus diversipapillatus* in Australia, as potential indicators of sewage effluent polution. Australian Journal of Marine and Freshwater Research 37: 159–166.

Been, T. H. & C. H. Schomaker, 2006. Distribution Patterns and Sampling. In Perry, R. N. & M. Moens (eds), Plant Nematology. CABI Publishing, Wallingford.

Beier, S. & W. Traunspurger, 2001. The meiofauna community of two small German streams as indicators of pollution. Journal of Aquatic Ecosystem Stress and Recovery 8: 387–405.

Beier, S. & W. Traunspurger, 2003a. Temporal dynamics of meiofauna communities in two small submountain carbonate streams with different grain size. Hydrobiologia 498: 107–131.

Beier, S. & W. Traunspurger, 2003b. Seasonal distribution of free-living nematodes in the Krähenbach, a fine-grained submountain carbonate stream in southwest Germany. Nematology 5: 113–136.

Beier, S. & W. Traunspurger, 2003c. Seasonal distribution of free-living nematodes in the Körsch, a coarse-grained submountain carbonate stream in southwest Germany. Nematology 5: 481–504.

Bert, W., A. Coomans, F. Claerbout, E. Geraert & G. Borgonie, 2003. Tylenchomorpha (Nematoda: Tylenchina) in Belgium, an updated list. Nematology 5: 435–440.

Bert, W., M. Messiaen, J. Manhout, W. Houthoofd & G. Borgonie, 2006. Evolutionary loss of parasitism by nematodes? Discovery of a free-living filarioid nematode. Journal of Parasitology 92: 645–647.

Biro, K., 1968. The nematodes of Lake Balaton. II. The nematodes of the open water mud in the Kesztely Bay. Annales Instituti Biologici Tihany Hungaricae Academiae Scientiarum 35: 133–140.

Bongers, T., 1990. The Maturity Index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83: 14–19.

Bongers, T., 1999. The Maturity Index, the evolution of nematode life history traits, adaptive radiation and cp-scaling. Plant and Soil 212: 13–22.

Bongers, T. & J. Van de Haar, 1990. On the potential of basing an ecological typology of aquatic sediments on the nematode fauna: an example from the river Rhine. Hydrobiological Bulletin 24: 37–45.

Bongers, T. & M. Bongers, 1998. Functional diversity of nematodes. Applied Soil Ecology 10: 239–251.

Bongers, T. & H. Ferris, 1999. Nematode community structure as a bioindicator in environmental monitoring. Tree 14: 224–228.

Callahan, C. A., V. R. Ferris & J. M. Ferris, 1979. The ordination of aquatic nematode communities as affected by stream water quality. In Cairns, J., G. P. Patil & W. E. Waters (eds), Environmental Biomonitoring, Assessment, Prediction and Management. International Co-operative Publishing House, Maryland, 101–116.

Clarke, K. R. & M. Ainsworth, 1993. A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series 92: 205–219.

Clarke, K. R. & R. N. Gorley, 2001. PRIMER v5: User manual/tutorial. PRIMER-E. Plymouth Marine Laboratory, UK.

Caveness, F. E. & H. J. Jensen, 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Proceedings of the Helminthological Society of Washington 22: 87–89.

Cobb, N. A., 1917. Notes on nemas. Contribution to a Science of Nematology 5: 117–128.

Declerck, S., T. De Bie, D. Ercken, H. Hampel, S. Schrijvers, J. Van Wichelen, V. Gillard, R. Mandiki, B. Losson, D. Bauwens, S. Keijers, W. Vyverman, B. Goddeeris, L. De meester, L. Brendonck & K. Martens, 2006. Ecological characteristics of small farmland ponds: Associations with land use practices at multiple spatial scales. Biological Conservation 131: 523–532.

De Ley, P. & W. Bert, 2002. Video Capture and editing as a tool for the storage, distribution and illustration of morphological characters of nematodes. Journal of Nematology 34: 296–302.

De Meester, L., S. Declerck, R. Stoks, G. Louette, F. Van De Meutter, T. De Bie, E. Michels & L. Brendonck, 2005. Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. Aquatic Conservation: Marine and Freshwater Ecosystems 15: 715–725.

Eyualem, A., J. Mees & A. Coomans, 2001. Nematode communities of Lake Tana and other inland water bodies of Ethiopia. Hydrobiologia 462: 41–73.

Eyualem, A., 2004. Status quo of limno-nematology: how close are we to understanding the ecology of free-living nematodes in inland water bodies. In Cook, R. & D. J. Hunt (eds), Proceedings of the Fourth International Congress of Nematology, 8–13 June 2002, Tenerife, Spain. Nematology Monographs and Perspectives 2, Brill, Leiden.

Gerlach, S. A. & F. Riemann, 1973/1974. The Bremerhaven Checklist of Aquatic Nematodes. Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven, Supplement 4 (Heft 1 1973, Heft 2 1974).

Giere, O., 1993. Meiobenthology: The Microscopic Fauna in Aquatic Sediments. Springer-Verlag, Berlin.

Hach, 1992. Water Analysis Handbook. The Hach Company, Loveland.

Heip, C., M. Vincx & G. Vranken, 1985. The ecology of marine nematodes. Oceanography and Marine Biology: An Annual Review 23: 399–489.

Höss, S., & W. Traunspurger, 2003. Nematodes. In Markert, B. A., A. M. Breure & B. V. Zechmeister (eds), Bioindicators, Biomonitors: Principles, Concepts and Applications. Elsevier, Amsterdam, 529–554.

Johnson, J. B. & K. S. Omland, 2004. Model selection in ecology and evolution. Trends in Ecology and Evolution 19: 101–108.

Murphy, J. & J. P. Riley, 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27: 31–33.

Neher, D. A., 2001. Role of nematodes in soil health and their use as indicators. Journal of Nematology 33: 161–168.

Neter, J., M. H. Kutner, C. J. Nachtsheim & W. Wasserman, 1996. Applied Linear Statistical Models. WCB McGraw-Hill, Boston.

Ocaña, A. & J. S. Picazo, 1991. Study on nematode species encountered in the Monachil River (Granada, Spain): Response to organic pollution. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 24: 2729–2737.

Pennak, R. W., 1988. Ecology of freshwater meiofauna. In Higgins, R. P. & H. Thiel (eds), Introduction to the Study of Meiofauna. Smithsonian Institution Press, Washington DC, 39–60.

Prejs, K., 1977. The littoral and profundal benthic nematodes of lakes with different trophy. Ekologia Polska 25: 21–30.

Prejs, K., 1983. Ecological characteristics of lakes in Nort-Eastern Poland versus their trophic gradient. XI. Meiobenthos and near-bottom meiofauna in 20 lakes. Ekologia Polska 31: 477–493.

Ritz, K. & D. L. Trudgill, 1999. Utility of nematode community analysis as an integrated measure of the functional state of soils: perspectives and challenges. Plant Soil 212: 1–11.

Schiemer, F. & A. Duncan, 1974. The oxygen consumption of a freshwater benthic Nematode, *Tobrilus gracilis* (Bastian). Oecologia 15: 121–126.

Seinhorst, J. W., 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4: 67–69.

Seinhorst, J. W., 1966. Killing nematodes for taxonomic study with hot F.A. 4:1. Nematologica 12: 178.

Talling, J. F. & D. Driver, 1963. Some problems in the estimation of chlorophyll a in phytoplankton. Proceedings of the conference on Phytoplankton—primary productive measurement, Marine and Freshwater, Hawaii, 1961, T.D. 7633: 142–146.

Tietjen, J. H., 1977. Population distribution and structure of free-living nematodes of Long Island Sound. Marine Biology 43: 123–136.

Traunspurger, W., 1996a. Distribution of benthic nematodes in the littoral of an oligotrophic lake (Königssee, National Park Berchtesgaden, FRG). Archiv Für Hydrobiologie 135: 393–412.

Traunspurger, W., 1996b. Distribution of benthic nematodes in the littori-profundal of an oligotrophic lake (Königssee, National Park Berchtesgaden, FRG). Archiv Für Hydrobiologie 135: 557–575.

Traunspurger, W., 1997a. Bathymetric, seasonal and vertical distribution of feeding-types of nematodes in an oligotrophic lake. Vie et Milieu 47: 1–7.

Traunspurger, W., 1997b. Distribution, seasonal occurrence and vertical pattern of *Tobrilus gracilis* (Bastian, 1865) and *T. medius* (Schneider, 1916). Nematologica 43: 59–81.

Traunspurger, W., 2000. The biology and ecology of lotic nematodes. Freshwater Biology 44: 29–45.

Traunspurger, W., 2002. Nematoda. In Rundle, S. D., A. L. Robertson & J. M. Schmid-Araya (eds) Freshwater Meiofauna: Biology and Ecology. Backhuys Publishers, Leiden, 63–105.

Tudorancea, C., A. Zullini, 1989. Associations and distribution of benthic nematodes in the Ethiopian rift valley lakes. Hydrobiologia 179: 81–96.

Vidaković, J. & I. Bogut, 2004. Aquatic nematodes of Sakadaš lake (Kopački rit Nature Park, Croatia). Biologia Bratislava 59: 567–575.

Vranken, G., P. M. J. Herman & C. Heip, 1988. Studies of the life-history and energetics of marine and brackish-water nematodes: 1. Demography of *Monhystera disjuncta* at different temperature and feeding conditions. Oecologia 77: 296–301.

Wetzel, R. G., 1983. Limnology, 2nd ed. Saunders College Publishing, Philadelphia.

Williams, P., M. Whitfield, J. Biggs, S. Bray, G. Fox, P. Nicolet & D. Sear, 2003. Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. Biological Conservation 115: 329–341.

Yeates, G. W., 1981. Nematode populations in relation to soil environmental factors: a review. Pedobiologia 22: 312–338.

Zullini, A., 1976. Nematodes as indicators of river polution. Nematologia Mediterranea 4: 13–22.

Zullini, A. & C. Ricci, 1980. Bdelloid rotifers and nematodes in a small Italian stream. Freshwater Biology 10: 67–72.

Zullini, A., 1988. The ecology of the Lambro river. Rivista di Idrobiologia 27: 39–58.