Chapter 2

Spatio-temporal variation in harpacticoid copepod assemblages and of their food sources in an estuarine intertidal zone

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Clio Cnudde, Willem Stock, Anne Willems, Annelien Rigaux, Marleen De Troch and Tom Moens. Spatio-temporal variation in harpacticoid copepod assemblages and of their food sources in an estuarine intertidal zone

ABSTRACT

Spatio-temporal patterns in harpacticoid assemblage composition and in individual abundances of harpacticoid species were studied in an estuarine intertidal area with high habitat heterogeneity and in relation to spatio-temporal patterns in environmental variables including sediment abiotic characteristics (granulometry, nutrient concentrations), organic matter availability, organic matter quality (chlorophyll a and pheophytine, protein content, lipid content), composition of microphytobenthos biofilms (carotenoid pigments) and sediment bacterial abundances. The tidal flat (a sand flat and a mud flat) and the salt marsh region (a *Spartina*-dominated sandy sediment, a *Spartina*-dominated muddy sediment and a muddy gully) were sampled over 4 sampling events. The two tidal flat stations have distinct harpacticoid assemblages while the harpacticoid assemblages of salt marsh stations were composed of the same abundant harpacticoid families. The influence of abiotic habitat characteristics (e.g. granulometry, inorganic nutrients) and biotic characteristics relating to food source availability and quality (total organic matter, microphytobenthic biofilms characterised by pigments and their degradation products, differences in detrital origin) on harpacticoid assemblage structure was determined and species-specific responses to environmental factors were revealed. Spatial harpacticoid assemblage variation was assigned to five variables: ammonium concentrations, total organic matter, abundance and composition of microbial biofilms (chlorophyll a proportion of total organic matter; proportion of diatoxanthin in the microphytobenthos) and abundance of detritus (pheophytine over chlorophyll a). However, harpacticoid assemblages of tidal flats were seemingly structured by abiotic factors (granulometry and tidal height) and especially copepod species from the sand flat (*Paraleptastacus spinicauda*, *Asellopsis intermedia*) were highly specific in space and constant over time. High intercorrelations between variables and especially granulometry presumably masked the role of granulometry. In contrast, the high resemblance among salt marsh harpacticoid assemblages is in spite of differences in salt marsh granulometry, and points towards a primary influence of food availability and food quality. Variability in *Microarthridion littorale* abundances related to microphytobenthos abundance. For Ectinosomatidae and *Tachidius discipes*, the low densities and low correlations over all environmental factors points towards a low affinity with the sediment surface and a generalistic occurrence, respectively. For some species, linkages between habitat characteristics and species distributions were little decisive (e.g. *Platychelipus littoralis*, *Paronychocamptus nanus*, *Amphiascus* sp. 1). Overall, it is not possible to denote with certainty the main variables regulating harpacticoid species distributions due to complex intercorrelations between environmental variables, including abiotic and food-related variables

INTRODUCTION

In many marine ecosystems, harpacticoids (Crustacea, Copepoda) are among the most abundant meiobenthic taxa, only outnumbered by nematodes. Their taxonomic diversity has been studied from intertidal and shallow waters (Chertoprud et al. 2013) to the deep sea (Gregg et al. 2010), and their large-scale distribution is reasonably well characterized (Chertoprud et al. 2010, Veit-Köhler et al. 2010). On the local scale, however, harpacticoid distributions reveal a high spatial-temporal variability, even at decimeter scales (≤ 10 cm^2)(Azovsky et al. 2004). The ecological mechanisms structuring local species diversity and patchiness remain insufficiently understood. Many studies have discussed the influence of habitat characteristics such as sediment type and heterogeneity, physico-chemical characteristics and hydrological conditions, food availability and biotic interactions on harpacticoid and other meiofaunal taxa abundances (e.g. Huys et al. 1992, Kotwicki et al. 2005, Rubal et al. 2012) and to a very limited extent on harpacticoid assemblage composition (Veit-Kohler 2005, Stringer et al. 2012).

Depending on their association with the sediment, harpacticoid species are divided into ecotypes. Interstitial copepods are small species living in the interstitial spaces between sand grains; epi- and endobenthic species live on top or in the upper centimeters of the sediment, respectively; and free-living species are swimming but remain in close contact with the sediment. The distribution of harpacticoid ecotypes strongly depends on the median sediment grain size and sediment sorting (Hulings & Gray 1976, Rybnikov et al. 2003, Giere 2009), which in turn is intricately linked to local hydrodynamics and tidal flat morphology. Besides a direct structuring role of grain size on the meiobenthos, sediment type also affects food availability and quality. Sediment organic matter content, density and diversity of microbial biofilms, and other biotic environmental variables are often correlated with grain size (e.g. Decho & Castenholz 1986, Stal & de Brouwer 2003). However, in sediments with similar granulometry, the structuring role of food resources becomes apparent: within a habitat type, harpacticoid distributions are conform the patterns of microbial food sources such as diatoms, ciliates and purple sulfur bacteria (Decho & Castenholz 1986, Azovsky et al. 2004). *Enhydrosoma littorale* had differential abundances in two adjacent tidal habitats with different granulometry but showed no preferences for either granulometry under laboratory conditions. However, the species responded only to sediment particles coated with microbial epigrowth (Ravenel & Thistle 1981). The relative importance of these different factors for harpacticoid distribution and community structure remains poorly understood. Distinct spatial heterogeneity and temporal dynamics of meiobenthos and harpacticoid assemblages are prominent in estuaries (Hicks & Coull 1983, Heip et al. 1985, Chertoprud et al. 2007). Estuarine sediments are often characterized by a mosaic of habitat types (Davidson et al. 1991) and, especially in intertidal areas, are subject to short-term fluctuations in interstital water content and temperature, salinity, dissolved nutrients, grain size, (Thomson-Becker & Luoma 1985) and resource availability, e.g. microphytobenthic biofilm production, organic matter deposition, etc. (Blanchard et al. 2002, Chen et al. 2005). Data on the simultaneous dynamics of environmental variables and multiple harpacticoid species from the same harpacticoid assemblage are scant (Veit-Kohler 2005, Stringer et al. 2012). In both cited studies, species distributions were predominantly impacted by physical factors (mainly grain size, water depth or tidal height) and by pH.

The present study investigated the spatio-temporal patterns of harpacticoid assemblages in an estuarine intertidal area composed of a range of habitats, in relation to variability in environmental factors. For this purpose, 5 habitats were sampled in a tidal flat-salt marsh area within an area of ca. 0.3 km^2 and at 4 sampling periods within one year. These habitats differed in sediment granulometry, tidal height, and vicinity and type of vegetation. The following questions were investigated: (1) Which environmental variables structure the horizontal distribution of harpacticoid assemblages in an estuarine intertidal area (between-habitat heterogeneity) and which variables account for temporal fluctuations in harpacticoid assemblages (within-habitat heterogeneity)? (2) Which environmental variables correlate with variability in abundances of individual harpacticoid species within and among habitats? We hypothesized that spatial harpacticoid heterogeneity would be mainly governed by granulometry and/or large differences in total

organic matter content due to presence of vegetation, and expected to observe differences in harpacticoid assemblages between habitats in the tidal flat and in the salt marsh ('vegetation-effect'), as well as between the habitats in the tidal flat (grain size-effect) and in the salt marsh (grain size-effect and effect of type and prominence of vegetation).

MATERIALS AND METHODS

Study area

The Paulina intertidal area is located along the southern shore of the polyhaline zone of the Westerschelde estuary (SW Netherlands, 51°20'55.4''N, 3°43'20.4''E) (Fig 1a). Mean tidal range is 3.8 m (low) (Claessens & Meyvis 1994) and hydrodynamic energy is relatively low. Five sampling stations were chosen, covering different intertidal habitats (Fig. 1b, c) in terms of, among other things, tidal height, granulometry and presence/absence of vegetation (Table 1; Fig. 1). These five stations were geographically oriented over an east-west distance range of approximately 750 m and a north-south distance range of approximately 350 m. Two stations (H1 and H2) are situated in the tidal flat area. Station H1 is located at the lower intertidal and exhibited a temporally variable granulometry, while station H2 is located in the mid-intertidal and was characterized by fine sandy sediment with a negligible silt fraction throughout the year. The other three stations H3, H4 and H5 are situated in or at the edge of the salt marsh. Station H3 is a bare sediment patch positioned at the mid to high intertidal amidst *Spartina anglica* vegetation. Samples were collected within less than half a meter of *Spartina* vegetation, in sediment dominated by fine sand and with a variable mud fraction (0 to 25 %). Station H4 is located in the high intertidal, near *Spartina* vegetation and bordering 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 major drainage gully in the salt marsh, which cuts through dense vegetation which, at the level of station H5, is dominated by a combination of *Spartina anglica*, *Aster tripolium* and *Atriplex portulacoides*. Samples were collected intermediate of the bed and the flank of the gully, on exposed horizontal sediment surfaces. Since stations H3, H4, and H5 were in close proximity of salt marsh vegetation, we henceforth refer to these as 'salt marsh' stations, while H1 and H2 are referred to as 'tidal flat' stations or more specific the 'mud flat' and 'sand flat', respectively.

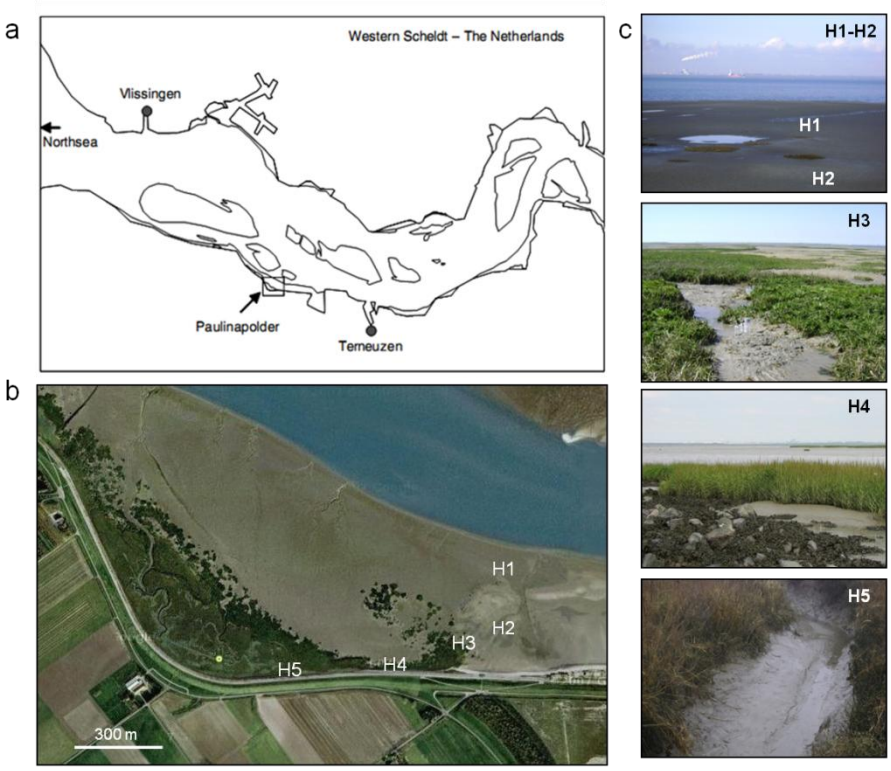

Fig. 1. Location of a) the Paulina intertidal area and b) sampling stations H1 to H5. The diversity of sampled habitats is shown in c). Coordinates of stations are given in Table 1.

Table 1. Geografic location and visual characteristics of the five sampling stations. (For detailed data on sediment characteristics, see table 3)

Station	Location	Tidal height* (m)	Tidal exposure* (% of tidal cycle)	Habitat type	Sediment type	Vegetation
H1	N 51°21'06.8" E 03°43'53.2"	-21	47	Tidal flat	Mud (though variable)	none
H2	N 51°21'00.7" E 03°43'52.2"	119	71	Tidal flat	Sand	none
H3	N 51°20'57.6" E 3°43'49.1"	237	93	Salt marsh	Sand (though variable)	*Spartina anglica*
H4	N 51°20'56.1" E 03°43'34.2"	143	78	Salt marsh	Mud	*Spartina anglica* and macroalgae
H5	N 51°20'55.7" E 03°43'30.6"	230	97	Salt marsh	Mud	Salt marsh vegetation

* data from 2008 (data source: Rijkswaterstaat Servicedesk

Sampling procedure

Four sampling campaigns were carried out in the year 2010-2011 with 3-month intervals, 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). Sediments of the five stations were sampled at low tide for harpacticoid assemblage analysis and for analyses of environmental biotic and abiotic sediment characteristics, by means of plexiglass cores with inner diameter = 3.6 cm (surface = 10 cm^2), except for samples for nutrient and bacterial analyses, which were collected with larger cores (i.d. = 6.2 cm, surface = 30.2 cm^2) and syringes (i.d. = 2.0 cm, surface = 3.1 cm^2), respectively. For each type of analysis, four replicate sediment cores were sampled within a surface of ca 1 m^2, replicate cores for abiotic and biotic

sediment analyses matching replicate cores for harpacticoid assemblage analysis. Exceptions are station H3 where no homogeneous horizontal sample area of 1 m^2 was available and replicates were hence taken over a slightly larger sampling area, and station H5, where sediments were sampled along the exposed sides of the gully bed over a distance of ca. 10 m.

Cores were sliced into sediment layers 0-0.5 cm, 0.5-1 cm and 1-3 cm. Sediments for nutrient analysis were sliced, after removal of any water layer on top of the sediment surface, in 0-1 cm and 1-3 cm, because insufficient pore water could be obtained from slices of half a cm thick. Sediment slices for bacterial analyses were (vertically) bisected to provide one subsample for bacterial cell counting and one matching subsample for genetic bacterial assemblage analysis. Samples for protein and lipid analysis were subdivided in the same way. All samples were cooled on ice during the sampling campaign, except for lipid and pigment samples which were frozen using dry ice; the latter were wrapped in aluminium foil to avoid photodegradation. For long-term storage, copepod samples were preserved in a 4% formaldehyde solution, lipid and pigment samples were stored at -80°C and other samples at -20°C.
Interstitial water temperature and salinity were measured at each station using a field electrode. Water temperature ranged from 1 °C to 27.5 °C (in November and June, respectively). Salinity varied from 20.7 to 27.9 (in February and June, respectively).

Harpacticoid assemblage analysis

Sediment slices were rinsed with tap water over a 38-µm sieve and copepods were extracted from the sediment by flotation with ludox (density 1.18 g/cm^3) using a 1:10 ratio sediment:ludox and centrifugation for 12 min at 3000 rpm. This procedure was repeated three times. After staining with Rose Bengal, copepods were enumerated and manually sorted under a Leica MZ stereomicroscope (125 x magnification) using an eyed needle. Only adult specimens were identified; they were mounted on glass slides in a drop of glycerin. From samples with a high number of copepods, only the first 100 randomly-picked adult specimens were identified; for samples with fewer than 100 specimens, all were identified. Harpacticoids were identified to species level using Lang (1948) and Boxshall and Halsey (2004). A taxonomic list of identified harpacticoid taxa is presented in addendum II Table S1. Data were obtained from three replicates.

Environmental variables

Sediment granulometry was analysed with a Malvern Hydro 2000G particle size analyser on sediment dried for 24h at 60°C. Grain size fractions (in vol %) were classified according to the Wentworth scale (Wentworth 1922). Characteristics used in this study were median grain size, percentage mud (clay-silt fraction, < 63 µm), sorting coefficient SC (QD_φ) and skewness Sk (Sk_φ). SC and Sk were calculated based on the statistical parameters median grain size (Md), the first (Q1) and the third (Q3) quartile (in mm) using the formula of Giere (2009):

$$QD_\varphi = \frac{(\varphi Q1 - \varphi Q3)}{2}$$

$$Sk_\varphi = \frac{(\varphi Q1 + \varphi Q3)}{2} - \varphi Md$$

with $\varphi = -(\log x / \log 2)$ and x = grain size (in mm)

Concentrations of nitrogenous nutrients (NO_x^-,NH_4^+), phosphate (PO_4^{3-}) and silicium (SiO_2) in the sediment pore water were measured using a SAN^{plus} Segmented Flow Analyser (SKALAR)

Various sediment characteristics were used to estimate food availability and quality: sediment total organic matter content (TOM), absolute and relative phytopigment concentrations, lipid and protein concentrations, and bacterial abundance and diversity.

TOM (total organic matter) was determined as the weight loss of sediment after combustion in a muffle furnace at 550 °C for 2 h. The photosynthetic component of TOM was quantified by means of phytopigment analysis. Pigments of lyophylised and homogenized sediments were extracted in 90% v/v aceton at 4°C in the dark and separated by reverse-phase high-performance liquid chromatography (HPLC Agilent 1100 Series) according to Wright and Jeffrey (1997). Chlorophyll pigments measured were chlorophyll *a* (chla), pheophytin *a* and pheophytin *a*-like (both were summed to a single value, pheo), and chlorophyll c_2 (chlc). Carotenoid pigments measured were fucoxanthin (fuc), zeaxanthin (zea), lutein (lut), diadinoxanthin (diadino), diatoxanthin (diato) and β-carotene (bcar). Pigment concentrations were expressed as micrograms per gram sediment dry weight. The ratio chla:TOM was used as an indication for the proportion of fresh photoautotrophic-derived organic matter in the total organic matter pool. The ratio pheo:chla was considered indicative of the turnover of photoautrotrophic matter (e.g. herbivory leading to high pheo:chla ratio, Cartaxana et al. 2003). Carotenoid pigments (and their ratios to chla) provide information on the taxonomic composition of the microphytobenthos (MPB) (Table 2).

Table 2. Taxa affinity of pigments, based on Barranguet et al. (1997), Lucas and Holligan (1999), and Buchaca and Catalan (2008).

Pigment	**Taxa**
Chlorophyll c_2	Chrysophytes and diatoms, cryptophytes and dinoflagellates
Fucoxanthin	Diatoms and chrysophytes
Zeaxanthin	Cyanobacteria (and chlorophytes)
Lutein	Chlorophytes
Diadinoxanthin	Diatoms, dinoflagellates, chrysophytes, euglenophytes
Diatoxanthin	Diatoms
β-carotene	Cyanobacteria, eukaryotic algae and vascular plants (or cosmopolitan)

Total proteins were extracted in accordance to Hartree (1972), as modified by Rice (1982) to compensate for phenol interference. Total lipids were extracted through elution with chloroform and methanol in accordance to Bligh and Dyer (1959), as modified by Marsh and Weinstein (1966). Protein (PRT) and lipid (LIP) concentrations, as proxy for organic matter quality, were measured spectrophotometrically and concentrations are expressed as albumin and tripalmitin equivalents, respectively. Protein and lipid data were normalized to sediment dry weight after dessication at 60°C (details see Pusceddu & Danovaro 2009). With PRT as a proxy for organic nitrogen and TOM as a proxy for total organic matter, the ratio PRT:TOM can be indicative of sediment organic matter quality.

Bacterial analysis

Bacterial cell abundances were determined using 4',6-diamidino-2-phenylindole (DAPI) staining and epifluorescence microscopic counting (Porter & Feig 1980). Bacteria were extracted from 0.5 – 2.0 g frozen sediment. Frozen sediments were impregnated overnight with 2 ml of ice cold 0.2-µm pre-filtered and borax buffered ($Na_2B_4O_7.10H_2O$) glutaraldehyde (final conc. 4%, salinity of 27). For detachment of cells from sediment and detritus particles, the sample was incubated for 1h with 0.2 µm pre-filtered tetrasodium pyrophosphate (Na_4P_2O, 10 mM final conc., salinity of 27). Separation of bacterial cells from the sediment was achieved by adding an additional volume of borax buffered seawater (salinity 27), followed by 3 x 30s sonication at 20% amplitude, short centrifugation and supernatant collection (containing the dislodged bacteria). This procedure was repeated 3 times resulting in a final volume of 40 ml of supernatant. 1 ml of supernatant was stained with DAPI 200 µg ml^{-1} (Sigma D9542, final conc w/v)

for 15 min, then filtered on a 0.2 µm black polycarbonate filter (Whatman) following the protocol of Porter and Feig (1980) and counted. At least 10 microscopic fields and a total of 300 bacterial cells were counted. Since bacterial samples were stored at -20°C without fixative, counted bacterial cell densities will be an underestimation of actual bacterial densities and data should be interpreted exclusively in a relative manner, i.e. to illustrate spatio-temporal changes.

Bacterial assemblage structure was analyzed using Denaturing Gradient Gel Electrophoresis (DGGE). Total bacterial DNA was extracted from approximately 3 - 5 g wet sediment following the phenol-based protocol of Muyzer et al. (1993). Prior to cell lysis and DNA extraction, cells were separated from the sediment and extracellular DNA was removed following Corinaldesi et al. (2005). The extracellular DNA pool is by far the largest DNA fraction in marine sediments (Frostegard et al. 1999). Despite natural fragmentation of extracellular 16S rRNA, preserved short sequences of the 16S rRNA gene might still interfere with the PCR-DGGE analysis since this electrophoresis technique specifically targets short DNA sequences (≤ 500 bp). PCR-DGGE of the variable V_3 region of the 16S rDNA and gel staining were performed as described in Cnudde et al. (2013, chapter 6). 800 ng purified PCR-product was loaded on DGGE. Gels were digitally visualized using a charge-coupled device (CCD) camera and the Bio-Rad Quantity One software program. On each DGGE gel, 3 reference lanes were included to allow digital normalization of the fingerprint profiles using the BioNumerics software version 5.10 (Applied Maths, St.-Martens-Latem, Belgium). As a measure of bacterial diversity, number of bands per sample (OTU richness, phylotype richness) was counted. The reference for DGGE analysis was composed of selected cultured bacterial strains originating from the Paulina tidal flat and salt marsh (addendum II, appendix 1). Bacterial abundance and diversity data were obtained from two replicates only.

Data analysis

Data from the depth layer of 1-3 cm were excluded from the data analysis because of very low copepod abundances or even complete absence beneath the top one cm. Additionally, since separate measurements of 0 - 0.5 cm and 0.5 – 1 cm could not be obtained for nutrients, data on copepods and other variables from the 0 - 0.5 cm and 0.5 – 1 cm slices were combined by averaging (for concentration data, e.g. nutrient and pigment concentrations) or by summation (for abundance data, e.g. copepod species and bacterial abundance).

Spatial heterogeneity and temporal fluctuations in environmental variables and in harpacticoid assemblage structure were analysed separately by multivariate ordination of all sampled stations over all four sampling campaigns. The environmental data matrix was composed of 22 variables (see Table 3, indicated by asterisks) with N = 4. Variables omitted from multivariate analysis were (i) bacterial abundances and diversity (number of OTU) because of N = 2 and (ii) highly collinear or redundant variables (see also results). Collinearity between variables was tested using Pearson pairwise correlations, applying a threshold of 90 % collinearity. Furthermore, carotenoid pigment ratios were used instead of concentrations. Preliminary testing showed that using individual pigment concentrations instead of ratios did not strongly affect the ordination (1.9 % increase in percent explained variation) and both concentrations and ratios contained no collinearities of > 90 % . All environmental variables were log(X+1) transformed, reducing the right-skewness of data for many variables, and the normalized matrix was analysed by Principal Component Analysis (PCA). The matrix of harpacticoid species abundances was standardized to relative abundances, overall square-root transformed and subjected to Principal Coordinates Analysis (PCO). The null hypothesis of 'no spatio-temporal differentiation' for environmental data and harpacticoid data was tested with a two-factorial, fully crossed Permutational ANOVA (PERMANOVA) with the factors station (St) and month (Mo). The significance level was set at 5% and p-values > 0.05 were marked as 'insignificant' (ns). Monte Carlo p-values (p_{MC}) were interpreted in case of low number of permutations (< 10). When the assumption of homogeneity of dispersion, as tested with PERMDISP, was rejected ($p < 0.05$), we report the exact p-value to indicate that PERMANOVA results

should be interpreted with caution. Differences in harpacticoid assemblage structure were further analysed by Hill's diversity indices (N_0, N_1, N_2, N_{inf}) (Hill 1973). As for total copepod abundance, these univariate data were analysed using crossed 2-way PERMANOVA based on Euclidian resemblance matrices. SIMPER analysis on the transformed relative copepod abundance matrix was conducted to identify the copepod species characteristic for each station. In addition, nMDS was performed on absolute species counts (with zero-adjusted Bray-Curtis matrix) and, by means of species bubble plots, distribution patterns of most abundant species in the Paulina intertidal area are visualized.

To determine the environmental variables which best explain the similarities between environmental and copepod assemblage patterns, BEST-BVSTEP analysis was used (Clarke & Warwick 2001). The combination of variables with maximum rank correlation coefficient (rho) is the subset of variables which best explain the observed copepod assemblage differences. The significance of the rho value was confirmed using the BEST permutation test using 999 permutations and applying a significance level of 1 %.

Relationships between environmental variables and harpacticoid abundances were analyzed for the most characterstic species, as previously determined by SIMPER, and using Spearman rank correlation analysis (correlation coeffient $-1 < r_s < 1$) and similarly, pairwise correlations between species were analysed. Only species-environmental variable interactions and species-species interactions with significant correlation coefficients were reported.

STATISTICA 7.0 (Microsoft, StatSoft ver. 7.0) was used for Spearman rank correlation analyses and Primer 6.0 for calculating diversity indices and for the uni- and multivariate analyses.

Table 3. Environmental variables for the top 1 cm sediment layer. Averaged values (N = 4 unless indicated otherwise) with standard deviations between brackets. *: variables included in multivariate statistical analysis. Abbreviations: MGS: median grain size; SC: sorting coefficient, Sk: skewness; LIP: lipids; PRT: proteins; TOM: total organic matter; Bact A: bacterial abundance; Bact D: bacterial diversity.

	Sediment				Nutrients				Bacteria					
	MGS* (µm)	Mud (%)	SC*	Sk*	NO_X* (µg l^{-1})	NH_4* (µg l^{-1})	PO_4* (µg l^{-1})	Si* (µg l^{-1})	Bact A (10^8 cells g^{-1} DW)	Bact D (# OTUs)	TOM* (%)	LIP* (mg g^{-1})	PRT* (mg g^{-1})	PRT:TOM*
H1 June	77.8 (3.2)	41.7 (1.7)	0.959 (0.045)	0.096 (0.019)	1263.8 (464.4)	7312.5 (936.1)	1737.8 (538.2)	2253.8 (114.3)	3.606 (1.236)	24 (0)	3.32 (1.14)	0.936 (0.317)	3.759 (0.884)	0.119 (0.038)
Aug	125.7 (22.6)	22.6 (8.4)	0.732 (0.053)	0.060 (0.015)	618.5 (341.5)	2306.5 (378.5)	541.3 (157.2)	2598.3 (505.7)	2.280 (0.382)	22 (4)	1.59 (0.36)	0.235 (0.149)	2.844 (1.202)	0.194 (0.123)
Nov	105.4 (20.4)	30.7 (8.7)	0.889 (0.116)	0.089 (0.027)	734.3 (56.2)	1625.8 (290.2)	495.0 (105.1)	2004.8 (536.6)	1.115 (0.178)	22 (4)	1.86 (0.30)	0.290 (0.143)	2.275 (0.810)	0.123 (0.038)
Febr	187.2 (38.5)	13.2(11.9)	0.514 (0.192)	0.034 (0.029)	889.0 (260.9)	1510.0 (570.1)	557.0 (357.4)	2395.5 (874.1)	0.848 (0.034)	24 (2)	1.40 (0.77)	0.157 (0.048)	3.310 (0.833)	0.284 (0.144)
H2 June	227.0 (3.2)	0.0 (0.0)	0.314 (0.001)	0.000 (0.000)	1239.5 (733.4)	3862.5 (960.7)	2389.0 (370.2)	888.8 (424.8)	2.779 (1.246)	21 (1)	0.74 (0.24)	0.111 (0.012)	0.605 (0.057)	0.086 (0.017)
Aug	221.3 (4.6)	0.4 (0.8)	0.330 (0.025)	0.001 (0.002)	971.8 (388.9)	10869.5 (11847.0)	1494.8 (439.6)	143.5 (92.0)	2.088 (0.531)	21 (0)	0.64 (0.04)	0.159 (0.083)	0.637 (0.334)	0.100 (0.053)
Nov	228.2 (1.0)	0.0 (0.0)	0.311 (0.002)	0.000 (0.000)	2190.0 (490.1)	2425.0 (209.1)	1961.5 (277.0)	1182.3 (354.7)	0.909 (0.128)	19 (1)	1.48 (0.74)	0.345 (0.199)	0.246 (0.073)	0.020 (0.010)
Febr	230.0 (2.8)	0.0 (0.0)	0.310 (0.008)	0.000 (0.000)	1881.8 (607.5)	1124.0 (55.9)	628.5 (110.8)	1754.3 (835.4)	0.860 (0.148)	20 (2)	0.46 (0.02)	0.076 (0.031)	0.876 (0.309)	0.190 (0.070)
H3 June	203.8 (37.9)	14.1 (9.4)	0.567 (0.227)	0.052 (0.046)	607.0 (314.8)	7230.0 (2190.8)	1921.5 (462.7)	2218.3 (164.5)	2.936 (1.627)	20 (5)	1.36 (0.54)	0.363 (0.126)	2.917 (1.825)	0.199 (0.056)
Aug	188.8 (7.5)	18.6 (2.6)	0.697 (0.102)	0.169 (0.077)	420.8 (356.2)	3689.0 (815.4)	732.0 (387.5)	2751.0 (503.0)	2.469 (0.951)	17 (1)	1.46 (0.23)	0.223 (0.119)	3.166 (1.886)	0.211 (0.112)
Nov	211.3 (14.6)	12.9 (4.1)	0.530 (0.098)	0.067 (0.045)	988.5 (518.2)	3988.8 (1110.5)	1176.0 (660.4)	2186.3 (1073.2)	1.360 (0.264)	20 (1)	2.16 (0.49)	0.340 (0.045)	2.259 (0.512)	0.105 (0.012)
Febr	205.0 (36.7)	6.9 (8.2)	0.465 (0.175)	0.039 (0.063)	426.5 (197.1)	3775.3 (1420.5)	1390.5 (480.4)	2991.8 (697.9)	1.101 (0.197)	19 (5)	2.01 (1.65)	0.310 (0.116)	2.823 (1.496)	0.175 (0.071)
H4 June	43.9 (4.1)	66.4 (4.6)	0.937 (0.067)	0.048 (0.021)	68.3 (75.3)	5870.0 (1937.1)	2069.5 (1190.5)	2573.3 (433.0)	3.681 (1.554)	23 (0)	5.55 (1.63)	0.919 (0.151)	5.923 (1.212)	0.114 (0.042)
Aug	48.8 (4.3)	66.0 (4.5)	0.642 (0.070)	0.052 (0.034)	1003.5 (1199.6)	6950.5 (1255.5)	825.8 (34.1)	2489.8 (915.7)	2.549 (0.014)	22 (2)	3.58 (1.14)	0.680 (0.261)	6.014 (3.340)	0.163 (0.074)
Nov	47.8 (1.9)	66.9 (2.1)	0.655 (0.019)	0.035 (0.012)	1070.5 (922.3)	4729.3 (538.9)	1106.8 (286.6)	1717.3 (135.9)	2.049 (0.013)	27 (0)	4.75 (0.91)	0.829 (0.189)	3.225 (0.432)	0.070 (0.019)
Febr	47.5 (2.8)	65.5 (3.3)	0.757 (0.008)	0.068 (0.015)	643.0 (205.2)	4049.0 (902.8)	2405.5 (2367.8)	3996.0 (1361.6)	1.754 (0.656)	26 (0)	3.11 (0.22)	0.797 (0.166)	8.030 (1.760)	0.261 (0.066)
H5 June	62.2 (12.3)	51.6 (5.1)	1.290 (0.128)	0.051 (0.120)	37.8 (28.7)	4571.0 (1147.3)	2242.0 (1400.0)	2735.8 (220.9)	3.396 (1.033)	23 (1)	7.07 (2.66)	1.058 (0.599)	7.877 (0.889)	0.123 (0.048)
Aug	126.1 (19.1)	30.4 (6.0)	1.064 (0.176)	0.385 (0.071)	645.3 (250.6)	3889.8 (496.1)	919.8 (221.1)	3474.5 (287.6)	2.213 (0.460)	22 (0)	3.67 (1.89)	0.604 (0.291)	5.688 (4.256)	0.144 (0.068)
Nov	90.6 (18.3)	42.2 (5.6)	1.367 (0.103)	0.378 (0.098)	1602.0 (553.3)	3068.0 (355.7)	681.3 (103.5)	3242.3 (942.0)	1.401 (0.071)	25 (1)	6.26 (0.77)	1.129 (0.571)	4.532 (1.528)	0.072 (0.020)
Febr	60.3 (13.4)	52.6 (6.6)	1.399 (0.108)	0.130 (0.069)	837.5 (162.8)	2320.8 (773.5)	451.0 (191.1)	4438.3 (375.2)	2.095 (0.986)	23 (0)	6.90 (2.80)	0.909 (0.268)	9.680 (7.068)	0.133 (0.049)
									N = 2	N = 2				

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		Chlorophyll			Carotene						Pigment ratios								
		Chla* ($\mu g\ g^{-1}$)	c2 ($\mu g\ g^{-1}$)	Pheo* ($\mu g\ g^{-1}$)	fuc ($\mu g\ g^{-1}$)	diadino ($\mu g\ g^{-1}$)	diato ($\mu g\ g^{-1}$)	zea ($\mu g\ g^{-1}$)	lut ($\mu g\ g^{-1}$)	b-car ($\mu g\ g^{-1}$)	chla:TOM*	pheo:chla*	chlc:chla*	fuc:chla*	diato:chla*	diadino:chla*	zea:chla*	lut:chla*	bcar:chla*
H1	**June**	15.62 (4.93)	1.17 (0.26)	1.35 (0.55)	7.24 (2.53)	1.48 (0.53)	0.50 (0.07)	0.176 (0.096)	0.052 (0.030)	0.65 (0.19)	0.481 (0.127)	0.085 (0.012)	0.0784 (0.0178)	0.459 (0.020)	0.431 (0.115)	0.0939 (0.0053)	0.0106 (0.0028)	0.0039 (0.0028)	0.0417 (0.0017)
	Aug	5.72 (1.39)	0.50 (0.06)	0.31 (0.05)	1.72 (0.39)	0.47 (0.18)	0.10 (0.02)	0.017 (0.023)	0.000 (0.000)	0.17 (0.04)	0.384 (0.170)	0.056 (0.008)	0.0898 (0.0116)	0.302 (0.008)	0.609 (0.233)	0.0802 (0.0169)	0.0025 (0.0031)	0.0000 (0.0000)	0.0292 (0.0032)
	Nov	7.65 (0.81)	2.35 (2.81)	0.48 (0.16)	2.72 (0.19)	0.71 (0.09)	0.21 (0.07)	0.041 (0.015)	0.064 (0.028)	0.35 (0.06)	0.419 (0.070)	0.064 (0.026)	0.2847 (0.3083)	0.356 (0.014)	0.744 (0.062)	0.0925 (0.0027)	0.0055 (0.0024)	0.0087 (0.0043)	0.0472 (0.0123)
	Febr	13.82 (0.87)	1.04 (0.14)	0.29 (0.03)	3.20 (0.30)	1.23 (0.13)	0.11 (0.12)	0.007 (0.006)	0.001 (0.002)	0.31 (0.04)	1.167 (0.472)	0.021 (0.001)	0.0749 (0.0072)	0.232 (0.014)	0.537 (0.610)	0.0889 (0.0069)	0.0005 (0.0004)	0.0001 (0.0002)	0.0226 (0.0021)
H2	**June**	5.63 (1.08)	0.49 (0.05)	0.10 (0.04)	1.63 (0.27)	0.59 (0.07)	0.05 (0.01)	0.188 (0.041)	0.091 (0.181)	0.22 (0.04)	0.808 (0.255)	0.019 (0.006)	0.0910 (0.0261)	0.306 (0.116)	0.466 (0.056)	0.1092 (0.0341)	0.0350 (0.0140)	0.0133 (0.0265)	0.0407 (0.0155)
	Aug	9.98 (1.31)	0.78 (0.25)	0.23 (0.06)	2.11 (1.94)	0.91 (0.48)	0.09 (0.04)	0.054 (0.074)	0.027 (0.025)	0.27 (0.08)	1.554 (0.196)	0.023 (0.003)	0.0770 (0.0188)	0.225 (0.220)	0.793 (0.445)	0.0951 (0.0583)	0.0060 (0.0081)	0.0026 (0.0024)	0.0272 (0.0100)
	Nov	11.74 (2.10)	2.09 (0.39)	0.13 (0.07)	3.86 (0.64)	1.33 (0.19)	0.06 (0.02)	0.057 (0.029)	0.000 (0.000)	0.35 (0.06)	0.935 (0.396)	0.011 (0.004)	0.1781 (0.0134)	0.330 (0.007)	0.713 (0.409)	0.1139 (0.0042)	0.0046 (0.0019)	0.0000 (0.0000)	0.0295 (0.0006)
	Febr	7.87 (0.65)	0.71 (0.12)	0.17 (0.00)	2.13 (0.27)	0.86 (0.09)	0.06 (0.00)	0.021 (0.002)	0.000 (0.000)	0.25 (0.02)	1.701 (0.105)	0.021 (0.002)	0.0903 (0.0129)	0.269 (0.016)	0.646 (0.048)	0.1088 (0.0044)	0.0027 (0.0005)	0.0000 (0.0000)	0.0313 (0.0012)
H3	**June**	4.18 (2.12)	0.22 (0.10)	0.70 (0.69)	1.11 (0.64)	0.28 (0.17)	0.21 (0.10)	0.145 (0.076)	0.355 (0.224)	0.29 (0.13)	0.348 (0.248)	0.165 (0.100)	0.0603 (0.0215)	0.256 (0.044)	0.511 (0.298)	0.0644 (0.0166)	0.0465 (0.0349)	0.1116 (0.0825)	0.0840 (0.0487)
	Aug	7.26 (2.73)	0.43 (0.22)	1.03 (0.08)	1.83 (0.69)	0.33 (0.07)	0.20 (0.05)	0.125 (0.125)	0.512 (0.420)	0.42 (0.10)	0.490 (0.117)	0.152 (0.037)	0.0571 (0.0085)	0.252 (0.007)	0.310 (0.089)	0.0495 (0.0170)	0.0155 (0.0156)	0.0873 (0.0784)	0.0631 (0.0257)
	Nov	11.42 (1.93)	1.54 (0.75)	1.76 (0.99)	3.61 (0.78)	0.97 (0.19)	0.31 (0.17)	0.143 (0.070)	0.592 (0.414)	0.65 (0.27)	0.542 (0.126)	0.146 (0.076)	0.1294 (0.0507)	0.315 (0.028)	0.320 (0.118)	0.0853 (0.0063)	0.0119 (0.0048)	0.0489 (0.0319)	0.0558 (0.0183)
	Febr	13.15 (2.19)	0.82 (0.16)	0.57 (0.25)	3.44 (0.60)	1.41 (0.28)	0.10 (0.02)	0.050 (0.012)	0.090 (0.034)	0.43 (0.08)	0.985 (0.642)	0.043 (0.015)	0.0626 (0.0105)	0.262 (0.013)	0.262 (0.074)	0.1065 (0.0058)	0.0038 (0.0004)	0.0068 (0.0019)	0.0325 (0.0014)
H4	**June**	13.94 (1.65)	0.61 (0.21)	3.49 (0.27)	3.53 (0.74)	0.82 (0.20)	0.79 (0.24)	0.576 (0.104)	0.335 (0.128)	0.68 (0.22)	0.269 (0.085)	0.252 (0.018)	0.0427 (0.0110)	0.254 (0.047)	0.250 (0.060)	0.0590 (0.0124)	0.0418 (0.0091)	0.0238 (0.0073)	0.0492 (0.0152)
	Aug	16.71 (5.65)	1.01 (0.27)	2.87 (1.52)	4.49 (2.66)	1.01 (0.42)	0.32 (0.18)	0.243 (0.133)	0.186 (0.062)	0.60 (0.25)	0.476 (0.129)	0.165 (0.046)	0.0617 (0.0079)	0.258 (0.100)	0.163 (0.035)	0.0618 (0.0203)	0.0137 (0.0042)	0.0122 (0.0067)	0.0350 (0.0039)
	Nov	22.51 (6.15)	2.39 (0.79)	4.08 (0.56)	7.49 (2.08)	2.14 (0.80)	0.72 (0.15)	0.400 (0.018)	0.368 (0.094)	1.02 (0.20)	0.505 (0.247)	0.188 (0.039)	0.1056 (0.0109)	0.333 (0.010)	0.232 (0.029)	0.0931 (0.0102)	0.0186 (0.0041)	0.0171 (0.0056)	0.0459 (0.0039)
	Febr	14.87 (2.61)	0.79 (0.08)	3.00 (0.94)	3.92 (0.64)	1.36 (0.12)	0.58 (0.20)	0.245 (0.044)	0.343 (0.119)	0.88 (0.23)	0.480 (0.093)	0.199 (0.042)	0.0545 (0.0102)	0.264 (0.021)	0.273 (0.055)	0.0926 (0.0102)	0.0166 (0.0025)	0.0226 (0.0053)	0.0586 (0.0076)
H5	**June**	20.44 (5.30)	0.78 (0.23)	5.97 (1.88)	5.43 (2.98)	1.21 (0.73)	0.94 (0.50)	1.021 (0.682)	1.066 (0.635)	1.20 (0.36)	0.309 (0.085)	0.291 (0.051)	0.0385 (0.0062)	0.247 (0.100)	0.182 (0.066)	0.0541 (0.0253)	0.0459 (0.0246)	0.0501 (0.0297)	0.0586 (0.0117)
	Aug	16.36 (12.84)	0.71 (0.32)	5.33 (4.77)	3.18 (1.00)	0.27 (0.19)	0.25 (0.28)	0.740 (0.630)	0.614 (0.640)	1.17 (1.23)	0.410 (0.097)	0.308 (0.041)	0.0504 (0.0122)	0.240 (0.085)	0.107 (0.107)	0.0280 (0.0203)	0.0440 (0.0234)	0.0327 (0.0147)	0.0625 (0.0233)
	Nov	11.45 (3.09)	1.41 (0.65)	3.29 (0.75)	4.40 (1.29)	0.94 (0.43)	0.45 (0.14)	0.179 (0.026)	0.463 (0.063)	0.63 (0.10)	0.189 (0.072)	0.291 (0.023)	0.1191 (0.0316)	0.383 (0.035)	0.201 (0.115)	0.0789 (0.0181)	0.0161 (0.0026)	0.0418 (0.0078)	0.0565 (0.0085)
	Febr	12.85 (2.23)	0.75 (0.11)	3.26 (0.99)	3.68 (0.44)	1.10 (0.11)	0.31 (0.09)	0.137 (0.043)	0.383 (0.186)	0.57 (0.15)	0.198 (0.042)	0.249 (0.043)	0.0583 (0.0014)	0.289 (0.019)	0.129 (0.013)	0.0865 (0.0087)	0.0105 (0.0019)	0.0286 (0.0097)	0.0436 (0.0045)

RESULTS

Environmental variables

Mutually redundant variables were (1) mud fraction and median grain size (negatively correlated) and (2) total nutrients, inorganic N and NH_4^+. From the intercorrelated variables, only median grain size and NH_4^+ were retained in the environmental data matrix.

The five stations clearly differed in sediment characteristics. In the PCA, where the first two axes explained 52.0 % of the total variation, samples grouped primarily according to location rather than time (Fig. 2). Nevertheless, both spatial and temporal differences were significant (PERMANOVA $p < 0.001$ for St, Mo and St x Mo; PERMDISP St x Mo: $p = 0.015$; Addendum II Table S2). Stations were consistently differentiated throughout the year (pairwise tests $p < 0.05$; addendum II Table S2), with few exceptions ($p > 0.05$ for H2-H3 and H4-H5 in June and H1-H3 in February; addendum II Table S2). Station differentiation was mainly located along PC1 which explained 39.1 % of the variation, with H4-H5 positioned on the negative side, H1-H3 around zero and H2 on the positive side of the axis. Ten variables correlated strongly with PC1 with $r_s > 0.75$ (Fig. 2). The prime three variables (highest correlation coefficients) contributing to station differentiation related to general OM availability (TOM, $r_s = - 0.88$) and to the amounts and proportions of photoautotrophic matter i.e. pheo ($r_s = - 0.90$), and pheo:chla ($r_s = - 0.94$); all were higher in stations H4 and H5. TOM ranged from 3 - 7 % at H5 to < 1 % at H2 (Table 3), and the pheo:chla ratio was five to ten times higher in the marsh stations H4 and H5 compared to tidal flat stations H2 and H1. The contributions of fresh photoautotroph-derived organic matter to the total organic matter pool (chla:TOM, $r_s = 0.76$) were consistently highest for H2 (≥ 0.81) and lowest for H4 (≤ 0.48) and H5 (≤ 0.41) (Fig. 2, Table 3). Nevertheless, stations H4 and H5 harboured higher concentrations of chlorophyll *a* (chla, Table 3). For lipids and proteins ($r_s = -0.78$ and $r_s = -0.85$), highest concentrations were measured in muddy marsh stations H4 and H5 (LIP = 0.60 – 1.13 mg g^{-1}, PRT = 3.22 – 9.68 mg g^{-1}) than in tidal flat stations and the sandy salt marsh station (H1-H2-H3: LIP = 0.08 – 0.36 mg g^{-1}, PRT = 0.25 – 3.31 mg g^{-1}), except for high values at station H1 in June (LIP = 0.94 mg g^{-1}; PRT = 3.76 mg g^{-1}).

There was no evidence of pronounced variability in sedimentary bacterial abundance among stations, but a temporal variability was apparent. Cell abundances were lower during November-February, ranging from 0.85×10^8 to 2.10×10^8 compared to 2.09×10^8 to 3.68×10^8 in June-August (Table 3). Bacterial diversity, in terms of number of phylotypes, did not show any spatial or temporal differences (Table 3).

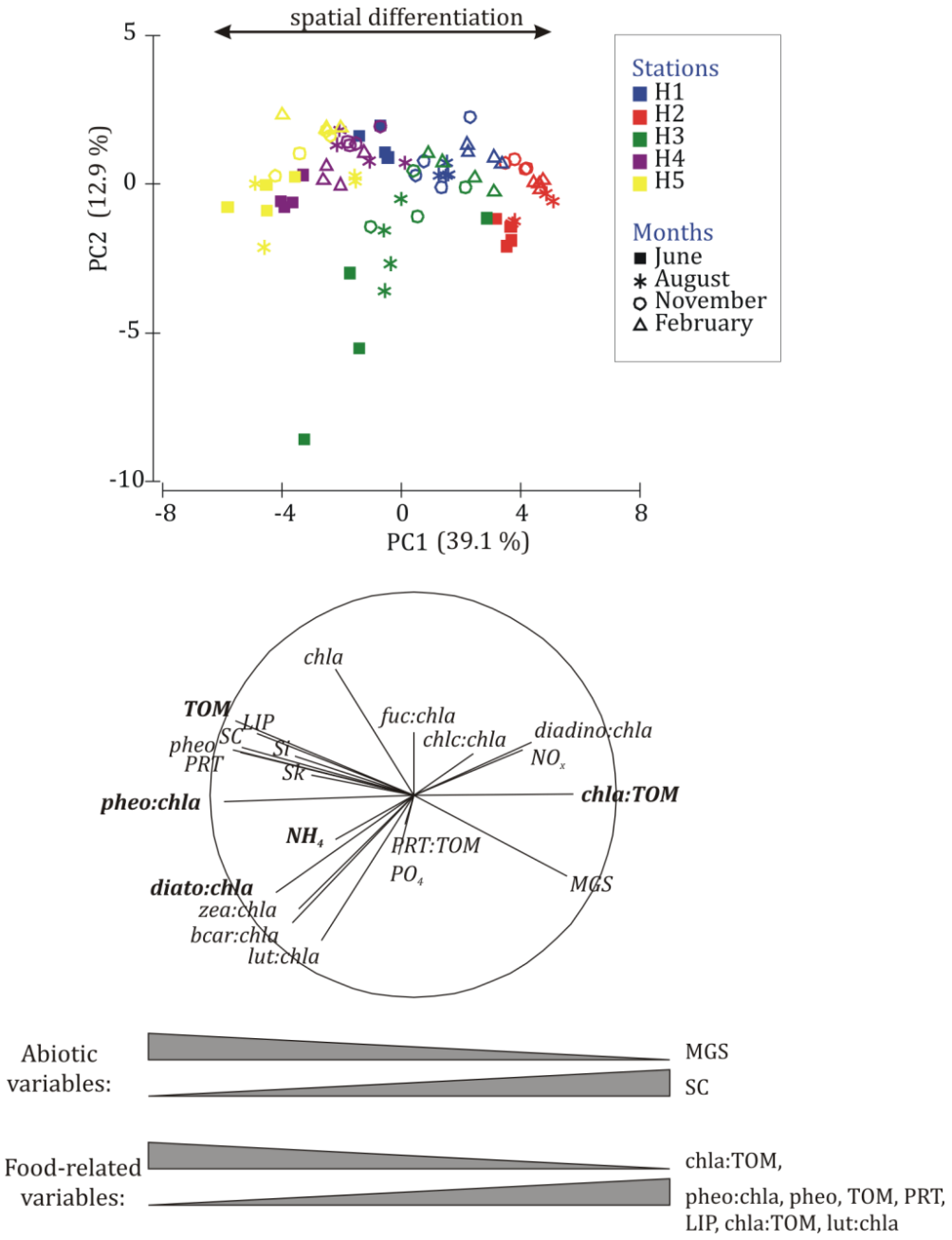

Fig 2. Principle component analysis (PCA) of normalized, log-transformed environmental variables from stations H1 to H5 over four sampling occasions (June, August, November, February). Underneath the PCA, eigenvectors of variables are presented, followed by the presentation of those variables with highest correlations to PC1 axis (r_s > |0.75|). The five vectors in bold are the BEST explanatory variables of spatial harpacticoid assemblage differentiation. Abbreviations: MGS: median grain size; SC: sorting coefficient, Sk: skewness; LIP: lipids; PRT: proteins; TOM: total organic matter.

Abiotic variables contributing to station differentiation were median grain size (r_s = 0.79) and the sorting coefficient (r_s = - 0.83). Median grain size ranged from silt in station H4 to fine sand in station H2 (from 44 to 221 µm, respectively) with corresponding mud fractions of 66 % in H4 and 0 % in H2 (Table 3). Silty sediments were moderately (0.64 ≤ SC ≤ 0.94, for H4) to poorly sorted (1.06 ≤ SC ≤ 1.40, for H5), while coarser sediment was very well sorted (SC ≤ 0.33, for H2). Furthermore, from the nutrients only NO_x^- and Si contributed to station differentiation (r_s = 0.54 and r_s = - 0.58, respectively).

In general, with the exception of bacterial abundance, temporal fluctuations in environmental variables in the Paulina area were limited relative to spatial heterogeneity, and were more located along axis PC2.

Only station H3 showed a large temporal variability. The variable most strongly correlated to PC2 was chla (r_s = - 0.51). However, chla concentrations showed temporal variability in all stations but not according to a uniform pattern (Fig. 3).

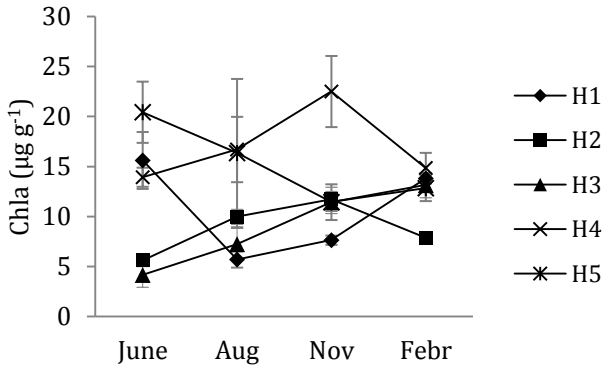

Fig.3. Temporal fluctuations in chl *a* concentrations (mean ± SE, N = 4) in Paulina stations (H1 to H5), from June to February.

Fucoxanthin, primarily indicative of diatoms, was consistently the main pigment in terms of absolute concentrations as well as proportions (Fig. 4 a, b), followed by chlc and diadinoxanthin, both with elevated concentrations in November (Fig. 5). The pigments with overall lower concentrations and ratios were zeaxanthin, lutein and diatoxanthin (which were nearly absent from stations H1 and H2) (fig. 4, 5). Concentrations of all pigments were higher in H4 and H5 compared to H2 and H3 (Fig. 4). At stations H1 and H5, fluctuations in fucoxanthin concentrations correlated significantly with fluctuations in chlc and diatoxanthin (Fig. 5; for H1: r_s = 0.70 and: r_s = 0.55; for H5: r_s = 0.79 and 0.54), which is consistent with their prominent presence in diatoms. However, in other stations, fucoxanthin and diatoxanthin concentrations did not correlate.

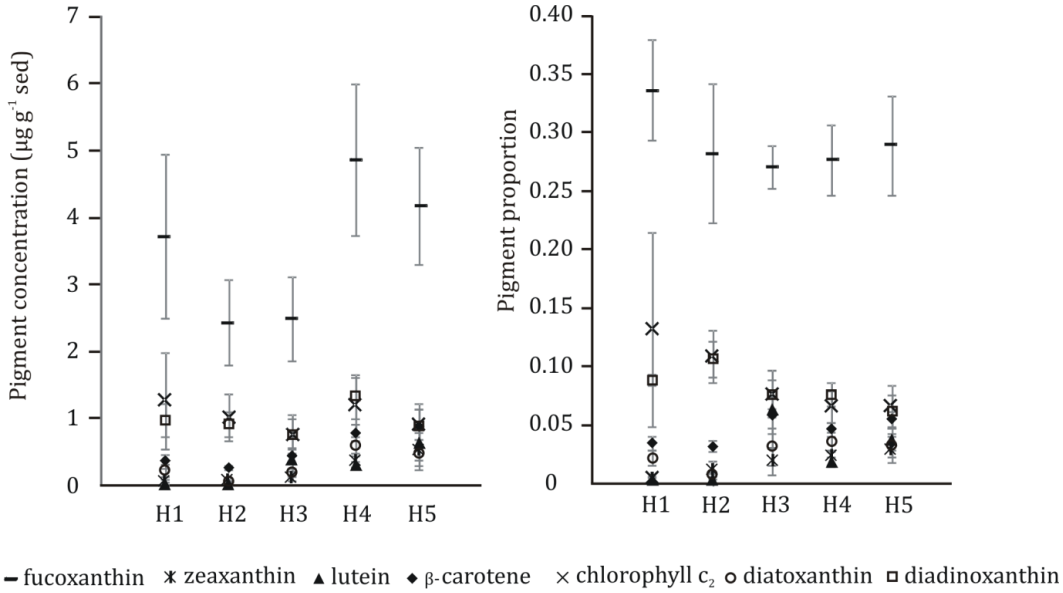

Fig. 4. Carotenoid pigments in each station (means over months ± SD, N = 4): (a) as absolute pigment concentrations and (b) as proportional (pigment concentration divided by chla concentration). Absolute concentrations are a proxy for taxon density of photoautotrophs, pigment proportions are a proxy for relative taxon abundance of photoautotrophs.

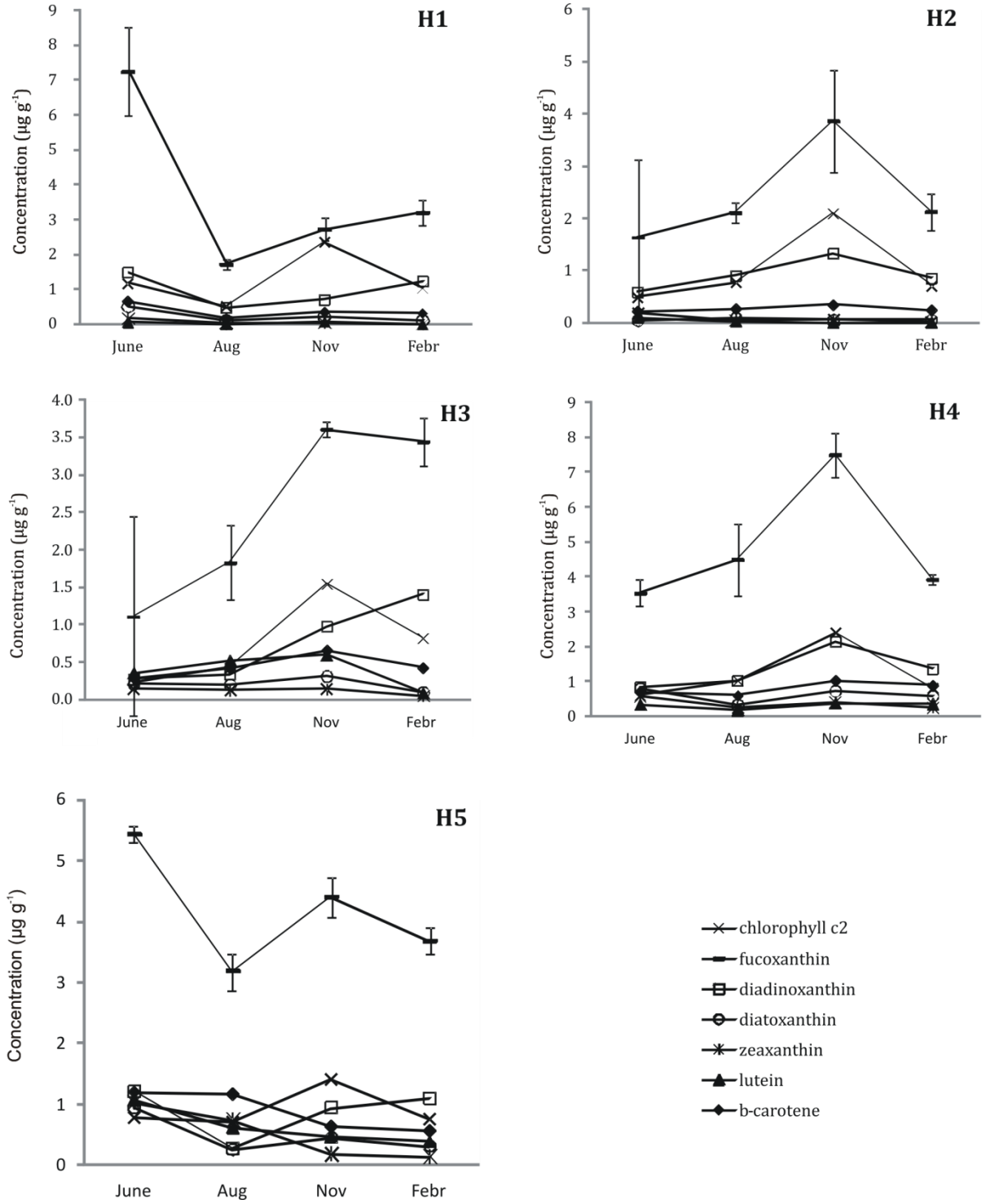

Fig. 5. Temporal fluctuations in carotenoid pigments per each station. Mean ± SE (N = 4). Only SE of fucoxanthin are presented. SE of other pigments are too small to present.

Harpacticoid copepods

Harpacticoid abundance

Total copepod densities in the top 3 cm ranged from 0 to more than 700 ind. 10 cm^{-2} (Addendum II Table S3). Densities varied among stations but not among seasons (PERMANOVA, St: $p<0.01$, Mo: ns, St x Mo: ns; addendum II Table S3). Harpacticoid densities were 171 ± 222, 230 ± 194, 161 ± 57 ind. 10 cm^{-2} (average ± SD, N = 12) in H3, H4 and H5, respectively, and were significantly higher in those three stations than at H1 (24 ± 21 ind. 10 cm^{-2}, p ranging from <0.05 to <0.001; addendum II Table S3) and H2 (68 ± 60 ind. 10 cm^{-2}, $p<0.05$; addendum II Table S3). With the exception of H5, copepod abundances were highly variable among replicates.. Copepods were largely confined to the upper 1 cm. The proportion of copepods in the 1 – 3 cm slice ranged from 1 - 3 % for stations H1, H3, H4 to 11 - 12 % for stations H2 and H5. Community analyses are based on adult specimens only and on the top-1 cm of sediment.

Harpacticoid assemblage structure and diversity

Identified adults over all stations and seasons comprised a total of 20 species from 16 genera and belonging to 8 harpacticoid families (addendum II, Table S1). An overview of absolute and relative species abundances is provided in Addendum II Table 5 and 6. The PCO plot, the first two axes of which explained 64.4 % of the variation, indicated strong spatial differentiation in harpacticoid assemblage structure (PERMANOVA: St, Mo, St x Mo, all $p < 0.001$, PERMDISP St x Mo: $p = 0.03$, addendum Table S4). This is in agreement with the observed spatial differences in environmental characteristics (Fig. 5, Fig. 6). Tidal flat stations H1 and H2, positioned at the positive side of PCO1, grouped separately, from each other (pairwise, $p < 0.05$; addendum II Table S4) and from salt marsh stations H3, H4 and H5 (pairwise, $p < 0.05$; addendum II Table S4). Harpacticoid assemblages of H3, H4 and H5 were similar to each other throughout the year ($p > 0.05$), except in November, when H3 separated from H4 and H5 (pairwise, both $p < 0.05$; addendum Table S4) (Fig. 6). Overall, the five stations comprised three different harpacticoid assemblages, i.e. the assemblage of H1, of H2 and of H3-4-5 (Fig. 6, encircled).

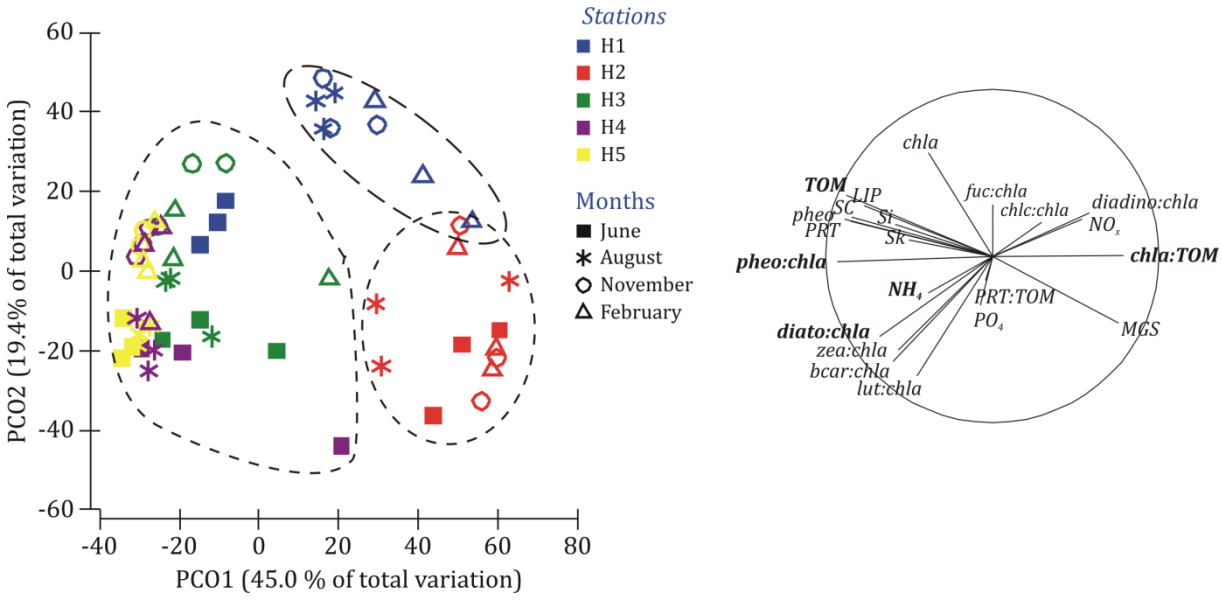

Fig. 6. Principle coordinates analysis (PCO) of harpacticoid species assemblages based on relative, square-root transformed species abundances from all stations over four sampling occasions. Environmental vectors from fig. 2 are repeatedly shown here for the ease of comparison between harpacitcoid data and environmental data.

Temporal variability was less obvious than spatial variability, but still significant (PERMANOVA, see above) (Fig. 6), again much like for the environmental variables. In salt marsh stations (H3, H4, H5), major changes in assemblage composition occurred between August and November (A-N, $p_{MC} < 0.05$; addendum II Table S4); November was the only moment where the copepod assemblage of H3 significantly differed from those of H4 and H5 (pairwise, $p_{MC} < 0.05$; addendum II Table S4). The post-hoc test revealed that the harpacticoid assemblage composition of H2 did not vary during the year (all $p_{MC} > 0.05$). However, the two most abundant and characteristic species, *Paraleptastacus spinic*auda and *Asellopsis intermedia*, showed clear variation in abundance (fig. 7). The low-tidal mud flat assemblage (station H1) showed major seasonal changes in composition between June and August and between November and February (pairwise, both $p_{MC} < 0.05$; addendum II Table S4). These patterns were equally observed using copepod family data as when using species data.

Hill's diversity indices also demonstrate structural differences in copepod communities in terms of richness and diversity (N_0, N_1) as well as evenness and dominance (N_2, N_{inf}). N_0 and N_1 fluctuated both spatially and temporally (for both N_0 and N_1, PERMANOVA St x Mo: $p < 0.05$; addendum II table S7). Tidal flat stations were characterized by lower copepod richness than salt marsh stations (pairwise, most $p < 0.05$; Addendum II Table S5) (Table 4). Temporal variability in species richness was present at H1 and H5 (pairwise, most $p < 0.05$; Addendum II Table S7). In H1, number of species was highest in June and lowest in February. In site 5, number of species was slightly higher in August and November. Evenness/dominance did not vary over time but differed between locations (for both N_2 and N_{inf}, PERMANOVA St: $p < 0.001$, Mo: ns, St x Mo: ns; Addendum II Table S7): the sandy sediment harpacticoid assemblage (H2) showed low evenness and high dominance compared to all other stations (pair wise, all $p < 0.05$; Addendum II Table S7).

Table 4. Copepod assemblage structure of the top cm (0-1 cm) presented by univariate Hill's diversity measures, averaged over time (N=12, standard deviations between brackets)

	N_0	N_1	N_2	N_{inf}
H1	5.08 (2.71)	3.91 (1.78)	3.41 (1.50)	2.50 (1.03)
H2	3.08 (1.38)	1.92 (1.05)	1.64 (0.86)	1.36 (0.45)
H3	8.27 (2.69)	4.33 (2.05)	3.38 (1.89)	2.33 (1.20)
H4	8.67 (2.23)	4.43 (0.96)	3.34 (1.00)	2.29 (0.70)
H5	8.83 (1.40)	5.30 (1.21)	4.16 (1.25)	2.70 (0.88)

Copepod species contributing to differentiation among stations, as indicated by their correlation to PCO1 (Fig. 6; threshold $r_s > |0.70|$) were *Microarthridion littorale* ($r_s = -0.86$), *Nannopus palustris* ($r_s = -80.0$) and *Enhydrosoma sp.* ($r_s = -0.78$) which were most abundant in stations H4 and H5 (and few individuals in H3), and *Paraleptastacus spinicauda* ($r_s = 0.72$) which was unique for H2. The species with the strongest correlation to PCO2 was *Paronychocamptus nanus* ($r_s = 0.68$), mainly present in H3, H4, and H5 and occurring in higher densities in November-February.

The copepod community of station H1 was characterized by the general presence of Laophontidae, Ectinosomatidae and Miraciidae, albeit with temporally variable abundances. Some families significantly contributed to the assemblage during one season only (Tachidiidae, Harpacticidae, Leptastacidae). The most characteristic species of station H1 was *Asellopsis intermedia* (SIMPER, 42 % contribution to similarities within station H1; see Fig. 7). However, similar abundances of this species were also found in

station H2 (Fig. 8). Station H2 was dominated by Leptastacidae, in particular *Paraleptastacus spinicauda* (SIMPER, 82.4 %; Fig. 8), and by considerable abundances of Laophontidae (species *A. intermedia*, Fig. 8).

All salt marsh stations H3, H4 and H5 primarily hosted the same four main families, i.e. Tachidiidae, Laophontidae, Cletodidae and Miraciidae. Their distribution varied among the stations and even within each station. In station H3, Laophontidae and in particular *Paronychocamptus nanus* dominated the assemblage during the colder period (November and February, Fig. 7, 8). In addition to *P. nanus*, *M. littorale* (SIMPER 17.0%) strongly contributed to the copepod community of station H3 (Fig. 7). In station H4, Tachidiidae were dominant during that period, and Cletodidae and Laophontidae in the other months. The most important species were *M. littorale* (SIMPER, 23.3 %) and *Platychelipus littoralis* (SIMPER, 20.0 %), although both were also abundant in station H5 (Fig. 8). Family composition and dominance of station H5 were largely similar to station H4. Furthermore, station H5 showed a fairly constant presence of Huntemanniidae. The most characteristic species here is also *Microarthridion littorale* (SIMPER, 29.7 %) followed by *Nannopus palustris* (SIMPER, 13.2 %) and *Enhydrosoma sp*.

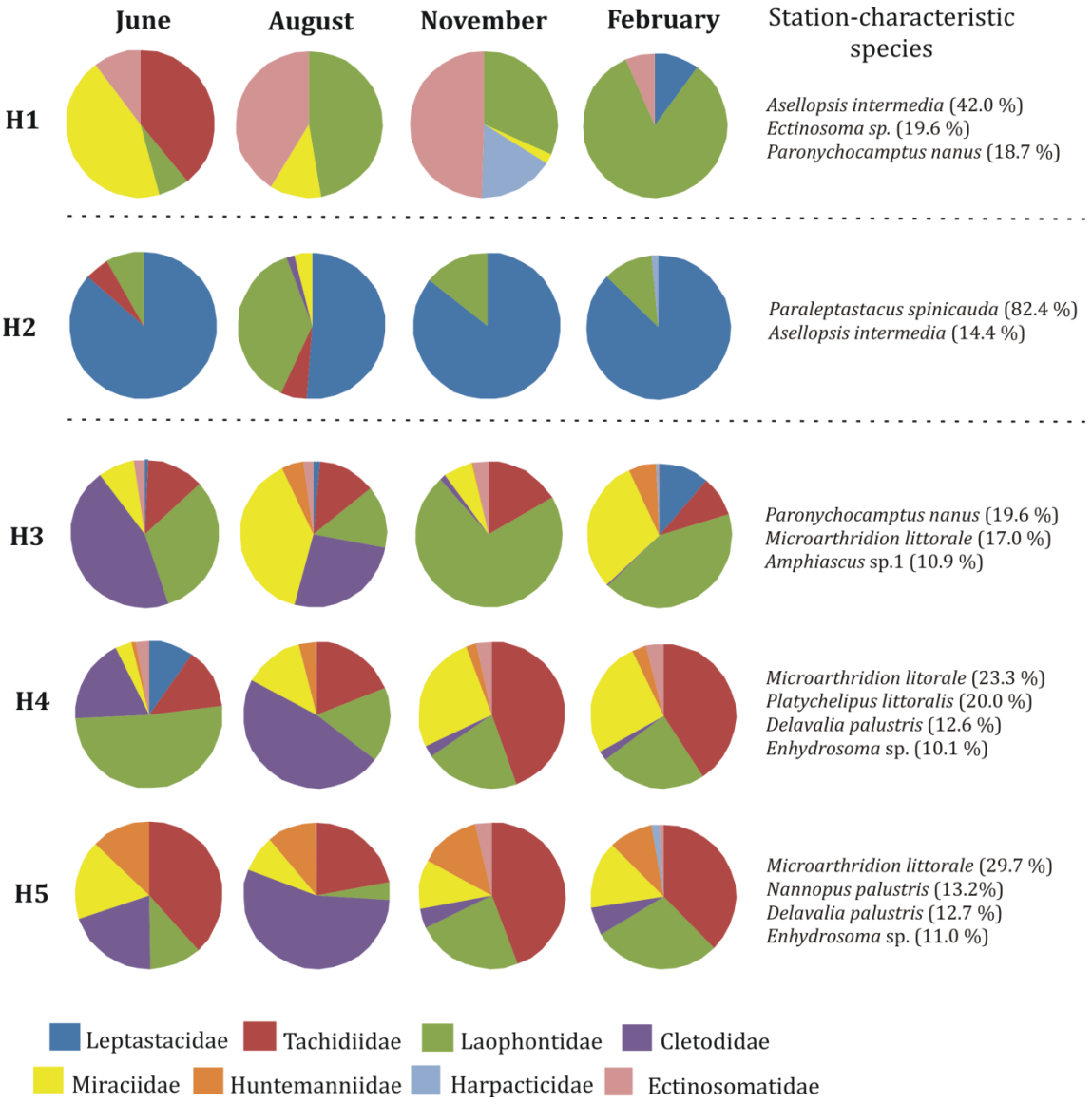

Fig. 7.. Spatio-temporal variability in harpacticoid communities based on relative family abundances, and an overview of the species indicated by PCO as characteristic for a particular station. Species contributions to 'station similarity', presented between brackets, were obtained by SIMPER analysis using a threshold contribution value of 10 %.

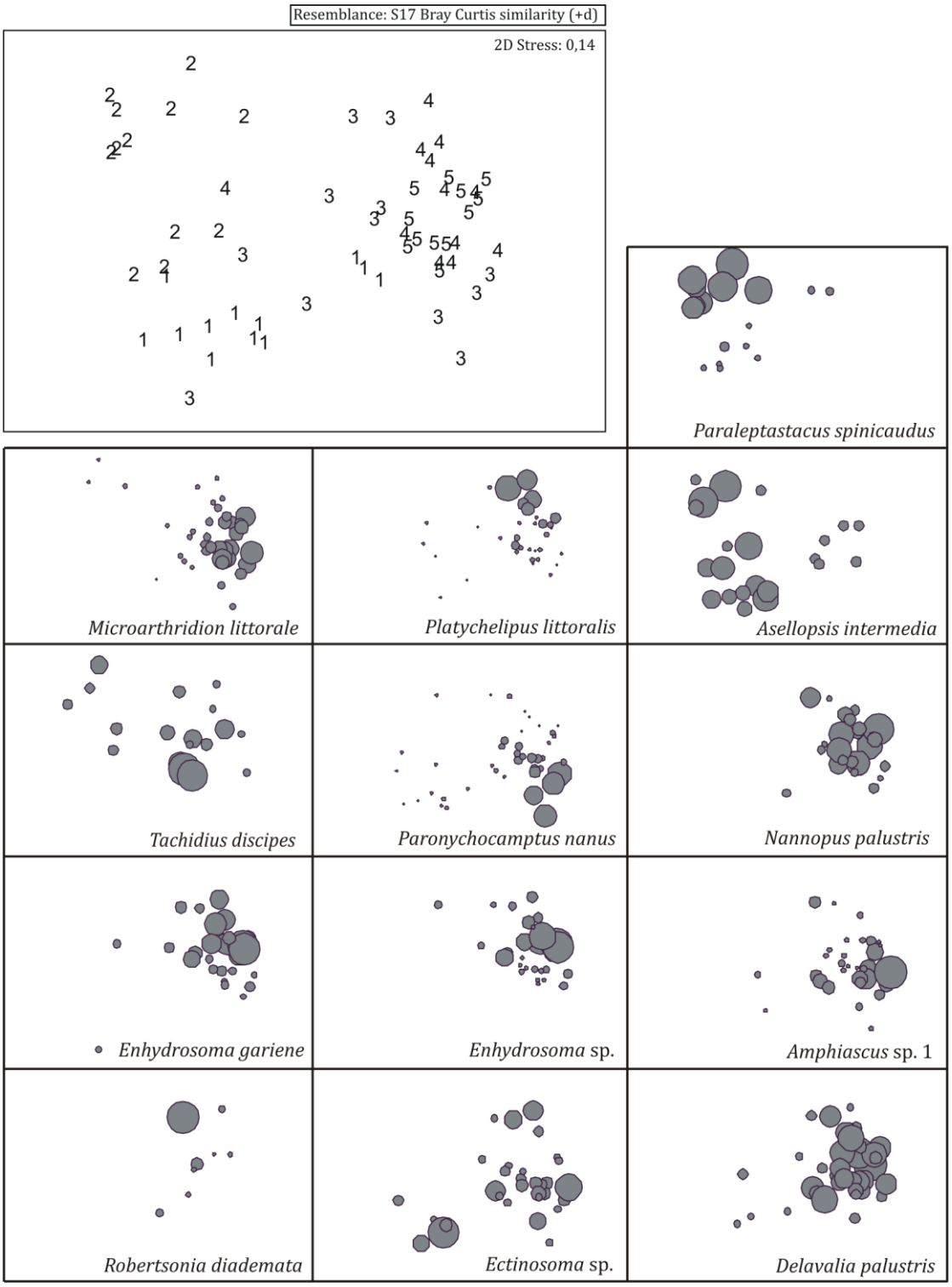

Fig. 8. nMDS presenting differences between harpacticoid communities from stations H1 to H5 (numbers 1 to 5) based on absolute species abundances, and species distributions along the stations.

Environmental variables explaining copepod assemblage differentiation

Since ordination of environmental variables (PCA) and copepod communities (PCO) showed similarities, it was expected that some of the measured environmental variables would contribute to the spatio-temporal variation in copepod assemblage composition. The subset of best explanatory variables, as determined by BV-STEP on the total dataset, was a combination of the following 6 variables (rho = 0.617, significance level < 0.1 %): TOM, NH_4^+, chla:TOM, pheo:chla, diato:chla and PRT. Four of these variables (TOM, pheo:chl a, diato:chla, PRT) were also important for station differentiation by ordination (PCA; Fig. 2).

The subset of environmental variables best explaining temporal shifts in copepod assemblage structure was determined by BV-STEP analysis per station to exclude the dominant spatial effect. Only for stations H1 and H5, a significant match between the copepod and environmental variables matrices was found (p < 1%, Table 4). The change in harpacticoid assemblages in these two stations related to changes in pigment ratios of fucoxanthin, diadinoxanthin and diatoxanthin (Table 4). In the other stations, except H2, carotenoid pigment ratios were also denoted as best explanatory variables, for instance zeaxanthin in station H4 (Table 4). More specifically, in station H1, the reduced copepod species richness in August concurred with reduced ratios of diatoxanthin and fucoxanthin to chl a (diato:chla and fuc:chla, fig. 9). Thus, the pigment diatoxanthin contributed to both spatial and temporal variation,

Table 5. Subsets of best explanatory variables for temporal fluctuation in harpacticoid community composition, with highest correlation coefficient (rho) and lowest number of variables (# var). Note that only for stations H1 and H5 the p values are low (< 1%).

	rho	p value (%)	# var	BEST var
H1	0.763	**0.1**	3	fuc:chla, diadino:chla, diato:chla
H2	0.452	52.0	2	MGS, LIP
H3	0.530	14.7	2	diadino:chla, diato:chla
H4	0.428	21.5	1	zea:chla
H5	0.618	**0.6**	2	diadino:chla, diato:chla

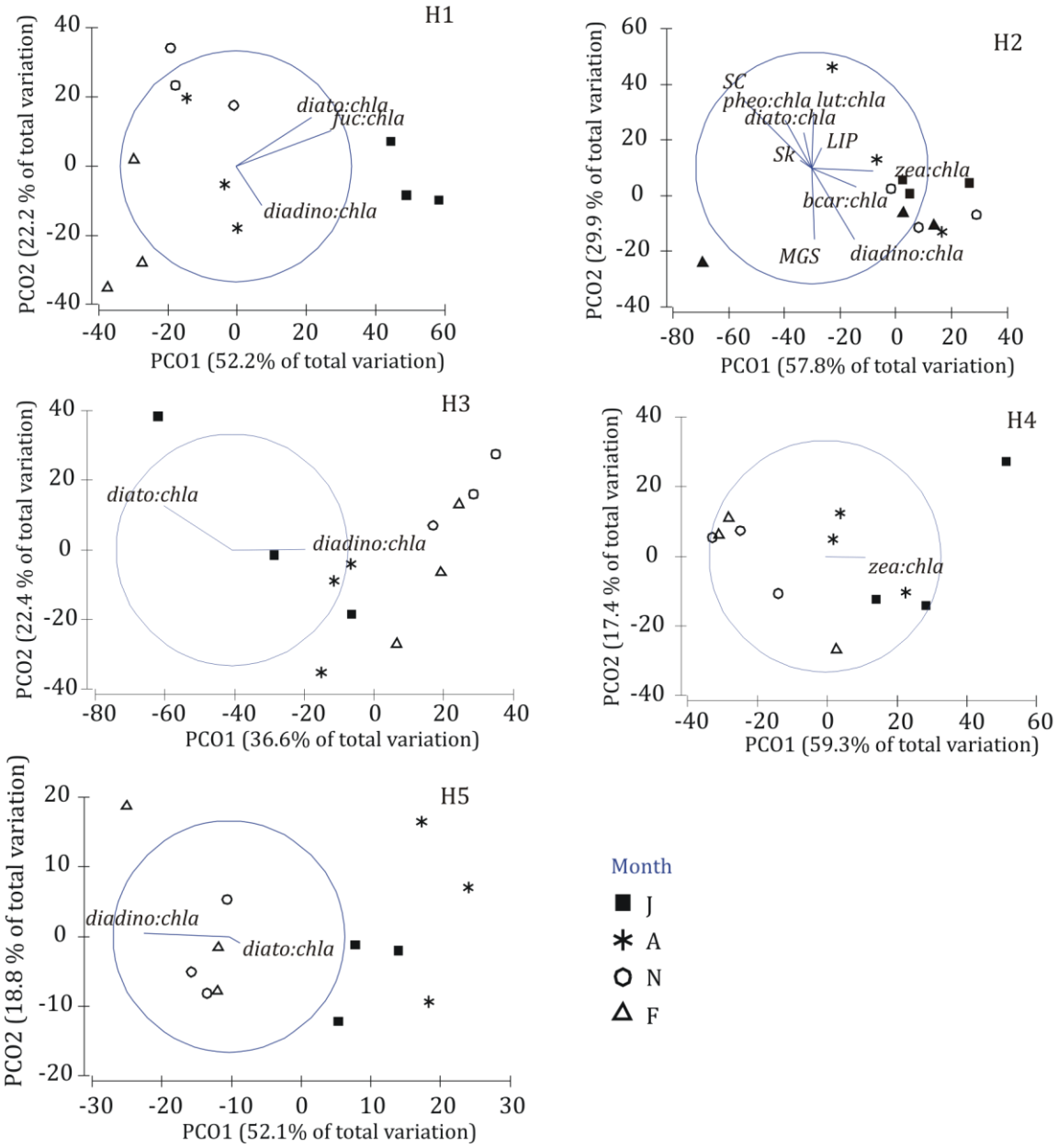

Fig. 9. Temporal variation in harpacticoid assemblages (PCO per station) and the best explanatory environmental variables (see Table 4). For station H2, other variables have been added which originate from other BEST subsets of variables though with equally high explanatory value (correlation coefficient rho). J, A, N, F (June, August, November, February)

Univariate correlation analyses of total copepod abundances with individual environmental variables (Table 6) also pointed at a substantial link with phytopigments. Total copepod abundances of stations H1, H2 and H3 related to fluctuations in pigments and pigment ratios. At the low-tide station H1, copepod abundances also correlated with median grain size and TOM. In contrast, at H4 and H5, copepod abundances did not significantly correlate to any of the environmental variables.

Species occurring in similar habitats, i.e. in salt marsh stations (H3, 4, 5) or tidal flat stations (H1 and H2), generally correlated in the same direction (positive or negative) to variables. Salt marsh species (e.g. *Microarthridion littorale*, *Platychelipus littoralis, Nannopus palustris, Enhydrosoma* sp. 1) correlated relatively strongly and positively with pheo:chla as well as with other pigment ratios. Species of tidal flat

stations and H3 generally showed negative correlations except with chla:TOM, indicative of food quality (fresh photoautotrophic production) and, for H2, also with median grain size.

Furthermore, within each station, the abundances of some species were mutually correlated (data not presented). At station H1, *Amphiascus* sp and *Tachidius discipes* abundances were highly correlated (r_s = 0.90) and additionally, a high abundance of these species alternated with a high abundance of *Asellopsis intermedia* (r_s = -0.72, r_s = -0.76). Also in station H2, *Asellopsis intermedia* abudances were inversely correlated to *Paraleptastacus spinicaudus* (r_s = -0.89). In H4, *Platychelipus littoralis* abundances were at their lowest when both *Amphiascus* sp 1 and *Microarthridion littorale* were at their highest (A-M: r_s = 0.81, P-A: r_s = -0.70, P-M: r_s = -0.67). This is a similar pattern to H1, where there was also a close coupling between a Tachidiidae species and Miraciidae species and a inverse relation of both with a Laophonthidae species. Finally, at H5, a significant correlation was found between *Paronychocamptus nanus* and *Enhydrosoma s*p (r_s = -0.73) and between *Delavalia palustris* and *Nannopus palustris* (r_s = 0.66).

Table 6. Significant spearman rank correlations between total harpacticoid abundances or relative species abundance and each environmental variable. The table includes only species which were highly specific for each habitat, as determined by SIMPER (see Fig. 7) or which were among the three most abundant in the habitat. Correlations between species and environmental variables were made on the largest spatial scale i.e. over all sampled stations where it occurred as well as on the scale of individual habitats. 'All' = between habitats (analysis of complete species dataset), 'H..' = within habitat fluctuation (analysis per station) and relation with environmental variables. Highest significant correlations (r_s > 70 %) are marked grey.

	Copepod abundance						*Paralaptastacus spinicauda*		*Asellopsis intermedia*				*Paronychocamptus nanus*		*Platychelipus littoralis*				*Ectinosoma sp.*	
	all	H1	H2	H3	H4	H5	all	H2	all	H1H2	H1	H2	all	H3	all	H3H4	H3	H4	all	H1
MGS	-0.30	-0.66					0.65		0.32		0.88				-0.49				-0.29	
mud	0.36	0.65					-0.70		-0.43		-0.85				0.56				0.31	
SC		0.61																		
Sk		0.60																		
NOx				0.75			0.26	0.56		-0.55				0.60	-0.36					
NH4									-0.42		-0.64			-0.66	0.45					
PO4							0.35		-0.27	-0.52	-0.61		-0.39						-0.35	
Si							-0.54		-0.49				0.28		0.44					
inorg. N	0.27								-0.42		-0.63				0.36					
nutrient	0.27								-0.47						0.42		0.61			
TOM	0.41	0.65					-0.65		-0.59		-0.93				0.59					
chla	0.30		-0.66				-0.30		-0.34					0.66	0.46					
pheo	0.50	0.62	-0.59				-0.67	-0.73	-0.59						0.64					
chlc		0.65												0.68						
fuc	0.32	0.58					-0.33		-0.38				0.31	0.66	0.27					
diadino													0.27	0.69						
diato	0.40	0.87	-0.64				-0.61	-0.68	-0.53		-0.80				0.52				0.41	
zea	0.57	0.79	0.62				-0.33		-0.68	-0.63	-0.68				0.45					
lut	0.53	0.82					-0.53		-0.71		-0.64				0.56					
bcar	0.49	0.65					-0.50		-0.58						0.52					
CPE	0.40		-0.66				-0.38		-0.45					0.69	0.52					
CPE:TOM							0.58	-0.56	0.40						-0.37					
chla:TOM							0.60	-0.56	0.48					0.59	-0.44					
pheo:chl a	0.47	0.63					-0.67		-0.62		-0.78				0.60				0.55	
chlc:chla									0.40						-0.55	-0.47	-0.59			0.62
fuc:chla	0.46	0.81		0.83							-0.88		0.31	0.63					0.32	
zea:chla	0.46	0.73	0.67						-0.61	-0.58	-0.68				0.34					
lut:chla		0.76					-0.50		-0.69						0.46					
diadino:chla							0.38		0.26	-0.42					-0.34					
diato:chla	0.27	0.74					-0.58		-0.44		-0.78				0.39				0.47	
bcar:chla	0.40	0.69	0.69				-0.46		-0.54	-0.53	-0.76				0.30					
LIP	0.38						-0.57		-0.55		-0.59				0.47					
PRT	0.29						-0.66	-0.60	-0.46						0.50					
PRT/TOM										0.54	0.72									
bact-abund									-0.34						0.38					
bact-div.															0.46	0.57			0.40	
# of samples	40	40	40	40	40	40 40	40	8	40	16	8	8	40	8	40	16	8	8	40	8

	Delavalia palustris					*Amphiascus* sp. 1					*Microarthridion littorale*					*Tachidius discipes*		*Nannopus palustris*		*Enhydrosoma sp.*			
	all	H4H5	H4	H5		all	H1H3H4	H1	H3	H4	all	H3H4H5	H3	H4	H5	all	H1	all	H5	all	H4H5	H4	H5
MGS	-0.52		0.72			-0.36		-0.64			-0.56	-0.38					-0.76	-0.41		-0.42	0.45		
mud	0.53		-0.58			0.43		0.64			0.61	0.38					0.76	0.47		0.47	-0.43		-0.59
SC								0.68									0.73				-0.41	-0.21	-0.58
Sk																							
NOx			0.71					0.72				0.35		0.66			0.76			-0.28			
NH4	0.49					0.29		0.62			0.26	-0.37				0.47	0.73	0.25		0.28			
PO4			-0.80					0.72					-0.70				0.76						
Si	0.39					0.41					0.51							0.58		0.45			
inorg. N	0.43					0.27	0.43	0.67								0.42	0.75						
nutrient	0.46					0.33	0.43	0.63			0.33					0.36	0.75	0.27					
TOM	0.59					0.42		0.67			0.73	0.55	0.56		0.63		0.75	0.67		0.50			
chla	0.36					0.38	0.36				0.46	0.40						0.42					
pheo	0.58					0.52	0.43	0.83			0.77	0.55	0.60				0.76	0.69		0.64			
chlc							0.33					0.50											
fuc	0.36					0.47	0.51	0.63			0.49	0.50					0.75	0.38					
diadino							0.34				0.28	0.42									-0.42		
diato	0.52		-0.66			0.51	0.39	0.79			0.65	0.43	0.57				0.76	0.50		0.47			
zea	0.37		-0.68			0.36	0.36	0.83			0.61						0.76	0.51		0.54			0.76
lut	0.38					0.46	0.35	0.77		0.59	0.74	0.38						0.61		0.64			
bcar	0.44					0.56	0.55	0.82		0.65	0.72	0.52	0.62				0.75	0.60		0.45			
CPE	0.43					0.43	0.36				0.59	0.49						0.53		0.34			
CPE:TOM	-0.40						0.37		0.85		-0.42							-0.40		-0.34			0.80
chla:TOM	-0.42								0.79		-0.50			0.59				-0.48		-0.44			0.76
pheo:chl a	0.55		-0.80			0.45		0.79			0.75	0.46					0.59	0.69		0.70			
chlc:chla			0.64								-0.275							-0.40		-0.49			
fuc:chla						0.28	0.33	0.82				0.43					0.75						
zea:chla			-0.71					0.84			0.48					0.29	0.75	0.40		0.50			0.82
lut:chla	0.31					0.39		0.62			0.67							0.55		0.62			
diadino:chla											-0.26			0.61		-0.28		-0.36		-0.58	-0.60		-0.78
diato:chla	0.37		-0.88			0.37		0.75			0.51							0.33		0.43			
bcar:chla						0.42		0.73			0.58							0.41		0.42		-0.69	
LIP	0.53					0.41		0.59			0.67	0.49	0.65				0.75	0.47		0.49			
PRT	0.46					0.38					0.58							0.56		0.45			
PRT/TOM																							
bact-abund	0.45				-0.87									-0.83		0.51				0.34			
bact-div.	0.33					0.34					0.42	0.52			0.74						-0.58		
# of samples	40	16	8	8		40	24	8	8	8	40	24	8	8	8	40	8	40	8	40	16	8	8

DISCUSSION

Overall, the spatial heterogeneity of harpacticoid copepod species in the estuarine intertidal area under study was high, and over the 5 stations, 3 significantly different harpacticoid assemblages were recognized. Differences among copepod assemblages in terms of composition and abundance were most extreme between the tidal flat and the salt marsh stations, but also among the two stations on the tidal flat, which mainly differed in granulometry and tidal height. Harpacticoid variability among the salt marsh habitats (H3, H4, H5) was smaller despite their differences in tidal height, median grain size and prominence of vegetation. Harpacticoid assemblages in the mud and sand flat (H1, H2) were characterized by lower abundances and species richness, and at the sandy station H1 also by a high dominance of *Paraleptastacus spinicauda* and to a lesser extent *Asellopsis intermedia*. Interstitial Leptastacidae are highly competitive in sandy intertidal flats characterized by strong physical impacts and reduced food availability, and they may even occur as a monospecific assemblage (George & Rose 2004). In contrast to epibenthic species, interstitial *Paraleptastacus* sp has high vertical dispersal ability and an extended breeding season which contributes to their steady occurrence in disturbed habitats (Hockin & Ollason 1981). In contrast, *A. intermedia* may have a short breeding season. Lasker (1970) observed only one generation for *A. intermedia* in a sand flat in Scotland, with mating in August and hatching of nauplii from the eggs only after May in the next year (Lasker et al. 1970). This can be an explanation for their increased abundances in August. Moreover, fish predation on *A. intermedia* in sand flats is known to be a down-regulating factor during most of the year, whereas fish predation in muddy sediments is less specific due to the larger range of suitably sized prey (Gee 1987). In the sandy station (H2), harpacticoid assemblages showed no significant temporal shifts, and neither did median grain size and mud fraction. Food availability (chla) did, however, change during the year. This contributes to the idea that the copepod assemblage at this station is primarily structured by hydrodynamics and its effects on sediment granulometry, and by predation pressure. Production of microphytobenthos in this type of sediment can be very similar to that in siltier sediments, but its turnover through hydrodynamics tends to be considerably higher (Middelburg et al. 2000, Herman et al. 2001, Stal 2003, Stal & de Brouwer 2003), resulting in lower MPB biomass. MPB of sandy habitats is diverse, with epipelic and epipsammic species, the latter lacking temporal shifts (de Jonge 1985, Ribeiro et al. 2013). At the same time, deposition and retention of organic matter from the water column are very limited, resulting in sediments with low organic matter content but high OM quality as indicated by chla/TOM and PRT/TOM ratios. In addition to their better adaptation to hydrodynamic disturbance, copepods like *P. spinicauda* and *A. intermedia* may be more efficient in feeding on epipsammic MPB resources. For *Paraleptastacus*, the latter scenario is supported by fatty acid and stable isotope data, which suggest a consistent (throughout the year) indirect dependence on MPB carbon through grazing on bacteria (Cnudde et al., chapter 3), while *Asellopsis* is a direct diatom grazer (Cnudde et al., chapter 3).

In all other stations harpacticoid assemblages exhibited pronounced temporal variability, both in species diversity (N_0, N_1) and, primarily, species composition, but not in total copepod abundances. This may indicate that overall, food availability does not limit copepod abundances in this intertidal area, but that shifts in resource composition and/or other environmental variables drive assemblage composition.

Temporal changes in the copepod assemblage were station-specific. Firstly, highest temporal variability was found in the low-intertidal mud flat station H1, with two clear temporal shifts (between June-August and between November-February) in copepod dominance i.e. from (1) a Tachidiidae-Miraciidae dominated assemblage to a Laophontidae-Ectinosomatidae dominated assemblage, and from (2) a Laophontidae-Ectinosomatidae-Harpacticidae assemblage to a Laophontidae assemblage (*Asellopsis intermedia*) with some Leptastacidae (*Paraleptastacus spinicauda*), and with a general species impoverishment. The latter assemblage resembled the sand flat assemblage. Indeed, this strong shift in

copepod assemblage composition coincided with a significant change in granulometry from silty in June to fine to medium sandy in February. Such pronounced changes in granulometry in the upper sediment layers are typical for many tidal flat stations in this part of the Westerschelde Estuary, which is characterized by substantial seasonal transport-deposition cycles of fine particulate matter (Herman et al. 2001). Concomitantly, these sediments can rapidly shift between two stable states as a result of a complex interplay between sedimentation-erosion, microphytobenthos development and biodeposition/bio-erosion from deposit-feeding macrofauna (the Molenplaat, Herman et al. 2001, van de Koppel et al. 2001, Weerman et al. 2011, Weerman et al. 2012). Compared to station MP2, however, our station H1 may be even more temporally variable, because its low intertidal location hampers development of stable microphytobenthos biofilms due to the shorter exposure time and to hydrodynamics. The two alternative stable states are easily noticeable in this tidal flat station, where a basically sandy sediment in winter, very comparable to mid-tidal sandy station H2, becomes covered with a layer of silt of variable thickness in summer (June). Accompanying this shift in sediment granulometry are increases in food availability (chla, LIP, fuc:chla) and in nutrient concentrations (NH_4^+, PO_4^+) towards summer.

The increased abundance of Laophontidae (*Asellopsis intermedia*) and Leptastacidae (*Paraleptastacus spinicauda*) when sediments turn more sandy relates to niche specialization. *Asellopsis intermedia* is typically a sand-dweller while the latter only occurs in the sediment interstices (see above). For these species, the presence of fine particulate matter may be unfavorable due to clogging of interstitial spaces (for *P. spinicauda*) or even of the feeding apparatus. By contrast, *Amphiascus* sp. 1 (Miraciidae) and *Tachidius discipes* (Tachiidae) attained high abundances when sediment was silty (June). Peak abundances in spring are typical for *T. discipes* (Heip 1979, Herman et al. 1984). In addition, *T. discipes* shows characteristics of a niche generalist: its temporal variability correlated to almost all environmental variables while its spatial distribution correlated to nearly none of the variables (Table 5), including food quantity and quality related factors which were highly different among stations. *Amphiascus* sp. 1 feeds on diatoms (Mine et al. 2005; see also intermediate levels of diatom-specific 16:1w7, chapter 3, De Troch et al. 2006) with a potentially high selectivity towards larger cell sizes (De Troch et al., 2005). With an increase in granulometry, MPB species and functional group composition may change (Hamels et al. 1998), and this may affect *Amphiascus* abundances. For Ectinosomatidae, the limited number of correlations with environmental factors (Table 5) suggests Ectinosomatidae to be largely unaffected by overall changes in sedimentary variables. We hypothesize that they are highly flexible, and this fits well with the fact that the epibenthic Ectinosomatidae are highly motile and occur in a range of habitats (mud, sand, phytal) (Hicks and Coull, 1983). Salt marsh stations (H3, H4 and H5) shared many harpacticoid species but their contributions to the copepod assemblages were highly variable over space and time. Their spatio-temporal patterns cannot be easily explained based on a few environmental variables. In the following discussion, we focus on the most striking patterns and on species with high variability in abundance.

For some species, temporal changes in abundances were similar among the different salt marsh stations: (1) presence of Cletodidae specific in June-August (in H3, H4, H5), (2) maximum *Platychelipus littoralis* abundances in June (in H3, H4, H5), (3) decreasing abundances of *Microarthridion littorale* from June to August (in H4, H5) followed by an increase in November (in H3, H4, H5), and (4) a *Paronychocamptus nanus* increase in November (in H3, H4, H5). Cletodidae were strongly restricted to the warmer June-August period. Considering their specialized trophic ecology with a high dependence on sulphur-oxidizing bacteria (Cnudde et al., chapter 3), we suggest that spatio-temporal shifts relate to the seasonality in physical sediment characteristics which in turn affects the sediment sulfur cycle.

We have no clear explanation for the peaking abundances of *Platychelipus littoralis* in the salt marsh stations in June, but this peak coincided with deviant values for several nutrient concentrations (increased NH_4^+ and PO_4^+, decreased NO_x). This species is unique in its non-swimming behavior and is therefore directly linked to the local conditions on a microspatial scale. Similarly, the causes of the spatio-temporal distribution for the species *Paronychocamptus nanus* en *Amphiascus* sp. 1, in H3 en in H3-H4 respectively,

remained unclear, though the contrasting median grain size in H3-H4 excludes predominant influences of sediment granulometry on the spatial distribution pattern for these two species. Furthermore, also tidal height and food availability differed among stations H3 and H4. Distribution patterns of *Microarthridion littorale* related to food availability, with high abundances in H5 relating to TOM and temporal variability within station H4 correlating to absolute quantities of autotrophic resources (chla, diadinoxanthin) and trophic interaction with diatoms supported by elevated proportions of diatom FA 16:1ω7 during November-February (Cnudde et al., chapter 3). The lack of clear explanations for the distribution of these species in the field should be searched in our limited knowledge on their individual (feeding) ecology. The correlations observed in the present study could be underpinned or rejected based on future data from lab experiments.

The distribution *of Robertsonia diademata* (Miraciidae) was highly specific, restricted to H3 and with a peak in August. Iwasaki (1999) similarly observed their specific occurrence in a sandy sediment compared to a vegetated salt marsh. Stringer et al (2012) found a negative correlation between *Robertsonia propinqua* and *Enhydrosoma* sp. (Cletodidae), and a negative correlation of the former to median grain size. This fits relatively well with our results, where a shift from a Cletodidae dominance in June to an *R. diademata* dominance in August at H3 coincided with a small decrease in median grain size (ca. 15 µm) and with changes in other sediment characteristics: in August the mud fraction, sorting sediment sorting and sediment skewness reached maximum values and especially skewness was twice as high compared to June.

The strong spatial dissimilarities among harpacticoid copepod assemblages correspond to strong differences among environmental conditions between habitats. Apart from a large difference in median grain size and mud content, habitat types differed substantially in resource quantity, quality and diversity. For instance, while diatoms are probably the dominant photoautotrophs at all stations (Sabbe & Vyverman 1991), abundances and proportions of Cyanobacteria and chlorophytes were higher in salt marsh stations than in tidal flat stations, thereby diversifying resource availability. In addition, the salt marsh stations are accretory stations which potentially receive and retain substantial inputs of organic matter from the water column. Together with the higher stability of these sediments, which allow a higher build-up of MPB biomass despite MPB productivity does not necessarily exceed that on tidal flat stations, this implies that food availability in the marsh stations is much higher than on the hydrodynamically more disturbed bare tidal flat stations. This is clearly translated in higher abundances and diversity of harpacticoid assemblages. At the same time, however, a considerable fraction of the deposited material consists of more refractory, low-quality detritus; as a consequence, the salt marsh stations are characterized by higher OM stocks but of lesser overall quality. Moreover, the availability of high-quality resources to consumers may be partly hampered by their mixing with silt and refractory detrital particles (Herman et al. 2001). BEST-BVSTEP analysis indicated that spatial heterogeneity in harpacticoid assemblage composition was primarily linked to total resource availability (TOM), OM turnover (pheo:chla ratio) and proportion of diatoms to total photoautotrophs (diato:chla). Temporal variation was linked to microphytobenthos (diato:chla, diadino:chla). While most of the factors listed above are intricately related to sediment granulometry, sediment grain size and silt content did not appear among the factors best explaining variation in copepod assemblage composition. Owing to the general variability of biotic factors over space and time, partly correlating to physical sediment characteristics but additionally affected by other non-sediment physical variables (temperature, pH), these may have had more weight in the statistical tests, overshadowing the potential primary role of sediment granulometry. The apparently high structuring role of food-related environmental variables was also illustrated at the species level. Significant positive correlations between the distribution of harpacticoid species in salt marsh stations and a large number of biotic variables, including proxies for food availability, food type (different photoautotropic taxa) and food quality were found. For harpacticoid species from the bare tidal flat stations, the lack of positive relationships with food-related variables is likely due to the direct and comparatively high physical impact in these more hydrodynamically controlled systems. But note that it is within the range of expectations that all food-related environmental variables are strongly linked to

sediment characteristics and correlations between harpacticoids distribution and certain food-related environmental variables do not necessarily imply causation. Actually, two studies from the same Paulina intertidal area, Gallucci et al. (2005) and Van Colen et al. (2010), have well demonstrated the structuring role of physical variables e.g. sediment grain size and hydrodynamic regime, on benthic assemblages, of predacious nematodes and deposit-feeding macrofauna, respectively. These studies stress the consequences of changing environmental conditions to biological traits and hence, ecosystem functioning.
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