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Nematode morphometry and biomass patterns in relation to community characteristics and environmental variables in the Mekong Delta, Vietnam

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Abstract. Nematode morphometry and biomass were investigated in the dry season of 2009 at the mouth of eight Mekong estuaries in Vietnam. We investigated how morphometry and biomass of nematodes were related to community characteristics and environmental gradients. Mean nematode lengths were significantly greater along the Co Chien estuary. In contrast, there were no significant differences in nematode width, length/width ratio, individual biomass and total biomass between stations at the estuarine mouth and along the Co Chien estuary. This study further confirmed that nematode morphometry and biomass distribution reflect specific modes of life in terms of feeding strategies, life history, and diversity. There were also particular morphotypes associated with specific environmental conditions. Over the whole area and especially along the mouth stations, nematodes were especially longer in sandy substrates where chlorophyll a concentrations were low, possibly due to the presence of predators and an associated reduction in the density of small opportunistic deposit feeders. An increase in the silt fraction together with pigment concentrations, especially along the Co Chien estuary, was positively correlated with the number, total biomass and abundances of small opportunistic genera resulting in a low maturity index and a high number of nematode genera.

Key words. Nematode morphometry; biomass; Mekong estuaries

INTRODUCTION

Morphometry and biomass are two important aspects to consider in ecological studies of free-living nematodes, which can be the dominant meiofauna in estuaries throughout the world. Biomass and size related studies have proved useful in elucidating the ecology and metabolism of nematodes (Warwick & Price, 1979), carbon partitioning within meiobenthic or nematode communities (Kennedy, 1994), ecological interpretation of size spectra, body width and the distribution of morphotypes of nematodes (Tita et al., 1999; Soetaert et al., 2002; Soetaert et al., 2009), feeding ecology (Moens & Vincx, 1997), experimental approaches using techniques of stable isotopes to unravel the feeding ecology (Moens et al., 2005, Moens et al., 2007) and the application of nematode biomass for anthropogenic impact or environmental assessment (Warwick, 1988; Clarke & Warwick, 2001; Vanaverbeke et al., 2003; Vidakovic & Bogut, 2004). However, whilst nematode biomass is very well documented elsewhere in the world (e.g., Dye & Furstenberg, 1978; Smol et al., 1994; Soetaert & Heip, 1995; Vanreusel et al., 1995; Vanreusel et al., 2000; Nozais et al., 2001, 2005; Tita et al., 2002; Boufahja et al., 2007; Leduc et al., 2010), little or no information of estuarine nematode biomass is available from Southeast Asia, including the Mekong delta.

The Mekong River is the largest river in the Southeast Asian region that forms a vast delta and complex estuarine system. The structure of nematode communities in this area showed a strong correlation with granulometry and chlorophyll a (Ngo et al, 2010; Ngo et al. 2013). However, nematode morphometry and biomass distribution patterns in relation to ecological gradients are poorly known. An understanding of such patterns can provide insights on the mode of life of dominant nematodes and nematode ecology. By relating nematode morphometry to measures of diversity, as well as their maturity index and the trophic composition on one hand and environmental conditions on the other, we can identify how particular types of nematodes are promoted in specific conditions. This in turn can help us determine which conditions favour specific feeding modes or life strategy characteristics. Therefore, the following research questions for this study were identified: 1) Do nematode morphometry and biomass distribution reflect specific modes of life or ecological interactions in terms of feeding strategies, life history and diversity of nematodes? 2) Are particular nematode morphotypes promoted in specific environmental conditions?

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MATERIAL AND METHODS

Sampling locations. Sampling was carried out during the dry season in March 2009. Nematode samples and environmental parameters were collected along the coast at the mouth of the Mekong estuarine system comprising eight stations (see Fig. 1): Cua Tieu estuary – ECT; Cua Dai estuary – ECD; Ba Lai estuary – EBL; Cung Hau estuary – ECH; Co Chien estuary – ECC; Ham Luong estuary – EHL; Dinh An estuary – EDA; Tran De estuary – ETD. Of these, seven stations (from north to south) ECT, ECD, EBL, EHL, ECH, EDA and ETD are referred to collectively as 'mouth stations' in the text.

Environmental parameters. In order to understand how environmental parameters vary in this area, physicochemical parameters in the water including temperature, pH, total dissolved solids (TDS), and dissolved oxygen (DO) were measured at each station.

Data of sediment associated environmental features such as nutrients (nitrite, nitrate, and ammonium), chlorophyll a, phaeopigment (CPE) concentrations and granulometry were also collected and analysed at the Marine Biology Section of the University of Ghent, Belgium. Coliform concentrations in the sediment were determined at the laboratory of Ho Chi Minh City Environmental Technology Center, Vietnam.

Nematode morphometry and biomass. Meiobenthos samples were collected using a corer 3.5 cm in diameter (10 cm^2 surface area) and 30 cm in length. The cores were pushed down into the sediment up to 10 cm deep. Three replicates were taken at each station and samples were collected in plastic bottles. The samples were all fixed in a 7% formalin in seawater solution at 60°C and gently stirred before transportation to the laboratory.

Samples for nematode analysis were extracted from the sediment using a 1 mm sieve and keeping the fraction retained on a 38 µm sieve. Nematodes were then separated and collected by the flotation technique using Ludox-TM50 (specific gravity of 1.18). In order to facilitate sorting of the nematodes, they were stained with 1% Rose Bengal solution. All nematodes in the samples were counted under a stereomicroscope. For each station, 200 nematode specimens were picked out randomly for making slides. These nematode specimens were measured in terms of maximum length (excluding filiform tail) and width using Leica Application Suite software integrated with a Leica microscope. Individual nematode wet biomass was calculated according to Andrassy's formula (Andrassy, 1956): Biomass (µg WW) = $L \times W^2/1{,}600{,}000$; where L is nematode length (in µm), and W is nematode width (in µm). Dry biomass was estimated to be 25% of the wet biomass (Wieser, 1960) and expressed in µg.

The nematodes were classified into four feeding categories, based on the structure of the buccal cavity according to Wieser (1953): (1A) the selective deposit-feeders are genera with a very small and unarmed buccal cavity, presumed to feed selectively on small particles including bacteria; (1B) non-selective deposit-feeders are genera with unarmed buccal cavity of moderate size, which feed less selectively so that also larger particles, such as diatoms can be ingested; (2A) epistratum (epigrowth) feeders are genera with a medium sized buccal cavity, provided with small teeth that are used to pierce food particles or to scrape them off a solid surface; (2B) predators or omnivores are genera with a wide buccal cavity and large teeth or other strong mouth structures for relatively large food items.

All nematode data encompassing length (L, in µm), width (W, in µm), ratio of length: width (L/W), mean nematode biomass (µg), total nematode biomass (µg 10 cm^{-2}) were analysed in relation to other characteristics of nematode communities, namely density, maturity index – MI (Bonger et al., 1991), percentage of feeding types, and index of trophic diversity – ITD (total square percentage of feeding types according to Heip et al., 1985). Statistical analyses of nematode variables between stations were carried out either by one-way ANOVA or non-parametric Kruskal-Wallis Rank Test.

In order to investigate the relationship between nematode size, shape and biomass with other nematode characters or environmental variables, the software STATISTICA 7.0 was used to calculate the Spearman Rank Correlation analysis (r coefficients) and multiple regressions. In the case of multiple regressions R^2 was adjusted for the number of independent variables (R^2 adj.).

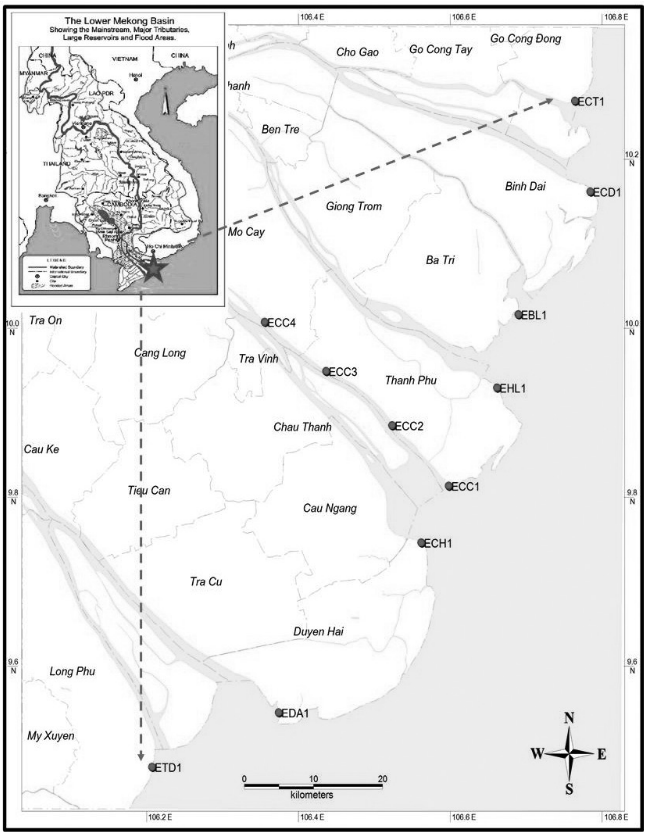


Fig. 1. Locations of sampling stations in the Co Chien estuary (ECC1 through ECC4) and at the mouth of Mekong Delta (from north to south, mouth stations ECT, ECD, EBL, EHL, ECH, EDA and ETD) in Vietnam.

Table 1. Environmental conditions at 11 sampling stations in the Mekong Delta, Vietnam. Abbreviations used: DO, dissolved oxygen; TDS, total dissolved solids; NO_3, nitrate; NO_2, nitrite; NH4, ammonium; Chla, chlorophyll a; CPE, chloroplastic pigment equivalents. n.d means not detected

Stations	DO (mg l^{-1})	pH	TDS (g l^{-1})	Coliform (MPN g^{-1})	NO_3+NO_2 (mg l^{-1})	NO_2 (mg l^{-1})	NH_4 (mg l^{-1})	Chla (µg g^{-1})	CPE (µg g^{-1})	Silt (%)	Sand (%)
ECT1	9.0	8.6	22.1	170	251.33	6.33	2749.50	0.00	0.00	0.0	100.0
ECD1	5.4	8.3	9	24000	151.50	3.67	270.67	0.16	0.19	0.0	100.0
EBL1	6.4	8.62	23.6	n.d	206.76	7.69	1134.44	0.40	0.54	0.0	100.0
EHL1	7.1	8.2	34.3	93	48.79	10.14	325.14	0.03	0.05	0.0	100.0
ECC1	5.9	8.12	8.2	330	47.20	5.00	1402.80	6.61	7.74	17.9	79.8
ECH1	6.8	8.2	42.8	45	626.00	8.17	9314.17	0.31	0.38	0.7	99.1
EDA1	6.7	8.51	40	n.d	99.60	4.80	1466.20	0.22	0.33	0.0	100.0
ETD1	5.7	7.4	35.8	270	148.50	10.67	591.83	1.73	3.67	76.1	2.9
ECC2	6.4	8.21	26.5	n.d	39.83	7.00	497.67	0.24	0.38	2.2	97.5
ECC3	6.1	7.7	17.91	270	3.00	2.83	1142.50	3.89	9.00	73.9	12.1
ECC4	5.6	7.2	7.33	340	6.00	4.33	3192.67	9.37	22.90	76.0	8.4

Apart from linear regression, other models of regression such as exponential or logarithmic models were also applied to explain the variation in nematode morphometry.

RESULTS

Environmental variables at the mouth stations and along the estuary ECC. The environmental variables measured are shown in Table 1. At the mouth of the estuarine systems, high values of dissolved oxygen and pH were observed. High coliform concentrations were measured at station ECD1.

In the sediment, chlorophyll and total chloro-pigment concentrations were relatively high along the estuary ECC compared to the mouth stations except at station ECC2 where values were less than 1µg g^{-1} (Fig. 2). Ammonium, nitrite and nitrate concentrations showed high values at ECH1 (Fig. 3).

Those stations had a high percentage of sand in the sediment except for station ETD1 and along the estuary ECC1.

Morphometry and biomass of nematode assemblages. The biomass of nematodes depends on the shape of the nematodes since it was calculated based on their length and width. In the samples, major genera in the mouth stations were *Desmodora*, *Halalaimus*, *Oncholaimellus*, *Daptonema*, *Thalassomonhystera*, *Theristus*, *Parodontophora*, *Paracanthonchus*, and *Omicronema*. Densities ranged between 1118 indiv. $10cm^{-2}$ and 6175 indiv. $10cm^{-2}$. The length of nematodes at the eight stations of the Mekong estuarine system ranged from 117.0 to 4302.3 µm but their arithmetic mean per replicate sample ranged from 538.4 to 1067.2 µm. Nematode widths ranged from 5.2 to 165.6 µm and their arithmetic mean varied from 18.9 to 39.6 µm.

Nematode length along the Co Chien estuary was shorter than those in mouth stations. The most abundant genera were *Daptonema*, *Terschellingia*, *Dichromadora*, *Halalaimus*, *Parodontophora*, *Theristus*, *Thalassomonhystera*, and *Desmodora* with densities ranging from 1033 indiv. $10cm^{-2}$ to 3568 indiv. $10cm^{-2}$. Their lengths ranged from 166.1 to

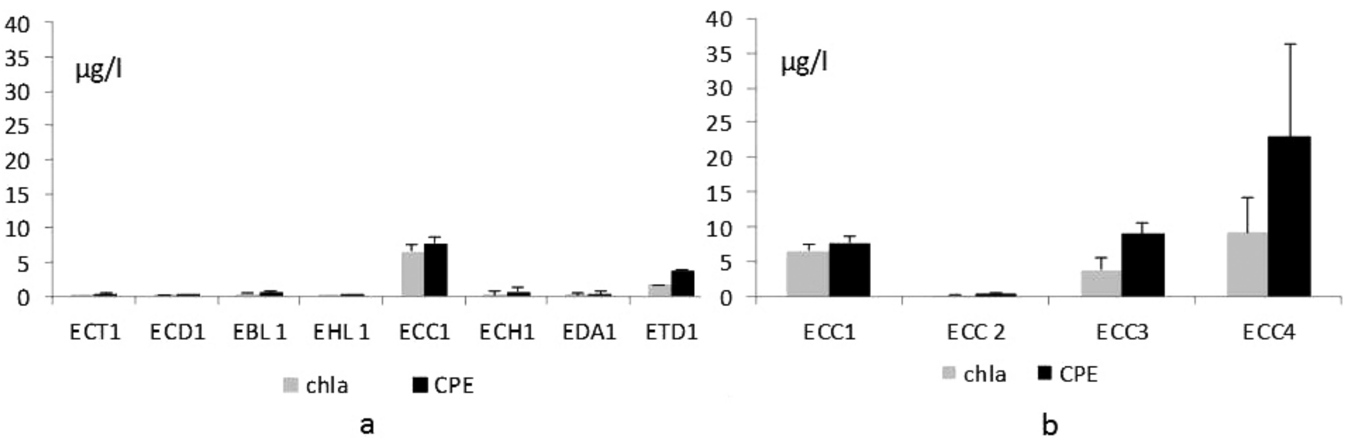


Fig. 2. Chloroplastic Pigment Equivalents (mean CPE±SD; µg L^{-1}) and chlorophyll a (mean±SD; µg L^{-1}) at the mouth stations (a) and along the Co Chien estuary (b).

3857.1 µm and their means varied from 538.4 to 989.9 µm per replicate sample. Nematode width ranged from 7.1 to 159 µm, and their means from 19 to 28.6 µm. A plot of nematode width against length of all measured nematodes in the study area is shown in Fig. 4.

The ratio of nematode length and width (L/W ratio) at the mouth stations ranged from 4.5 to 149.3 (mean from: 20.6 to 48.6; Fig. 5b). Their frequency distribution over all stations at the river mouth is shown in Fig. 5a. The L/W ratios at the Co Chien estuary varied from 3.9 to 183.6 (mean from: 20.6 to 48.6; Fig. 5b). Individual nematode biomass ranged between 0.041 and 0.167 µg and total biomass ranged from 9.08 to 706.3 µg $10cm^{-2}$.

Nematode lengths were significantly different between mouth stations (ANOVA test: $F_{(16, 7)} = 14.13$, $p < 0.01$). There was also a significant difference in nematode widths (log transformed) between sampling localities. Pairwise comparisons indicated that only nematodes from station ECD1 differed from those at ECT1, EHL1, ECH1, and EDA1 ($F_{(16, 7)} = 5.38$, $p < 0.01$). The log transformed total biomass also differed significantly between stations, with nematodes from ECT1 differing from all remaining stations.

Significant differences were also found for L/W ratio between ECC1 and EHL1 and ECH1, as suggested by Kruskal-Wallis rank test ($H_{(7, n=24)} = 20.68$, $p < 0.01$). The individual biomass was not different between mouth stations (p = 0.1, Kruskal-Wallis ANOVA by rank test).

For stations along the Co Chien estuary (ECC stations), nematode lengths and L/W ratios showed significant differences between station ECC2 and remaining stations when tested by one-way ANOVA followed by a post hoc pairwise comparison test (HSD) (length: $F_{(8,3)} = 15.21$, $p = 0.001$; ratio L/W: $F_{(8,3)} = 18.54$, $p < 0.01$). The log-transformed total biomass of nematodes was significantly different between ECC2 and ECC3, ECC4; ECC1 and ECC3 ($F_{(8,3)} = 18.61$, $p < 0.01$). There was no significant difference between stations for nematode width and individual nematode biomass ($p_w = 0.07$, $p_{ind.bio.} = 0.118$, Kruskal-Wallis rank test).

Mean nematode lengths at all mouth stations were significantly higher than at stations along the ECC estuary (ANOVA analysis with log (x+1) transformed data: $F_{(34,1)} = 4.42$, $p = 0.042$). There were no significant differences between mouth stations and estuarine ECC stations in nematode width, L/W ratio, individual biomass and total biomass (Fig. 6).

Correlation between nematode morphometric data and other characteristics of nematode communities. Correlation coefficients based on nematode size and biomasses with other nematode community characters are shown in Table 2. Fig 7a shows graphs of Nematode length was significantly negatively correlated with genera richness (S) but positively correlated with the maturity index (MI).

In addition, nematode lengths also showed a significant negative correlation with percentage of feeding type 1A and a positive correlation with 2B (Fig. 7b). Nematode widths showed a significant positive correlation with the percentage of feeding type 2A (Fig. 7c). The ratio between nematode lengths and widths were also significantly correlated with genera richness (negative), maturity index (positive) and feeding types 2A (negative) and 2B (positive) (Fig. 7d). Individual and total biomass were also positively and significantly correlated with nematode densities (N). The polynomial regression of % 2B with nematode length was strong (70% of the variation explained). Table 3 shows the results of significance tests for these regressions.

At stations near the river mouth, the correlation pattern between nematode morphometric data with other nematode aspects was similar to the results for the whole area.

Along the estuary ECC, the length of nematodes was negatively correlated with the percentage of feeding 1B ($r = -0.82$, $p = 0.002$) but positive correlated with the feeding type 2B ($r = 0.7$, $p = 0.01$). The ratio L/W showed a significant negative correlation with ITD ($r = -0.6$, $p = 0.037$) and percentage of feeding type 1B ($r = -0.83$, $p = 0.001$) whereas it was positive correlated with 2B ($r = 0.74$, $p = 0.006$). The widths of nematodes were not significantly correlated with any measured data of nematodes. Nematode densities had a significant positive correlation with both individual

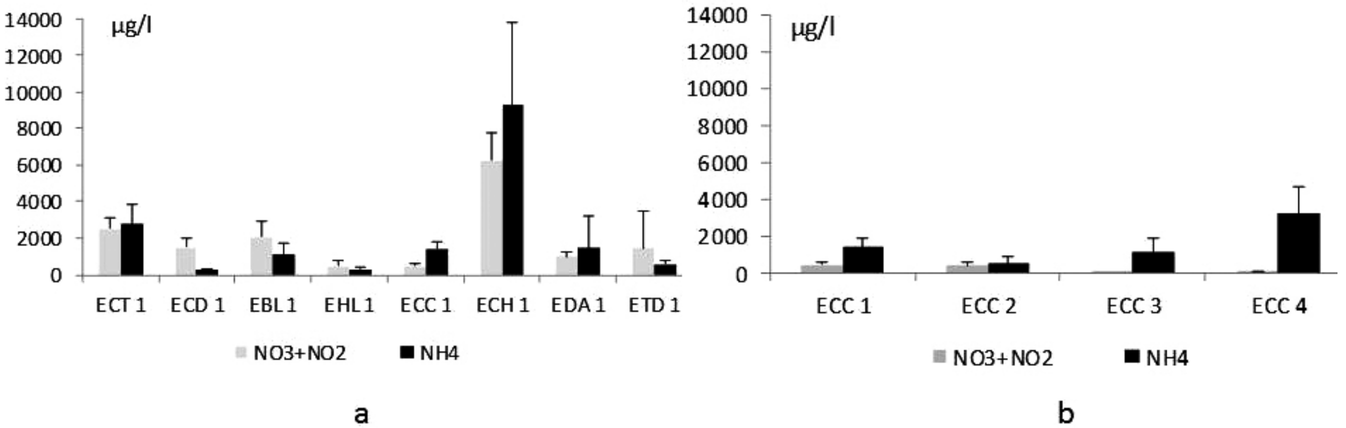


Fig. 3. Nitrite and nitrate concentrations (mean±SD; raw data multiplied by 10) and ammonium concentrations across a vertical sediment profile at the mouth of the Mekong delta (a), and along the Co Chien estuary (b).

Table 2. Correlations between nematode morphometric data and other nematode community characteristics (n= 24). S, genera richness; N, densities; MI, Maturity Index; %1A, %1B, %2A, %2B, percentage of feeding types in nematode communities: the selective deposit-feeders with a very small and unarmed buccal cavity, presumed to feed selectively on small particles including bacteria (1A); non-selective deposit-feeders with unarmed buccal cavity of moderate size, which feed less selectively so that also larger particles, such as diatoms can be ingested (1B); epistratum (epigrowth) feeders with a medium sized buccal cavity, provided with small teeth that are used to pierce food particles or to scrape them off a solid surface (2A); predators or omnivores with a wide buccal cavity and large teeth or other strong mouth structures for relatively large food items (2B).

Morphometric Variables		S	N	MI	%1A	%1B	%2A	%2B
Mean Length	r	−0.6537	−0.3402	0.6079	−0.5150	−0.3874	−0.1823	0.7997
	p	0.001	0.104	0.002	0.010	0.061	0.394	0.000
Mean Width	r	0.0981	0.1087	−0.3299	−0.2648	0.1708	0.7154	−0.2702
	p	0.648	0.613	0.115	0.211	0.425	0.000	0.202
Length/Width ratio	r	−0.6523	−0.3168	0.7341	−0.3015	−0.4631	−0.5501	0.8620
	p	0.001	0.131	0.000	0.152	0.023	0.005	0.000
Individual biomass	r	0.0417	0.4851	−0.0567	0.1272	−0.0816	0.2914	−0.2283
	p	0.847	0.016	0.792	0.554	0.705	0.167	0.283
Total biomass/10cm^2	r	0.0928	0.7270	−0.1047	0.3592	−0.0476	0.1429	−0.3729
	p	0.666	0.000	0.626	0.085	0.825	0.505	0.073

Table 3. Regression coefficients and their statistical significance between genera richness (S), maturity index (MI), percentage occurrence of feeding types 1A (%1A), 2A (%2A), 2B (%2B) and nematode lengths (L), length/width ratio (L/W). Models are shown in Fig. 8.

Regression test	R^2	F	df	p
S & L	0.294	12.900	1,31	0.0011
MI & L	0.246	11.489	1,31	0.0019
%1A & L	0.178	6.720	1,31	0.0144
%2A & W	0.163	6.070	1,31	0.0195
%2B & L	0.705	35.930	1,1,30	<0.000001
%2B & L/W	0.533	35.488	1,31	<0.000001

biomass ($r = 0.63$, $p = 0.028$) and total biomass ($r = 0.91$, $p < 0.001$). Fig. 8 shows the best described functions for feeding types 1B and 2B based on length and for total and individual biomass based on densities N.

Correlation between nematode morphometric data and environmental variables. Nematode lengths showed a significant correlation with a number of environmental variables as shown in Table 5. In particular, there was a strong positive correlation with chlorophyll a and concentrations NO_3+NO_2. There was also a significant though weak correlation with % silt (negative) and sand (positive).

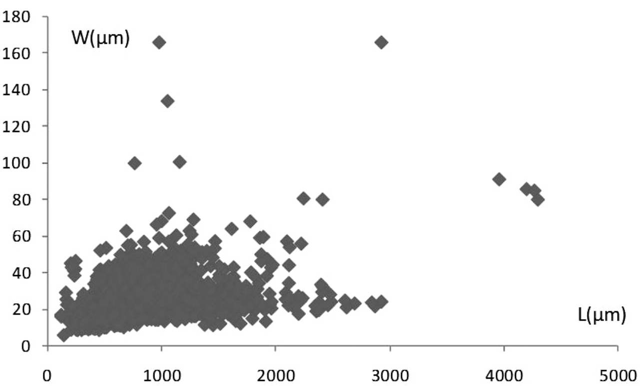


Fig. 4. Nematode length (L) and width (W) at all mouth stations and along the Co Chien river estuary.

Nematode widths were significantly correlated with coliform and NO_2 concentrations.

The ratio L/W was also significantly correlated with a number of environmental variables as shown in Table 5. Strong positive correlations with TDS, NO_3+NO_2, and NO_2 concentrations were observed. The ratio L/W was also negatively correlated with the percentage of silt and the chlorophyll a concentration (Table 4).

Individual nematode biomass was not significant correlated with environmental variables except for dissolved oxygen. Biomass decreased when dissolved oxygen increased significantly ($r = -0.51$, $p = 0.012$). Similar to the case of individual biomass, total nematode biomass was significantly negatively correlated with dissolved oxygen and also with pH and percentage of sand. Total biomass increased significantly with percentage of silt (Tables 4, 5).

Considering the mouth stations separately, the nematode morphometric data showed a similar pattern in correlation with several environmental variables as for the whole area. The strongest significant correlations are found between length and pigments ($r = -0.73$, $p = 0.001$), width and coliform concentrations ($r = 0.81$, $p < 0.001$) and L/W and TDS ($r = 0.82$, $p < 0.001$). The main difference is that percentage of silt and sand were not correlated with nematode length.

Table 4. The significant regression of percentage of feeding type 1B (%1B), 2B (%2B) with nematode lengths and total biomass (Total bio.), individual biomass (Individual bio.) and densities (N) of nematode communities along the estuary ECC. Significant regressions of nematode lengths (L) and chlorophyll a concentration (Chl.a); length – width ratio (L/W) and TDS; total biomass (Total bio.) and percentage of silt in the sediment (% silt) were detected in whole area.

Regression test	R^2	F	df	p
%1B & L	0.672	20.49	1, 10	0.0011
%2B & L	0.55	5.5	1, 1, 9	0.027
Total bio. & N	0.825	47.32	1, 10	0.00004
Individual bio. &N	0.63	7.688	1, 1, 9	0.011
L & Chl. a	0.622	46.1	1, 28	<0.000001
L/W & TDS	0.418	22.29	1, 31	0.000048
Total bio. & % silt	0.296	13	1, 31	0.00106

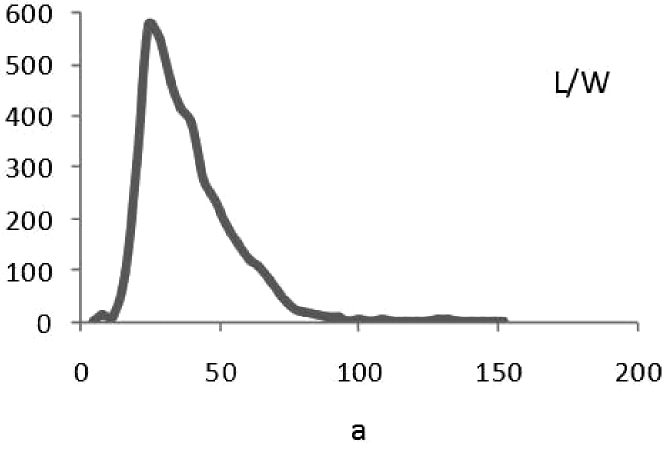
Along the estuary ECC, the lengths, widths and ratio L/W of nematode were not correlated with any environmental variable. Individual biomass showed significant correlations with variables TDS ($r = 0.69$, $p = 0.038$), coliform ($r = -0.68$, $p = 0.042$) and NO_2 ($r = -0.697$, $p = 0.037$). Similar to individual biomass, total biomass of nematode communities in this estuary was significantly correlated with the variables TDS ($r = 0.81$, $p = 0.008$), coliform ($r = -0.79$, $p = 0.01$) and NO_2 ($r = -0.86$, $p = 0.003$).

DISCUSSION

Nematode morphometry in the Mekong estuaries compared with other study areas. Our study showed that nematode length and width in the Mekong estuaries varied greatly. The length of nematodes was generally more than 100 µm but less than 3000 µm. Only a few nematodes were more than 3000 µm long. This range in nematode lengths was smaller than those observed by Romeyn and Bouwman (1983) in the Ems-Dollard estuary (England) who reported that the lengths of estuarine nematodes varied between 500 and 5000 µm. However, our results overlapped with the range of nematodes observed in the deep sea and ocean margins (Soetaert et al., 2002; Soetaert et al., 2009) where nematodes were up to 5000 µm long. Mean nematode lengths at the mouth station were significantly higher than those along the estuary ECC. This may relate to the sediment features at those stations. The mouth stations were characterised by a sandy habitat in general while along the Co Chien estuary (CC stations) the sediment showed a higher silt fraction. The ECC stations also all showed higher chlorophyll a concentrations. These results support earlier conclusions of Warwick (1971) and Heip et al (1985) that nematodes in sandy sediments are longer in length.

The nematode widths are mainly less than 70 µm and greater than 5 µm. Mean nematode widths along the estuary ECC were not different from nematodes at the mouth stations. However, these results are in contrast to those of Tita et al., (1999) who studied nematode assemblages in the St Lawrence Estuary, Quebec, Canada and reported that nematode widths ranged from 22.6 to 32.0 µm.

The length:width (L/W) ratio of nematodes ranged in value between 20 and 70. The frequency distribution of the L/W ratio showed that the peak L/W ratio along the estuary ECC was lower than at the river mouth stations. According to Schratzberger et al. (2007), the L/W ratio is a measure of a nematode's body shape with long/thin animals having high ratios, and stout animals' low ratios. In addition, Soetaert et al. (2002), Vanaverbeke et al. (2004) and Schratzberger et al. (2007) suggested that three morphologies have developed over evolutionary time-scales: stout, slender and long/thin. Slender and long/thin nematodes are able to move swiftly through the sediment but the stout morphotype in contrast may have evolved towards reducing the predation pressure



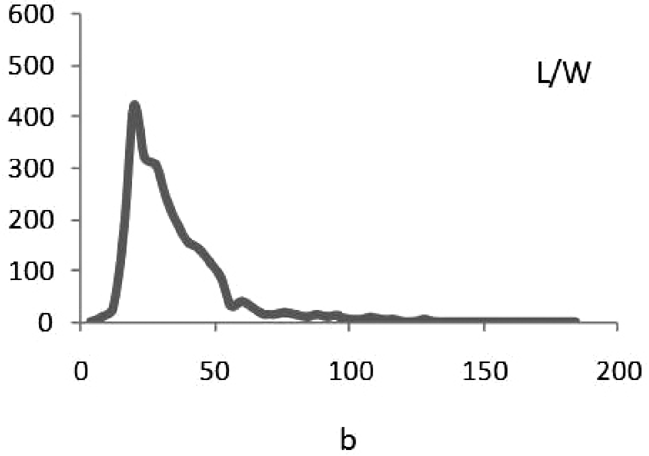


Fig. 5. Frequency distribution of L/W ratios at the mouth stations (a) and Co Chien estuary (b).

Table 5. Correlation coefficients of nematode morphometric data with environmental variables in the Mekong estuaries (n=24).

Morphometric Variables		DO (mg l^{-1})	pH	TDS (g l^{-1})	Coliform (MPN g^{-1})	NO_3+NO_2 (mg l^{-1})	NO_2 (mg l^{-1})	NH_4 (mg l^{-1})	chla (µg g^{-1})	CPE (µg g^{-1})	Silt (%)	Sand (%)
Mean Length	R	0.2300	0.3998	0.6424	0.1726	0.6118	0.4685	0.4208	−0.6682	−0.5665	−0.5631	0.5510
	P	0.280	0.053	0.001	0.420	0.001	0.021	0.041	0.000	0.004	0.004	0.005
Mean Width	R	−0.4416	0.1209	−0.3891	0.7299	−0.1698	−0.4565	−0.3144	−0.1019	−0.1265	−0.1029	0.1096
	P	0.031	0.574	0.060	0.000	0.428	0.025	0.135	0.636	0.556	0.632	0.610
Length/Width ratio	R	0.4119	0.2814	0.7794	−0.2105	0.6457	0.6476	0.5507	−0.5442	−0.4367	−0.4393	0.4250
	P	0.046	0.183	0.000	0.323	0.001	0.001	0.005	0.006	0.033	0.032	0.038
Mean biomass/ind	R	−0.4909	−0.2571	−0.0147	0.4040	−0.1320	−0.1248	−0.2676	−0.1245	−0.0624	0.2971	−0.3032
	P	0.015	0.225	0.946	0.050	0.539	0.561	0.206	0.562	0.772	0.159	0.150
Total biomass/10cm^2	R	−0.5063	−0.4216	−0.0832	0.2786	−0.2430	−0.1784	−0.3151	0.0266	0.1021	0.5060	−0.5110
	P	0.012	0.040	0.699	0.187	0.253	0.404	0.134	0.902	0.635	0.012	0.011

on these small individuals but these adaptations undoubtedly bring along reduced mobility. Schratzberger et al. (2007) classified nematode shapes to three shape categories based on the following criteria: stout with a L/W ratio < 18, slender with a L/W ratio of 18–72 and long/thin with a L/W ratio > 72. In the case of the Mekong estuaries, we see that 16.2% of the nematodes have a stout shape, 3.6% have a long/thin shape and more than 80.2% of the nematodes have a slender shape. These percentages showed differences between the estuary ECC and the river mouth stations. At these stations, the percentage of slender nematodes was 86.9%, a value that was much higher than along the estuary ECC with only 66.7% but the percentage of stout nematodes along the estuary ECC (26.3%) was higher than in the mouth stations (10.3%). The long and thin nematodes were present in low percentages at all stations: 2.8% at the mouth stations and 5.0% along the ECC estuary.

Schratzberger et al. (2007) recorded that the majority of nematodes were slender (82%) while stout animals accounted for 6% and long/thin animals for 12% of the nematode communities in the southwestern subtidal North Sea. These percentages were explained by the fact that most of the sediments consisted of moderately to poorly sorted muddy sands. The percentage of slender nematodes was not much different from the Mekong estuarine nematodes but the percentage of stout nematodes was lower while the percentage of long/thin nematodes was higher. The proportion of slender nematodes is lower while the stout nematodes increase in relative abundance when the silt fraction is higher.

The individual biomass of the Mekong estuarine nematodes expressed in dry weight ranges from 0.041 to 0.167 µg. This range overlapped with the ranges for individual dry weight of nematodes in the deep sea reported by Soetaert et al. (2002) which measured from 0.038 to 0.205 µg, by Soetaert et al. (2009) which measured from 0.067 to 0.18 µg, and from a tropical lagoon reported by Boufahja et al. (2007) which measured between 0.0525 and 0.2625 µg. But they were lower than the individual biomass in a temperate estuary reported by Smol et al. (1994) (0.38 ± 0.02 to 0.46 ± 0.02 µg). It seems that nematodes in the Mekong estuaries were characterised to have a similar weight and size compared to nematodes in other habitats, although they are lower than in the Oosterschelde estuary, Belgium.

However, the total biomass of Mekong nematodes showed rather high values from 9.08 to 706.3 µg dwt 10 cm^{-2} in comparison with some other studies. Dye and Furstemberg (1978) in their study on the Swartskop estuary, South Africa found values for total biomass ranging from 0.1–0.4 µg 10cm^{-2}. Van Damme et al. (1980) showed values from 0.03–4.58 µg 10cm^{-2} in the Western Scheldt estuary. Tita et al. (2002) showed mean total nematode biomass values in five intertidal estuarine assemblages ranging between 96±14 and 248±86 µg 10 cm^{-2}. They were also considerably higher than the total biomass in the deep sea reported by Vanreusel et al. (1995) from 2.13–13.54 µg 10 cm^{-2} (8.52–54.16 µg wwt 10cm^{-2}) and Vanreusel et al. (2000) from <1 to 48 µg 10 cm^{-2}. Nevertheless, our results are still low compared

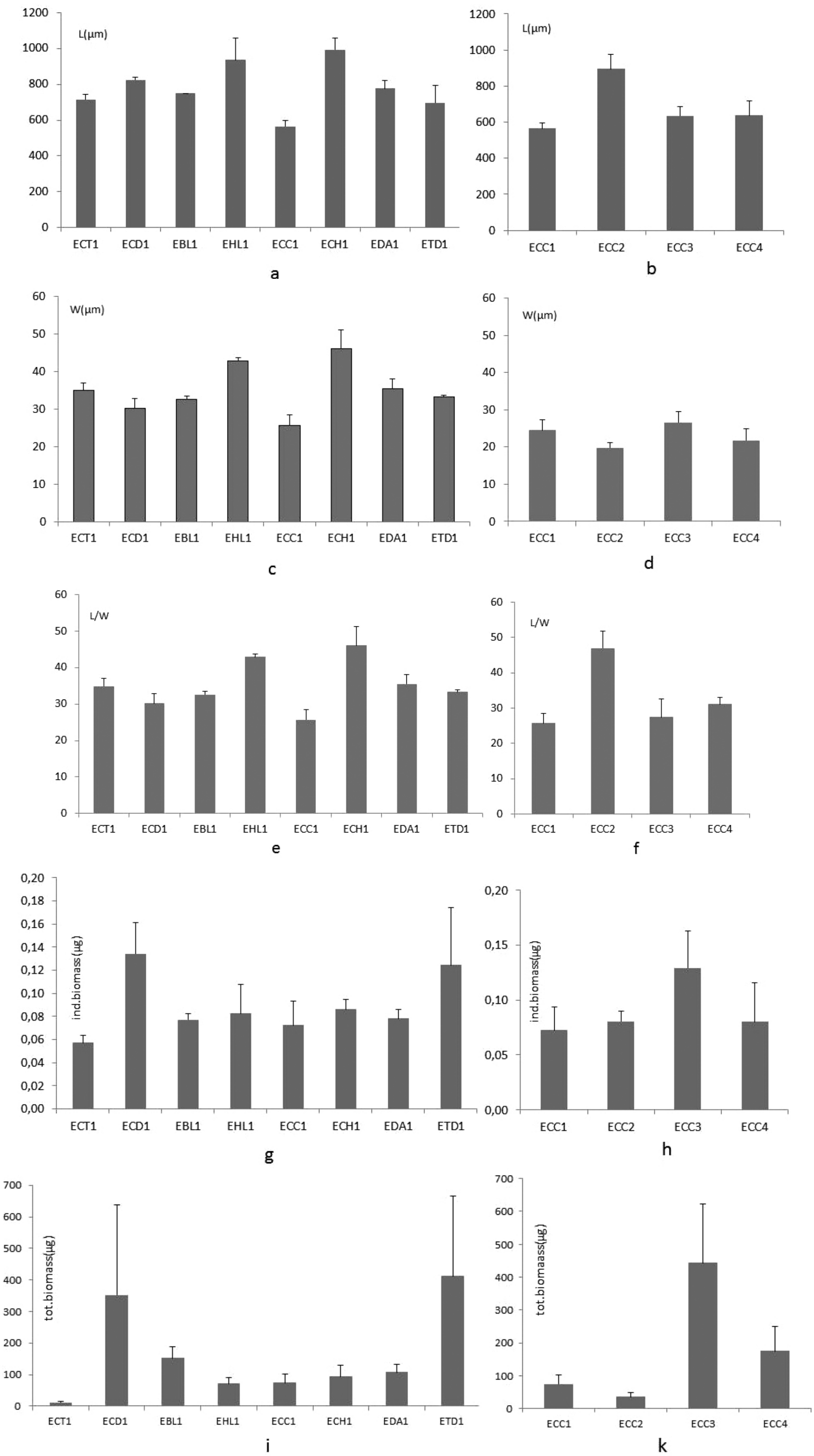


Fig. 6. Variation in the morphology of nematodes in the Mekong Delta, Vietnam. mean (± SD) nematode length in the Mekong Delta (a); mean (± SD) nematode length along the Co Chien estuary (b); mean (± SD) nematode width in the Mekong Delta (c); mean (± SD) nematode width along the Co Chien estuary (d); ratio L/W (the mouth stations [e], along estuary ECC [f]), individual biomass (µg) (the mouth stations [g], along estuary ECC [h]) and total biomass (µg 10 cm−2) (the mouth stations [i], along estuary ECC [k]).

to a study from the Oosterschelde, an intertidal estuary by Smol et al. (1994) who showed nematode total biomass values ranged from 49 to 7044 μg 10 cm^{-2}.

Do nematode morphometry and biomass distribution reflect specific modes of life? There were strong relationships between nematode morphometry and other characteristics of the nematode communities representing specific modes of

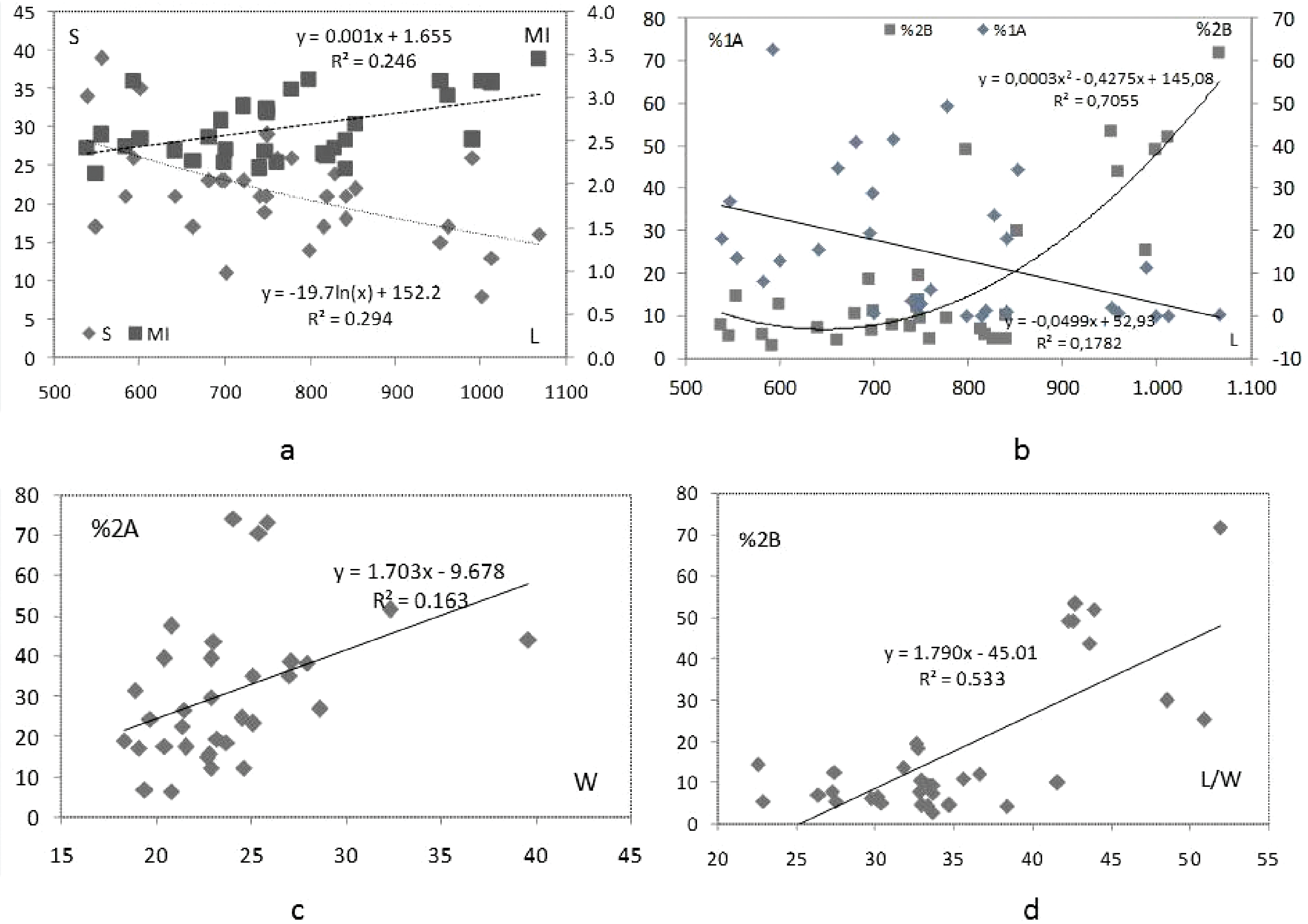


Fig. 7. Regression functions between the length and ratio L/W with other characters (maturity index, feeding types) of nematodes at all stations. length L and genera richness S, maturity index MI (a); length L and percentage % of feeding type 1A and 2B (b); nematode width W with percentage of feeding type 2A (c); ratio L/W with the percentage of feeding type 2B (d).

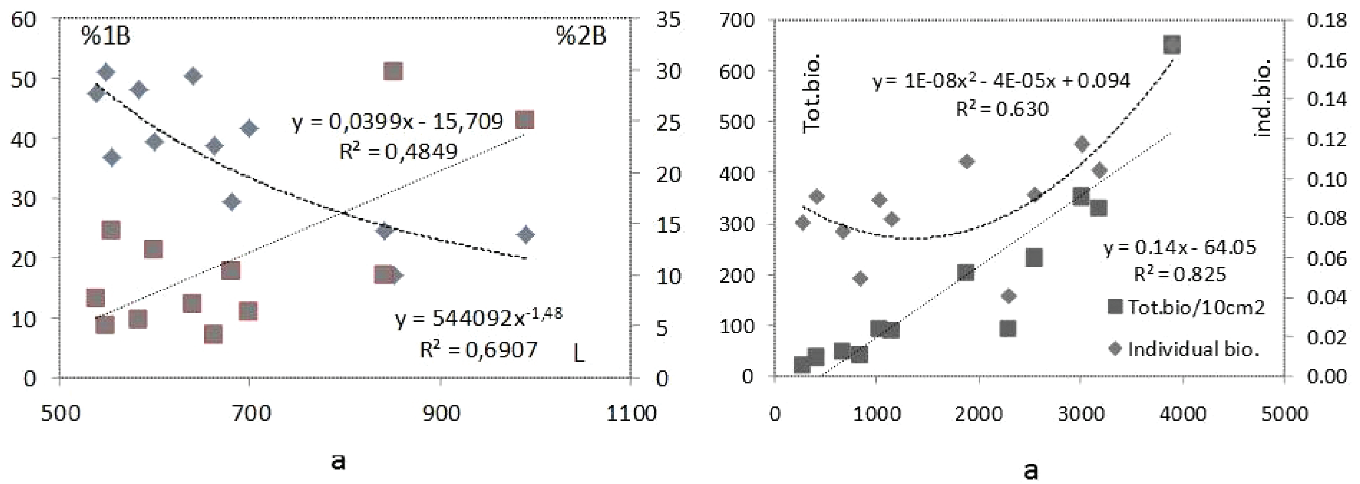


Fig. 8. Regression model functions between nematode length and feeding types/biomass along the Mekong estuary ECC nematode length L and percentage of feeding types 1B and 2B (a); nematode densities of individual biomass and total biomass (b).

life in this study. Our results showed that nematode lengths were correlated with genus richness, the maturity index, and the percentages of feeding types 1A and 2B. The relationship between average lengths on one hand and species richness and maturity index on the other was opposite. Species richness decrease when average length increase and maturity index increase, so it is suggested that smaller nematodes were more important in diverse communities with a lower maturity index. These smaller nematodes represented mainly the feeding types of non-selective feeders (1A) while longer nematodes were more representative of the 2B group of predators/omnivores. According to Bongers (1999) and Ferris & Bongers (2006), there is presumably a relation between body length and generation time. Large nematodes have a longer generation and a higher "coloniser – persister" value. Under stressed conditions, body size reduces and there is a shift towards opportunists. In the opposite case, when the average nematode lengths increase, they can lead to a higher maturity index and indicate the success of more persisting nematodes. The opposite trends that the two feeding types showed with length points to the fact that nematodes that are feeding on deposits and bacteria (group 1A) have mostly shorter bodies and a more opportunistic behavior than nematodes that feed as predators or omnivores (group 2B) which are big and rather invest in growth than in reproduction. The correlation between length and genus richness only holds within the group of mouth stations, not along the estuary, and suggests that in case of a decrease in average length (and thus more opportunistic deposit feeders) there are more genera present. These obervations appear controversial since so called opportunistic species tend to dominate and therefore reduce the diversity. However, stations belonging to the silty habitat (like station ECC1 and ETD1) showed the highest densities and diversity but not the highest maturity index due to the proliferation of different species of Monhysterida. Nematode widths had a strong correlation with the percentage of epistratum feeding nematodes. It seems that this feeding group including the dominant genus *Desmodora* and less dominant genera such as *Dichromadora*, *Neochromadora*, *Ptycholaimellus*, *Spilophorella*, *Paracanthonchus* which are characterised by large body widths.

In addition, the L/W ratios followed the same trend as observed for the length and width separately. The correlations as found for the mouth stations also apply over the whole area but they are somehow different along the estuary ECC. Length and L/W ratios are positively correlated with the predators/omnivores but there was also a negative correlation with the non-selective deposit feeders (1B), instead of the selective deposit feeders (1A). In this view, we can assume that the predators/omnivores tend to have slender or longer bodies while non-selective or selective deposit feeders tend to be shorter. This may be a special adaptation by developing in opposite ways to maintain the natural predator-prey interaction. The predators have longer bodies to move faster and to prey better on smaller nematodes. According to Soetaert et al. (2002), the longer and more mobile nematodes penetrate much deeper into the sediment than their plump and presumably rather immobile relatives. The vulnerability of individuals to a predator attack is often inversely related to the size of the animals (Peters 1983; Soetaert et al., 2002). In addition, Soetaert et al. (2002) also mentioned that predators must have a larger mouth cavity to swallow a fat nematode compared to a thin nematode and therefore prey nematodes with thicker bodies will survive better because when predators attack the head, tail or from the side, thick nematodes may not break as easily as very thin ones.

However, our results were in contrast to the study of Tita et al. (1999) at three intertidal assemblages of the St Lawrence Estuary, Quebec, Canada. The authors found nematode morphotypes (body width/body length ratio = W/L ratio) associated with feeding groups. Small W/L ratios mean high L/W ratio was typical for microvores, while greater W/L ratios were typical for epigrowth feeders and predators.

The nematode individual and total biomass did not have any correlation with feeding types but they both had a positive relationship with total densities. This kind of relationship enforces each other since nematodes become bigger when densities increase. The correlation between length and L/W ratio with the maturity index and species richness was not significant along the estuary, in contrast to the mouth stations. It seems that the correlations between nematode community characteristics were different between mouth stations and along the estuary ECC, likely because different environmental gradients were present in both groups, influencing the nematode community structure in different ways.

Are there shifts in nematode morphometry associated with specific environmental conditions? The nematode morphometry and biomass in the Mekong estuaries were correlated with several environmental factors over the whole study area and river mouth separately. Our results showed that nematode lengths and L/W ratios have strong positive relationships with total dissolved solids in the water and NO_3^- $+NO_2^-$ concentrations in the sediment. The nematode lengths further showed a positive correlation with percentage of sand and a negative correlation with silt. Also Heip et al. (1985) and Soetaert et al. (2009) both mentioned that nematode lengths were affected by grain size, with longer nematodes associated with coarser grain size. This also explains why nematode lengths at the mouth stations are larger than at stations along the estuary ECC. Warwick (1971) also showed that nematodes in muddy habitats have small sizes with short setae but in sandy habitats they were often longer and represented by large predators and epistratum feeders.

Chlorophyll a and total pigment concentration showed a negative correlation with lengths and L/W ratios. This can be easy understood when we link this with the increase in small, occasionally stout deposit feeders (when averaged length or L/W ratio's decreases), since they mainly feed on detritus or fresh micro-algae. The L/W was further strongly positive correlated with TDS and to a lesser extent with nitrite + nitrate, and ammonium concentrations. This means that nematodes become thinner and/or longer when those environmental factors increase. Factors such as TDS, nitrite, nitrate and ammonium were all considered as disadvantageous for aquatic animals. A number of studies have indicated that the various aquatic species' reactions range from intolerance to outright toxicity when TDS increase. However, most aquatic ecosystems involving mixed fish fauna can tolerate TDS levels of 1000 mg l^{-1} (Boyd, 1999, Masters & Ela, 2007). High nitrite, nitrate, and ammonium concentrations were also found to have a negative effect on aquatic invertebrates and other animals (Beketov, 2004; Camargo et al., 2005; Tilak et al., 2007). When these chemical substrates increase in the water and sediment they may generate stress full conditions for nematodes. Why being longer gives advantages in these conditions remains however unclear.

The nematode widths had a strong positive correlation with coliform concentrations. Nematodes had stouter bodies where coliform concentrations were higher. They also showed negative correlation with nitrite. Although nitrite is present as a transition phase in the water and sediment but there are some disadvantage effects of nitrite to aquatic animals (Margiocco et al., 1983; Jensen, 2003). A research of Tita et al. (1999) found that silt-clay proportions were also an influential factor in determining the mean nematode body width.

Moreover, nematode widths were also negatively correlated with dissolved oxygen. Nematodes had been reported to inhabit in muds apparently devoid of oxygen (Atkinson, 1973) but in this case they seem to have thinner bodies in the condition of high oxygen. Rogers (1962) and Atkinson (1973) mentioned that the major factors influencing the nematode's ability to obtain oxygen by diffusion are the environmental oxygen tension and its body size—especially its body radius. Jensen (1987) who studied nematodes living in sandy bottoms in the northern part of Oresund, Denmark noted that thiobiotic (deeper living) species are significant slender than oxybiotic species.

Individual biomass and total biomass both showed a negative correlation with dissolved oxygen. It seems that nematodes are well adapted to conditions of low oxygen. Atkinson (1973) performed an experiment with two species *Enoplus brevis* and *E. communis* and found clearly that the rate of oxygen consumption all of those nematodes were influenced by oxygen tension. Nematodes with a higher dry weight required lower oxygen consumptions since the oxygen consumption per unit weight decreased with increasing body size. Moreover, total biomass showed a negative correlation with percentage of sand. Total biomasses of nematodes increased when percentage of sand decreases or increase silt in the sediment. This was contrary to individual biomass that increase when sand increase due to large size. This can be explained by high densities of nematode communities in the silty sediment. These observations were in contrast with the results of Tita et al. (1999) based on three intertidal nematode assemblages in the St. Lawrence estuary, Canada where the mean nematode individual biomass in sandy sediments was smaller than in muddy sediments.

CONCLUSIONS

Nematodes in the Mekong estuary were characterised by medium sizes in both length and width. Individual biomass was low in general but total biomass was relatively high comparing to other studies in the discussion. Over the whole area and especially at the river mouth, nematodes were longer in sandy substrates where chlorophyll concentrations were low. This could be due to increased predation and ac concomitant decrease in small opportunistic deposit feeders. When silt fraction with pigment concentrations increased the number and abundance of small opportunistic genera increases resulting in a lower maturity index and a higher number of genera.

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LITERATURE CITED

Andrassy I (1956) The determination of volume and weight of nematodes. Acta Zoologica, 2: 1–15.

Atkinson HJ (1973) The influence of oxygen tension and body size. The respiratory physiology of the marine nematodes *Enoplus brevis* (Bastian) and *E. communis* (Bastian). Journal of Experimental Biology, 59: 355–266

Beketov MA (2004) Different sensitivity of mayflies (Insecta, Ephemeroptera) to ammonia, nitrite and nitrate: linkage between experimental and observational data. Hydrobiologia, 528: 209–216

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, Alkemade R & Yeates GW (1991) Interpretation of disturbance-induced maturity decrease in marine nematode assemblages by means of the Maturity Index. Marine Ecology Progress Series, 76: 135–142.

Boufahja F, Beyrem H, Essid N, Amorri J, Mahmoudi E & Aïssa P (2007) Morphometry, energetics and diversity of free-living nematodes from coasts of Bizerte lagoon Tunisia: an ecological meaning. Cahiers de Biologie Marine, 48: 121–137.

Boyd CE (1999) Water Quality: An Introduction. Kluwer Academic Publishers Group, The Netherlands, 330 pp.

Camargo JA, Alonso A & Salamanca A (2005) Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere, 58: 1255–1267.

Clarke KR & Warwick RM (2001) Change in marine communities: an approach to statistical analysis and interpretation. 2nd Edition. PRIMER-E: Plymouth, 175 pp.

Dye AH & Furstenberg JP (1978) An ecophysiological study of the meiofauna of the Swartkops estuary. 2. The meiofauna: composition, distribution, seasonal fluctuation and biomass. Zoology Africa, 13: 19–32

Ferris H & Bongers T (2006) Nematode indicators of organic enrichment. Journal of Nematology, 38: 3–12.

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

Jensen FB (2003) Nitrite disrupts multiple physiological functions in aquatic animals. Comparative Biochemistry and Physiology, Part A, 135: 9–24.

Jensen P (1987) Difference in microhabitat, abundance, biomass and body size between oxybiotic and thiobiotic free living marine nematode. Oecologia, 71: 564–567.

Kennedy AD (1994) Carbon partitioning within meiobenthic nematode communities in the Exe Estuary, UK. Marine Ecology Progress Series, 105: 71–78.

Leduc D, Probert PK & Nodder SD (2010) Influence of mesh size and core penetration on estimates of deep-sea nematode abundance, biomass, and diversity. Deep-Sea Research I, 57: 1354–1362.

Margiocco AA, Mensi AP & Schlnoni G (1983) Nitrite bio-accumulation in salmo garidneri and hematological consequences. Aquatic Toxicology, 3: 2611–2620.

Masters G & Ela W (2007) Environmental Engineering and Science. 3rd Edition. Prentice Hall. Arizona, USA, 720pp.

Moens T & Vincx M (1997) Observations on the feeding ecology of estuarine nematodes. Journal of the Marine Biological Association of the UK, 77: 211–227.

Moens T, Vanhove S, De Mesel I, Kelemen B, Janssens T, Dewicke A & Vanreusel A (2007) Carbon sources of Antarctic nematodes as revealed by natural carbon isotope ratios and a pulse-chase experiment. Polar Biology, 31: 1–13

Moens T, Bouillon S & Gallucci F (2005) Dual stable isotope abundances unravel trophic position of estuarine nematodes. Journal of the Marine Biological Association of the UK, 85: 1401–1407.

Ngo XQ, Vanreusel A, Smol N & Nguyen NC (2010) Meiobenthos in the Mekong estuarine system with special focus on free living marine nematodes. Ocean Science Journal, 45: 213–224.

Ngo XQ, Smol N & Vanreusel A (2013) The meiofauna distribution in correlation with environmental characteristics in 5 Mekong estuaries, Vietnam. CBM - Cahiers de Biologie Marine, 54: 71–83.

Nozais C, Gosselin M, Michel C & Tita G (2001) Abundance, biomass, composition and grazing impact of the sea-ice meiofauna in the North Water, northern Baffin Bay. Marine Ecology Progress Series, 217: 235–250.

Nozais C, Perissinotto R & Tita G (2005) Seasonal dynamics of meiofauna in a South African temporarily open/closed estuary (Mdloti Estuary, Indian Ocean). Estuarine, Coastal and Shelf Science, 62: 325–338.

Peters RH (1983) The Ecological Implications of Body Size. Cambridge University Press, Cambridge, 329 pp.

Rogers WP (1962) The Nature of Parasitism. Academic Press, New York, 287 pp.

Romeyn K & Bouwman LA (1983) Food selection and consumption by estuarine nematodes. Aquatic Ecology, 17: 103–109.

Schratzberger M, Warr K & Rogers SI (2007) Functional diversity of nematode communities in the southwestern North Sea. Marine Environmental Research, 63: 368–389.

Smol N, Willems KA, Govaere JC & Sandee AJJ (1994) Composition, distribution and biomass of meiobenthos in the Oosterschelde estuary SW Netherlands. Hydrobiologia, 282: 197–217.

Soetaert K, Muthumbi A & Heip C (2002) Size and shape of ocean margin nematodes: morphological diversity and depth-related patterns. Marine Ecology Progress Series, 242: 179–193.

Soetaert K & Heip C (1995) Nematode assemblages of deep-sea and shelf break sites in the North Atlantic and Mediterranean Sea. Marine Ecology Progress Series, 125: 171–183,

Soetaert K, Franco M, Lampadariou N, Muthumbi A, Steyaert M, Vandepitte L, Vanden Berghe E & Vanaverbeke J (2009) Factors affecting nematode biomass, length and width from the shelf to the deep sea. Marine Ecology Progress Series, 392: 123–132.

Tilak KS, Veeraiah K & Milton Prema Raju J (2007) Effects of ammonia, nitrite and nitrate on hemoglobin content and oxygen consumption of freshwater fish, *Cyprinus carpio* (Linnaeus). Journal of Environmental Biology, 28: 45–47

Tita G, Vincx M & Desrosiers G (1999) Size spectra, body width and morphotypes of intertidal nematodes: an ecological interpretation. Journal of the Marine Biological Association of the UK, 79: 1007–1015.

Tita G, Desrosiers G, Vincx M & Clément M (2002) Intertidal meiofauna of the St Lawrence estuary Quebec, Canada: diversity, biomass and feeding structure of nematode assemblages. Journal of the Marine Biological Association of the UK, 82: 779–791.

Van Damme D, Herman R, Sharma Y, Holvoet M & Martens P (1980) Benthic studies of the Southern Bight of the North Sea and its adjacent continental estuaries: Progress Report 2. Fluctuation of the meiobenthic communities in the Westerschelde estuary, in 1980. In: Coordinated Research Actions Interuniversitary Actions Oceanology: Symposium Reports. Pp. 131–170.

Vanaverbeke J, Steyaert M, Vanreusel A & Vincx M (2003) Nematode biomass spectra as descriptors of functional changes due to human and natural impact. Marine Ecology Progress Series, 249: 157–170

Vanaverbeke J, Soetaert K & Vincx M (2004) Changes in morphometric characteristics of nematode communities during a spring phytoplankton bloom deposition. Marine Ecology Progress Series, 273: 139–146.

Vanreusel A, Vincx M, Schram D & Van Gansbeke D (1995) On the vertical distribution of the metazoan meiofauna in shelf break and upper slope habitats of the NE Atlantic. International Review of Hydrobiology, 80: 313–326.

Vanreusel A, Clough L, Jacobsen K, Ambrose W, Jivaluk J, Ryheul V, Hermana R & Vincx M (2000) Meiobenthos of the central Arctic Ocean with special emphasis on the nematode community structure. Deep-Sea Research, 147: 1855–1879.

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

Warwick RM & Price R (1979) Ecological and metabolic studies on free-living nematodes from an estuarine mudflat. Estuarine Coastal Marine Science, 9: 259–271.

Warwick RM (1971) Nematode associations in the Exe estuary. Journal of the Marine Biological Association, 51: 439–454.

Warwick RM (1988) Effects on community structure of a pollutant gradient: introduction. Marine Ecology Progress Series, 46: 149

Wieser W (1953) Die Beziehung zwischen Mundhfhlengestalt, Ernhrungsweise und Vorkommen bei freilebenden marinen Nematoden. Arkiv for Zoology, 2: 439–484.

Wieser W (1960) Benthic studies in Buzzards Bay. II. The meiofauna. Limnology and Oceanography, 5: 121–137.