View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Open Marine Archive

Cah. Biol. Mar. (2013) 54 : 71-83

The meiofauna distribution in correlation with environmental characteristics in 5 Mekong estuaries, Vietnam

Xuan Quang NGO[1,3], Nic SMOL[2] and Ann VANREUSEL[3]

(1) *Department of Environmental Management and Technology, Institute of Tropical Biology, Vietnamese Academy of Science and Technology. 85, Tran Quoc Toan, Dist.3, Ho Chi Minh city, Vietnam*

Email: ngoxuanq@gmail.com

(2) *Postgraduate International Nematology Course, Vakgroep Biologie, Ledeganckstraat 35, B-9000 Gent, Belgium*

(3) *Marine Biology Research group, Biology Department, Ghent University, Krijgslaan 281, S8, B-9000 Ghent, Belgium*

Abstract: Meiofauna assemblages in 5 estuaries of the Mekong river system (Cua Tieu, Cua Dai, Ba Lai, Co Chien and Dinh An) were sampled for community analysis in March 2009, the dry season. The objectives of this research were to provide the first base line survey of meiofauna assemblages in the 5 Mekong estuaries, and to understand how environmental characteristics affect the densities, diversity and structure of the meiofauna. In each estuary, three to four sampling stations were chosen along the salinity gradient from the river mouth to the fresh water part. Besides the meiofauna also sediment- and water column-related environmental characteristics were identified such as dissolved oxygen, pH, salinity, temperature, median grain size, density of coliform bacteria, nutrient and pigment concentrations. Twenty-three major taxa of meiofauna were recorded over the 19 sampling stations. Nematoda, Copepoda, Turbellaria and Oligochaeta dominated with varying densities, but without any clear correlation with the salinity gradient present along the estuaries. The densities of the meiofauna ranged from 105 to 3678 ind.10 cm^{-2} on average. Nematodes were always dominant with relative abundances ranging from 40-98% of the total meiofauna. Meiofauna densities were significantly correlated with sediment pigment concentrations but also other factors may play a role. Diversity showed a positive correlation with dissolved oxygen in the overlying water. The observed densities of the intertidal meiofauna in the Mekong delta are high compared to other estuaries worldwide.

Résumé : *Répartition de la méiofaune en fonction des caractéristiques environnementales dans cinq estuaires du Mekong, Vietnam.* Les assemblages de méiofaune de 5 estuaires du fleuve Mékong (Cua Tieu, Cua Dai, Ba Lai, Co Chien et Dinh An) ont été échantillonnés en mars 2009, pendant la saison sèche. Les objectifs de cette recherche étaient de fournir une base de référence des communautés de méiofaune dans les 5 estuaires du Mékong et de comprendre comment les caractéristiques environnementales affectent les densités, la diversité et la structure de la méiofaune. Dans chaque estuaire, trois ou quatre stations d'échantillonnages ont été choisies le long du gradient de salinité de l'embouchure vers la partie eau douce. Les caractéristiques environnementales du sédiment et de l'eau ont été identifiées comme l'oxygène dissous, le pH, la salinité, la température, la taille médiane du grain, la densité des bactéries coliformes et les concentrations en pigments et en nutriments. Vingt trois taxons majeurs de la méiofaune ont été identifiés sur les 19 stations d'échantillonnage. Les nématodes, copépodes, turbellariés et oligochètes dominent avec des densités variables mais sans aucune correlation claire avec le gradient de salinité présent le long des estuaires. Les densités de la méiofaune varient de 105 à 3678 ind.10 cm^{-2} en moyenne. Les nématodes sont toujours dominants avec des abondances relatives représentant 40 à 98 % de la méiofaune

Reçu le 24 octobre 2011 ; accepté après révision le 1 juin 2012.
Received 24 October 2011; accepted in revised form 1 June 2012.

totale. Les densités de méiofaune sont significativement corrélées avec les concentrations de pigment dans les sédiments mais d'autres facteurs peuvent également jouer un rôle. La diversité montre une corrélation positive avec l'oxygène dissous dans l'eau au-dessus du sédiment. Les densités observées de la méiofaune intertidale du delta du Mékong sont fortes, comparées aux autres estuaires mondiaux.

Keywords: Meiofauna assemblage ● Mekong estuary ● Environmental parameters ● Diversity

Introduction

Estuaries occur at the mouth of rivers where fresh and marine water mixes. These transition zones are characterized by a salinity gradient and are subject to both marine and freshwater influences. Most estuaries are under strong anthropogenic pressure. Especially water quality degradation due to increased nutrient, pesticide and heavy metal inputs are strongly affecting estuarine ecosystems in the past and the present. Also habitat destruction and reduced freshwater flows due to water level regulation and unsustainable use of estuarine resources may alter the estuarine ecosystem.

The Mekong River is the largest river in the Southeast Asia and carries about 550 billions m^3 of water into the Southeast Asia Sea (The South China Sea) together with 4-5 kg alluvium.m^{-3} rich in nitrogen and minerals. Due to the slower current velocities downstream, the alluvium quickly accumulates, thereby creating islands within the estuaries. These islands are flooded by high tide creating a suitable environment for pioneer mangrove forest (Phan & Hoang, 1993). The Mekong River originally divides into 6 large rivers but finally reaches the East Sea by 8 estuaries, all characterized by different landscape morphologies.

The Mekong River system provides a high diversity of bioresources for local people. However, because of a rapid increasing human population density in this region, the natural resources are facing overexploitation. Many natural areas, such as mangroves, were converted into agricultural fields or aquaculture farms. This has led to a decrease in the ecological quality of the estuaries. Some natural aquatic habitats were completely destroyed by industrial shrimp farming activities. Also the organic pollution load of the whole Mekong River System has increased seriously due to urbanization, industrialization and the higher agricultural production (Mekong River Commission, 2007). The Mekong River Delta is therefore facing serious chemical contamination and nutrient pollution (wisdom.caf.dlr.de, 2009), so that the anthropogenic pressure influences strongly the status of its environmental quality.

Not only anthropogenic pressure, but also natural variability affects the Mekong Estuarine System. The tidal regime for instance is causing a heterogeneous and unstable biotope characterized by strong environmental gradients to which the biota is adapted. Especially the tidal flats are daily subjected to great fluctuations in salinity and humidity. The combined impacts of natural and anthropogenic stressors on the estuarine system are responsible for a continuous change in the biochemical, physical and biological characteristics of the sediment including the distribution of aquatic animals along the estuarine gradient. Therefore the benthic meiofauna, which lives in close association with the sediment and which represents the numerically dominant faunal group, was studied in relation to relevant environmental factors in order to establish a base line against which past and future changes can be measured.

Studies on meiofauna from estuaries were performed worldwide (reviewed in detail by Heip et al., 1985 and Giere, 2009) including Vietnam (Olga et al., 2008). However, so far only one study was done on the marine meiofauna from the vast Mekong area (Ngo et al., 2010), not including the estuarine gradient. Therefore the goal of this study was to investigate and compare the ecological quality of the sediments in 5 out of the 8 estuaries of the Mekong River System based on the analyses of the meiofauna communities in relation to their physical and biochemical environment. This study provides the first base line survey of meiofauna assemblages in the Mekong estuaries by assessing their composition, densities and diversity at the higher taxon level.

Materials and Methods

Meiofauna samples were collected in the estuaries Cua Tieu (ECT), Cua Dai (ECD), Ba Lai (EBL), Co Chien (ECC) and Dinh An (EDA) during the dry season in March 2009 (Fig. 1).

Per estuary, 4 low intertidal sampling stations were identified representing a comparable salinity gradient and distance from the mouth. Only in estuary Ba Lai, 3 sampling stations were chosen because of a dam dividing the estuary in 2 parts. In each estuary, the stations 1 and 2 were targeting the polyhaline part (18-30), the station 3 the mesohaline part (5-18) and the station 4 the oligohaline part (0.5 to 5). At each station sediment samples were taken for

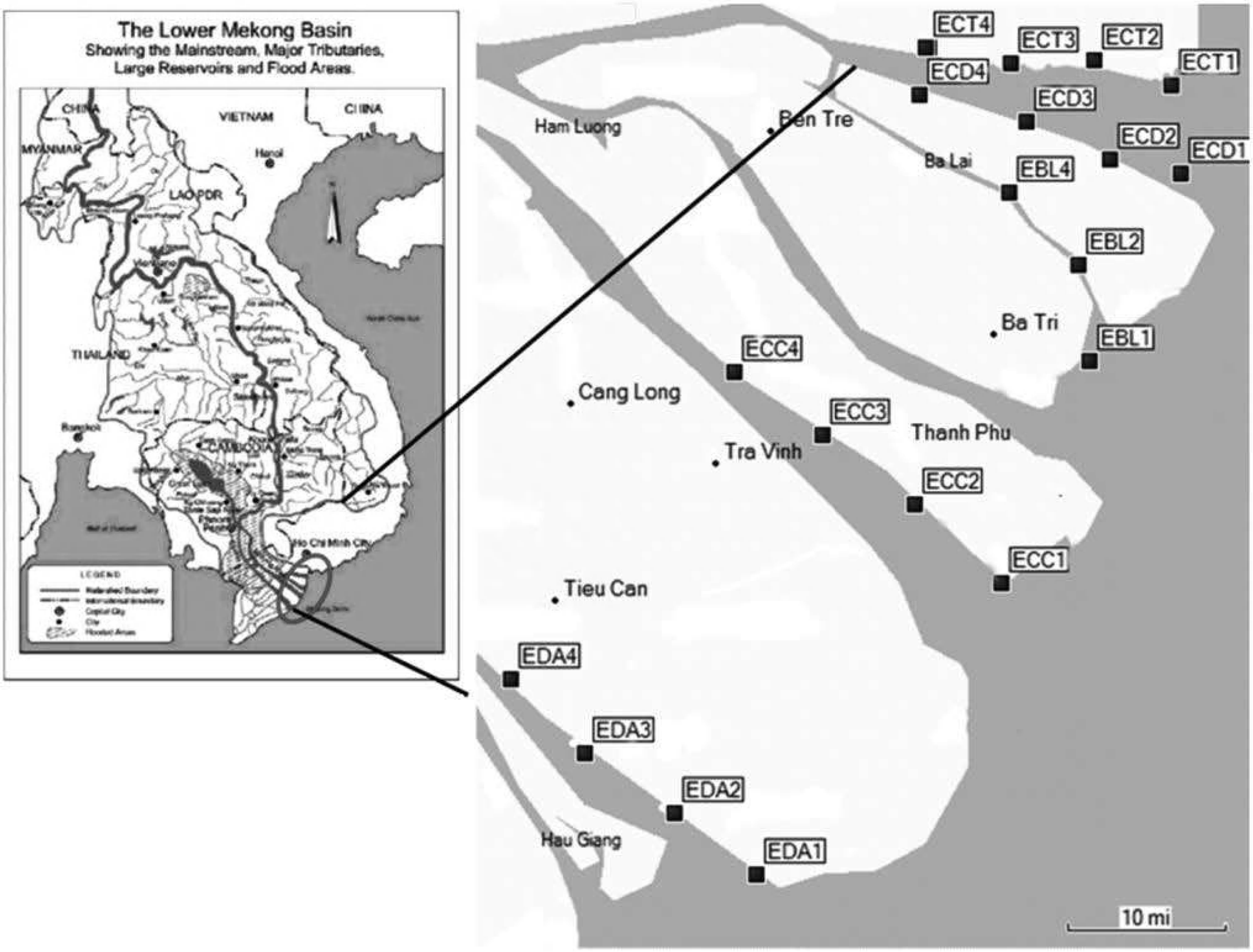

Figure 1. Map of the 19 sampling stations in 5 estuaries of the Mekong delta.

analysis of the meiofauna, granulometric analysis, concentrations of nutrients, coliform bacteria and pigments. Overlying water samples were taken for chemical and physical characterization. Temperature, salinity, pH, electric conductivity, dissolved oxygen, and turbidity were measured in situ by a Multiparameter Water Quality Meter Model WQC22A.

Coliform concentrations in sediments which are a measure for organic pollution were identified based on the standard Most Probable Numbers (MPN) method (Olson, 1978). The MPN technique estimates microbial population sizes by dilution and incubation of replicated cultures across several serial dilution steps. This technique relies on the pattern of positive and negative test results following the inoculation of a suitable test medium. For each sample 300 gram of surface sediments were collected and preserved cool before being analysed in the Ho Chi Minh City Laboratory of Environmental Technology Centre (ETC).

The samples for nutrient concentration of the pore water, pigments (Chloroplastic Pigments Equivalent (CPE) including phaeopigments) and grain size composition were analysed in the Marine Biology research group, University of Ghent, Belgium. One core was collected at each station for pigment analysis. The surface sediment layers were collected by slicing the core respectively 0-1 cm and 1-2 cm deep. For each sediment layer, 1 cm^3 sediment was collected and frozen. Before analysing, the samples were freeze dried. Then, 10 ml acetone 90% was added and the sample was sonicated during 30 seconds. The samples were filtered on a Milex-SR 0.2 μm filter (Milipore), and then injected immediately into the chromatography system for pigment analysis. The High-Performance Liquid Chromatographic system of Gilson was used.

Also for sediment grain size, one sample was collected per station by means of a cut of syringe of 3 cm in diameter, and sliced per cm until 5 cm deep. The rest was kept bulk (5-10 cm). The granulometry was analysed by a Coulter counter type Mastersizer 2000 (Model APA2000 with support equipment Hydro 2000G model AWA2000 of The Malvern Instruments). The grain size composition of the sediment was represented as different proportions of sand, silt and clay (clay < 4 µm, silt = 4-63 µm, sand > 63 µm).

Nutrient samples were collected by means of a core with 6 cm diameter pushed in the sediment up to 10 cm deep, sliced per cm until 5 cm deep, but kept bulk for the 5-10 cm sediment depth, and preserved at 4°C until arrival at the laboratory where they were frozen at -20°C until further analysis. Then pore water samples were extruded under N_2 atmosphere and passed through Whatman GF/C filters. These water samples were stored at -20°C, then thawed and processed for measurements of the concentration of nitrite (NO_2^-), nitrate (NO_3^-) and ammonia (NH_4^+), by using A_{II} automatic chain (SAN^{plus} Segmented Flow Analyser, SKALAR).

Per station, three replicate samples were collected for meiofauna by means of cores of 10 cm^2 in surface. They were fixed and preserved in the field in 7% neutralized formalin (heated to 60-70°C). In the laboratory, meiofauna taxa were extracted from the sediment fraction using Ludox HS-40 colloidal silica at a specific gravity of 1.18 g.cm^{-3} and a 38 µm sieve (Vincx, 1996). In order to facilitate sorting of the meiofauna, the samples were stained with 1% solution of Rose Bengal. Major taxa were identified by using the meiofauna handbook of Higgins & Thiel (1988) and Giere (2009).

Different diversity indices (e.g. Shannon-Wiener diversity H' (log_e), Hill indices) were calculated by means of the PRIMER VI software. Multivariate statistics like multidimensional scaling ordination (MDS), species "best explaining" community pattern (BEST-BIOENV), Similarity/distance percentages (SIMPER) and Principal Component Analysis (PCA) were also performed by the same software. The Spearman rank correlation, multiple linear regressions, analysis of variance (ANOVA) and the non-parametric Kruskal-Wallis test were calculated using the software STATISTICA 7.0 in order to identify respectively significant correlations between biotic and abiotic factors and significant differences for these factors between stations and estuaries. Abiotic data such as nutrient, chlorophyll and phaeo-pigments were used as a mean of the individual sediment layers in the PCA analysis.

Results

Water and sediment environmental characteristics.

Water temperature ranges from 29.3-35°C over all stations. The variation is related to the time of sampling: cooler temperatures at early morning and late afternoon, higher temperatures at midday. The pH and the salinity in general decrease from the river mouth to inland in each of the estuaries. The salinity ranges from 0.3 to 30 but reaches different levels in each of the estuaries related to the distance from the mouth. However the tidal regime also influences the salinity at each sampling station. The salinity measurements done during the moment of sampling confirmed earlier identified salinity regions of the estuaries (stations 1 and 2 are both polyhaline, stations 3 are all mesohaline and the stations 4 are oligohaline) except that EDA4 and ECC4 showed salinities in the mesohaline class. The values of pH in all sampling stations are higher than 7. They were rather low in those stations far from the sea but they were all high in the river mouth (pH > 8, alkaline environment). Dissolved oxygen (DO) shows different patterns for different estuaries since high values were recorded at ECT, but the DO concentrations were rather lower along ECD (Fig. 2).

The sediment in all but one of the stations situated at the river mouth consisted of 100% sand. Only the sediment of station ECC1 had 80% sand. From the second stations inland, the percentage of sand decreased (except for station ECC2) and was mainly replaced by silt and for a small part by clay (Fig. 3).

The coliform bacteria concentrations (Fig. 4) show a high variation between estuaries and sampling stations. In some stations, mainly from the polyhaline part, no coliforms were detected such as in EBL1, EBL2, ECC2 and EDA1. The estuary ECD presents on average the highest concentrations comparing the 5 estuaries except for its freshwater station. Most coliform concentrations range from 0-35000 MPN.100 ml^{-1}. Only at ECD1 and EDA4, the coliform concentrations appeared very high (35000 MPN.100 ml^{-1}). In the other stations, the coliform concentrations were much lower (from 68-2400 MPN.100 ml^{-1}).

The total concentration of Chloroplastic Pigments Equivalent (CPE) including phaeopigments in the surface sediments (0-2 cm) over the 19 sampling stations ranged from 0.4-45.8 µg.g^{-1} over the total sediment profile (Fig. 5A). The amount of chlorophyll a ranges from 0.1-22 µg.g^{-1} sediment (Fig. 5B). The sediment pigment concentration showed for three out of the five estuaries highest values in the middle of the sampled part of the estuaries (stations 2 or 3). In most of the stations situated at the river mouth, or near the freshwater part, the pigment concentrations were rather low, except for station 1 and 4 of the Co Chien estuary (ECC) and station 4 of EBL. The percentage of chlorophyll a (fresh pigments) per total pigment concentrations in the sediment is ranging from 2.7-85.4%. Estuary EBL shows the highest values with respectively 74.4, 69.1 and 83.4% from the mouth to inland. In 3 estuaries ECD, ECC and EDA, the percentage of chlorophyll a reduces from the mouth stations toward

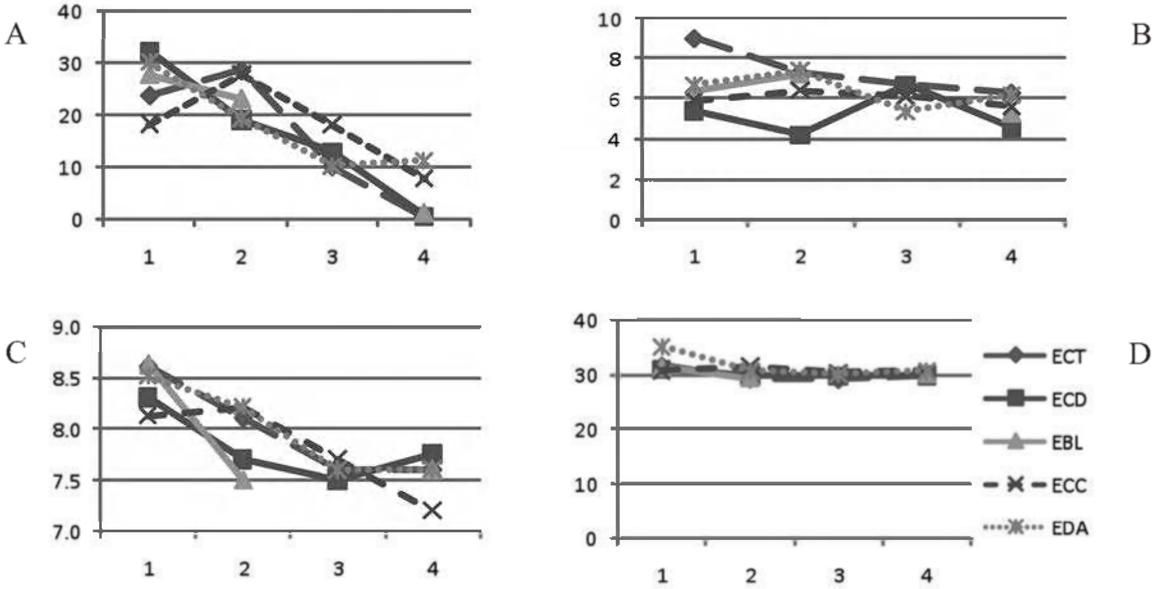

Figure 2. A. Salinity. **B.** Dissolved oxygen (mg.L-1). **C.** pH. **D.** Temperature (°C) of the overlying water in all stations from the 5 estuaries.

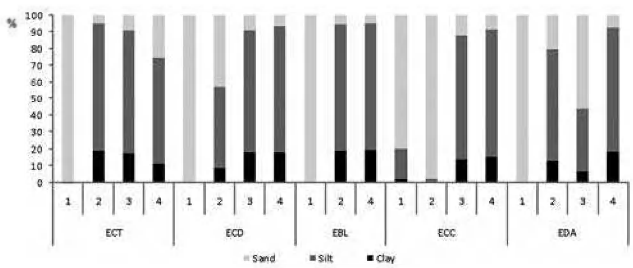

Figure 3. The grain size composition expressed as % sand, silt and clay in all stations from the 5 estuaries.

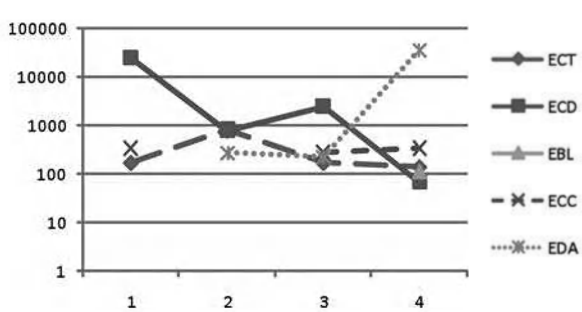

Figure 4. The concentrations of coliform bacteria (log scale MPN.100 mL-1) in the sediment in all stations from the 5 estuaries.

inland (Table 1) while ECT shows the highest value at the middle part of the estuary, whereas high values are found through the estuary in EBL (Table 1).

Table 1. The percentage (%) of chlorophyll a per total pigments in the sediment.

Station	ECT	ECD	EBL	ECC	EDA
1	2.7	84.5	74.4	85.4	66.7
2	47.8	29.7	69.1	63.4	52.6
3	48.2	31.9	-	43.2	65.7
4	12.5	17.5	83.4	40.9	7.6

The NH_4^+ concentration (Fig. 6) increased with depth in the sediment in various stations from all estuaries. Stations ECT1, ECD2, EBL4, ECC4, and EDA1 all showed maxima of more than 3000 µg.g-1 at 5-10 cm depth. In most other stations the depth profiles were much less pronounced or flat. The stations EDA3, EBL2 showed relatively high values (> 2000 µg.g-1) over the whole sediment profiles. In contrast, some stations as ECD1, ECD4, ECC2 and EDA4 showed only values lower than 1000 µg.g-1.

Nitrite (NO_2^-) concentrations (Fig. 7) were always lowest in the estuaries ECT and ECC (< 12 µg.g-1). In ECT, nitrite increased with depth in the sediment until 3-4 cm, except for ECT2 where there is a decrease with depth in the sediment. In ECC nitrite showed a high variability with depth in the sediment in all stations. In the 3 other estuaries, nitrite was occasionally high, up to 40 µg.g-1, or exceptionally even 100 µg.g-1 at 5 to 10 cm depth in ECD2.

In contrast to nitrite, the nitrate (NO_3^-) concentrations (Fig. 8) generally decreased from the poly- to the meso- and

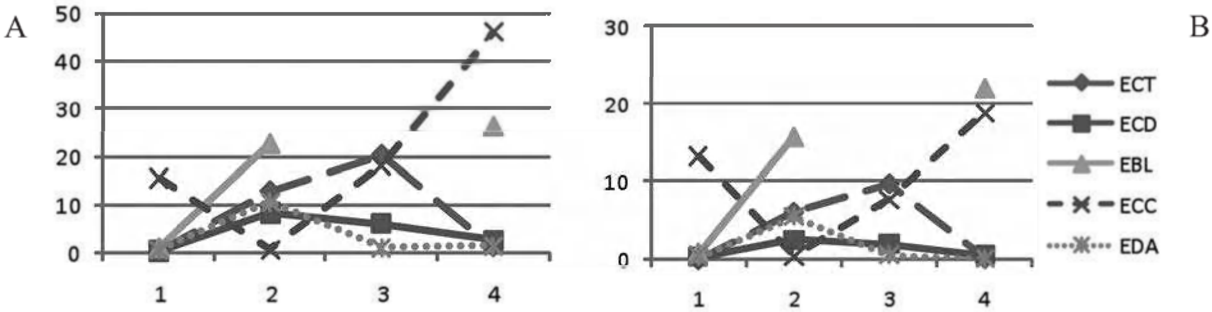

Figure 5. A. Amount of total CPE ($\mu g.g^{-1}$). **B.** Chlorophyll a ($\mu g.g^{-1}$) in the sediments of all stations from the 5 estuaries.

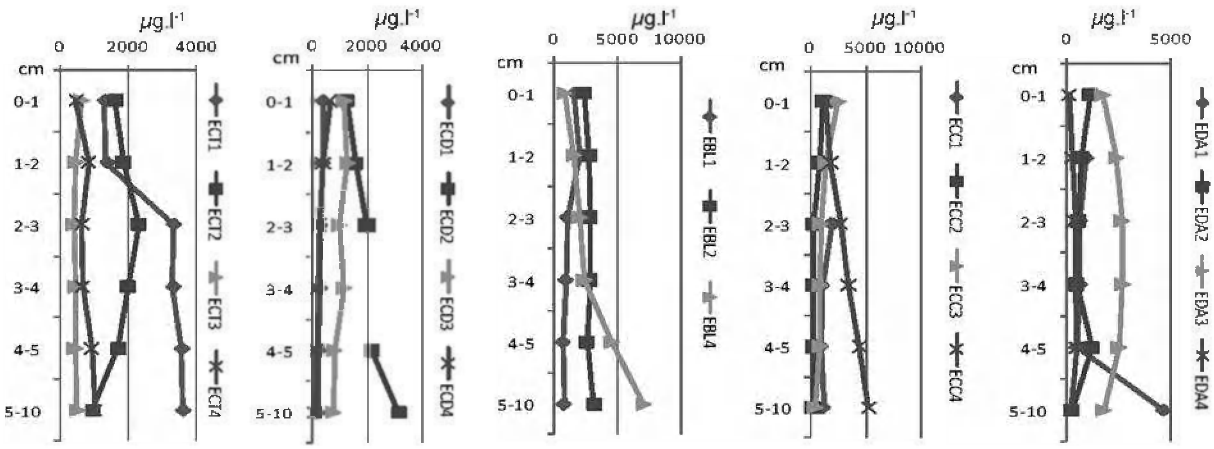

Figure 6. NH_4^+ concentration ($\mu g.l^{-1}$) between 0-10 cm deep in the 5 estuaries.

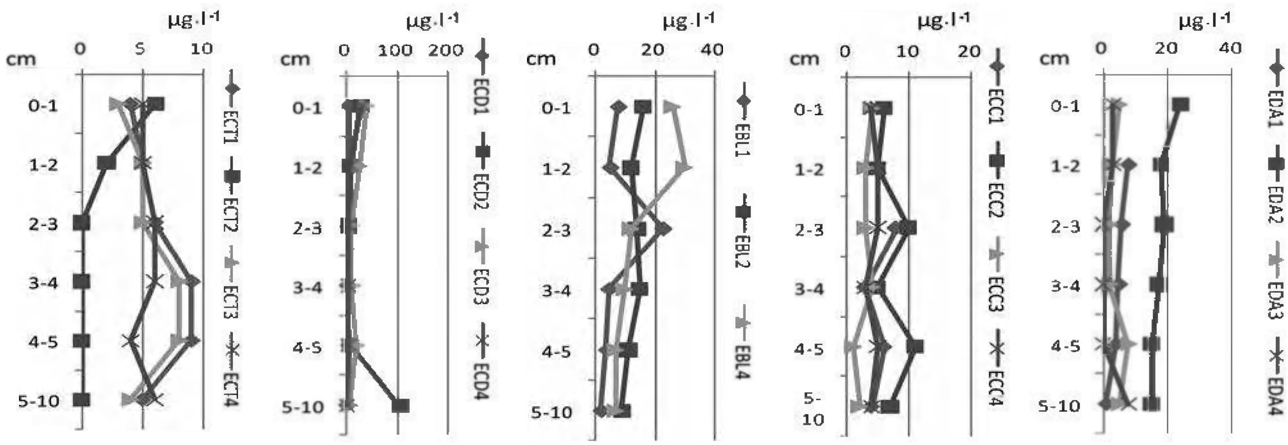

Figure 7. NO_2^- concentration ($\mu g.l^{-1}$) between 0-10 cm deep in the 5 estuaries.

oligohaline part of the estuaries. When the estuaries were compared, the highest concentrations are found at ECD, ECT and EBL. Much lower values are found at EDA and ECC. The depth profiles in the sediment are highly variable from surface maxima in some stations to subsurface maxima at various depths for other stations.

In order to visualize the most prominent environmental gradient, a principal component analysis (PCA) (Fig. 9) was made based on all the environmental characteristics with the exception of coliform concentrations and dissolved oxygen. It shows two main gradients more or less parallel to PCA axis 1 and 2 which explain respectively 53 and 17 % of the variation. The main gradient (axis 1) is present along the course of the estuaries and illustrates the positive correlation between grain size, pH, salinity and NO_3^- $+NO_2^-$, and temperature; the second one which is more parallel to the second PCA axis separates stations like ECC4, EBL4 and EBL2 by their higher chlorophyll a and Ammonium concentrations. Coliform concentrations and dissolved oxygen were excluded from this analysis since they were not significantly correlated to any of the other environmental characters.

Composition and densities of meiofaunal assemblages in the 5 Mekong estuaries

In total 23 higher meiofauna taxa were recorded in the 5 estuaries. Nematoda dominated the meiofauna with 40-98% but Copepoda, Turbellaria, Oligochaeta, Polychaeta, Sarcomastigophora, Bivalvia and Ostracoda were also represented by several individuals. Some taxa such as Bivalvia, Tardigrada, Amphipoda, Syncarida, Tanaidacea were only recorded with a very small number of individuals. The same occurs for Cumacea, Isopoda, Gastrotricha, Gastropoda, Kinorhyncha and aquatic insect larvae such as Diptera, Coleoptera and Trichoptera, which were found in some brackish or fresh water stations. All Crustacea larvae identified as nauplii stages were classified in one group present in almost all stations of the 5 estuaries.

Small differences in total number of taxa were found between the different estuaries: ECC has the highest total number of taxa (18), followed by EDA and ECD with 16 taxa, then ECT with 14 taxa and finally EBL with 13 taxa. The subdominant taxa (> 100 ind.10 cm^{-2}) were also different for the different estuaries. Next to the most dominant position of Nematoda, the following taxa were most abundant in different estuaries: Sarcomastigophora, Ostracoda, Amphipoda, Copepoda and Turbellaria in ECC, Copepoda, Turbellaria and Ostracoda in EDA; Oligochaeta, Turbellaria in ECT; Turbellaria, Oligochaeta, Sarcomastigophora, Ostracoda in ECD; and Copepoda, Turbellaria, Polychaeta, Tardigrada in EBL.

A SIMPER analysis (Bray-Curtis similarity) also illustrated that within each estuary particular trends in (dis)similarity could be observed following the present salinity gradient. In estuary ECT, the oligohaline station ECT4 is far most distinguished from the other stations (dissimilarity value range from 37.8 to 45.4%). This higher dissimilarity is based on the higher abundance of Nematoda, Oligochaeta, Turbellaria and Polychaeta in the poly- and mesohaline stations, whereas Copepoda, Sarcomastigophora, Ostracoda and Nauplii were more abundant in the oligohaline station. The same tendency was recorded in estuary EDA where again the oligohaline station is most distinguished from the other stations (dissimilarity ranged from 34.0-38.4%). Here Copepoda and Nematoda are more abundant in the poly- and mesohaline stations, whereas Nauplii, Ostracoda, Turbellaria are more abundant in the oligohaline station. The other estuaries also showed a similar difference between the oligohaline and the remaining stations but there was not a clear pattern because all stations showed almost the same level of dissimilarity. Some meiofauna taxa were only found in the polyhaline part such as Tardigrada, Cumacea and Syncarida, whereas other taxa were exclusively found in the oligohaline stations such as the larvae of Diptera, Coleoptera and Trichoptera.

The densities of the meiofauna in the 5 estuaries range from 105 ind.10 cm^{-2} to 3678 ind.10 cm^{-2} on the average. Densities increase in most estuaries from the polyhaline part towards the mesohaline part, and then decrease again towards the oligohaline stations except for EBL (Fig. 10). There are

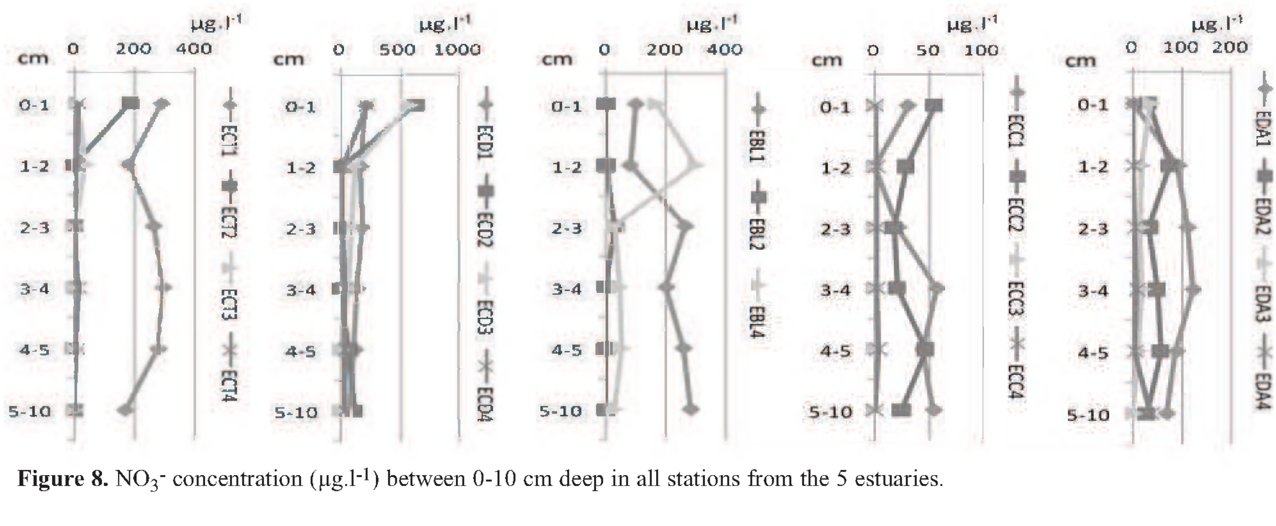

Figure 8. NO_3^- concentration ($\mu g.l^{-1}$) between 0-10 cm deep in all stations from the 5 estuaries.

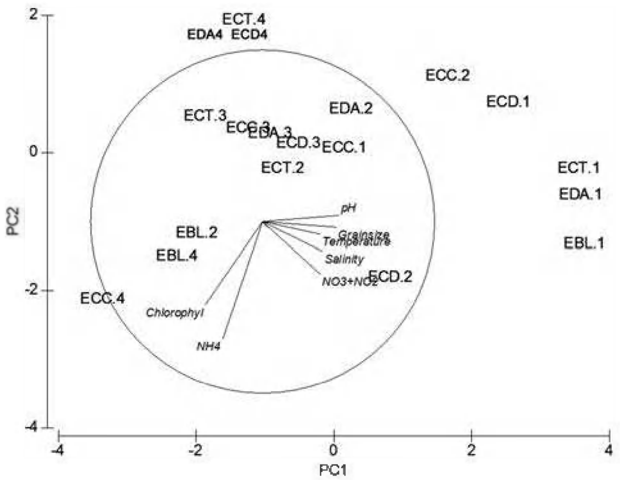

Figure 9. PCA of normalized environmental variables showing the main environmental gradients along the 5 estuaries.

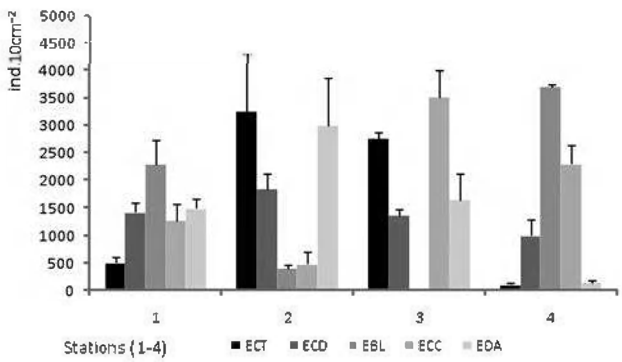

Figure 10. The densities of total meiofauna (ind.10cm^{-2}) per station in all stations from the 5 estuaries. (average ± standard deviation).

some sampling stations with very high densities. Especially ECT2, ECD2, ECC3 and EBL4 present densities of more than 3000 ind.10 cm^{-2}. In contrast, some stations in the oligohaline region were represented by rather low densities (less than 200 ind.10 cm^{-2}) such as ECT4 and EDA4. An ANOVA with log (x + 1) transformed data test ($F_{(38,18)} = 59.38$, $p < 0.01$) and post hoc comparison confirmed the significant higher densities in the middle part of the estuaries and the lower densities in the oligohaline stations

Diversity of meiofauna assemblages in the 5 Mekong estuaries

The diversity of the meiofauna assemblages was calculated as Shannon-Wiener (H') and Hill indices (N_1, N_2 and N_{inf}). The H' index ranged from 0.2-1.14 on average. The H' diversity value was generally highest in the polyhaline stations such as ECT1, ECC1, EBL2 and reduced towards the oligohaline stations (except ECT4, EDA4). The same trend is followed by the Hill indices (Fig. 11).

A Kruskal-Wallis test confirmed significant differences between stations for meiofauna diversity (Table 2) as expressed by the Shannon-Wiener index. A multiple comparison test indicated that station ECT1 was different from EBL4 and ECD4. In addition, also Hill indices showed significant differences between station ECT1 and the three freshwater stations ECC4, EBL4 and ECD4.

Table 2. The Kruskal-Wallis coefficient and p-values for different diversity indices.

Index	p	$H_{(18, N=57)}$
H'	< 0.001	49.55
N_1	< 0.001	49.54
N_2	< 0.001	49.64
N_{inf}	< 0.001	49.37

Correlation between the environmental characteristics and the structure of the meiofauna assemblages

The MDS (Fig. 12) based on the Bray-Curtis similarity of the meiofauna assemblage (not shown) did not show a clear pattern related to salinity, nor were the different estuaries separated (confirmed by ANOSIM). Furthermore the stress value exceeded 0.2 (0.21).

However, the BEST analysis showed that a combination of 4 variables Grain size, DO, Coliform densities and NH_4^+ concentrations correlate best with the meiofauna assemblage composition ($r_s = 0.426$).

The diversity index H' showed a significant positive correlation with dissolved oxygen ($N = 57$, $r = 0.47$, $p < 0.001$) (Fig 13A). There was also a weaker but still significant negative correlation of the diversity index H' with Chlorophyll a ($N = 57$, $r = -0.279$, $p = 0.035$). Also the total meiofauna densities were positive correlated with Chlorophyll a ($N = 57$; $r = 0.46$ $p < 0.001$) (Fig. 13B).

Discussion

Environmental gradient in the 5 Mekong estuaries

One of the most prominent and important environmental characteristic in estuaries is the presence of a salinity gradient. The Mekong estuarine system, as many tropical estuaries, is a temporarily "negative estuary" in the dry season. It means that evaporation from the surface exceeds the freshwater runoff entering the estuaries (McLusky & Elliott, 2004). The salinity pattern in the estuaries ECC and ECT was somehow conflicting with the observations in the other estuaries, in the sense that the first station at the river

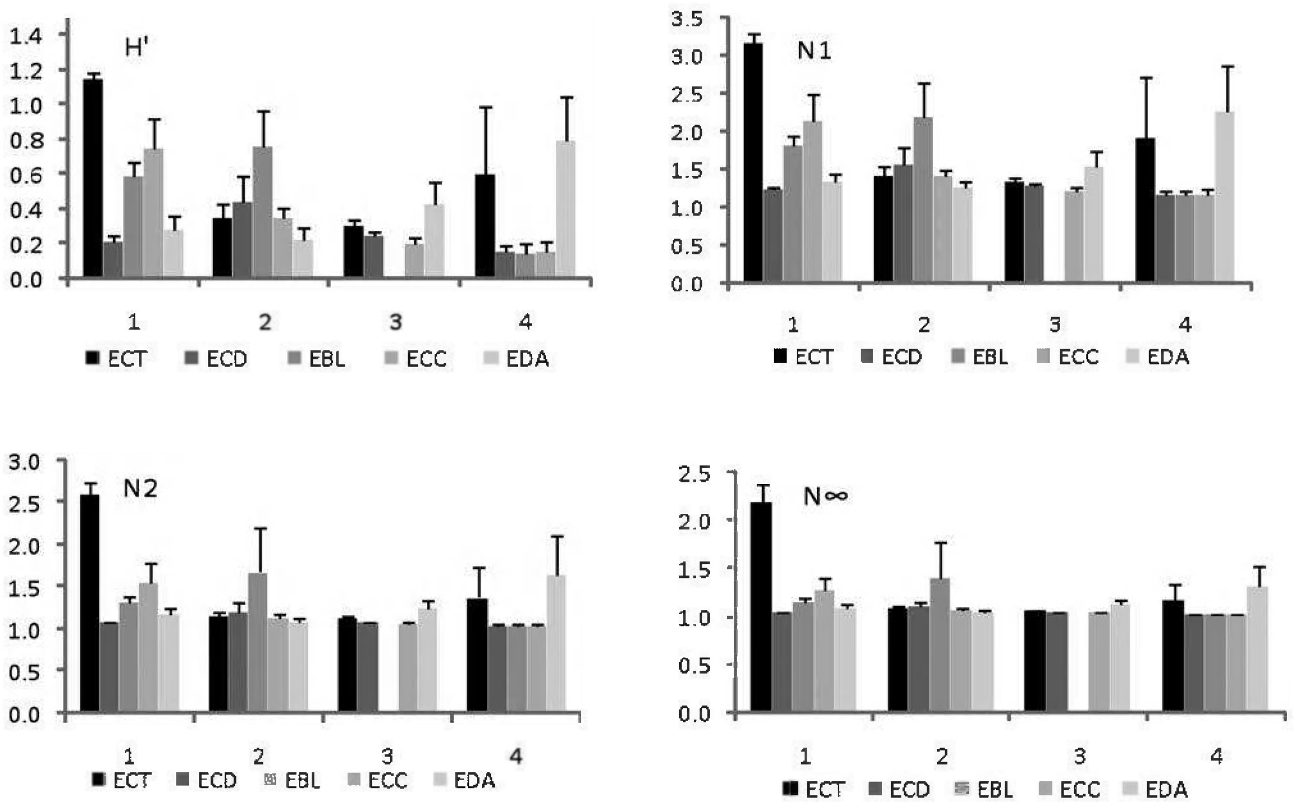

Figure 11. Biodiversity indices (H', N_1, N_2, N_∞) of meiofauna in all stations of the 5 estuaries (average ± standard deviation).

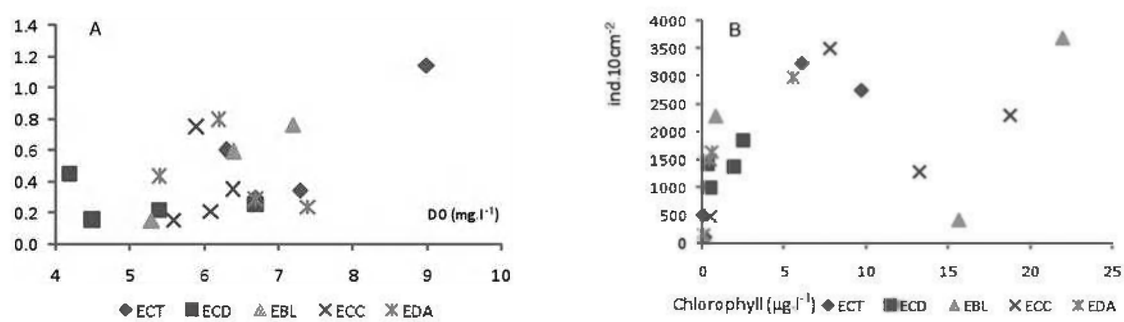

Figure 12. The relationship between (**A**) diversity (H') and DO and (**B**) meiofauna densities (ind.10 cm^{-2}) and chlorophyll a (b).

mouth showed lower salinity than the second station situated farther away from the sea at around 15 km land inward. The tidal level at the time of sampling is considered as the main responsible factor for these aberrant salinity data.

As reported by McLusky & Elliott (2004), an estuary is a very dynamic ecosystem connected with the open sea, through which seawater enters according to the semidiurnal rhythm of the tides, and with rivers providing freshwater. The seawater is then measurably diluted with freshwater resulting in many processes influencing the environmental characteristics in an estuary. In the Mekong estuaries some water and sediment related environmental characteristics, such as grain size, pH and to a lesser extent dissolved oxygen showed an increasing trend along the salinity gradient from the oligo- to the polyhaline part. However, the results also indicate a large variation in environmental characteristics such as pigment and nutrient concentrations especially ammonium in the sediment, which are not correlated to the estuarine gradient.

The Mekong River carries about 4-5kg alluvium.m^{-3} of water rich in nitrogen and minerals which explains the high percentage of silt and clay (Phan & Hoang, 1993). This

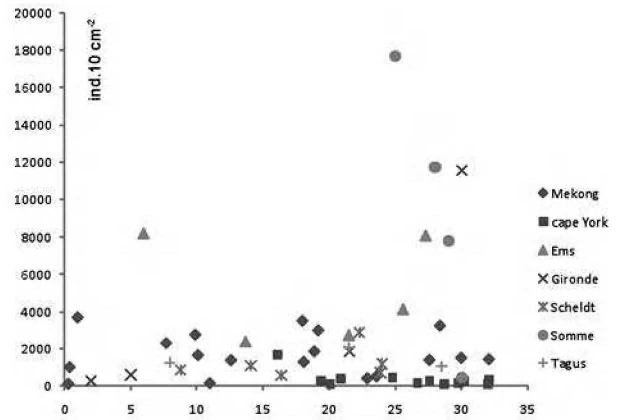

Figure 13. The densities of meiofauna (ind.10 cm^{-2}) in the intertidal zone of the 5 Mekong estuaries and other estuaries worldwide plotted against salinity.

matter deposits and quickly accumulates down into sediment at estuaries where opposite currents from in- and outflowing water mixes. Those processes usually take place in the brackish water region where the two water bodies (saltwater and river-flow) meet (McLusky & Elliott, 2004). Therefore, the percentage of silt and clay is rather high from the middle part toward inland of the estuaries where deposition is highest. In this way many fertile alluvial islands were created in the estuarine area to form a delta. The sediments of the stations at the river mouth mainly consist of sand (up to 100%, except ECC1) due to the tidal action that cleans the sand from small particles. The sediment grain size is an important environmental factor since it provides the physical and chemical habitat of the benthic meiofauna.

The Mekong delta was reported before as organically polluted (Minh et al., 2007; Ikemoto et al., 2008). Therefore, the coliform concentration were analysed in order to identify areas of higher pollution (Gerba & McLeod, 1976; Labelle & Gerba, 1980; Farrapeira et al. 2010). The concentrations of coliform bacteria in the sediment were very variable over the 5 estuaries. Some stations, such as ECD1, EDA4, showed very high values (> 10000 MPN) whereas in other stations (EBL1, EBL2, ECC2 and EDA1) coliform bacteria were not detected. Estuary ECD had overall the highest concentrations of coliform bacteria and the estuaries EBL and ECT contained the lowest concentrations of coliform bacteria. In a study by Gerba & McLeod (1976) on the estuaries of Galveston Bay and West Bay in Texas, the concentrations of coliform bacteria in the water column ranged from 540-35000 MPN.100 ml^{-1} in the mesohaline part down to 2-240 MPN in the polyhaline parts of the estuaries. Remarkably, the concentrations of coliform bacteria in the sediment of the same estuaries increased dramatically in the mesohaline part (from 16000-2400000 MPN.100 ml^{-1}) whereas they decreased down to 2-7 MPN.100ml^{-1} in the polyhaline area. The much higher coliform concentrations in the sediment compared to the water column in the middle of estuaries are also confirmed by a study of Labelle & Gerba (1980) in the same bay (Galveston Bay, Texas) where they found that coliform concentrations in 3 sampling stations range from 15-1100 coliform units per 100 ml in the water, while in the sediment 15000-46000 coliform units per 100 ml were counted. The same study also indicates that viruses were present at higher concentrations in polluted estuarine sediments than in the overlying seawater. It has been demonstrated previously that viruses and bacteria are present in high numbers on a volume basis in sediment. Hence, the concentrations of coliform bacteria in the Mekong estuarine sediment are not so high compared with some other estuaries in the world except for the stations ECD1 and EDA 4.

The concentration of chlorophyll a is indicative for the amount of fresh plant material present in the sediments. In the estuarine area, a large portion of plant detritus derives from phytoplankton and phytobenthos, as well as from terrestrial plants and it is a measure for the potential fresh carbon sources for bacteria, meiofauna, protozoa and other benthic organisms, whose feeding activities may convert the chlorophyll to phaeo-pigment (Tietjen, 1968).

The amount of chlorophyll a and CPE differs between the 5 estuaries. The estuaries ECT, ECD and EDA have high amounts of chlorophyll a and CPE concentrations in the middle parts (stations 2 and 3) and low concentrations at the mouth and the freshwater stations. The pattern in ECC is however opposite with much higher values in stations 1 and 4, whereas estuary EBL shows an increase from the mouth station toward inland. In addition, the highest percentage of chlorophyll a, relative to the total CPE, is found in the estuary EBL indicating that the photosynthetic primary production in this estuary is high; whereas in the other 3 estuaries ECD, ECC and EDA, this percentage is only high in the polyhaline stations and reduces inland. This indicates that the fresh chlorophyll is reduced from the high salinity zone to the freshwater part and is replaced by degraded detritus. ECT is the only estuary that shows a low percentage of chlorophyll on the total CPE in the mouth and freshwater station.

The amount of sediment pigments in the 5 Mekong estuaries is not so different from the concentrations in the Niantic River, United States (both North and South Shoals) ranging from 5.9-31.6 µg.g^{-1} sediment, or the concentrations in the Pettaquamscutt river (both east and west) with 6.6-23.9 µg.g^{-1} sediment (Tietjen, 1968). The amount of chlorophyll a is also in the same range as found in the Western Scheldt estuary (the Netherlands) with values of 0.3-27 µg.g^{-1} sediment (Jong & Jonge, 1995). Although many factors influence estuarine and coastal primary productivity, the rates of supply of nutrients are fundamentally important in regulating this process. In estuarine ecosystems, nitrogen (N) is commonly the most limiting nutrient for phytoplankton production (Pinckney et al., 2001). As nitrogen passes through the estuarine environment it undergoes a complex series of transformations, with nitrification and denitrification being essential microbiological processes (Niels et al., 2004). The organic matter bound nitrogen accumulates at the bottom and is either buried permanently or undergoes decomposition resulting in the release of NH_4^+ from the process of bacterial reduction-oxidation reactions in the case of oxygen depletion. The nitrification process in aerobic conditions oxidizes NH_4^+ to NO_2^- and NO_3^- before the denitrification process convert them back to N_2 and N_2O in anaerobic conditions. These processes explain the vertical profiles of NH_4^+, NO_2^- and NO_3^- in the sediments of the 5 Mekong estuaries. For instance, NH_4^+ concentration shows generally an increasing concentration with depth in the sediment in most of estuaries because of the lack of oxygen in the deeper layers. It is also indicated that organic matter bound nitrogen increases in deeper sediments. Two estuaries ECT and ECD showed that the NH_4^+ concentrations decreased from the marine part towards the oligohaline stations which could indicate that the buried organic matter decreases in the sediment when salinity decreases. In the other 3 estuaries the pattern is opposite.

NH_4^+ is oxidized to NO_2^- by ammonium oxidizing bacteria but NO_2^- is a polyatomic ionic compound that is oxidized on its turn to NO_3^- by nitrite oxidizing bacteria (Niels et al., 2004) which means that NO_2^- is a transitional phase in the nitrification process. That is why the values of NO_2^- concentration in the 5 Mekong estuaries are rather low compared to NH_4^+ and NO_3^-. The vertical profiles of NO_2^- and NO_3^- in the Mekong estuaries also show the expected pattern of lower concentrations in the deeper layers except for some stations such as the polyhaline, sandy stations ECT1, ECD2, EBL1 and ECC1, but also ECT3. This is explained by the fact that in most silty sediments nitrification takes place in the oxygenated zone just a few centimeters below the surface sediment since the aerobic litotrophic process of nitrification is dependent on the supply of both NH_4^+ and oxygen (Niels et al., 2004). This fits also to the observation by Caffrey et al. (1993) that nitrification rates decrease with sediment depth, associated with the decrease of oxygen and with the amount of organic matter available. So the polyhaline sandy stations do not show a decrease in nitrate concentrations with increasing sediment depth suggesting that these stations are well oxygenated along the total sediment profile.

Meiofauna assemblages in correlation with environmental characteristics

The total number of higher taxa in the meiofauna assemblages of the 5 Mekong estuaries in the dry season amounts 23 major taxa. The number of meiofauna taxa observed in the Mekong is much higher compared to the number found in 5 European estuaries studied by Soetaert et al. (1995), where 13 taxa were found. Also the number of meiofauna taxa per Mekong estuary ranging from 13 to 18 taxa is higher than the values recorded for several other estuaries. In the Cua Luc estuary (North Vietnam), Olga et al. (2008) recorded 11 taxonomical groups. The number of taxa in the present study is similar to the 17 taxa found in the study on the Mira estuary by Adao (2003) but higher than the 12 meiofauna taxa found by Alves et al. (2009) for the Mira and Mondego estuaries.

Not only the number of taxa, also the densities of the meiofauna in the 5 Mekong estuaries are high compared to the average recorded densities worldwide. Figure 14 shows the intertidal meiofauna densities in relation to the salinity gradient in different estuaries from different parts of the world such as Cape York in Australia (Alongi, 1987), the Ems and the Scheldt (Westerschelde) in the Netherlands, the Somme and the Gironde in France, the Tagus in Portugal, and the Tamar in England (Soetaert et al., 1995), and the 5 Mekong estuaries from this study. It shows that the meiofauna densities in most of the stations in the 5 Mekong estuaries are lower than the counts in the Somme and Ems estuary but higher than that in Cape York estuary as well as the Scheldt and Gironde.

In estuaries, the tidal regime, the salinity and the sediment characteristics are typically the main determinants of the meiofauna distribution (Soetaert et al., 1995). In contrast to other estuarine studies (Soetaert et al., 1995; Udalov et al., 2005; Alves et al., 2009), there is not a clear tendency of decreasing densities along the salinity gradient in the Mekong estuaries. In addition to salinity, the structure of the meiofauna assemblages in the 5 Mekong estuaries was influenced by a combination of many environmental factors such as grain size, chlorophyll a, ammonium, coliform concentrations in the sediment and dissolved oxygen of the water column.

Although no prominent pattern in meiofauna community structure was observed, the BEST analysis selected grain size, coliform concentrations, ammonium and DO as the best combination of environmental variables that correlated with the meiofauna. Beside salinity, also NH_4^+ and coliform, oxygen were considered before as the pre-dominant factors among the abiotic parameters determining the habitat conditions, and so the presence and abundance of different meiofauna taxa (Giere, 2009). Also Patricio et al. (2012) found by a similar approach that nutrient concentration and grain size were explaining meiofauna communities. In the Mekong estuarine system, the taxa diversity tends to decrease with decreasing oxygen concentrations in the overlying water column. It demonstrates that likely several meiobenthic taxa have higher oxygen demands. It was indeed demonstrated before that several meiofauna groups such as copepods and ostracods are more sensitive than nematodes to low oxygen concentrations (Moodley et al., 1997; Wetzel et al., 2001), and may lower in abundance or disappear leading to an overall dominance of nematodes and a reduced diversity in areas with lower oxygen supply. Both diversity and densities only increased significantly though weak with chlorophyll a concentrations in the Mekong which was in accordance with previous observations. Also Tolhurst et al. (2010) and Netto & Gallucci (2003) found in general no or weak correlations between meiofauna community characteristics and a whole range of relevant environmental variables. The most siginificant correlations were found between densities and detritus biomass in the South Brazilian mangrove area (Netto & Gallucci, 2003). Tolhurst et al. (2010) suggested that localized factors are driving patterns in fauna at small scales, which complicates the correlation between biota and abiota at larger scales like in this study.

Conclusion

These first records of Meiofauna assemblages in 5 Mekong estuaries are characterized by a high number of taxa and high densities. Nematodes play an important dominant position controlling the characters of the meiofauna assemblage. The distribution of the meiofauna expresses large variation between sampling stations within and between the estuaries. There is no clear response between the meiofauna densities and diversity in relation to the salinity gradient but there are trends related to a combination of environmental factors. The highest densities and lowest diversity seems to be associated with the most organically enriched stations, as reflected in the lower concentrations of dissolved oxygen and higher chlorophyll concentrations.

References

Adão H. 2003. *Dynamic of meiofauna communities in association with Zostera noltii seegrass beds in the Mira SW Portugal.* PhD thesis. University of Evora. 328 pp.

Alongi D.M. 1987. Intertidal zonation and seasonality of meiobenthos in tropical mangrove estuaries. *Marine Biology*, **95**: 447-458.

Alves S.A., Adão H., Patricio J., Neto J.M., Costa M.J. & Joao C.M. 2009. Spatial distribution of subtidal meiobenthos along estuarine gradients in two southern European estuaries Portugal. *Journal of the Marine Biological Association of the United Kingdom*, **10**: 1-12.

Caffrey J.M., Sloth N.P., Kaspar H. & Blackburn T.H. 1993. Effect of organic loading on nitrification and denitrification in a marine sediment microcosm. *FEMS Microbial Ecology*, **12**: 159-16

Farrapeira C.M.R., Mendes E.S., Dourado J., Guimarães J. 2010. Coliform accumulation in Amphibalanus amphitrite (Darwin, 1854) (Cirripedia) and its use as an organic pollution bioindicator in the estuarine area of Recife, Pernambuco, Brazil. *Brazilian Journal of Biology*, **70**: 301-309.

Gerba C.P. and McLeod J.S. 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. *Applied Environmental Microbioly*, **32**: 114-120.

Giere O. 2009. *Meiobenthology. The microscopic motile fauna of aquatic sediments*. 2nd edition. Springer Verlag: Heidelberg. 527 pp.

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

Higgins R. & Thiel H. 1988. *Introduction to the study of meiofauna*. Smithsonian Institution Press: Washington DC. 488 pp.

Ikemoto T., Nguyen P.C.T., Michio X.W., Noboru O., Koji O., Shinsuke T., Bui C.T. & Ichiro T. 2008. Analysis of biomagnification of persistent organic pollutants in the aquatic food web of the Mekong Delta, South Vietnam using stable carbon and nitrogen isotopes. *Chemosphere*, **72**: 104-114.

Jong D.J. & Jonge V.N. de 1995. Dynamic and distribution of microphytobenthic Chlorophyll-a in the Western Scheldt estuary SW Netherlands. *Hydrobiologia*, **311**: 21-30.

LaBelle R.L. & Gerba C.P. 1980. Influence of estuarine sediment on virus survival under field conditions. *Applied and Environmental Microbiology*, **39**: 749-755.

McLusky D.S. & Elliott M. 2004. *The Estuarine Ecosystem Ecology, Threats, and Management*, 3rd edition. Chapman & Hall: London, 224 pp.

Mekong River Commision 2007. *Diagnostic study of water quality in the Lower Mekong Basin.* MRC Technical Paper N° 15, Mekong River Commission. 57 pp.

Minh N.H., Minh T.B., Natsuko K., Tatsuya K., Hisato I., Pham H.V., Nguyen P.C.T., Bui C.T. & Shinsuke T. 2007. Pollution sources and occurrences of selected persistent organic pollutants (POPs) in sediments of the Mekong River delta, South Vietnam. *Chemosphere*, **67**: 1794-1801.

Moodley L., Van der Zwaan G.J., Herman P.M.J., Kempers L. & Van Breugel P. 1997. Differential response of benthic meiofauna to anoxia with special reference to Foraminifera (Protista: Sarcodina). *Marine Ecology Progress Series*, **158**: 151-163.

Netto S.A. & Gallucci F. 2003. Meiofauna and macrofauna communities in a mangrove from the island of Santa Catarina, South Brazil. *Hydrobiologia,* **505**: 159-170.

Ngo X.Q., Vanreusel A., Smol N. & Nguyen N.C. 2010. Meiobenthos assemblages in the Mekong estuarine system with special focus on free-living marine nematodes. *Ocean Science Journal*, **45**: 213-224.

Niels S.L., Banta G.T. & Pedersen M.F. 2004. The influence of Primary Producers on estuarine nutrient cycling. *Aquatic ecology book series*, 303 pp. Kluwer Academic Publishers.

Olga Y.T., Nguyen V.T. & Nguyen D.T. 2008. Meiobenthos in Estuary Part of Ha Long Bay Gulf of Tonkin, South China Sea, Vietnam. *Ocean Science Journal*, **43**: 153-160.

Olson B.H. 1978. Enhanced accuracy of coliform testing in seawater by a modification of the most-probable-number method. *Applied and Environmental Microbiology*, **36**: 438-444.

Patrício J., Adão H., João M. N., Alves A. S., Traunspurger W. & Marques J.C. 2012. Do nematode and macrofauna assemblages provide similar ecological assessment information? *Ecological Indicators*, **14**: 124-13.

Phan N.H. & Hoang T.S. 1993. *Mangroves of Vietnam*. IUCN wetlands programme. IUCN publisher. 173 pp.

Pinckney J.L., Paerl H.W., Tester P.A. & Richardson T.L. 2001. The role of nutrient loading and eutrophication in estuarine ecology. *Environmental Health Perspectives*, **109**: 699-706.

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

Tietjen J.H. 1968. Chlorophyll and pheo-pigments in the estuarine sediments. *Limnology & Oceanography*, **13**: 189-192.

Tolhurst T.J., Defew E.C. & Dye A. 2010. Lack of correlation between surface macrofauna, meiofauna, erosion threshold and biogeochemical properties of sediments within an intertidal mudflat and mangrove forest. *Hydrobiologia,* **652**:1-13.

Udalov A.A., Mokievskii V.O. & Chertoprud E.S. 2005. Influence of the salinity gradient on the distribution of meiobenthos in the Chernaya River Estuary White Sea. *Oceanology,* **45**: 680-688.

Vincx M. 1996. Meiofauna in marine and freshwater sediments. In: *Methods for the examination of organismal diversity in soils and sediments* (G.S. Hall ed), pp. 187-195. Cabi International: Wallinfort, UK.

Wetzel M.A., Fleeger J.W. & Powers S. 2001. Effects of hypoxia and anoxia on Meiofauna: a review with new data from the Gulf of Mexico. In: *Coastal hypoxia: consequences for living resources and ecosystems* (N.N. Rabalais & R.E. Turner eds), pp 165-184. American Geophysical Union: Washington D.C.

Website:http://wisdom.caf.dlr.de/en 2009.