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Seasonal dependence on seagrass detritus and trophic niche partitioning in four copepod eco-morphotypes

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Abstract :

Benthic copepods dominate meiofaunal communities from marine phytodetritus, both in terms of numerical abundance and species diversity. Nevertheless, ecological factors driving copepod co-existence and population dynamics are still largely unknown. Here, we aimed to explore feeding habits of four copepod species commonly found in Mediterranean seagrass detritus accumulations, representing distinct eco-morphotypes (planktonic, phytal, epibenthic and mesopsammic). Joint use of fatty acid and stable isotope trophic markers showed that co-occurring harpacticoid copepods have diversified diets. Contrary to what was expected, microphytobenthos does not serve as their main food source. Instead, we found evidence from both techniques that major food items include heterotrophic biomass, macro-epiphytes and, depending on eco-morphology and season, of seagrass detritus-derived organic matter. Isotopic niches suggested that eco-morphotypes showed resource segregation. This segregation varies temporally, and partial overlap occurs between niches of phytal and epibenthic eco-morphotypes in some seasons. Our results highlight that, contrary to what is often assumed for meiofaunal consumers, considerable trophic diversity exists among copepod assemblages. They also indicate that, through multiple non-exclusive possible mechanisms, copepods could constitute a major link between seagrass detritus and associated biomass and higher trophic levels (namely macroinvertebrates and juvenile fish).

Keywords : Macrophytodetritus, *Posidonia oceanica*, Meiofauna, Stable isotopes, Fatty acids, Isotopic niche, Mixing models, Mediterranean Sea

1. INTRODUCTION

Seagrass meadows are net autotrophic ecosystems and key components of carbon cycle in marine coastal areas (Champenois and Borges, 2012). An important proportion of seagrass and macroalgae productivity is exported as shed biomass, accumulating on the sea bottom to form habitats called 'exported macrophytodetritus accumulations' (hereafter EMAs) (e.g. Vetter, 1995, Hyndes & Lavery, 2005, Lepoint et al., 2006; Boudouresque et al., 2016) and fuelling the detrital pool (Cebrian, 2002). The endemic and highly productive Neptune grass, *Posidonia oceanica* (L.) Delile, covers from 25 to 45.10^3 km² and the meadows it forms represent one of the dominant ecosystems found in the coastal Mediterranean (Pasqualini et al., 1998). EMAs formed by *P. oceanica* dead leaves are ubiquitous features of shallow areas of the Mediterranean Sea (Boudouresque et al. 2016). These patches of necromass accumulation are heterogeneous in their composition, being variable in thickness, size, and persistence in the environment (i.e. from very ephemeral to year-along presence) (Boudouresque et al., 2016). Their occurrence and persistence in the environment are determined by the local *P. oceanica* biomass cycle, by the local hydrodynamics and by the sea bottom morphology (Ricart et al., 2015).

Marine macrophytodetritus is considered an important trophic subsidies for food webs in many marine, estuarine, salt marsh, or terrestrial systems, both in temperate and tropical areas (Bouillon and Connolly, 2009; Heck Jr et al., 2008), providing habitats for many organisms (Como et al., 2008; Duggins et al., 2016; Mancinelli and Rossi, 2002; Vetter, 1995). Nevertheless, most literature focuses on macro- and megafauna, and smaller animals (i.e. meiofauna, animals with a body size between 38 µm and 1 mm) have received comparatively little attention. Small crustaceans (i.e. harpacticoid copepods) are the dominant taxa of meiofauna colonizing EMAs and represent up to 10^5 individuals per square meter (Mascart et

al. 2015b). The copepod assemblages found in *P. oceanica* EMAs are diverse, and different morphotypes (*sensu* Noodt (1969)) can be found among the necromass. These morphotypes have different biological traits, behavioural patterns, and occupy different micro-habitats. Phytal (i.e. often flattened copepods, mobile but strongly associated to macrophyte substrate, often grasping dead or living plant) and epibenthic eco-morphotypes (i.e. free-living benthic copepods, less associated to a substrate, able to live on and often in sediment) are dominant, but truly planktonic (i.e. copepods living in the water column) and mesopsammic (i.e. copepods living inside sediment but sometimes able to live as epibenthic copepods) species are also encountered (Mascart et al. 2015b). Given those differences, it is likely that feeding habits, and therefore the role of these copepods in EMAs' food web functioning differs. In other environments, trophic niches of copepod species belonging to the same eco-morphotype can be different (Arroyo et al., 2006; Azovsky et al., 2005; Carman and Fry, 2002; Carman and Thistle, 1985; De Troch et al., 2006b; Pace and Carman, 1996; Steinarsdóttir et al., 2010). How resource partitioning determines co-existence of dominant eco-morphotypes in EMAs remains unknown.

Because of their short life cycle and high turnover rates, harpacticoid copepod communities respond rapidly to organic matter inputs, and their life cycles are closely coupled to these inputs (Danovaro et al., 2002). In addition, EMAs are seasonally pulse-sourced by dead seagrass leaves and the epiphytic community covering them (Mascart et al., 2015b; Remy et al., 2017). Therefore, food item availability fluctuates over time, and this could have consequences for copepod trophic ecology and food partitioning between the different copepod eco-morphotypes (Mascart et al., 2015b).

Stable isotope (SI) analyses of carbon and nitrogen allow identification and quantification of food sources that are assimilated into the tissues of consumers over time. Fatty acid (FA) profiling complements stable isotope analysis as a second biomarker, providing additional

information on the feeding ecology of meiofauna (Cnudde et al., 2015; De Troch et al., 2012; Leduc et al., 2009), as several FAs can be used as markers for specific food sources (Dalsgaard et al., 2003; El-Sabaawi et al., 2009).

By combining stable isotope ratios and fatty acid profiles, we aimed to study the trophic ecology of four co-occurring species of copepods, representing four dominant eco-morphotypes of *Posidonia* litter meiofauna (Noodt, 1969): *Diosaccus tenuicornis* (phytal type, harpacticoid), *Tisbe furcata* (epibenthic type, harpacticoid), *Ectinosoma dentatum* (mesopsammic type, harpacticoid) and *Calanus arcuicornis* (water column type, calanoid) (Fig. 1). Specifically, we addressed 4 questions and put forward the following hypotheses.

1) What are the food sources sustaining copepod consumers in seagrass detritus accumulations? While copepods are typically regarding as depending mostly on microphytobenthos, we hypothesized that their food items in EMAs are diverse because available food sources are diverse.

2) Do different copepod eco-morphotypes exhibit resource segregation? Despite the "black box" approach generally applied to meiofaunal consumers in ecological literature (i.e. meiofaunal consumers are considered ecologically redundant and feeding on the same items regardless of consumer species) (Danovaro et al., 2002), we hypothesized that the 4 species studied here can have different diets and occupy different niches, and that this could facilitate co-existence of these abundant consumers.

3) Does copepod trophic ecology vary seasonally? Given the high turnover of copepod populations, we expect their feeding habits in EMAs to change temporally as food availability and composition varies seasonally.

4) Do copepods feed on dead seagrass tissue? In saltmarsh ecosystems, copepods assimilate detritus-derived organic matter and, like macrofauna, depend on dead plant material not only as a shelter but also as a food source (Couch, 1989). Therefore, we expect that it is also the

case in seagrass detritus accumulation. Using trophic markers, we aim to explicitly test that, and to propose mechanisms through which copepods could feed on and assimilate seagrass detritus.

2. MATERIALS AND METHODS

2.1 Study site and field sampling

A sandy patch close to a continuous *P. oceanica* seagrass meadow was located near the STARESO marine research station (University of Liège) in the Revellata Bay (Calvi Bay, Corsica, France, NW Mediterranean; 42°35'N, 8°43'E). Sampling of consumers and their potential food sources (macrophytodetritus, epiphytes, drifted macroalgae and particulate organic matter) was carried out at a depth of 10 m on a seasonal basis. Four sampling events were conducted, each representing a season, namely winter (February 2012), spring (May 2012), summer (August 2011) and autumn (October 2011). 30 L plastic bags were used to hand-collect copepod consumers and food sources, as well as the seagrass detritus, with which they are associated. Subsequently, the collected material was kept alive in a 0.75 m^3 aquarium with 38 µm filtered seawater. The content of the aquarium was sequentially rinsed over three mesh size sieves: a 10 mm mesh size to collect vegetal fraction (i.e. potential food sources), a 1 mm mesh size to exclude macrofauna, and finally a 38 µm mesh size to retain copepods. The sampled EMA food sources consisted of a heterogeneous mixture of (1) dead *P. oceanica* leaf litter fragments (leaves with attached epiphytes), (2) drift (epilithic) macroalgae, and (3) living shoots of *P. oceanica* comprising rhizomes and living leaves. Epiphytes present on *P. oceanica* leaves were scraped off using razor blades. The fourth food source, the suspended particulate organic matter (SPOM), was collected about 1 m above the seafloor with Niskin bottles (2.5 L) handled by SCUBA divers. The content was afterwards vacuum-filtered onto pre-combusted glass fibre filters (Whatman GF/F, diameter 47 mm). In

Revellata Bay, SPOM composition is seasonally variable, but generally dominated by phytoplankton biomass, except during and after storm when re-suspended detritic material may represent a large proportion of its composition (Dauby et al., 1995).

In a second stage, the 38 µm fraction holding the copepods was kept in a 20 L aquarium with an air stone to collect living copepods using positive phototactic attraction, in a setup similar to that used by Svensson et al. (2010). By means of a stereomicroscope, individuals were subsequently determined using the identification keys and reference books by Boxshall & Hasley (2004) and Lang (1948, 1965), and separated by species. The copepods were placed in filtered seawater overnight to empty their gut contents. The four most abundant copepod species (Mascart et al., 2015b) (Fig. 1), each belonging to a different eco-morphological type (Mascart et al., 2015a) were selected: *Diosaccus tenuicornis* (phytal type harpacticoid), *Tisbe furcata* (epibenthic type harpacticoid), *Ectinosoma dentatum* (mesopsammic type harpacticoid) and *Calanus arcuicornis* (water column type calanoid). Replicates were realised by pooling individuals, to have enough biomass for reliable measurements (i.e. for stable isotopes: 60-100 individuals per replicate, fatty acids: 120-200 individuals). We have excluded gravid female as eggs may affect the FA content of the copepods. All consumer and food source samples were stored at -80°C for FA profiling and -20°C for SI analyses.

2.2 Lipid extraction and fatty acid analysis

Prior to the lipid extraction, food sources and copepod samples were freeze-dried and transferred to glass vials. Lipid extraction, fatty acid methylation, and analysis of fatty acid methyl esters (FAMEs) were executed according to the methods used in De Troch et al. (2012), including a lipid hydrolysis and fatty acid methylation achieved by a one-step derivatization method modified after Abdulkadir & Tsuchiya (2008). FAMEs were injected at a temperature of 250°C in splitless mode (1 µl for food sources and 5 µl for copepods) into a gas chromatograph (HP 6890N, Agilent, USA) with a capillary column (J&W HP88, Agilent,

USA), coupled to a mass spectrometer (HP 5973, Agilent, USA). Quantification of individual FAMEs was accomplished with internal standard C19:0 (Fluka 74208, Sigma-Aldrich, USA). Concerns about analytical error were raised in the profiles yielded for the *Ectinosoma dentatum* copepod, and the latter were thus not taken into account. Therefore, the FA profiles of only 3 copepod species are shown in this study: *Calanus arcuicornis*, *Tisbe furcata* and *Diosaccus tenuiremis*. The FA shorthand 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). FAs were reported as percentage of the total fatty acids (%TFA ± SD) and grouped as saturated (SAFA), monounsaturated (MUFA), and polyunsaturated (PUFA) FAs.

2.3 Stable isotope ratio analysis

Food sources were dried at 60°C for 96 h, and, with the exception of SPOM filters, ground to a homogenous powder using a ball-mill (Retsch Mixer Mill MM301). The attached epiphytes and drift macroalgae material were subdivided into two parts for acidification, to remove inorganic carbonates prior to carbon measurements. Acidification was done by fumigation of HCl vapours (fuming HCl, 37%, overnight). Stable isotope ratios of carbon (hereafter, $\delta^{13}C$) were measured on acidified material, and stable isotope carbon ratios of nitrogen (hereafter, $\delta^{15}N$) on non-acidified material. Ground food source samples (2-3 mg) were subsequently loaded into tin capsules for isotopic measurements. Regarding the consumers, pooled copepod individuals were transferred into a droplet of MilliQ water in a tin capsule (8x5 mm, Elemental Microanalysis), and subsequently dried at 60°C for 24 h. They were then precisely weighed (± 0.001 mg) (Metler Toledo, XS3DU). Copepods were not acidified, considering the very low carbonate content of their exoskeletons.

Isotopic ratios and elemental content measurements were performed using an isotopic ratio mass spectrometer (IsoPrime100, Isoprime, UK) interfaced in continuous flow with an

elemental analyzer (vario MICRO cube, Elementar, Germany). Isotope ratios of C and N were reported conventionally (Coplen, 2011), using standard delta (δ) notation relative to their respective international standards, Vienna-Pee Dee Belemnite (V-PDB) and atmospheric N_2.

Reference gases CO_2 and N_2, as well as certified reference materials (i.e. sucrose (IAEA-C6; $\delta^{13}C = -10.8 \pm 0.3‰$) and ammonium sulfate (IAEA-N2; $\delta^{15}N = 20.3 \pm 0.3‰$) obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria)), were used for calibration. The analytical precision was assessed by procedural blanks, internal replicates (i.e. glycine and in-house crustacean and seagrass reference materials) and isotopic certified materials (i.e. IAEA-C6 and IAEA-N2). Standard deviations on replicate measurements presented hereafter were 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. Neither chemical lipid extractions nor *a posteriori* lipid corrections were performed; this is due to the often limited relevance of *a posteriori* corrections for aquatic invertebrates containing high proportions of chitin in addition to lipids and proteins (Logan et al. 2008).

2.4 Data analyses

Stable isotope data were normalised by subtracting means and dividing by SD, in order to place them on comparable measurement scales and to homogenize variances between groups. The variance of sources and consumers was then compared using a two-way multivariate PERMANOVA with fixed factors (Month-Source or Month-Species, respectively) to determine whether the food sources or consumer species differed significantly through time. A PERMANOVA model generated pseudo F-statistics and p-values based on 999 permutations of the data, computed from a Euclidean distances resemblance matrix (Anderson et al. 2008). Prior to making the decision to use this analysis, normality and homoscedasticity assumptions were tested (p-values of the Hawkins test < 0.05). The disadvantage of this analysis of variance is the difficulty in distinguishing the source of the

variation (due to location or dispersion). Therefore, homogeneity of dispersion was tested with a PERMDISP procedure, using distances amongst centroids calculated at the lowest level (Quinn & Keough 2002).

All data analyses for FAs were performed on relative (%) FA concentrations. Therefore, a similarity percentages (SIMPER) analysis was preferred to identify the main harpacticoid copepod species, primarily providing differentiation between the seasons. An analysis of similarity (ANOSIM) was carried out to test whether the defined communities were significantly different. All the above-mentioned analyses were performed with the Primer 6.1.11 software (Clarke & Gorley 2006) with the PERMANOVA add-on (Anderson et al. 2008). A significance level of $P < 0.05$ was used in all tests.

To estimate the consumer's assimilated diet, several Bayesian mixing models have been developed (reviewed by Phillips et al. (2014). The stable isotope mixing model, SIAR (Stable Isotope Analysis in R; (Parnell et al., 2010), was used to estimate the relative contribution of different food sources to the diet of the four copepod species. SIAR 4.2 was fitted in R 3.2.2 (R Core Team 2014), including isotopic compositions of each individual, isotopic compositions of food sources (mean ± SD), and trophic enrichment factors (TEFs; expressed as mean ± SD) that correspond to the net isotopic composition change between a consumer and its ingested food source(s). Some sources were combined, as isotopic composition of autotrophs can overlap (Phillips et al., 2005). Accordingly, some food items did not display significant differences in their isotopic composition. Therefore, the following potential sources were combined: dead leaf litter and living *P. oceanica* shoots (PERMANOVA for every month: for $\delta^{13}C$, all $P > 0.061$ and for $\delta^{15}N$, all $P > 0.196$); and the drift macroalgae and scraped epiphytes from the *P. oceanica* leaves (PERMANOVA for every month: for $\delta^{13}C$, all $P > 0.097$ and for $\delta^{15}N$, all $P > 0.348$). After combination, three significantly different potential food sources (PERMANOVA per month for $\delta^{13}C$ and $\delta^{15}N$ $P = 0.001$)

were used in the mixing models: (1) macrophytodetritus, i.e. primarily dead and living *P. oceanica* seagrass leaves without epiphytes; (2) epiphytes, i.e. the scraped leaf epiphytes and drift (epilithic) macroalgae; and (3) suspended particulate organic matter.

Since there are no specific TEFs for the taxa studied here, we used widely applicable values from (McCutchan et al., 2003), i.e. 0.40 ± 1.20‰ for C and 2.30 ± 1.61‰ for N (mean ± SD, in each case).

In addition, it has been proposed that the variability of isotopic composition of a population or a species (i.e. its isotopic niche) can be used as a proxy to assess the trophic niche of this population or species, and/or the degree of individual specialisation in the population (Bearhop et al., 2004; Jackson et al., 2011). This concept of isotopic niche has also been developed through numerical methods (Jackson et al., 2011). The Stable Isotope Bayesian Ellipses in R (SIBER) set of functions from the aforementioned SIAR 4.2 R package was used to describe isotopic niches and quantify their essential parameters. The yielded standard ellipses, containing ~40% of the data (centred on the mean and SDs of the bivariate data as semi-axes), and standard ellipse area (SEA), were used to delineate an isotopic niche space for each species. The areas of ellipses were estimated using the SEA_c correction for small sample size, as outlined in Jackson et al. (2011). The areas of these ellipses were also estimated using Bayesian modelling (SEA_B, 10^6 iterations), and direct inter-group pairwise comparisons of SEA_B were performed. Model solutions were presented using credibility intervals of probability density function distributions. Pairwise comparisons were considered meaningful when the probability of occurrence exceeded 95%.

3. RESULTS

3.1 Fatty acid profiling

A total of 17 FAs were identified in the potential food sources, i.e., those yielding a fatty acid percentage above 0.5% in at least one of the samples (Table 1). The relative FA contribution

of sources showed a similar composition, with relatively high amounts of SAFAs, especially in the macrophytodetritus, compared to the epiphyte samples (mean of 72.4 ± 9.3% and 55.4 ± 5.1%, respectively). The most abundant SAFA was 16:0 (palmitic acid) with mean contributions of 30.8 ± 2.0% for macrophytodetritus and 36.0 ± 3.7% for epiphytes. Other abundant SAFAs were 26:0 (cerotic acid) and 28:0 (montanic acid), especially in macrophytodetritus, with a respective contribution of 11.3 ± 5.2% and 13.6 ± 4.5%. The MUFAs and PUFAs of the macrophytodetritus represented a similar share (12.1 ± 4.0% and 14.0 ± 5.0%, respectively). Conversely, in the epiphytes, PUFAs were more abundant than MUFAs (27.5 ± 7.5% and 19.7 ± 3.4%, respectively). The three most abundant MUFAs were 16:1ω7 (palmitoleic acid), 18:1ω7 (*cis*-vaccenic acid), and 18:1ω9 (oleic acid) (Table 1). The most frequently-encountered PUFAs in the epiphyte source were 20:4ω6 (arachidonic acid or ARA) and 20:5ω3 (eicosapentaenoic acid or EPA) (9.4 ± 3.2% and 8.5 ± 4.2%, respectively). The most abundant PUFAs in the macrophytodetritus were 18:2ω6 (linoleic acid; 5.0 ± 2.9%) and 18:3ω3 (α-linolenic acid; 3.7 ± 1.5%). Both sources were clearly separated according to their PUFA profile (ANOSIM, R = 1, P <0,001). A less pronounced distinction between months was visible (ANOSIM, R = 0.667, P <0,001). SIMPER analysis showed a dissimilarity of 26.6% between these sources. The main FAs responsible (48.6 cumulated %) for the dissimilarity were long-chained: 26:0 (14.2%), 28:0 (13.1%), 20:4ω6 (10.9%) and 20:5ω3 (10.5%). Regarding the different months, the macrophytodetritus collected in October differed most from the other months, with the largest dissimilarity between October and August (21.8%). For the epiphytes, May was separated most clearly from the other months, with the largest dissimilarity between May and August (15.9%).

In the four consumer copepod species, a total of 19 FAs were identified (Table 2). Compared to the food sources, three FAs were not present in the consumers: 26:0, 28:0, and 16:1ω6. Four FAs were restricted to the copepods' profiles: 18:4ω3 (stearidonic acid), 20:0

(eicosanoic acid), 22:6ω3 (docosahexaenoic acid or DHA), and 23:0 (tricosylic acid). The SAFAs were the most important class, accounting on average for 92.0 ± 5.1% in *C. arcuicornis*, 78.7 ± 7.5% in *D. tenuiremis*, and 62.0 ± 15.8% in *T. furcata*. The predominant SAFAs were 16:0 and 18:0, representing on average 39.7 ± 12.8% and 21.1 ± 5.1%, respectively, across all species. The MUFAs were more abundant in *T. furcata*, where they accounted for 17.5 ± 4.5% of the total FA composition. Palmitoleic acid (16:1ω7) was the most abundant MUFA in this species, with 10.0 ± 2.7%. It also dominated the MUFA composition of *D. tenuiremis* (7.1 ± 1.33%). A very low and variable amount of MUFAs was found in *C. arcuicornis* (9.9 ± 7.0%). *C. arcuicornis* presented an even lower and more variable amount of PUFAs (1.2 ± 1.6%). Strangely, no DHA (22:6ω3) was found in *C. arcuicornis*, contrasting with *T. furcata* and *D. tenuiremis*, where it was the most important PUFA, with 16.8 ± 9.8% and 5.7 ± 4.2%, respectively. ANOSIM revealed a subtle difference among months for all species (ANOSIM, R = 0.691, P <0,001). In addition, a stronger difference among species was visible (ANOSIM, R = 0.893, P <0,001), separating *C. arcuicornis* from the other two species, mainly based on the DHA levels, which contributed most to the dissimilarities with *T. furcata* and *D. tenuiremis* (20.3% and 13.3%, respectively). Conversely, differences in the FA of *T. furcata* and *D. tenuiremis* were less clear, with dissimilarity between the two species being only 17.39%. Moreover, these two species FA compositions only differed between May and February (PERMANOVA, pair-wise, P(MC) = 0.018 and 0.016, respectively). Those relatively weak differences were mostly driven by the lower MUFA and PUFA content of *T. furcata* during those months (Table 2).

3.2 Stable isotope compositions

δ^{13}C values of food sources ranged from -28.1‰ (SPOM February) to -11.3‰ (macrophytodetritus August). On an annual basis, epiphytes (δ^{13}C = -17.2 ± 1.9‰) exhibited

the largest temporal difference in their δ^{13}C values. The highest values were found in October (-13.2‰) and the lowest values were noted in August (-20.4‰). Macrophytodetritus showed the highest δ^{13}C values, with a mean isotopic composition of -13.1 ± 0.8‰. SPOM was always the most ^{13}C-depleted food source (average -25.4 ± 1.4‰), with significant differences among months, except between May and August. The two-way PERMANOVA showed significant effects of both month and source factors for carbon composition (Table 3).

δ^{15}N values of food sources ranged from 0.2‰ (epiphytes, August) to 4.6‰ (Epiphytes, May). Food sources displayed significantly different isotopic composition across months, except for SPOM, where no difference occurred (annual mean: 1.7 ± 0.2‰). The epiphyte and macrophytodetritus sources showed a similar annual mean δ^{15}N with values of 2.0 ± 1.1‰ and 2.1 ± 0.9‰, respectively. However, epiphytes showed a large fluctuation among months (Pair-wise, P = 0.467).

δ^{13}C values of consumers ranged from -24.9‰ (*Clausocalanus arcuicornis,* February) to -15.3‰ (*Diosaccus tenuiremis,* February) (Fig. 2). On an annual basis, *C. arcuicornis* displayed the lowest δ^{13}C values, and showed no significant difference in δ^{13}C values among months (annual mean of -21.4 ± 1.4‰) except between August and October (Table 3). The other three species did not show any significant difference in their annual mean δ^{13}C. However, temporal δ^{13}C fluctuations, especially for *D. tenuiremis* and *Ectinosoma dentatum* (Table 3). These species had similar δ^{13}C in August and October. *D. tenuiremis* reached its lowest δ^{13}C values in February and its highest in May. On the contrary, *E. dentatum* showed its lowest δ^{13}C values in February.

δ^{15}N values of consumers ranged from 0.9‰ (*C. arcuicornis,* May) to 4.5‰ (*E. dentatum,* May) (Fig. 2). *Tisbe furcata* showed the most constant δ^{15}N value over the year, in contrast to *C. arcuicornis* and *E. dentatum* (Table 3). Both these species showed their lowest δ^{15}N values

in May and their highest δ^{15}N values in August.

3.3 Isotopic niches

Bivariate standard ellipses (Fig. 2), representing core isotopic niches of consumers, indicated that, except for *D. tenuiremis* and *T. furcata* in May and October, ellipses occupied different parts of the isotopic space, without overlapping. Along the carbon axis, ellipse position in the isotopic space of *D. tenuiremis* and *E. dentatum* changed drastically according to sampling dates. In May and February, SEA_B calculations suggested that, in over 99.90% of model runs, ellipse area (Fig. 3) of *C. arcuicornis* was greater than those of the other three species. However, in August, *D. tenuiremis* showed the smallest ellipse areas in over 99.95% of model runs, when all other ellipses showed similar areas.

The ellipse area of *C. arcuicornis* decreased from February to August in over 99.98% of model runs. They were more constant for the other three species (except *E. dentatum* in February and *D. tenuiremis* in October, when they were larger).

3.4 Stable isotopic mixing model

SIAR modelling results showed that *C. arcuicornis* mainly assimilated food sources coming from a planktonic environment (Fig. 4) (upper and lower limit of a 95% credibility interval – hereafter C.I. 95% – for all seasons: 35-82%). This contribution was the lowest in August (C.I. 95%: 35-63%; mode = 46%) and the highest in October (C.I. 95%: 66-82%; mode = 75%). Although sometimes contributing to the diet of *C. arcuicornis*, the other two food sources showed a wide range of possible contribution (i.e. very wide 95% C.I.). Nevertheless, contributions by macrophytodetritus were generally low (upper and lower limit of C.I. 95%: 0-30%). They were the lowest in October (0-19%; mode = 4%) and the highest in August (C.I. 95%: 0-30%; mode = 13%). All three food sources seemed to contribute significantly to the diet of *T. furcata, D. tenuiremis* and *E. dentatum*, except in May, when epiphytes seemed to contribute only a little. However, the credibility intervals of model solutions were often

very wide. Macrophytodetritus seemed to contribute significantly to the diet of these three species, as the model never gave contributions equal to zero. Strong seasonal variability seemed to be present for *D. tenuiremis* and *E. dentatum* (Fig. 4).

4. DISCUSSION

4.1 What are the food sources sustaining copepod consumers in seagrass detritus accumulations?

Stable isotope and fatty acid profiling indicated that the food sources of this copepod assemblage are relatively diverse and not dominated by microphytobenthos. Isotopic modelling showed that the diet of the four eco-morphotypes analysed was unlikely dominated by only one food source, and was clearly different between benthic (i.e. the 3 harpacticoid) and planktonic species.

The planktonic species (calanoid) found in the EMA clearly showed isotopic compositions and fatty acid profiles which reflect feeding on phytoplankton in the water column. Indeed, in the Calvi Bay, δ^{13}C of SPOM always show more negative values than benthic primary producers found in the bay, excepted for sciaphilous algae (Lepoint et al., 2000). Nevertheless, even for this species, our results showed potential contribution of benthic food sources to their diet.

According to literature, harpacticoid copepods can consume a large spectrum of food sources, depending on food availability in a particular habitat. In intertidal and subtidal habitats, copepod diet mostly consists of microscopic algae assemblages, i.e. microphytobenthos (De Troch et al., 2005; Evrard et al., 2010; Rzeznik-Orignac et al., 2008). Here, FA profiles of copepods indicate that feeding on diatoms – which are part of the sessile epiphytes – could occur, as 16:1ω7 and 20:5ω3 marker compounds (Dalsgaard et al. 2003) were present. However, the ratio between 16:1ω7 and 16:0, often used as an indicator of high diatom

contribution to diet, was rather low compared to intertidal copepods, who mainly feed on microphytobenthos (Kharlamenko et al., 2001). Contrary to the suggestion by Couch (1989), this indicates that, in EMAs, diatoms are not dominant in the diet of copepods.

In our study, macroepiphytes (both sessile fauna and flora) and drift epilithic macroalgae (i.e. macroalgae ripped from surrounding rocks) seemed to be part of the copepods' diet. In the study site, epiphytes and drift algae represented around 10% of EMA biomass each (Mascart et al. 2015b). Both food sources could be valuable for copepods in terms of nutrient and fatty acid content, as they are not necromass in the litter. Epiphytes were already previously found as food source for copepods living in *P. oceanica* canopy (Mascart et al., 2013) and for many other crustaceans, such as amphipods (Michel et al., 2015). In Australian coastal waters, macroalgae consumption in seagrass-dominated litter has also been documented (Hyndes and Lavery, 2005).

Uprooted living seagrass, often found in high abundance in EMA, were not consumed by copepods, as no typical FAs from living seagrass were found in copepods' tissues (Kharlamenko et al., 2001). FA profiles of live seagrass blades differ significantly from seagrass detritus, due to the loss of some FAs during decay and the presence of bacterial FAs in detritus (Kharlamenko et al., 2001; Leduc & Probert, 2009; Michel et al., 2015).

Our isotopic modelling indicated that carbon coming from seagrass detritus often constituted a non-negligible part of the copepods' diets (particularly in the case of harpacticoids). This is in accordance with the diet of copepods in *P. oceanica* meadow sediments (Danovaro et al., 2002), *Zostera noltii* meadow sediments (Lebreton et al., 2012; Vafeiadou et al., 2014), and in salt marshes (Couch, 1989), or in *Amphibolis* sp. and *Posidonia* spp. detritus accumulations (Hyndes & Lavery, 2005).

Seagrass leaf detritus it-self is mostly constituted of carbon and refractory nitrogen associated with structural carbohydrates. In this refractory material, microbial biomass can constitute the

majority of the nitrogen and fatty acids available for consumers (Newell et al., 1989). Even if photo-autotrophic organisms are present, heterotrophic biomasses (eukaryotic and bacterial) are also very abundant on and inside degrading leaves (see scanning electron microscope images in (Lepoint et al., 2006). Bacteria are a possible food source for harpacticoid copepods (Rieper, 1978). In our study, the presence of 14:0, 15:0 and 17:0, and 18:1ω7 could indicate a contribution of bacterial biomass to copepod diet (Cnudde et al., 2015; Jaschinski et al., 2008). Other heterotrophs (fungi, protists) are also potentially ingested by copepods, but our approach cannot determinate this potential contribution to the copepod diet.

The overall picture painted by our data is that harpacticoid copepods inhabiting detritus accumulations display a relatively diverse diet, dominated by heterotrophic, epiphytic/algae, and seagrass detritus food items. On the contrary, the planktonic eco-morphotype found in the litter appears to mostly rely on the water column for its nutrition.

4.2 Do different copepod eco-morphotypes exhibit resource segregation?

Our data indicated that different eco-morphotypes can exhibit different isotopic niches and different diets, meaning that there is in partitioning of resources offered by EMAs among the four investigated species. Food partitioning and dietary specialisation is often observed in meiofauna inhabiting coastal sediments (Rzeznik-Orignac et al. 2008) or macrophyte habitats (Steinarsdóttir et al. 2010). High feeding selectivity has been documented for nematodes grazing on bacteria and microphytobenthos (Moens et al., 2006), relating to a specific diversity of prey and consumers (De Meester et al., 2016). Specific feeding preferences have also been demonstrated for copepods (e.g. Carman and Thisle 1985), sometimes with a selectivity at species level (i.e. diatom species) or related to particle characteristics (i.e. diatom sizes) (De Troch et al., 2006a). This resource partitioning is one of the mechanisms sustaining high biodiversity and high consumer biomass at a local scale.

The planktonic copepods analysed here had markedly separated isotopic niches as a

consequence of both distinct diets and/or habitats (Fig. 2). Indeed, isotopic niches are related both to trophic niche (i.e. when different diets at population or species level reflect different isotopic niches) and to the habitats in which animals feed (i.e. when different habitats have different isotopic baselines, and animals shifting between habitats may integrate these different isotopic baselines) (Flaherty and Ben-David, 2010 and Phillips et al. 2014). *C. arcuicornis* individuals regularly found in the litter would probably shift between water column and litter (e.g. diel migration, or refuge during windy events). Nevertheless, isotopic data evidenced significant contributions of benthic food sources in the diet of *C. arcuicornis* too. This means that, in shallow coastal areas, EMAs support coupling between planktonic and benthic food webs. This coupling does not necessarily imply an important transfer of seagrass detritus carbon to the pelagic ecosystem as SIAR modelling showed a very low contribution of seagrass material to the diet of *C. arcuicornis*. More likely, epiphytic and microphytobenthic biomass appear as the preferential benthic food sources used by *C. arcuicornis*. Nevertheless, considering its abundance in litter accumulations (10^3 ind.m^{-2} during zooplankton bloom) (Mascart et al. 2015b), this species could channel significant matter fluxes between benthic and pelagic compartments.

The mesopsammic species *E. dentatum* also had a distinct isotopic niche compared to other harpacticoid eco-morphotypes. As with *C. arcuicornis*, this species may be found in two habitats, namely EMA and sediment under the EMAs. This may explain the particular δ^{15}N value observed for this species, which is representative of sediment organic matter in the study area (Michel et al. 2015).

Isotopic niches of the other two eco-morphotypes, which are more associated with substrate surface (sediment or phytal), were partly overlapping or were completely separated, depending on the season. These may be related to the relative abundance of the food sources they exploited (i.e. epiphytes, seagrass detritus) and the relative level of competition between

these two species (i.e. their relative abundance, which was not assessed here).

Generally speaking, the fact that isotopic niches differed between copepods showing different body morphologies indicated segregation of resources in this particular environment.

4.3 Do copepod trophic ecology vary seasonally?

The four eco-morphotypes investigated displayed a certain level of variability in their respective isotopic composition according to sampling period. A part of this variability is linked to baseline shifts (i.e. temporal variability of isotopic composition of food sources, cf. Tables 3 and 4), independent of actual trophic shifts. This confirms the importance to sample food sources in a period compatible with consumer assimilation to assess this baseline shift and properly parameterize the mixing model, as we have done here.

Application of a mixing model using season-specific data for food sources also showed that contributions of food sources to diet varied seasonally. Seasonal diet shifts in benthic copepods are probably related to food availability (i.e. microbial or epiphytic biomass vs. detritus biomass) or nutritive quality. The strongest seasonal shift in isotopic composition was observed for the mesopsammic *E. dentatum*, and this could be explained by a seasonal shift in both trophic resources and habitat (sediment vs. EMA). Indeed, Mascart et al. (2015a) have shown an active and rapid colonisation of EMA from sediment by this species.

Phytal and epibenthic eco-morphotypes shared part of their isotopic niches in May and October but not in February and August. In terms of diet, our data indicated species-specific changes between the relative importance of epiphytic resources *sensu lato* (i.e. epiphytic macroalgae, microbial biomass, etc.), and seagrass. Such segregation/overlap between trophic niches has already been documented in phytal habitats (e.g. Arroyo et al. 2007, Steinarsdóttir et al. 2010), suggesting that harpacticoid copepods colonizing phytal environments interact and, potentially, compete for food sources (Arroyo et al. 2007). Temporal changes in food availability (i.e. epiphytic and microbial biomass) (Mascart et al., 2015b), but also quality

(detritus ageing, enrichment by microbial biomass, fatty acid composition), are probably the main drivers of trophic competition and trophic niche partitioning between these two eco-morphotypes, tightly associated to phytal habitats. These resource pulses have been demonstrated to have an important effect on macrofauna biodiversity in studied EMAs (Remy et al., 2017).

In addition, our data indicate that species found in litter showed high trophic plasticity, regardless of their eco-morphotype. Indeed, all species observed in the litter were also found on living *P. oceanica* leaves in the seagrass bed, where food source composition, availability, and quality are different, compared to seagrass litter accumulations (Mascart et al. 2013). In the canopy of *P. oceanica*, diatoms colonizing leaves as microepiphytes are likely a major food source for phytal and epibenthic eco-morphotypes, and the contribution from seagrass organic matter is probably reduced.

4.4 Do copepod feed on dead seagrass material?

All 3 harpacticoid copepods analysed here seemed to rely, to some extent, on organic matter originating from seagrass detritus. This conclusion arises from our isotopic data, as $\delta^{13}C$ allowed us to distinguish seagrass carbon from other sources. . Harpacticoid copepods are known to bioconvert short-chain FA into long-chain FA (De Troch *et al.* 2012). This mechanism of bioconversion is an essential tool in case of low food quality as it allows them to make their own PUFA or increase their PUFA level. This may represent a possible explanation why some copepod species can deal better with detritus as possible food than other species. Three mechanisms could explain this important observation in terms of energy flow (Fig. 5). The first hypothesis would be that direct copepod grazing on macrophytodetritus could occur. However, copepods are considered to feed selectively on individual particles (e.g. plankton, microphytobenthos) or to browse on the substrate surface, as the morphology of their mouth are not adapted to cutting large pieces of material. Detritus

material nevertheless fragments rapidly due to physical effect of water motion and consumption by detritivores; therefore, fine material is often the majority of the *Posidonia* detritus pool (Mateo and Romero, 1997). This size is potentially compatible with direct ingestion by copepods; particularly epibenthic and mesopsammic eco-morphotypes, or planktonic ones when particles are resuspended in the water column (following a storm for example).

Second, seagrass carbon could be assimilated indirectly by copepods via microbial intermediates. Seagrass detritus are colonised by various microorganisms (bacteria, fungi, protists) that degrade detritus biomass. The isotopic composition of carbon assimilated by microbial biomass is only slightly affected by isotopic fractionation, meaning that the isotopic composition of heterotrophic microbial biomass could be very close to that of detritus biomass (seagrass detritus, in this case) (Bouillon and Boschker, 2006). Such indirect transfer is often considered to be dominant in seagrass meadow sediments, for example, because of the refractory character of detritus itself (Danovaro, 1996). This has also been hypothesised for salt marshes (Couch, 1989). Indirect transfer of seagrass carbon would add a trophic step between detritus biomass and copepods, but this trophic step between microbial biomass and copepods would not necessary result in an increase of δ^{15}N as demonstrated for planktonic copepods (Gutiérrez-Rodríguez et al., 2014).

The third hypothesis is that a second indirect routing could happen via the consumption of faeces produced by detritus-feeding macrofauna. Indeed, this compartment is dominated by detritus-feeding amphipods (i.e. *Gammarella fucicola* and *Gammarus aequicauda*) that ingest large quantity of seagrass detritus (Lepoint et al., 2006; Remy 2016). Their faeces, partly digested and enriched by microbes and peritrophic membranes, could be a valuable food source for copepods. Coprophagy is documented in harpacticoid copepods, particularly ones with small mandibular processes (De Troch et al., 2005). Arroyo et al. (2007) observed phytal

harpacticoid copepods swimming around their own faecal mounds, and carrying considerable amount of faecal material within their furcae. This is also a feeding mechanism found in planktonic copepods, particularly in the oligotrophic area where this study was done (Frangoulis et al., 2011).

This third hypothesis would also introduce (at least) one trophic step between seagrass detritus and copepods (Fig 5). Therefore, these three mechanistic hypotheses do not have the same efficiencies in terms of energetic transfer of seagrass material to upper trophic levels (such as macrofauna and fish juveniles). Nevertheless, considering the abundance of copepods in the litter ($10^4 – 10^5$ individuals.m^{-2} or 10-100 individuals.g^{-1}DM) (Mascart et al., 2015b), and the fact they are an important prey for many fish juveniles, this could constitute an important transfer of seagrass C to higher trophic levels. In *Amphibolis* sp. and *Posidonia* spp. detritus accumulations, detrital seagrass material may be specifically fuelled into the food web by harpacticoid copepods, rather than by all detritivores (Hyndes & Lavery, 2005). This highlights the fact that meiofaunal populations are often key players in seagrass organic matter transfer to higher trophic levels, both in seagrass meadow sediments and in detritus accumulations (Danovaro et al., 2002).

To conclude, our data demonstrated that, in this detritus environment, different copepod species and eco-morphotypes feed on various sources and adopt different trophic strategies. The relatively diverse diet was dominated by heterotrophic food items, epiphytes and/or algae, and seagrass detritus. Copepod feeding strategies change over time. This means that the food web associated with litter accumulations is more complex than previously thought, and can change according to seagrass litter composition and availability. Copepods could contribute importantly to the transfer of seagrass organic matter to higher trophic levels, and, as in pelagic ecosystems, constitute a key link between microbial biomass and bigger animals (such as macrofauna and fish larvae or juveniles).

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FIGURE LEGENDS

Figure 1. Pictures of focal copepods: a. *Tisbe furcata*; b. *Ectinosoma dentatum*; c. *Diosaccus tenuicornis*; d. *Clausocalanus arcuicornis*.

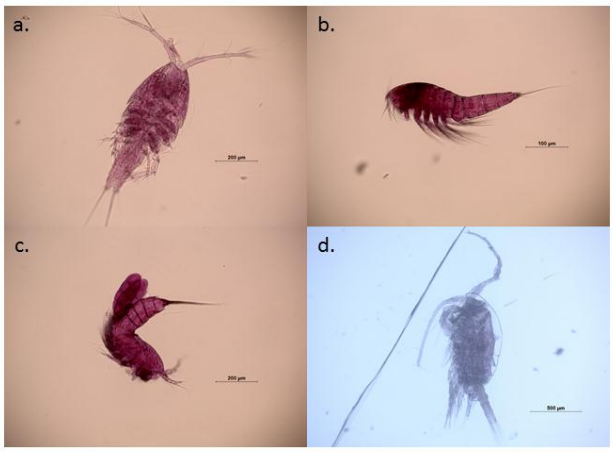


Figure 2. Stable isotope compositions of the four copepod species sampled in macrophytodetritus accumulations. Points are measurements on copepods pools (n = 30-100 depending upon copepod size), and solid lines are bivariate standard ellipses that represent core isotopic niches of consumers. a. February; b. May; c. August; d. October.

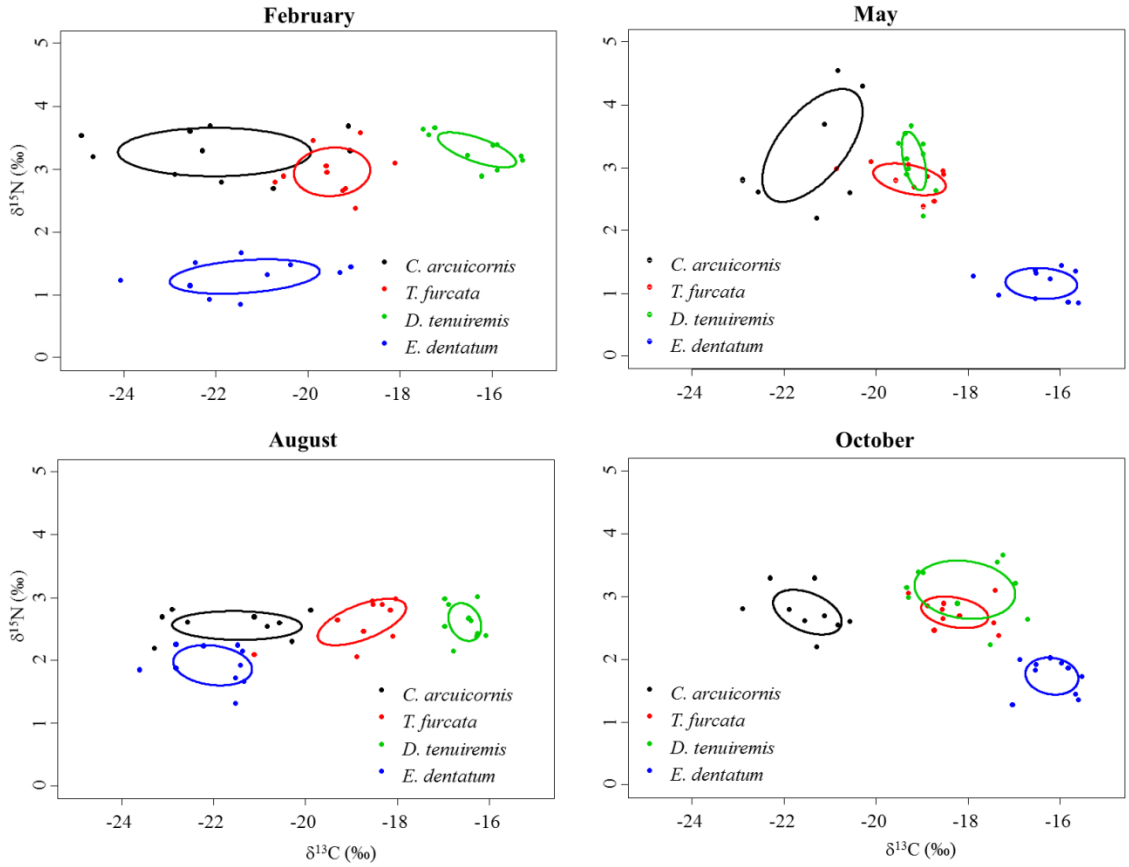


Figure 3. Boxplots of model-estimated bivariate standard ellipse area (SEA_B) for each copepod species in each season. Dark, median and light grey boxes are respectively the 50%, 75% and 95% credibility intervals of the probability density function distributions of the model's solutions, and black dots are the modes of these distributions. Red dots are the SEA_C values associated with each group. a. February; b. May; c. August; d. October.

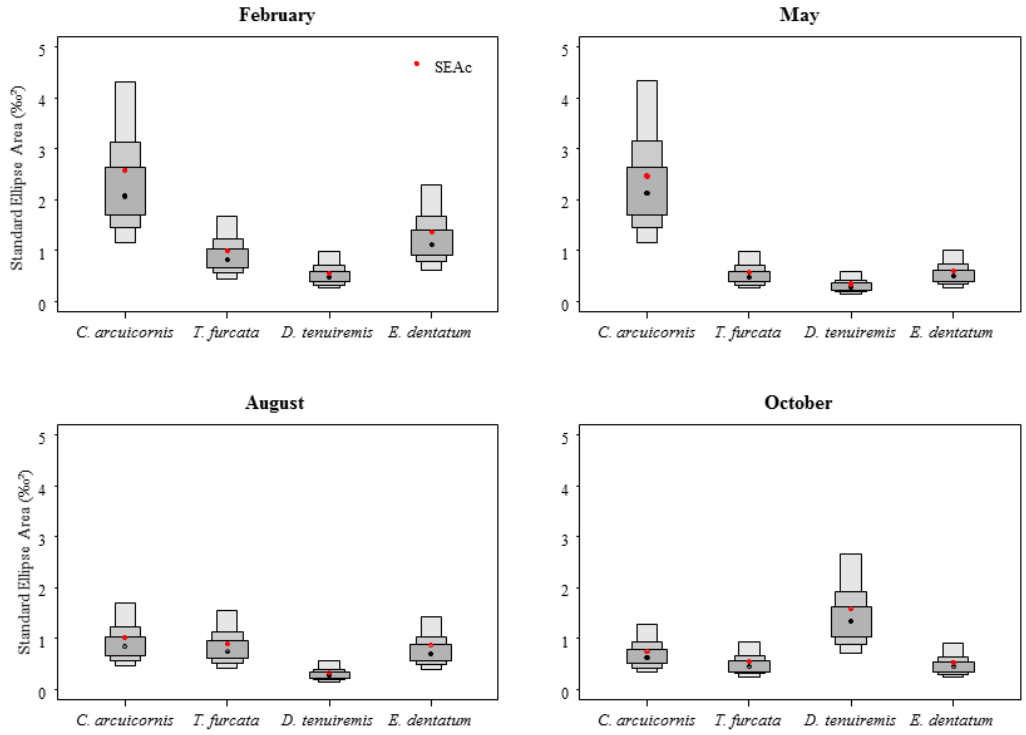


Figure 4. Distribution of posterior probability density function of the relative contribution of macrophytodetritus (MPD), epiphytic and drift macroalgae (EPI), and suspended particulate organic matter (SPOM) for each copepod (line) at each sampling date (column), computed using the SIAR model.

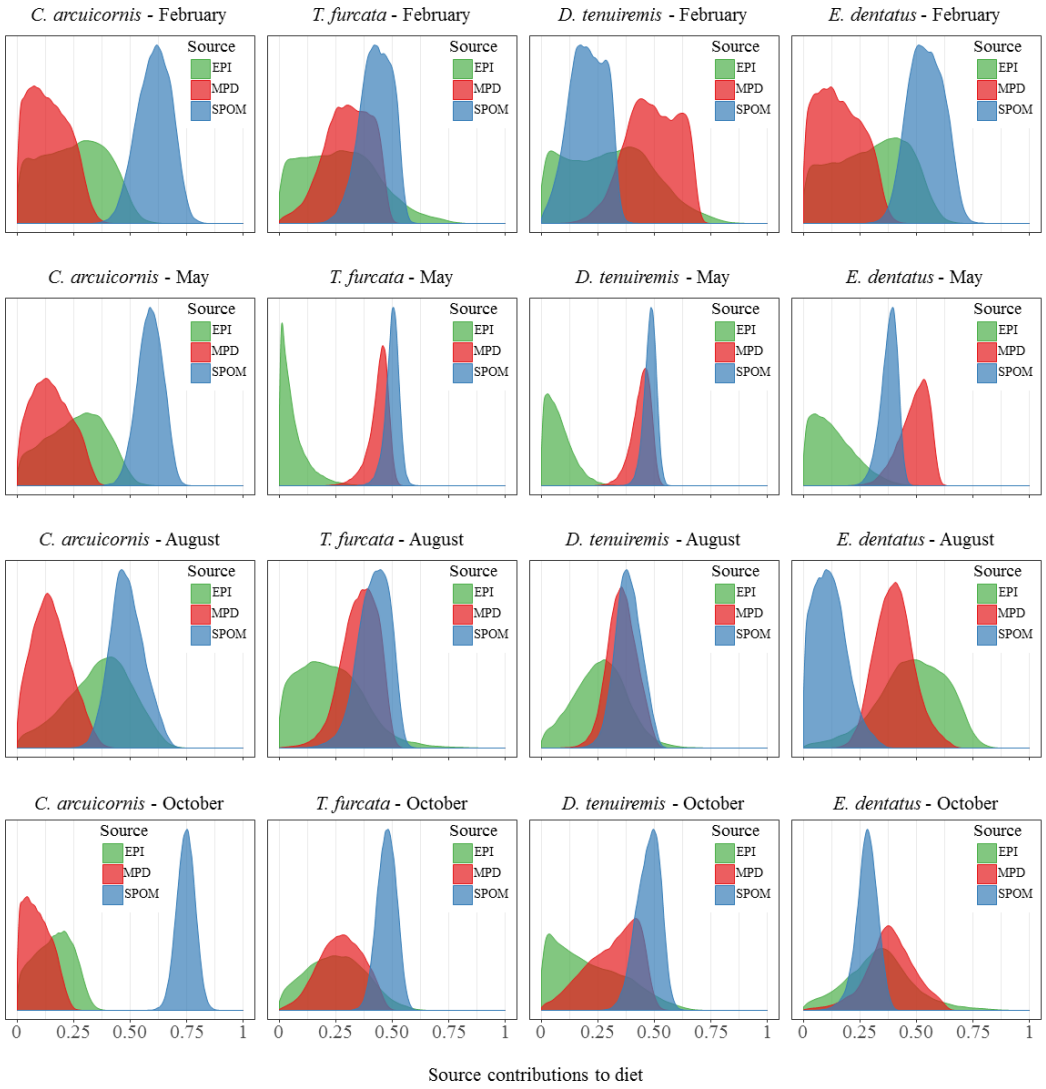
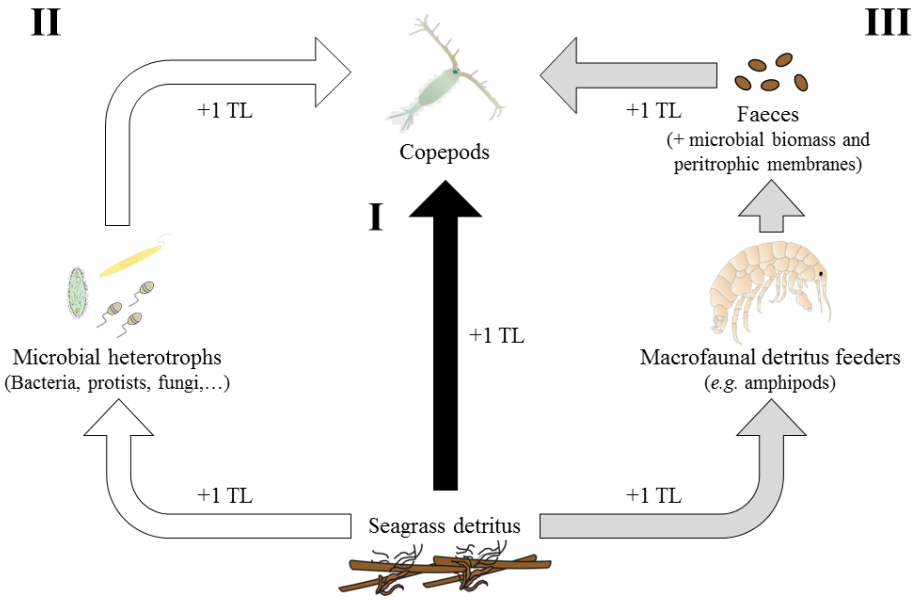


Figure 5. Possible mechanisms explaining the assimilation of seagrass detritus organic matter by benthic copepods. 1. Direct ingestion of fine detritus particles; 2. Indirect assimilation of processed material via microbial biomass; 3. Indirect assimilation of processed material via faeces of detritus-feeding macrofauna. TL: Trophic level



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