Effect of nutrient enrichment on seagrass associated meiofauna in Tanzania

4 authors:

Lillian Nduku Daudi
Kenya Marine and Fisheries Research Institute (KMFRI)
3 PUBLICATIONS   **14** CITATIONS

Jacqueline Uku
Kenya Marine and Fisheries Research Institute (KMFRI)
12 PUBLICATIONS   **339** CITATIONS

Charles Lugomela
University of Dar es Salaam
118 PUBLICATIONS   **453** CITATIONS

Marleen De Troch
Ghent University
212 PUBLICATIONS   **1,557** CITATIONS

Some of the authors of this publication are also working on these related projects:

An ecosystem approach to fisheries management: The Atlantic seabob shrimp (Xiphopenaeus kroyeri) in Suriname View project

Marine Antarctic Food Webs: complexity, structure and stability View project

Effect of nutrient enrichment on seagrass associated meiofauna in Tanzania

Lillian Nduku Daudi [a,b,*], Charles Lugomela [c], Jacqueline Nduku Uku [a], Marleen De Troch [b]

[a] Kenya Marine and Fisheries Research Institute, P.O. Box 81651(80100), Mombasa, Kenya
[b] Ghent University, Biology Department, Marine Biology, Krijgslaan 281 − S8, B-9000 Ghent, Belgium
[c] Department of Aquatic Sciences and Fisheries, University of Dar es Salaam, P.O. Box 35064, Dar es Salaam, Tanzania

ABSTRACT

Abundance, diversity and community structure of meiofauna, with special emphasis on epiphytic harpacticoid copepods, occurring in Tanzanian seagrass beds under various nutrient inputs was determined. All measured parameters for epiphytic meiofauna and diatoms (fucoxanthin) were negatively affected by nutrient input and this was detected even at the higher taxonomic levels of meiofauna, supporting the validity of higher taxon surrogacy in environmental impact studies. However, benthic meiofauna and other biofilm characteristics (chlorophyll a) did not show any difference between sites suggesting that nutrient enrichment had less impact on these variables. This indicates a differential impact of pollution on epiphytic vs. benthic communities. Consequently, different trophic levels will be impacted in various ways and hence the effects of pollution on the overall ecosystem functioning of seagrass beds are complex and not straightforward. Although the seagrass plants themselves don't show any major changes under different nutrient input, associated organisms that guarantee energy flow at basal levels of the food web in this ecosystem can be largely impacted.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Anthropogenic disturbance of nearshore ecosystems appears to be the most widespread phenomena threatening coastal ecosystems (Short and Wyllie-Echeverria, 1996). In coastal marine environments, sewage discharge is a common practice which can affect several ecosystems in multiple ways by changing structural and functional attributes of biodiversity (Pearson and Rosenberg, 1978; Short and Wyllie-Echeverria, 1996).

In East-African coastal cities domestic sewage infrastructures are in general poorly developed and can be considered as a major source of pollution to nearshore coastal waters. Impacted areas are mainly those that border major towns and cities from which they receive untreated municipal and industrial wastes due to high population densities and industrial aggregation. A good example along the East-African coast is the city of Dar es Salaam where the main sources of water pollution include sewage, heavy metal pollution, hydrocarbon pollution, solid wastes, agrochemical pollution and sedimentation, with domestic waste being the most serious source of pollution (Machiwa, 1992). As a result, the coastal waters, especially in the vicinity of the city, are heavily polluted.

The Msimbazi River and Msimbazi Creek meander through the city centre and are the most polluted water bodies ultimately discharging their waters into the sea (Mohammed, 2000). The river and the creek receive large quantities of untreated domestic waste water from the city in addition to industrial wastes from various industries and drains into the ocean near Ocean Road. These waste waters influence not only the water quality but also the coastal ecosystems including seagrass beds, mangrove forests and the reef fringing the coast.

Along tropical coasts, seagrass beds typically support a large diversity of associated fauna and flora (Hemminga and Duarte, 2000). They provide food, shelter and nurseries for a variety of animals, including many commercially important fish and shellfish species (Bell and Pollard, 1989). In addition, seagrass beds form an important link between mangrove and coral reef ecosystems. Seagrass communities are however subjected to frequent disturbance by anthropogenic (e.g. shoreline construction, eutrophication, mechanical damage) or natural (sand wave motion, storms and hurricanes/typhoons, overgrazing) sources which can lead to alterations in vegetation complexity (De Troch et al., 2001a; Gray, 1997; Snelgrove et al., 1997). The main impact is of course visible on the plants and the size of the seagrass beds. However, the impact can also be evaluated as being less severe when plants remain intact but yet the associated fauna can be influenced, although often neglected in impact studies.

* Corresponding author. Kenya Marine and Fisheries Research Institute, P.O. Box 81651(80100), Mombasa, Kenya.
   E-mail addresses: lillynduku@yahoo.com, ldaudi@kmfri.co.ke (L.N. Daudi).

The present study aimed to analyse seagrass associated meio-faunal organisms (Metazoa that pass through a 1 mm sieve but are retained on a 38 μm sieve). There are numerous advantages to use meiofauna in pollution studies including their widespread occurrence, high diversity and densities, permanent and intimate contact with contaminated sediment, high sensitivity and fast response, and their short generation time allows for tests of sensitive reproductive stages (Heip, 1980; Vincx and Heip, 1991; Warwick and Clarke, 1993; Giere, 2009). The present study focussed on harpacticoid copepods (Crustacea, Copepoda) since they are abundant in seagrass beds (Bell et al., 1988; Bell and Hicks, 1991; De Troch et al., 2001a,b; Hicks, 1977a,b,c, 1980) but also play a pivotal role in the energy flow from primary producers to higher trophic levels such as fish (Sogard, 1984; Fujiwara and Highsmith, 1997).

The impact of habitat modifications of seagrass beds for the associated meiofauna is far from being well documented. In order to unravel the impact of nutrient enrichment on ecosystem functioning of seagrass beds and their associated fauna and flora, the present study analysed the abundance, diversity and community structure of meiofauna, with special emphasis on epiphytic harpacticoid copepods, occurring in Tanzanian seagrass beds under contrasting nutrient levels.

2. Materials and methods

2.1. Study area

The study was conducted at Ocean road and Mbweni area off Dar es Salaam City, Tanzania (Fig. 1). Ocean Road is located close to the city centre just outside the entrance of the Dar es Salaam harbour (about 6°48′21.2″S; 39°17′41.9″E). This beach extends northwards to the mouth of Msimbazi creek. To the south, the beach is protected from the open sea by the Kigamboni headland as a result of change of the coastal alignment across the harbour, and
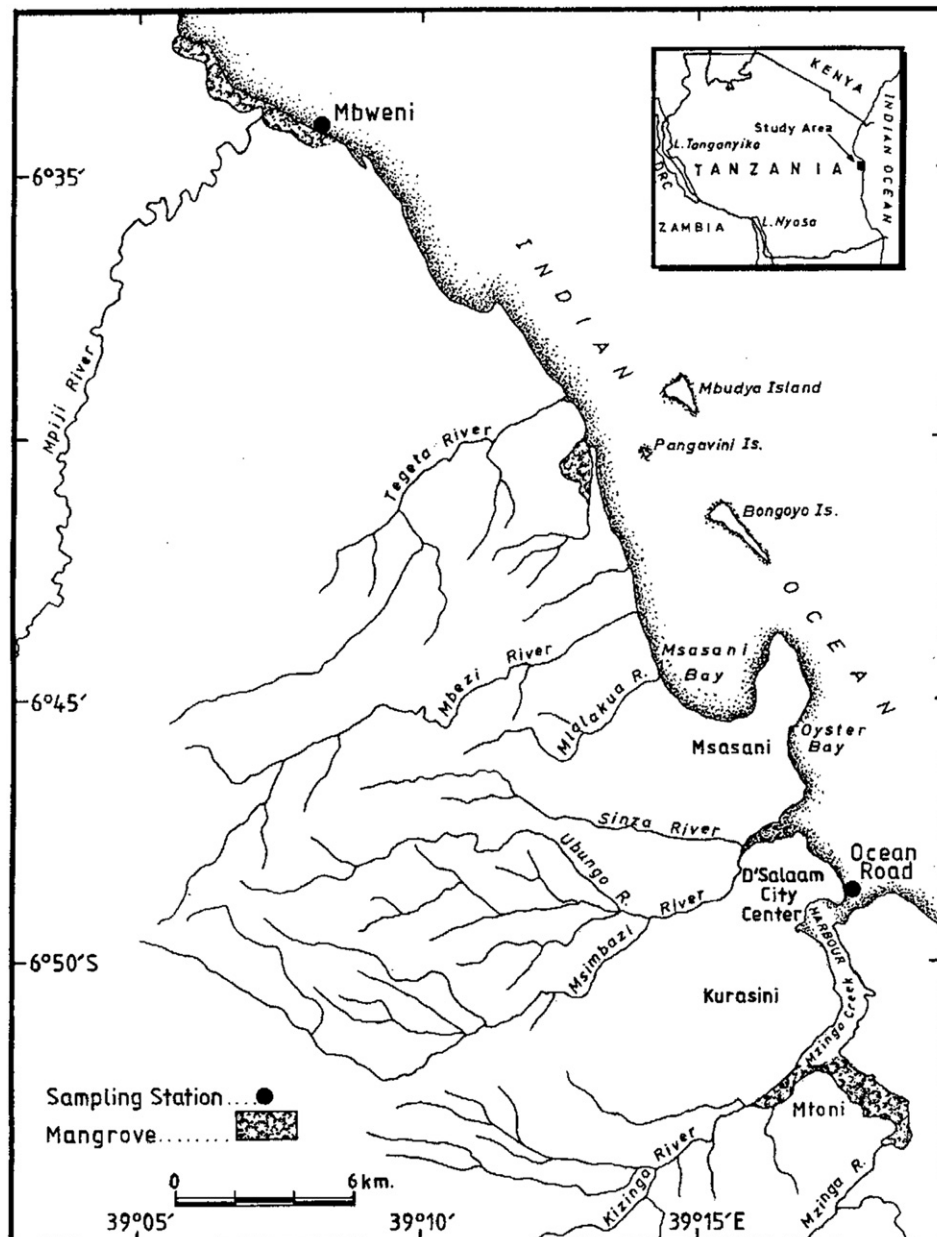

Fig. 1. Map of the study area with indication of the sampling sites.

the Inner and Outer Makatumbe islands. Ocean Road receives sewage from the city centre through pipes and the Msimbazi River and faces heavy sedimentation due to harbour activities. The beach is characterized by sandy/muddy habitats and seagrasses occur as small patches from the lower littoral zone and extend towards the sublittoral zone on a harder substrate of calcareous gravel (Hamisi et al., 2004).

Mbweni (6°34′23.7″S; 39°8′09.3″E) is located 30 km north of Dar es Salaam and Ocean Road beach. The area is characterized by a large sand flat which is mostly exposed at low tide. Seagrasses flourish all over the lower reaches of the sand flat while on the upper reaches a small mangrove forest fringes the area. There is a village nearby and a landing site for the fishermen. Mpiji River passes through less populated areas of the city and empties its waters on the northern side of the beach. There are no sewage effluents into the beach so this area is less polluted compared to the Ocean Road site (Lyimo, 2009).

Several studies in the area of Dar es Salaam showed that the main sources of pollution include sewage, heavy metals, hydrocarbons, industrial pollution, solid wastes, agrochemicals and sedimentation (Machiwa, 1992). The Dar es Salaam city sewage dumps raw sewage into the shallow subtidal ocean through a pipe at Ocean Road beach that is located near the city centre (Wagner, 2007). From previous studies nutrient levels (nitrate, nitrite and phosphate), have been found to be significantly higher near this discharge point at Ocean road than in other sites studied (Hamisi et al., 2004). Msimbazi River, which drains from the Dar es Salaam city centre is the main source of domestic and industrial contaminants for Ocean Road. Heavy metals of anthropogenic origin have been reported to be higher in sediments of Msimbazi (near Ocean Road) in comparison to Mbweni (Okuku et al., 2010; De Wolf et al., 2001). A recent study by Rumisha et al. (2012) reported trace metal pollution in sediments of Msimbazi (near ocean road) and Mbweni. The study used an enrichment factor to determine sources of trace metal concentrations (whether natural or from anthropogenic sources) and categorize contamination of these trace metals. Significant enrichment was observed at Msimbazi for As (17.5), Cd (9.0), Mn (7.5), Pb (7.9) and Zn (12.8); very high enrichment for Mo (27.2) and extreme high enrichment for Sb (54.3). Mbweni showed significant enrichment for As (6.9), Mn (6.3) and Sb (5.0) and extreme high enrichment for Mo (95.0). Therefore, significantly more enrichment of most trace metals was observed at Msimbazi than at Mbweni. However, the concentration of most of the trace metals in sediments was within recommended sediment quality standards. These sites were therefore characterized by a different status of environmental quality based on data independently assessed in previous studies mentioned above.

In both sampling sites, the seagrass beds are mainly composed of mixture of seagrass species. *Thalassia hemprichii* only becomes exposed to air at spring tides while *Syringodium isoetifolium*, *Thalassodendron ciliatum* and *Enhalus acroides* occur in deeper water. Seagrasses in Ocean Road occur in patches with no uniform pattern in distribution and *T. hemprichii* and *Cymodocea rotundata* are the dominant seagrass species (Lugendo et al., 1999). In Mbweni, *S. isoetifolium* is the dominant species followed by *T. hemprichii* (Mamboya et al., 2009).

2.2. Sampling design

Sampling was conducted in October 2009 during spring low tides at two locations near the sewage discharge point, Ocean Road (Polluted) and at a relatively pristine site, Mbweni (Non-polluted), about 30 km away from the discharge point. Sampling occurred while snorkeling at a water depth of approx. 0.5 m. Four seagrasses were sampled: *E. acroides* (Ea), *S. isoetifolium* (Si) *T. hemprichii* (Th)

and *T. ciliatum* (Tc). In each location, three patches for each seagrass species were sampled and within each patch three random replicates were collected for each species. This resulted into a total of 72 samples (2 locations × 4 seagrass species × 3 patches × 3 replicates). The sampling design incorporated two factors: location (Polluted and Non-polluted) and seagrass species (Ea, Si, Th and Tc).

2.3. Meiofauna collection

2.3.1. Epiphytic meiofauna

Harvesting was done by placing plastic bags over an average of 5–10 seagrass shoots, closing the bag with the leaves underwater and bringing it to the surface. The bags were closed with rubber bands to avoid loss of the water hence loss of meiofauna. Then, 8% MgCl$_2$ solution was added to the bags for 15 min to detach the meiofauna from the leaves prior to rinsing off the leaves over a 1 mm and 38 μm sieve using filtered seawater. The epiphytic samples were stored in 4% formaldehyde (final concentration). The seagrasses were then preserved on ice for later collection of the biofilm and determination of the surface area.

2.3.2. Benthic meiofauna

Polyvinyl chloride (PVC) cores (3.6 cm inner diameter, 10 cm^2 surface) were used to collect meiofauna from the sediment (referred to as benthic meiofauna) in the vicinity of the seagrass plants where epiphytic meiofauna was collected. Sediment cores were sliced into two depth layers (0–1 cm, 1–2 cm) and preserved in 4% formaldehyde (final concentration).

2.4. Processing of meiofauna samples

Benthic samples were decanted 5 times over a 38 μm mesh sieve, centrifuged 5 times with Ludox (specific density 1.18) and stained with Rose Bengal (Heip et al., 1985). The epiphytic meiofauna were directly stained with Rose Bengal. Copepods and nauplii were counted separately in view of their different ecology and dispersal abilities (Hicks and Coull, 1983). Meiofauna was counted and identified at higher taxonomic levels according to Higgins and Thiel (1988).

Copepods were sorted from the epiphytic samples using an 'Irwin loop'. Per sample, a total of 100 harpacticoid copepods (including copepodites) were picked out as they were encountered in a counting tray. Copepods were stored in 75% ethanol and later on mounted in toto on glycerin slides for identification. For samples with less than 100 individuals, all copepods were identified. Planktonic copepods (orders Calanoida and Cyclopoida) occurred only occasionally in the samples and were excluded from further analyses. They can be recognized based on their long first antennae (A1) and typical body tagmosis.

Copepods belonging to the order Harpacticoida were identified to family level under a stereo microscope using the keys provided by Boxshall and Hasley (2004), Huys et al. (1996) and Lang (1965). The use of family level was justified by higher taxon surrogacy as validated by an earlier study on intertidal copepod communities along the Kenyan coast (De Troch et al., 2008; see also Discussion).

2.5. Biofilm collection

The biofilm from the leaves was scraped off using cover glasses, stored in Eppendorf vials at −20 °C for further pigment and fatty acid analysis. Collection of the biofilm was done for three seagrass species as it was hard to obtain biofilm from the cylindrical leaves of *S. isoetifolium*.

2.6. Biochemical analyses

Pigment analysis (Chlorophyll *a* and fucoxanthin) was done using High-Performance Liquid Chromatography (HPLC) techniques according to a modified protocol after Wright et al. (1991). Hydrolysis of total lipid extracts (in triplicate) of the biofilm and methylation to Fatty Acid Methyl Esters (FAMEs) was achieved by a modified one-step derivatisation method after Abdulkadir and Tsuchiya (2008). The boron trifluoride-methanol reagent was replaced by a 2.5% H_2SO_4–methanol solution since BF_3–methanol can cause artefacts or loss of Poly Unsaturated Fatty Acids (PUFAs) (Eder, 1995). The FAMEs thus obtained were analysed using a Hewlett Packard 6890N GC coupled to a mass spectrometer (HP 5973). The samples were run in splitless mode, at an injector temperature of 250 °C using an HP88 column (Agilent J&W; Agilent Co., USA). The oven temperature was programmed at 50 °C for 2 min, followed by a ramp at 25 °C min^{-1} to 175 °C and then a final ramp at 2 °C min^{-1} to 230 °C with a 4 min hold. The FAMEs were identified by comparison with the retention times and mass spectra of authentic standards and available spectra in mass spectral libraries (WILEY), and analysed with the software MSD Chem-Station (Agilent Technologies). Quantification of individual FAMEs was accomplished by the use of external standards (Supelco # 47885, Sigma–Aldrich Inc., USA). The quantification function of each FAME was obtained by linear regression applied to the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 250 μg ml^{-1}).

2.7. Statistical analyses

Multivariate and univariate techniques were used for data analysis using the software PRIMER 6 (Clarke and Gorley, 2006) and STATISTICA 8.0 from Statsoft. Data were standardized to leaf area prior to statistical analysis since the surface area of leaves collected was not the same for all samples. Statistical differences in number of meiofauna taxa, epiphytic harpacticoid copepod taxa (family level) and densities were tested. Levene's test was used to test for homogeneity of variances of the data. All data that were not normally distributed were log transformed prior to variance analysis in order to achieve normality and homogeneity of variance. Factorial ANOVA was used to test for significant differences in densities and diversities using STATISTICA 8, tested factors were location and seagrass species. Post-hoc analysis was done using the Tukey HSD test. Kruskal–Wallis test was performed for data that did not achieve homogeneity of variance even after transformation.

Multivariate patterns were visualized by non-metric multidimensional scaling (nMDS) on the basis of a Bray–Curtis similarity matrix on fourth-root transformed data in PRIMER 6 software. Non-parametric analysis of similarity (ANOSIM) was used to test for differences in multivariate structure of assemblages. Data were fourth-root transformed to down weigh the contribution of dominant species to community structure.

Diversity of meiofauna was expressed by means of Hill's indices N_0, N_1, N_2 and N_{inf} (Hill, 1973). In order to detect any correlations between the biofilm present and the meiofauna, linear regressions between meiofauna densities (and more specifically copepod densities) and concentrations of pigments were performed.

3. Results

3.1. Epiphytic meiofauna

Total epiphytic meiofauna densities ranged from 30 individuals/100 cm^2 (*T. ciliatum* in the polluted site) to 939 individuals/100 cm^2 (*S. isoetifolium* in the non-polluted site). Non-polluted samples
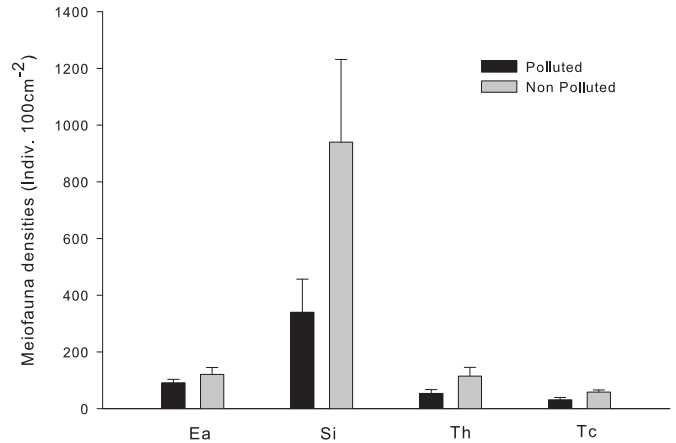

Fig. 2. Average (±standard error) meiofauna densities for all seagrasses sampled in both locations (polluted vs. non-polluted). Ea: *Enhalus acroides*, Si: *Syringodium isoetifolium*, Th: *Thalassia hemprichii*, Tc: *Thalassodendron ciliatum*.

showed higher meiofauna densities in comparison to the polluted ones for all seagrass species sampled (Fig. 2). Overall, *S. isoetifolium* harboured the highest meiofauna densities compared to other seagrass species. Factorial ANOVA of total meiofauna densities showed a significant difference between both locations ($p = 0.001$), i.e. an effect of pollution, and also between seagrass species ($p < 0.001$) as shown in Table 1.

A total of 20 higher meiofauna taxa were present in the epiphytic samples and in general more taxa were found in the non-polluted site, except for *E. acroides* (Fig. 3). The taxa included Insecta, Ostracoda, Copepoda, Nematoda, Amphipoda, Polychaeta, Mysida, Cumacea, Isopoda, Oligochaeta, Gastropoda, Turbellaria, Tanaidacea, Kinorhyncha, Ciliophora, Nemertinea, Tardigrada, Holothuroidea, Appendiculata, Priapulida and Bivalvia. Copepods in general were the dominant taxon (>40% relative abundance) followed by nematodes (>11%) in all sites and on all seagrasses sampled. Amphipods were twice as abundant in the polluted compared to the unpolluted site while polychaete densities were four times higher in the unpolluted than the polluted site.

Diversities of epiphytic meiofauna showed significant differences between the polluted and non-polluted location (N_0, N_1, and N_2; $p < 0.050$). Meiofauna diversity was generally higher in the non-polluted site compared to the polluted site for all seagrass species except for *E. acroides* where the reverse pattern was observed. There were no differences in the dominance structure (N_{inf}) of the meiofauna in the different sites ($p > 0.010$) but differences in meiofauna dominance between seagrass species were observed. The meiofaunal assemblages differed between both

Table 1
Factorial ANOVA for epiphytic meiofauna densities and taxa. Significant *p*-values are printed in bold.

| Source of variance	df	SS	MS	F	p-Level
Meiofauna densities (log$_{10}$ *y* transformed)					
Site	1	1.4793	1.4793	11.236	**0.001350**
Seagrass	3	10.7532	3.5844	27.225	**0.000000**
Interaction	3	0.2798	0.0933	0.708	0.550529
Error	64	8.4260	0.1317		
Meiofauna major taxa					
Site	1	120.125	120.125	25.532	**0.000004**
Seagrass	3	46.264	15.421	3.278	**0.026536**
Interaction	3	123.153	41.051	8.725	**0.000062**
Error	64	301.111	4.705		

53
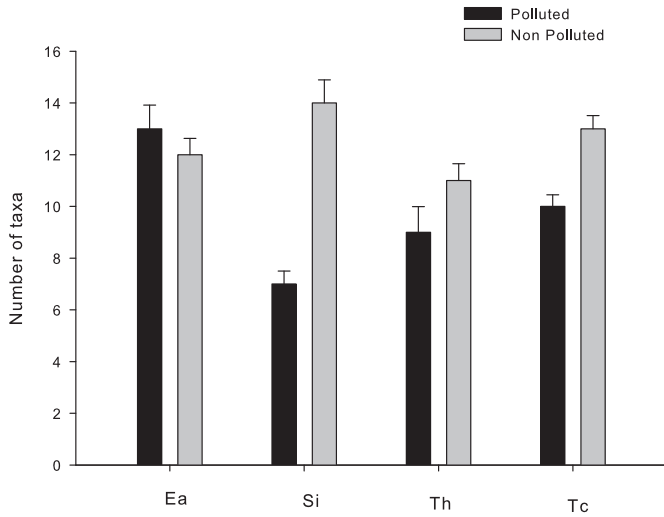
Fig. 3. Average number (±standard error) of meiofauna taxa for all seagrasses sampled in both locations (polluted vs. non-polluted). Ea: *Enhalus acroides*, Si: *Syringodium isoetifolium*, Th: *Thalassia hemprichii*, Tc: *Thalassodendron ciliatum*.

locations for all seagrass species (ANOSIM, $R = 0.205$; $p = 0.006$; $R = 0.630$; $p = 0.001$; $R = 0.190$; $p = 0.018$ and $R = 0.516$; $p = 0.001$ for *Enhalus acoroides*, *S. isoetifolium*, *T. hemprichii* and *T. ciliatum*, respectively) (Fig. 4).

3.2. Benthic meiofauna

Benthic meiofauna densities ranged from 100 to 1500 individuals/10 cm². Factorial ANOVA of densities showed significant differences between locations, depths and seagrass species ($p < 0.001$). Meiofauna densities in sediments were generally higher in the top centimetre than at 2 cm depth and overall higher in the non-polluted than the polluted sites ($p < 0.010$) (Table 2). Independent of the sampling site, meiofauna densities within *S. isoetifolium* beds were higher compared to the other seagrass species (Fig. 5).

A total of 16 taxa were identified with more taxa being enumerated in the non-polluted samples. The taxa included Amphipoda, Bivalvia, Ciliophora, Copepoda, Cumacea, Gastropoda, Insecta, Isopoda, Kinorhyncha, Nematoda, Nemertinea, Oligochaeta, Ostracoda, Polychaeta, Tanaidacea and Turbellaria. Nematodes were the dominant taxon (>75% in relative abundance) in general followed by copepods at both locations and for all seagrass species.

Diversities of benthic meiofauna differed in relation to sediment depth and the interaction of seagrass and depth (Kruskal–Wallis, $p < 0.001$ for N_0: seagrass and depth; for N_1: depth and for N_2: seagrass). There was no observed difference in the dominance structure (N_{inf}) of benthic meiofauna. An MDS (not shown) showed that benthic assemblages did not differ between the locations.

3.3. Harpacticoid copepods

Total epiphytic copepod densities ranged from 20 individuals/100 cm² (*T. ciliatum*, polluted) to 532 individuals/100 cm² (*S.*
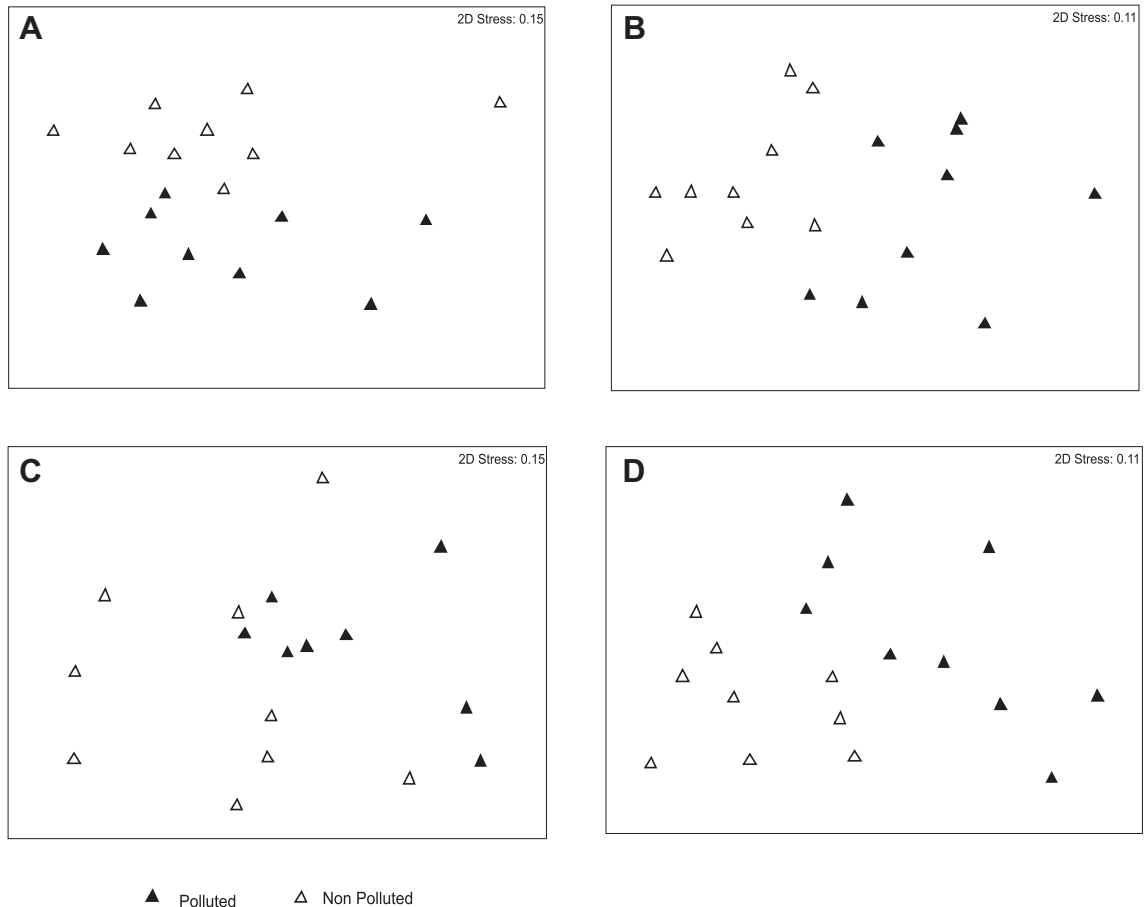

Fig. 4. MDS of meiofauna composition for all seagrasses in both locations (polluted vs. non-polluted). Polluted: closed symbols, non-polluted: open symbols. A – *Enhalus acroides*, B – *Syringodium isoetifolium*, C – *Thalassia hemprichii* and D – *Thalassodendron ciliatum*.

Table 2
Factorial ANOVA for benthic meiofauna. Significant *p*-values are printed in bold.

Source of variance	df	SS	MS	F	p-Level
Sediment meiofauna ($\log_{10} y$ transformed)					
Site	1	1.0926	1.0926	11.393	**0.002504**
Seagrass	2	1.9589	0.9794	10.213	**0.000618**
Depth	1	0.7971	0.7971	8.312	**0.008180**
Site*seagrass	2	0.5057	0.2528	2.636	0.092254
Site*depth	1	0.1378	0.1378	1.437	0.242272
Seagrass*depth	2	0.0990	0.0495	0.516	0.603319
Site*seagrass*depth	2	0.1832	0.0916	0.955	0.398846
Error	24	2.3016	0.0959		

isoetifolium, non-polluted). In general, copepod densities were higher in the non-polluted than in the polluted site with *S. isoetifolium* also having higher copepod densities than the other seagrass species (Fig. 6).

Non-parametric tests (Kruskal–Wallis) showed significant differences in copepod densities among seagrass species ($H = 36$, $N = 72$, $p = 0.000$). However, there were no significant differences between locations ($p = 0.090$). Also, copepod diversities differed between sites with higher diversities observed in non-polluted compared to polluted site ($p < 0.050$). However, the number of copepod taxa was not different between sites. Copepod assemblages differed between polluted and non-polluted sites for all seagrass species (ANOSIM, $R = 0.718$; $p = 0.001$; $R = 0.286$; $p = 0.010$ and $R = 0.681$; $p = 0.002$ for *S. isoetifolium*, *T. hemprichii* and *T. ciliatum*, respectively) (Fig. 7).
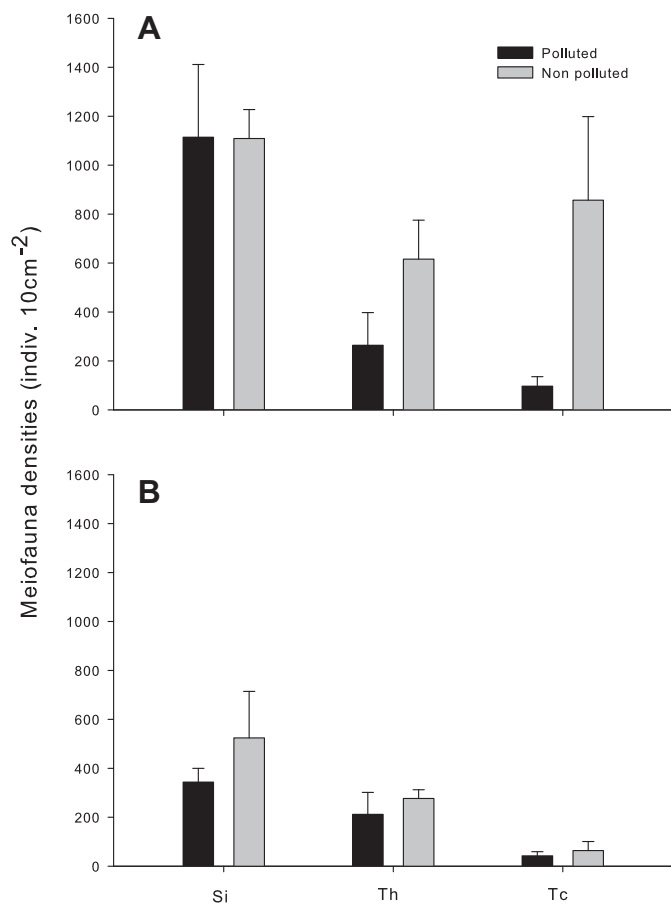
Fig. 5. Benthic meiofauna densities (±standard error) for seagrass species for two depth layers sampled in both locations (polluted vs. non-polluted). A) 0–1 cm layer, B) 1–2 cm layer. Si: *Syringodium isoetifolium*, Th: *Thalassia hemprichii*, Tc: *Thalassodendron ciliatum*.
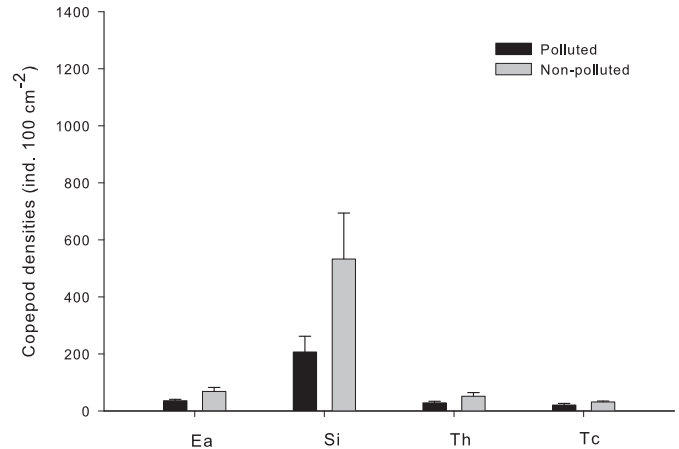
Fig. 6. Epiphytic copepod densities (±standard error) for seagrass species in both locations (polluted vs. non-polluted). Ea: *Enhalus acroides*, Si: *Syringodium isoetifolium*, Th: *Thalassia hemprichii*, Tc: *Thalassodendron ciliatum*.

3.4. Biofilm characteristics

Chlorophyll *a* ranged between 300 and 400 μg/g of biofilm and the values were not significantly different for seagrass species or locations. Fucoxanthin ranged between 10 and 35 μg/g of biofilm. Significant differences were observed for fucoxanthin (seagrass species and location, two-way ANOVA, $p < 0.050$) (Fig. 8). However, no strong correlations were found between meiofauna and biofilm ($R = 0.001$ for chl *a*; $R = 0.064$ for fucoxanthin).

The fatty acids 16:0 and 18:3ω3 (α-linolenic acid, ALA) were present in relatively high amounts in all biofilm samples (Fig. 9). Significant differences of fatty acids 14:0, 16:1ω7, 18:0, 18:1ω9c and 18:2ω6t were observed between locations and seagrasses and combinations of both factors (two-way ANOVA, $p < 0.050$). Significant differences between seagrass species were observed for fatty acids 16:0, 18:1ω9t, 18:3ω3, 20:4ω3 and 20:5ω3 (eicosapentaenoic acid, EPA) (Kruskal–Wallis, $p < 0.050$) while fatty acid 20:4ω6 (arachidonic acid, ARA) also showed significant differences between locations (Mann–Whitney test, $p < 0.050$). An MDS plot (stress: 0.06) of the fatty acid profiles of biofilm on seagrass leaves revealed a difference between seagrass species but not between locations (Fig. 10).

4. Discussion

In general, the results of the present study show a decrease in meiofaunal abundance and a change in the structure of the meiofauna assemblages resulting from the nutrient load that distinguishes the polluted site from the pristine one. This impact was consistent for all seagrass species that were included in the present study. Nutrient enrichment in seagrass meadows has typically been associated with the alteration of the structure and biomass of epiphytic assemblages supported by the plants (Cambridge et al., 2007). Biomass of microalgal epiphytes as expressed by chlorophyll *a* did not show significant variations between both sampling sites in the present study. However, fucoxanthin (for diatoms) differed between sites but not between seagrass species. The characteristics of the microalgal epiphytes were mainly attributed to the host plant rather than to nutrient enrichment as shown by the fatty acid profiles. However, composition and abundance of microalgal epiphytes were not determined within the scope of the present study; biomass was used as a proxy.

Epiphytic meiofauna, especially meiofauna on subtidal seagrasses, is typically structured by plant related environmental
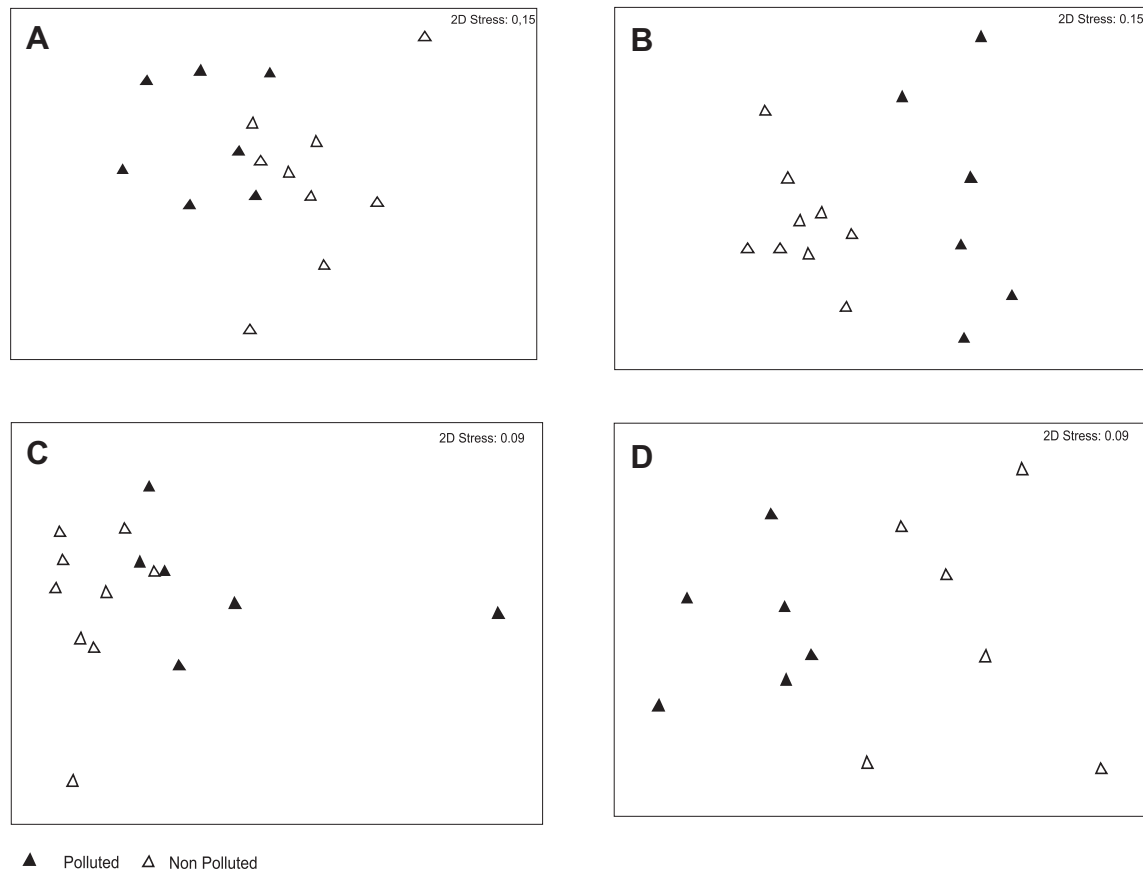
Fig. 7. MDS of copepod composition associated to the different seagrasses in both locations (polluted vs. non-polluted). Polluted: closed symbols, non-polluted: open symbols. A) *Enhalus acroides*, B) *Syringodium isoetifolium*, C) *Thalassia hemprichii*, D) *Thalassodendron ciliatum*.

factors such as associated diatoms (De Troch et al., 2001b). This was also found in the present study as higher concentrations of fuco-xanthin (typically higher in diatoms) were found in biofilms on subtidal seagrass species.

Based on simulated nutrient enrichment experiments, previous studies reported an impact of eutrophication on epiphytic primary production (Wear et al., 1999; Bucolo et al., 2008). Moderate enrichment is known to promote epiphytes to a certain extent, whereas high nutrient concentrations reduce epiphyte diversity (Jaschinski et al., 2010). Since the present study focused on the impact of nutrient enrichment without any field manipulations, a clear quantification of the spatial extent and magnitude of nutrient enrichment owing to ocean dynamics such as tides and wave regimes was limited.

Similar microalgal epiphytes on the seagrasses at both sampling locations let us expect to find similar assemblages of meiofauna on both locations. However, this was not the case since there was a clear discrepancy between primary producers and consumers in terms of functional response towards nutrient enrichment. However, differences in fucoxanthin pigments imply an effect on diatoms between the sites as higher values were recorded in the non-polluted site compared to the polluted site. The minimal response of microalgal epiphytes in combination with the highly sensitive diatoms and consumers (meiofauna) towards nutrient enrichment was a major finding of the present study and underlines the differential impact of pollution on different levels of marine food webs. Furthermore, any additional pollutant discharged at the polluted site could have contributed to this finding.

The higher meiofauna densities in the unpolluted site in contrast to the polluted site suggest a more disturbed environment for meiofauna in the polluted site. This could be a result of physical disturbance attributed to sewage discharge around the polluted area (Hamisi et al., 2004). Sewage discharge may also result in the accumulation of deposits on the leaf surfaces that may reduce the habitable area and structural complexity for phytal meiofauna leading to a decrease in abundance and diversity. However, this did not seem to affect harpacticoid copepod densities. It could be attributed to their modified appendages and adapted body shape that allow them to holdfast onto the surfaces of the leaves even with the accumulation of deposits.

In terms of diversity, however, both meiofauna taxa and har-pacticoid copepod families showed that they were negatively affected by nutrient enrichment, except for the meiofauna communities associated with the seagrass *E. acroides*.

Meiofauna and copepod community structures (polluted vs. non-polluted) were more pronounced for *E. acroides*, *S. iso-etifolium* and *T. ciliatum* than for *T. hemprichii*. This could suggest that pollution affects the structure of meiofaunal communities in the first three seagrass species more in comparison to the ones on *T. hemprichii*. Previous studies have shown that expo-sure and tidal stresses are factors that influence the composition and microdistribution of phytal meiofauna (Hooper and Davernport, 2006). *T. hemprichii* is a typical intertidal species that remains exposed especially during spring low tides and experiences high wave energy in the intertidal zone while the other species found in the subtidal zone undergo low wave energy (L.N., pers. observ.).
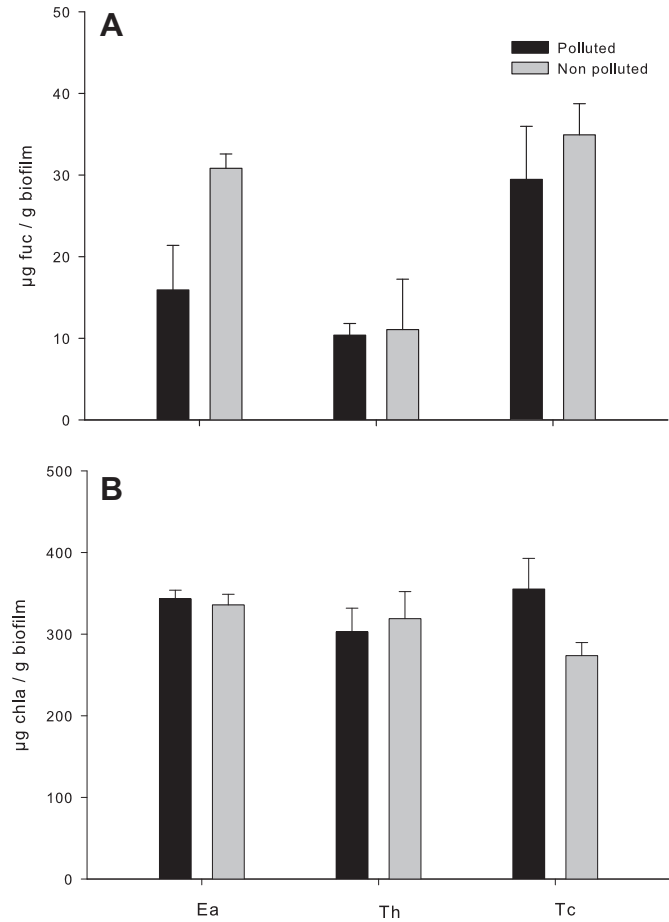
Fig. 8. Average levels (±standard error) of A) fucoxanthin and B) chlorophyll *a* in biofilms from seagrasses in both locations (polluted vs. non-polluted). Ea: *Enhalus acroides*, Th: *Thalassia hemprichii*, Tc: *Thalassodendron ciliatum*.

Fig. 9. Fatty acid profiling of the biofilm on different seagrass species in both locations. A) Polluted site. B) Non-polluted site. Ea: *Enhalus acroides*, Th: *Thalassia hemprichii*, Tc: *Thalassodendron ciliatum*.

At the level of enrichment in the analysed sites, it appears that meiofaunal abundance and community structure in general is indicative of enrichment. Consequently, we can state that higher taxon surrogacy is valid in this situation as higher meiofauna taxa show the main impact of nutrient enrichment (De Troch et al., 2008; Kallimanis et al., 2012). De Troch et al. (2008) showed that high taxon surrogacy is applicable for copepods i.e. the same trends were found at higher taxonomic level for both copepods and nematodes in coastal ecosystems along the Kenyan coast. In previous studies, focussing on the drawback of the difficulties to determine meiofauna, it has been stated that it may not be necessary to work at the species level to be of value in a pollution assessment context (Warwick, 1988).

However, since this level of enrichment did not clearly affect the microalgae it can be concluded that the effects of nutrient enrichment are small compared to the effects of other pollutants discharged within the site.

The nutrient organic enrichment in this case is one factor affecting meiofauna structures and diversities but there could also be sources of inorganic pollutants through the sewage input such as heavy metals and hydrocarbons that could also have contributed to the effect. Detailed information on the distribution of pollutants (organic and inorganic) along the East African coast is therefore needed in order to determine the levels of pollution to enable mitigation of these negative effects.

There was no variation in the benthic community structure and diversities for both locations other than for depth suggesting that
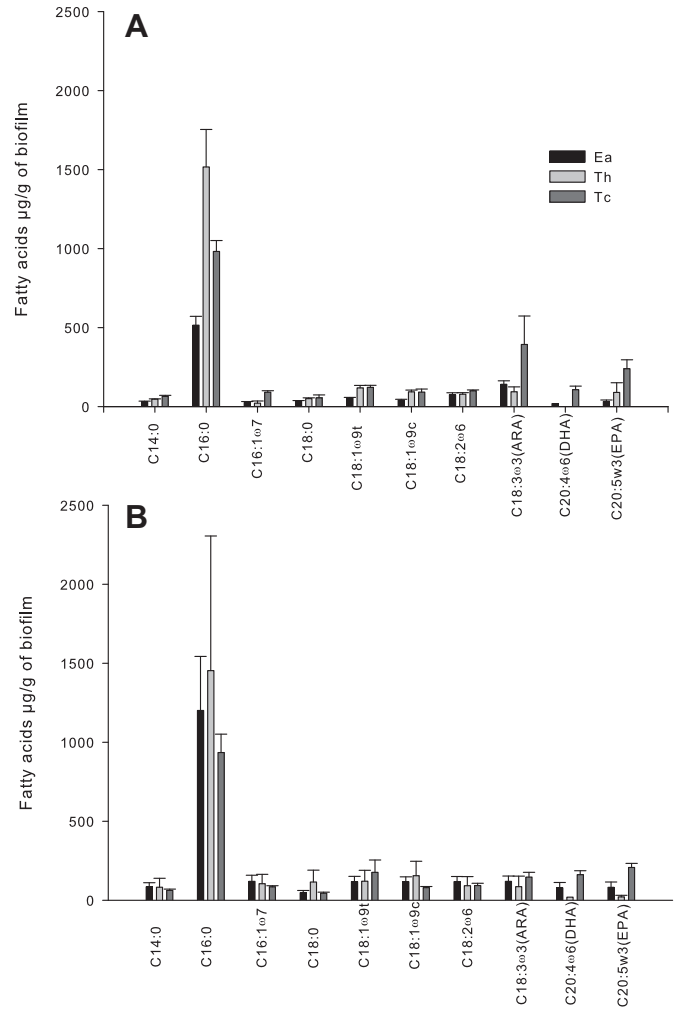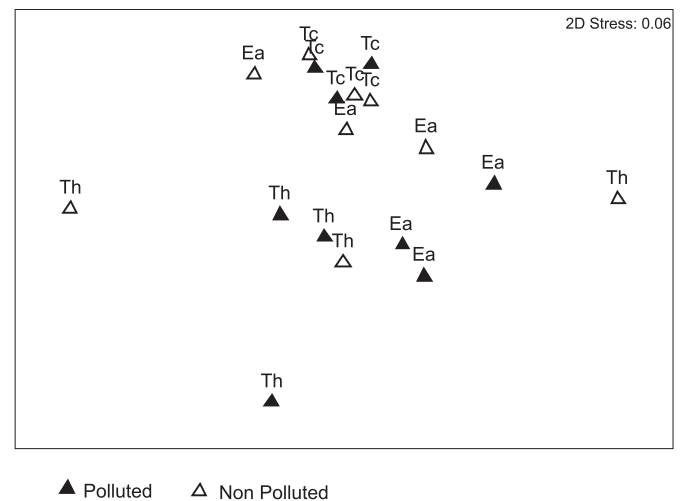

Fig. 10. MDS plot of fatty acid profiles of biofilms on seagrasses from the polluted and non-polluted site. Ea: *Enhalus acroides*, Th: *Thalassia hemprichii*, Tc: *Thalassodendron ciliatum*.

the impact of pollution was less effective on the benthic meiofauna communities in comparison to the epiphytic ones. The reason for this was not quite apparent; however, other studies have reported negative effects of nutrient enrichment on benthic meiofauna (Armenteros et al., 2010; Webb, 1996; Austen and Widdicombe, 2006). Meiofauna taxa were generally higher in the upper centimetre than in lower sediment layers. In the present study the disturbance may have only led to enrichment of organic matter suitable as food supply especially for a number of deposit feeding nematode species. The latter often become dominant when concentrations of organic matter increase (Moens and Vincx, 1997). This enrichment did not seem to be sufficient to affect the benthic meiofauna community structure. However, other factors such as variation of the physical properties and unequal distribution of food items in the sediment affect the vertical distribution of meiobenthic animals (Jansson, 1966; McLachlan et al., 1977) which was not considered in the present study.

The present study underlines the use of epiphytic meiofauna assemblages in seagrass ecosystems as general indicators of natural and anthropogenic disturbance to seagrass meadows. From these findings it was clear that the effect of nutrient loading could be depicted in the community structure and diversities of seagrass associated meiofauna and harpacticoid copepods. Moreover, the results showed that nutrient enrichment had a clear effect on the composition of the epiphytic meiofauna while their main food source (biofilm on the seagrass leaves) was not impacted. Especially in view of their pivotal role as trophic link between primary producers and secondary consumers, the results further suggest that any impact on the seagrass associated meiofauna will inevitably be transferred up to higher trophic levels. The energy transfer in coastal systems like seagrass beds will therefore be largely impacted and by extension also the overall ecosystem functioning e.g. in terms of the use of seagrass beds as nursery and feeding grounds for young fish. The study therefore illustrates how the food web structure and functioning of seagrass habitats can change with human impacts.

Acknowledgements

References

Abdulkadir, S., Tsuchiya, M., 2008. One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. Journal of Experimental Marine Biology and Ecology 354, 1–8.

Armenteros, M., Pérez-García, J.A., Ruiz-Abierno, A., Díaz-Asencio, L., Helguera, Y., Vincx, M., Decraemer, W., 2010. Effects of organic enrichment on nematode assemblages in a microcosm experiment. Marine Environmental Research 70, 374–382.

Austen, M.C., Widdicombe, S., 2006. Comparison of the response of meio- and macrobenthos to disturbance and organic enrichment. Journal of Experimental Marine Biology and Ecology 330, 96–104.

Bell, S.S., Hicks, G.R.F., 1991. Marine landscapes and faunal recruitment: a field test with seagrasses and copepods. Marine Ecology Progress Series 73, 61–68.

Bell, J.D., Pollard, D.A., 1989. Ecology of fish assemblages and fisheries associated with seagrasses. In: McComb, A.J., Larkum, A.W.D., Shepherd, S.A. (Eds.), The Biology of Seagrasses: an Australian Perspective. Elsevier, Amsterdam, pp. 565–609.

Bell, S.S., Hicks, G.R.F., Walters, K., 1988. Active swimming in meiobenthic copepods of seagrass beds: geographic comparisons of abundances and reproductive characteristics. Marine Biology 98, 351–358.

Boxshall, G.A., Hasley, S.H., 2004. An Introduction to Copepods Diversity, vol. xv. Henry Ling Ltd.

Bucolo, P., Sullivan, M.J., Zimba, P.V., 2008. Effects of nutrient enrichment on primary production and biomass of sediment microalgae in a subtropical seagrass bed. Journal of Phycology 44, 874–881.

Cambridge, M.L., How, J.R., Lavery, P.S., Vanderklift, M.A., 2007. Retrospective analysis of epiphyte assemblages in relation to seagrass loss in a eutrophic coastal embayment. Marine Ecology Progress Series 346, 97–107.

Clarke, K.R., Gorley, R.N., 2006. Primer V6: User Manual/Tutorial. Primer-E, Ltd, Plymouth.

De Troch, M., Fiers, F., Vincx, M., 2001a. Alpha and beta diversity of harpacticoid copepods in a tropical seagrass bed: the relation between diversity and species' range size distribution. Marine Ecology Progress Series 215, 225–236.

De Troch, M., Gurdebeke, S., Fiers, F., Vincx, M., 2001b. Zonation and structuring factors of meiofauna communities in a tropical seagrass bed (Gazi Bay, Kenya). Journal of Sea Research 45, 45–61.

De Troch, M., Raes, M., Muthumbi, A., Gheerardyn, H., Vanreusel, A., 2008. Spatial diversity of nematode and copepod genera along the coral degradation zone along the Kenyan coast, including a test for the use of higher-taxon surrogacy. African Journal of Marine Science 30, 25–33.

De Wolf, H., Ulomi, S.A., Backeljau, T., Pratap, H.B., Blust, R., 2001. Heavy metal levels in the sediments of four Dar es Salaam mangroves: accumulation in and effect on the morphology of the periwinkle, *Littoraria scabra* (Mollusca: Gastropoda). Environment International 26, 243–249.

Eder, K., 1995. Gas chromatographic analysis of fatty acid methyl esters. Journal of Chromatography 671, 113–131.

Fujiwara, M., Highsmith, R., 1997. Harpacticoid copepods: potential link between inbound adult salmon and outbound juvenile salmon. Marine Ecology Progress Series 158, 205–216.

Giere, O., 2009. Meiobenthology, second ed.. In: The Microscopic Motile Fauna of Aquatic Sediments, 528 pp.

Gray, J.S., 1997. Marine biodiversity: patterns, threats and conservation needs. Biodiversity and Conservation 6, 153–175.

Hamisi, M.I., Lyimo, T.J., Muruke, M.H.S., 2004. Cyanobacterial occurrence and diversity in seagrass meadows in Coastal Tanzania. Western Indian Ocean Journal of Marine Science 3, 113–122.

Heip, C., Vincx, M., Vranken, G., 1985. The ecology of marine nematodes. Oceanography and Marine Biology: An Annual Review 21, 67–175.

Heip, C., 1980. Meiobenthos as a tool in the assessment of the quality of the marine environment. Rapports et Proces-verbaux des Réunions. Conseil International pour l'Éxploration de la Mer 179, 182–187.

Hemminga, M.A., Duarte, C.M., 2000. Seagrass Ecology. Cambridge University Press, Cambridge.

Hicks, G.R.F., 1977a. Species composition and zoogeography of marine phytal harpacticoid copepods from Cook Strait, and their contribution to total phytal meiofauna, New Zealand. Journal of Marine Freshwater Research 11, 441–469.

Hicks, G.R.F., 1977b. Species associations and seasonal population densities of marine phytal harpacticoid copepods from Cook Strait New Zealand. Journal of Marine Freshwater Research 11, 621–643.

Hicks, G.R.F., 1977c. Observations on substrate preference of marine phytal harpacticoids (Copepoda). Hydrobiologia 56, 7–9.

Hicks, G.R.F., 1980. Structure of phytal harpacticoid copepod assemblages and the influence of habitat complexity and turbidity. Journal of Experimental Marine Biology and Ecology 44, 157–192.

Hicks, G.R.F., Coull, B.C., 1983. The ecology of marine meiobenthic harpacticoid copepods. Oceanography and Marine Biology: An Annual Review 21, 67–175.

Higgins, R.P., Thiel, H., 1988. Introduction to the Study of Meiofauna. Smithsonian Institution Press, 388 pp.

Hill, M.O., 1973. Diversity and evenness: a unifying notation and its consequences. Ecology 54, 555–557.

Hooper, G.J., Davernport, J., 2006. Epifaunal composition and fractal dimensions of intertidal marine macroalgae in relation to emersion. Journal of the Marine Biological Association of the United Kingdom 86, 1297–1304.

Huys, R., Gee, J.M., Moore, C.G., Hamond, R., 1996. Marine and brackish water harpacticoid copepods. Part 1 keys and notes for identification of the species. In: Barnes, R.S.K., Crothers, J.H. (Eds.), Synopses of the Britain Fauna (New Series) No. 51. Field Studies Council, Shrewsbury.

Jansson, B.O., 1966. Microdistribution of Factors and Fauna in Marine Sandy Beaches, vol. 2. Veröffentlichungen des Instituts für Meeresforschung, Bremerhaven, pp. 77–86.

Jaschinski, S., Flöder, S., Sommer, U., 2010. Consumer identity, abundance and nutrient concentration affect epiphyte diversity in an experimental eelgrass system. Oikos 119, 1745–1754.

Kallimanis, A.S., Mazaris, A.D., Tsakaniras, D., Dimopoulos, P., Pantis, J.D., Sgardelis, S.P., 2012. Efficient biodiversity monitoring: which taxonomic level to study? Ecological Indicators 15, 100–104.

Lang, K., 1965. Copepoda: Harpacticoidea from the Californian Pacific Coast. Kungliga Svenska Vetenskapakademiens.

Lugendo, B.R., Mgaya, Y.D., Semesi, A.K., 1999. The seagrass and associated macroalgae at selected beaches along Dar es Salaam Coast. In: Richmond, D.M.,

Francis, J. (Eds.), Marine Science Development in Tanzania and East Africa, pp. 359–373.

Lyimo, T.J., 2009. Microbial and nutrient pollution in the coastal bathing waters of Dar es Salaam. Aquatic Conservation: Marine and Freshwater Ecosystems 19, 27–37.

Machiwa, J.F., 1992. Heavy metal content in coastal sediments off Dar es Salaam, Tanzania. Environment International 18, 409–415.

McLachlan, A., Winter, P.E.D., Botha, L., 1977. Vertical and horizontal distribution of sublittoral meiofauna in Algoa Bay, South Africa. Marine Biology 40, 355–364.

Mamboya, F., Lugomela, C., Mvungi, E., Hamisi, M., Kamukuru, A.T., Lyimo, T.J., 2009. Seagrass–sea urchin interaction in shallow littoral zones of Dar es Salaam, Tanzania. Aquatic Conservation: Marine and Freshwater Ecosystems 19, 19–26.

Moens, T., Vincx, M., 1997. Observations on the feeding ecology of estuarine nematodes. Journal of the Marine Biological Association of the United Kingdom 77, 211–227.

Mohammed, S.M., 2000. The Assessment of Water Quality and Pollution in Tanzania. http://hdl.handle.net/1834/183.

Okuku, E.O., Mubiana, V.K., Hagos, K.G., Peter, H.K., Blust, R., 2010. Bioavailability of sediment-bound heavy metals on the East African coast. Western Indian Ocean Journal of Marine Science 9, 31–42.

Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology: An Annual Review 16, 229–311.

Rumisha, C., Elskens, M., Leermakers, M., Kochzius, M., 2012. Trace metal pollution and its influence on the community structure of soft bottom molluscs in intertidal areas of the Dar es Salaam coast, Tanzania. Marine Pollution Bulletin 64 (3), 521–531.

Short, F.T., Wyllie-Echeverria, S., 1996. A review of natural and human-induced disturbance of seagrasses. Environmental Conservation 23, 17–27.

Sogard, S.M., 1984. Utilisation of meiofauna as a food source by a grassbed fish, the spotted dragonet *Callionymus pauciradsiatus*. Marine Ecology Progress Series 17, 183–191.

Snelgrove, P.V.R., et al., 1997. The importance of marine sediment biodiversity in ecosystem processes. Ambio 26, 578–583.

Vincx, M., Heip, C.H.R., 1991. The use of meiobenthos in pollution monitoring studies: a review. ICES Techniques in Marine Environmental Science 16, 50–67.

Wagner, G.M., 2007. The Dar es Salaam Seascape: a case study of an environmental management 'hotspot'. Western Indian Ocean Journal of Marine Science 6, 85–109.

Warwick, R.M., Clarke, K.R., 1993. Increased variability as a symptom of stress in marine communities. Journal of Experimental Marine Biology and Ecology 172, 215–226.

Warwick, R., 1988. Analysis of community attributes on the macrobenthos of Frierfjord/Langesundfjord at taxonomic levels higher than species. Marine Ecology Progress Series 46, 167–170.

Wear, D.J., Sullivan, M.J., Moore, A.D., Millie, D.F., 1999. Effects of water-column enrichment on the production dynamics of three seagrass species and their epiphytic algae. Marine Ecology Progress Series 179, 201–213.

Webb, D.G., 1996. Response of macro- and meiobenthos from a carbon-poor sand to phytodetrital sedimentation. Journal of Experimental Marine Biology and Ecology 203, 259–271.

Wright, S.W., Jeffrey, S.W., Mantoura, R.F.C., Llewellyn, C.A., Bjsrnland, T., Repeta, D., Welschmeyer, N., 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Marine Ecology Progress Series 77, 183–196.