

Chapter 3

Trophodynamics of intertidal harpacticoid copepods based on stable isotopes and fatty acid profiling

In preparation:

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ABSTRACT

Lower food-web interactions between the meiobenthos and basal food sources are key drivers of benthic energy fluxes. Yet, they are most challenging to reveal due to the complexity of benthic resources and small sizes of interacting organisms. By means of stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and fatty acids, we examined the variability of *in situ* diets of harpacticoid species and families from a heterogeneous tidal flat – salt marsh area (5 stations sampled). This was done to describe trophic heterogeneity among harpacticoid species and spatio-temporal dietary shifts of individual species. At all stations, microphytobenthos played a central role in harpacticoid feeding although the pathway (direct/indirect) was uncertain. For a limited number of species, dietary contributions of suspended particulate matter and bacterial-derived energy were found. In salt marsh stations, consumption of *Spartina alterniflora* detrital matter was low, and in the sand flat station with poor harpacticoid diversity, co-occurring species showed dietary differentiation. Copepod taxa with complete trophic independence of microphytobenthos were *Paraleptastacus spinicauda*, Cletodidae and potentially also Ectinosomatidae. Moreover, Cletodidae were highly specialist feeders of chemoautotrophic matter.

Key words: harpacticoid copepods, intertidal, fatty acids, stable isotope analysis

INTRODUCTION

Harpacticoid copepods often comprise an important fraction of the meiofauna in marine sediments, usually only surpassed in abundance by nematodes (Hicks & Coull 1983). Harpacticoid assemblages are structured by a range of abiotic and biotic habitat characteristics, such as temperature, salinity, food availability, biogenic structures, predation, etc. (e.g. Chandler & Fleeger 1987, Azovsky et al. 2004, Giere 2009). In spite of their abundance, their roles in energy transfer in marine sediments remain unclear. Harpacticoids transfer primary production to higher trophic levels, mainly to juvenile fish (Gee 1989). Their main food sources, in turn, probably consist of microalgae, mainly diatoms (Montagna et al. 1995, Buffan-Dubau & Carman 2000), but cyanobacteria, ciliates, phytoflagellates, heterotrophic bacteria, detritus, exopolymeric mucus and fungi have also been reported as food for harpacticoids (for overview see Hicks & Coull 1983). Despite this broad dietary spectrum, there is little evidence to suggest that harpacticoids would be generalist feeders, and rather little is known about species-specific differences in their nutritional requirements, and hence on resource partitioning (Lee et al. 1976, Carman & Thistle

1985, Pace & Carman 1996, Buffan-Dubau & Carman 2000). When feeding conditions are unfavorable, harpacticoids survive on their lipid reserves (Weiss et al. 1996), adjust their feeding rate (Montagna et al. 1995), or shift to alternative food sources as observed for planktonic copepods (Ger et al. 2011). Furthermore, the flexibility of their diet in response to spatial and temporal environmental variation has important consequences for the copepod's value as food for higher trophic levels (John et al. 2001). Much of the available information on harpacticoid feeding selectivity and flexibility is derived from lab experiments, whereas studies revealing the *in situ* contribution of different food sources to the diet of harpacticoids are few (Carman & Fry 2002, Rzeznik-Orignac et al. 2008).

Trophic biomarkers, such as stable isotopes and fatty acids (FAs), are highly suitable to investigate trophic interactions *in situ* (Carman & Fry 2002, Kelly & Scheibling 2012). Complementary use of stable isotopes and FAs to disentangle meiofaunal trophic interactions, is highly recommended (El-Sabaawi et al. 2009, Leduc et al. 2009). Consumer fatty acid composition may clarify ambiguities on food source consumption that remain as a consequence of overlap of carbon isotopic signatures between resources (e.g. benthic microalgae and *Spartina anglica* detritus). For instance, a strong reliance on microphytobenthos (MPB) can be deduced from a high polyunsaturated fatty acid (PUFA) content of the consumers (indicator FA), with eicosapentaenoic acid (EPA, 20:5 ω 3) and decosahexaenoic acid (DHA, 22:6 ω 3) characteristic for the diatom and flagellate component of the MPB, respectively. Highly specific FA or 'marker FA' of food sources are, for instance, FA 16:1 ω 7 for diatoms and low-chained odd-numbered FAs 15:0 and 17:0 for bacteria (Kelly & Scheibling 2012, and references therein).

In highly dynamic ecosystems like temperate intertidal zones, small-scale habitat heterogeneity and temporal variability in environmental parameters result in high spatio-temporal variability of harpacticoid community structure (Azovsky et al. 2004, Cnudde et al. chapter 2). Whether and how these structural shifts are accompanied by shifts in resource utilization and partitioning has not been properly investigated yet while this is crucial for understanding the role of harpacticoids in benthic energy fluxes. Using both natural isotopic signatures and FA composition of harpacticoid copepods, this paper focuses on spatial and temporal dietary variability of harpacticoid species in a temperate intertidal zone with high habitat heterogeneity. Harpacticoids from five stations on a tidal flat and salt marsh area, differing in tidal height, granulometry and vegetation, were sampled on 4 occasions with a 3-month interval. The first aim of this study was to investigate the relative importance of different food sources such as microphytobenthos (MPB), suspended particulate organic matter (SPOM), bacteria, vascular-plant and macroalgal detritus and epiphytes for intertidal harpacticoids. This would give an impression of the trophic diversity of Harpacticoida in a heterogeneous ecosystem. The second aim was to examine dietary variability of species in space and time, which is an indication of their trophic plasticity.

MATERIAL AND METHODS

Study area

Harpacticoid copepods were collected from five stations in the intertidal zone of the Paulina tidal flat and salt marsh, located along the southern shore of the polyhaline zone of the Westerschelde Estuary (SW of The Netherlands, 51°20'55.4"N, 3°43'20.4"E). The five stations differed in terms of intertidal position (tidal height), granulometry and vegetation and therefore we presumed food availability and diversity to differ among habitat types. The hydrodynamic disturbance of the sediment surface and light exposure time (both related to hydrodynamics) is expected to impact microbial biofilm formation and stability as well as deposition of detrital matter from different origins (Herman et al. 2001). The five stations were geographically oriented over an east-west distance range of approximately 670 m and a north-south distance range of approximately 550 m. The first two stations (H1 and H2) were situated in the tidal flat area. Station H1 was located in the lower intertidal and exhibited a temporally variable granulometry in

the upper cms of sediment, while station H2 was located in the mid-intertidal and was characterized by fine sandy sediment with a negligible silt fraction. The other three stations H3, H4 and H5 are situated in or at the edge of the marsh area, although samples were always collected in unvegetated sediment spots. Station H3 is a sediment patch positioned in the mid to high intertidal surrounded by *Spartina anglica*. Samples were collected within 10 cm of the *Spartina* vegetation, in sediments dominated by the fine sand fraction and with a variable mud fraction of 0 to 25 %. Station H4 is located in the high intertidal, near *Spartina* vegetation as well as a small area with stones covered by *Fucus vesiculosus*. Samples were collected at about 1 m from the *Fucus* vegetation. Station H5 is positioned in a marsh gully surrounded by dense vegetation, dominated by a combination of *Spartina anglica*, *Aster tripolium* and *Atriplex portulacoides*. Samples were collected at the bed of the gully. Since stations H3, H4, and H5 were in close proximity of salt marsh vegetation, we refer to these as 'salt marsh stations'.

Sampling procedure

Four sampling campaigns at the Paulina salt marsh were performed in the year 2010-2011, covering the four calendar seasons: 2-3 June 2010 (spring), 31 August - 1 September 2010 (summer), 29-30 November 2010 (autumn) and 7-8 February 2011 (winter). Intertidal sediments were sampled for analysis of harpacticoid and sediment fatty acids and stable isotopes. Additionally, samples were taken for the analysis of harpacticoid communities, and of biotic and abiotic sediment characteristics including sediment granulometry, dissolved nutrients, total organic matter, phytopigment concentrations, lipid and protein concentrations, and bacterial abundances and diversity (see Cnudde et al., in preparation, chapter 2)

Harpacticoid copepods for isotopic and fatty acid analyses were sampled qualitatively by collecting the top 1 cm of the sediment (approx. 1 m²) during low tide. Copepods were extracted by rinsing the sediment with fresh water over a 250 µm sieve. The harvested copepods were divided in two samples: one from which copepods were collected alive for fatty acid analysis; the other was stored at -20°C until processing for later stable isotope analyses. We also collected triplicate sediment samples for isotope and fatty acid analysis of bulk sediment particulate organic matter (SOM) by means of 3.5-cm diam. plexiglass cores. These sediment cores were sliced into 0-0.5 and 0.5-1cm layers. Suspended particulate material (SPOM) was obtained through filtration on a precombusted GF/F Whatman glass fibre filter of surface water collected near the low water level. Fresh and decaying leaves or thalli of cordgrass, *Spartina anglica*, and of the macroalga *Fucus vesiculosus* were collected, rinsed with MQ water to remove adhering sediment particles, and their epigrowth scraped off using a glass slide cover slip. This 'biofilm' material was collected in MQ water and then concentrated through centrifugation. Epiphytic biofilm samples and (biofilm-free) cordgrass/macrophyte material were stored at -20°C prior to isotopic analysis, and so were SOM and SPOM samples for isotope analysis. Copepod and sediment samples for fatty acid analysis were stored at -80°C.

Fatty acid analysis

Fatty acid samples were prepared (personal protocol) from living copepod specimens within max. 2 days after field sampling to minimize FA losses. On the first day, copepods were sorted from the sediment under a Leica dissecting microscope (magnification 180 x) using a Pasteur pipette. Batches of different copepod taxa were washed three times in 0.2 µm filter-sterilized and autoclaved artificial seawater (Instant Ocean, salinity of 28) (ASW) to remove external, cuticle-attached particles, and were stored overnight in a climate room (15 °C, 12h:12h light:dark) to allow defecation. The following day, copepods were given a final wash by transferring them through sterile ASW, and collected on a precombusted GF/F Whatman filter (diameter 25 mm). Filters were stored in Eppendorf tubes at -80°C until FA extraction. Target sample size was usually 100 specimens per filter, but actual sample size and number of replicates

depended on the abundance and biomass of the copepod taxa: down to 60 specimens per sample for the largest taxa (e.g. *Platychelipus littoralis* and *Harpacticidae*), and up to 500 specimens for *Paraleptastacus spinicaudus*. Ca. ten specimens of each copepod taxon that was sampled for FA analysis were preserved on ethanol for later species identification. For FAME (fatty acid methyl ester) analysis of sediments, 1 – 1.5 g of lyophilized and homogenized sediment was used.

Lipid extraction, fatty acid methylation and analysis of fatty acid methyl esters (FAMES) were executed according to De Troch et al. (2012a). Lipid hydrolysis and fatty acid methylation were achieved by a modified 1-step MeOH-H₂SO₄ derivatisation method after Abdulkadir and Tsuchiya (2008). Aside from dilution (FAMES in 300 µl and 750 µl hexane for copepods and sediment, respectively), FAME extraction and analysis was similar for copepod and sediments. FAMES were separated using a gas chromatograph (HP 6890N) with a mass spectrometer (HP 5973) based on a splitless injection (i.e. 1 µl and 5 µl of extract for sediment and copepods, respectively) at a temperature of 250°C on a HP88 column (Agilent J&W; Agilent). FAMES were identified based on comparison of relative retention time and on mass spectral libraries (FAMES, WILEY) by means of the software MSD ChemStation (Agilent Technologies). Calculation of FAME concentrations (µg FA per g sediment dry weight) was based on the internal standard 19:0. The FA short hand notation A:BωX was used, where A represents the number of carbon atoms, B gives the number of double bounds and X gives the position of the double bound closest to the terminal methyl group (Guckert et al. 1985).

Since sampled copepods were collected and combined over the top one cm of the sediment, sediment FAs of the two depth layers (0-0.5 cm and 0.5-1 cm, originating from the same sediment core) were combined by summing the raw FA data of the depth layers (i.e. surface areas of chromatogram peaks) and converting these to FA amounts (in µg FA) per g sediment dry weight based on the internal standard (19:0). Absolute FA concentrations of sediment and copepods were converted to proportions of total sample FA content (in %).

Several potential resources for harpacticoid copepods have unique FAs (marker FA) or are characterized by a specific combination of FA (indicator FA) (Table 1a). The presence of marker FAs in the copepods and certain FA ratios (Table 1b) can specify the type(s) of food that were consumed. The long-chained poly-unsaturated FA (PUFAs), EPA (20:5ω3) and DHA (22:6ω3) are essential FA for consumers. Ratios EPA/DHA and 16:1ω7/16:0 ratios in excess of 1 are indicative of diatom feeding (herbivory), while low EPA/DHA and high PUFA/SFA (saturated fatty acids) ratios are characteristic of carnivory, although a low EPA/DHA ratio may also point at the relative importance of dinoflagellates. A dietary contribution of bacteria can be deduced from the sum of odd-chained FA and from 18:1ω7c (see Table 1).

Stable isotope analysis

Harpacticoids for stable isotope analysis were obtained by handpicking and washing specimens thoroughly in ASW using an eyed needle. The copepod samples were processed within maximum 2-3 days; they were maintained at 4°C for most of this time and kept cool during handling using pre-cooled ASW. Triplicate samples of each harpacticoid taxon were prepared for carbon isotope analysis. Generally each sample was composed of at least 20 specimens in a precombusted (450°C, 3h) aluminium capsule (2.5 x 6 mm, Elemental Microanalysis). For smaller species (e.g. *Paraleptastacus spinicauda*), considerably more individuals (typically 100) were collected in order to obtain sufficient biomass for reproducible measurements. For the most abundant harpacticoid taxa, we prepared one or more sample(s) for dual (i.e. carbon and nitrogen) isotope analysis (personal protocol). Such samples usually contained 60 to 150 specimens, but up to 500 for *Paraleptastacus spinicauda*. Samples of plant material, epiphytes, SPOM and SOM were dried at 60°C and ground with mortar and pestle for homogenisation. Samples of 5-6 mg of plant material were prepared in tin capsules. Epiphytes (4-6 mg), SPOM (4-6 mg) and sediment samples (40-80 mg) were prepared in silver capsules and acidified *in situ* with dilute HCl (1% v/v) to remove

carbonates (Nieuwenhuize et al. 1994). Capsules were dried overnight at 60°C, closed and stored in a desiccator until analysis. Stable carbon and nitrogen isotope ratios were analysed using a C-N-S elemental analyser coupled to an isotope ratio mass spectrometer (V.G. Optima, Micromass, UK and Sercon Ltd., Cheshire, UK). Isotopic ratios were expressed as δ values (‰) with respect to the Vienna PeeDee Belemnite carbon and atmospheric N₂ standards: $\delta X = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 10^3$, where X is ¹³C or ¹⁵N and R is the isotope ratio (Post 2002). Similar as for sediment FA, sediment isotopic data shown here represent the top 1 cm, and have been obtained by averaging the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of the 0-0.5 and 0.5-1 cm layers originating from the same sediment core.

Data analysis

Spatio-temporal differences in sediment resource availability and composition were analysed based on the univariate data including sediment $\delta^{13}\text{C}$, sediment $\delta^{15}\text{N}$ and total (absolute) FA content, as well as on the multivariate relative FA composition data. After log-transformation, total FA content matched the assumptions of normality and homogeneity of variances (tested with the Shapiro-Wilk test and the Levene test, respectively) and two-way ANOVA was performed using stations (stat) and months (mo) as fixed factors. Tukey's HSD-test was used for *a posteriori* pairwise comparisons. Isotopic data did not match the requirements for parametric ANOVA, even after log-transformation and in some cases also suffered from low replication. Therefore, these data were analysed with two-way Permutational ANOVA (PERMANOVA, main test and pair wise test) with stations (stat) and months (mo) as fixed factors. Variability in the fatty acid composition as well as variability in the proportions of individual FAs or in FA ratios were also inspected using multivariate or univariate PERMANOVAs. PERMANOVAs were performed with 9999 permutations and were based on a Euclidian distance or Bray-Curtis resemblance matrix, for univariate or multivariate tests, respectively. Homogeneity of dispersions was checked via the PERMDISP routine. When this homogeneity is not met, interpretation of significant factor effects should be done with due caution. For pair wise tests with less than 10 unique permutations, Monte Carlo p-values were interpreted (Anderson & Robinson 2003).

As for sediment isotopes, spatio-temporal variation in copepod $\delta^{13}\text{C}$ signatures was tested with two-way PERMANOVA because assumptions for parametric tests were not met. Since PERMDISP often indicated heterogeneity of dispersions, any significant differences between these copepod isotope data need to be interpreted with caution. No estimation of the contributions of food sources to copepod diet was performed using an isotope mixing model such as SIAR (Parnell et al. 2010, Fry 2013). The accuracy of model-fitting is expected to be low for spatio-temporal studies where sampling of potential food sources was incomplete (not at all times and all places) (Dethier et al. 2013), given the likelihood of substantial spatio-temporal variation in natural isotopic signatures of potential food sources (e.g. marine macrophytes, Dethier et al. 2013).

Spatio-temporal variability in relative FA composition of sediment and copepods were visualized by non-metric Multidimensional Scaling (nMDS) based on a Bray-Curtis resemblance matrix of untransformed relative FA profiles. Spatial and temporal differences in most abundant FA in sediments as well as FA contributing to the unique character of stations (% contribution to group similarity) or to differences among stations or sampling dates (% contribution to dissimilarity) were determined using a two-way Similarity Percentages (SIMPER) analysis. Additionally, a one-way SIMPER (factor month) was executed for each station to denote more specifically which FAs changed over time within that station. Variability in the proportions of individual FA was inspected using univariate PERMANOVAs. Copepod FA compositions were further compared non-statistically, by describing those marker FA or FA ratios with striking values.

Parametric analyses (assumption testing, ANOVA and post-hoc test) were performed in R. All other analyses were conducted in Primer V6 (Clarke & Gorley 2006), using the PERMANOVA + add-on package (Anderson et al. 2008).

Table 1. Literature-based overview of (a) indicator fatty acids of marine resources and of (b) some FA markers or ratios regularly applied to indicate consumers' diet. Selected literature primarily based on benthic resources and consumers. Strongly modified from Leduc et al. (2009). LC-SFA = long-chained saturated FA; PUFA = poly-unsaturated FA

a	Resource	Reference
16:1 ω 7c 20:5 ω 3 (EPA) limited in C ₁₈ -PUFA	Diatoms	Graeve et al. (1997), Ackman et al. (1968), Volkman et al. (1980), Kharlamenko et al. (1995)
18:2w6, 18:3w3	Cyanobacteria, ciliates, vascular plant/terrestrial detritus, green algae (Chlorophyceae), macrophyta, (non-diatom sources)	Boschker et al. (2005), Kharlamenko et al. (2001), Graeve et al. (2002), Nelson et al. (2002), Cook et al. (2004)
15:0, 17:0, 15:1 ω 1, 17:1 ω 1 18:1 ω 7c	Bacteria	Volkman et al. (1980); Findlay et al. (1990)
16:1 ω 7c, 18:1 ω 7c	Chemoautotrophe bacteria	Van Gaever et al. (2009)
18:1 ω 9c	Bacteria, Phaeocystis (marine phytoplankton), green algae	Nichols et al. (1982), Volkman et al. (1980), Sargent and Falk-Petersen (1981)
20:4 ω 6	Protists, macroalgae, cyanobacteria	Graeve et al. (2002), Zhukova and Kharlamenko, (1999), Howell et al. (2003)
22:6 ω 3 (DHA) C ₁₈ -PUFA	Dinoflagellates	Sargent et al. (1987)
14:0	Prokaryotes (also diatoms)	Volkman et al. (1980); Findlay et al. (1990)
LC-SFA (with > C ₂₀)	Terrestrial plant debris	Douglas et al. (1970)

b	Diet	Reference
C16:1 ω 7/16:0	Diatoms	Ackman et al. (1968) (planktonic)
EPA/DHA	Diatoms/dinoflagellates	Kelly and Scheibling (2012)
C ₁₈ PUFA	Non-diatom feeding	Kayama et al. (1989)
Σ C15:0-C17:0	Bacteria	Kelly and Scheibling (2012)
20:1 ω 9	Carnivorous feeding (or de novo biosynthesis)	Graeve et al. (1997)
C18:1 ω 9c (oleic acid)	Carnivorous/detritivorous copepod	Sargent and Falk-Petersen (1981)
PUFA/SFA DHA/EPA	Carnivorous	Cripps and Atkinson (2000)

RESULTS

Characterization of sedimentary organic matter

Isotopic signature

Carbon isotopic signatures of sediment particulate organic matter (POM) differed between stations but not between sampling dates (main test: stat: $p < 0.001$, mo and stat x mo: ns) (Fig. 1). The sandy station H2 was ^{13}C -enriched compared to other stations (pairwise, all $p < 0.001$) with a $\delta^{13}\text{C}$ value of -18.3 ± 1.0 ‰ (mean \pm SD), while stations H1 and H5 were ^{13}C -depleted (pairwise, all $p < 0.05$ except H1-H5: ns) with $\delta^{13}\text{C}$ value of -22.6 ± 0.5 ‰. Nitrogen isotopic signatures also differed mainly between stations but the interaction effect station x month was also significant (main test, stat: $p < 0.001$, mo: ns, stat x mo: $p < 0.01$; PERMDISP for stat x mo impossible – due to less than three replicates per group), with a clear difference between the isotopically heavier muddy salt marsh stations H4 and H5 and the other stations (pairwise, all $p < 0.01$, but H4-H5: ns, H1-H2-H3: ns). $\delta^{15}\text{N}$ values in H4 and H5 averaged 8.2 ± 0.6 ‰ and in other stations 6.4 ± 1.1 ‰. The significant interaction term mainly reflects the following differences in temporal behavior of sediment organic matter $\delta^{15}\text{N}$ between stations: no significant temporal variation at all for station H2, while stations H4, H5 and also H1 increased in $\delta^{15}\text{N}$ during the warmer period (pairwise, $p < 0.05$ for Aug-Febr in H4 and H5, and $p < 0.05$ for June-Febr in H1), and H3 decreased in $\delta^{15}\text{N}$ in late spring compared to late winter (pairwise, $p < 0.05$ for June-Febr in H3).

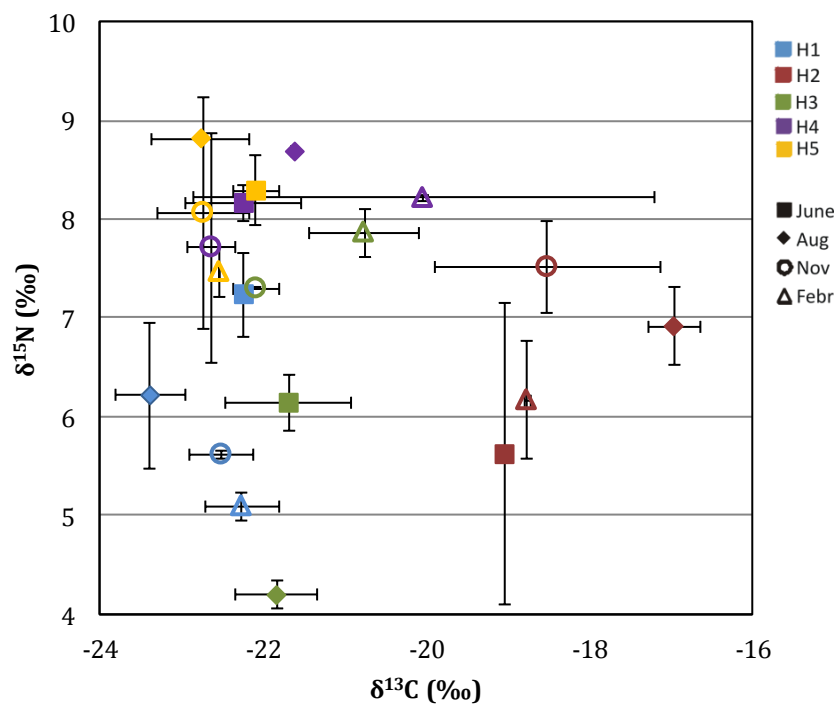


Fig. 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of the sediment top 1 cm (mean \pm SD, $n = 2$): stations are indicated by colors, sampling dates are indicated by symbols.

Fatty acid content

Considerable variability in sediment FA among replicates (small-scale patchiness) was present in both total FA content (Fig. 2; error bars) and FA composition (Fig. 3; sample spread), but variability was low for station H2. Total FA content showed complex spatio-temporal variation (main test: stat: $p < 0.01$, mo: $p < 0.05$, stat x mo: $p < 0.001$). There was a tendency of higher total FA amounts in stations H4, H5 and H1 ($921 \pm 297 \mu\text{g/g}$ and $793 \pm 350 \mu\text{g/g}$, $620 \pm 420 \mu\text{g/g}$, respectively) and lowest FA amounts, together with lowest temporal variability, in sandy station H2 ($613 \pm 196 \mu\text{g/g}$) (Fig. 2). In addition, temporal changes within stations were only significant for H1 ($p < 0.05$ for June-Aug, June, Nov and Nov-Febr), and the exact timing of maximum and minimum FA content was station-specific (Fig. 2).

Similarly, H1 and H2 had the highest and lowest temporal variability in FA composition, respectively (Fig. 3). Overall, FA composition exhibited significant spatio-temporal differences (main test: stat, mo and stat x mo: all $p < 0.001$; PERMDISP of stat x mo: $p = 0.009$), but visually observed trends were not always strongly confirmed by the significance levels from pair wise PERMANOVA tests. nMDS (Fig. 3) did not clearly aggregate sediments according to station or month, but there were some trends: on the spatial scale, differences in FA composition were most explicit between the sand flat (H2) and salt marsh stations H3, H4 and H5 (pairwise within each month, most $p < 0.05$), positioned at the left and right side of the nMDS, respectively. Again, station differentiation was time-dependent. For instance, the sediment of H1 had a unique FA pattern in June only (pairwise within June, H1 versus all other stations, all $p < 0.05$), and the often quite similar sediments from the salt marsh area did differ from each other in November (H3, H4 and H5, all pair wise combinations, $p < 0.05$). Temporal fluctuations in FA composition were station-specific (main test: stat x mo: $p < 0.001$), but a general trend was noticeable with a shift in FA composition between warmer and colder periods: for stations H1, H2 and H3, February samples aggregated, and for stations H4 and H5 November-February samples were separated from June-August samples. EPA was not a characteristic FA in June and August (see further, $< 10\%$ contribution to group similarity) as a result of low EPA relative contributions ($< 10\%$ abundance) compared to November-February sediments.

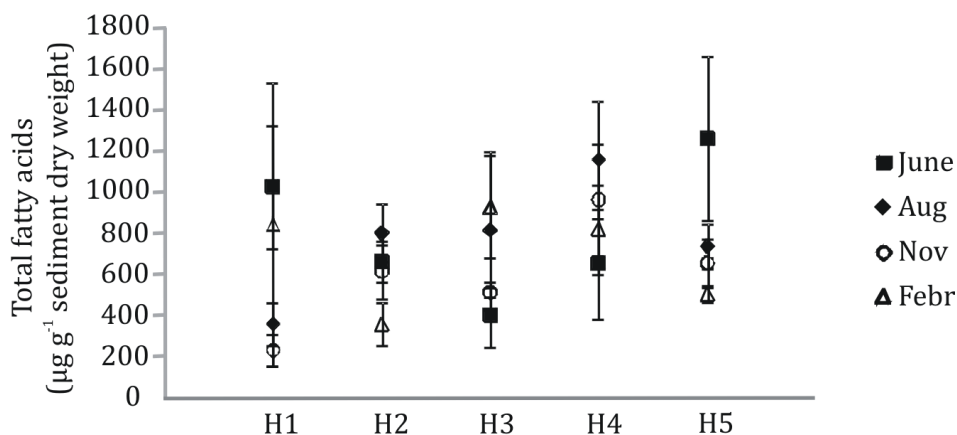


Fig. 2. Spatio-temporal variation in total fatty acid content of sediments (mean \pm SD, $n = 3$)

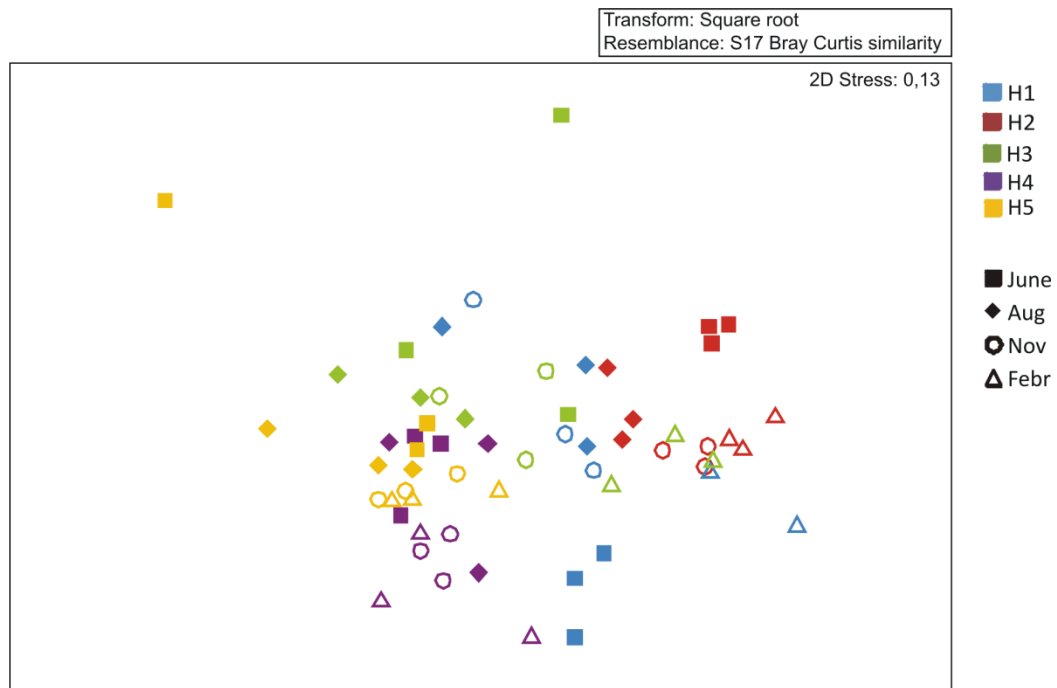


Fig.3. nMDS of sediment fatty acid composition, based on untransformed data: stations are indicated by colors, sampling dates by symbols.

Overall, the most characteristic sediment fatty acids were 16:1 ω 7 (diatom-specific), 16:0 and EPA (20:5 ω 3) ($\geq 10\%$ contribution to similarity within stations and to similarity within months, based on 2-way and 1-way SIMPER, respectively) and generally, their % contributions were a reflection of their relative abundances. These three FA constituted up to 78.0 % (cumulative abundance) of the FAs in H2, considerably more than in salt marsh stations H4 and H5, where they constituted ca. 57.0 %. The latter two stations further differentiated from tidal flat sediments H1 and H2 by the bacteria-specific FA 15:0 ($\geq 10\%$ contribution and $\geq 10\%$ abundance). Highest proportions (and absolute concentrations) of bacteria-specific FA 15:0 and also 15:1 ω 5 were found in H4, containing 6 and 10 times higher levels, respectively, than at station H2. Station H5 is further characterized by FA 18:1 ω 9, which could originate from bacteria or phytoplankton (Table 1a). When present, C24:0 from vascular plant litter/detritus was only a minor component of sediment FA content, with relative abundances of $< 1.3\%$.

Spatial and temporal variability in sediment FA were often accounted for by the same characteristic FA. FA differences between station H2 and stations H3, H4 and H5 were attributed to higher levels of diatom-related FA 16:1 ω 7 and EPA in H2, and to higher levels of 15:0, 15:1 ω 5 and 18:1 ω 9 in the other stations. FA % contributions to dissimilarity among months revealed the following patterns: EPA generally increased in relative abundance towards winter (February), but contributed only little to temporal variation at station H4. Similarly, the other main PUFA, DHA, showed an increased relative abundance in colder months. Bacteria-specific FA (15:0 and also 15:1 ω 7) showed a reverse trend (e.g. station H3, H5), i.e. decreasing in February. In station H5, the temporal changes in EPA and 18:1 ω 9 were opposite.

Isotopic signatures of candidate resources

Potential food sources were characterized by specific $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures, irrespective of spatial or temporal variability (Fig. 4; and data shown in addendum III - Table S1). Fresh *Spartina* and *Spartina* detritus in an early stage of decomposition were isotopically the heaviest carbon sources; while strongly decomposed, fibrous *Spartina* detritus was slightly more depleted in ^{13}C and its $\delta^{13}\text{C}$ overlapped with MPB

(ca. -17 to -14 ‰) (Fig. 4). MPB signatures of the Paulina tidal flat – salt marsh area were obtained from the study of Moens et al (2005a). Epiphytic biofilms had intermediate $\delta^{13}\text{C}$ (ca. -20 ‰), whereas *Fucus* detritus and SPOM were more depleted in ^{13}C (ca. -23 ‰). Nitrogen isotopic signatures increased from SPOM and MPB (ca. 6 ‰) to epiphytes (ca. 10 ‰), *Spartina* (ca. 13 ‰) and *Fucus* (ca. 15 ‰). $\delta^{15}\text{N}$ of *Fucus* even exceeded that of copepods (Fig. 4).

The spatial variability in POM carbon isotope signatures mentioned before, spanned a range of 4 ‰ in $\delta^{13}\text{C}$ values, from -18.3 ± 1.0 ‰ (H2, n = 8) to -22.5 ± 0.4 ‰ (H5, n = 8), the latter revealing a predominant contribution of settled SPOM to bulk sediment OM.

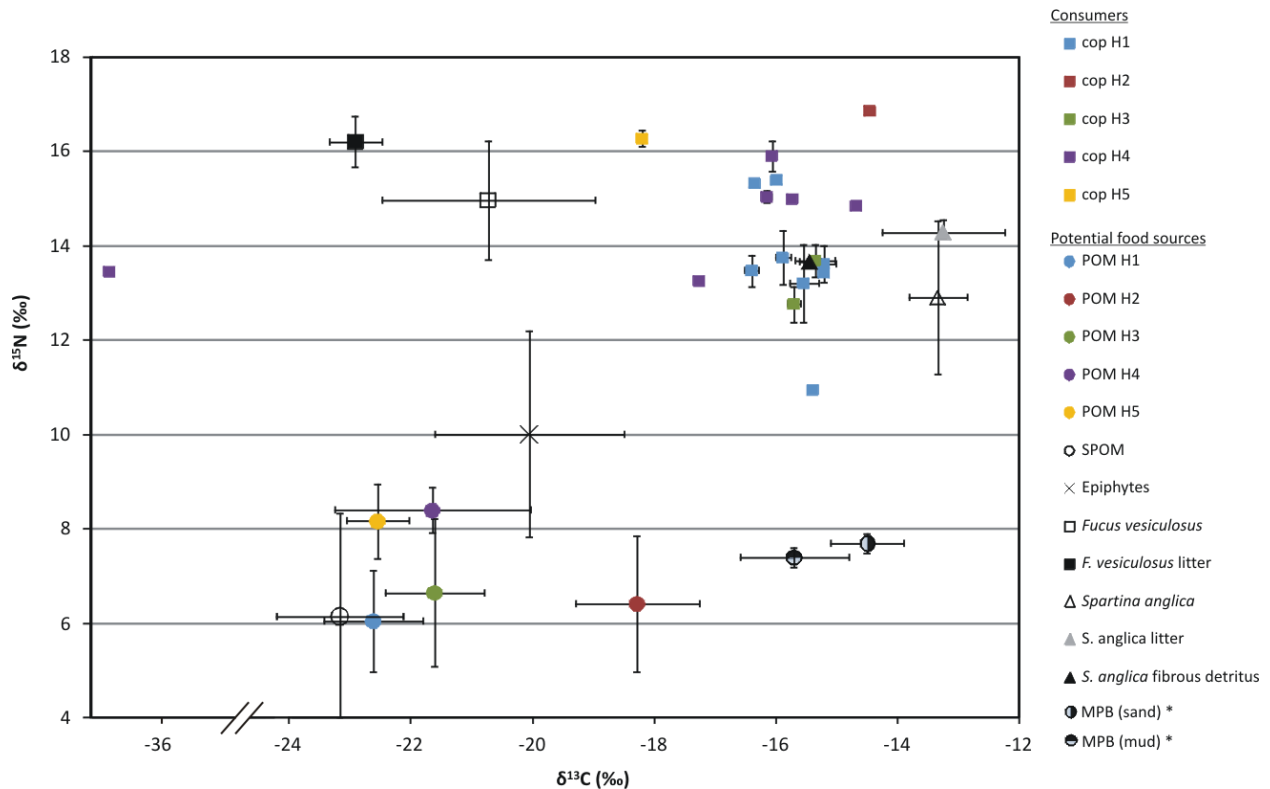


Fig. 4. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (mean \pm SD) of copepod as consumers (cop) and its candidate food sources in the Paulina study area. POM = sediment particulate organic matter, SPOM = suspended particulate organic matter, Epiphytes = epiphytes from *Fucus vesiculosus* and *Spartina anglica*, MPB = microphytobenthos. Asterisks (*) indicates data that originated from the study of Moens et al. (2005). MPB data of Moens et al (2005) were collected in 2004, from a sandy and muddy flat, having similar granulometry and tidal position as stations H2 and H4 in current study. Copepod data point represent individual species from a certain month and station. If replicates were present, these were averaged (and indicated by error flags).

Isotopic signatures of harpacticoid copepods

Harpacticoid $\delta^{13}\text{C}$ values in the Paulina tidal area ranged from -40.3 to -12.1 ‰, with Cletodidae having extremely depleted values (mean $\delta^{13}\text{C}$ = -36.0 ± 2.7 ‰). Even though Cletodidae $\delta^{13}\text{C}$ values spanned a range of 6 ‰ (Table S2), compared to all other copepod taxa, their ^{13}C depletion was clearly consistent over all stations (H3, H4 and H5) and times. When considering all copepod taxa, copepod carbon isotopic data differed among stations and months (stat, mo: $p < 0.001$, stat x mo: ns, PERMDISP for stat and mo: $p = 0.018$, $p = 0.0001$). Highest $\delta^{13}\text{C}$ values were found in H2 (all $p < 0.001$) and lowest values in stations H3, H4 and especially H5 (compared to other stations, all $p < 0.05$, in between H3-H4-H5, all $p \geq 0.05$) (Fig.

5a). This pattern was still present, though less outspoken, when excluding the values of Cletodidae (Fig 5b). The absence of a correlation between the standard deviations on the $\delta^{13}\text{C}$ per station and time, and the number of species analysed (S) (Spearman rank correlation for S), strongly indicates that despite dissimilarity among data sets of the stations, in terms of species richness, spatial and temporal variability in $\delta^{13}\text{C}$ values is primarily caused by interspecific differences.

The majority of harpacticoid copepod species had average $\delta^{13}\text{C}$ signatures between -14 and -18 ‰ (Fig. 6). Aside from Cletodidae, the copepod taxa with lowest $\delta^{13}\text{C}$ values were *Paronychocamptus nanus* (mean $\delta^{13}\text{C} = -16.5 \pm 2.8$ ‰, with lowest value of -23.5 ± 1.2 ‰ at H5 in November), *Amphiascus* sp. 1 (mean $\delta^{13}\text{C} = -17.0 \pm 1.8$ ‰ with lowest value of -20.3 ± 0.7 ‰ at H5 in November) and *Microarthridion littorale* (mean $\delta^{13}\text{C} = -17.5 \pm 1.7$ ‰ with lowest value of -20.5 ± 1.7 ‰ at H4 in August). However, the $\delta^{13}\text{C}$ of these species was not consistent over time and stations. *P. nanus* $\delta^{13}\text{C}$ varied significantly (stat, mo, stat x mo: all $p < 0.01$), but this variation was largely limited to deviant values in H5 in November (spatial dissimilarity among H5 and the other stations: all $p < 0.01$). *Amphiascus* sp. 1 $\delta^{13}\text{C}$ did not significantly change over time but did consistently exhibit spatial differences (stat: $p < 0.05$, mo and stat x mo: ns) between station H5 and stations H1 and H4 (H5-H1 and H5-H4, $p < 0.05$). Finally, significant temporal variability was found for *M. littorale* (stat: ns, mo: $p < 0.01$ and stat x mo: $p < 0.05$), a species which we found in generally high abundances in most stations and at most times. Temporal differences for *M. littorale* were, however, restricted to stations H4 (between June-Aug and Aug-Nov, both $p_{\text{MC}} < 0.05$) and H5 (between Aug-Nov and Febr-Nov, both $p_{\text{MC}} < 0.05$).

Copepod taxa with highest $\delta^{13}\text{C}$ values were *Asellopsis intermedia* (mean $\delta^{13}\text{C} = -14.3 \pm 1.2$ ‰, with highest value of -12.6 ± 0.1 ‰) and *Paraleptastacus spinicauda* (mean $\delta^{13}\text{C} = -14.1 \pm 1.2$ ‰ with highest value of -12.7 ± 0.6 ‰), for both at station H2 in February. The latter species did not exhibit significant spatial or temporal variation in $\delta^{13}\text{C}$ (stat, mo: ns, stat x mo: not tested due to limited dataset), while the former did (stat, mo: both $p < 0.01$, stat x mo: ns). In fact, a majority of copepod taxa showed significant spatial and/or temporal variation in $\delta^{13}\text{C}$. From the taxa with clear shifts mentioned earlier, the maximal temporal range of $\delta^{13}\text{C}$ was up to 7.8 ‰ for *P. nanus* in station H5 (between Nov-Febr) and its maximal spatial range of $\delta^{13}\text{C}$ was also 7.8 ‰, between H1 and H5 (in November). Copepod taxa with no spatio-temporal variability were restricted to *P. spinicauda* (statistical significance see above, range in $\delta^{13}\text{C} = 4$ ‰) and Harpacticidae (range in $\delta^{13}\text{C} = 1.9$ ‰; station, month, station x month, all $p > 0.05$). An overview of $\delta^{13}\text{C}$ values per copepod taxon over all stations and sampling months is given in Table S2 (addendum III).

The carbon isotopic ratios of copepods most closely resembled those of microphytobenthos (MPB) and the fibrous *Spartina anglica* detritus (Fig. 4). Comparatively ^{13}C -depleted signatures, primarily observed for copepod taxa from H5, may result from the consumption of epiphytic biofilms, but a mixture of MPB/*Spartina* detritus and SPOM may equally yield such an intermediate consumer $\delta^{13}\text{C}$. $\delta^{15}\text{N}$ of copepods covered a range of ca 5.5 ‰ (from 11.0 ‰ for *Tachidius discipes* at H1 to 16.9 ‰ for *P. spinicauda* at H2) (Fig. 4), which is equivalent to two or three trophic levels. Among H1 and H4 copepods, variability in $\delta^{15}\text{N}$ was relatively small (2-3 ‰). The $\delta^{15}\text{N}$ of many copepod samples was at least 5 ‰ higher than that of MPB. The restricted dataset of copepod $\delta^{15}\text{N}$ data does not allow to draw conclusions about species-specific and spatio-temporal variability in nitrogen isotope signature.

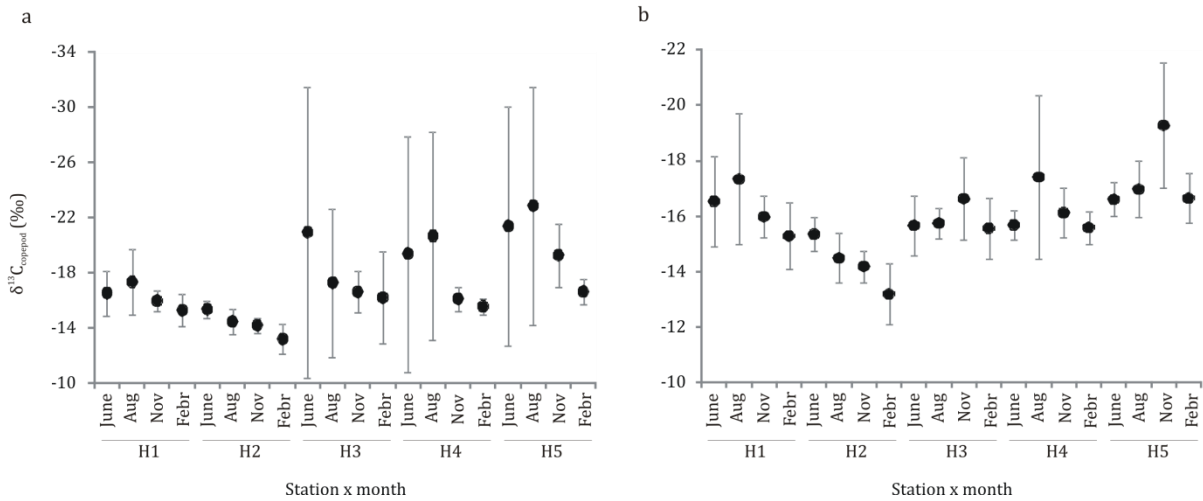


Fig. 5. Spatio-temporal $\delta^{13}C$ signatures of copepod communities as the average of $\delta^{13}C$ values of the participating copepod species (mean \pm SD, n = variable), (a) with and (b) without inclusion of Cletodidae (present in H3, H4 and H5 only)

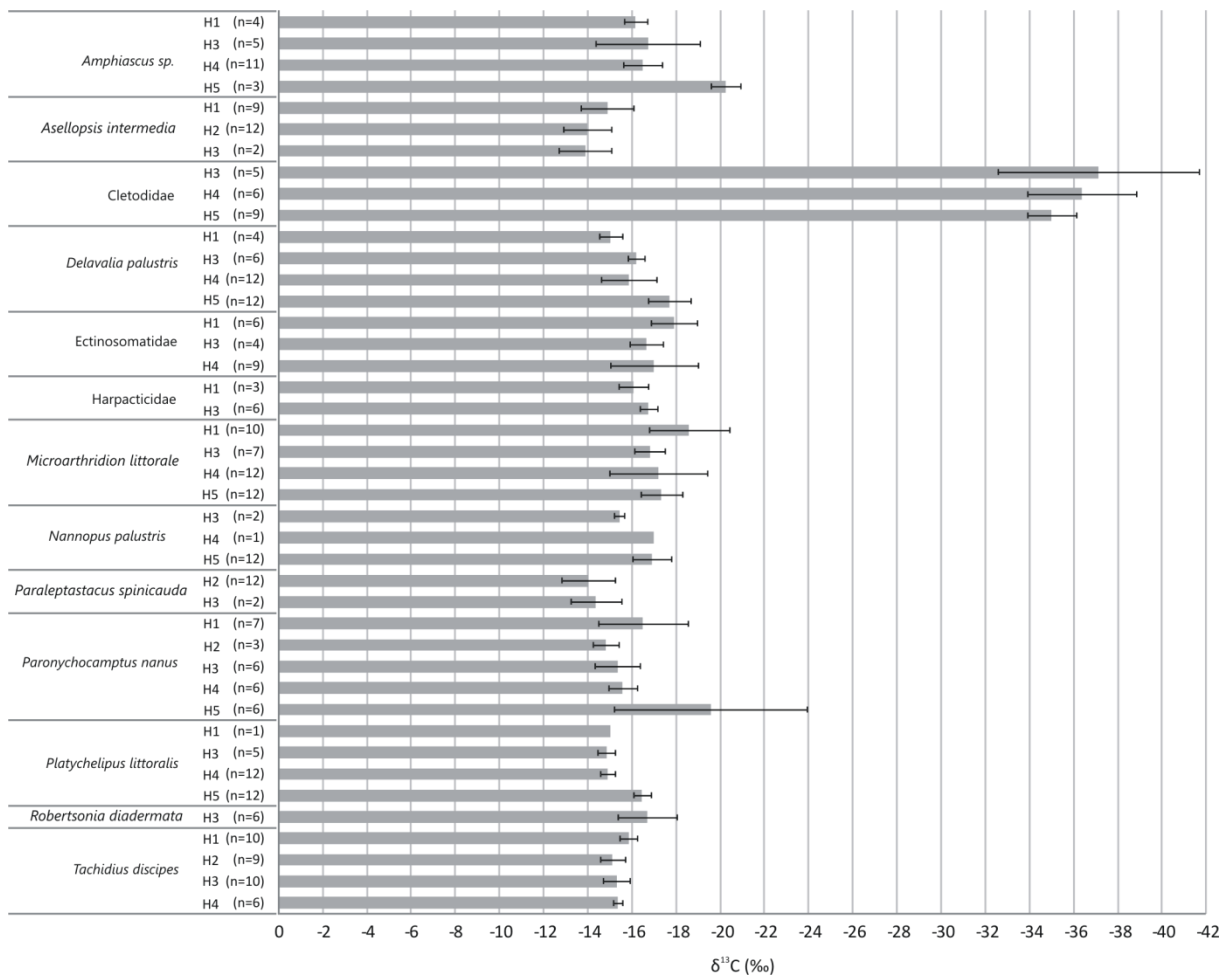


Fig. 6. Stable carbon isotope signatures of harpacticoid taxa from different stations (mean \pm SD). Presented data are averaged values (\pm SD) of samples from the four sampled months.

Harpacticoid FA profiles

An nMDS showed no clear grouping of copepod samples by station or by sampling date (Fig. 7), although there was a tendency for copepods sampled in June-August (positioned at the left side) to be separated from copepods sampled in the colder period November-February (at the right side of the nMDS) (Fig.7), in line with the results of a two-way PERMANOVA (stat: ns, mo: $p < 0.001$, stat x mo: $p < 0.01$, PERMDISP: $p = 0.0003$). Total FA content of copepods varied over species and months (PERMANOVA; spec, mo: both $p < 0.001$, species x month: $p < 0.01$), with species being 2 to 10 times more FA-depleted during spring (air temperature of 30°C) compared to winter (pairwise, for each species, $p < 0.05$ for June-Febr). The highly abundant PUFAs, DHA (stat: ns, mo: $p < 0.001$, stat x mo: $p < 0.05$) and EPA (stat, stat x mo: ns, mo: $p < 0.001$) strongly differed over time. Copepods sampled in November and February showed higher amounts of DHA (pairwise, multiple $p < 0.05$, Nov-Febr: $p > 0.05$). We need to note here that due to this general temporal change in copepod FA composition, especially the PUFAs, dietary ratios FA/PUFA (e.g. PUFA/SFA used as indicator for carnivory) must be interpreted with caution.

Furthermore, copepods did not group by copepod taxon (taxa not shown in fig. 7). Exceptions to this rule were *Paraleptastacus spinicauda* and *Nannopus pallustris* (Fig. 7), the former also lacking variation in $\delta^{13}\text{C}$ values which are, in addition, positioned vary opposed from each other in the nMDS (fig. 7., encircled samples).

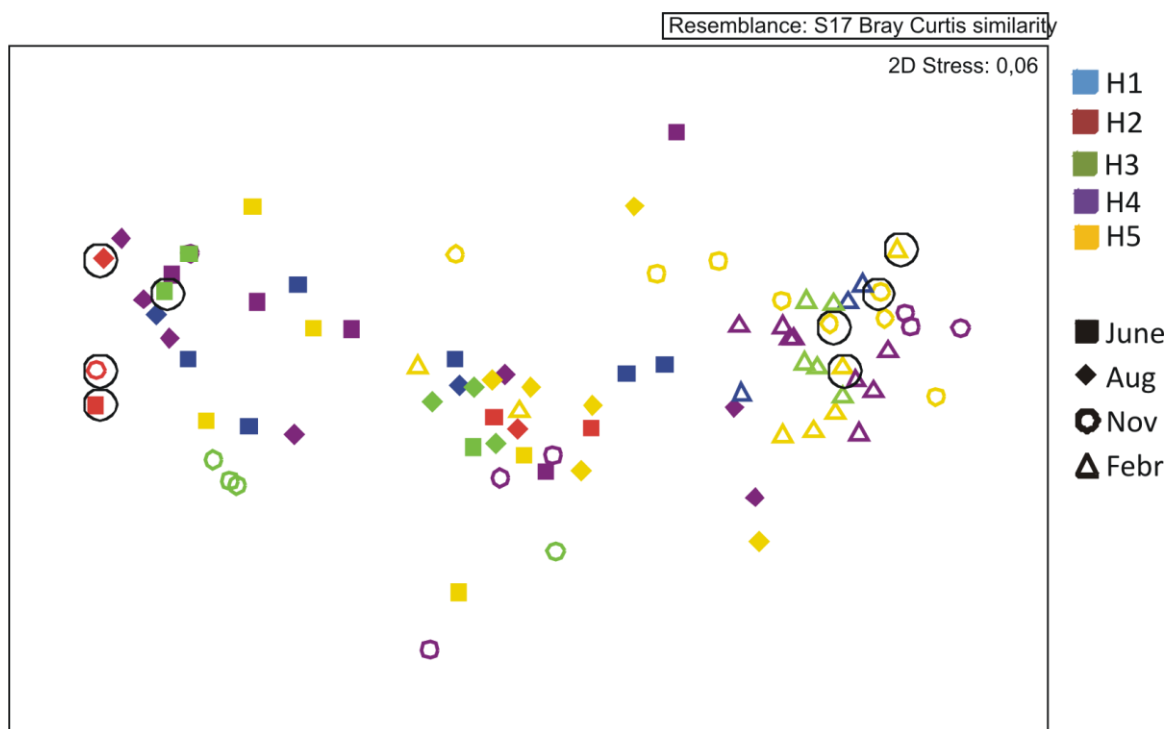


Fig. 7. nMDS based on relative FA profiles from copepods originating from different stations (indicated by colors) during 4 months (indicated by symbol). Encircled symbols at left and right side are *Paraleptastacus spinicauda* and *Nannopus pallustris* samples.

The FA profile of *P. spinicauda* was characterized by (1) the (nearly) complete absence of conventional diatom FAs, i.e. PUFAs (EPA, DHA) and 16:1 ω 7, (2) the high abundance of total bacterial FA Σ 15:0-17:0, and (3) considerable proportions of 14:0 (Table 2; Table 3). FA profiles of *N. palustris* were dominated by PUFAs (> 50 % of total FA), with a predominance of EPA, intermediate levels of 16:1 ω 7 (9-12%) and the

presence of C₁₈-PUFA (Table 2; Table 3). More generally, C₁₈-PUFA were present in low proportion (≤ 2.3 %, constituting 18:2 ω 6 and 18:3 ω 3) and in nearly all species, but were absent in *P. spinicauda*, *Cletodidae* and *Ectinosomatidae*. Highest values were measured in *Amphiascus sp. 1* and *Nannopus palustris*. Spatially, C₁₈-PUFA were absent or low in copepods from stations H1 and H2. Copepods can only obtain C₁₈-PUFA through uptake of non-diatom food sources (Table 1a).

16:1 ω 7 (characteristic for diatoms) was present in nearly all copepod species, except for *Cletodidae*, *P. spinicauda* and *Ectinosomatidae*, and at considerably higher levels (on average 11.7 %) than the C₁₈-PUFA, albeit with high spatio-temporal variability for most species. Copepods generally attained high EPA/DHA ratios (characteristic for herbivory), with values larger than 1 and were highest for *Platychelipus littoralis* and *D. palustris* (> 2.1 , with few exceptions). In addition, the latter contained remarkably high proportions of bacterial FA ($\Sigma 15:0-17:0$). Values of ratio 16:1 ω 7/16:0 were lower than 1. PUFA were nearly absent in *Cletodidae*, *P. spinicauda* and *Ectinosomatidae*. In these species, other trophic biomarker FA (ratio), e.g. PUFA/SFA and FA 20:1 ω 9, were present in low levels. However, *P. spinicauda* contained up to 15 % bacterial FA ($\Sigma 15:0-17:0$, Table 3), which in all other copepods except *D. palustris* contributed < 4 %. The one sample of *Cletodidae* did not contain substantial levels of bacterial FA. FA 18:1 ω 7 is a potential bacterial marker (Table 1a). Because of its similar retention time as FA 18:1 ω 9c, this FA could not be separately identified. FA 18:1 ω 7 was part of the reported 18:1 ω 9c levels, which constituted less than 2 % of *Cletodidae* FA. Next to EPA-rich diatoms, DHA-rich dinoflagellates are an important component of the MPB and preferential feeding on dinoflagellates would result in EPA/DHA < 1 . Low values of 18:1 ω 9c occurred for *Tachidius discipes*, *Microarthridion littorale* and *P. spinicauda*.

Variability in copepod FA profiles cannot easily be associated with observed spatio-temporal shifts in copepod $\delta^{13}\text{C}$ since not only those species with high $\delta^{13}\text{C}$ variability, but most copepod species showed a certain level of variation in the marker/indicator FA (proportions of 16:1 ω 7, EPA, DHA C₁₈-PUFA and bacterial FA and ratio EPA/DHA). The strongest $\delta^{13}\text{C}$ -depleted *Amphiascus sp. 1*, observed in November at H5, showed lowest EPA/DHA and lowest proportion of bacterial FA (Table 2 and 3). For the highly $\delta^{13}\text{C}$ -depleted *P. nanus* and $\delta^{13}\text{C}$ -enriched *A. intermedia*, in November at H5 and in February respectively, no complementary FA samples are present. However, for *P. nanus* in February, we noticed a lower 16:1 ω 7 proportion and remarkably low PUFA content (EPA + DHA = 17%) considering the pattern of PUFA-enrichment of most copepods during colder months, which could be indicative for copepods' independence of microphytobenthos during that period.

		Relative fatty acid profile																
		C14:0	C15:0	C15:1 ω 5	C16:0	C16:1 ω 7	C17:0	C17:1 ω 7	C18:0	C18:1 ω 9t	C18:1 ω 9c*	C18:2 ω 6	C18:3 ω 3	C20:1 ω 9	20:4 ω 6	20:5 ω 3	22:6 ω 3	
<i>Amphiascus</i> sp.	H1 June	2.14	0.44		28.87	11.39	1.52		11.16	0.84	5.73		0.64	0.59	24.35	12.35	n = 2	
	H3 Febr	1.13	0.63		16.86	12.34	1.10		4.14	1.62	9.99	1.49	0.68	0.64	0.52	32.5	16.33	
	H4 Febr	1.08	1.83	0.27	9.99	7.06	1.49	4.52	2.19	2.00	5.79	1.18	0.53	0.30	0.54	35.66	25.58	
	H4 Nov	0.93	0.69		15.30	8.06	1.28	1.63	4.07	1.4	7.29	1.45	0.40	0.41	0.40	34.95	21.73	
	H5 Nov	1.00	0.58		14.64	7.3	0.83		4.94	2.44	5.88	1.72	0.62	0.47	0.65	33.01	25.93	
<i>Asellopsis intermedia</i>	H1 Febr	1.36	0.86		23.10	19.84	0.85	0.50	4.11	1.24	2.45	0.56		0.36	1.87	24.83	18.09	n = 2
	H2 June	2.49	0.76		35.71	19.00	0.88		10.34	0.78	2.33				1.09	13.21	13.41	
	H5 Nov	2.03	0.39		21.97	9.32	0.39		6.31	1.24	1.11	0.50			0.86	30.89	24.98	
Cletodidae	H4 Aug	1.81	0.49		54.72	0.53	0.58		40.21		1.67							
<i>Delavalia palustris</i>	H1 June	1.89	1.01		28.83	9.57	1.51		11.95	1.21	4.77	0.61		1.69	1.96	23.80	11.19	n = 2
	H4 Aug	2.74	4.94	0.71	35.93	8.65	4.89	4.22	16.61	0.87	4.09	0.84	1.30	1.02	3.36	25.04	11.04	
	Nov	4.58	9.82	0.39	33.59	17.07	5.08	10.44	7.87	0.77	0.84	1.04	0.82	0.74	0.33	5.84	0.77	
	Febr	1.23	1.95	0.48	15.74	9.24	2.42	3.07	4.47	1.12	7.60	1.09	0.39	2.30	1.37	32.13	15.42	
	H5 June	3.44	9.67	0.96	32.98	15.4	5.37	6.14	10.31	0.36	3.61			0.78	0.70	7.74	2.52	
	Aug	1.24	4.21	0.82	21.45	7.00	5.33	3.95	10.46	0.72	4.76	0.63	0.93	1.34	4.20	21.68	12.36	n = 2
	Nov	1.29	1.25	0.33	11.15	9.41	1.07	3.20	2.58	0.60	5.13	1.20	0.39	1.57	1.55	40.96	18.32	
Febr	2.41	1.40	0.29	19.20	12.08	0.75	1.32	5.00	0.90	7.13	1.84	0.38	2.15	1.30	33.19	11.30	n = 2	
Ectinosomatidae	H1 June	2.33	1.02		51.72	2.46	2.14		24.16							10.52	5.65	
	H4 June	2.65	1.37		55.96	1.76	7.00		32.31		2.86					2.22		
<i>Microarthridion littorale</i>	H1 Aug	4.16	1.31		57.81	4.35	1.63		29.82	0.92								
	June	5.46			58.60	15.49			16.21							4.24		
	H3 Febr	1.41	0.39		18.98	14.5	0.43		3.60	0.74	3.07	0.37	0.20		0.86	25.46	29.98	
	H4 June	0.58	0.38		54.65	8.09	0.56		26.23		1.34					4.68	3.49	
	Aug	3.51	1.10		58.28	11.1	0.81		21.81	0.56	1.36					3.30	1.54	n = 2
	Nov	3.33	1.07		43.13	16.00	0.72		15.87	1.01	2.30	0.49			0.36	9.21	6.68	n = 3
	Febr	1.51	0.58		19.18	14.87	0.80	1.38	3.34	1.07	4.79	1.11	0.22	0.49	0.51	28.81	22.59	n = 3
	H5 June	3.10	0.75		44.21	18.86	0.53	0.15	10.8	0.66	2.02	0.17			0.35	9.69	8.98	n = 3
	Aug	3.07	0.96		39.13	14.37	0.83		9.11	1.35	1.67	0.71			0.90	13.85	14.58	n = 3
Nov	1.55	0.39		30.59	6.79	0.37		15.25	0.81	1.19	0.57	0.20		0.59	18.26	23.37	n = 3	
Febr	3.48	0.75		39.70	16.04	0.76		9.71	1.68	3.03					14.37	10.49		
<i>Nannopus palustris</i>	H5 Nov	1.31	0.50	0.07	16.75	8.80	0.43		4.51	1.37	4.17	1.25	0.17	0.58	0.65	30.89	28.53	n = 2
	Febr	1.76	0.46		17.11	12.92	0.45		3.24	1.60	5.96	1.86	0.23	0.84	0.47	33.37	19.71	n = 2
<i>Paraleptastacus spinicauda</i>	H2 June	6.90	6.30		58.59		8.72		19.49									
	Aug	0.93	0.42		57.76	0.28	1.09		37.93	0.18	0.12					0.37	0.44	
	Nov	4.49	4.41		58.40		10.05		22.64									
	H3 June	2.77	1.68		55.67	2.84	2.99		31.05	0.60	2.40							
<i>Paronychocamptus nanus</i>	H3 Aug	3.13	1.03		37.53	20.11	1.31		11.46	0.88	2.52	0.39	0.63		0.93	11.13	8.94	n = 3
	Nov	5.89	2.00		63.00	15.68	1.63		11.60	0.60								
	Febr	1.75	0.42		19.62	14.81	0.47		3.52	1.34	2.51	0.36	0.22	0.52	0.76	34.12	19.83	n = 2
	H5 Febr	4.00	0.61		45.62	11.8	0.54		15.77	2.60	1.96					13.51	3.59	
<i>Platychelipus littoralis</i>	H4 June	2.34	0.68		41.83	15.85	0.54		15.28	1.25	4.47	0.41		0.53	0.89	11.16	4.98	n = 2
	Aug	1.33	0.86		23.69	16.68	0.88	0.37	5.47	1.15	5.90	0.48	0.22	0.58	1.24	28.35	12.8	
	Nov	1.33	0.70	0.12	13.38	9.39	0.62		2.86	1.06	3.60	0.50	0.13	0.71	1.26	38.97	25.43	n = 2
	Febr	1.48	0.42		16.02	12.04	0.59		2.70	0.99	6.21	0.92	0.22	0.81	0.73	40.37	16.5	
	H5 June	4.96	1.41		43.06	6.68	0.96		29.78		13.14							
Febr	1.63	0.36		18.12	14.13	0.39		3.28	0.91	6.16	1.03	0.21	0.80	0.65	38.68	13.65		
<i>Tachidius discipes</i>	H1 June	5.23	1.54		58.33	9.07	1.2		24.63									
	Febr	2.30	0.47		14.92	13.75	0.23		2.62	1.21	3.98	0.65	0.23	0.25	0.56	27.52	31.33	n = 2
	H2 June	2.11	1.42		38.74	8.05	1.70		17.33	0.68	1.36				0.95	9.4	19.09	n = 2
	H3 June	1.71	1.18		45.49	18.33	1.70		12.08	0.35	2.83				0.90	7.62	7.81	
	Aug	3.63	1.37		43.57	14.73	1.33		14.54	1.46	1.61					10.00	7.76	
	Nov	5.32	1.63		31.99	28.63	0.69		5.34	2.99	3.74				0.42	10.86	8.39	
	Febr	1.74	0.05		16.64	12.51	0.29		4.32	2.09	3.94	0.44	0.14	0.20	0.41	27.23	30.00	
H4 Febr	2.43	0.51		21.65	12.40	0.45	0.47	5.62	1.85	4.27	0.35	0.21	0.28	0.23	23.63	25.90	n = 2	

* or C18:1 ω 7c**Table 2.** Relative fatty acid profiles of harpacticoid species. If more than one replicate, the number of replicates is indicated (right).

		Diatoms 16:1 ω 7/16:0	Diat./Flagel. EPA/DHA	non-diatom C ₁₈ PUFA	Bacteria Σ 15:0-17:0	Bacteria (partially) C18:1 ω 9c*	Carnivorous C20:1 ω 9	Carnivorous PFSA/SFA	
<i>Amphiascus sp.</i>	H1 June	0.4	2,00		1.96	5.73	0.64	0.85	n = 2
	H3 Febr	0.731	1.99	2.17	1.73	9.99	0.64	2.16	
	H4 Nov	0.71	1.39	1.71	3.31	5.79	0.3	3.83	
	Febr	0.53	1.61	1.85	1.98	7.29	0.41	2.65	
	H5 Nov	0.5	1.27	2.33	1.41	5.87	0.47	2.82	
<i>Asellopsis intermedia</i>	H1 Febr	0.86	1.37	0.56	1.71	2.45	0.36	1.5	n = 2
	H2 June	0.54	1,00		1.64	2.33		0.57	
	H5 Nov	0.42	1.24	0.5	0.79	1.11		1.84	
Cletodidae	H4 Aug	0.01			1.06	1.67			
<i>Delavalia palustris</i>	H1 June	0.33	2.13	0.61	2.52	4.77	1.69	0.83	n = 2
	H4 Aug	0.33	2.27	2.14	9.83	4.09	1.02	1.13	
	Nov	0.51	7.59	1.86	14.9	0.84	0.74	0.14	
	Febr	0.59	2.08	1.48	4.37	7.6	2.3	1.95	n = 2
	H5 June	0.47	3.07		15.04	3.61	0.78	0.18	
	Aug	0.34	1.81	1.24	9.54	4.76	1.34	0.94	n = 2
	Nov	0.84	2.24	1.59	2.32	5.13	1.57	3.6	n = 2
Febr	0.63	2.94	2.23	2.15	7.13	2.15	1.68		
Ectinosomatidae	H1 June	0.05	1.86		3.16			0.2	n = 2
	H4 June	0.03			2.24	2.86		0.02	
<i>Microarthridion littorale</i>	H1 Aug	0.08			2.94				n = 2
	June	0.26						0.05	
	H3 Febr	0.76	0.85	0.58	0.82	3.07		2.29	n = 3
	H4 June	0.15	1.34		0.94	1.34		0.1	
	Aug	0.2	2.14		1.92	0.68		0.06	n = 2
	Nov	0.41	1.6	0.49	1.79	2.3		0.32	n = 3
	Febr	0.77	1.31	1.33	1.38	4.79	0.49	2.1	n = 3
	H5 June	0.44	1.13	0.17	1.28	2.02		0.36	n = 3
	Aug	0.37	0.95	0.48	1.79	1.67		0.57	n = 3
	Nov	0.24	0.78	0.77	0.75	1.19		1.00	n = 3
Febr	0.4	1.37		1.5	3.03		0.46		
<i>Nannopus palustris</i>	H5 Nov	0.53	1.1	1.42	0.93	4.17	0.58	2.66	n = 2
	Febr	0.76	1.69	2.1	0.91	5.96	0.84	2.42	n = 2
<i>Paraleptastacus spinicauda</i>	H2 June				15.02				n = 2
	Aug		0.84		1.51	0.12		0.01	
	Nov				14.46				
	H3 June	0.05			4.67	2.4			
<i>Paronychocamptus nanus</i>	H3 Aug	0.54	1.24	1.02	2.34	2.52		0.4	n = 3
	Nov	0.25			3.63				
	Febr	0.76	1.72	0.59	0.89	2.51	0.52	2.15	n = 2
	H5 Febr	0.26	3.77		1.15	1.96		0.26	
<i>Platychelipus littoralis</i>	H4 June	0.41	2.17	0.41	1.22	4.47	0.53	0.32	n = 2
	Aug	0.7	2.21	0.7	1.74	5.9	0.58	1.34	
	Nov	0.7	1.53	0.63	1.33	3.6	0.71	3.51	n = 2
	Febr	0.75	2.45	1.14	1.01	6.21	0.81	2.77	
	H5 June	0.16			2.38	13.14			
Febr	0.78	2.83	1.24	0.75	6.16	0.8	2.28		
<i>Tachidius discipes</i>	H1 June	0.16			2.74				n = 2
	Febr	0.92	0.88	0.87	0.7	3.98	0.25	2.94	
	H2 June	0.21	0.51		3.12	1.36		0.48	n = 2
	H3 June	0.4	0.98		2.88	2.83		0.26	
	Aug	0.34	1.29		2.71	1.61		0.28	n = 2
	Nov	0.89	1.29		2.32	3.74		0.44	
	Febr	0.75	0.91	0.58	0.34	3.94	0.2	2.53	
	H4 Febr	0.58	0.91	0.56	0.96	4.27	0.28	1.65	

* or C18:1 ω 7c**Table 3.** Marker fatty acids (in %) or fatty acid ratios in harpacticoids, indicative for copepods' diet.

DISCUSSION

Spatio-temporal variability in resource availability

The biggest contrasts in sediment isotopic signatures and FA profiles were found between the sandy station (H2) and the muddy salt marsh stations (H4, H5). Bulk organic matter at H2 had an MPB-dominated isotopic signature. Comparatively higher hydrodynamic disturbance of the sandy sediment minimizes accumulation of silt and retention of ^{13}C -depleted detrital organic matter. Hence, the $\delta^{13}\text{C}$ signature of the sediment surface is mainly a reflection of the autochthonous primary production by MPB. However, this MPB does not accumulate as stable biofilms as it does on nearby siltier and less hydrodynamically disturbed sediments, leading to lower MPB biomass in sandy sediment, even though the overall rates of primary productivity may be very comparable in both sediment types (Herman et al. 2001). MPB isotopic data used in the present study were coined from previous work in the Paulina intertidal area (Moens et al. 2002, Moens et al. 2005a). They are in the range of typical saltmarsh and tidal flat MPB (Currin et al. 1995, Riera et al. 1996, Deegan & Garritt 1997) and overlap with isotopic values of decomposed *Spartina* (Middelburg et al. 1997). Fresh *Spartina anglica* tissue, SPOM and bulk sediment $\delta^{13}\text{C}$ data (POM) from the current study matched well with earlier measurements from the polyhaline part of the Schelde Estuary (Middelburg et al. 1997, Middelburg & Nieuwenhuize 1998, Moens et al. 2002). At most stations, with the exception of H2, sediment organic matter $\delta^{13}\text{C}$ closely resembled that of SPOM (see Fig. 4), illustrating the strong retention of deposited phytoplankton and other detritus. SPOM deposition appeared most pronounced in June and could be clearly observed in the field: the upper few cms were siltier and richer in detritus than deeper sediment layers (Cnudde et al., 2013, in preparation, chapter 2).

Especially stations H4 and H5 (^{13}C -depleted and ^{15}N -enriched as a result of oxic organic matter degradation (Lehmann et al. 2002) can be considered mainly detritus-based systems with high and more diverse resource availability, as also shown by their higher FA content and FA diversity. In such environments, there is a positive feedback between MPB biofilms and silt deposition, but the MPB has lower turnover rates and is often less available to grazers than in sandier sediments (Herman et al. 2001). In terms of sediment granulometry, station H3 resembled more closely H2, indicating a higher hydrodynamic activity. At the same time, it is situated amidst pioneer *Spartina* vegetation, and its POM $\delta^{13}\text{C}$ was not significantly different from that of the siltier sediments. Concentrations of bacterial FA at this station were, however, comparatively limited. *Spartina anglica* was the dominant vegetation in the immediate vicinity of H3, H4 and H5, but our $\delta^{13}\text{C}$ demonstrate that its detritus input at these stations is limited, in line with data from other salt marshes (Middelburg et al. 1997). The high $\delta^{15}\text{N}$ in the muddy salt marsh stations suggest intensive microbial nitrification-denitrification processes (Lehmann et al. 2002, and ref herein). These stations were also characterized by high harpacticoid abundances and biomass (Cnudde et al., in preparation, chapter 2), suggesting high food availability and/or quality (Ahlgren et al. 1997, de Skowronski & Corbisier 2002, Sevastou et al. 2011).

Copepod resource utilization

Considering the substantial habitat and temporal coverage of the present sampling campaign, the variation in natural stable carbon isotope signatures of harpacticoid copepods was relatively small. With a $\delta^{13}\text{C}$ range of -18 to -12.5 ‰ and a high prominence of diatom-specific FA (e.g. 16:1 ω 7), we can conclude that the majority of copepod species rely predominantly on MPB. Significant contributions of ^{13}C -depleted sources, i.e. SPOM or detrital vascular plant material (C_{18} -PUFAs), were more rare. These sources were, however, manifest in *Paronychocamptus nanus* (particularly at H5 in February, with a $\delta^{13}\text{C}$ value of -23.52 ± 1.18 ‰), *Amphiascus* sp. 1 (at H5 in November, with $\delta^{13}\text{C}$ value of -20.3 ± 0.7 ‰) and, to a lesser degree, *Microarthridion littorale* (at H4 in August, with $\delta^{13}\text{C}$ value of -20.5 ± 1.7 ‰). *P. nanus* and *Amphiascus* sp. 1 were previously considered detritus and diatom feeders, respectively (Hicks 1971, Heip 1979), but no

study has specifically tackled their *in situ* feeding habits. *M. littorale* has been well studied because of its near omnipresence in tidal flats worldwide. At the Paulina tidal flat, it was present at all stations (except H2) and at all times. It can feed on benthic and planktonic microalgae (Decho 1986, Decho & Fleeger 1988, Santos et al. 1995) but also on bacteria. Their ability to bioconvert essential PUFA such as DHA (De Troch et al. 2012a) may be an important aspect behind this 'generalist' feeding behaviour. For other harpacticoid species, the smaller significant variability in $\delta^{13}\text{C}$ signatures (narrow range of 2‰) could result from a more selective feeding behaviour on different microbenthic algal species, depending also on spatial-temporal changes in MPB composition. Lab experiments by De Troch et al. (2006, 2012b) and Wyckmans et al. (2007) have documented that harpacticoid species can select among diatoms by diatom size, age or species. A high dependence of harpacticoids on MPB carbon is in line with previous researches (Santos et al. 1995, Riera et al. 2004, Galvan et al. 2008). Importantly, however, our stable nitrogen isotope data showed a spread of 5.5 ‰ among different harpacticoid species, and a mean nitrogen isotopic fractionation of 6 ‰ between harpacticoid copepods and diatoms, which is nearly twice the expected value for a single trophic step (Post 2002, McCutchan et al. 2003). The latter result suggests harpacticoid copepods can obtain MPB carbon indirectly, through one or even two trophic intermediates, rather than by direct grazing on MPB. Caution is due when interpreting this fractionation between MPB and copepods, because the MPB isotopic data used here have not been obtained at the same time as the copepod data. $\delta^{15}\text{N}$ signatures of marine sources can exhibit substantial spatio-temporal variation (Riera et al. 2000, De Brabandere et al. 2002, Dethier et al. 2013). Nevertheless, the explicit variation in $\delta^{15}\text{N}$ among copepod species strongly suggests that they span more than one trophic level. For the two copepod samples with $\delta^{15}\text{N} > 16$ ‰ (Fig. 4), there is no obvious indication of a higher trophic level (e.g. carnivorous feeding) in their FA profiles, although their FA profiles indicate a lower dependence on MPB: (1) *P. spinicauda* (station H2, red data point) showed no affinity at all with the FA characteristics of primary producers, and (2) for *M. littorale* (station H5 – November, yellow data point), FA composition had a predominance of DHA instead of EPA, which indicated dinoflagellate feeding or carnivory; 16:1 ω 7 proportions were intermediate compared to other species. For nematodes from the Paulina area, $\delta^{13}\text{C}$ also generally point at MPB as the main basal carbon source. $\delta^{15}\text{N}$ data, however, demonstrate that several species obtain this carbon indirectly, and not always in accordance with expectations from morphology-based feeding types (Moens et al. 2005a, *in press*). For harpacticoid copepods, however, mouth parts provide only little information on their feeding strategy (De Troch et al. 2006). There are several potential scenarios which may help to explain the spread in $\delta^{15}\text{N}$ in harpacticoid copepods in our study. First, some harpacticoid copepods may feed partly or predominantly on bacteria (Rieper 1982), which in turn may derive a considerable portion of their carbon from MPB, for instance from their exopolymer secretions (EPS) (Decho & Moriarty 1990). In our study, the FA patterns of *D. palustris* and *P. spinicauda*, for instance, clearly indicate at least a partial dependence on bacterial food sources. The carbon isotopic signature of heterotrophic bacteria is often very similar to that of their carbon sources (Boschker & Middelburg 2002), but can also deviate by up to 11 ‰ (Macko & Estep 1984). In contrast, nitrogen fractionation by sedimentary bacteria is poorly predictable and depends on the molecular nature of the organic nitrogen source (including C:N ratio, biosynthetic and metabolic pathways e.g. degree of transamination). Even among similar nitrogen sources, ^{15}N fractionation can vary from strongly negative (Macko and Epstein 1984) to strongly positive (McCarthy et al. 2007). Second, some harpacticoid copepods may feed on heterotrophic ciliates and/or flagellates, which in turn consume MPB, be it through grazing on cells or consumption of EPS (Rieper 1985). With DHA levels dominating over EPA, consumption of dinoflagellates by *M. littorale* and *Tachidius discipes* is, for instance, plausible. Third, some harpacticoids may be predators of other, MPB-grazing metazoans, such as nematodes, ostracods or harpacticoid nauplii (Lazzaretto & Salvato 1992, Lehman & Reid 1992, Kennedy 1994, Seifried & Dürbaum 2000, Dahms & Qian 2006). However, copepod FA profiles did not reveal carnivorous feeding (PUFA/SFA and 20:1 ω 9 low). Fourth, harpacticoids may re-utilize their own fecal pellets and associated microbes (e.g. De Troch et al. 2009).

When comparing stable isotope data from different stations in our study, the dependence of sandy-sediment copepods on MPB was more pronounced than that of copepods from other, more accretory

stations. However, FA data did not fully support this conclusion, in that the abundant interstitial copepod *P. spinicauda* had very low contributions of MPB-characteristic FA. The other two dominant species from station H2 were *Asellopsis intermedia* and *Tachidius discipes*. FA data support the view that the former is primarily a diatom-feeder, at least in station H2 (and H1) where 16:1 ω 7 contributed 19 % to copepod FA and contrasting to only 9% in H5 (based on one sample only), while the latter species is not (low EPA/DHA ratio, low 16:1 ω 7).

No *in situ* data of these harpacticoid species are available from other biomarker studies, but our results line up with trophic knowledge obtained from more classical approaches. Interstitial copepods are considered as non-diatom feeders (Joint et al. 1982), browsing on sediment grains and scraping the epiflora which is largely composed of epipsammic bacteria (Noodt 1971, Feller 1980). For species from the genus *Paraleptastacus* specifically, previous suggestions of bacterial feeding were based on the absence of diatoms in the gut of *P. klei* (Azovsky et al. 2005) and the tolerance of *P. espinulatus* to high organic inputs with high bacterial activity (Hockin 1983). In contrast, the sand dwelling *A. intermedia* showed tidal migration, moving to the sediment surface during low tide and hence to MPB biofilms (Joint et al. 1982). *T. discipes* is the only species where DHA levels exceeded EPA and partial trophic reliance on protists is indicated.

The few species from muddy sediments for which our stable isotope data indicated variable or no reliance on MPB were *Paronychocamptus nanus* and *Amphiascus* sp. 1, both from station H5 in November, and Cletodidae. To our knowledge, no empirical evidence about their trophic ecology has hitherto been published. The more depleted $\delta^{13}\text{C}$ of *P. nanus* and *Amphiascus* sp. 1 ($\delta^{13}\text{C} \leq -20$ ‰) compared to that of co-occurring species ($\delta^{13}\text{C} \geq -18.5$ ‰) suggests a stronger reliance on SPOM ($\delta^{13}\text{C}$ averages -22.8 ‰; Table S1). For *P. nanus*, however, the more depleted $\delta^{13}\text{C}$ was not consistent over time, the data in February ($\delta^{13}\text{C} = -15.7$ ‰) resembling those of other species and suggesting a closer link with MPB. For *Amphiascus* sp 1, a lower presence of diatoms indicators (16:1 ω 7, EPA/DHA) and a small increase in C₁₈-PUFAs in station H5, indeed support the idea of higher contribution to SPOM to their diet.

Cletodidae are clearly specialist feeders relying on a completely different carbon source than the other copepods that were analysed here. Their very light carbon isotopic signatures are characteristic for chemoautotrophic bacteria. Sulphide production in these largely anoxic marsh sediments is high, even though sulphide concentrations in the Paulina intertidal area are on average low because of rapid scavenging by the high Fe²⁺ and Mn²⁺ concentrations. Interestingly, our results on Cletodidae are confirmed by a report on Cletodidae from seagrass-vegetated stations in the Mira estuary, Portugal (Vafeiadou et al. in prep). To our knowledge, there have been only two reports of harpacticoid copepods from shallow-water environments with similar strong reliance on chemoautotrophic carbon, but the identity of the species were unknown in these cases: one at a mudflat station in the Eastern Scheldt estuary (Moens et al. 2011) and one from shallow subtidal sediments in the North Sea (Franco et al. 2008). Van Gaever et al. (2006) found ¹³C-depleted values of -51 ‰ for a species morphologically similar to the harpacticoid *Tisbe wilsoni* at a cold methane-venting seep in the Barents Sea, demonstrating reliance on methanotrophic bacteria. These few published results on harpacticoid copepods are in accordance with similar results on particular nematode species from shallow waters (Ott et al. 2004), deep-sea (Van Gaever et al. 2006, 2009, Tchesunov et al. 2012), mangrove (Kito & Aryuthaka 2006, Moens et al. unpubl., Bouillon et al. 2008) and seagrass (Vafeiadou et al. in prep) sediments, confirming that chemoautotrophic carbon may be an important energy source for several meiofaunal taxa. Whether in Cletodidae this reflects some sort of symbiotic relationship, as reported in several marine nematode genera (e.g. Polz et al. 1992, Riemann et al. 2003), or rather a direct and selective grazing (as in *Halomonhystera disjuncta* from an active, methane-venting mud volcano, Van Gaever et al. 2006, 2009) remains to be established. Unfortunately, we could only obtain a single successful FA profile of Cletodidae, yielding somewhat equivocal results: the lack of essential PUFA such as EPA and DHA, confirms their independence of MPB. But at the same time, the abundances of bacterial-specific FA (Kharlamenko et al. 1995) and those

specifically for chemoautotrophic bacteria (Table 1a; Van Gaever et al., 2006) were low, with levels comparable to other species and lower than in bacterivorous *D. palustris* and *P. spinicauda*.

Most of the interspecific, spatial and temporal variation in $\delta^{13}\text{C}$ was in the range of 2 ‰. Whether this points at shifts in the copepod diets, for instance with increasing or decreasing contributions of MPB, or at different taxa of MPB being consumed, cannot be derived from our data. Small spatio-temporal variations in resource $\delta^{13}\text{C}$ (Dethier et al, 2012), and/or shifts in the composition of these resources (for instance the MPB community) may equally explain the spatio-temporal variation in consumer isotopic data. A second difficulty, related mostly to the use of FA as trophic biomarkers, is the fact that species consuming the same food source may differentially assimilate and metabolize trophic markers.

For some species, observed $\delta^{13}\text{C}$ variation in combination with changes in relative FA proportion did illustrate shifts in diet. For instance, the spatial variation in $\delta^{13}\text{C}$ values of *Asellopsis intermedia* related to a different reliance on benthic (epipsammic) diatoms among the stations: diatom reliance was clearly higher in stations H1 and H2 (tidal flats) compared to H5. For *Microarthridion littorale* at station H5, the isotopic difference between November and February was ascribed to a change from flagellate consumption (DHA>EPA) in combination with some assimilation of vascular plant detritus (C₁₈-PUFA), to a higher diatom grazing (EPA>DHA, 16:1ω7, no C₁₈-PUFA). For *Delavalia*, FA data suggest the use of two food sources, i.e. bacteria (15:0, 17:0) and diatoms (high EPA/DHA).

Copepods showed a high FA diversity in February, accompanied by the highest proportions and absolute concentrations of PUFAs EPA and DHA), in accordance with sediment organic matter PUFA content at this moment. Possible explanations include (1) an early MPB bloom at the end of February, and/or (2) an increased PUFA accumulation by copepods for overwintering during November (winter). With a seasonal change in temperature, copepods produce a FA reserve for overwintering and copepods overwinter in diapauses with a reduced metabolic rate (Kattner & Krause 1989, Lee et al. 2006, Falk-Petersen et al. 2009). However, exact hibernating strategy differences among copepods are unknown, some species will end up being lipid poor after winter while others were able to maintain enough lipid reserves to spawn in spring (Kattner & Krause 1989). Moreover, copepod lipid content before overwintering can be highly variable, depending on whether the copepod species still produced a late-summer generation (Kattner & Krause 1989).

General conclusion

MPB, mainly diatoms, was of high dietary importance for the majority of intertidal harpacticoid taxa over the entire tidal flat – salt marsh area. Copepods spanned at least two trophic levels, and whether MPB carbon is mainly channeled directly or indirectly to harpacticoids remains unclear. There was little evidence for a role of *Spartina* detritus as a resource for copepods. SPOM contributed significantly to the diets of a limited number of species. In spite of the general importance of MPB as a major carbon source for a majority of our copepod species, food source utilization patterns were diverse and species-specific. In addition to species-specific trophic differences, spatio-temporal patterns also occurred, and particular species had at least partly different diets depending on the station where they were found.

Cletodidae consistently used chemoautotrophic energy, a trophic link which for harpacticoid copepods had hitherto only been reported from a deep-sea vent system and a subtidal flat.