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The role of meiofauna in the energy transfer in a Mediterranean seagrass bed (Calvi, Corsica)

by
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Abstract

Meiofaunal communities of five different habitats characterised by different qualities of macrophytodetritus were sampled in a Mediterranean seagrass bed. Two different kinds of meiofauna communities were distinguished amongst the five habitats. A benthic community of meiofauna living on a sediment substrate or in highly fragmented macrophytodetritus and a foliar, epiphytal community associated with seagrass leaves and low fragmented macrophytodetritus leaves. The diversity index amongst these communities was comparable, but the composition in harpacticoid copepods families was different. Trophic biomarkers such as stable isotopes and fatty acids were combined to identify the major sources of organic matter contributing to the diet of these marine invertebrates. Harpacticoid copepods are very likely to feed on the biofilm on the plant material and hence, copepods use the seagrasses and detritus merely as substrate.

In addition to the field data, an experimental setup was conducted where detritus biofilm was enriched with ^{13}C stable isotopic carbon. Harpacticoid copepods and *Gammarus aquicauda* amphipods were inserted in the incubation to observe their interaction and difference in uptake. No interaction between the two invertebrates was observed. The amphipods preferably feed on the detritus. The harpacticoid copepods on the other hand preferred and assimilated more biofilm than the amphipod.

Content

Introduction

Photosynthesis by marine organisms produces 30 to 60 10^{15} g organic carbon per year (Duarte et al., 1996). This is approximately 40% of the total earth primary production. In coastal oceans, autotrophic carbon production is mainly completed by marine macrophytes such as mangroves (Twilley et al., 1992), kelp forests (Duggins, 1989), macroalgae (Duarte, 1996), salt marshes (Currin et al., 1995) and seagrasses (Duarte, 1989). These macrophytic primary producers represent 75% of the autotrophic biomass (Smith, 1981) and are characterised by a high growth rate and production rate comparable to a tropical forest (Pergent et al., 1997). Marine vegetation only covers a very small part of the total world ocean surface, but accounts for a high fraction of carbon storage (Duarte, 1996) and sustains a very high biodiversity (Duarte, 2000).

Seagrasses contribute to the food web by direct consumption (Pergent, 1997) but this is only a modest contribution given the poor nutritional value of the living tissues (Keegan, 1977; Dauby, 1989).

Studies on macrophytodetritus pathways regarding the carbon budget of these benthic communities suggest that on average 25% of the organic carbon produced is being exported to adjacent ecosystem by hydrodynamic forces (Romero et al., 1992).

The remaining part, on average 50.3% accumulates and decomposes as refractory material within the meadow. As such, seagrass ecosystems hold a significant fraction of autotrophic biomass (Duarte et al., 2005) and it can be assumed that these ecosystems are detritus based (Mateo et al., 1997).

Mediterranean seagrass ecosystems are dominated by an endemic seagrass, *Posidonia oceanica*, hereafter referred to as *Posidonia*. Since, *Posidonia* are long living organisms with a very slow turn-over time, a constant supply of detached leaf material is provided as detrital material (Smith, 1981; Hemminga, 2000).

The *Posidonia* leaves are colonised by epiphytes which account up to 30% of the seagrass canopy biomass and use the leaf surface merely as substratum to attach. The *Posidonia* canopy with its epiphytes support an important community of mesograzers and displays a high species diversity (Lepoint et al., 1999; Hemminga, 2000). Kitting et al. (1984) suggested that the indirect grazing on *Posidonia* contributed to the energy flow of the ecosystem.

Macro- and meiofaunal invertebrates living on this canopy form the primary food source for juvenile fish and thus play an indispensable role in transferring the energy from primary producers to higher trophic levels (Sogard, 1984). Beside living in the canopy, those benthic invertebrates are also associated with the refractory material or detritus accumulations in the

meadow (Gallmetzer et al., 2005). In this detritus accumulations was found a significant relationship between bacteria and epifaunal meiofauna (Danovaro, 1996). Bouillon et al. (2004) looked at epifaunal mangrove communities and came to the conclusion that these epifaunal organisms used a wide variety of carbon sources. This variety of carbon sources fuels the indication that seagrasses are dominated by bottom-up processes. Vizzini (2009) state that bottom-up effects are recognizable in food webs where seagrasses limit the herbivory level through their low nutritional quality.

The epifaunal invertebrates, like harpacticoid copepods play thus a key role in the organic matter transportation inside the ecosystem (Jernakoff, 1996). They largely influence the energy flow to higher trophic levels and hence the productivity of the ecosystem, but little is known about their feeding behaviour, except that they are known to feed on a wide variety of food sources (Hicks et al., 1983). Some studies presume a preferential grazing upon the biofilm (De Troch et al., 2008).

One of the central questions in this research will be, to find out what type of carbon source the harpacticoid copepods prefer as major food source. Is it the detrital matter? Do they prefer the biofilm on living leaves or on detritus? Do they primarily feed on bacteria? Which food source, related to different potential habitats can support the highest population densities? Do these different potential habitats create diversity amongst the ecosystem and thus different meiofaunal population composition?

In order to answer these questions, up-to-date tracer techniques are used as they provide a useful tool to unravel trophic interactions in complex ecosystems with multiple potential trophic pathways like seagrass beds. Two trophic biomarkers were combined in this study. Stable isotope tracers ($\delta^{13}C$ and $\delta^{15}N$) have been used to follow the organic matter fluxes into food webs and try to resolve the food web structure (Fry, 1987; Lepoint et al., 2000; Vizzini et al., 2002). Fatty acid trophic biomarkers have also been used to unravel the interactions between trophic levels and identify major sources of organic matter contributing to detrital food webs (Kharlamenko et al., 2001). The essential fatty acids are incorporated into consumers in a conservative way, thereby providing information on predator-prey relations (Dalsgaard et al., 2003).

Materials and methods

The sampling for this study was carried out at the Punta Oscellucia site in the Revellata Bay (Calvi, NW Corsica, France) (42 °35'N, 8 °43'E) near the oceanographic station of STARESO (University of Liège) (**Fig.1**).

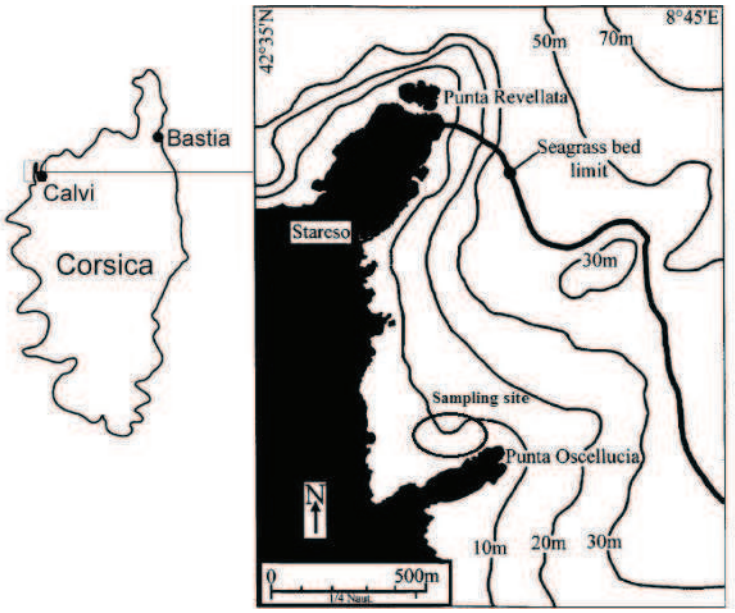

Fig. 1 Location of the Revellata bay, the sampling site and the limits of the *P. oceanica* meadow in the bay.

In situ characterisation

All samples were taken by scuba divers at 10 m depth in August 2009. Five potential habitats for meiofauna representing different detritus states were collected in three replicates each: living stems with leaves of *Posidonia* (abbreviation: LL), low fragmented macrophytodetritus of *Posidonia* (abbreviation: LF), highly fragmented macrophytodetritus of *Posidonia* (abbreviation: HF), uncovered sediments (abbreviation: US) and sediments covered by phytodetrital material of *Posidonia* (abbreviation: CS). The living leaves were cut off at the sediment-water interface and put in a plastic jar closed under water. The macrophytodetritus samples were scooped by hand off the seafloor and collected in a 1L jar and closed under water. For the sediment samples, the top 4 cm of the sediments on a 20 cm long tract were collected in a 0.5L jar that was closed under water.

In the field research lab for the community composition, an 8% $MgCl_2$-solution was added to the epiphytic samples (the two LF and HF detritus samples and the LL living leaves samples) to facilitate the collection of epiphytic organisms (Hulings, 1971; Hicks, 1977). All samples were rinsed over a 1-mm mesh size sieve to exclude the macrofauna and on a 38-µm mesh size sieve to retain the meiofauna before fixation with formaldehyde (4% final concentration).

The 38 µm fractions were centrifuged three times at 3000 rpm for 15 min with Ludox HS40 (density of 1,18) in order to extract meiofauna. The supernatants containing the meiofauna were stained with Roze Bengal. All the meiofauna was counted at higher taxon level based on Higgins and Thiel (1988) using a binocular. The first hundred copepods encountered were picked out at random and stocked in 75% ethanol. They were afterwards identified to order level and family level for the order Harpacticoida using the identification keys and reference books by Boxshall (2004) and Lang (1948; 1965).

In relation to the central research question, three environmental factors were considered: the total organic carbon, the epiphytic samples dry weight and the sediment grain size. To measure the total organic carbon, a part of all the 1mm fraction samples were dried at 60°C for 48h and afterwards pulverised for 30 minutes after acidification with HCl. Analysis were carried out with a ThermoFinnigan Flash1112 elemental analyzer using the method of Nieuwenhuize et al. (1994). The epiphytic samples dry weight were measured as remaining weight after drying in an oven at 60°C for 72h. Sediment grain size was analysed using a sieving tower set up of nine sieves. A series of 125 µm, 250 µm, 500 µm, 710 µm, 10000 µm, 1400 µm, 2000 µm, 2800 µm, 4000 µm mesh sizes were used in order to classify the sediments according to the Udden/Wentworth grain size classes (Blair et al., 1999).

To understand ecological processes and trophic relations, samples were taken for stable isotopes and fatty acids. Two 50L bags were filled with low fragmented litter and highly fragmented litter under water. These bags were put in separate aquaria and copepods were caught using phototaxis. Afterwards the copepods were washed, put in petriplates per 100 specimens and directly frozen in liquid nitrogen. The three epiphytic habitats plus the associated biofilm, scraped with a razor blade, were collected in petriplates and stored in liquid nitrogen (except for the highly fragmented habitat where no biofilm could be sampled). Part of the leaves, detritus, biofilm and copepods were dried for 24 h at 60 °C, ground and loaded into tin capsules for isotopic measurements (see further). The other part was extracted for fatty acid measurements (see further).

Experimental design

In order to test the grazing activity, a thirty litre jar was filled with low fragmented detritus and seawater during an extra field campaign in March 2010 at the Punta Oscellucia sampling site. The organisms used in this microcosm experiment were harpacticoid copepods and *Gammarus aequicauda*, hereafter referred to as *Gammarus*. They were collected amongst the detritus of the extra field campaign. The experimental design consisted of four different treatments: detritus (control), detritus + copepods, detritus + *Gammarus*, detritus + copepods

+ *Gammarus.* Each treatment was replicated six times in bottles with 250 ml Punta Oscellucia water. In total the experimental set-up consisted of 6x4 jars (Fig. 2). From each sample, detrital leaves with their epiphytic biofilm were rinsed to eliminate fauna. These defaunated leaves were labelled with 200 µg ^{13}C ($NaH^{13}C0_3$ 99% ^{13}C) and 100 µg ^{15}N ($^{15}NH_4$) through impregnation. The tracers and the litter were incubated in a 4L aquarium for 48h. Afterwards each leaf was cut into five pieces (four treatments + one t_0) to obtain pieces of 145 mm². The t_0 was divided in three equal parts for a control stable isotope analysis, quantitative epiphytic flora analysis and fatty acid analysis. The analysis of the epiflora and the fatty acid analysis were not conducted in the present study due to time constrains.

The remaining four pieces were weighed and photographed with a DP 200 camera mounted on a Zeiss Stemi 2000-C binocular. The foliar surfaces were calculated by means of Deltapix viewer pro software.

A micro-respiration chamber system (Unisense A/S, Aarhus, Denmark) was used in the respiration experiments. Litter fragments were placed in a 2 ml glass respiration chamber in a thermostatically controlled water bath (13 ± 0.1 °C). The chambers contained 0.2 µm filtered Punta Oscellucia sea water. Dissolved oxygen content was measured with a calibrated Clark-type oxygen microsensor (Unisense A/S, model OX-MRCh) during ten minutes.

Twelve *Gammarus* adults (ten males and two females) were manually caught from the 30L macrophytodetritus jar. The meiofauna present in the jar was sieved on a 38 µm mesh size sieve. In addition, 720 random copepods (60 per replicate) were attracted by phototaxis and were collected with a plastic pipette.

Fig. 2 Experimental set up scheme of the treatment bottles placed in a temperature regulated aquarium. Each bottle was filled with 250ml Punta Oscellucia seawater and the treatment.

The whole set up was incubated during four days in an aquarium at real time Calvi photoperiod (approximately 12h/12h light/dark regime) and temperature (13°C for end March 2010).
After the incubation, the respiration was measured during ten minutes. The weight and foliar surface area of the remaining detritus was measured. The life status of the amphipods and the copepods was observed. The copepods were rinsed and transferred into tin capsules with a picking needle. The amphipods were ground after gender identification and loaded in tin capsules. The capsules with copepods and amphipods were desiccated at 60°C for 24h before conducting isotopic measurements (see further).
Additional samples for fatty acids and electronic microscopical observations in order to identify the epiphytic flora were taken. Due to time constrains in the present study, the analysis of the epiflora and fatty acids were not conducted.

Analytical techniques and data treatment

The diversity indices of Hill (Hill, 1973) were calculated using the PRIMER 5 software (version 5.2.8) with N_0= number of taxa or families; N_1= exp (H'), with H' the Shannon-Wiener diversity index based on the natural logarithm (ln); N_{inf}= the reciprocal of the proportional abundance of the most common taxa or family (reciprocal of the Berger-Parker index).
Community structure similarity was analyzed through clustering using the Bray-Curtis similarity index after being square-root transformed in the statistical software PRIMER 5. The relative abundance was expressed as percentages. In order to highlight the most characteristic and contributing harpacticoid copepod families a SIMPER (SIMilarity PERcentage) analysis has been carried out with the same PRIMER 5 software. Differences amongst treatments were tested by means of a one-way analysis of variance (ANOVA) with the statistical Statistica software (version 7.0, Statsoft Inc.).
The isotopic measurements were performed with a mass spectrometer (VG Optima, Micromass, UK) coupled to a C-N-S elemental analyzer (Carlo Erba, Italy) at the University of Liège. Isotopic data were expressed as δ value (‰) relative to the VPDB (Vienna Peedee Belemnite) standard and to atmospheric N_2 for carbon and nitrogen, respectively.
Before the analyse, the biofilm was not treated with HCl to eliminate the calcium carbonate. As a result, all the biofilm ^{13}C values show presence of $CaCO_3$ and organic material and thus cannot be used to discuss their carbon contribution. Data from (Lepoint et al., 2006) and (Dauby 1989) on the ^{13}C values of the biofilm will be used for the discussion.
The isotopic composition ($\delta^{13}C$) of the experiment gave a qualitative measure for the uptake

of labelled biofilm. A quantitative measure for uptake was calculated through the differences in atom^{13}C%. That is the proportion of ^{13}C atoms relative to total C atoms (^{12}C+^{13}C) after the experiment minus their natural value. This excess in ^{13}C multiplied by the organisms dry weight and their carbon proportion from Avery et al. (1996) for *Gammarus* and from Mauchline et al. (1998) for copepods gives the total ^{13}C uptake . Afterward transformation to uptake per day (μg^{13}C.day^{-1}) was done.

The fatty acid extraction or total lipid hydrolysis and methylation to fatty acid methyl esters (FAMEs) for FA-specific δ^{13}C analysis were carried out at the Ghent University. A one step derivatisation method with H_2SO_4-methanol (Guckert et al., 1985) with a modified Bligh and Dyer method, according to (Findlay et al., 1989), was used. These FAME's were analysed in a Hewlet Packard 6890N GC with a mass spectrometer (HP 5973). The samples were run in splitless mode, at an injector temperature of 250°C using a HP88 column (Agilent Co., USA). Identification of all FAMEs was based on the comparison of their retention times relative to an authentic standard and comparison with mass spectra of an authentic standard. The fatty acid C19:0 was used as an internal standard for the quantification. The composition data was afterwards transformed to proportions of total fatty acid.

Results

In situ characterisation

Community composition

Meiofauna

A total of 36802 organisms, belonging to 19 taxa have been identified over the five sampled habitats. Based on relative composition, 88% of all the organisms belonged to three taxa*:* Polychaeta, Nematoda and Copepoda. Copepods showed the highest relative abundance (49.4% ± 3.4% SD) with a maximum in the LF sample. Nematodes accounted for 23.3% ± 6.8% SD with a maximum in the HF sample. Polychaetes accounted for 15.0% ± 5.1% SD with a maximum in the LL sample. The remaining 12% were made of, in order of decreasing abundance: Amphipoda, Nauplii, Ostracoda, Tanaidacea, Isopoda, Holacarida, Turbellaria, Leptostraca, Oligochaeta, Tardigrada, Decapoda, Chaetognatha, Cnidaria, Pycnogonida, Cumacea and Paguroidea.

After standardisation to dry weight and volume (depending on the sampling method: sediment and plant material, respectively) two groups were defined arbitrarily: an epiphytic group including the HF, LF and LL samples and a sediment group including the US and CS samples. The mean densities for the epiphytic group were standardised towards gram seagrass material dry weight (Fig 3 A) while the mean densities for the sediment group were shown per litre sediment (Fig 3 B).

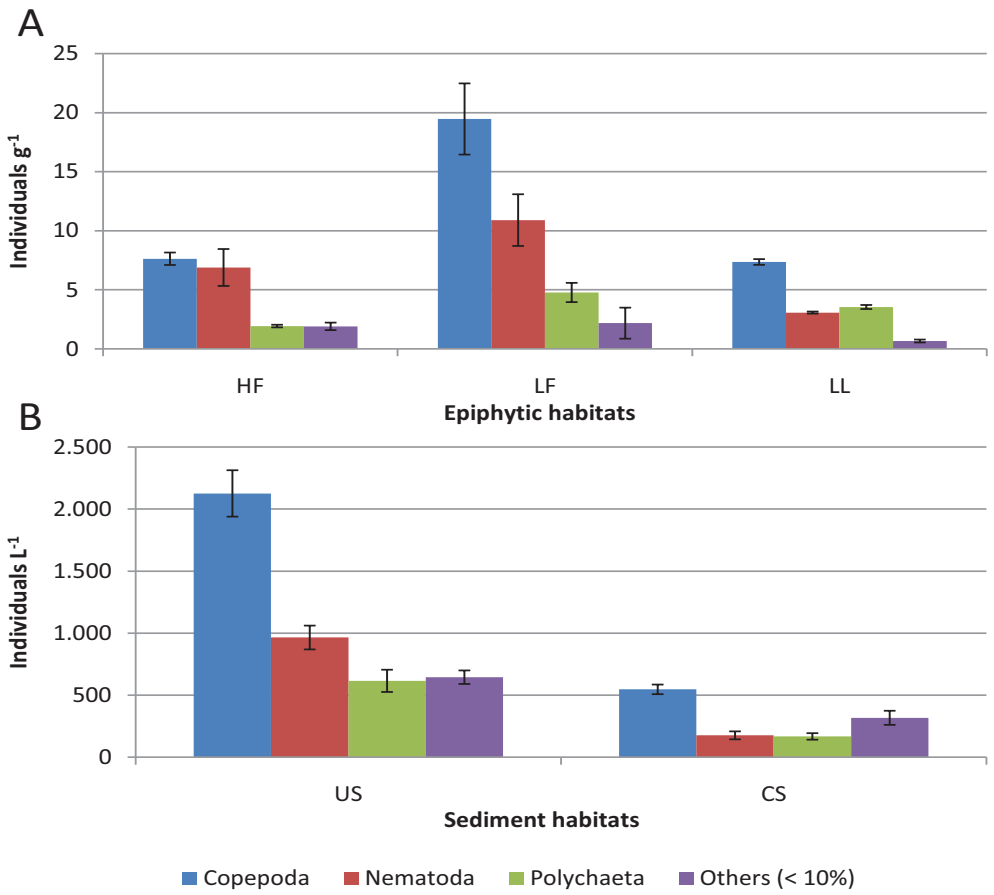

Fig. 3 Densities per habitat type. *A*: Mean densities per gram dry weight epiphytic habitats. *B*: Mean densities per litre sediment habitats. Error bars represent the standard error. Abbreviations are LL: Living leaves; LF: Low fragmented phytodetritus; HF: Highly fragmented phytodetritus; CS: Covered sediments; US: Uncovered sediments

The low fragmented litter harboured a higher density per gram dry weight than the highly fragmented litter and living leaves in the epiphytic group. In the sediment group a total density of 4351 organisms in the uncovered sediment was much higher than the total density of 1207 organisms in the covered sediment sample.

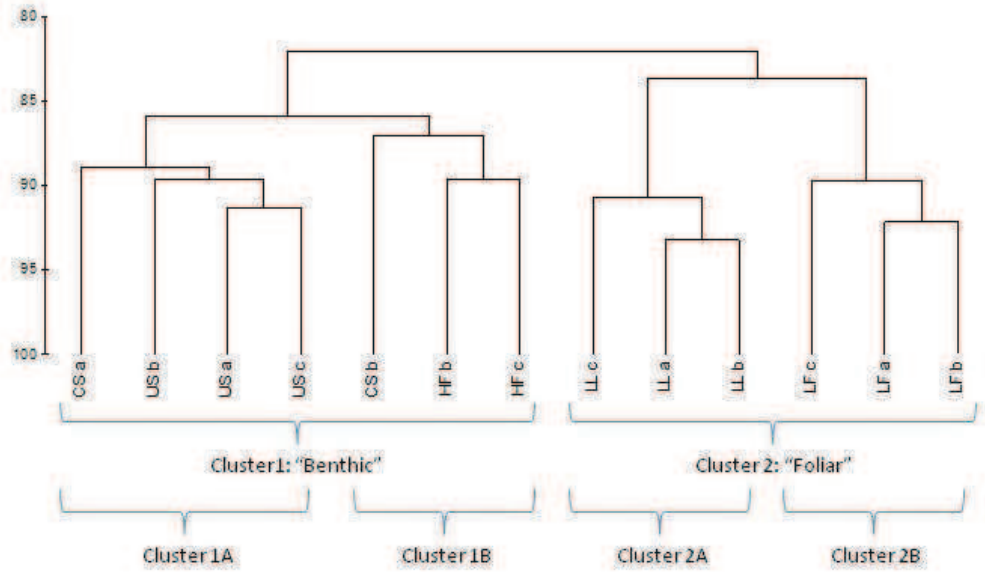

Fig. 4 Cluster analysis per habitat sample, without HF a and CS c for the meiofauna. Composition based on the Bray-Curtis similarity coefficient (in % on y-axis).

The cluster similarity dendogram (Bray-Curtis similarity index) shows that all meiofaunal samples have a similarity of 82.03% (Fig. 4). The samples CS c and HF a were left out of the analysis because they were biased due to a very high amount of nauplii and nematodes in these samples, respectively.

The dendogram show two main clusters. Cluster 1 with a similarity of 85.87% groups all the sediment samples and the highly fragmented litter, these samples are further referred to as the benthic samples. Cluster 2 with a similarity of 83.7 % regroups the LF and LL samples, these will be referred to as the foliar samples. Cluster 1 subdivides in a cluster 1A (similarity of 88.9 %) and groups all the US samples and the CS a sample. The cluster 1B (similarity of 87.1 %) groups the HF samples with the CS b sample. The cluster 2 splices into a cluster 2A grouping all LL samples (similarity: 90.7%). The cluster 2B groups all LF samples (similarity: 89.7%).

Hill's diversity indices (Table 1) showed a higher meiofaunal taxa diversity in the foliar habitats than in the benthic habitats.

	N_0	N_{inf}	H'		N_0	N_{inf}	H'
US	12.00	2.02	1.49				
CS	11.50	2.06	1.45	Benthic	11.86	2.03	1.46
HF	12.00	2.03	1.43				
LF	16.67	1.86	1.21				
LL	11.67	1.99	1.24	Foliar	14.17	1.93	1.23

Table 1 Meiofauna Hill diversity indices (N_0 = number of taxa; N_1 = exp H', N_{inf} = dominance index) Benthic and foliar regrouping was based on the cluster analysis (Fig. 4)

Copepoda

In total, 4 orders and 16 harpacticoid families were found in 1500 copepod specimens that were identified. The majority of the copepods (90.6% ± 3.0% SD) belong to the order Harpacticoida. The remaining 9.4% ± 3.0% SD belongs to the order Cyclopoida (6.1% ± 2.8% SD) or is copepodites (2.9% ± 2.8% SD). The remaining specimens (0.4% ± 0.3% SD) were representatives of the order Calanoida and the order Misophrioida.

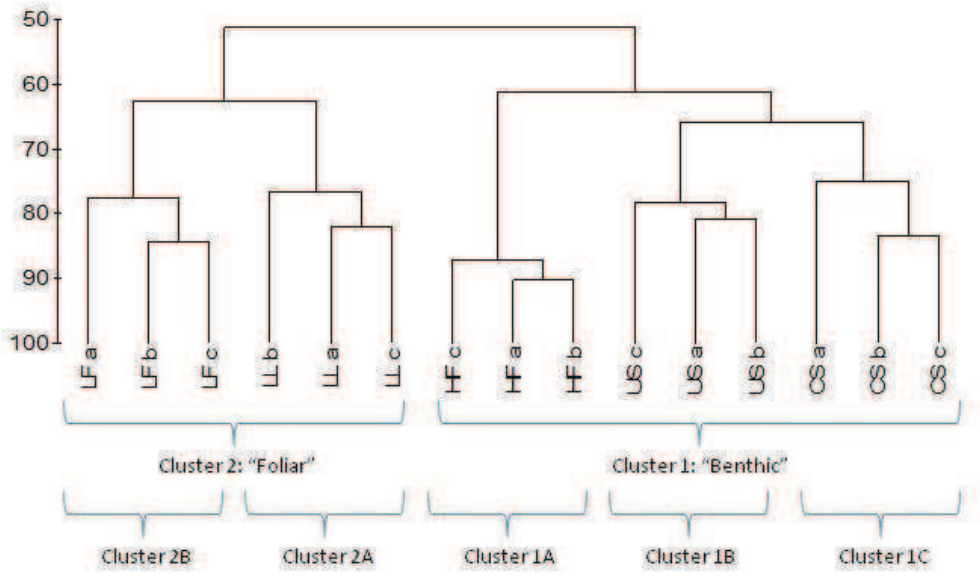

Fig. 5 Cluster analysis per habitat sample for harpacticoid copepods. Composition based on the Bray-Curtis similarity coefficient (in % on y-axis)

The overall harpacticoid copepod family similarity was 51.1%. The same two main clusters as in the meiofaunal dendogram (see Fig. 4) were found at 61.1% (Fig. 5): foliar (cluster 2) and benthic cluster (cluster 1). In the benthic cluster a sediment cluster (cluster 1B & 1C) and a cluster with the highly fragmented detritus (cluster 1A) were detected with a similarity of 65.7%. In the foliar cluster, the living leaves (cluster 2A) separated from the low fragmented phytodetritus (cluster 2B). All 5 sub-clusters matched the five potential habitats.

The relative composition histogram of the harpacticoid copepods (Fig. 6) shows the 4 harpacticoid families that contribute for more than 5% in the composition in all samples.

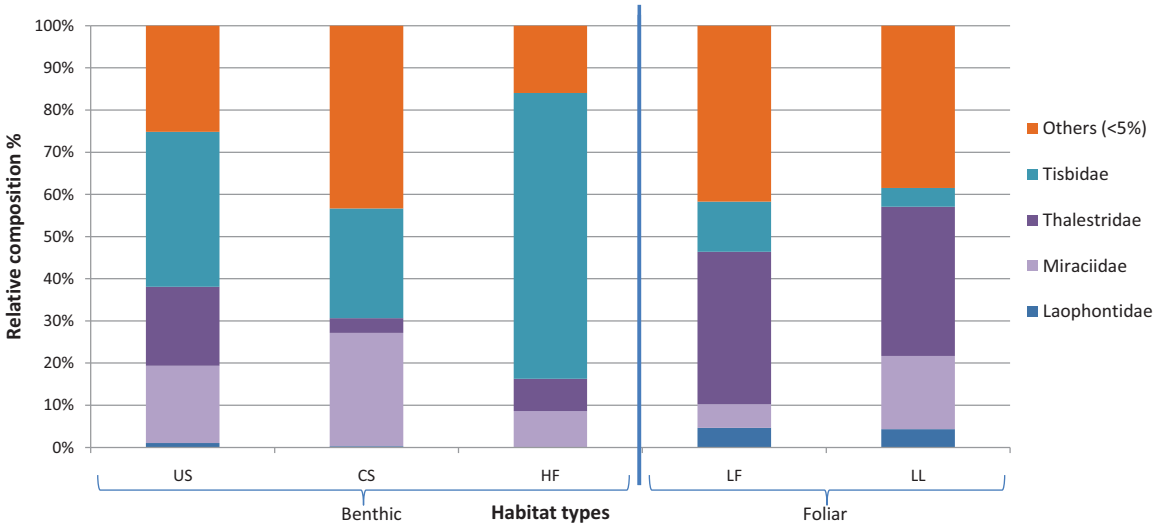

Fig. 6 Relative composition of the harpacticoid copepod families

A SIMPER analysis showed that the benthic cluster was mainly characterised by three families (in decreasing order of average contribution): Tisbidae (31.9%), Miraciidae (17.6%) and Thalestridae (12.9%). In the foliar cluster a different decreasing contribution order was seen: Thalestridae (23.8%), Laophontidae (15.0%), Tisbidae (10.4%)/Miraciidae (9.6%).

	N_0	N_{inf}	H'		N_0	N_{inf}	H'
US	10.00	2.68	1.68	Benthic	8.00	2.07	1.35
CS	9.00	2.20	1.53				
HF	5.00	1.32	1.11				
LF	8.67	2.57	1.70	Foliar	9.50	2.52	1.72
LL	10.33	2.47	1.73				

Table 2 Hill diversity indices (N_0 = number of taxa; N_1 = exp H', N_{inf} = dominance index) for the harpacticoid copepod data. Benthic and Foliar regrouping was based on the cluster analysis

Hill's diversity indices showed a slightly higher diversity in the foliar habitats (Table 2)

Environmental factors

Both sediment samples consist mainly of granule gravel material (>2mm). The uncovered sediments have proportionately more gravel and less coarse sand. A small increase in fine fraction for the covered sediment samples was noted. The total organic carbon content in the sediment samples was very low (0.18 % for the uncovered sediments and 0.07 % for the covered sediments).

A linear correlation between the total organic carbon amount in the epiphytic samples and the number of harpacticoid copepods per gram dry weight gave an R^2 of 0.7988. This

correlation results in an average abundance of 28 copepods for the HF samples, 20 copepods for the LL samples and a maximum of 32 copepods in the LF samples per gram carbon.

Trophic biomarkers

Fatty acids

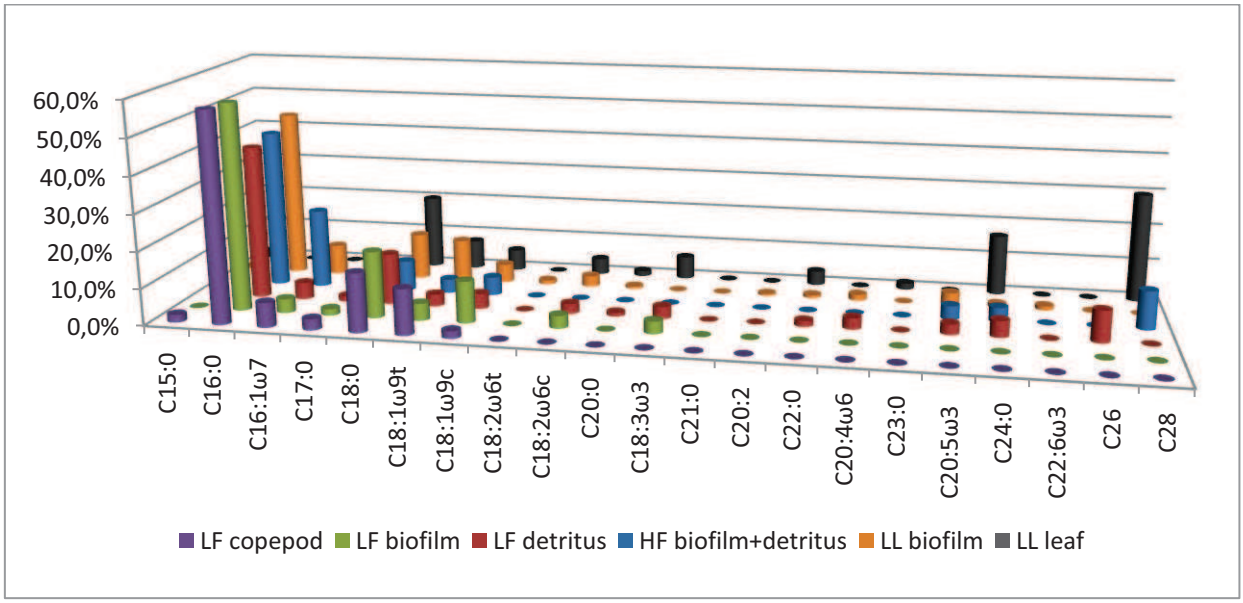

Fig. 7 Representation of the fatty acid composition (as % of total fatty acids on the y-axis). Abbreviations are *LF copepod*: copepods found on low fragmented macrophytodetritus; *LF biofilm*: the biofilm on low fragmented macrophytodetritus*; LF detritus*: low fragmented macrophytodetritus without biofilm; *HF biofilm + detritus*: highly fragmented macrophytodetritus biofilm and detritus; *LL biofilm*: the biofilm on living leaves; *LL leaf*: living leaves without biofilm

The bacterial fatty acids C15:0 and the C17:0 were present in all samples but in very low amounts of a few percent with a maximum in the LF copepod sample. The saturated fatty acids C16:0 and the C18:0 were most abundant followed by their unsaturated forms. There was no data for the C16:0, C16:1ω7 and C17:0 in the LL leaf sample. This is presumably due to an error. Long chain fatty acids (>20C) were not detected in the LF biofilm and LF copepod samples. This could be biased by some errors in the measurement owing to a too low amount of material. These long chain FA are however present in the other samples, especially in the LL leaf sample. A big similarity is present between the LF biofilm and LF copepod, especially in the C16:0 proportions. The LF detritus and HF biofilm + detritus seem very similar. The LL leaf seems to have a larger proportion of long chain FA.

Stable isotopes

More different samples could be analysed for the stable isotopes in comparison to the fatty acids. All values in this paragraph are average $\delta^{13}C$ values ± standard deviation in ‰. For the *Posidonia* living leaves, two different parts were analysed: the biofilm (LL biofilm) = -11.2‰ ± 2.4‰ and the leaves without biofilm (LL leaf) = -9.1‰ ± 3.4‰. For the low fragmented litter three different parts were analysed: the biofilm (LF biofilm) = -11.0‰ ± 0.8‰, the litter without biofilm (LF detritus) = -14.5‰ ± 1.5‰ and the litter with biofilm (LF detritus + leaf) = -17.1‰ ± 3.1‰. For the highly fragmented macrophytodetritus it was impossible to separate the biofilm from their substrate. The highly fragmented macrophytodetritus with biofilm (HF detritus + biofilm) had a value of -12.7‰ ± 1.0‰. For further comparison we will use the biofilm values of (Lepoint, 2006) for the biofilm on low fragmented detritus (new LF biofilm) = -19.6‰ ± 2.3‰. For further comparison we will use the biofilm values of (Dauby 1989) for the living leaves biofilm (new LL biofilm) = -19‰.
The copepods collected in the field from the low fragmented detritus (LF copepod) had a value of -18.5‰ ± 1,2‰ and a value of -17.9‰ ± 1,4‰ was found for the highly fragmented detritus (HF copepod) .

Lab experiment

Interactions

After 4 days of incubation, all amphipods were found alive. For the copepods, all 60 specimens were still alive in the detritus + copepod + *Gammarus* replicates except for the E replicate containing one female *Gammarus*. Some antenna remnants were found back in the gut analysis of the female amphipod. In the detritus + copepod treatment, a survival of approx 80% was encountered. Some empty moults and carcasses were found.

Litter

Grazing on the *Posidonia* litter leaves by *Gammarus* was clearly visible after four days at the end of the experiment. There was no significant decrease in litter weight after the experiment ($p>0.8$). Nevertheless, there was almost no weight loss in the detritus (control) and detritus + copepod treatments. This was the case in the treatments with *Gammarus*. A loss of approximately 25 mg ± 8 mg out of the original 900 mg of detrital material was observed which equals a loss of 3% ± 1%.

The foliar surface comparison showed no significant decrease in surface ($p>0.1$). However, in the *Gammarus* present treatments, a loss of 2% to 5% was noticeable.
In the respirations measurements no significant change in oxygen consumption was perceptible ($p>0.9$). None of the treatments showed a specific decreasing trend in respiration whatsoever, except in a few *Gammarus* treatment replicates.

Stable isotopes

The copepods showed a measurable increase in ^{13}C but the detection level of ^{15}N was not reached. The *Gammarus* showed measurable ^{13}C and ^{15}N levels. In the following paragraph only the $\delta^{13}C$ values will be assessed in ‰ ± standard error.
There was a highly significant difference between copepods and *Gammarus* ($p<0.0001$), with higher values for copepods. No significant difference was found between the two *Gammarus* treatments ($p>0.82$) neither between the two copepods treatments ($p>0,82$). The $\delta^{13}C$ value for copepods in the detritus + copepod treatment was 15.5‰ ± 8.9‰ and 17.5‰ ± 7.1‰ for the detritus + copepod + *Gammarus* treatment. For the detritus + *Gammarus* treatment the value was -9,0‰ ± 0,2‰ and -8,8‰ ± 0,8‰ for the detritus + copepod + *Gammarus* treatment. This means that meiofauna and amphipods have significantly incorporated the label, as their natural isotopic delta is -17.8‰ and -16.4‰ respectively.
To calculate the uptake, carbon proportions of the organisms were needed. The value of 31% carbon content from (Avery, 1996) for the *Gammarus* was used and the value of 44% carbon content from (Mauchline, 1998) was used. The difference in uptake between the copepods (1,8 µg ± 0,5 µg on average) and *Gammarus* (1,0 µg ± 0,2 µg on average) treatments was significant ($p<0.05$). No significant effect was seen between the two copepod treatments ($p>0.11$) neither between the two *Gammarus* treatments ($p>0.11$). In the following paragraph the uptake values will be expressed in $\mu g^{13}C\ d^{-1}$ ± standard error.
The daily ^{13}C uptake for the detritus + copepod treatment was 1.7 ± 0.5 $\mu g^{13}C\ d^{-1}$, 1.9 ± 0.5 $\mu g^{13}C\ d^{-1}$ for the detritus + copepod + *Gammarus* treatment, 1,0 ± 0,1 $\mu g^{13}C\ d^{-1}$ for the detritus + *Gammarus* treatment and 1,0 ± 0,2 $\mu g^{13}C\ d^{-1}$ for the detritus + copepod + *Gammarus* treatment.
It was worth to notice that during the experiment one female *Gammarus* gave birth to juveniles that were also analysed. Their $\delta^{13}C$ value was about 1.66 ‰, meaning these juveniles also incorporate biofilm.

Discussion

In situ characterisation

In the present study, it was remarkable to see that harpacticoid copepods dominated the meiofauna samples. Half of the meiofaunal organisms were harpacticoid copepods and a quarter were nematodes. So far, no studies on harpacticoid copepods diversity and density were carried out on the living leaves or in the litter of *Posidonia*. Studies on sediment in the seagrass meadow report that the most abundant meiofaunal taxon is not or very rarely harpacticoid copepods (Danovaro et al., 2002; De Troch et al., 2007; Grego et al., 2009). Other studies on macroalgae thali from Norderhaug et al. (2007) and on leaves of salt-march grass from Rutledge et al. (1993) confirm the finding of the sediment studies mentioned above.

The high densities in the uncovered sediment samples in comparison to the covered sediment samples might not be due to the light grain size difference or light TOC difference. It is most probably due to the anoxicity of the lower sediment layer in the covered sediment sample. No oxygen penetration measurements were conducted, but during the covered sediments sampling there was a clear visible anoxic, black coloured, sediment at 2 cm depth. This effect was not observed in the uncovered sediment samples.

For the epiphytic sample, there was a clear positive correlation between the TOC and density per gram dry weight. To conclude, the low fragmented habitat has the highest density per gram dry weight and per gram carbon of the three epiphytic habitats.

Both meiofaunal and harpacticoid copepod clusters analysis displayed the same two main clusters: a foliar and a benthic one. This indicates that the highly fragmented macrophytodetritus habitat behaves more like a sediment habitat in terms of composition of the associated epifauna. The foliar habitat displays the most diverse community, especially the low fragmented detritus samples. Since the LF habitat has the highest density and diversity it can be presumed that the food quality and/or diversity is the highest in this habitat. This still has to be confirmed by habitat composition analysis.

The harpacticoid copepod family composition between the habitats change, and the overall diversity remains the same. Except for the highly fragmented sample which has a very low diversity, as a result of the very high abundance of Tisbidae. The composition vary due to some indicator harpacticoid copepod families. For the benthic habitat this is the dominant Tisbidae family and for the foliar habitat this is the dominant Thalestridae family. The shift in dominant family composition could indicate that functional redundancy occurs and thus both

families use similar food sources in the respective habitats. So far, nothing confirms that if the dominant family would be removed, a less dominant would take his place without diversity loss. Duffy et al. (2001) proposes a better approach which sees the shift of dominance towards habitat type as an optimalisation. In that case, there is no functional redundancy but more functional specialisation for a certain habitat. Beside those typical families, discriminating families were also present. Such families are only present in one certain habitat. E.g. Porcellidiidae for the foliar habitats and Ancorabolidae for the sediment habitat. Harpacticoid copepods are a diverse and well adapted group (Hicks, 1983; Huys et al., 1991; Boxshall, 2004). That is also reflected in a high morphological diversity. The discriminating and typical families reflect preferences in a certain habitat, by their morphological adaptation, which can be seen as niche differentiation (Price, 1983; Loreau, 1998).

Beside the structural difference of habitats (e.g. benthic versus foliar) important functional difference in food availability, quality and accessibility also play a role. The latter one is dependent on the habitat structure.

Studies show that harpacticoid copepods are likely to feed on benthic or epiphytic biofilms (De Troch, 2008). To find out what the exact food source is of harpacticoid copepods, stable isotopes and fatty acids were used to identify trophic interactions and dietary relationships (Graeve et al., 1994). Fatty acid compositions were analyzed for the copepod, biofilm and leaf substrate in the epiphytic samples. The fatty acids 15:0 and 17:0 originated from bacteria (Rajendran et al., 1994) were found in higher proportions in the copepods in comparison to fauna. Long-chain fatty acids, like e.g. EPA (C20:5ω3) and DHA (C22:6ω3) are formed by primary producers (Dalsgaard, 2003). They are present in both leaf material and biofilm material. They should decrease towards higher trophic levels (Falk-Petersen et al., 2002). No peak has been measured in the copepod samples. But since the copepod sample could be biased for long chains, this is not conclusive. The saturated fatty acid C16:0 and C18:0 proportions are quasi the same in the LF biofilm and LF copepod samples (57%). This indicates that the biofilm the main food source is of the copepods. Copepods can't get that proportion of C16:0 on another food source since these have a lower content.

The recorded stable isotope data correspond to the values in literature. Seagrasses show a rather broad range in isotopic values between –23 and –3‰ (δ^{13}C notation). However most ratios vary around –10‰ (Hemminga 1996). These relatively high δ^{13}C ratios compared to other potential source materials make it possible to trace seagrass material (Fry, 1987). Fry (2006) state that in order to define trophic position in the ecosystem the following can generally be assumed. The consumer reflects the assimilated diet with a slight increase in δ^{13}C value of about 1‰. In our present study harpacticoid copepods living on and around the macrophytodetritus showed more negative values than the *Posidonia* and their biofilm. The

Harpacticoid copepods were more ^{13}C depleted in comparison to *Posidonia* detritus, which had relatively less negative values. But when compared to the new, from (Lepoint, 2006) and (Dauby, 1989) biofilm values, the ^{13}C level of copepods shows only a small negative increase. This reflects that harpacticoid copepods feed more on biofilm than on the detritus itself. This implicates that the harpacticoid copepods preferably graze upon the biofilm which uses the detritus as substrate and not the detritus itself. The biofilm consists of different food sources ranging from cyanobacteria to diatoms and microalgae. This is congruent with the hypotheses that harpacticoid copepods use a variety of food sources (Hicks, 1983).

The variety of food sources is linked to the food quality. All organisms prefer feeding on material rich in organic matter. The nitrogen content of *Posidonia* detritus is lower than in living leaves because nitrogen is resorbed in senescing leaves before abscission and because labile nitrogen is quickly mobilized by bacterial degradation (Mateo, 1997). This implies that (1) the bacterial and microbial loop is quite important for the degradation of detritus and (2) harpacticoid copepods prefer feeding on the biofilm containing bacteria rich in assimilated nutrients.

Lab experiment

It was surprising that no predation of *Gammarus* on copepods was observed in the experimental units. This could mean that *Gammarus* preferably feeds on the litter rather than on copepods. This explains the high amounts of *Posidonia* litter in their gut content (Lepoint, 2006). In that study, a low amount of unknown animal material was found back in their guts. *Gammarus* may be opportunistic feeders when energetic needs are high. This was maybe the case in the one replicate containing a female who ingested some copepod.

In the detritus + copepod treatments, a certain level of mortality of copepods was noted. Since predators were excluded, only cannibalism amongst copepods or lack of food can be seen as a possible explanation. So far, copepod cannibalism is not proven amongst adults and parental care is present (Gallucci et al., 2007). In general, a lack of appropriate food can be another option but harpacticoid copepods are known to feed on a variety of food sources (Hicks, 1983). Some studies even advance microbial gardening on faecal pallets as one of their main food sources (De Troch et al., 2005). In presence of *Gammarus*, faecal pellets were present and this could explain their higher survival rate in comparison to treatments without *Gammarus*.

No significant decrease in litter leaf weight, foliar surface or respiration was noticed. However, there was an indication of decrease in litter in the *Gammarus* treatments. This was visually observed by the grazing marks of the gammarids on the borders of the litter

fragments. A possible explanation might be the relative short duration of the experiment.

A more reliable manner to evaluate actual uptake and assimilation of litter material was to measure the stable isotope $^{13}C/^{14}C$ and $^{15}N/^{14}N$ ratio of the fauna after ^{13}C and ^{15}N labelling of the biofilm on detritus as potential food source. The results showed a significant grazing on the biofilm by the copepods but to a far lesser extent for the amphipods. This leads to the conclusion that harpacticoid copepods graze upon the biofilm and that the amphipods graze on the *Posidonia* tissue. In contrast to the amphipod adults, their juveniles were found to take more labelled biofilm. This could indicate that the juveniles preferably graze upon the biofilm or that during their juvenile stage their feeding apparatus is only developed for biofilm feeding.

Conclusion

Harpacticoid copepods account for half of the meiofauna proportion in this *Posidonia* ecosystem. The structural diversity of harpacticoid copepods remains the same over the different habitats but the functional diversity changes. The foliar habitats have a slightly higher diversity than the benthic habitats. The low fragmented macrophytodetritus supports a higher density of organisms. Stable isotopes and fatty acids as trophic biomarkers showed that harpacticoid copepods preferably graze upon biofilm. Amphipods (*Gammarus Aequicauda*) however feed preferentially on *Posidonia* detritus and have a lower uptake of biofilm than harpacticoid copepods. This supports the role of the macrophytodetritus as substrate for biofilm food sources that feeds copepods and act as a direct food source for amphipods. Future research should be focussed to investigate in detail the higher meiofaunal diversity and density in the low fragmented detritus habitat. The microcosm experiment should be done again, since it was a success, but it would be better to have specific harpacticoid copepods and a longer incubation time.
The role of meiofauna in the energy transfer in thus a key role. They influence the energy flow of organic matter to higher trophic levels since they feed on diverse and rich nutritional sources originated from the detrital food web.

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