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Restoration of Kenyan seagrass beds: a functional study of the associated fauna and flora



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Dedication

To my loving family that made me what I am today, Dad, Mum, Janet, Geoffrey and Mercy and for all the inspiration you have accorded to me, always believing in me, not forgetting my sweet nephew Ian Mutisia whom I cherish so much.

Abstract

Seagrass communities are subject to frequent anthropogenic and natural disturbances that can lead to alterations in vegetation complexity and hence may affect associated fauna. Seagrass loss in Kenya has been mainly due to extensive grazing by the sea urchin Tripneustes gratilla which has affected almost the entire coastline. This has led to habitat fragmentation and sometimes vast areas of defoliated beds that were formerly covered with seagrass. The most affected species has been Thalassodendron ciliatum. Diani beach, south of Mombasa, is an area that has been typically affected by seagrass depletion. Natural recovery has been reported in certain areas and transplantation projects were started. The challenge is to see if the system can recover fully and will be able to function as before. To test this, the current study focused on the density, diversity and community structure of meiofauna, and more specifically of harpacticoid copepods as a measure of the ability of the system to recover. Artificial seagrass mimics were planted in natural, replanted and areas of bare sand and harvested in a series of 2, 4, 6, 10, 14 and 21 days in order to collect the associated meiofauna. Related environmental parameters were collected at the same time intervals except for the sediment samples for environmental analysis that were sampled once during the study period at the last day of collection of mimics.

Significant differences of meiofauna densities between the sites and the colonization days were found but for harpacticoid copepods there was only a significant effect of the colonisation time. The densities of meiofauna reached those of natural seagrasses by day 4 but most of them were opportunistic species and not true phytal dwelling meiofauna. Both passive migration from neighbouring seagrass patches and active migration from sediments were observed, based on the harpacticoid copepod family composition. In the bare and replanted sites similar community structures of harpacticoid copepods were observed from day 6 onwards while for the healthy site it was from day 10. Colonization by epiphytic biofilm was collected from day 10 in the bare and replanted sites but not the healthy site. In the previous days it was too negligible to be collected. The results thus suggest possible recovery of harpacticoid copepods after disturbance thanks to their mobility and ability to colonize new areas quickly. However, this may depend largely on the time the epiphytic flora are able to recover as well as the recovery time of the seagrass plants which may take approximately 4 years.

Keywords: Disturbance, Ecosystem recovery, Seagrass beds, Meiofauna

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1. Introduction and Literature Review

Seagrass beds in tropical regions support a large variety of associated fauna and flora with several ecological characteristics (Hemminga & Duarte, 2000). They provide food, shelter and nurseries for several animals, including many commercially important fish and shellfish species (Bell et al. 1989) and create remarkably high rates of secondary productivity (Edgar, 1990; Fredette et al. 1990; Valentine & Heck 1993). The 3-dimensional structure of seagrass beds contains a broad spectrum of microhabitats and niches making them convenient as permanent and transient residences for various benthic, demersal and pelagic organisms (Kikuchi & Peres 1997; Robbins & Bell 1994). Animals living within the sediment are dominated by invertebrate species of crustaceans (harpacticoid copepods, amphipods, and ostracods), bivalves, polychaetes, nematodes, cumaceans, holothuroids and phoronoids (Howard et al. 1989). Seagrasses attract an assortment of organisms to proliferate attached to the stem and leaf canopy. Tropical seagrass plants are often inhabited by colonies of sessile fauna like bryozoans and hydroids (Kikuchi & Peres 1997), in association with epiphytic algae (Kitting, 1984; Montfrans et al. 1984). The fouling community also includes motile meiofaunal organisms such as amphipods, harpacticoid copepods. ostracods, nematodes. turbellarians. polychaetes. foraminiferans and gastropods (Bell et al. 1984; Kitting, 1984; Kikuchi & Peres 1997). Occasionally, substantial quantities of suspension feeding ascidians are attached to seagrass leaves (Lemmens et al. 1996). The major taxa living on the sediment surface in seagrass beds are echinoderms (starfish, sea urchins, brittle stars and sea cucumbers), crustaceans (crabs) and molluscs (bivalves and snails) (Kirkman et al. 1991; Kalk, 1995). The epibenthic fauna also consist of mobile animals inhabiting the water over and under the seagrass leaf canopy, and includes fish, decapods, crustaceans (prawns and shrimps) and cephalopods, as well as small crustaceans like mysids, copepods (cyclopoids and calanoids), amphipods and isopods (Kikuchi & Peres 1997; Pollard, 1984; Sanchez et al. 1996). Many species spend their post larvae and juvenile stages in the seagrass beds before they migrate into other habitats. The presence of juveniles of commercially important penaeid prawns, with peak abundances during relatively short periods of the year, have been reported in a

number of studies (Staples *et al.* 1985; deFreitas, 1986; Duke, 1987; Sheridan, 1992; Coles *et al.* 1993).

Seagrasses are a valuable habitat for marine fauna as it provides the basis of a detrital food chain, sediment stabilisation, nutrient cycling and a refuge from predation for small and juvenile fish and macroinvertebrates (Orth *et al.* 2006).

Seagrasses provide habitat for a large set of organisms which can not live in unvegetated bottoms. The leaf canopy and the network of rhizomes and roots provide substratum for attachment, which is scarce in unconsolidated bottoms, stabilize the sediment, and reduce irradiance producing an array of microhabitats not present in unvegetated bottoms. In addition, the three-dimensional structure of seagrasses creates hiding places to avoid predation. As a result, the abundance and diversity of the fauna and flora living in seagrass meadows are consistently higher than those of adjacent unvegetated areas hence increase habitat diversity and contribute to the overall biodiversity of the coastal zone.

Seagrass communities are subject to frequent disturbance, whether anthropogenic (e.g. shoreline construction, eutrophication, mechanical damage) or natural (sand wave motion, storms and hurricanes/typhoons, overgrazing) that lead to alterations in vegetation complexity (Snelgove *et al.* 1997; De Troch *et al.* 2001a; Gray, 2004). Grazing, a factor which can influence seagrass production and distribution is a natural disturbance in tropical seagrass meadows (Heck & Valentine 2006). Two key parameters of disturbances in general and grazing in particular are their intensity and frequency.

Overgrazing of seagrasses by sea urchins may be triggered by reduced predation by fish and eutrophication. In severe cases, such overgrazing could decimate entire seagrass meadows (Eköf *et al.* 2008). Theory predicts that increasing herbivore diversity should reduce plant community biomass as the most efficient grazers come to dominate a system, leading to overgrazing (Holt & Loreau 2002). This can also depress plant diversity and facilitate invasion of grazing-resistant species (Leibold *et al.* 1997).

Intensive grazing by sea urchins is important in determining the structure and abundance of both macrophyte and seagrass assemblages in many marine littoral ecosystems. Decline of seagrasses has occurred as a result of sea urchin grazing where macroalgal kelp forests have been converted to grazer resistant coralline dominated algal pavements in temperate and boreal settings (Lawrence, 1975).

In marine kelp forests over harvesting of predatory sea otters was observed to lead to large increases in the density of their sea urchin prey, which subsequently brought about the loss of kelps as they were overgrazed by the urchins (Estes & Palmisano 1974; Duggins, 1980).

Nevertheless, in the tropical western Atlantic, studies have reported sea urchin overgrazing of large meadows of *Thalassia testudinum* (Camp *et al.* 1973) and *Syringodium filiforme* (Maciá & Lirman, 1999; Rose *et al.* 1999).

The ocean's predators have been greatly reduced by fishing, and many popular articles have increased the public's awareness and concern about the consequences of removing great numbers of predators from the world's oceans (Parfit, 1995; Safina, 1995). The drastic reductions in many species of preferred fishes may be extensive enough to endanger the function of entire marine ecosystems. Fishing activities have been ranked as the most serious threat the oceans now face (Sciences, 1995).

The seagrass community of the East African coastline is composed of 12 species, belonging to 8 genera, and each of these supports on their leaves a diverse phyllosphere microbiology or epiphytic community composed of a variety of macroalgae as well as faunal associations (Isaac, 1968a; Bandeira, 1995; Lindow & Brandl 2003).

Some signs of seagrass recovery where overgrazing had taken place have been seen in areas of *Thalassodendron ciliatum* along the Kenyan coast but this is not the case everywhere (Uku 2005). Seagrass transplantations trials have also been tried to establish whether seagrass restoration is also possible in Kenya (unpublished data).

Therefore seagrass ecosystems provide lots of ecosystem functions that could be lost with an increasing degradation of seagrasses which partly is due to overgrazing but also includes so many other factors that are anthropogenic.

Diversity patterns are essential to understand the organization and functioning of organisms present in an ecosystem and their interaction with the environment (Duarte, 2000). Loss of biodiversity may result in the loss of ecosystem functions and the many services they provide to society (Constanza *et al.* 1997). Testing the link between biodiversity and ecosystem functions and services is essential to demonstrate a significant ecosystem role for biological diversity (Tilman, 1997).

The present study dealt with faunal biodiversity as a parameter of ecosystem functioning and whether there is possibility of maintaining this after a natural disturbance attributed to overgrazing. The study looked at meiofauna with special emphasis on harpacticoid copepods. This is because they play an important functional role and because a large part of the energy flow passing the community is dissipated through epiphytic systems and is thus transferred to higher trophic levels (Asmus, 1985; Klumpp, 1992; Asmus 2000). They also represent an important link between primary producers and higher trophic levels (Sogard, 1984; Fujiwara & Highsmith 1997; Sutherland *et al.*2000) and are abundant in seagrass beds (Hicks, 1977a, b, c; Hicks, 1980; Bell *et al.* 1988; Bell & Hicks 1991; De Troch *et al.* 2001a, b). Studies have also been done on harpacticoid copepods and their response to small scale natural disturbance (Thistle, 1980), species diversity changes within and between habitats in tropical seagrass beds (De Troch *et al.* 2001a), colonization and recruitment in seagrass mimics (Bell & Hicks 1991; Walters & Bell 1994; De Troch *et al.* 2001b).

1.1 Objectives

The main objective of the study was to determine the abundance composition and biodiversity of meiofauna with special emphasis on harpacticoid copepods in tropical seagrass beds in Diani, Kenya.

Specifically the study aimed to determine

- epiphytic meiofauna (copepods) on seagrass leaves
- epiphytic plants (primary producers) on seagrass leaves
- benthic meiofauna on seagrass sediments
- Water parameters (nutrients, pigments, fatty acid, C/N)
- · Sediment parameters (nutrient, grain size, porosity, organic matter)

2. Materials and methods

2.1 Site Description

The Kenyan coast has a rich and diverse fauna of marine algae and seagrasses (Isaac, 1968b). Twelve seagrass species are present in Kenya in extensive beds that cover the largest proportion of shallow reef slopes, both the back-reef and fore-reef. The dominant seagrass species is *Thalassodendron ciliatum* which attaches to both hard and soft substrates. Its structure provides habitat for small and juvenile fish and invertebrates, making seagrass beds important habitats for many coral reef species (see introduction).

Diani Beach is situated at approximately 72 km south of Mombasa at latitude $4^{\circ}21$ 'S – $39^{\circ}33$ 'E on the coast of Kenya separated from the main body of the Indian Ocean by a fringing coral-reef platform that is about 0.9 km wide. Surveys carried out in the Diani-Chale lagoon area indicated a loss of up to 50% seagrass cover and an increase in the proportion of sand within the lagoon (unpublished).



Figure 1: Map of the Kenyan coast with indication of the sample site Diani

2.2 Field sampling and Experimental Set-up

The sampling campaign was conducted between 15th August and 6th September 2008 in Diani, Kenya. The study focused on the biodiversity of the meiofauna occurring on seagrass leaves in three different sites within the southern coastal zone of Kenya (Diani): bare sand (approx. 240m from shore), healthy seagrasses (approx 500m from shore), and an area with recently replanted seagrasses (approx. 230m from shore). The replanted seagrasses had been there for about one year though most of them were lost during the intermonsoon season around July 2008. The healthy and replanted seagrasses belonged to the species Thalassodendron ciliatum, a seagrass species that had been adversely affected by sea urchin population explosions. These were found in the subtidal waters in the study area. In addition to an evaluation of the field situation. seagrass mimics (plastic seagrasses, Biomodels http://cgbiomodels.com/) were planted in the field close to the three selected sites. The seagrass mimics however resembled Thalassia hemprichii. The mimics were light green in colour and were 35.7 ± 2.3 cm and 0.8 ± 0.1 cm long and wide, respectively. Each mimic plant consisted of 4 green and 1 brown leaf resembling natural plants with fresh (green) and dead (brown) leaves. Total leaf length per plant (5 leaves) therefore was 178.4 ± 12.9 cm corresponding to an average plant surface area of 146.8 \pm 17.3 cm. The total surface area for each replicate was 293.7 \pm 34.7 cm since two clumps (10 leaves) were used per replicate. For the natural seagrass the average length of leaves was 12.8 ± 1.5 cm and the average total length was 104.3 ± 4.4 cm. The surface area for the shoots collected was approximately 1304.4 ± 238.9 cm per replicate.

The artificial (plastic) seagrasses represented a disturbed ecosystem at T_0 ready to be colonized by epiphytic meiofauna and flora. The planted mimics were to be harvested at different time intervals with a maximum of 21 days.

Three sets of mimics were available where the first set was for Day 2, 6 and 10, the second for Day 4 and 14 and the last set for Day 21 (Fig. 2). For each day 3 replicates were planted/ harvested for each site. Each replicate consisted of 2 clumps of 10 'leaves' each (20 in total). To make efficient use of the mimics they were harvested in three series (see colour code in Fig. 2) and were replanted again after collection and

cleaning using a soft kitchen scouring pad (see Fig 2). Only the set for Day 21 was left in the field for the whole period. Sampling was done during low tides and the average water cover at sampling was 0.7m.



Figure 2: Experimental set-up of the colonisation experiment



Figure 3: Harvesting schedule

2.2.1 Planting seagrass mimics

Due to the loose sediment metal rods of about two inches in height were used to 'plant' the seagrass mimics in the sea. These rods were hammered deep into the sediment and only part of the hook was above the sediment to attach the mimics. Cable ties were used to tie the mimics to the hook and ribbons were used to identify the three sets of mimics.



Figure 4: a) Metal rods used to attach seagrass mimics b) Planted seagrass mimics

2.2.2 Epiphytic meiofauna

Triplicate samples of the mimics were harvested after 2, 4, 6, 10, 14, 21 days to follow the colonisation in time. Harvesting was done by snorkelling and placing plastic bags over the mimics and cutting the cable ties. The mimics together with the water in the plastic bags were brought to the boat closed with rubber bands to avoid loss of the water hence loss of meiofauna. 8 % of magnesium chloride was added to the bags for 15 minutes to detach the meiofauna from the leaves and the contents of the plastic bags was collected over a 38 μ m sieve. The mimics were rinsed thoroughly using filtered sea water over the 38 μ m sieve and a funnel used to collect epiphytic fauna. The samples were collected in plastic bottles and 8% formalin was added to bring the solution (sample mixed with formalin) to a final concentration of 4%.

The same was done for meiofauna on natural seagrasses that were collected during the last day of the sampling period.

2.2.3 Epiphytic plants

The biofilm was removed after collecting the epiphytic fauna by scraping the leaves using the blunt side of a surgical blade. The blades were cleaned carefully on preweighed GF/F filters of known weight. The filters were folded into two (material inside) and wrapped in aluminium foil. They were labelled and brought back to the lab where they were frozen as soon as possible. For the natural seagrasses the same was done and the length and widths of the leaves was determined as 12.8 ± 0.3 cm and 1.5 ± 0.07 cm, respectively. The wet weight of the leaves were also determined as well as the weight of the leaves after the removal of the epiphytes. The leaves were then dried in an oven at 60°C.

2.2.4 Sediment Meiofauna

Polyvinyl chloride (PVC) cores (3.6 cm inner diameter, 10 cm² surface) were used to collect meiofauna from the sediment. Sediment cores were sliced to analyze meiofauna at different depths (0-1cm, 1-2cm, 2-5 cm). These were also preserved in 4% formalin (final concentration). Sediment samples were collected at the beginning of the experiment before planting the mimics.

2.2.5 Grain size analysis

Two replicates of sediment were collected from the healthy, bare and replanted sites for grain size analysis using a core. They were then stored in plastic bags and frozen in the lab for further analysis. The samples were collected at the end of the sampling period.

2.2.6 Sediment nutrients

PVC cores were used to collect the sediments for nutrient determination from three depths (0-1cm, 1-2cm and 2-5 cm). Two replicate samples were collected for the healthy, bare and replanted site respectively. The samples were put in a coolbox in the field and transported to the lab where they were stored frozen. These were also collected at the end of the experiment.

2.2.7 Water Nutrients

Water was collected in small scintillation jars at ³/₄ volumes and frozen for further nutrient analysis. This was done during each sampling occasion. Additional nutrient samples were collected for analysis in KMFRI (Kenya) where 500 ml of water was collected once during the sampling period.

2.2.8 Other biochemical analysis

A known volume of water was filtered through a pre-weighed GF/F filters and the filters were fold into two (material inside) and put in a coolbox. They were later frozen in the lab. These were collected during all sampling days.

2.3 Laboratory analysis

2.3.1 Sediment nutrient analysis

Nutrients in the pore water in the sediment was first extracted by taking approximately 10 g of sediment then adding 40 ml 1M KCl flash with nitrogen gas (2 minutes) and shaking for 2 hours, to ensure maximum extraction. The sample was then centrifuged at a speed of 2000x g.r.m. for 10 minutes. The extract was decanted and diluted with distilled water and used for the determination of nutrients.

 NH_4^+ was determined according to the procedure of Parsons *et al* (1984). By this method ammonium ion in the sample is buffered in alkaline citrate medium and then treated with sodium nitroprusside (which acts as a catalyst). The reaction in this mixture gives a complex, indophenols whose blue colour intensity is measured calorimetrically at 630 nm.

 NO_3^- was determined according to the procedure of ALPHA (1992) in which NO_3 is reduced to NO_2 by passing through a reduction column packed with cadmium filing coated with copper. The nitrite is reacted with sulfanilamide in acidic solution where the resulting diazo compound was complexed with N-(-1-naphthyl) – ethylenediamine to form a highly coloured azo dye whose intensity is measured at 543 nm.

For phosphates the sample was mixed with a phosphate reagent containing molybdic add, ascorbic acid and potassium antimony III tart rate. The resulting complex formed was reduced by ascorbic acid with trivalent antimony ion as catalyst to give a blue colour solution whose intensity is measured at 885nm (Parsons *et al* 1984).

Standards were prepared for all the nutrients.

2.3.2 Water nutrient analysis

Frozen samples were thawed and analysed for NO_2 , NO_3 , NH_4 , PO_4 and SiO_2 concentrations using an A_{II} automatic chain (SANplus Segmented Flow Analyzer, SKALAR, UGent).

2.3.3 Grain size analysis

Grain size composition was determined using the dry sieving method. This is because the sediment samples had low or no percentage of fine silts and clays. 100 g of the wet sediment sample was spread over an enamel pan and dried in an oven at 105°C until the weight was constant. The sediment was then passed through a series of sieves (2.00 mm, 1.60 mm, 1.00 mm, 500 μ m, 250 μ m, 125 μ m, 63 μ m, 38 μ m). The remainder, if any was collected in a pan below. The sieves were then removed and their content weighed and noted down.

2.3.4 Organic matter content

A known weight of sediment sample was put in aluminium of foil known weight and was ashed. The organic matter content was determined as (dry weight – ash weight)/ dry weight and expressed as a percentage.

2.3.5 Porosity

To calculate porosity water content was determined by putting sediment samples of known weight on aluminium foil also of known weight and keeping them in the oven for 12 hours and transferring them to a dessicator. The % water content was calculated from the wet weight (Sample and foil weight before drying – foil weight) and dry weight (Sample and foil weight – foil weight after drying). The porosity was calculated as water content and the product of % sediment density.

2.3.6 Pigment analysis

Pigments from water and epiphytic algae were extracted in 90% acetone at 4°C in the dark and separated by reverse phase liquid chromatography on a Gilson C-18 HPLC-chain (spectrophotometrical and fluorometrical detection) according to a modified protocol of Montoura and Llewellyn (1983).

2.3.7 Meiofauna

Benthic samples were decanted 5 times over a 38 μ m mesh sieve, centrifuged five times with Ludox (specific density 1.18) and stained with Rose Bengal solution. The epiphytic meiofauna were not centrifuged but directly stained with Rose Bengal. The meiofauna was counted and identified at high taxon level based on Higgins and Thiel (1988) using a binocular. Copepods were collected from the samples by an 'eye shaped' needle to be identified to the lowest taxon level possible (specifically for harpacticoid copepods). At least 100 individuals were collected for identification where the numbers exceeded 100 individuals. For samples with less then 100 individuals, all copepods were picked. The copepods were stored in 75% ethanol in

small glass tubes and later they were mounted in glycerine (5 individuals per slides) with the swimming legs facing upwards for ease of identification. The copepods were then identified using a stereo microscope. Harpacticoid copepods were identified to the family level using Boxshall and Hasley (2004), Lang (1948; 1965) and original species description.

2.4 Calculating Biodiversity

Biodiversity was calculated for both epiphytic and benthic meoifauna using the indices of Hill (N_0 , N_1 , N_2 and N_{∞}) (Hill, 1973).

2.5 Statistical Analysis

Assumptions of analysis of variance (ANOVA) were examined using box and normal probability plots. To test for homogeneity of variances which is an assumption for using ANOVA Levene's test was used. Factorial ANOVA was used to test for significant differences in the densities and diversities of the different sites and harvest days using STATISTICA 8. Non parametric tests were used to analyze the benthic harpacticoid samples. For the mimics the two factors used were colonization days and site. For factorial ANOVA post hoc analysis was done using the Tukey HSD test. The community structure of the different samples was analysed by means of MDS plots in PRIMER 6 software. Testing relationship between community composition and environmental variables was done using BioEnv.

3. Results

3.1 Meiofauna composition, diversity and community structure

3.1.1 Epiphytic meiofauna

A total of 21 groups including both epiphytic and benthic meiofauna were counted in the samples. These groups were Nematoda, Isopoda, Copepoda, Ostracoda, Polychaeta, Amphipoda, nauplii, Gastropoda, Tanaidacea, Cumacea, Turbellaria, Cnidaria, Rotifera, Tardigrada, Oligochaeta, Halacaridae, Cladocera, Insecta, Thermosbaenacea, halacarid mites and Ciliophora.



The densities of the epiphytic meiofauna were as shown in the figure 5 below.

Figure 5: Total meiofauna densities (± standard error) in three sites at different colonization days (left figure) and on natural seagrass sampled (right figure)

Epiphytic meiofauna densities ranged between 5 and 97 $ind/100cm^2$ during the colonisation experiment. The densities of the epiphytic meiofauna were low during day 2 and increased towards day 4. However the densities dropped at day 6 (~ by a factor of 3 for all sites) and on day 10 they increased ten times for the healthy site and doubled for both the bare and replanted sites. The densities stabilized for all sites on day 14 but on day 21 they reduced 1.5 times for the healthy and bare sites and six times for the replanted site. The highest densities for the bare and replanted sites were observed at day 4 while for the healthy site it was at day 10.

The natural seagrass species *Thalassodendron ciliatum* was sampled (three replicates) once during the sampling period and the average epiphytic meiofauna was 83 ± 21.1 ind/100cm² (fig. 4). The highest average densities observed on the mimics at the

replanted and bare site were 76 and 96 ind/100 cm² respectively at day 4 while for the healthy site it was 62 ind/100 cm² at day 10 (fig 5). The densities were on average higher in the natural seagrass compared to the densities on seagrass mimics. However, the bare site at day 4 recorded higher densities than the natural situation (76 ind/100 cm²).

Table 1: ANOVA Univariate analysis of meiofauna densities collected from seagrass mimics inDiani, Kenya

Factors	df	SS	MS	F	р
Intercept	1	4003893	4003893	154.1246	0.000000
Day	5	648427	129685	4.9921	0.001634
Site	2	187271	93636	3.6044	0.038387
Day*Site	10	294661	29466	1.1343	0.367727
Error	33	857283	25978		
Total	50	1970629			

Bold characters imply significance at p < 0.05

Statistical analysis showed significant differences in meiofauna densities that were mainly due to the site effect and the sampling day (duration of the colonisation) as shown in the table above (p<0.05). Post hoc Tukey HSD indicated that only day 4 (replant) and day 6 (healthy) were significantly different from each other (p<0.05).

	$N_{0 \pm SE}$	$N_{1\pm SE}$	$N_{2\pm SE}$	$N_{inf \pm SE}$
Bare	9.12 ± 3.02	3.82 ± 1.07	0.07 ± 0.57	1.95 ± 0.72
Replant	10.31 ± 0.64	3.95 ± 0.27	0.01 ± 0.00	1.93 ± 0.12
Healthy	10.28 ± 0.70	4.02 ± 0.27	0.01 ± 0.00	1.92 ± 0.10
Natural	11 ± 1.53	4.6 ± 0.39	3.34 ± 0.55	2.23 ± 0.4

Table 2: Table showing the calculated Hill's indices for the sites sampled

Mean Hill diversity indices for the whole colonization period were calculated and are shown in the table above. Taxa richness (N₀) was higher for healthy and replanted site on average as compared to the bare site. It was however high for the natural seagrass samples when compared to mimics. N₁ was higher in the healthy site followed by the replanted and the bare site but high for the natural seagrasses compared to the mimics. There was high dominance on the mimics compared to the natural seagrass sampled while the healthy site had the highest dominance followed by the replant then the bare site. A parametric test did not show any significant difference between either sites or days for taxa richness (N₀) and Shannon diversity (N₁) and dominance index (N_{inf}). N₂ did not pass the Levene's test hence Kruskal-Wallis was used which showed no statistical significant difference between the sites but for the days there was significant difference (p< 0.05). Dunn's post-hoc tests revealed a significant difference between day 2 and day 4.



Figure 6: Relative abundance of dominant meiofauna groups at different colonization days. A-Bare; B- Replant; C- Healthy; D-Natural

Nauplii dominated the meiofauna community for the replant and healthy sites initially (until day 6) and later on the dominance shifted towards adult copepods and copepodites. For the bare sediment at day 2 (one replicate) copepods dominated but at day 4 nauplii were the dominant group. From day 6 onwards, copepods dominated in relative abundance as in the other two sites explained above. Nematodes were of second importance after copepods. For the healthy site, day 6 had the lowest abundance of nauplii but increased on day 10 and 14 and finally reduced at day 21. For the bare and replanted sites the highest abundance of nauplii was at day 4 (>50%) and the abundance reduced further towards the last harvest day (day 21).

The natural seagrasses harboured high relative copepod abundance (47%), followed by nematodes (24%).



A





Figure 7: Community structure of epiphytic meiofauna in the three sampling sites, A-Bare; B - replant; C- Healthy (numbers represent the day of harvest and the last letter represents the replicate)

There was no defined community structure for the bare areas in the different colonisation days (Fig. 7A). However, especially in the healthy site (Fig. 7C) the communities that colonised for a longer time (from day 10 onwards) grouped together and differed from the early colonisation phase (day 2 to day 6). This was also true but to a lesser extent for the replanted site (Fig. 7B).



3.1.2 Benthic Meiofauna

Figure 8: Average meiofauna densities (\pm standard error) in the different sites at different sediment layers

Benthic meiofauna densities ranged from 200- 600 ind/10cm² in the top layer (0-1cm), 377-705 ind/10cm² in the middle layer (1-2cm) and 490-685 ind/10cm² in the lowest layer sampled (2-5cm). Meiofauna densities were high on the top sediment layer in the bare site while the replanted site had the highest density in the deepest sediment layer (2-5cm). The healthy site had the highest number on the middle layer (1-2cm). ANOVA results did not show any statistical significant differences in the benthic meiofauna densities between sites and layers.

Diversity of meiofauna was calculated using Hill's diversity indices (N_0 , N_1 , N_2 , and N_{∞}).

Table 3: Table showing the calculated Hill's indices for the benthic meiofauna of the sites sampled averaged for the different layers

	$N_{0 \pm SE}$	$N_{1 \pm SE}$	$N_{2 \pm SE}$	$N_{inf \pm SE}$
Bare	6.63 ± 0.50	2.4 ± 0.23	1.91 ± 0.21	1.5 ± 0.14
Replant	4.67 ± 0.5	2.03 ± 0.09	1.25 ± 0.24	1.33 ± 0.05
Healthy	6 ± 0.6	1.89 ± 0.19	1.54 ± 0.16	1.32 ± 0.12

Taxa richness was highest in the bare site followed by the healthy and the replanted site (Table 3). Shannon diversity (N_1) was also highest in the bare site followed by the replanted site and was lowest in the healthy site. N_2 was also high in the bare site followed by the healthy and the replanted site. There was higher dominance (N_{inf}) in the healthy site than the replant and the bare sites.

All data was parametric and ANOVA results showed no significant statistical differences between the sites and sediment layers for all Hill's indices calculated.



Figure 9: Relative abundance (%) of meiofauna between sediment layers in all the sites. A-Bare; B- Replant; C- Healthy

For benthic meiofauna abundant groups included Polychaeta, Copepoda, Turbellaria, and Nematoda as dominant group in terms of relative abundance (fig.9 above).



Figure 10: Community structure for the benthic meoifauna at different depths (1:0-1 cm; 2: 1-2 cm and 3: 2-5 cm; A, B, C represent replicates) Fig A- Bare; B- Replant and C- Healthy site

There was no clear structure in the communities with the different depths other than in the replanted site where the communities for the different depths grouped together.

3.2 Copepod composition, diversity and community structure

3.2.1 Epiphytic copepods

A total of 17 families of the order Harpacticoida were identified at all sites in the study area. Other orders like Cyclopoida and Calanoida were also observed but only identified at the order level.



Figure 11: Average epiphytic copepod densities (± standard error) at the different sites for the different colonization days

Densities of copepods increased from day 2 at all sites to day 4 but declined at day 6(one and a half times). From day 10, densities increased again (by a factor of one and a half) and were stable until day 21. However, densities for the replanted site declined (2.5 times lower). There was a significant difference in the copepod densities at the different days and the interaction between day and site as shown (table 4, p < 0.05).

Factors	df	SS	MS	F	р
Intercept	1	183056.8	183056.8	992.4337	0.000000
Day	5	9303.1	1860.6	10.0873	0.000013
Site	2	1115.3	557.7	3.0234	0.064737
Day*Site	10	5763.2	576.3	3.1245	0.008428
Error	28	5164.7	184.5		
Total	45	22215.8			

Table 4: ANOVA results for copepod densities

Bold characters imply significance at p < 0.05

Factorial ANOVA for Hill's indices showed only a significant difference for taxa richness (N_0) and for the factor site (P<0.05).

	$N_{0\pmSE}$	$N_{1\pmSE}$	$N_{2\pm SE}$	$N_{inf \pm SE}$
Bare	8.21 ± 0.54	6.33 ± 0.59	5.01 ± 0.54	3.07 ± 0.31
Replant	9.47 ± 0.53	$6.47~\pm~0.50$	5.27 ± 0.49	3.40 ± 0.32
Healthy	9.53 ± 0.61	6.62 ± 0.56	$5.33 \ \pm 0.48$	3.26 ± 0.25
Natural	9.33 ± 0.33	5.09 ± 0.22	3.65 ± 0.2	2.34 ± 0.17

Table 5: Mean Hill diversity indices for harpacticoid copepods at the different sites

The taxa richness was high on the mimics in the healthy site followed by the replant and natural seagrass. Taxa richness was lowest in the bare area. Diversity of copepods was highest in the healthy site followed by the replanted and the bare site but was lowest in the natural samples. Dominance was high in the natural samples followed by the mimics in the bare area and the healthy site. The least dominance was observed in the replanted site. Relative abundance of the epiphytic copepods is as shown below including the calanoids and cyclopoids.



Figure 12: Relative abundance of copepod families at different colonization days. A- Bare; B-Replant; C- Healthy and D-Natural (Calanoids and cyclopoids were at the order level but harpacticoids at the family level, copepodites were not included)

The most abundant families in the natural samples collected were Thalestridae and Ectinosomatidae.

Cyclopoids and calanoids were generally abundant at the three sites in the first two colonization days (day 2 and 4). From day 6 onwards, harpacticoid copepods were seen to colonize the seagrass mimics and the cyclopoids and calanoids reduced in abundance (Fig. 12 above).



А



В



Figure 13: Community structure of copepods for the three sites, A-Bare; B- Replant; C- Healthy (numbers represent the day of harvest and the letters represent the replicate)

There was a clear community structure from day 6-14 for the bare site (Fig. 13A). The replanted site had a community structure that was similar from day 6-21(Fig. 13B). For the healthy site (Fig. 13C) a similar structure was established later; namely at day 10-21.

3.2.2 Benthic copepods

A total of 8 families of the order Harpacticoida were identified at all sites in the study area. Other orders like Cyclopoida and Siphonomastoida were also observed but only identified at the order level.



Figure 14: Average benthic copepod densities (± standard error) at the different sites for the different sediment depths sampled

The healthy and replanted sites seemed to have high copepod densities on the deepest sediment depth sampled (2-5cm) while the highest densities in the bare area was found at 0-1cm depth.

ANOVA tests did not show any difference in terms of densities of copepods between sites or sediment layers.

	N ₀	N ₁	N ₂	N _{inf}
Bare				
0-1cm	5.33± 0.7	3.76 ± 0.7	3.15 ± 0.6	2.20 ± 0.3
1-2cm	5 ± 0	3.5 ± 0.9	3.19 ± 0.9	2.58 ± 0.6
2-5cm	5 ± 0	2.19 ± 0	1.75 ± 0	1.39 ± 0
Replant				
0-1cm	1.50 ± 0.5	1.38 ± 0.4	1.30 ± 0.3	1.17 ± 0.2
1-2cm	2 ± 0	1.52 ± 0.5	1.51 ± 0.5	1.50 ± 0.5
2-5cm		1	1	1
Healthy				
0-1cm	3 ± 1.7	2.42 ± 1.4	0.14 ± 0.1	1.60 ± 0.9
1-2cm	4 ± 0.6	3.29 ± 0.3	0.13 ± 0.1	2.42 ± 0.1
2-5cm	4 ± 1.0	2.41 ± 0.6	0.15 ± 0.1	2.10 ± 0.9

Table 6: Mean Hill diversity indices for benthic harpacticoid copepods at the different sites in the different depths

In the course of analysis of the benthic copepods, most of them were lost in the slide making process hence these are results for the few that were analyzed. All the diversity indices were statistically significant between sites (Kruskal-wallis test). Diversity indices calculated are shown in table 6.

There was generally a higher taxa richness in the bare site (5) that was followed by the healthy site (3). The replanted site had the lowest number of taxa (2). Taxa richness is much dependent on the number of the species sampled hence the low taxa richness could generally be as a result of loss of many copepods when making the slides. There was high dominance of species in the bare site on the lowest sediment layer (2-5cm) as compared to the other layers. For the healthy site it was the reverse as high dominance was observed in the top layer (0-1cm) as compared to the other layers below. For the replanted site high dominance occured in the lowest sediment layer (2-5cm) followed by the top (0-1cm) and middle layer (1-2cm).

 N_0 , N_1 and N_2 did not pass the normality tests hence Kruskal-wallis test was performed which showed significant difference between the sites but not for depth. N_{inf} was normally distributed and ANOVA results did not show any significant difference between the sites and the depth.

Relative abundance of copepods at the different sites is as shown in the figure 15 below.



Figure 15: Relative abundance of copepod families at different colonization days. A- Healthy; B-Bare; C- Replant

For the healthy site Ectinosomatidae were abundant at the sediment surface while Cletotidae and Ameiridae were abundant in the middle and lower layer, respectively. In the bare site Ameiridae were abundant in the top and lower layer while Miiracidae were abundant in the middle layer. For the replanted site Ectinosomatidae were abundant on the surface layer while Ameiridae were dominant for the middle and lower layers.



Figure 16: Figure showing the community structure of copepods for the three sites, A-Bare; B-Replant; C-Healthy in the different sediment depths (surface, 0-1cm; Mid, 1-2cm; Low, 2-5cm).

There was no defined community structure for the benthic copepods in all the sites (see Fig. 16).
3.3 Environmental factors

3.2.1 Biofilm characteristics and water nutrients

Biofilm was only collected from day 10 in the bare and replanted site but not in the healthy site. Other water environmental parameters (nutrients, fatty acid, pigments) did not show any significant difference between the sites.

BIOENV analysis was done on the water parameters and the biofilm data to see the relationship between community composition and environmental variables. This showed a poor relationship.

3.2.2 Sediment characteristics and nutrients

The environmental parameters for sediments for different depths were as in the table 7 below. Phosphates were generally higher in the healthy sites than the other sites. Nitrates were higher on the top sediment depth (0-1cm) for the replanted site and higher in the sediment layer (2-5cm) in the bare area and also generally higher in the bare site.

Total organic matter was higher in the healthy site (upper layer) and lowest in the replanted site. In the bare site total organic matter was higher deeper into the sediments than on the upper sediment layer. There was no difference in porosity between the sites and the replant site had more sand than the bare and healthy.

	Bare	Replant	Healthy
		-	-
Sediment Nutrients(µmoles/g Dry wt)			
Phosphates			
0-1cm	1.3 ± 0.18	1.2 ± 0.28	2.8 ± 0.65
1-2cm	1.3 ± 0.05	1.2 ± 0.43	1.4 ± 0.30
2-5cm	1.6 ± 0.32	1.0 ± 0.40	0.6 ± 0.03
Nitrates			
0-1cm	1.3 ± 0.16	1.7 ± 0.78	2.3 ± 0.65
1-2cm	2.9 ± 0.52	1.0 ± 0.22	0.9 ± 0.11
2-5cm	3.5 ± 1.03	0.8 ± 0.34	0.7 ± 0.08
TOM(%)			
0-1cm	3.0 ± 0.86	2.7 ± 1.02	6.3 ± 0.21
1-2cm	2.8 ± 1.11	2.9 ± 0.33	4.3 ± 1.16
2-5cm	4.2 ± 1.26	2.1 ± 0.65	5.6 ± 0.48
Porosity			
0-1cm	0.4 ± 0.09	0.4 ± 0.04	0.4 ± 0.08
1-2cm	0.5 ± 0.03	0.4 ± 0.05	0.5 ± 0.03
2-5cm	0.5 ± 0.02	0.5 ± 0.002	0.5 ± 0.03
Sediment grain size (%)			
Gravel	5.0 ± 1.84	1.0 ± 0.08	6.7 ± 2.17
Sand	89.0 ± 7.44	98.5 ± 0.17	93.1 ± 2.19
Silt	0.3 ± 0.06	0.2 ± 0.03	0.2 ± 0.11

Table 7: Summary of sediment environmental parameters

Statistical analysis showed that the bare and replant sites were significantly different in nitrates but nitrates were not significantly different between the sites. Total organic matter was also significantly different between sites but not between depth. Sediment grain size did not have any significant difference between the sites or the depth

4. Discussion

4.1 Meiofauna in seagrass beds

In general, meiofauna is known as an important component of heterotrophic assemblages in the aquatic environment ranging from marine to freshwater habitats and from soil to pelagic systems (Kemp 1990; Giere 1993, 2009; Freckman et al 1997). They often occur in high densities and represent a high diversity of species in different marine habitats. Due to their small size, high turnover, lack of larval dispersion and sensitivity to environmental changes, they have been thought of as a good tool for environmental monitoring (Higgins and Thiel, 1988; Coull and Chandler, 1992; Kennedy and Jacoby, 1999). Meiofaunal assemblages in seagrass habitats can be found on both the blades and in the surrounding sediments and can migrate actively between these sub-habitats and the overlying water column (Bell *et al.*, 1984).

The average densities of epiphytic meiofauna found in this study on the natural seagrasses (*Thalassodendron ciliatum*) were about 83 individuals/ 100cm². There are few studies that have recorded epiphytic meiofauna densities as most of them have dealt with benthic meiofauna. Comparing meiofauna densities should be linked to the mesh size of the sieve used since meiofauna is defined as those metazoans passing through a 1 mm sieve but are retained on a 32 or 38 µm sieve. Different studies have used different mesh sizes within this range to define meiofauna. In this study a 38 µm sieve was used as lower size limit. Studies using comparable mesh sizes have recorded average epiphytic meiofauna densities of 400 individuals/100 cm² for Thalassia testudinum (De Troch et al., 2005) and total epiphytic meiofauna densities of 300-8000 individuals/10 cm² for Halodule wrightii and Halophila stipulacea, respectively (De Troch et al., 2001a). The densities in the present study were much lower in comparison to these studies although the seagrass species in question here was different from the one in the other studies. This could probably be attributed to the season since it was the intermonsoon season that is normally rough. Other studies that have used a minimum mesh size of 63 µm obtained meiofauna densities ranging between 600 and 3000 individuals/100cm² (Hicks 1986; Hall and Bell 1988).

Total benthic meiofauna densities obtained in this study ranged between 1300 and 1700 individuals/10 cm^2 in the top 5 cm in all sampled sites with nematodes having

the largest proportion followed by copepods. These densities were highest in the healthy site compared to the bare and replanted site. Other studies have reported ranges of meiofauna between 100-1600 individuals/10 cm² in tropical seagrass beds (Decho *et al.* 1985; Danovaro and Gambi 2002; De Troch *et al.* 2006) but the maximum densities found in the healthy site in our study were higher. This corresponds to high densities that have been reported from Kenyan seagrass beds in previous studies (De Troch *et al.* 2006). However, it should also be noted that the depths for the studies should be taken into account. Other studies have used depth ranges of 0-10cm while this study only considered depth ranges between 0-5cm in order to study the similarity between species present in the sediment and those found on the mimics. A study integrating edge effects into studies of habitat fragmentation reported densities ranging between 1000-3000 individuals/core on a 63 μ m sieve (Wary *et al.* 2009).

Other studies have used biomass as a measure to quantify meiofauna instead of densities. Where biomass was used fluctuations between seagrass meadows and seasons were observed (Paula *et al.* 2001). The biomass can be related to densities since higher densities would mean a higher biomass. Therefore the values reported here could be different from other studies due to the sampling season and the type of seagrass meadow studied. This poses problems in comparing the densities and diversity of different studies.

The abundance of benthic invertebrates including copepods have been reported to be higher in seagrass beds than in bare areas (Boström and Bonsdorff 1997). This was also true in the present study.

4.2 Meiofauna on seagrass mimics

On the seagrass mimics the densities ranged between 5-97 individuals/ 100cm² during the whole colonisation experiment period for all the three sites. These densities were comparable to that of the undisturbed healthy seagrass beds after four days of colonisation though the densities varied with colonisation days and were lower than that after day 4. Studies have shown that there is no significant difference in the abundance of fauna between natural and artificial seagrasses when both have the same sizes and shapes of leaves (Bell et al., 1985). The mimics used in this study resembled *Thalassia hemprichii* which has longer and narrower leaves as compared to the natural seagrass in question in this study, *Thalassodendron ciliatum* which has broader leaves than the latter seagrass mentioned. Densities of meiofauna on artificial substrates are known to increase with increasing colonization time (De Troch *et al.,* 2005; Mirto and Danovaro, 2004; Atilla and Fleeger, 2000) and also irregardless of the season. However, this depends on the meiofauna composition since the first colonizing meiofauna will normally be opportunistic species followed by the true species associated with the substrate in question. Colonization could also depend on the carrying capacity of the artificial substrate and other factors as the presence of food (prey) as well as predators. Some studies have shown that variation in the densities of meiofauna in seagrass beds are affected by epiphytic algae (Hall and Bell 1993). Densities of harpacticoid copepods can also reduce during the pre-monsoon period (June-September) as illustrated for the seagrass *Halophila ovalis* along the South-west coast of India (Arunachalam and Balakrishnan 1988).

The colonization time of three weeks (21 days) was chosen to be able to compare the results with other studies that have used this time frame (De Troch *et al.* 2005). Others have used an even much shorter period (e.g. two weeks) to study colonisation (Mirto and Danovaro, 2004; Atilla and Fleeger, 2000).

The results therefore indicate that colonisation of mimics was rapid in all the sites studied. Initial colonisers however, seemed to be planktonic copepods and nauplii which are not true phytal dwelling copepods on seagrass blades since epiphytic copepods are mainly harpacticoid copepods. This could suggest that the initial colonizers were probably opportunistic species associated with the water column (planktonic) and probably looking for food and the harpacticoid copepods colonized fully from day 6 onwards for the bare and replanted site and day 10 for the healthy site.

Other studies have also shown that immediate adjacency of dense seagrass vegetation is not a prerequisite for the recruitment of high densities of copepods, a pattern also previously detected for insects on host plants (Kareiva 1987). High abundance of copepods has been recorded outside bed margins although some studies recorded high densities within the seagrass beds (Bell and Hicks, 1991).

4.3 Community structure and diversity of meiofauna and harpacticoid copepods

Meiofauna community structure on the mimics in the replanted and bare sites did not change with time. There was a clear community structure for the meiofauna in the healthy site as the communities colonizing in the early days were different from the late colonisers. Studies have shown distinct differences in faunal communities in seagrass areas and unvegetated habitats over small spatial scales (Bostrom and Bonsdorff 1997; Connolly, 1997). In this case the bare site had no vegetation while the replanted site had very patchy vegetation since most transplanted seagrasses have been washed off during the intermonsoon period characterized by strong currents and winds just before the start of this study. Nauplii and planktonic copepods seemed to be the dominant colonisers at initial stages but harpacticoid copepods increased in relative importance in later colonisation stages. The benthic communities for all the sites were dominated by nematodes. This has been so in earlier studies which found nematodes as the dominant taxon in sediments.

Harpacticoid copepods are of importance in seagrass beds. As harpacticoids are important grazers on primary production, they represent an important link between microalgal primary production and higher trophic levels (e.g. Coull 1990; De Troch *et al.* 1998; Turner, 2004; Andersen *et al.* 2005). They comprise by far the dominant fraction of the diet of fishes in seagrass beds (Sogard, 1984). They are found in high abundance in seagrass beds (Bell and Hicks 1991; De Troch *et al.*, 2001 a, b) and can also actively migrate from sediments to the water column (Commito and Tita, 2002). In the absence of vegetation passive recruitment processes dominate (Palmer, 1988).

Further analysis of harpacticoid copepods showed that the common ones at the initial stages were cyclopoids and calanoids as Harpacticoida increased their relative importance in the later colonisation stages. Community structure for the bare and replanted site was established from day 6 while for the healthy site it was later at day 10. The bare site had its structure excluding day 21. The first colonization phase was characterized by planktonic copepods (mainly cyclopoids and calanoids) while harpacticoids formed the main group as the colonization progressed. Communities were not similar in the first phase of colonisation probably because these were opportunistic species that did not establish themselves since cyclopoids and calanoids

are known to be planktonic and not epiphytic. Harpacticoids were only analyzed at the family level but this was appropriate for the study in order to document the phytal and sediment dwelling harpacticoid copepods as has been seen in other studies (Vincx, 1990, De Troch et al. 2008). The results of copepods from day 6 in the bare and replanted sites and day 10 for the healthy site suggest that recolonization levels of harpacticoid copepods in this system starts around this time. This is supported by the temporal difference observed for the main families of copepods. However, no spatial difference was observed at all sites implying that colonization is possible in bare and replanted areas just as in the healthy site. The mimics on the healthy sites were planted within a seagrass canopy while the mimics on the bare and replant sites were planted on areas further away from the seagrass patches but the distance to other seagrass patches was equally not very far though it was not determined in this study. Some studies have also shown that recolonization on seagrass mimics depend on the distance from the natural seagrasses that are assumed to be the source for recruitment of the adult copepods. However, it has also been shown that some species may colonize mimics at consistent rates irrespective of the distance from that source (Bell and Hicks, 1991).

True phytal dwelling harpacticoids have been known to belong to the families Harpacticidae, Tisbidae, Porcellidiidae, Tegastidae, Miracidae (formerly Diosaccidae), Peltidiidae and Thalestridae (Hicks and Coull 1983; Hicks 1985; 1977a, b; 1986; Johnson and Scheibling 1987a, b). All these families except Peltidiidae were observed in the sites studied. The relative abundance of these families increased from colonization day 6 onwards suggesting that phytal harpacticoid copepods were colonizing the mimics. These were also observed from the natural seagrasses apart from Harpacticidae and Porcellidiidae. This could be a result of the sampling because not all the families might have been represented since natural samples were collected in triplicate but only once at the end of the study period. More families on the bare and replanted sites than the healthy site could also suggest the potentiality of the sediments on these canopy deficient areas to act as pools for copepods by upward movement from the sediment. This is because families such as Cletodidae, Longipediidae and Laophontidae were also observed in underlying sediments in the bare and replanted sites.

The present study only focused on the identification of adult harpacticoid copepods hence the copepodites and nauplii were not identified to family level. Ectinosomatidae and Thalestridae were the abundant harpacticoid families present in all sampling points during the colonization period. Ectinosomatidae are mainly known to be itinerant species, occupying both the blade surfaces and sediment hence its presence on the mimics could suggest that the surrounding sediments were the main origin of the copepods. Ameiridae, a sediment-dwelling family, was also found in high abundance on the mimics planted and on natural plants in the healthy site. These densities were higher in the sediments of the bare and replanted sites also but not as high as on the mimics at these sites especially at the last colonization day (day 21). These opportunistic sediment dwelling species maybe found their way to the seagrass blades due to the complexity of the seagrass bed allowing actively migration towards the canopy. The bare and replanted areas were characterized by fewer plant structures which might explain the lower densities of this family on the mimic blades.

From the results it is clear that the source of the harpacticoid copepods could have been both the sediment (active migration) and also the natural seagrass blades especially for the phytal families found in the bare and replanted sites since these were not so far from other healthy seagrass patches around the bare and replanted sites (passive migration). Studies have shown that it is likely that many harpacticoid copepods are capable of actively departing the sediment (Teasdale *et al.* 2004).

Biodiversity and community composition can be affected positively or negatively through the changes in size, shape and location of the remaining habitat patches (Fahrig, 1997) especially in cases of fragmentation that could be caused by natural as well as anthropogenic factors. Differences in infaunal assemblages have been found between fragmented and continuous seagrass habitats (Frost *et al.* 1999) and also in seagrass patches of differing sizes (Bowden *et al.* 2001).

Heterogeneity in terms of physical structuring of seagrass canopies could also play a role in determining biodiversity which in turn determines the ecosystem functioning.

This could explain the higher Hill diversity indices found on natural seagrasses for total meiofauna although they were low for harpacticoid copepods.

Predators may often exert a 'top-down' control thus affecting dominance of species such that with high predation species have low chances of becoming dominant. In this study predation was not considered. However, this is an important factor to be considered as far as the copepods were concerned.

4.4 Biofilm characteristics and meiofauna

There was no biofilm collected until day 10 of colonisation and this was only for the bare and replanted site since for the healthy site no biofilm was observed even on day 21. The variation of meiofauna and late biofilm colonisation could suggest an unstable community implying that the colonization time considered here did not enable the meiofauna to reach stable communities and could suggest that the system needs more than the 21 days to stabilise since the maximum colonisation for the biofilm may not have been reached in 21 days. This can also imply that the epiphytic meiofauna colonisation could not have reached its maximum and, hence, this might be the reason for not achieving a stable community. Absence of biofilm in the healthy site could be explained by the high energy and also the fact that the mimics on the healthy sites were planted in between natural vegetation that could have reduced the chances of settlement of epiphytic flora. The late biofilm colonisation that was observed also suggests that this system needs more than the 21 days to recover since the maximum colonisation for the biofilm may not have been reached in 21 days. This can imply also that the epiphytic meiofauna colonisation could not have reached its maximum and, hence, this might be the reason for not achieving a stable maximum community as explained by the low densities of meiofauna and copepods. However, this set up was done to be able to compare with other studies on meiofauna colonisation for three weeks (21 days) in seagrass beds.

The nutrient, pigment, biofilm and fatty acid samples were transported from Kenya for analysis in the University of Ghent laboratory. These were frozen though by the time they arrived at the lab they were a bit defrosted. The temperature used to store the samples in Kenya during the sampling period was -20°C but they were supposed to be stored at -80°C. This could have affected the results of the analysis but was the best possible storage and transport of the samples. The environmental parameters in this study showed poor correlation.

4.5 Threats of seagrass in the study area and recovery

Diani beach is part of the larger Diani– Chale lagoon dominated by touristic activities and the area has seagrass beds which form fishing grounds for the artisanal fishermen. It is an area that has been affected by seagrass depletion. Extensive seagrass decline especially for the dominant species, *Thalassodendron ciliatum* has been reported (Uku *et al.* 2005).

The main cause of the decline has been the increased sea urchin abundance that has been reported by fishermen in Diani – Chale and other areas along the south coast. A study done in Diani established that there was overgrazing on seagrasses due to population explosions of the sea urchin *Tripneustes gratilla* (L.). Natural recovery has been reported to take place in certain areas (Uku *et al.* 2005).

A transplantation study was done to determine the potential transplantation success (Unpublished data, 2009). The transplantation involved the planting of $5m^2$ plots of seagrass (*Thalassodendron ciliatum*) with sods from healthy sites bearing 20 shoots. An experiment was also done for *Thalassia hemprichii*. The plots were doing well until a month before the start of this study when most of them were washed to the shore due to a rough intermonsoon season. The outcome of the study is affected such that there was not much difference in the bare site and replanted site since the replanted site had very few and sparse seagrass cover. This was beyond our control due to the rough weather.

5. Conclusion

Biodiversity can influence several aspects of ecosystem structure and function since each species or group of species has a functional role to play in the ecosystem. To be able to quantify ecosystem functioning biodiversity and community structure are thus important to establish. Seagrass beds play an important role in stabilising sediments and protecting the coral reefs among other functions. Harpacticoid copepods are a very important group in contributing to the functioning of seagrass ecosystem by providing the link between primary producers and secondary organisms higher on the food chain. Loss of seagrass would mean loss of these copepods and this could impact the populations of higher animals like fish.

Since disturbances are bound to occur in seagrass ecosystems whether anthropogenic or natural, it is interesting to find out how this affects the functioning of the system by looking at associated fauna (in this case harpacticoid copepods). Here we showed that the seagrass ecosystem is bound to recover in terms of associated fauna if the seagrass plants recover naturally or are even transplanted into defoliated areas.

However, the recovery of the functioning of the ecosystem will depend much on the recovery of the seagrass plants themselves especially since the phytal-dwelling meiofauna requires seagrass blades to attach to.

The seagrass species of interest in this study has been shown to take approximately 4 years to recover from disturbance (Alcovero and Mariani, 2002) and this would mean that the associated functions of a seagrass ecosystem will be affected. However, the transplantation experiments that have been tested to be successful could accelerate the recovery of the system and thus prevent any possible loss of biodiversity.

It is therefore of importance that the anthropogenic causes of seagrass depletion be dealt with to avoid further loss and solutions to the natural disturbance be employed to avoid more detrimental effects that come about as a result of seagrass loss.

From the current study it is clear that disturbance of seagrass by urchins did not affect the densities of harpacticoid copepods across the sites since there was no spatial difference between the vegetated and unvegetated sites. This could mean that natural recovery or transplantation of seagrass into bare areas could result in the restoration of the meiofauna community comparable to the neighbouring natural undisturbed patches.

However, predators and prey also affect the densities of especially the harpacticoid copepods on seagrass blades. The late colonization of epiphytic flora observed from the experiment could on the long-term affect the densities of copepods on seagrass blades since they mainly feed on these. Recovery of the functions of the seagrass ecosystem would therefore take a longer period than the studied colonization time due to the late colonization by epiphytic flora. However, this could also be attributed to the season when the samples were collected i.e. after strong intermonsoon conditions. The scale of the study was on a local basis at the site and the sampling sites were less than 500 m apart. To try and improve this I would recommend a study that would include a greater spatial scale since this may influence greatly the interpretation of the seagrass ecological system as a whole. Seasonality would also be a good point to include when doing such a kind of study to be able to identify any seasonality pattern in the meiofauna densities and their community structure.

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ANNEXES

Draft Manuscript

Restoration of Kenyan seagrass beds: a functional study of the associated fauna and flora

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ABSTRACT

Seagrass communities are subject to frequent anthropogenic and natural disturbances that can lead to alterations in vegetation complexity and hence may affect associated fauna. Seagrass loss in Kenya has been mainly due to extensive grazing by the sea urchin Tripneustes gratilla which has affected almost the entire coastline. This has led to habitat fragmentation and sometimes vast areas of defoliated beds that were formely covered with seagrass. The most affected species has been Thalassodendron ciliatum. Diani beach, south of Mombasa, is an area that has been typically affected by seagrass depletion. Natural recovery has been reported in certain areas and transplantation projects were started. The challenge is to see if the system can recover fully and will be able to function as before. To test this, the current study focused on the density, diversity and community structure of meiofauna, and more specifically of harpacticoid copepods as a measure of the ability of the system to recover. Artificial seagrass mimics were planted in natural, replanted and areas of bare sand and harvested in a series of 2, 4, 6, 10, 14 and 21 days in order to collect the associated meiofauna. Related environmental parameters were collected at the same time intervals except for the sediment samples for environmental analysis that were sampled once during the study period at the last day of collection of mimics.

Significant differences of meiofauna densities between the sites and the colonization days were found but for harpacticoid copepods there was only a significant effect of the colonisation time. The densities of meiofauna reached those of natural seagrasses by day 4 but most of them were opportunistic species and not true phytal dwelling meiofauna. Both passive migration from neighbouring seagrass patches and active migration from sediments were observed, based on the harpacticoid copepod family composition. In the bare and replanted sites similar community structures of harpacticoid copepods were observed from day 6 onwards while for the healthy site it was from day 10. Colonization by epiphytic biofilm was collected from day 10 in the bare and replanted sites but not the healthy site. In the previous days it was too negligible to be collected. The results thus suggest possible recovery of harpacticoid copepods after disturbance thanks to their mobility and ability to colonize new areas quickly. However, this may depend largely on the time the epiphytic flora are able to recover as well as the recovery time of the seagrass plants which may take approximately 4 years.

Keywords

Disturbance, Ecosystem recovery, Seagrass beds, Meiofauna

Introduction

Seagrass beds in tropical regions are an example of a complex, variable and diverse ecosystem that supports a large variety of associated fauna and flora with several ecological characteristics (Hemminga & Duarte, 2000). Seagrass communities are subject to frequent anthropogenic and natural disturbances that can lead to alterations in vegetation complexity (DeTroch *et al.*, 2001a; Gray, 2004; Snelgrove *et al.*, 1997) hence affecting the associated fauna. Grazing is a natural factor which can influence seagrass production and distribution in tropical seagrass meadows (Heck & Valentine, 2006)

) and its impact depends on the intensity and frequency. Overgrazing of seagrasses by sea urchins may be triggered by reduced predation by fish and eutrophication. In severe cases, such overgrazing could decimate entire seagrass meadows (Eklöf *et al.*, 2008). Theory predicts that increasing herbivore diversity should reduce plant community biomass as the most efficient grazers come to dominate a system, leading to overgrazing (Holt & Loreau, 2002). This can also depress plant diversity and facilitate invasion of grazing-resistant species (Leibold *et al.*, 1997). The drastic reductions in many species of preferred fishes may be extensive enough to endanger the function of entire marine ecosystems.

The seagrass community of the East African coastline is composed of 12 species, belonging to 8 genera, and each of these supports on their leaves a diverse phyllosphere microbiology or epiphytic community composed of a variety of macroalgae as well as faunal associations (Bandeira, 1995; Isaac, 1968; Lindow & Brandl, 2003). Seagrass loss has been reported by fishermen in several areas along the Kenyan coast due to population explosions of sea urchins (especially *Tripneustes gratilla*). Natural recovery is reported from certain areas although not all areas have recovered (Uku *et al.*, 2005).

Seagrass recovery, the ability to restore damaged beds, can be enhanced by transplantation from other seagrass sources (Paling *et al.*, 2001) other than the natural recovery. Attempts have been done on transplantation which has shown success with survival levels up to 90% (Paling *et al.*, 2003). Seagrass transplantations trials have also been tried to establish seagrass restoration in Kenya which showed positive

results apart from the harsh weather conditions that have been detrimental to the trials (unpublished data).

In contrast to the transplantation efforts, little research has been done to look at the recovery of the functions of the seagrass ecosystem other than the plant itself since the associated flora and fauna also have to recover for the system to assure its full functioning. It is therefore important to look at recovery of the system as a whole since processes are normally dependent on each other. Diversity patterns are essential to understand the organization and functioning of organisms present in an ecosystem and their interaction with the environment (Duarte, 2000). Loss of biodiversity may result in the loss of ecosystem functions and the many services they provide to society (Constanza *et al.*, 1997). Testing the link between biodiversity and ecosystem functions and services is essential to demonstrate a significant ecosystem role for biological diversity (Tilman, 1997).

Meiofauna especially the harpacticoid copepods are known to form an important link between primary producers and higher trophic levels (Coull 1990; De Troch *et al.* 1998; Turner 2004; Andersen *et al.* 2005) like fish (Sogard 1984). They are found in high abundance in seagrass beds (Bell and Hicks 1991; De Troch *et al.* 2001 a, b) and can also actively migrate from sediments to the water column (Commito and Tita 2002). Due to their small size, high turnover, lack of larval dispersion and sensitivity to environmental changes, they have been thought of as a good tool for environmental monitoring (Coull and Chandler 1992; Kennedy and Jacoby 1999).

The present study dealt with faunal biodiversity as a parameter of ecosystem functioning and whether there is a possibility of maintaining this after a natural disturbance attributed to overgrazing. The study looked at meiofauna with special emphasis on harpacticoid copepods. This is because they play an important functional role, and because a large part of the energy flow passing the community is dissipated through epiphytic systems and is thus transferred to higher trophic levels (Asmus, 1985; Asmus, 2000; Klumpp, 1992) and are abundant in seagrass beds (Bell & Hicks, 1991; Bell *et al.*, 1988; DeTroch *et al.*, 2001a; DeTroch *et al.*, 2001b; Hicks, 1977b; Hicks, 1977a; Hicks, 1977c; Hicks, 1980).

Studies have also been done on harpacticoid copepods and their response to smallscale natural disturbance (Thistle, 1980), species diversity changes within and between habitats in tropical seagrass beds (DeTroch *et al.*, 2001a), colonization and recruitment in seagrass mimics (Bell & Hicks, 1991 ; DeTroch *et al.*, 2005; Walters & Bell, 1994) as well as in terms of biodiversity and evolution (DeTroch *et al.*, 2001b). The main objective of the study was to determine the biodiversity of meiofauna with special emphasis on harpacticoid copepods in tropical seagrass beds in Diani, Kenya and relate this to the possibility of the seagrass system's functional recovery.

Materials and Methods

Sampling strategy

The sampling campaign was conducted between 15^{th} August and 6^{th} September 2008 in Diani, Kenya. Diani Beach is situated at approximately 72 km south of Mombasa at latitude $4^{\circ}21$ 'S – $39^{\circ}33$ 'E on the coast of Kenya separated from the main body of the Indian Ocean by a fringing coral-reef platform that is about 0.9 km wide.

The study focused on the biodiversity of the meiofauna occurring on seagrass leaves in three different sites along the southern coastal zone of Kenya (Diani): bare sand (approx. 240m from shore), healthy seagrasses (approx 500 m from the shore), and an area with recently replanted seagrasses (approx. 230 m from the shore). The replanted seagrasses have been there for about one year though most of them were lost during the intermonsoon season around July 2008. The healthy and replanted seagrasses belonged to the species *Thalassodendron ciliatum*, a seagrass species that had been adversely affected by sea urchin population explosions. These were found in the subtidal waters in the study area. In addition to an evaluation of the field situation, seagrass mimics (plastic seagrasses, Biomodels http://cgbiomodels.com/) were planted in the field close to the three selected sites. The seagrass mimics however resembled *Thalassia hemprichii*. The mimics were light green in colour and were 35.7 \pm 2.3 cm and 0.8 \pm 0.1 cm long and wide, respectively. Each mimic plant consisted of 4 green and 1 brown leaf resembling natural plants with fresh (green) and dead (brown) leaves. Total leaf length per plant (5 leaves) therefore was 178.4 ± 12.9 cm corresponding to an average plant surface area of 146.8 ± 17.3 cm. The total surface area for each replicate was 293.7 ± 34.7 cm since two clumps (10 leaves) were used per replicate. For the natural seagrass the average length of the leaves was $12.8 \pm$ 1.5cm and the average total length was 104.3 ± 4.4 cm. The surface area for the shoots collected was approximately 1304.4 ± 238.9 cm per replicate.



Figure 17: Map showing the study area

Epiphytic meiofauna

The artificial (plastic) seagrasses represented a disturbed ecosystem at T_0 ready to be colonized by epiphytic meiofauna and flora. The planted mimics were to be harvested at different time intervals (2, 6, 10, 14 and 21 days). To make efficient use of the mimics they were harvested in three series and were replanted again after collection and cleaning using a soft kitchen scouring pad. Three sets of mimics were available

where the first set was for Day 2, 6 and 10, the second for Day 4 and 14 and the last set for Day 21. Only the set for Day 21 was left in the field for the whole period. For each day 3 replicates were planted/ harvested for each site. Each replicate consisted of 2 clumps of 10 'leaves' each (20 in total). Sampling was done at low tide and the average water cover at sampling was 0.7m.

Harvesting was done by snorkelling and placing plastic bags over the mimics and cutting the cable ties. The mimics together with the water in the plastic bags were brought to the boat closed with rubber bands to avoid loss of the water hence loss of meiofauna. 8 % of MgCl₂ was added to the bags for 15 minutes to detach the meiofauna from the leaves and the contents of the plastic bags was collected over a 38 μ m sieve. The mimics were rinsed thoroughly using filtered seawater over the 38 μ m sieve and a funnel was used to collect epiphytic fauna. The samples were collected in plastic bottles and 8% formalin was added to bring the solution (sample mixed with formalin) to a final concentration of 4%. The same was done for meiofauna on natural seagrasses that were collected during the last day of the sampling period.

Benthic meiofauna

Polyvinyl chloride (PVC) cores (3.6 cm inner diameter, 10 cm² surface) were used to collect meiofauna from the sediment. Sediment cores were sliced to analyze meiofauna at different depths (0-1 cm, 1-2 cm, 2-5 cm). These were also preserved in 4% formalin (final concentration). Sediment samples were collected at the beginning of the experiment before planting the mimics.

Biofilm samples

The biofilm was removed after collecting the epiphytic fauna by scraping the leaves using the blunt side of a surgical blade. The blades were cleaned carefully on preweighed GF/F filters of known weight.

Environmental samples

Sediment was collected for grain size analysis (2 replicates) and for nutrient for all the depths considered only at the last day of the experiment. Water samples for nutrients and samples for chlorophyll and fatty acid analysis were also collected for all the colonization days of the experiment.

Laboratory analysis

Meiofauna samples processing

Benthic samples were decanted 5 times over a 38 µm mesh sieve, centrifuged five times with Ludox (specific density 1.18) and stained with Rose Bengal solution. The epiphytic meiofauna were not centrifuged but directly stained with Rose Bengal. The meiofauna was counted and identified at higher taxon level based on Higgins and Thiel (1988) using a binocular. Copepods were collected from the samples by an 'eye shaped' needle to be identified to the lowest taxon level possible (specifically for harpacticoid copepods). At least 100 individuals were collected for identification where the numbers exceeded 100 individuals. For samples with less then 100 individuals, all copepods were picked. The copepods were stored in 75% ethanol in small glass tubes and later they were mounted in glycerine (5 individuals per slides) with the swimming legs facing upwards for ease of identification. The copepods were identified to the family level using Boxshall and Hasley (2004), Lang (1948; 1965) and original species description.

Environmental samples

Sediment and water nutrients (NO₃⁻, NH₄⁺, and PO₄³) were determined using spectrophotometric methods (KMFRI, Kenya) while an A_{II} automatic chain (SANplus Segmented Flow Analyser, SKALAR) was used for the water samples analyzed in Ghent. Organic matter was determined by using a known weight of sediment sample in an aluminium of foil known weight and was ashed hence difference between the dry and the ash weight were expressed as a ration of dry weight to determine organic matter. Grain size composition was determined using the dry sieving method where a known weight of sediment was dried in an oven at 105°C until the weight was constant. The sediment was then passed through a series of sieves (2.00 mm, 1.60 mm, 1.00 mm, 500 μ m, 250 μ m, 125 μ m, 63 μ m, 38 μ m). Pigments were extracted in 90% acetone at 4°C in the dark and separated by reverse phase liquid chromatography on a Gilson C-18 HPLC-chain (spectrophotometrical and fluorometrical detection) according to a modified protocol of Mantoura & Llewellyn (1983).

Porosity of water content was determined by putting sediment samples of known weight on aluminium foil also of known weight and keeping them in the oven for 12 hours and transferring them to a dessicator. The % water content was calculated from the wet weight (Sample and foil weight before drying – foil weight) and dry weight

(Sample and foil weight – foil weight after drying). The porosity was calculated as water content and the product of % sediment density.

Statistical analyses

Assumptions of analysis of variance (ANOVA) were examined using box and normal probability plots. To test for homogeneity of variances which is an assumption for using ANOVA Levene's test was used. Factorial ANOVA was used to test for significant differences in the densities and diversities of the different sites and harvest days using STATISTICA 8. Non parametric tests were used to analyze the benthic harpacticoid samples. For the mimics the two factors used were colonization days and site. For factorial ANOVA post hoc analysis was done using the Tukey HSD test. The community structure of the different samples was analysed by means of MDS plots in PRIMER 6 software. Testing relationship between community composition and environmental variables was done using BioEnv.

Results

Meiofauna in general

A total of 21 groups including both epiphytic and benthic meiofauna were counted in the samples collected. Meiofauna densities ranged between 5 and 97 ind/100cm² during the colonisation experiment (Fig. 2). The densities of the epiphytic meiofauna were low during day 2 and increased towards day 4. However, the densities dropped at day 6 (~ by a factor of 3 for all sites) and on day 10 they increased ten times for the healthy site and doubled for both the bare and replanted sites. The densities stabilized for all sites on day 14 but on day 21 they reduced one and a half times for the healthy and bare sites and six times for the replanted site. The highest densities for the bare and replanted sites were observed at day 4 while for the healthy site it was at day 10. The natural seagrass species *Thalassodendron ciliatum* was also sampled (three replicates) once during the sampling period and the average epiphytic meiofauna was 83 ± 21.1 ind/100cm². The highest average densities observed on the mimics at the replanted and bare site were 76 and 96 ind/100cm² respectively during day 4 while for the healthy site it was 62 ind/100cm² during day 10 (fig 2). The densities were higher on average in the natural seagrasses as compared to the densities on seagrass mimics.



Figure 18: Total meiofauna densities (\pm standard error) in three sites at different colonization days (left) and on the natural seagrasses (right)

 Table 8: ANOVA Univariate analysis of meiofauna densities collected from seagrass mimics in Diani, Kenya

Factors	df	SS	MS	F	р
Intercept	1	4003893	4003893	154.1246	0.000000
Day	5	648427	129685	4.9921	0.001634
Site	2	187271	93636	3.6044	0.038387
Day*Site	10	294661	29466	1.1343	0.367727
Error	33	857283	25978		
Total	50	1970629			

Bold characters imply significance at p < 0.05

Statistical analysis showed significant differences in meiofauna densities were mainly due to the site effect and the sampling day (duration of the colonisation) (p<0.05, table 1). Post hoc Tukey HSD indicated that only day 4 (replant) and day 6 (healthy) were significantly different from each other (p<0.05).

No significant differences were observed for Hill's diversity indices other than N_2 which showed a significant difference for the factor day (non parametric tests used).

Benthic meiofauna densities ranged from 200- 600 ind/10cm² in the top layer (0-1cm), 377-705 ind/10cm² in the middle layer (1-2cm) and 490-685 ind/10cm² in the lowest layer sampled (2-5cm). Meiofauna densities were high on the top sediment layer in the bare site while the replant site had the highest density in the deepest sediment layer (2-5cm). The healthy site had the highest number on the middle layer (1-2cm). ANOVA results did not show any statistical significant differences in the benthic meiofauna densities and Hill's diversity indices between sites and layers.



Figure 19: Relative abundance of dominant meiofauna groups at different colonization days. A-Bare; B- Replant; C- Healthy; D-Natural

Nauplii dominated the meiofauna community for the replant and healthy sites initially (until day 6) and later on the dominance shifted towards adult copepods and copepodites. For the bare sample day 2 (one replicate analyzed) copepods dominated but at day 4 nauplii were the dominant group. From day 6 onwards, copepods dominated in relative abundance as explained for the other two sites. Nematodes were second in abundance after copepods. For the healthy site, day 6 had the lowest abundance of nauplii but increased on day 10 and 14 and finally reduced at day 21. For the bare and replanted sites the highest abundance of nauplii was at day 4 (>50%) and the abundance reduced further towards the last colonisation day (day 21).

The natural seagrasses harboured high relative copepod abundances (47%), followed by nematodes (24%). There was no defined community structure for the bare areas in the different colonisation days but in the healthy site the communities that colonised for a longer time (from day 10 onwards) grouped together and differed from the early

colonisation phase (day 2 to day 6). This was also true but to a lesser extent for the replanted site.

Harpacticoid copepods

A total of 17 families of the order Harpacticoida were identified at all sites in the study area. Other orders like Cyclopoida and Calanoida were also observed but only identified at the order level.



Figure 20: Average epiphytic copepod densities (\pm standard error) at the different sites for the different colonization days

Densities of copepods increased from day 2 at all sites to day 4 but declined at day 6 (1.5 times). From day 10 onwards, densities increased again by a factor of 1.5 and remained stable until day 21. However, densities for the replanted site declined (2.5 times lower). There was a significant difference in the copepod densities in the different days and the interaction between day and (p < 0.05, table 2).

Factors	df	SS	MS	F	р
Intercept	1	183056.8	183056.8	992.4337	0.000000
Day	5	9303.1	1860.6	10.0873	0.000013
Site	2	1115.3	557.7	3.0234	0.064737
Day*Site	10	5763.2	576.3	3.1245	0.008428
Error	28	5164.7	184.5		
Total	45	22215.8			

Table 9: ANOVA results for copepod densities

Bold characters imply significance at p < 0.05

Taxa richness (Hill index) differed only significantly between colonization days (time) but not between sites.



Figure 21: Relative abundance of copepod families at different colonization days. A- Bare; B-Replant; C- Healthy and D-Natural (calanoids and cyclopoids were at the order level but harpacticoids at the family level, copepodites were not included)

The most abundant families in the natural samples were Thalestridae and Ectinosomatidae. Cyclopoids and calanoids were generally the dominant copepods at the three sites in the first two colonization days (Day 2 and 4). From day 6 onwards harpacticoid copepods were seen to be colonising the seagrass mimics and the cyclopoids and calanoids reduced in abundance (Fig 5).

There was a defined community structure from day 6-14 for the bare site while the replanted site had a community structure that was similar for day 6-21. For the healthy site a similar structure was established from day 10-21 which was later than for the two sites mentioned above. ANOVA test done on the densities of copepods did not show any difference between sites or the sediment layers.

Environmental parameters

Biofilm data was collected only in the bare and replanted site during the colonization day 10 onwards. BIOENV analysis done showed poor correlation of environmental parameters to the structure of meiofauna.

Discussion and conclusion

In general, meiofauna is known as an important component of heterotrophic assemblages in the aquatic environment ranging from marine to freshwater habitats and from soil to pelagic systems (Kemp 1990; Giere 1993; Freckman et al 1997). The densities in the present study were much lower in comparison to these studies although the seagrass species in question here was different from the one in the other studies. The values reported here could be different from other studies due to the sampling season and the type of seagrass meadow studied. On the seagrass mimics the densities ranged between 5-97 individuals/ 100cm² during the whole colonisation experiment period for all the three sites. These were comparable to that of the undisturbed healthy seagrass beds after four days of colonisation though the densities varied with colonisation days and were lower than that after day 4. Studies have shown that there is no significant difference on the abundance of fauna between natural and artificial seagrasses when both have the same sizes and shapes of leaves (Bell et al. 1985). The mimics used in this study resembled Thalassia hemprichii which has longer and narrower leaves as compared to the natural seagrass in question in this study, Thalassodendron ciliatum which has broader leaves than the seagrass mimics. Densities of meoifauna on artificial substrates have been known to increase with increasing colonization days from previous studies (Atilla and Fleeger, 2000; Mirto and Danovaro, 2004; De Troch et al. 2005) and also regardless of the season. This depends however on the meiofauna since the first colonizing meiofauna will normally be opportunistic species followed by the 'true phytal' species associated with the substrate in question later. Colonization could also depend on the carrying capacity of the artificial substrate and other factors as the presence of food (prey) as well as predators. Some studies have shown that variation in the densities of meiofauna in seagrass beds are affected by epiphytic algae (Hall and Bell 1993). There was no biofilm collected until day 10 of colonisation and this was only for the bare and replanted site since for the healthy site no biofilm was observed even on day 21. The variation of meiofauna and late colonisation by biofilm could suggest an

unstable community implying that the colonization days studied did not enable the meiofauna to reach stable communities and could suggest that the system needs more than the 21 days to stabilise since the maximum colonisation for the biofilm may not have been reached in 21 days. This can also imply that the epiphytic meiofauna colonisation could not have reached its maximum and, hence, this might be the reason for not achieving a stable community. Absence of biofilm in the health site could be explained by the high energy and also the fact that the mimics on the healthy sites were planted in between natural vegetation that could have reduced the chances of settlement of epiphytic flora. The late biofilm colonisation that was observed also suggests that this system needs more than the 21 days to recover since the maximum colonisation for the biofilm may not have been reached in 21 days. This can imply also that the epiphytic meiofauna colonisation could not have reached its maximum and, hence, this might be the reason for not achieving a stable maximum community as explained by the low densities of meiofauna and copepods. However, this set up was done to be able to compare with other studies on meiofauna colonisation for three weeks (21 days) in seagrass beds. Studies have also shown densities of harpacticoid copepods to reduce during the pre-monsoon period (June-September) along the Southwest coast of India for Halophila ovalis seagrass species (Arunachalam & Balakrishnan, 1988).

The results therefore indicate that colonisation of mimics was rapid in all the sites studied. Initial colonisers however, seemed to be planktonic copepods and nauplii which are not true phytal dwelling copepods on seagrass blades since epiphytic copepods are normally mainly composed of harpacticoid copepods. This could suggest that the initial colonizers were probably opportunistic species associated with the water column (planktonic) and probably looking for food and the harpacticoid copepods colonized fully from day 6 onwards for the bare and replanted site and day 10 for the healthy site.

Other studies have also shown that immediate adjacency of dense seagrass vegetation is not a prerequisite for the recruitment of high densities of copepods, a pattern also previously detected for insects on host plants with higher abundances of copepods having been recorded outside bed margins although some studies recorded high densities within the seagrass beds (Kareiva 1987; Bell and Hicks 1991).

There was a clear structure for the meiofauna in the healthy site as the communities colonizing in the early days were different from the late colonisers. Studies have
shown distinct differences in faunal communities in seagrass areas and unvegetated habitat over small spatial scales (Bostrom and Bonsdorff 1997; Connolly, 1997). In this case the bare site had no vegetation while the replanted site had very patchy vegetation since most transplanted seagrass had been washed off during the intermonsoon period characterized by strong currents and winds just before the start of this study. The first colonization phase was characterized by planktonic copepods (mainly cyclopoids and calanoids) while harpacticoids formed the main group as the colonization progressed. Communities were not similar in the first phase of colonisation probably because these were opportunistic species that did not establish themselves since cyclopoids and calanoids are known to be planktonic and not epiphytic. Harpacticoids were only analyzed at the family level but this was appropriate for the study in order to document the phytal and sediment dwelling harpacticoid copepods (Vincx, 1990, De Troch et al. 2008). Other studies dealing with colonization have also analyzed them to the family level (De Troch *et al.* 2005; Warry *et al.* 2009).

The results of copepods from day 6 in the bare and replanted sites and day 10 for the healthy site suggest that recolonization levels of harpacticoid copepods in this system starts around this time. This was seen by the temporal differences observed of the main groups of copepods. However, no spatial difference was observed implying that there is possibility of colonization in bare areas and replanted areas just as the healthy site. The mimics on the healthy sites were planted within a seagrass canopy while the mimics on the bare and replant sites were planted in areas further away from the seagrass patches but the distance to other seagrass patches was equally not very far though it was not determined in this study. Some studies have also shown that recolonization on seagrass mimics depend on the distance from the natural seagrass blades that are assumed to be the source of the colonizing adult copepods. However, it has also been shown that some species may colonize mimics at consistent rates irrespective of the distance from the source (Bell and Hicks, 1991).

True phytal dwelling harpacticoids have been known to belong to the families Harpacticidae, Tisbidae, Porcellidiidae, Tegastidae, Miracidae (formerly Diosaccidae), Peltidiidae and Thalestridae (Hicks and Coull 1983; Hicks 1985; 1977a, b; 1986; Johnson and Scheibling 1987a, b). All these families except Peltidiidae were observed in the sites studied. The relative abundance of these families increased from colonization day 6 onwards suggesting that phytal harpacticoid copepods were

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colonizing the mimics. These were also observed from the natural seagrasses apart from Harpacticidae and Porcellidiidae. This could be a result of the sampling because not all the families might have been represented since natural samples were collected in triplicate but only once at the end of the study period. The more families on the bare and replanted sites than the healthy site could also suggest the potentiality of the sediments on these canopy deficient areas to act as pools for copepods by upward movement from the sediment. This is because the families such as Cletodidae, Longipediidae and Laophontidae were also observed in underlying sediments in the bare and replanted sites.

The study only focused on the identification of adult harpacticoid copepods hence the copepodites and nauplii were not identified to family level. Ectinosomatidae and Thalestridae were the dominant harpacticoid families present in all sampling points during the colonization period. Ectinosomatidae are mainly known to be an itinerant form, occupying both the blade surfaces and sediment hence its presence on the mimics could suggest that the main origin of the copepods was from the surrounding sediments. Ameiridae, a sediment-dwelling family, was also found in high abundance on the mimics planted in the healthy site and on natural plants from the healthy site. It was found in high densities in the sediments in the bare and replanted sites also but not as much on the mimics at these sites especially at the last colonization day (day 21). This suggests that they like the seagrasses in healthy conditions since they form a canopy structure that enables them to actively move from the sediments inspite of their sediment-dwelling ecology.

From the results it is clear that the source of the harpacticoid copepods could have been both from sediment (active migration) and also from natural seagrass blades especially for the phytal families found in the bare and replanted sites since these were not so far from other healthy seagrass patches around the bare and replanted sites (passive migration). Studies have shown that it is likely that many harpacticoid copepods are capable of actively departing the sediment (Teasdale *et al.* 2004).

Biodiversity and community composition can be affected positively or negatively through the changes in size, shape and location of the remaining habitat patches (Fahrig, 1997) especially in cases of fragmentation that could be caused by natural as well as anthropogenic factors. Fragmented and continuous seagrass habitats have been found to have significant differences in infaunal assemblage (Frost *et al.* 1999) and also in different sized seagrass patches (Bowden *et al.* 2001).

The results thus suggest possible recovery of harpacticoid copepods after disturbance thanks to their mobility and ability to colonize new areas quickly. However, this may depend largely on the time the epiphytic biofilm is able to recover as well as the recovery time of the seagrass plants which may take approximately 4 years (Alcovero and Mariani 2002). Recovery of the associated meiofauna is thus possible and will also depend on variation of seasons. Recovery of the functions of the seagrass ecosystem would therefore take a longer period than the studied colonization time due to the late colonization by epiphytic flora. However, this could also be attributed to the season when the samples were collected i.e. after strong intermonsoon conditions. The scale of the study was on a local basis at the site and the sampling sites were less than 500 m apart. To try and improve this I would recommend a study that would include a greater spatial scale since this may influence greatly the interpretation of the seagrass ecological system as a whole. Seasonality would also be a good point to include when doing such a kind of study to be able to identify any seasonality pattern in the meiofauna densities and their community structure.

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Raw Data

Annex 1: Epiphytic meiofauna data for healthy site

neurity	д, 0 ,0-10р	neutes 1,2		.cuvciy, 2,	4,0,10,14,21		st augs									
Major Taxa	2A	2B	2C	4A	4B	4C	6A	6B	6C	10A	10B	10C	14A	14B	14C	21A
Nematoda	4	5	0	6	4	3	8	5	3	42	43	21	63	33	C	20
Isopoda	C	0 0	2	0	0	1	2	1	5	6	18	2	7	8	C	1
Copepoda	47	35	32	38	92	54	14	46	49	157	194	135	235	138	77	69
Ostracoda	4	4 O	1	2	1	2	9	1	6	15	24	19	13	14	11	5
Polychaeta	C	0 0	1	1	2	1	6	3	4	4	2	2	12	7	7	5
Amphipoda	2	2 2	1	0	4	3	4	6	1	18	8	10	12	10	6	c C
Nauplii larvae	81	35	117	109	66	160	0	1	3	187	97	30	66	78	122	28
Gastropoda	5	5 4	0	2	1	0	0	0	1	1	0	7	7	4	1	5
Tanaidacea	C	0 0	0	0	0	0	0	0	0	1	0	8	8	1	C	2
Cumacea	C	0 0	2	0	0	0	0	2	0	0	5	0	0	0	C	C
Turbellaria	C	0 0	0	0	0	1	0	0	0	1	3	1	7	4	1	8
Cnidaria	C) 1	0	0	1	0	0	0	0	5	2	2	4	17	2	4
Rotifera	C	0 0	0	0	0	0	0	0	0	1	0	0	0	0	C	0
Tardigrada	C	0 0	0	0	0	0	0	0	0	2	0	0	0	0	C	0
Oligochaeta	C	0 0	0	0	0	0	0	0	0	1	0	0	0	0	C	0
Halacaridae	C	0 0	0	0	0	0	0	0	1	1	16	3	3	2	1	0
Cladocera	C	0 0	1	1	3	0	0	1	1	5	3	0	6	0	C	0
Insecta	C	0 0	0	0	0	0	0	0	1	0	0	1	6	0	7	1 1
Thermosbaenacea	1	0	0	0	0	0	0	0	0	0	0	0	0	0	C	0
Water mite	C	0 0	0	0	0	0	0	0	0	0	0	0	0	0	C	0
Total Density	144	82	157	159	174	225	43	66	75	447	415	241	449	316	235	148
Number of Taxa	7	' 6	8	7	9	8	6	9	11	16	12	13	14	12	10	11

Healthy A.B.C-replicates 1,2 and 3 respectively; 2,4,6,10,14,21 are harvest days

Annex2: Epiphytic meiofauna data for Replant site

Ropiant	7 ij D j O 10 p	iloatoo iji t		, _ ,	i,e, ie, i=,=	ale naive	otaayo									
Major Taxa	2A	2B	2C	4A	4B	4C	6A	6B	6C	10A	10B	10C	14B	14C	21A	21B
Nematoda	10	8	17	12	22	19	39	64	39	88	40	36	94	35	34	37
Isopoda	0	0	1	0	2	0	1	4	5	5 2	6	2	19	1	5	1
Copepoda	86	54	31	81	72	119	99	79	91	477	187	252	286	118	60	28
Ostracoda	7	2	9	2	10	10	14	13	9	44	20	17	88	37	4	4
Polychaeta	4	5	3	8	0	1	0	4	5	5 2	11	1	26	5	14	0
Amphipoda	7	7	4	10	1	6	8	3	4	24	7	12	10	8	5	2
Nauplii larvae	284	223	107	133	661	512	105	25	30	93	53	72	80	91	7	9
Gastropoda	12	6	12	7	16	0	5	4	1	0	10	4	6	7	1	3
Tanaidacea	0	0	0	0	1	0	0	0	0) 11	7	0	0	2	0	0
Cumacea	3	0	0	1	0	0	0	0	0) 4	1	0	2	0	0	0
Turbellaria	0	0	0	0	0	0	0	1	0	16	1	2	8	3	0	1
Cnidaria	0	0	0	0	1	0	0	2	0	2	0	1	4	3	4	0
Rotifera	0	0	0	0	0	0	0	0	0	0 0	0	1	1	0	0	0
Tardigrada	0	0	0	0	0	0	0	4	0	22	0	7	0	0	0	0
Oligochaeta	0	0	0	0	0	0	0	0	0	0 0	0	2	2	0	0	0
Halacaridae	0	29	1	0	1	0	1	1	0) 4	2	0	1	0	0	0
Cladocera	0	0	0	0	0	0	1	2	4	0	1	0	0	0	0	0
Insecta	0	0	0	0	0	0	0	0	0) 3	0	0	0	2	0	0
Thermosbaenacea	0	0	0	0	0	0	0	0	0	0 0	0	0	0	0	0	0
Water mite	0	0	0	0	0	2	0	0	0	0 0	0	0	1	0	0	0
Total Density	413	334	185	254	787	669	273	206	188	792	346	409	628	312	134	85
Number of Taxa	8	8	9	8	10	7	9	13	9	14	13	13	15	12	9	8

Replant A,B,C-replicates 1,2 and 3 respectively; 2,4,6,10,14,21 are harvest days

Annex3: Epiphytic meiofauna data for bare site

Bare	A,B,C-rep	licates	1,2 a	nd 3 respe	ectively; 2,4	4,6,10,14,2	1 are harve	st days									
Major Taxa	2A	2B		4A .	4B	4C	6A	6B	6C	10A	10B	10C	14A	14B	14C	21A	21B
Nematoda	0		1	19	14	22	33	25	5 3 [.]	31	34	16	23	41	62	1	0
Isopoda	0		2	0	0	1	12	1	1	s 0	3	2	7	5	12	2	2 1
Copepoda	0		38	125	232	150	79	49	96	5 106	291	112	93	122	383	1	144
Ostracoda	0		6	4	4	4	31	6	6	6 9	22	36	22	49	44	1	23
Polychaeta	0		2	5	10	9	2	0		8 7	12	2 2	1	7	9	0	0 0
Amphipoda	0		3	0	1	0	9	6	5 2	2 5	7	13	31	12	32	0	0 0
Nauplii larvae	0		1	143	396	198	13	30	22	2 36	20	80	23	20	55	0	0 0
Gastropoda	3		0	1	4	8	6	0) 2	2 4	8	0	0	0	0	0) 1
Tanaidacea	0		0	1	0	0	0	0		8 1	8	0	0	0	0	0	0 0
Cumacea	0		0	2	0	1	1	1		0 0	2	3	19	4	0	0	0 0
Turbellaria	0		0	0	0	1	3	0) -	0	C	0 0	0	0	0	0	0 0
Cnidaria	0		0	0	0	1	2	0) -	1	2	0	1	5	7	0	0 0
Rotifera	0		0	0	0	0	3	6	6 (0 0	C) 1	0	0	0	0	0 0
Tardigrada	0		0	0	0	0	2	3	3	0	C	0 0	0	6	0	0	0 0
Oligochaeta	0		0	0	0	0	2	1		0 0	1	0	0	1	0	0	0 0
Halacaridae	0		0	0	0	0	2	0) -	0	1	0	0	0	0	0	5
Cladocera	0		0	0	0	0	0	0) (0 0	C	0 0	0	0	0	0) 1
Insecta	0	1	0	0	0	0	0	0) (0 0	4	0	0	0	0	0	0 0
Thermosbaenacea	0		0	0	0	0	0	0) (0 0	C	0 0	0	0	0	0	0 0
Water mite	0		0	0	0	0	0	() (0 0	C	0 0	0	0	0	0	0 0
Total Density	3		53	300	661	395	200	128	3 172	200	415	265	220	272	604	5	5 175
Number of Taxa	1		7	8	7	10	15	10	13	8 9	14	9	9	11	8	4	6

Annex4: Epiphytic harpacticoid families in the healthy site

nd 21 for the days

Family	2A	2B	2C	4A	4B	4C	6B	6C	10A	10B	10C	14A	14B	14C	21A	21B
Thalestridae	7	0	1	2	11	4	15	4	19	20	17	15	22	14	5	18
Tisbidae	12	12	5	3	9	2	12	7	2	6	8	4	0	5	4	5
Ectinosomatidae	2	7	1	1	15	5	7	10	9	20	24	11	33	7	8	11
Ameiridae	0	1	0	0	5	1	1	6	4	9	17	8	15	2	17	29
Laophontidae	2	0	0	0	1	0	0	1	2	1	2	4	1	2	1	3
Longipediidae	0	0	0	0	0	0	0	2	1	0	3	3	2	0	0	0
Miiracidae	0	1	0	0	0	0	0	2	7	1	7	9	5	9	6	10
Porcellidiidae	3	1	0	1	2	0	0	3	5	2	2	7	5	2	3	2
Unidentified	0	0	0	0	0	0	0	3	1	0	0	3	0	1	0	0
Harpacticidae	1	0	1	0	1	0	2	0	0	0	0	3	0	1	0	0
Tetragonicipidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tegastidae	1	0	0	0	0	0	0	0	0	0	3	2	1	0	2	2
Cleotidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Canuellidae	0	1	0	0	0	0	0	0	3	0	0	1	0	0	0	0
Rhizotrichidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Canthocamptidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Total Density	28	23	8	7	44	12	37	38	53	59	83	72	84	43	46	80
Number of families	7	6	4	4	7	4	5	9	10	7	9	14	8	9	8	8

Annex5: Epiphytic harpacticoid copepods in the replant site

Femily	24		100			6.4	6P	60	40.4	100	100	440	440	24.4	240
Family	ZA	2B	20	4A	4B	6A	6B	6C	10A	108	100	14B	140	21A	218
Thalestridae	3	1	1 5	5 2	2	8	4	24	31	20	15	14	18	15	2
Tisbidae	0	13	3 3	5 5	4	11	10	10	5	1	14	4	4	0	0
Ectinosomatidae	10	2	2 5	5 3	7	13	7	15	3	13	12	15	12	11	6
Ameiridae	1	1	I C) 1	2	4	14	6	9	3	9	6	11	3	0
Laophontidae	0	(0 0) 1	1	6	2	6	7	12	3	10	5	6	0
Longipediidae	0	(0 0	2	0	1	3	0	6	8	4	2	1	0	1
Miiracidae	0	(0 0	2	0	4	1	6	10	7	6	12	2	4	2
Porcellidiidae	1	1	I C) 3	0	1	4	0	0	0	1	0	0	0	0
Unidentified	0	(0 0	0 0	0	1	0	0	1	0	0	1	1	0	0
Harpacticidae	0	(0 0) 1	1	2	2	0	3	0	0	1	0	3	0
Tetragonicipidae	0	(0 0	0 0	0	0	0	0	0	0	0	0	0	0	0
Tegastidae	1	(0 0	0 0	0	0	0	0	0	3	4	1	6	1	2
Cleotidae	0	(0 0	0 0	0	0	0	0	0	0	0	0	0	0	0
Metidae	0	(0 0	0 0	0	0	0	0	0	0	0	0	0	0	0
Canuellidae	5	1	I C	0 0	1	0	0	0	0	0	0	0	0	0	0
Rhizotrichidae	0	(0 0	0 0	0	0	0	0	0	0	0	0	0	0	0
Canthocamptidae	0	(0 0	0 0	0	0	0	0	0	0	0	0	0	0	0
Total Density	21	19	9 13	3 20	18	51	47	67	75	67	68	66	60	43	13
Number of families	6	6	6 3	9 9	7	10	9	6	9	8	9	10	9	7	5

Replant A.B.C for replicate and 2.4.6.10.14 and 21 for the days

Annex6: Epiphytic harpacticoid families in the bare site

Bare	A,B,C for	replicate al	na 2,4,6,10,	14,and 21 1	for the days	S								
Family	2B	4A	4B	4C	6A	6B	6C	10A	10B	10C	14A	14C	21B	21C
Thalestridae	1	5	4	2	9	7	9	39	8	8	8	15	46	31
Tisbidae	3	6	3	3	3	7	15	5	5	8	6	3	0	1
Ectinosomatidae	4	. 10	2	7	13	5	21	10	7	17	23	18	1	23
Ameiridae	1	0	0	1	15	5	13	10	7	8	6	6	3	5
Laophontidae	0	2	0	1	7	2	4	2	4	3	1	3	7	4
Longipediidae	0	2	0	1	1	2	1	2	2	4	6	1	2	4
Miiracidae	0	1	0	0	5	3	0	7	3	9	6	8	20	13
Porcellidiidae	0	2	0	2	1	0	1	0	0	0	0	0	0	0
Unidentified	0	1	0	0	0	1	0	0	1	0	5	1	0	0
Harpacticidae	0	0	2	1	0	1	2	1	1	2	6	5	1	2
Tetragonicipidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tegastidae	0	0	0	0	0	0	1	0	0	0	1	6	6	10
Cleotidae	0	0	0	0	0	0	0	13	4	0	1	0	0	0
Metidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total Density	9	29	11	18	54	33	67	89	42	59	69	66	86	93
Number of families	4	. 8	4	8	8	9	9	9	10	8	11	10	8	9

Bare A B C for replicate and 2 4 6 10 14 and 21 for the da

Annex7: Benthic meiofauna in the healthy site

Healthy	A,B,C-repl	icates; 1,2	and 3 are 0)-1cm, 1-2 (cm and 2-5	cm respec	tively		
Major Taxa	1A	1B	1C	2A	2B	2C	3A	3B	3C
Polychaeta	7	25	0	22	53	9	31	129	
Copepoda	18	12	13	27	33	8	13	445	
Nauplis larvae	4	0	1	1	11	0	0	14	
Tubellaria	6	0	0	319	10	0	28	0	
Young crab	0	0	0	0	0	0	0	0	
Rotifera	0	0	0	0	0	0	0	0	
Amphipoda	1	0	3	2	0	0	0	0	
Nematoda	257	359	159	413	359	837	417	651	
Oligochaeta	13	4	2	6	1	2	0	5	
Ostracoda	0	1	0	1	0	0	1	0	
Cnidaria	1	0	0	1	0	0	0	0	
Tanaidacea	3	2	0	0	0	0	0	0	
Total Density	310	403	178	792	467	856	490	1244	
Number of Taxa	9	6	5	9	6	4	5	5	

Annex8: Benthic meiofauna in the replant site

Replant	A,B,C-replicates; 1,2 and 3 are 0-1cm, 1-2 cm and 2-5 cm respectively
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Major Taxa	1A	1B	1C	2A	2B	2C	3A	3B	3C
Polychaeta	0	0	0	1	2	4	0	2	3
Copepoda	50	10	6	120	35	120	270	58	31
Nauplis larvae	0	0	1	11	0	62	0	0	0
Tubellaria	55	8	9	10	51	2	0	0	7
Young crab	0	0	0	0	0	0	0	0	0
Rotifera	0	0	0	0	0	0	0	0	0
Amphipoda	0	0	0	0	0	0	1	0	0
Nematoda	247	123	91	321	287	419	521	753	197
Oligochaeta	0	0	3	0	0	1	2	53	4
Ostracoda	0	0	0	0	0	0	0	0	0
Cnidaria	0	0	0	0	0	0	0	0	0
Tanaidacea	0	0	3	0	0	0	0	0	0
Gastropoda	0	0	0	0	0	1	0	0	0
Halacaridae	0	0	1	0	0	0	0	0	0
Total Density	352	141	114	463	375	609	794	866	242
Number of Taxa	3	3	7	5	4	7	4	4	5

Annex9: Benthic meiofauna in the bare site

Bare	A,B,C-repl	icates; 1,2	and 3 are 0)-1cm, 1-2 d	cm and 2-5	cm respec	tively	
Major Taxa	1A	1B	1C	2A	2B	2C	3B	3C
Polychaeta	3	3	1	1	8	4	7	4
Copepoda	35	140	12	10	77	37	87	10
Nauplis larvae	20	14	0	56	2	1	9	1
Tubellaria	2	1	7	2	26	59	30	29
Young crab	0	0	0	0	0	0	0	0
Rotifera	0	0	0	0	0	0	0	0
Amphipoda	0	0	0	0	0	0	0	0
Nematoda	529	245	301	273	285	271	671	102
Oligochaeta	288	3	1	17	0	0	5	13
Ostracoda	1	0	0	1	0	1	2	2
Cnidaria	0	0	0	0	0	0	0	0
Tanaidacea	0	0	0	0	0	0	10	0
Tardigrada	384	0	0	0	0	0	0	0
Halacaridae	0	0	0	0	0	0	1	0
Total Density	1262	406	322	360	398	373	822	161
Number of Taxa	8	6	5	7	5	6	9	7

Annex10: Benthic harpacticoid copepods in the healthy site

lthv	A.B.C-replicates:	1.2 and 3 are 0-1cm.	1-2 cm and 2-5 cm respective	elv
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Healthy A,B,C-replicates; 1,2 and 3 are 0-1cm, 1-2 cm and 2-5 cm respectively									
Family	1A	1B	1C	2A	2B	2C	3A	3B	3C
Ameiridae	0	0	2	1	11	1	120	74	1
Laophontidae	0	0	0	0	0	0	0	0	0
Longipediidae	1	1	0	0	0	0	0	0	0
Miiracidae	0	0	0	0	1	0	5	20	0
Tetragonicipidae	0	0	0	0	0	0	0	8	0
Tegastidae	0	0	0	0	0	0	0	0	0
Cletodidae	0	0	0	2	12	2	0	4	1
Canuellidae	0	0	0	0	0	0	0	0	0
Canthocamptidae	0	6	0	1	0	0	0	0	0
Ectinosomatidae	11	2	7	0	1	2	0	0	0
Total Density	12	9	9	4	25	5	125	106	2
Number of families	2	3	2	3	4	3	2	4	2

Annex11: Benthic harpacticoid copepods in the replant site

Replant	A,B,C-replicates; 1,2 and 3 are 0-1cm, 1-2 cm and 2-5 cm respectively											
Family	1B	1C	2A	2B	2C	3A	3B	3C				
Ameiridae	0	0	0	1	0	0	0	0				
Laophontidae	0	1	0	0	1	0	0	0				
Longipediidae	0	0	0	1	0	0	0	0				
Miiracidae	0	0	0	0	0	0	0	0				
Tetragonicipidae	0	0	0	0	0	0	0	0				
Tegastidae	0	0	0	0	0	0	0	0				
Cletodidae	0	3	0	0	122	30	56	83				
Canuellidae	0	0	0	0	0	0	0	0				
Canthocamptidae	0	0	0	0	0	0	0	0				
Ectinosomatidae	3	0	0	0	0	0	0	0				
Total Density	3	4	0	2	123	30	56	83				
Number of families	1	2	0	2	2	1	1	1				

Annex12: Benthic harpacticoid copepods in the bare site

Bare	A,B,C-repl	icates; 1,2	and 3 are 0)-1cm, 1-2 (cm and 2-5	cm respec	tively
Family	1A	1B	1C	2A	2B	2C	3B
Ameiridae	0	0	0	0	0	9	17
Laophontidae	4	0	2	0	0	0	C
Longipediidae	1	1	0	0	0	0	C
Miiracidae	1	16	6	0	29	7	2
Tetragonicipidae	0	0	0	0	0	0	C
Tegastidae	0	0	0	0	0	0	C
Cletodidae	9	29	0	0	23	0	54
Canuellidae	0	0	0	0	0	1	1
Canthocamptidae	0	0	1	0	0	0	C
Ectinosomatidae	5	0	1	0	1		1
Total Density	20	46	10	0	53	17	75
Number of families	6	4	5	0	4	4	6

Annex13: Sediment environmental data

Site	Gravel %	Sand %	Silt %	Porosity	TOM %	PO₄µg/l	NO ₃ +NO ₂ µg/I
Healthy	8.824815	90.8676	0.101029	0.427333	6.273261	2.757947	2.284128789
Healthy	4.480675	95.239	0.316978	0.536333	4.289008	1.367053	0.900886034
Healthy	0	0	0	0.5343	5.631323	0.637022	0.736100842
Replant	0.918488	98.6797	0.177002	0.4284	2.683461	1.239111	1.650372606
Replant	1.077877	98.3467	0.229605	0.443033	2.908065	1.230096	0.97152193
Replant	0	0	0	0.468567	2.133565	0.95613	0.772263048
Bare	6.810116	81.5118	0.384716	0.3767	2.979027	1.316532	1.308446623
Bare	3.139854	96.4013	0.269528	0.511	2.807685	1.283768	2.863149501
Bare	0	0	0	0.506033	4.160155	1.592269	3.469594292

Annex14: Water environmental parameters and biofilm data

Site	Day		NO ₃ +NO ₂ µ	NO₂µg/l	NH₄µg/l	PO₄µg/l	Siµg/l	C:14	C:16	C:18	µgchlc2/l	µgfuco/l	µgchla/g	Epiphytes
Healthy		2	0	0	0.00	0.00		0.979521227	6.130612	5.562833	0.520596	0.058536	0.081173	0
Healthy		4	99	0.666667	280.00	18.67	78.00	1.288325148	9.454728	5.121784	0			0
Healthy		6	147	1.333333	121.00	42.33	317.33	1.785536107	9.184452	3.80864	0.34	0.00	0.00	0
Healthy		10	106.6667	2.333333	189	91.33333	304	1.593326424	9.401315	3.447883	1.396802	0.34754	1.621806	0
Healthy		14	106.6667	2.333333	189	91.33333	304	2.301094652	8.222038	2.937361	1.396802	0.34754	1.621806	0
Healthy		21	30.5	1.5	291	26	45	2.691878421	9.174649	3.551019	1.410272	0	0.253443	0
Replant		2	741.3333	1.333333	60.33	1.67	30.67	1.2	8.128668	4.510434	0	0	0.290246	0
Replant		4	1153.667	0.666667	154.00	28.33	463.00	1.0	6.128363	3.201396	0.302959	0	0	0
Replant		6	39.5	0	46.00	27.50	0.00	2.0	8.365992	3.319652	0.27429	0	0	0
Replant		10	127	0	45.33	2.67	84.00	2.0	11.79107	3.623516	0.663239	0	0.450942	166.3636
Replant		14	127	0	45.33	2.67	84.00	3.5	8.705695	3.4719	0.663239	0	0.450942	181.3695
Replant		21	10	0	105.50	8.00	20.00	3.559470622	13.51144	5.91595	4.209196	0.251389	1.388125	108.8674
Bare		2	179.6667	0	22.00	0.00	6.33	0.768252762	4.703392	1.870669	0	0		0
Bare		4	2642	0.333333	217.00	4.33	343.33	0.725999375	5.277668	3.611006	0.429426	0	0	0
Bare		6	39.5	0	46.00	27.50	0.00	2.504836474	12.93073	3.968579	0.27429	0	0	0
Bare		10	127	0	45.33	2.67	84.00	2.333008581	15.20844	4.304834	1.002231	0	0.580003	494.6025
Bare		14	127	0	45.33	2.67	84.00	2.446710745	7.031509	2.68426	1.002231	0	0.580003	297.4778
Bare		21	6	0	60.50	14.50	32.50	3.303494261	7.450202	2.568566	3.770871	5.296437	24.16792	9597.523