Doctoral dissertation

SEAGRASS MACROPHYTODETRITUS:

A COPEPOD HUB

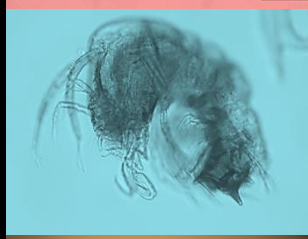
-

Species diversity, dynamics and trophic ecology of the meiofauna community in Posidonia oceanica *leaf litter accumulations*

THIBAUD MASCART





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Marine Biology Ghent University

Seagrass macrophytodetritus: a copepod hub

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Species diversity, dynamics and trophic ecology of the meiofauna community *in Posidonia oceanica* leaf litter accumulations

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Front cover:
Microscopic pictures of harpacticoid copepods. From top to bottom: *Alteutha depressa*, *Phyllopodopsyllus bradyi*, *Paralaophonte brevirostris, Longipedia minor*, *Tegastes falcatus*, *Porcellidium ovatum*, *Laophontodes bicornis* and *Laophonte cornuta.*

Back cover:
Underwater photography of a macrophytodetritus accumulation on a sand patch adjacent to a *Posidonia oceanica* meadow, Calvi, Corsica.

Photographs back cover and between chapters:
Courtesy of underwater photographer Arnaud Abadie

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En mémoire de maman,
car baisser les bras n’est jamais une option.

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Summary

Macrophytodetritus is a heterogeneous mixture of detrital material that accumulates on submerged unvegetated sand patches amid vast Mediterranean *Posidonia oceanica* seagrass meadows. Several vagile invertebrates are present in substantial biomass and biodiversity. Among these invertebrates, meiofauna (fauna between 38µm and 1mm) is ubiquitous and seems to play a key-feature in this dynamic and patchy system. Coastal ecosystems are under the direct effect of anthropogenic disturbance and degradation. Extra research is crucially needed to understand better the dynamics of coastal vegetation, in order to have a more successful restauration of these regressing ecosystems. In this context, the main goal of this PhD research was triple: (1) characterising *in situ* the physico-chemistry and the composition of the macrophytodetritus accumulations in the Calvi Bay, Corsica, (2) identifying the diversity of the associated meiofauna communities, especially harpacticoid copepods together with unravelling the origin of the present copepods and (3) characterizing the trophic ecology of the copepod communities in the macrophytodetritus at the specific and eco-morphological level.

This research showed that macrophytodetritus biomass is composed on average for 75% of dead *P. oceanica* seagrass leaves shed after senescence. Attached to the surface of the seagrass leaves numerous micro- and macroepiphytes are present, representing on average 10% of the total biomass. The remaining part is mainly constituted of drift material, like detached *P. oceanica* shoots and epilithic macroalgae. A seasonal pattern is observed regarding the amount of accumulated material and the physico-chemical composition inside the accumulation. Wind-induced hydrodynamics is the responsible driver behind the variability of the macrophytodetritus and consequently it has a major impact on the faunal communities already present in a macrophytodetritus accumulation.

Previous studies showed that the presence of macrofaunal invertebrates (> 1 mm) in high amounts contributes to the degradation of the detritus. Similarly, this study proves the ubiquitous presence of meiofauna in macrophytodetritus. Depending on the season, densities from 20.10^3 to 160.10^3 meiofaunal organisms per square metre of accumulation were recorded. Copepods were the most abundant taxon (> 50%) of which 87% belonged to the order Harpacticoida. Nematodes were the second most abundant taxon, representing on average 18% of the total meiofauna densities. A total of 61 copepod species were found in Calvi Bay macrophytodetritus accumulations and adjacent habitats (bare sand, seagrass and water column), wherefrom 85% were shared amongst these habitats, underlining the high colonization capabilities of copepods. Active colonization occurred within 24h through species-specific dispersal pathways. Certain species were more avid to colonize, resulting in a colonizer-competitor trade-off among the copepod community. Eco-morphological characteristics seemed to be responsible for the dispersal potential. However, the variety of the composition of the copepod community suggested that other factors also contributed to the attractiveness of the structurally complex macrophytodetritus habitat.

The isotopic niches of four abundant copepod species, representing four different eco-morphological groups were identified: *Ectinosoma dentatum* (mesopsammic-type), *Diosaccus tenuiremis* (phytal-type), *Tisbe furcata* (epibenthic-type) and *Clausocalanus arcuicornis* (water-column-type). Based on stable isotope analysis, fatty acid profiling and Bayesian mixing model, results suggested an interspecific diversity which would indicate a species-specific resource partitioning. *C. arcuicornis* mainly fed on suspended organic matter, while *D. tenuiremis* thrived mainly on epiphytes (mostly diatoms). *E. dentatum* was dependent on the seasonal availability of food sources, while *T. furcata* fed on a heterogeneous mixture of sources. Presumably none of the species directly assimilated dead seagrass leaf litter.

Overall, by combining *in situ* sampling, novel mesocosm experiment, biomarkers and mixing models, this study displayed the carrying capacity of macrophytodetritus to support a large amount of meiofauna and a wide diversity of copepod species. The morphological differences among copepod species seem to allow specialization towards habitat preferences, (physical habitat preferences and colonization potential) and towards resource preferences (food partitioning). Macrophytodetritus seems thus to be a suitable home, or a temporary hub for a diverse copepod community. Finally, this dynamic and patchy habitat, prone to swift changes and situated at the crossing of different ecosystems, plays a major role in coastal ecology.

Résumé

Les macrophytodetritus (ou litière) sont un mélange hétérogène de matériel détritique qui s'accumule sur des zones de sable sans végétation au milieu de vastes herbiers submergés de *Posidonia oceanica* en Méditerranée. Plusieurs invertébrés vagiles sont présents en importantes quantités. Parmi ces invertébrés, la meiofaune (faune entre 38 microns et 1 mm) est omniprésente et semble jouer une fonction clé dans cet habitat dynamique. Les écosystèmes côtiers sont sous l'influence directe de perturbations anthropogéniques et de dégradation. Des recherches supplémentaires sont cruciale pour mieux comprendre tous les compartiments de la végétation côtière, afin d'avoir plus de succès l'ors de restauration de ces écosystèmes en régression. Dans ce contexte, l'objectif principal de cette thèse de doctorat était triple: (1) la caractérisation *in situ* de la physico-chimie et la composition des accumulations de macrophytodetritus en baie de Calvi, Corse, (2) l'identification de la diversité de la communauté de meiofaune associée, en particulier les copépodes harpacticoides en retraçant leur et (3) la caractérisation de l'écologie trophique des communautés de copépodes présents dans les macrophytodetritus au niveau spécifique et éco-morphologique.

Cette étude à montrer que les macrophytodetritus sont composés en moyenne de 75% des feuilles mortes de *Posidonia oceanica* provenant d'une senescence naturelle. Associé à la surface de la feuille de nombreux micro- et macroepiphytes sont présents, représentant en moyenne 10% de la biomasse totale. Du matériel en dérive, principalement arraché, comme des pousses de *P. oceanica* et des macroalgues épilithique forment les principaux composés restants. Un cycle saisonnier est observé à l'égard de la quantité de matière accumulé, ainsi que des compositions physico-chimiques dans la litière. L'hydrodynamisme induit par le vent est la force principale lié aux variabilités des accumulations et en conséquent a un

impact majeur sur les communautés fauniques présents dans les accumulations de litière.

Des études antérieures ont montré la présence de quantités élevées d'invertébrés de macrofaune (> 1mm) qui contribuent directement à la dégradation de la litière. De même, cette étude prouve la présence omniprésente de la meiofaune dans les accumulations de macrophytodetritus. Dépendant de la saison, des densités de 20.10^3 à 160.10^3 organismes meiofauniques par mètre carré d'accumulation ont été enregistrées. Les copépodes sont le taxon le plus abondant (> 50%) dont 87% des espèces appartenaient à l'ordre, Harpacticoida. Les nématodes sont le deuxième taxon le plus abondant représentant en moyenne 18% des taxa représentés. Un total de 61 espèces de copépodes a été identifié dans les accumulations et habitats adjacents (sable, herbier, colonne d'eau) en baie de Calvi. Jusque 85% des espèces ont été retrouvée dans divers habitats, soulignant les capacités de colonisation de copépodes. La colonisation active à lieu endéans les 24h par des voies de dispersion spécifiques par l'espèce. Certaines espèces de copépodes sont plus avides à coloniser, résultant en un trade-off entre colonisateurs et compétiteurs. Les caractéristiques éco-morphologiques semblaient être à la base du potentiel de dispersion, mais la variété des espèces présentes suggéré que d'autres facteurs contribuent à l'attrait de cet l'habitat, structurellement complexe, de macrophytodetritus.

Les niches isotopiques des quatre espèces de copépodes abondantes représentant quatre groupes éco-morphologique différents ont été identifiés: *Ectinosoma dentatum* (de type mesopsammic), *Diosaccus tenuiremis* (de type phytal), *Tisbe furcata* (de type epibenthic) et *Clausocalanus arcuicornis* (de type colonne d'eau). Suite au analyses d'isotopes stables, de profiles d'acide gras et aux modèles de mélange Bayésien, les résultats suggèrent une diversité interspécifique ce qui indiquerait un partitionnement des ressource spécifiques par espèce. *C. arcuicornis* était principalement alimenté en matière organique en

suspension, tandis que *D. tenuiremis* prospérait sur des épiphytes (surtout des diatomées). *E. dentatum* était dépendant de la disponibilité saisonnière des sources de nourriture, tandis que *T. furcata* était alimenté par un mélange de sources. Aucune des espèces n'a vraisemblablement directement assimilé de la litière morte.

Ainsi, en combinant l'échantillonnage *in situ*, une nouvelle expérience en mésocosme et à l'aide de biomarqueurs ainsi que de modèles de mélange, cette étude doctorale a montré que les accumulations de litière soutiennent une grande densité de meiofauna et de diversité d'espèces de copépodes. Les différences morphologiques entre les espèces de copépodes semblent permettre la spécialisation vers une préférence d'habitat, (préférences physique et par le potentiel de colonisation), ainsi que vers une préférence en matière de ressources (partitionnement nutritionnel). Les macrophytodetritus semblent donc être un nid douillet, ou un hub temporaire pour une communauté de copépodes diversifiée. Par conséquent, cet habitat dynamique et variable, assujetti à des changements rapides et situé au croisement de différents écosystèmes, joue un rôle majeur dans l'écologie côtière.

Samenvatting

Macrofytodetritus is een heterogeen mengsel van verschillend organisch detritus dat zich ophoopt op zanderige zeebodems ten midden van grote *Posidonia oceanica* zeegrasvelden in de Middellandse Zee. Verschillende mobiele ongewervelden komen er voor in aanzienlijke biomassa en biodiversiteit. Onder andere meiofauna (fauna tussen 38µm en 1mm) zijn alomtegenwoordig en lijken een sleutelrol te spelen in dit dynamische en onregelmatige systeem. Kustecosystemen zijn onder de directe invloed van antropogene verstoring en degradatie. Om tot een succesvol herstel en behoud van kustecosystemen te komen, is er extra onderzoek nodig om een beter inzicht te vergaren omtrent alle compartimenten van kustvegetaties. In deze context is het doel van dit doctoraatsonderzoek drieledig: (1) het karakteriseren van de fysico-chemische samenstelling van de macrofytodetritus accumulatie in de baai van Calvi, Corsica (2) het doorgronden van de bijhorende meiofauna gemeenschap, met name harpacticoide copepoden, en hun oorsprong en (3) het ontrafelen van de trofische ecologie van de copepode gemeenschap geassocieerd met het macrofytodetritus op een specifiek en eco-morfologisch niveau.

Did onderzoek heeft aangetoond dat macrofytodetritus biomassa gemiddeld bestaat voor 75% uit afgeworpen dode *P. oceanica* zeegras bladeren. Op deze bladeren bevinden zich vele micro- en macroepifyten, die samen ongeveer 10% van de totale biomassa uitmaken. Losgerukt materiaal, zoals *P. oceanica* scheuten en epilitische macroalgen, vervolledigt de lijst van belangrijkste componenten van macrofytodetritus. Een seizoensgebonden patroon is aanwezig omtrent de hoeveelheid afgezet material en physico-chemische compositie binnen de accumulatie. Wind-geïnduceerde hydrodynamica is de drijvende kracht achter de variabiliteit van het macrofytodetritus en heeft dus een directe impact op de aanwezige gemeenschappen binnenin de macrofytodetritus accumulaties.

Waar eerder onderzoek vooral de aanwezigheid van macrofauna (fauna > 1mm) en hun bijdrage in de afbraak van het detritus heeft aangetoond, documenteert deze studie de alomtegenwoordigheid van meiofauna in macrofytodetritus. Dichtheden van gemiddeld 20.10^3 tot 160.10^3 meiofauna organismen per vierkante meter accumulatie werden geregistreerd. Roeipootkreeftjes waren de meest abundante taxon (> 50%), waarvan 87% tot de orde van de Harpacticoida behoorde. Nematoden waren het tweede meest voorkomende taxon met een gemiddelde van 18% van de densiteit. Een totaal van 61 copepode soorten werd geïdentificeerd in de macrofytodetritus accumulaties en aangrenzende habitats van de baai van Calvi. Hiervan kwam 85% voor in meer dan één habitat. Dit onderstreept het hoge kolonisatievermogen van roeipootkreeftjes. Actieve kolonisatie trad op binnen 24 uur door middel van soortspecifieke verspreidingspaden. Bepaalde soorten waren betere kolonisatoren, waardoor een kolonisatie-competitie trade-off tot stand kwam. Eco-morfologische kenmerken bleken verantwoordelijk te zijn voor het verspreidingspotentieel van de Harpacticoida, hoewel de verscheidenheid aan soorten geassocieerd met macrofytodetritus suggereerde dat ook andere factoren bijdragen aan de aantrekkelijkheid van dit structureel complexe habitat.

Daarom werden de isotopische niches bepaald van vier eco-morfologisch verschillende copepode soorten, allen abundant aanwezig in de macrofytodetritus accumulaties: *Ectinosoma dentatum* (mesopsammisch type), *Diosaccus tenuiremis* (epifytisch type), *Tisbe furcata* (epibenthisch type) en *Clausocalanus arcuicornis* (waterkolom type). De stabiele isotope analyses, de vetzuurprofielen en de Bayesiaanse mixing models doen een interspecifieke diversiteit met soortspecifieke voedselbehoeftes vermoeden. *C. arcuicornis* voedde zich voornamelijk met suspended organic matter, terwijl dit voor *D. tenuiremis* hoofdzakelijk epifyten (meestal diatomeeën) waren. Het dieet van *E. dentatum* varieerde naargelang de beschikbare voedselbronnen in ieder seizoen, en *T. furcata*

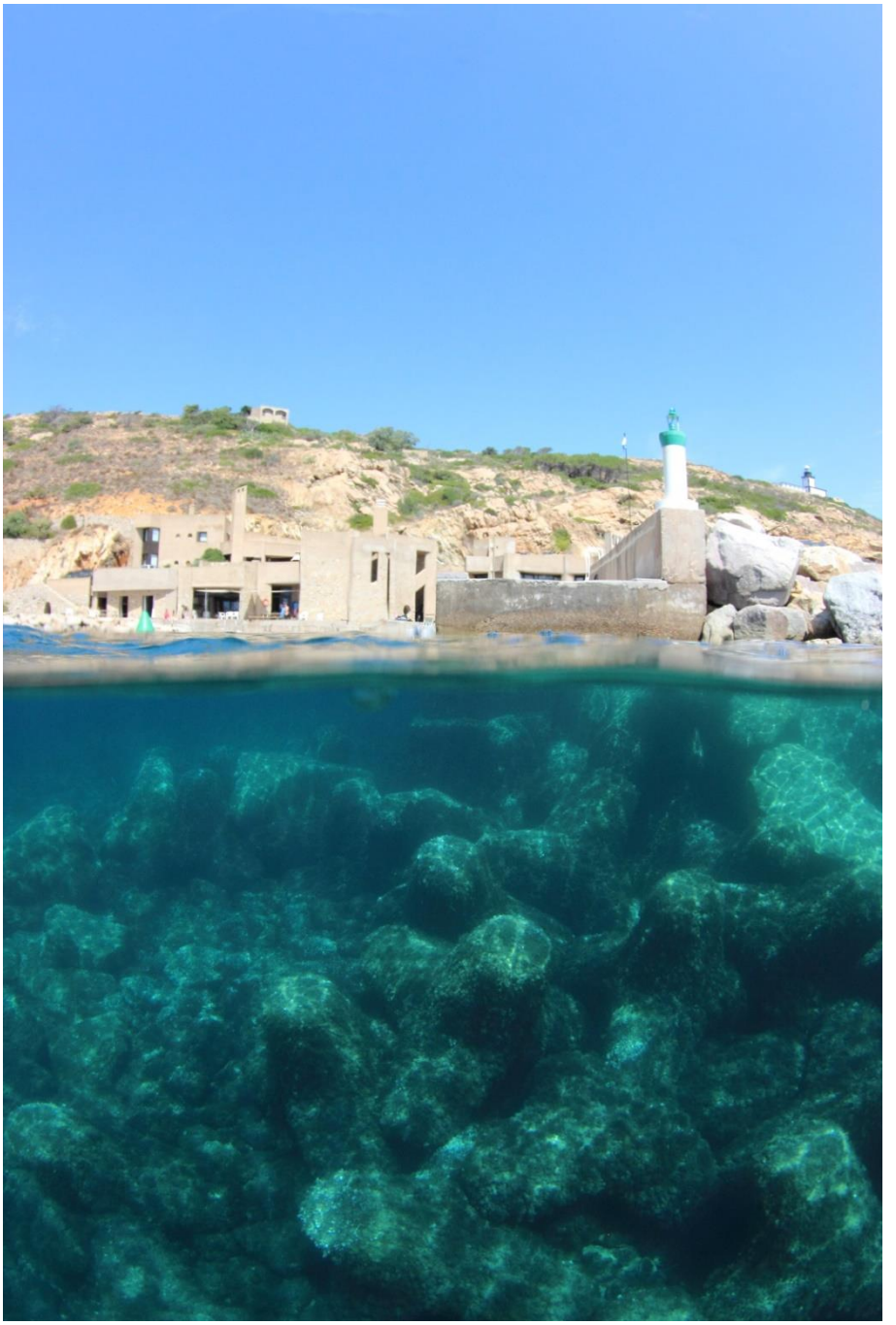
voedde zich met een mengsel van voedselbronnen. Naar alle waarschijnlijkheid gebruikte geen enkele soort rechtstreeks dode zeegrasbladeren als voedselbron.

Door het combineren van *in situ* staalnames, een mesocosm experiment, en het gebruik van biomerkers en mixing modellen, toont deze studie de draagkracht aan van macrofytodetritus voor grote meiofauna densiteiten en een grote diversiteit aan copepoden. De morfologische verschillen tussen copepode soorten laten specialisatie toe naargelang de verschillende habitatpreferenties (fysieke leefomgeving en kolonisatie potentieel), en de verschillende voedselbronnen (voedsel partitionering). Macrofytodetritus lijkt dus een geschikte woonplaats, of een tijdelijke draaischijf voor een gevarieerde copepode gemeenschap te zijn. Hierdoor blijkt dat dit dynamische en onregelmatig verdeeld habitat, gelegen op de kruising van verschillende ecosystemen, een belangrijke rol speelt in kustecologie.

List of abbreviations

1-λ': Simpson's evenness index
ANOSIM: Analysis of similarity
ARA: Arachidonic acid
AU: Approximately unbiased
Aug: August
BP: Bootstrap probability
C: Carbon
CA: *Calanus arcuicornis*
DHA: Docosahexaenoic acid
DISTLM: Distance-based linear model
DM: Dry Mass
DOC: Dissolved organic carbon
DT: *Diosaccus tenuicornis*
ED: *Ectinosoma dentatum*
E: Epibenthic-type
E_H: Heip's evenness
EPA: Eicosapentaenoic acid
EPI: Epiphytes
FA: Fatty acids
FAME: Fatty acid methyl esters
Feb: February
GUI: Graphical user interface
H': Shannon-Wiener diversity index
I: Infaunal colonizers
Indiv.: Individual
J' : Pielou evenness index
M: Mesopsammic-types
MPD: Macrophytodetritus
MUFA: monounsaturated fatty acid

N: Number of individuals / nitrogen
N_1: exp (H')
Oct: October
OSCE: Oscelluccia sampling site
P: Phytal-types
PCO: Principal coordinates analysis
PERMANOVA: Permutational analysis of variance
PORT: Stareso harbour sampling site
PUFA: polyunsaturated fatty acid
S: Number of species / Suspension-colonizers
SD: Standard deviation
SEA: standard ellipse area
SED: Sediments
SAFA: Saturated fatty acid
SI: Stable isotope analysis
SIAR: Stable isotope analysis in R
SIBER: Stable isotope Bayesian ellipse in R
SIMMs: Stable isotope mixing models
SIMPER: Similarity percentage
SOM: Suspended organic matter
TEF: Trophic enrichment factor
TF: *Tisbe furcata*
W: Water-column-types
WC: Water column



Picture: Entrance of the STARESO harbour

Chapter 1

General introduction

1. Preface and research framework

Coastal areas support a large variety of habitats, in comparison to the open ocean. Some of these harbour a very high biodiversity, such as mangroves, coral reefs, salt marshes, macroalgae ecosystems and seagrass meadows (Costanza *et al.,* 1997) providing important resources and services for the coastal human population. For instance: (1) biodiversity promotion, since many economically important organisms use it as feeding, nursery or refuge area; (2) coastline protection, by stabilizing the sediment and reducing the erosive forces of wave action; (3) bio filtration, by uptake of nutrients and other contaminants and finally (4) indirect benefits, such as tourism and fisheries (Pérez-Lloréns *et al.,* 2013; Vassallo *et al.,* 2013). As a negative consequence of human population density, coastal areas are impacted by the direct effect of anthropogenic disturbance and degradation like pollution of chemical contaminants and high amount of nutrients, boat anchoring, coastal aquaculture, trawling fisheries, sediment flow modification, etc. (Orth *et al.,* 2006; Boudouresque *et al.,* 2012). Decline of coastal areas due to human influence has significantly accelerated in the last 150-300 years. Lotze *et al.* (2006) stated that 65% of wetland and seagrass habitats have been destroyed, water quality has decreased significantly, and 90% of marine species have disappeared. For instance, more recent studies estimated that about one-third of the global seagrass area has already been lost and that the losses are accelerating from 0.9% $year^{-1}$ in the 1970's to more than 7% $year^{-1}$ since 2000 (Waycott *et al.,* 2009). In the Mediterranean basin, between 13 to 50% of the areal extent of the most abundant seagrass *Posidonia oceanica* (L.) Delile appears to be lost due to anthropogenic degradation during the last 50 years (Marbà *et al.,* 2014). Therefore, marine biodiversity is a priority for conservation and management (IUCN 2012). Unfortunately, the seagrass species occurring in the Mediterranean are assigned to the "least concern" category of the IUCN

Red list (Short *et al.,* 2011; IUCN 2012). Nonetheless, because of the growing concern about the regression of Mediterranean seagrass habitats, special protection under the auspice of the OSPAR or the EU habitat directive, is considered and restauration measures are taken (see Orth *et al.,* 2006). However, worldwide success of seagrass transplantation and restauration is only around 30%, mostly at small scales (Fonseca *et al.,* 1998). Therefore, next to reducing the cost for large-scale implementation (Duarte 2002), a better understanding of the factors controlling seagrass habitats could bring new insights for more efficient management and conservation strategies. On their turn, these strategies could reduce the trend of biodiversity loss and enhance economical ecosystem services.

Next to the high biodiversity and economical resource potential, coastal areas play a substantial role in the global carbon cycle (Wollast 1998). Together with mangroves (Bouillon *et al.,* 2008; Donato *et al.,* 2011) and salt marshes (Chmura *et al.,* 2003), coastal vegetated areas are sites of high carbon sequestration by sequestering carbon far more effectively (up to 100 times faster) and more permanently than terrestrial forests (Pendleton *et al.,* 2012). These coastal carbon stocks, increasingly referred to as ''Blue Carbon'' (Nellemann *et al.,* 2009), potentially support blue carbon strategies to mitigate anthropogenic CO_2 emissions (Fourqurean *et al.,* 2012; Marba *et al.,* 2015). Although seagrasses cover less than 0.15 % of the world's seabed (Pérez-Lloréns *et al.,* 2013), their net carbon production is estimated at approximately 35 ± 15 to 71 ± 30 Tg C yr^{-1} (average ± 95 % confidence limit) depending on the estimate of the global seagrass area (Duarte *et al.,* 2010). Therefore, ranking among the most productive ecosystems worldwide (Duarte and Chiscano 1999; Fourqurean *et al.,* 2012). A considerable part of the seagrass annual production is buried in marine sediments (Lo Iacono *et al.,* 2008; Duarte *et al.,* 2010), mainly in the form of rhizomes and secondarily as fragmentized leaf litter. This burial sink is estimated at 27-44 Tg C yr^{-1} or 10-18% of all organic seagrass carbon ("blue carbon") on a global scale (Kennedy *et al.,* 2010). The degradation rate and subsequent recycling of this carbon sink depends on

the nutritional quality of the organic matter and the dynamics of associated microbial and detritivores communities. For instance, in terrestrial systems (Hättenschwiler *et al.,* 2005) and mangroves (Alemanno *et al.,* 2007) the quality of the detritus (i.e. elemental composition and presence of refractory molecules) and the presence of detritivores communities are essential in the degradation process. In subtidal ecosystems, such as seagrass beds, the influence of these factors on the degradation rates is poorly understood (Mateo *et al.,* 2006). Macrophytodetritus dynamics is thus a major component of the carbon cycle and often determines the sink or source status of vegetated coastal ecosystems (Bouillon *et al.,* 2008; Champenois and Borges 2012).

This PhD research was conducted in the extensive *P. oceanica* seagrass beds in the Mediterranean Sea and focusses on the macrophytodetritus habitats resulting from the exportation of its primary production. The research project "Implications of *Posidonia oceanica* detritus and its microbial and faunal associated communities in the carbon cycle of a coastal oligotrophic area (FRFC 2.4511.09)" funded by the FRS-FNRS started in 2009 and aimed to obtain more insight into seagrass litter dynamics. Its objectives were triple: (1) to determine faunal diversity, dynamics and trophic interactions in *P. oceanica* detritus accumulations, (2) to evaluate the importance of endogenous (i.e. C/N/P content, age, etc. of litter) and exogenous (i.e. microbial and faunal dynamics and trophic interactions) factors on litter degradation and (3) to measure carbon fluxes associated to litter re-mineralization in order to estimate its importance for the carbon cycle. This PhD study fits within the framework of that research project and focusses on the first objective. The contributions to this project were concentrated on (1) characterising the physico-chemistry and the composition of the macrophytodetritus accumulations, (2) identifying the diversity of the associated meiofauna community, especially harpacticoid copepods together with unravelling the origin of the present copepods and

(3) characterizing the trophic ecology of the copepod communities in the macrophytodetritus at the specific and eco-morphological level.

This PhD research is a joint project between the Laboratory of Oceanology (University of Liège) and the Marine Biology Research Group (Ghent University).

2. Seagrass ecosystems

The term seagrass is defined as all Magnoliophyta growing and reproducing in the marine submerged photic zone (Den Hartog 1970). This ecological definition excludes mangrove, freshwater and saltmarsh higher plants. Seagrasses are descending from terrestrial plant lineages (Tracheophyta) belonging to the herbaceous flowering plants of aquatic and marsh habitats (order Alismatales) (Larkum and den Hartog 1989; Boxshall *et al.,* 2015). These seagrasses are confined to the worldwide shallow coastal areas with the exception of the Antarctic coast. These are found to tolerate fresh water for instance in estuaries and brackish water (Den Hartog 1970; Hemminga and Duarte 2000; Green and Short 2003). Seagrasses encompass 50 to 60 species which are grouped in four families: Zosteraceae, Cymodoceaceae, Hydrocharitaceae and Posidoniaceae. The latter comprises one genus *Posidonia* which includes eight Australian species and the Mediterranean species *Posidonia oceanica*, (L.) Delile, 1813 (Den Hartog and Kuo 2006).

In the Mediterranean, five species forming seagrass meadows are found (Short *et al.,* 2007). Contrary to tropical areas, Mediterranean seagrass meadows are generally monospecific, but some exceptions exist. One of the main climax stage species, endemic to the Mediterranean Sea is *P. oceanica* (Pérez-Lloréns *et al*., 2013). It has a slow growth rate and distinct preferences for sandy substrates (sometimes rocky), forming large underwater meadows (for details see Boudouresque *et al.*, 2006a). The other species, smaller in size and fast growing are seen as pioneers on unvegetated sand or on dead mattes of *P. oceanica* (see further in 3.1). Two of those are *Cymodocea nodosa*, (U.) Ascherson, 1870 and *Halophila stipulacea*, (F.) Ascherson, 1867 (De Troch *et al.,* 2001a; Boudouresque *et al.,* 2009). The latter is an Indo-Pacific species (Gambi *et al.,* 2009) introduced from the Red Sea through the Suez canal (Lipkin *et al.,* 2003). The final two, *Zostera (Zosterella) noltii*, Hornemann, 1832 and *Zostera (Zostera) marina*, Linnaeus, 1753 (Den Hartog 1970) show a preference for

more sandy and sheltered sites (Lipkin *et al.,* 2003; Procaccini *et al.,* 2003).

3. *Posidonia oceanica* ecosystem

3.1. Biology and morphology

The Neptune grass *P. oceanica* is the dominant seagrass in the Mediterranean Sea. It is composed of belowground rhizomes with roots and aboveground leaves. Two types of rhizomes, with two different growth patterns are present: (1) plagiotropic or horizontal in search for space and (2) orthotropic or vertical in search for light. Each end of a rhizome supports through a strong, lignified petiole (base attached to the rhizome) on average 4 to 8 ribbon-shaped leaves, forming a shoot (Fig. 1.1).

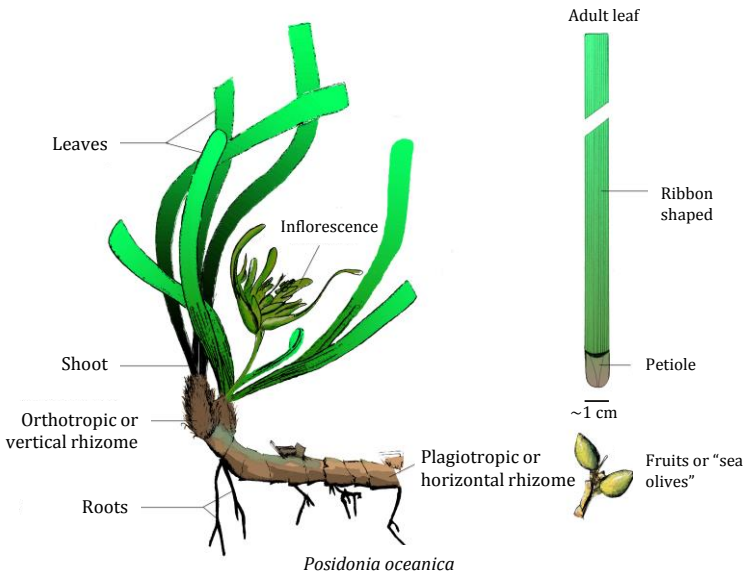


Fig 1.1*: Morphology of Posidonia oceanica, with one horizontal and two vertical rhizomes and one inflorescence as well as two mature fruit (right), called «sea olives». Adapted from pictures in Boudouresque et al. (2012)*

Leaves are on average 9.5 ± 1.5 mm wide (pers. obs.) and 75 cm long (Pérez-Lloréns *et al.,* 2013) with a maximum up to 130 cm (Gobert 2002). New leaves are formed all year long with a succession pattern from the inside to the outside of the shoot (Giraud *et al.,* 1979; Boudouresque and Meinesz 1982). Leaves, often covered with epiphytes are shed all year long, with an acceleration of leaf fall in late spring and, particularly, in autumn. Only the limb (leaf without the basal petiole) is deciduous, consequently a scale (i.e. petiole of the abscised leaf) remains attached to a rhizome. These scales are very resistant to degradation and form a sheath around its shoot (Gobert *et al.,* 2006a). Since dead rhizomes, roots and leaf sheaths are persistent organic materials, this form a "matte" which supports the horizontally growing surface rhizomes and shoots. Matte consists of interlaced living and dead rhizomes or roots, seagrass leaf litter, deposited suspended organic particulate matter and sediment filling the interstices (Gobert *et al.,* 2006a). The growth towards the surface can range several meters in height. "Dead matte" on the other hand is a matte devoid of living seagrass shoot, where the matte reached an eroded peneplain status (a low-relief plain representing an advanced stage of erosion, geological terminology) and consequently the accretion stopped (Romero *et al.,* 1994; Mateo *et al.,* 1997). *P. oceanica* blooms in autumn (September-November), although not every year, especially in the North-Western Mediterranean Sea (Boudouresque and Meinesz 1982; Gobert *et al.,* 2005). The subsequent fruits (1.5 to 2 cm long), called "sea olives" take several months to ripen and by the end of spring (May-June) these are cast off to new colonisable habitats or washed ashore (Molinier and Picard 1952; Den Hartog 1970).

Neptune grass is eurytherm and supports temperatures ranging from 9 to 29°C (optimum around 17-20°C) and optimal salinity around 36-39 (Boudouresque and Meinesz 1982; Gobert *et al.,* 2006a). It forms extensive underwater meadows covering a vast surface to a maximum depth of 40-50m in clear waters. It means that light availability limits the depth distribution (Duarte 1991). The meadow are estimated to cover about 1-2% of the total Mediterranean sea bottom surface, which equals an

estimated surface of 25 - 50.10^3 km^2 (Fig 1.2). The seagrass beds are sculptured by natural events like hydrodynamics (i.e. currents & waves) and water temperature (Marba and Duarte 1997; Borg *et al.,* 2005). The majority of the beds form continuous meadows interrupted by unvegetated sandy sediments or dead matte due to erosive currents or anthropogenic degradation (Molinier and Picard 1952).

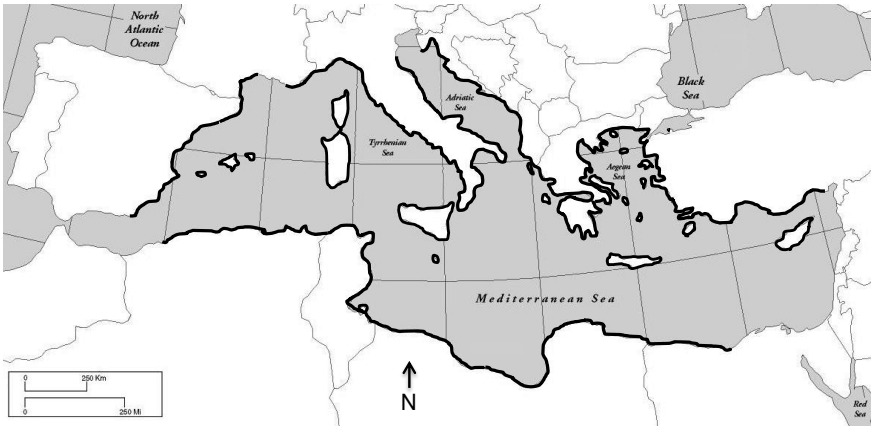


Fig 1.2*: Geographical distribution (Solid black line) of Posidonia oceanica, adapted from Michel (2011) using data from Lipkin et al. (2003); Procaccini et al. (2003); Boudouresque et al. (2012); Gobert et al. (2006a) and Meinesz et al. (2009).*

P. oceanica meadows provide important economic and ecological ecosystem services by harbouring highly diverse faunal and floral communities (Costanza *et al.,* 1997; Duarte 2000; Vassallo *et al.,* 2013). The meadow prevents, amongst others, coastal erosion, provides food for herbivores and detritivores, offers nursery areas, facilitates the accumulation of particulate and dissolved organic matter in sediments, increases the diffusion of oxygen through the rhizomes in the sediments and has an essential position in the biogeochemical carbon cycle (Gobert *et al.,* 2006a; Duarte *et al.,* 2010).

3.2. Fate of primary production

Seagrass meadows have very high levels of primary production, owing to the high turnover of seagrass leaves themselves and their associated epiphytes (Orth *et al.,* 2006). Nevertheless, slow growing *P. oceanica* displays a lower average above ground production compared to smaller and faster growing *Z. noltii*, of respectively 392 gC m^{-2} $year^{-1}$ and 875 gC m^{-2} $year^{-1}$ (see Pérez-Lloréns *et al.*, 2013). Conceptual models have been generated to quantify the fate of the primary production of seagrass ecosystems (e.g. Zupo *et al.*, 1997; Duarte and Chiscano, 1999; Elkaley *et al.*, 2000; Duarte *et al.*, 2005; Mateo *et al.*, 2006).

The majority of these models and contemporary updated models (Hyndes *et al.,* 2014) highlight the potential fates, but mainly quantitatively estimate the net primary production of the community (Fourqurean *et al.,* 2012; Pendleton *et al.,* 2012). In the light of the "Blue Carbon" coastal carbon stock story (Nellemann *et al.,* 2009), seagrass meadows are seen as carbon sinks. In other words, that the gross primary production is higher than the respiration rate, reflecting a net primary production and therefore driving seagrass ecosystems to be autotrophic communities (Duarte *et al.,* 2010; Kennedy *et al.,* 2010; Champenois and Borges 2012). Nevertheless, when compiling several estimates, 30-50% of the global seagrass primary production is buried *in situ* and preserved over long timescales in the sediment (Mateo *et al.,* 1997; Kennedy *et al.,* 2010). Consequently, the remaining fraction of the production is available for consumption and channelling through the food web. However, seagrasses are relatively recalcitrant for direct consumption by herbivores, since tissues have relatively high C/N/P ratios with median values of 474/24/1 (Duarte 1990) and high lignin and phenolic contents (Cebrian *et al.,* 1996; Agostini *et al.,* 1998). Hence, direct grazing is only performed by a few specialized herbivores (Valentine and Heck 1999; Peirano *et al.,* 2001), e.g., a sea urchin, *Paracentrotus lividus* (Tomas *et al.,* 2006) and a sparid fish *Sarpa salpa* (Cebrian *et al.,* 1996; Havelange *et al.,* 1997) graze on *P. oceanica*. As a

result, 5-20 % of the primary production is channelled via direct herbivory. Similar amounts have been found for numerous *P. oceanica* seagrass meadows (Mateo and Romero 1997; Pergent *et al., 1997;* Luque del Villar and Templado, 2004, Fig 1.3). However, this grazing estimate can be underestimated due to inadequate indirect measurement technique and variability in time and space (Heck and Valentine 2006). The grazing variability lays in the high levels of functional redundancy present, the effects of nutritional quality and chemical defences on the consumption patterns of diverse types of herbivores and their interaction the plants.

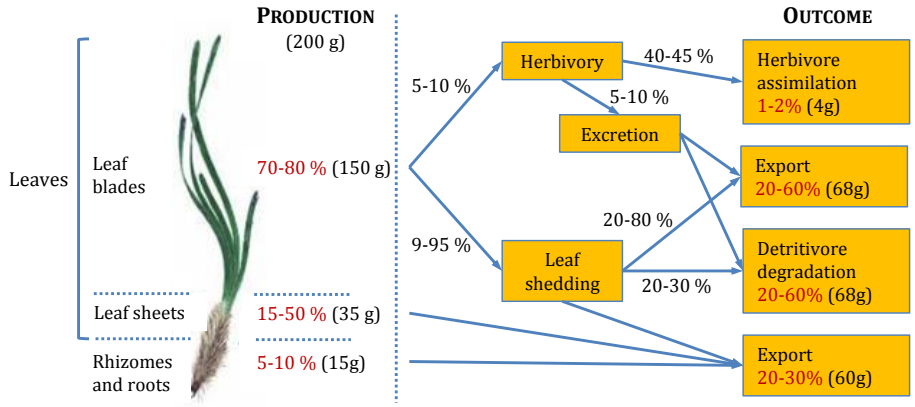


Fig 1.3*: Fate of P. oceanica primary production. Adapted from Luque del Villar and Templado (2004).*

Moreover, most models neglect or underestimate the contribution of associated epiphytic algal communities. These can contribute up to 30-40% of the total canopy biomass (Mazzella and Ott 1984; Hemminga and Duarte 2000) and up to 50% of the meadows' productivity (Borowitzka *et al.,* 2006). For that reason, epiphytes play an important trophic role sustaining a wide range of grazing organisms such as fishes and small invertebrates that in turn sustain larger consumers (Valentine and Duffy 2006; Marco-Méndez *et al.,* 2015).

Thus far, the fate of a large fraction of the production (10-60%) is unaccounted for and seen as either degraded within the meadow or

exported to adjacent habitats where it will eventually decompose (Duarte and Cebrian 1996; Mateo *et al.,* 2006). This organic carbon fuels the detrital pool under the form of leaf litter (Heck *et al.,* 2008). The leaf litter and its attached epiphytes are seen as important trophic subsidies for food webs since degraded material may be more accessible and the epiphytes, compared to fresh leaf material, possess a greater nutritional value (Dethier *et al.,* 2014). Seagrass detrital decomposition and burial outside the meadow contribute to the sequestration of "blue carbon" along the burial of rhizomes inside the meadow. The absolute exported decaying mass represents an enormous transfer of organic carbon and nutrients to the coastal ocean that sustains a wide array of consumers (Duarte *et al.,* 2005; Nellemann *et al.,* 2009). Furthermore, decomposing tissues of seagrasses leach dissolved organic carbon (DOC), which feeds bacterial communities (Velimirov 1986; Barron *et al.,* 2014) and supports the microbial food web (Peduzzi and Herndl 1991).

4. Macrophytodetritus decomposition regulators

In terrestrial environments decomposition of plant litter is considered as a two-staged process (Aerts 1997; Hättenschwiler *et al.,* 2005). Firstly, litter is mechanically fragmented by climate and detritivores (Hunter *et al.,* 2003). Secondly, microorganisms (bacteria and fungi) reduce and mineralize the small pieces into basic inorganic molecules (Romani *et al.,* 2006). The decomposition rate is regulated on three levels: environmental conditions, soil organisms and chemical composition of the litter (Barajas-Guzman and Alvarez-Sanchez 2003; Gartner and Cardon 2004; Cornwell *et al.,* 2008; Chapman and Newman 2010). In woodland streams, leaf litter associated algal biofilm assemblages' vary according to different light intensities affecting associated detritivores biomass (Friberg and Jacobsen 1994; Franken *et al.,* 2005). Therefore, considering the quality of litter leaves as food for detrital organisms, one should take into account both the

leaf and its attached biofilms (Cummins and Klug 1979).

In marine environments similar regulators of decomposition rate apply: hydrodynamics, detritivore communities and chemical composition of the macrophytodetritus, including microbes (e.g. Peduzzi and Herndl 1991; Mateo and Romero 1996).

4.1. Effects of hydrodynamics on macrophytodetritus

Leaves of *P. oceanica* with their associated epiphytes are shed all year long, with an acceleration of leaf fall in late spring and, mostly, in autumn (Cebrian *et al.,* 1997; Mateo and Romero 1997). The shed leaves stay within the canopy of the meadow and decay *in situ*. After the decay, leaves from late summer and early fall are usually exported out of the meadow by the fall and winter storms (Lepoint *et al.,* 2006; Champenois and Borges 2012). The generated macrophytodetritus accumulation is mainly composed of senescent or detached seagrass leaf litter, drift epilithic macroalgae, uprooted living seagrass shoots with rhizomes, seeds, dead macrofauna, faecal pellets and fine sediment (Lepoint *et al.,* 2006; Mascart *et al.,* 2015b). The resulting macrophytodetritus accumulations can thus provide shelter and food resources, that otherwise are not available. The exported leaf litter can end-up in (1) adjacent sandy areas, (2) deep-sea systems or as (3) supra-littoral beach-cast litter "banquettes" (Hyndes and Lavery 2005; Heck *et al.,* 2008), hence, modifying the habitat function of these unvegetated areas (Lenanton *et al.,* 1982; Robertson and Lenanton 1984).

Most leaf litter (10-60% of the primary production) will be deposited inside the meadow or will be exported to adjacent sand patches. The intensity of the hydrodynamic flow and bottom morphology are the major forces driving the exportation (Vetter and Dayton 1999). Besides the adjacent exportation both factors regulate the physical degradation of the macrophytodetritus and their exportation over greater distances down to submarine canyons (Vetter and Dayton 1999) or up to supra-littoral

beaches forming "banquettes" (Pergent *et al.,* 1997; Cebrian and Duarte 2001; Mateo *et al.,* 2003). The beach cast detritus will stabilize shorelines by reducing wave action and erosion (Simeone and De Falco 2012). Depending on the exposure, the beach wrack might be fragmented by beach organisms (e.g. talitrid amphipods) and be decomposed by microbes (Kirkman and Kendrick 1997; Ince *et al.,* 2007; Cardona and Garcia 2008). In contrast, the deposition can be temporary and the macrophytodetritus, under the influence of the wind and tides can return to the sea to a submerged state.

4.2. Macrophytodetritus nutritional composition

In order to identify the composition of shed *P. oceanica* leaf litter, one must look at the formation of living leaves. The dynamics of light intensity, water temperature and nutrient (un)availability act as factors for the leaf production (Alcoverro *et al.,* 1995; Marba *et al.,* 1996). The North-Western Mediterranean Sea and especially the Revellata Bay (Calvi, Corsica) where this work was done are considered as a typical oligotrophic area with a low nutrient availability and low planktonic primary production (Estrada 1996; Lepoint *et al.*, 2004; Romero *et al.*, 2006). Nevertheless, *P. oceanica* has developed strategies (Hemminga *et al.*, 1999; Lepoint *et al.*, 2002b) in order to cope with the low nutrient concentrations and therefore present very high production values (Gobert *et al.,* 2002). Aside from biomineralization of the sediments, nutrient re-mobilization prior to abscission of adult leaves (Lepoint *et al.*, 2002a) is the main strategy used. Subsequently, shed litter leaves are from the start depleted in nitrogen and phosphorus compared to old senescent leaves (Mateo and Romero 1996). Furthermore, it could be assumed that the decomposition rate could be correlated to the nutrient content of the leaf. Enriquez *et al.* (1993) found no correlation between the nutritional quality of the litter and the decomposition rate. However, Mateo and Romero (1996) demonstrated a

faster decay of old living leaves compared to shed litter. Litter contains more refractory materials (C/N = 35-40) than senescent leaves (C/N = 17-25) making it nutritionally less attractive for bacteria (Godshalk and Wetzel 1978). This was confirmed by a more recent study by Apostolaki *et al.* (2009) where decomposition was, next to the origin of the seagrass litter, related to the nutrient availability of seagrass tissue, pore water and sediment organic matter.

4.3. Macrophytodetritus detritivores community

Two communities should be differentiated in association with *P. oceanica* litter: the sessile and vagile communities (Lepoint *et al.,* 2006).

The sessile epiphytic community

An abundant and diversified community (i.e. bacteria, protists, fungi, plants, animals) lives fixed on the detritus leaf surface. This epiphytic community are not necessarily new colonizers of the litter surface, as these could already be present on the shed senescent leaf, and therefore are exported together with its substrate. Temporal variability and succession are present in epiphytic living leaf communities (see Borowitzka *et al.*, 2006 and Gobert *et al.*, 1995). Consequently, on shed leaf litter, microepiphytes can also be seen as settlement pioneers and the biofilm these create is mainly composed of diatoms (*Cocconeis* spp.), bacteria (rod, vibrionid, coccoid-shaped and filamentous) and marine filamentous unseptate fungi (Lepoint *et al.*, 2006). Similar microepiphytic communities are found in other seagrasses (Fenchel 1970) and mangrove litter (Gatune *et al.*, 2012). It is similar to terrestrial submerged macrophytes litter where microbial biofilms mainly consist of fungi and bacteria (Kominkova *et al.*, 2000; Findlay *et al.*, 2002). Macroepiphytes are composed of crustose and erected macroalgae, Hydrozoa, Bryozoa and sedentary polychaetes, however not as abundant as in the adjacent meadow canopy (Gambi *et al.*, 1992; Lepoint *et al.*, 1999; Lepoint *et al.*, 2014).

The vagile associated faunal community

Vagile associated fauna are primary consumers, secondary consumers or detritivores and consequently play a key role in the transfer of organic matter in the food chain. Several size classes (for instance meiofauna: 38 µm – 1 mm; macrofauna: > 1 mm and megafauna > 10 mm) are well represented in the accumulations (see Mascart 2010; Remy 2010). Megafauna, is most often referred to as juvenile and adult fishes, echinoderms and crustaceans. Macrofauna reside in the macrophytodetritus and they predate on meiofauna (Gibbons 1988). Next to fishes, invertebrates like decapods or holothurians or ophiuroids are present in the accumulations. Holothurians mainly feed on *P*. *oceanica* detritus (Lepoint *et al.*, 2000). Other megafauna seem to feed on a wide variety of food sources present in the detritus going from seagrass sheaths to meiofaunal nematodes and macrofauna (Gambi 2002; Ha *et al.*, 2014). Several studies investigated various macrofaunal crustaceans (e.g. amphipods and isopods) in the submerged *P. oceanica* litter (Gallmetzer *et al.*, 2005; Dimech *et al.*, 2006; Lepoint *et al.*, 2006; Sturaro *et al.*, 2010). Most encountered macrofauna species are present in the living seagrass canopy or macroalgae biota, however, in different assemblage compositions and diversities (Como *et al.*, 2008; Michel *et al.*, 2014). Smaller meiofaunal organisms are conjointly omnipresent and are dominated by a few taxa, mainly copepods and nematodes (Danovaro 1996; Mascart *et al.*, 2013). In marine detrital systems those meiofauna are seen as opportunistic and are able to utilize a wide variety of food sources sustaining rapid turn-over rate (Warwick 1987). Meiofauna have been shown in high densities on decaying mangrove leaves (Gee and Somerfield 1997; Torres-Pratts and Schizas 2007) utilizing the biofilm as a food source (Faust and Gulledge 1996; Gwyther 2003). In salt-marshes these seem to have a predominant role in the early phase of decomposition (Sanmarti and Menendez 2007).

5. Meiofauna and Copepoda

Derived from the Greek word "μείοσ" meaning "smaller", meiofauna are subjectively defined by size range (<1 mm) (Mare 1942) and consist of a taxonomically diverse group of metazoans smaller than macrofauna and larger than microfauna (Higgins and Thiel 1988). In other words these organisms pass through a 1 mm mesh sieve and are retained on a 38 μm mesh sieve. This arbitrary definition implies that some species are members of the "permanent meiofauna" and others belong to the "temporary meiofauna" as their immature stages fall within the meiofauna and the sexually mature stage belongs to the larger macrofauna size class. The permanent fauna is composed of various higher taxa amongst which Nematoda and Copepoda are the most abundant (Holme and McIntyre 1984; Coull 1987). Habitat-specificity and regulation by abiotic (e.g. physical disturbances) and biotic factors (e.g. ontogeny and food availability) are crucial factors in the distribution of meiofauna (Hicks 1979; Hicks 1984; MacIntyre *et al.,* 1996; Middelburg *et al.,* 2000). In marine and freshwater sediments, where the majority of the meiofauna live in the interstitial spaces (Giere 2009), characteristics of the granulometry are important.

5.1. Harpacticoida

In seagrass beds, harpacticoid copepods are the dominant taxon on the phytal leaf substratum (Hicks 1977b; Novak 1982; De Troch *et al.,* 2003; Giere 2009). Those meiobenthic copepods (for details on their taxonomic position see Addendum II) are mainly free-living species and comprise well over 4500 species (Boxshall and Hasley 2004; Wells 2007). Their body morphology is diverse (Fig 1.5) and well-adapted to their environment and life-mode (Noodt 1971; Bell *et al.,* 1987). Several basic forms or 'Lebensformtypen' (Remane 1952) can be distinguished in relation to a

particular habitat, for instance: interstitial (living in sandy sediments), burrowing (muddy sediments) and epiphytic (phytal). Species adapted to interstitial spaces in fine sediment are cylindrically elongated to vermiform with minute appendages and are mostly of small size: e.g. *Leptostacus, Leptopontia*. Species adapted to muddier substrates can be characterised by large dorsal spines, spinous appendages and thick body shields: e.g. *Ancorabolis*, *Laophonte, Cletodes, Rhizotrix*. Slender and spindle shaped copepods without strongly reduced appendages or spines are found in sediments and in phytal environments: e.g. *Ameira*, *Ectinosoma*. Species adapted to the phytal environment are diverse: dorso-ventrally flattened with a short and broad body (e.g. *Alteutha*, *Porcellidium, Sacodiscus*), laterally compressed of an unusual shape (e.g. *Tegastes*) and with an elongated body, somewhat tampering caudally, mostly of a bigger size than the previous with an extensive cephalothorax (e.g. *Amphiascus, Stenhelia, Diosaccus, Thalestris, Harpacticus*). The mentioned phytal species can also be found on mud and in the lowest part of the water column. The last group, mostly droplet shaped can be seen as really epibenthic with active emergence capabilities and good swimming capacities (e.g. *Tisbe*).

This makes the specificity of the community assembly remarkable, yet similar in different parts of the world since most families occur worldwide from littoral to deep-sea bottoms (Hicks and Coull 1983; Coull 1987). The concept of parallel ecological communities or 'isocommunities' is supported for meiofauna in sandy micro-tidal beaches (Gheskiere *et al.,* 2005). In the phytal community, two categories should be taken into consideration (Hicks 1977c): a true phytal type, clinging to the foliar substrate (mostly dorso-ventrally flattened or laterally compressed), and an epibenthic dwelling type, seemingly exhibiting better swimming abilities (Bell *et al.,* 1988; Hicks 1988). Not only epiphytic copepods possess well-developed swimming abilities. Some interstitial species have been found to actively emerge into the water, especially at night and in calm waters (Armonies 1988). A wide species-specificity is present in copepods and therefore to gain a deeper understanding of the mobility

behaviours of harpacticoids, experiments should be performed (Kurdziel and Bell 1992; Boeckner *et al.,* 2009; Arroyo *et al.,* 2013).

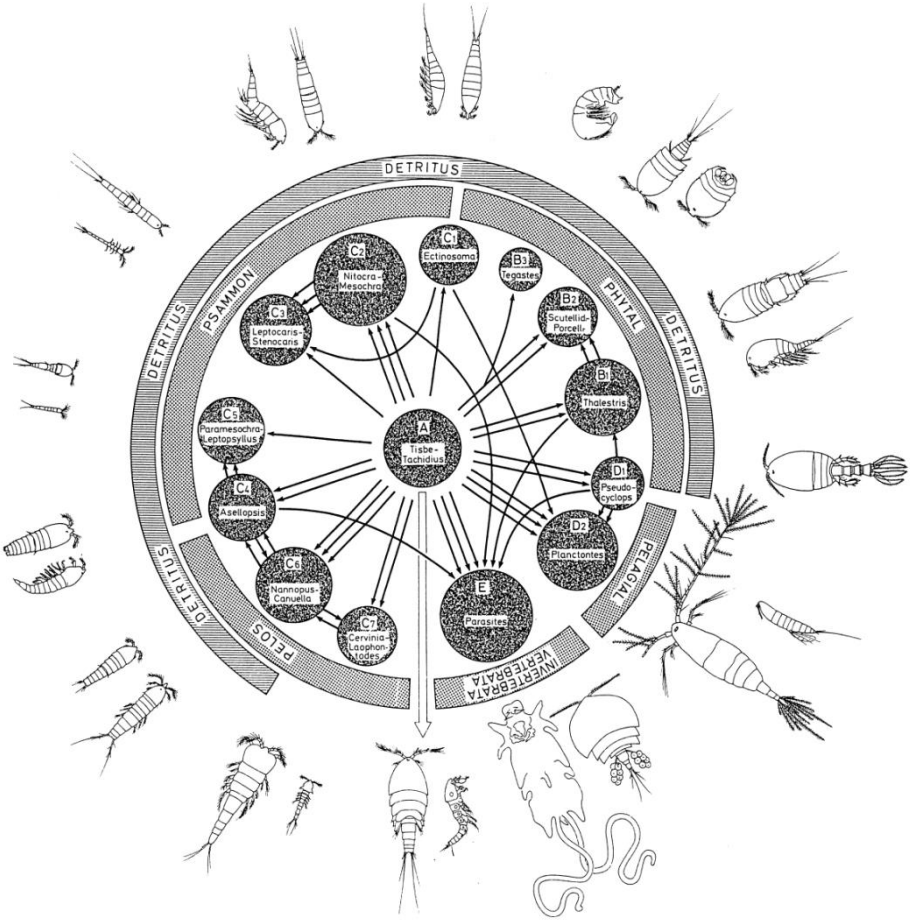


Fig 1.5: *Scheme of interrelationships between the most important types of morphological adaptations of the Copepoda with each type classified within its presumed preferred habitat from Noodt (1971). Pelos-types are associated to muddy substrates; Psammon-types are associated to sediments; phytal-types are associated to submerged vegetation; pelagial-types are associated to the water column; invertebrate/vertebrata-types are parasitic species and the central-type with the arrow is seen as the ancestral form in copepod evolution and is associated to an epibenthic life style. See Fig 5.2B for microscopic pictures of 4 abundant copepods belonging to different eco-morphological groups.*

Besides harpacticoids, the orders of Calanoida and Cyclopoida display

migrational and resettling behaviour. Vertical migration occurs following a diurnal pattern, with an abundance decrease among the seagrass canopy or upper sediment layer at night (Walters 1988; Sanchez-Jerez *et al.,* 1999a). Consequently, even though calanoids and cyclopoids are widely seen as exclusively planktonic, freshwater cyclopoids live epibenthically among macrophytes. Therefore, it is common to encounter cyclopoids or calanoids in the benthos. The buccal morphology of harpacticoids reveals a selective grazing on bacteria, protozoa and especially diatom cells derived from a substrate surface (Coull 1999; De Troch *et al.,* 2005a). Nevertheless, sinking planktonic diatoms, bacterial exudates, faecal pellets and sometimes also carcasses are on the menu (Decho and Fleeger 1988; Decho and Moriarty 1990; Seifried and Durbaum 2000; Dahms *et al.,* 2007; Gallucci and Ólafsson 2007; Frangoulis *et al.,* 2011). Moreover, particular feeding modes such as cannibalism (Daan *et al.,* 1988; Gallucci and Ólafsson 2007) and bacterial farming on faecal pellets (De Troch *et al.,* 2009; De Troch *et al.,* 2010) occur. Harpacticoid copepods are known to locate food by means of water-borne chemical cues at distances of many body lengths (Fechter *et al.,* 2004).

Trophic competition amongst benthic copepods has not yet been empirically demonstrated (Pace and Carman 1996; De Troch *et al.,* 2005a) in contrast to pelagic copepods (Guisande *et al.,* 2002; Laakmann *et al.,* 2009). Observations on congeneric benthic species (Vanden Berghe and Bergmanns 1981) suggested resource partitioning of photoautotrophic (herbivore) and bacterial food sources (bacterivore). However, food preferences in close relationship to food distribution patterns seem to stimulate trophic specialization. The species-specific induced preference niche can lead to a microscale distribution that will change according to food availability and seasonal fluctuations (Pace and Carman 1996; De Troch *et al.,* 2003; Azovsky *et al.,* 2005). Whether trophic niche separation reduces resource competition and contributes to the co-existence of harpacticoid species is still unclear.

6. Harpacticoid copepods in food webs studies

6.1. The potential trophic role of harpacticoids

Meiofauna and especially harpacticoid copepods are known to play a significant role in various benthic habitats (Coull 1990; Pinckney *et al.,* 2003; Van Gaever *et al.,* 2009). Copepods, to some extent, facilitate biomineralization of organic matter and enhance nutrient regeneration (Hicks 1980; Coull 1999). Conversely, a direct and important role as food for epibenthic predators like macrofauna (e.g. shrimps) and juvenile demersal fishes exists (Bell 1980; Gee 1989; Coull 1990). In the Mediterranean, Danovaro *et al.* (2007) estimated that 75% of total metazoan meiofaunal production can be channelled to higher trophic levels through predation on soft-bottoms. In contrast, other studies suggest that predation on meiofauna can be almost completely negligible, since prey populations are large and the number of predators comparatively small (Gibbons 1988; Shaw and Jenkins 1992). Nevertheless, habitat complexity can alter predation efficiency and influence faunal distribution and diversity (Coull and Wells 1983), compromising proper estimates of trophic specialization and habitat complexity.

Early experimental studies have demonstrated that macro- and megafauna meet their nutritional needs by feeding on microfauna and meiofauna associated with macrophytodetritus rather than directly on poor detrital plant tissues (Tenore *et al.,* 1984; Coull 1990). As mentioned by Vizzini (2009) and tackled by Holmer and her team (Holmer *et al.,* 2001; Holmer and Olsen 2002) seagrasses leach and exudate dissolved organic matter (DOM) contributing to an increase in bacteria and microbial loop. Not only living or dead vascular material leaches organic matter, also the associated epiphytes contribute. Higher excretion rates were found for *Z. marina* and associated epiphytes compared to epiphyte free leaves (Penhale and Smith 1977). The exact leaching quantities vary depending, for instance, on

material composition, season and light regime (Barrón *et al.,* 2006; Barrón and Duarte 2009; Apostolaki *et al.,* 2010a). This emphasises the importance of indirect consumption of vascular plant material compared to the direct exploitation of living tissues by animals. The proportion and assimilation success of direct consumption of the litter compared to the indirect consumption (i.e. ingestion of biofilm, faecal pellets of litter consumers, etc.) is unknown. Subsequently, how harpacticoid copepods exactly proceed in the macrophytodetritus habitat is unidentified. Therefore, a conceptual scheme should evaluate the role of the different food source compartments of the macrophytodetritus and the different assimilation of different copepod species in order to unravel the fate of macrophytodetritus accumulations.

6.2. Food web analysis: Trophic biomarkers

The drawbacks of the traditional methods in studying food webs (e.g. stomach contents) have led to techniques that utilize naturally occurring biomarkers, such as the determination of fatty acid profiles and stable isotopes analyses. Fatty acid profiling is a technique which associates consumers to a particular food source (Hobson 1999) by the discrimination of certain unusual fatty acids that are not synthesized by most marine consumers and are thus unique and characteristic food web markers (Smith *et al.,* 1996). FAMEs analyses were performed at the Ghent University.

The second technique, stable isotope analysis, is one that has been used in various disciplines (e.g. archaeology, geology and ecology) for many years (DeNiro and Epstein 1981; Fry *et al.,* 1987; Fry 2006). Elements having more than one stable isotope that are most commonly utilized are hydrogen (H), oxygen (O), carbon (C), sulphur (S) and nitrogen (N). In this study we use isotopes of two elements: Carbon and Nitrogen.

7. PhD outline

Chapter 1 introduces the scientific setting of the PhD thesis. Emphasis is drawn on the studied environment, the definition and role of macrophytodetritus, the fauna and flora associated to the detrital accumulations together with general aspects of food-web ecology. Small invertebrates like meiofauna and especially Copepoda are omnipresent in this system (Danovaro 1996; Mascart 2010) and are assumed to play an important part in the degradation dynamics of detritus. For instance: by directly feeding on detritivores microorganisms communities and thus indirectly influencing the speed of degradation or by raising the nutrient availability and turnover rate by enhancing fragmentation (Hyndes and Lavery 2005; Lillebo *et al.,* 2007). However, the ecology and composition of these meiofaunal organisms, mainly harpacticoid copepods on macrophytodetritus remain unknown. Moreover, their role in the food web is understudied as mostly multiple species pooled data have been used, generating a loss of resolution in comparison to species-specific data. In order to fill in those caveats, this study focuses on the diversity, the dynamics and the trophic role of copepods in the *Posidonia oceanica* macrophytodetritus accumulation.

Chapter 2 gives an overall insight into the multiple techniques used during this PhD and into the chosen sampling sites and time frames. Even though accumulated *P. oceanica* macrophytodetritus on sand patches is seen as an important trophic subsidy for food webs, it remains an understudied habitat. Macrophytodetritus is formed by decaying leaves and other degraded material from adjacent habitats, which may be of greater nutritional value than fresh material (Dethier *et al.,* 2014). Temporal variations of macrophytodetritus accumulations are driven by direct and indirect linked factors. Therefore, its relationship to physicochemical and hydrodynamical forcing needs to be unravelled. A seasonal (**Chapter 3**) and interannual (**Chapter 4**) follow-up has been carried out in order to discover the factors responsible for the variations in time and space of the

exported seagrass leaf litter meiofaunal community.

The faunal communities present in the accumulations experience drastic habitat variability in relation to the same forcing as litter accumulation dynamics. Ensuing, qualitative and quantitative responses to this variability are expected and therefore, the compositions of meiofauna and copepod communities are described at a spatial (two sites) and temporal (seasonal) scale (**Chapter 3**). Since these communities are known to be very variable in time (Giere 2009), controlling the consistency of the one-year seasonal scale was necessary. Therefore, a second year (interannual) was sampled and compared (**Chapter 4**). *In situ* collection of samples and identification of the present fauna allowed the creation of extensive species lists (Addendum I&II) providing new insights on the scattered and poor knowledge on harpacticoid copepods in the coastal Mediterranean (Chertoprud *et al.,* 2010). The variability of the community composition might impact the energy transfer pathway of the food web. Hence, in order to assess the trophic diversity among the community, species-specific biomarker analyses were performed. In **Chapter 5** two trophic biomarkers were used to identify interspecific diversity and trophic positions of the dominant copepods (Fig 5.2B) in a seagrass food web on a seasonal basis. The techniques applied were stable isotope analysis (SI) of Carbon and Nitrogen, and fatty acid profiling (FA). Stable isotope mixing models were applied (1) to assess the contribution of every potential food source present in the macrophytodetritus matrix to copepod diets, (2) to quantify and qualify the main trophic pathways. Isotopic niche models were used to unravel and compare seasonally the trophic niches for different species among season and to assess trophic plasticity of these species.

Copepods are present in high numbers in the dynamic macrophytodetritus accumulations (Mascart *et al.,* 2013). Subsequently, the question arose on how and from where copepods arrive in this very dynamic habitat. The macrophytodetritus by its intrinsic traits is situated at the crossroad of several habitats known to hold distinct communities (e.g. seagrass canopy,

unvegetated habitat, epilithic algae, pelagic water column, etc.). Given its patchy and refractory character, macrophytodetritus accumulations form a particular habitat. Therefore, in **Chapter 6**, an *in situ* mesocosm experiment was carried out to elucidate the exact colonization pathway and source pool of colonizing copepods present in the macrophytodetritus accumulations. Not much is known on the migratory pathway and dispersal activities of such small organisms (Palmer 1988; Fleeger *et al.,* 1995), therefore the current species-specific dispersion pathway knowledge (Noodt 1971) was revised. Finally, in the last chapter (**Chapter 7**) an extensive discussion is presented to reflect on all outcomes, key issues and considerations.

Each chapter is proposed to read as an autonomous part, which can be read separately from the other chapters. Inevitably, there is an overlap between the general introduction and materials & methods section and the chapter specific sections. In addition, a species list of all encountered Copepoda per site (Addendum I) and as a full classification list (Addendum II) is available. Furthermore, in Addendum III, an updated statistical approach of Chapter 3 is highlighted. Finally, the meiobenthic and harpacticoid copepod community structure in different habitats of a Mediterranean seagrass meadow is described in Addendum IV based on a dataset collected as part of the master thesis of the first author (Mascart 2010; Mascart *et al.,* 2013).

8. Research objectives

The general purpose of this research is to assess and better understand the ecological role and diversity of meiofauna, especially harpacticoid copepods in seagrass macrophytodetritus accumulations. Therefore, it is crucial to quantify the natural variability of this habitat and its associated meiofauna community. Furthermore, the dynamics of on one hand the macrophytodetritus and on the other hand the most dominant taxa needs to be investigated. Hence, the potential energy present in this detrital system and the exact channelling pathway towards higher trophic levels, mediated by copepods can be understood.

In this context, three specific objectives are put forward:

I. Identifying the factors controlling the dynamic and variability of macrophytodetritus accumulation at different spatio-temporal scales.

II. Characterizing the associated meiofauna taxa and copepods species communities at different spatio-temporal scales, and unravelling the colonization potential of the copepods and their origin.

III. Assessing the trophic ecology of the copepod communities present in the macrophytodetritus at the specific level and eco-morphological level and placing them in a macrophytodetritus food web conceptual model.

Picture: Submerged detritus *sensu lato*

Chapter 2

Sampling locations and sampling processes

In this chapter more information on the respective sampling sites is given, in particular on the sand patches and their dynamics. Hence, some site description overlap will occur. Regarding detailed information on specific techniques used during this PhD, an in-depth materials and methods section will be presented in each subsequent chapter.

1. Study site description

1.1. Revellata Bay

Samples were collected in the Revellata Bay in the Bay of Calvi, Corsica, northwest Mediterranean (42°34'48.4"N 8°43'27.8"E, Fig 2.1) near the oceanographic station of STARESO (Station de Recherches Sous-marines et Océanographiques, University of Liège). In the Gulf, *P. oceanica* seagrass meadows cover about 50% of the total surface down to a depth of 38 m (Bay 1984) and are ranked among the most productive *P. oceanica* beds in the North-Western Mediterranean (Pergent-Martini *et al.,* 1994). Nevertheless, the Mediterranean Sea is considered an oligotrophic environment which is characterised by low nutrient concentrations and a low particle load in the water column (Gobert *et al.,* 2002). Annual surface temperatures have a classical summer maximum (26°C in August) and winter minimum (13°C in March) and a fairly constant salinity of 38, stable throughout the year. Tidal variation amplitude (1-20 cm) and currents (4-5 $cm.s^{-1}$) are weak in the Calvi Bay (Vieira and Toro y Llaca 1992; Dauby *et al.,* 1995; Cazenave *et al.,* 2002). The dominant winds on the Bay originate from South-West (Libeccio, 200-250°) and North-East (Mistral and Tramontane, 320-60°) sectors (Bay 1984; Dauby *et al.,* 1995).

1.2. Sampling events

The samples for spatio-temporal characterization of the system were taken in the months February, May, August and October representing the winter, spring, summer and autumn conditions, respectively. It was done in the year 2011 (see Chapter 3). Since copepod communities are known to be very variable in time (Giere 2009) and that physicochemical and hydrodynamical forcing varies in time and space, assessing the consistency of the one-year seasonal dynamics was necessary. Therefore, a second year (interannual) was sampled and compared. In that year (2012) two seasons (spring and summer) were selected to compare with 2011 (see Chapter 4). All above mentioned samples were done in two sites PORT and OSCE separated 1km from each other (see paragraph 1.3 and Chapters 3 & 4). Next to the spatio-temporal characterisation samples an *in situ* experiment was carried out in autumn 2012 at the BANANE site to unravel the colonization pathways of copepods (see paragraph 1.3).

1.3. Sampling sites description

All sampling and experimental sites were located on the eastern side of the Revellata peninsula between the Punta Revellata in the North and the Punta di Oscelluccia in the South, at a depth of 8 to 15m depth. Three sites were used during the different sampling campaigns of this PhD: The PORT-site (blue), the OSCE-site (red) and the BANANE-site (green) (Fig 2.1).

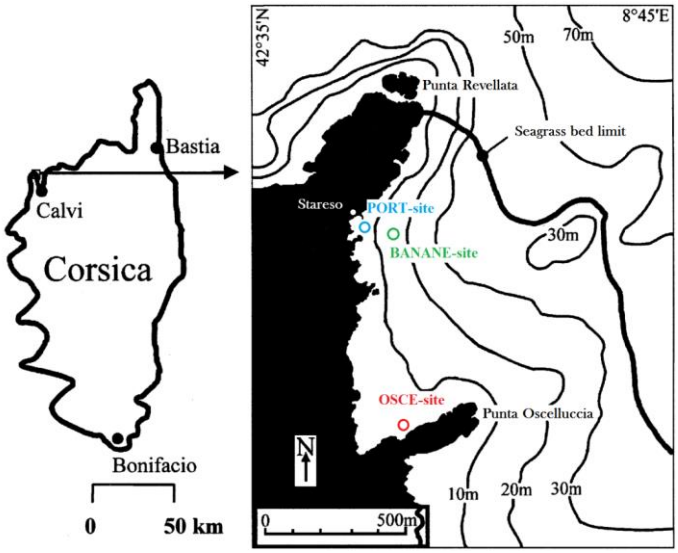


Fig 2.1 *Location of the sampling sites within the Revellata Bay in colour and the P. oceanica meadow isobaths with lower depth distribution limit. Adapted from Gobert et al. (2003)*

The PORT-site:

Fig 2.2 *Satellite image on the location of the PORT sampling site with indication of the location of the 3 sampling points/sand patches (Source: Google earth).*

The first sampling site was located at the entrance of the STARESO harbour at a depth of ~8 m and will further be referred to as 'PORT' (Fig 2.2). The site was characterised by two sand patches separated by a *P. oceanica* meadow (sampling point 1 & 2) stretching parallel and south from the STARESO jetty. The sampling point 3 (stretching in a SW-NE axis) was located 5-6 m south of point 2 with a *P. oceanica* meadow in between (Fig 2.3). Each sand patch was large enough to harbour macrophytodetritus all year long (Fig 2.3)

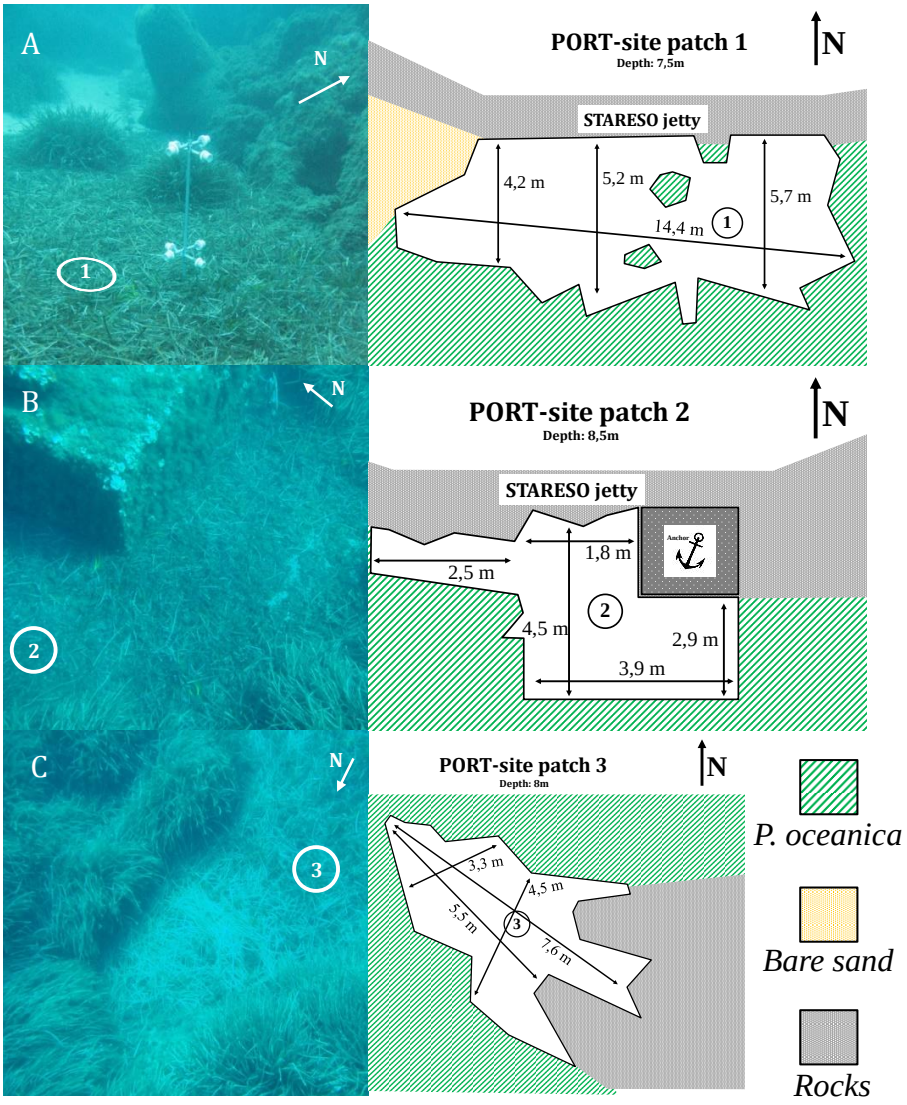


Fig 2.3 Descriptive pictures (left, taken in October) and schemes (right) of the PORT-sites with sampling points indicated as circles. A: Port-site sampling point 1; the experimental structure in the middle of the picture is from an unpublished plaster experiment of T. Mascart. B: PORT-site sampling point 2. C: PORT-site sampling point 3.

The OSCE-site:

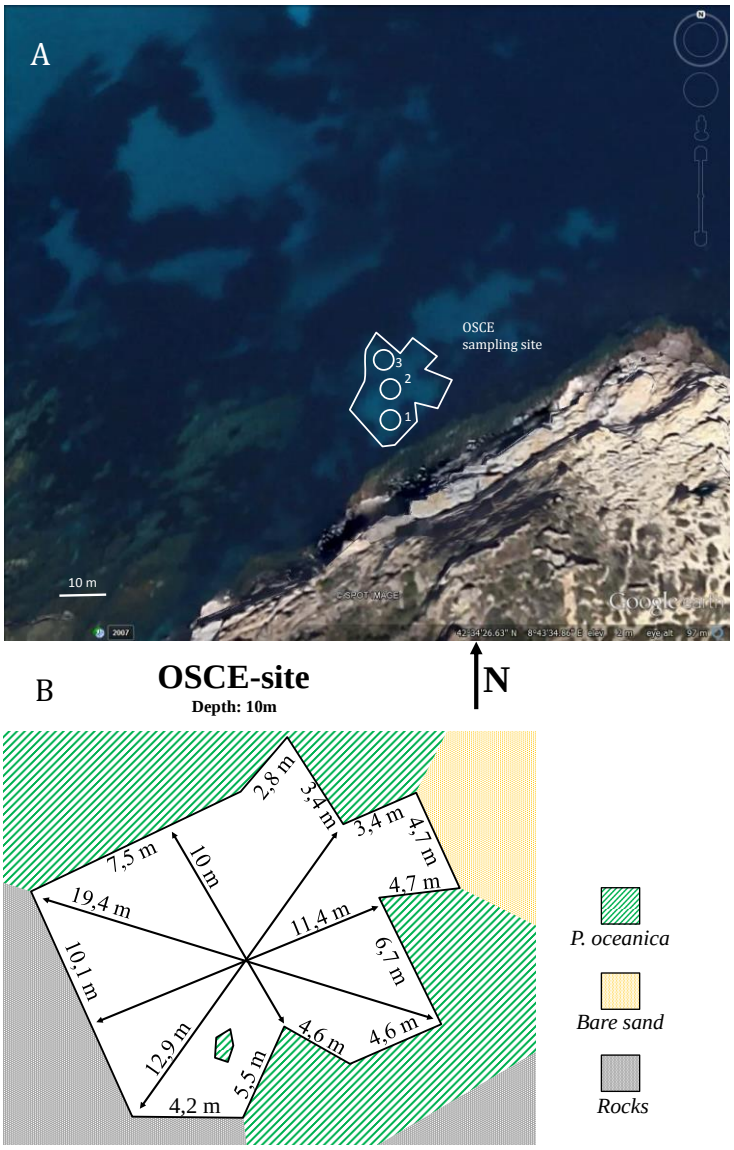


Fig 2.4 *A: Satellite image on the location of the OSCE sampling site and sapling points (circles). Source: Google earth. B: Scheme of the OSCE-site with length measurements.*

The second sampling site was located on the north-west side of the Oscelluccia peninsula at a depth of 10m and will hereafter be referred to as 'OSCE' (Fig 2.1, red circle). The site was characterised by the close vicinity of the Oscelluccia peninsula rock and by the underwater rock formation on its west side (Fig 2.4 B) protecting the bare sand patch from underwater returning currents (Blanc and Jeudy de Grissac 1984) of the Punta Oscelluccia north-side Bay (visible as sand patch in the upper left-corner, Fig 2.4 A). All 3 sampling points were located on a south-north gradient (Fig 2.4 & 2.5). The present dynamic macrophytodetritus accumulation varied in circumference and thickness according to the investigated season. Wind-induced hydrodynamic effects seemed to be at the basis of critical changes. In the November month of 2012, storms occurred and in a time laps of 4 days, the accumulations changed significantly. To illustrate such 'storm effect' before-after pictures were taken from three cameras on a 2.5 m high stainless steel foot in the middle of the OSCE-site at a fixed position (A: 0°; B: 120° and C: 240°). This was part of the 'pulse' follow up of F. Remy (unpublished data) (Fig 2.5).

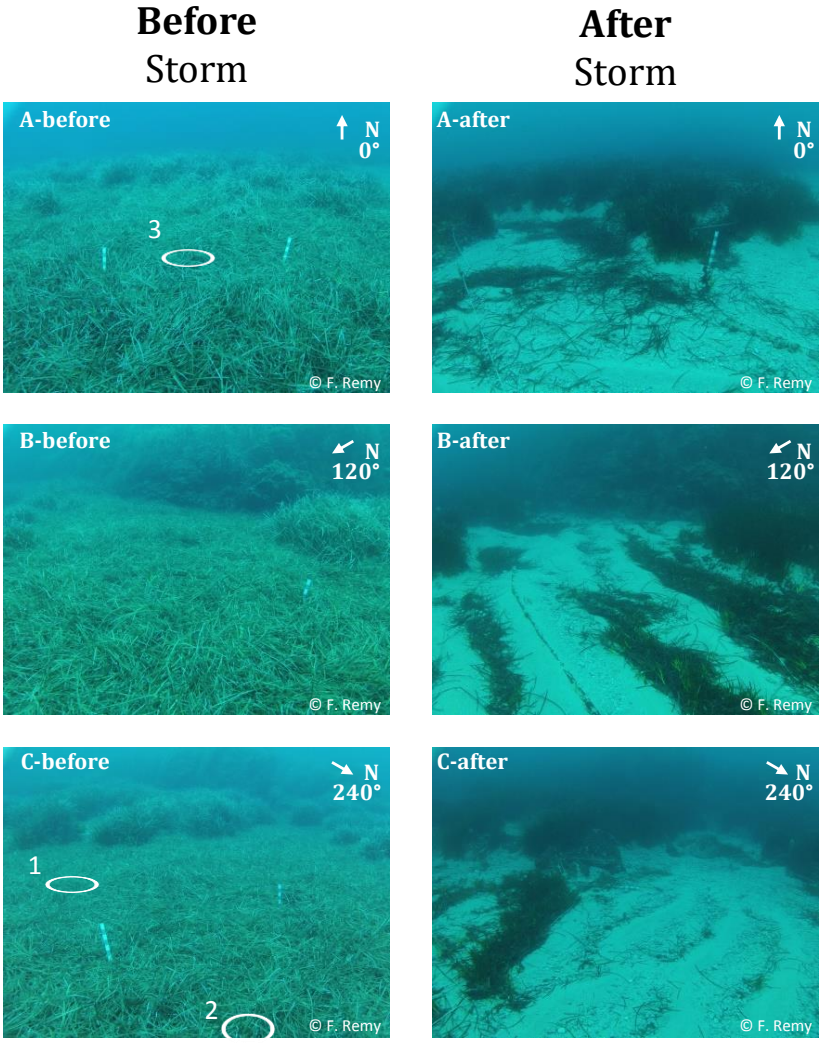


Fig 2.5 *Descriptive pictures of the 'storm effect' at the OSCE-site. Three cameras on a 2.5m foot in the middle of the OSCE-site at a fixed position (A: 0°; B: 120° and C: 240°) took at 12h00 the before and after series of pictures. Left-side: pictures before an autumnal storm taken at 12h on October 30th 2012. Right-side: pictures after an autumnal storm taken four day later at 12h on November 3th 2012. Sampling points 1-3 are indicated (circles) on pictures A and C before. On the A and C pictures, the P. oceanica meadow is visible in the upper part and on the B pictures, the foot of the Oscelluccia rock is visible in the upper part.*

The BANANE-site:

Fig 2.6 *Satellite image on the location of the BANANE sampling site (square). Source: Google Earth*

The *in situ* experimental site 'BANANE' was located about 100m east of the Stareso research facility at a depth of 15m (Fig 2.1, green circle). The continuous meadow present at this depth was interrupted by a naturally occurring band of 3-5 m wide bare sand in the shape of a banana (Fig 2.6). Towards the West, the sand patch was bordered by a 1.5 m high 'wall' of 'dead matte' with a *P. oceanica* meadow on top (Fig 2.7A) and on the eastern side by a ground level *P. oceanica* meadow. At the time of the experiment (see Chapter 6) the sand patch was partially covered with macrophytodetritus and the experimental units were deployed randomly (Fig 2.7B).

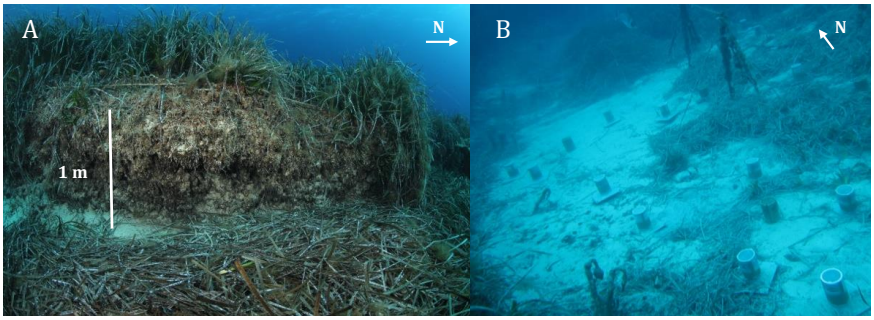


Fig 2.7 *Descriptive pictures of the BANANE-site. A: the 'dead matte wall' creating the sand patch's western border in time of high accumulation amounts. B: diver's perspective overview of the random deployment of the cylindrical experimental units on the sand patch.*

1.4. Site patchiness, edge effect and small-scale variability

To our best knowledge, no information is available on edge or patchiness effect on meiofauna or macrofauna inhabiting seagrass macrophytodetritus. On the other hand, such studies on edge effect and patch size effect in seagrass meadows have found few patterns that are consistent among vagile faunal species and over time, emphasizing the variability in species-specific responses to patchiness (Bostrom *et al.,* 2006; Connolly and Hindell 2006). In seagrass habitats, those patterns can be related to the interaction between, or to the combination of several factors. These factors may include the proximity to adjacent habitats (Skilleter *et al.,* 2006), the habitat configuration (Healey and Hovel 2004) and the patch-specific physical environment (Bologna and Heck 2002; Tanner 2006). A habitat fragmentation experiment on meiofauna, numerically dominated by harpacticoids, in artificial seagrass patches by Warry *et al.* (2009) revealed an edge effect with increased densities in the outer 0.5 m edge of a seagrass patch. Murphy *et al.* (2010) also reported an edge effect in seagrass beds for harpacticoid copepods of the family Porcellidiidae. Therefore, to avoid a potential edge effect in macrophytodetritus

accumulations and to exclude possible effects of the proximity to an adjacent *P. oceanica* seagrass edge, all samples were taken at a minimum of 1 m from the edge of the surrounding seagrass meadow.

As mentioned, seascape structure, e.g. patch dynamics, edges, and proximity of one patch type to another is known to influence coastal fauna (see Bostrom *et al.*, 2011 for an overview). Consequently, the sampling designs in the sites PORT and OSCE could be prone to underlying effects of sampling scales and patchiness influencing the meiofaunal characterisation. In the PORT-site, all three sampling points were separated by a stretch of *P. oceanica* seagrass. In the OSCE-site sand patch, point 2 was more central/inwards than point 1 and 3. Therefore, a hierarchical clustering of the multivariate copepod species composition was performed through pvclust in R (R Core Team 2014). The aim was to assess the uncertainty through *P*-values indicating how strong the cluster was supported by data. The output displayed the results of the cluster analysis with BP (Bootstrap Probability) values and AU (Approximately Unbiased - computed by 10000 multiscale bootstrap resampling) values (Fig 2.8). It can be concluded here that all clusters made by bootstrap are highly trust worthy (AU > 75%), except maybe the October PORT point 2 and 3 (AU = 67%). In general, the three sampling points within a site in a particular season of the year clustered together with a high degree of certainty. It can therefore be concluded that there is no bias at the level of the selected sampling points due to any potential patch size effect or small-scale variability (metres) of the macrophytodetritus accumulation, neither at the PORT-site nor at the OSCE-site.

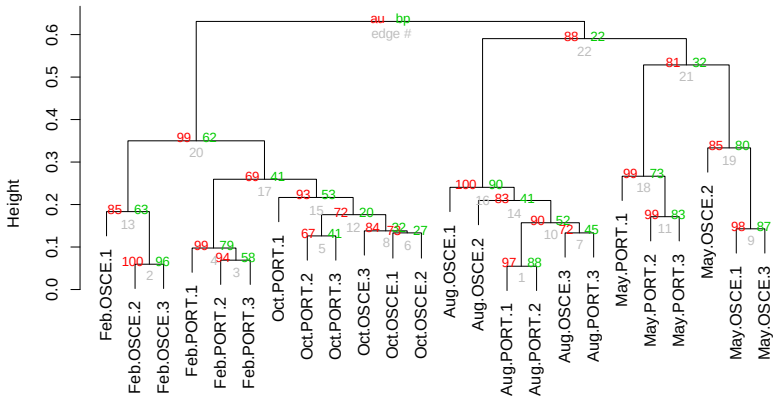


Fig 2.7 *Cluster dendogram of multivariate species composition per sampling point with indication of BP % (green, Bootstrap Probability) and AU % (red, Approximately Unbiased) values.*

Finally, small-scale variability on a scale of a few cm's is known to occur in meiofauna communities (Li *et al.,* 1997). The present variability can occur due to e.g. presence of predators, individual behaviour or patchy food distribution (Lee *et al.,* 1977; Hicks and Coull 1983; Heip *et al.,* 1985). Thus, small-scale variability of meiofauna spatial distribution can be related to individual behaviour, rather than taxonomic composition. In meiobenthic studies, the used standard meiocores have a diameter of 2.3 cm yielding a sampled surface of 10cm^2. Li *et al.* (1997) concluded that either replicated subsample meiocores or a sampling core larger than 10cm^2 was required to reduce sampling error. Therefore, a 'detritus core' (314 cm^2) was used during macrophytodetritus sampling to encompassing the small-scale spatial distribution variability present in a meiofauna community.

2. Samples collection and treatment

2.1. Macrophytodetritus and environmental samples collection

Macrophytodetritus accumulation was quantitatively sampled using a 314 cm^2 (20 cm diameter) core pushed into the macrophytodetritus accumulations until the bare sand was reached (Fig 2.9 A). Prior to collection of the macrophytodetritus and associated fauna, accumulation height was measured with a ruler stick pushed through the detritus alongside the core. The collection of the macrophytodetritus and associated fauna was completed by gently scooping the material off the seafloor bed by hand (without sediment) and putting it into sealed plastic jars. The qualitative sampling for the trophic biomarker study (stable isotope analyses and fatty acid profiling) was completed using 30 L plastic bags where the macrophytodetritus material and associated fauna was scooped into by hand and sealed under water (Fig 2.9 B). Subsequently, the collected material was kept alive at the research facility in 0.75 m^3 aquaria filled with 38 µm filtered seawater from the Bay in order to prevent deterioration for later analyses. Further preparations of samples were described in detail in Chapter 5.

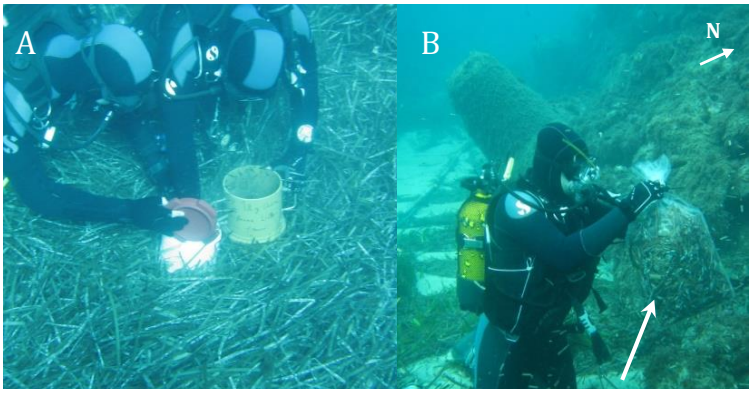


Fig 2.9 *Descriptive pictures of the macrophytodetritus collection. A: Quantitative sampling of macrophytodetritus by using a detritus core (right side) and transferring the content into a sealed jar (left side). B: Qualitative sampling for trophic biomarker analysis by means of a plastic bag (arrow). Both pictures were taken at PORT-site point 1.*

Meteorological data were recorded in order to map the effect of the weather on the local hydrodynamics and thus on the accumulation of macrophytodetritus. The data were acquired at the STARESO meteorological station located on top of the Punta Revellata and logged through the open source RACE (Rapid Assessment of the Coastal Environment) database (Binard *et al.,* 2008). For the purpose of this study only wind gusts, blowing from the 1st quadrant (0-90°), coming from the North and East, were selected (see Chapter 3).

Nutrient and oxygen contents and concentrations were collected using a 60 ml direct-suction filter sampler (Fig 2.10 A & B) from Gobert *et al.* (2006b) at different positions: the water column (WC), the water just above the detritus (WJA), the water inside the detritus (WI) and the interstitial water of the underlying sediments (IW). Nutrient concentrations, nitrogen (NH^{4+} and NO_3 + NO_2, hereafter NO_x) and phosphate (HPO_4^{2-}) were analysed with an autoanalyser (SKALAR San$^+$ continuous flow analyser) based on the method of Grasshoff *et al.* (2007) adapted for oligotrophic (low nutrient content) seawater (detection limits: 0.1, 0.04 and 0.05 µM for ammonium, NO_x and phosphates, respectively). Oxygen concentrations were measured using the Winkler method (Strickland and Parsons 1968).

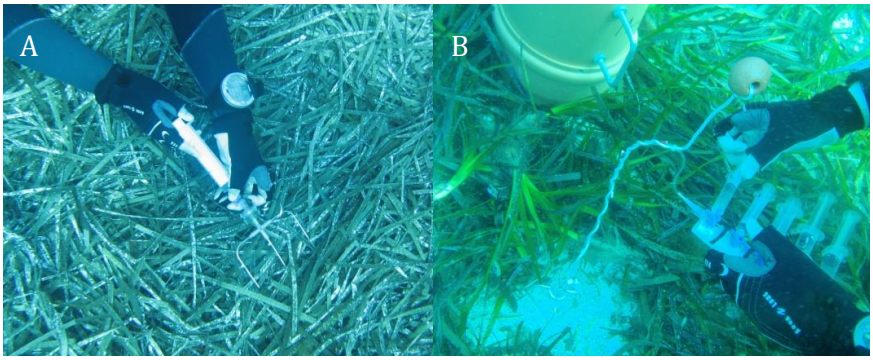


Fig 2.10 *Descriptive pictures of sampling. A: a direct suction filter sampler pushed into the macrophytodetritus to take a 'Water inside detritus (WI)' nutrient sample. B: a direct suction filter sampler pushed into the underlining sediment to take an 'interstitial water (IW)' nutrient sample based on Gobert et al. (2006b).*

SOM samples were collected from the water column 1m under the macrophytodetritus accumulations. Seawater (2.5 L) was collected above the sampling sites with a SCUBA hand-held Niskin-bottle. The content was afterwards filtered on glass fibre filters (47 mm, GF/F Whatman) and stored frozen at -20°C for further SI analysis or at -80°C for FA analyses.

2.2. Subsequent on site and lab treatment

Macrophytodetritus sampled during seasonal events or at the end of the experimental incubation were afterwards rinsed with an 8% $MgCl_2$-solution (Hulings and Gray 1971) and fresh water in order to separate meiofauna from the macrophytodetritus. The rinsed samples were sieved over a 1 mm and 38 µm mesh sieve to exclude macrophytodetritus and retain meiofauna, respectively. The heterogeneous defaunated macrophytodetritus was subsequently sorted in categories since the detritus accumulation was composed of mainly three types of material: (1)

the dead *P. oceanica* leaf litter or detritus fragments, (2) the drift epilithic macroalgae and (3) the drift living shoots of *P. oceanica* comprising rhizomes and living leaves (Fig 2.11).

Fig 2.11 *Pictures describing the heterogeneity of the macrophytodetritus accumulations. Left: a top-view picture at the centre of an accumulation and right: a picture taken at the edge of the accumulation where intermittent bands of bare sand were visible. The 3 main composition categories were dead P. oceanica leaf litter (triangle), drift epilithic macroalgae (ellipse) and drift living shoots of P. oceanica (rectangle).*

All categories were afterwards separately stored frozen for further processing. The meiofauna were preserved in a 4% formaldehyde seawater solution. The meiofauna present in the sediment were afterwards extracted by three centrifugation cycles with Ludox HS40 (density of 1.18 $g.dm^{-3}$) and stained with Rose Bengal. If the supernatant was too voluminous and subsampling was necessary, a Motoda (1959) splitter box was used. Meiofauna was sorted, counted and identified under a stereomicroscope to higher taxa based on Higgins & Thiel (1988), while copepods were, picked out and stored in 75% ethanol. These were later mounted on slides for microscopic identification to species level based on identification keys of Lang (1948, 1965), Huys and Boxshall (1991) and Boxshall & Hasley (2004). Due to time-consuming identification we restricted ourselves, in Chapter 3 and 4, to a random subsampling of the first one hundred twenty adult harpacticoid copepods encountered (De Troch *et al.,* 2001b). One

might expect an underestimation of the species richness since the identified subsample represented on average 25 ± 16% of the sampled individuals. Comparing the total number of individuals to the species richness revealed no correlation (R^2 = -0.11). In other words, no increase in species richness was observed with increasing amounts of sampled individuals. Moreover, a species-accumulation plot (Fig 2.11) with asymptotic species richness estimator for 95% contributing species abundance data ($Chao_1$), was created with 999 permutations in PRIMER (Clarke and Gorley 2006; Magurran and McGill 2011). By comparing it to the observed species richness (S_{obs}) it can be concluded (Gotelli and Colwell 2011) that a subsample of one quarter is enough to represent all species with a potential underestimation of maximum 2 species. This result is comparable to the conclusion of De Troch *et al.* (2001a) stating that the overall dominance structure of the sample remained stable irrespective of the size of the subsample that was identified. Nonetheless, for the colonization experiment of Chapter 6 all encountered adult copepod individuals were identified in order to remove any potential bias..

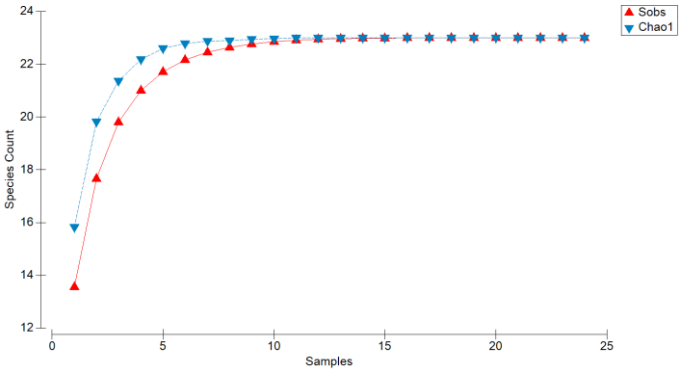


Fig 2.11 *Species-accumulation plot with S_{obs} (red triangle) observed species richness and $Chao_1$ (blue reverse triangle) predicted species richness.*

Picture: View on the Punta Revellata from “le sentier des douaniers”

Chapter 3

Seasonal variability of macrophytodetritus, meiofauna and copepod community

Adapted from:

Mascart T., Lepoint G., Deschoemaeker S., Binard M., Remy F., De Troch M. (2015). Seasonal variability of a meiofauna community, especially harpacticoid copepods, in *Posidonia oceanica* macrophytodetritus accumulations. *Journal of Sea Research 95, 149-160*

1. Abstract

The overall aim of this study was (1) to assess the diversity and density of meiofauna taxa, especially harpacticoid copepod species, present within accumulated seagrass macrophytodetritus on unvegetated sand patches and (2) to elucidate the community structure of detritus-associated harpacticoid copepods in relation to natural temporal variability of physico-chemical characteristics of accumulations. This was investigated in a *Posidonia oceanica* (L.) Delile seagrass ecosystem in the northwest Mediterranean Sea (Bay of Calvi, Corsica, 42°35'N, 8°43'E) using a triplicate macrophytodetritus core field sampling in two contrasting sites over the four seasons of 2011. Meiofauna higher taxa consisted of 50% Copepoda, of which 87% belonged to the Harpacticoida order. Nematoda was the second most abundant taxon. The copepod community displayed a wide variety of morphologically different species (i.e. mesopsammic, phytal, phytal-swimmers, planktonic and parasitic) (see Fig 1.5). The harpacticoid copepod community followed a strong seasonal pattern with highest abundances and species diversity in May-August, revealing a link with the leaf litter epiphyte primary production cycle. Aside from the important role in sheltering, housing and feeding potential of macrophytodetritus, a multivariate harpacticoid community composition BEST analysis demonstrated a positive correlation of abundances and diversity with habitat complexity and a negative correlation with wind induced hydrodynamics and *P. oceanica* leaf litter accumulation.

2. Introduction

In the Mediterranean Sea, seagrass meadows of *Posidonia oceanica* (L.) Delile cover vast areas of sea bottom. Yearly at the end of summer, the seagrass loses major part of its leaf biomass after senescence. The fate of these *P. oceanica* dead leaves, also called leaf litter, varies (Pergent *et al.*, 1997): a part of the leaf litter decays slowly or is buried within the meadow, another part is exported to other adjacent habitats where it may represent a considerable organic material input (Pergent *et al.,* 1994; Romero *et al.,* 1994; Duarte and Cebrian 1996; Cebrian *et al.,* 1997). Such exported leaf litter mixes with drift epilithic macroalgae, uprooted living seagrass shoots with rhizomes, other seagrass litter, seeds, dead macrofauna and fine sediment to form detritus. The exported detritus form dense accumulations, especially on adjacent unvegetated sand patches, in relation to local hydrodynamics and sand patch morphology (Vetter and Dayton 1999). The macrophytodetritus host many organisms which can participate in the degradation of this organic material, such as bacteria, fungi, diatom microalgae and invertebrates (Danovaro 1996; Graca 2001; Danovaro *et al.,* 2002; Mancinelli and Rossi 2002; Gallmetzer *et al.,* 2005). Especially motile macro- (> 1 mm) and meiofauna (38 µm–1 mm) invertebrates were revealed to be important in the shredding, degrading and decomposing of the organic wrack (Wittman *et al.,* 1981; Vetter 1995; Mancinelli and Rossi 2002; Hyndes and Lavery 2005; Lillebo *et al.,* 2007). Several studies in coastal ecosystems compared motile invertebrate communities in living seagrass habitats with communities present in directly adjacent habitats (unvegetated sand, root-rhizome matte and macrophytodetritus accumulations). Unvegetated sand showed a lower abundance of associated motile macro- and meiofauna than the foliar substrata of living seagrasses (Edgar *et al.,* 1994; Bostrom and Bonsdorff 1997; Connolly 1997; Sanchez-Jerez *et al.,* 1999b; Fonseca *et al.,* 2011). In the *P. oceanica* ecosystem, the root-rhizome layer mat supports diverse macro-invertebrate assemblages (Harmelin 1964). These mats occur with

live rhizomes or naturally dead rhizomes. The comparison between the dead and the living habitat yielded a higher total number of species and abundance in the dead detrital mat (Borg *et al.*, 2006). However, certain randomness in the species assemblages was present depending on subliminal parameters such as the substrate compactness, bacterial growth, depth, e.a. (Harmelin 1964; Abada Guerroui and Willise 1984). Macrofaunal communities in macrophytodetritus accumulations on unvegetated sand patches were, in terms of diversity low, but in terms of total abundance equal or higher than living seagrasses (Mancinelli and Rossi 2002; Gallmetzer *et al.,* 2005; Dimech *et al.,* 2006; Como *et al.,* 2008). Consequently, dead habitats and especially macrophytodetritus accumulations seem to supports a unique macro-invertebrate assemblage.

Meiofauna are said to play an important role in the degradation of leaf litter (Hyndes and Lavery 2005; Lillebo *et al.,* 2007). In some habitats, studies were made and clear associations between detritus and meiofauna assemblages were established, such as in mangrove leaf litter (Gee and Somerfield 1997; Gwyther 2003; Torres-Pratts and Schizas 2007) or in terrestrial forest (Dumont and Maas 1988; Fiers and Ghenne 2000). In seagrass ecosystems, food webs are mainly seen as detrital (Pergent *et al.,* 1994; Duarte and Cebrian 1996; Mateo and Romero 1997), but many potential food sources coexist (Lepoint *et al.*, 2000). No study, to our current knowledge, was ever performed on the harpacticoid copepod assemblage associated with seagrass macrophytodetritus. Mascart *et al.* (2013) compared *P. oceanica* meadows, sediment and two types of macrophytodetritus accumulations. The macrophytodetritus accumulations showed higher meiofauna abundances than living seagrasses, without expressing a higher diversity at the taxonomic level of copepod families. Consequently, do macrophytodetritus accumulations support a unique meiofauna community, in particular harpacticoid copepods and what is the origin and variability of this community?

The overall aim of this study was to assess the diversity and density of

meiofauna taxa, especially harpacticoid copepod species, present within macrophytodetritus wrack accumulations on unvegetated sand patches. A second aim was to elucidate the community structure of associated meiofauna and harpacticoid copepods to natural temporal variability of physico-chemical characteristics of macrophytodetritus accumulations. We investigated this by collecting triplicate macrophytodetritus core samples in the four seasons of the year in two contrasting sites. We addressed the following specific questions: (1) Do wind gusts, acting as a factor for near bottom currents, control the dynamics of the macrophytodetritus? (2) Does the temporal dynamics of environmental factors and composition of macrophytodetritus have an effect on the meiofauna and copepod community composition and density in two hydrodynamical contrasting sites? (3) What are the ecological groups of copepods present in the litter accumulation, i.e. planktonic, phytal or mesopsammic?

3. Material and methods

3.1. Sampling sites and strategy

Samples were collected in the Revellata Bay in the Calvi Bay, Corsica, northwest Mediterranean (42°35'N, 8°43'E). At the study site, *P. oceanica* seagrass meadows cover about 50% of the total bay surface down to a depth of 38 m (Bay 1984) and are ranked among the most productive *P. oceanica* beds in the north west Mediterranean (Pergent-Martini *et al.*, 1994). Annual surface temperatures have a classical summer maximum (26°C in August) and winter minimum (13°C in March). Currents are weak ($\leq$ 5 $cm.s^{-1}$) and the salinity is 38 and stable throughout the year. The dominant winds on the Bay originate from South-West (Libeccio, 200-250°) and North (Mistral and Tramontane, 320-60°) sectors (Bay 1984; Dauby *et al.,* 1995).

Samples were taken seasonally, i.e. in the months February, May, August and October of the 2011 year representing the winter, spring, summer and autumn, respectively. Sampling was carried out at a depth of 10 m by scuba divers during day time and calm sea conditions. Two contrasting sampling sites at about 1 km from each other were selected. Both sampling sites offered sandy patches with different local hydrodynamic conditions and variable shapes and patch sizes. The first sampling site was located in front of the harbour of the STARESO research facility and was referred to as PORT. The second sampling site was situated in front of the Punta Oscellucia peninsula and was referred to as OSCE. In each site, triplicate PVC cores were randomly pushed into the macrophytodetritus accumulation (inner diameter = 20 cm, surface = 0.0314 m^2) until reaching the sand layer. All detritus contained in the tube was gently scooped off the seafloor bed by hand and put into 6L sealed plastic jars. Sediment was not taken. In order to ensure no loss of material or contamination, all jars were closed under water. In order to separate meiofauna from the macrophytodetritus, an 8% $MgCl_2$-solution was added (Hulings and Gray 1971) and fresh water rinsing was used to stun the organisms. The samples were rinsed twice over a 1 mm mesh sieve to exclude detritus. Meiofauna was retained on a 38 µm mesh sieve and preserved in a 4% formaldehyde seawater solution. The defaunated detritus was stored frozen (-18°C).

3.2. Abiotic factors

Meteorological data were recorded during the entire year in order to map the effect of the weather on the local hydrodynamics. Previous studies stated that currents in the study site are weak (≤ 5 cm.s-1) and circulation mainly consists of a local residual gyre (Dauby *et al.*, 1995). According to ocean surface mixed layer models (see Cushman-Roisin and Beckers, 2011 and references therein) it is generally accepted that the surface winds, next to other factors like off-shore generated swell, have a direct influence on

the bottom currents. Shallow regions (typically with a depth of 10m) are considered to be dominated by shear turbulence and friction (Cushman-Roisin and Beckers 2011). Therefore the surface wind can be used as proxy for the near bottom currents. For the purpose of this study, only wind gusts, i.e. maximum wind speed over a two-second period at any time during 20 min, higher than 3.06 $m.s^{-1}$ were taken into account. This arbitrarily chosen limit corresponds to a gentle breeze (3 Beaufort) where crests begin to break and whitecaps are formed. Due to the geographical location and orientation of the bay, east-southerly to westerly winds are sheltered and thus have almost no effect on the local sea surface of the sampling sites. Therefore only wind gusts blowing from the 1st quadrant (0-90°), coming from North to East, were selected. In order to characterise and relate the selected wind gusts, two factors were included in the analysis: (1) Wind gust velocity, i.e. the median speed of the wind gusts during the time frame (2) Wind gust quantity, i.e. the percentage of time gusts were blowing during the time frame. The selected timeframe relevant to the sampling scale was four weeks prior to sampling, to map the long-term effects of the wind.

For each sample collection site at each season (N = 24), water was sampled using a 60 ml direct-suction filter sampler from Gobert *et al.* (2006b) at different positions: the water column (WC), the water just above the detritus (WJA), the water inside the detritus (WI) and the interstitial water of the underlying sediments (IW). Nutrients concentrations, nitrogen (NH_4^+ and NO_3 + NO_2, hereafter NO_x) and phosphate (HPO_4^{2-}) were analysed with an autoanalyser (SKALAR San+ continuous flow analyser) based on the method of Grasshoff *et al.* (2007) adapted for oligotrophic (low nutrient content) seawater (detection limits: 0.1, 0.04 and 0.05 µM for ammonium, NOx and phosphates, respectively). Oxygen concentrations were measured using the Winkler method with 13 ml biological oxygen demand (BOD) bottles. The Winkler method titration of iodine with a thiosulfate solution was adapted for micro volumes (Strickland and Parsons 1968). Oxygen

concentration was not measured in interstitial water of the underlying sediments. Oxygen values under 63 µM were defined as hypoxic (Middelburg and Levin 2009).

3.3. Macrophytodetritus characterization

During sampling, the macrophytodetritus accumulation height (Detritus height) was measured with a ruler stick pushed through the detritus alongside the core. The detritus accumulation was constituted of heterogeneous material, therefore after thawing, the defaunated macrophytodetritus was sorted in three categories: (1) the dead *P. oceanica* leaf litter fragments, (2) the drift epilithic macroalgae (Drift macroalgae) and (3) the living shoots of *P. oceanica* comprising rhizomes and living leaves (Living *P. oceanica*). In order to display the different contributions, all categories were dried at 60°C for 96h. Prior to dry massing of the leaf litter category, the 25 first fragments were scraped according to Dauby and Poulicek (1995) to remove the epiphytes which would bias the weight of the leaf litter fragments. Afterwards, for calculation purposes, the total epiphyte dry mass (Leaf litter epiphytes DM) and netto leaf litter dry mass (Leaf litter DM) was extrapolated from the measurements of the first 25 fragments. Standardization of dry mass was done towards $gDM.m^{-2}$ extrapolated from the core surface. An extra detritus characterization factor (Epi / Lit ratio) was mathematically added for the BEST analysis (see further). The leaf litter epiphytes DM / leaf litter DM ratio was created (Epi / Lit ratio), since the seasonal fluctuation of the epiphytic primary production (read: leaf litter epiphytes DM) and the *P. oceanica* leaf senescence (read: leaf litter DM) don't follow the same pattern.

3.4. Meiofauna community characterization

In the lab, the 38 µm-1 mm fraction of each replicate was centrifuged three times with Ludox HS40 (specific density of 1.18 g.dm^{-3}) in order to extract meiofauna from the macrophytodetritus derived organic material. Meiofauna was stained with Rose Bengal before being sorted and enumerated at a higher taxon level based on Higgins & Thiel (1988). Harpacticoid copepods were picked out and stored in 75% ethanol. Due to time-consuming identification we restricted ourselves to the first one hundred twenty adult harpacticoid copepods (De Troch *et al.,* 2001a), representing 15 to 95% of the total adult copepod amount. Copepods were mounted *in toto* on glycerine slides for identification at species level using the identification keys and reference books by Boxshall & Hasley (2004) and Lang (1948, 1965). The number of individuals was standardized by area m^2 and towards dry mass g extrapolated from the core surface and leaf litter dry mass, respectively.

3.5. Data analysis

A fully crossed 2-factor design was performed in PERMANOVA with fixed factors month and site for the multivariate harpacticoid copepod species composition and univariate diversity indices and environmental variables (excluding nutrients and oxygen). A fully crossed 3-factor design was performed in PERMANOVA with fixed factors month, site and position for the environmental variables nutrients and oxygen. A Bray-Curtis and Euclidean distance based resemblance matrix was used for untransformed multivariate and normalised univariate measures, respectively. Significant differences between groups can be shown by PERMANOVA, but no difference due to location (factor effect) or due to dispersion (variance) can be distinguished. Therefore, homogeneity of dispersion was tested with a PERMDISP, using distances among centroids calculated on the lowest level

(Quinn and Keough 2002). For univariate Euclidian distance the PERMDISP test is equivalent to the traditional univariate Levene's test (Anderson *et al.*, 2008). Post-hoc comparisons were performed using Pair-wise tests type III.

Copepoda species diversity was measured as species richness and Hill's diversity indices (Hill 1973): S = number of different species; N_1 = exp (H'), where H' is Shannon-Wiener diversity index based on the natural logarithm (ln); $N_2 = 1/\lambda$, where λ is Simpson's index.

Within the multivariate analysis, a SIMPER (Similarity Percentages) analysis was done to identify the main harpacticoid copepod species primarily providing the discrimination between the groups. A Principal coordinate analysis (PCO) based on a Bray Curtis similarity resemblance matrix of untransformed relative data of meiofauna taxa or harpacticoid copepod species was performed to visualise the community structure among the different months and sites (Anderson *et al.*, 2008). In order to find the best explanatory environmental variable for the meiofauna and harpacticoid copepod community structure, a multivariate BEST analysis with the BIOENV algorithm based on the Spearman rank correlation coefficient was performed (Clarke and Gorley 2006). The same BEST analysis was performed on the univariate data of the five most dominant harpacticoid copepods, representing each more than 5% of the total relative densities, to reveal the best explanatory variable of their distributions and abundances. After a skewness check through a Draftsman plot, the variables NO_x and PO_4 were log-transformed prior to the analysis. Several significant Spearman correlations were found: accumulation height and leaf litter DM (r_s = 0.81, N = 24, P = 0.022) and wind gust velocity and wind gust quantity (r_s = 0.96, N = 24, P < 0.001). Therefore accumulation height and wind gust quantity were excluded from the BEST analysis.

All the above mentioned analysis were performed with the Primer 6.1.11 software (Clarke and Gorley 2006) with PERMANOVA add-on software (Anderson *et al.*, 2008). A significance level of P < 0.05 was used for

univariate analysis and of $P < 0.001$ for multivariate analysis, due to the numerous comparisons in the multiple analyses of variance. Graphs were constructed in GraphPad 5.03 for Windows (GraphPad Software, San Diego California USA).

4. Results

4.1. Environmental data: Macrophytodetritus characterization

The month of October had the highest detritus accumulation height of 27 ± 4.6 cm (average ± standard deviation, henceforth used as notation) in OSCE compared to 5.0 ± 0.0 cm at the same site in August (Fig. 3.1). Detritus accumulation height differed significantly over months (Table 3.1). Pair-wise post-hoc tests for detritus accumulation height revealed that October differed significantly from all other months and that May and August also significantly differed (Table 3.1).

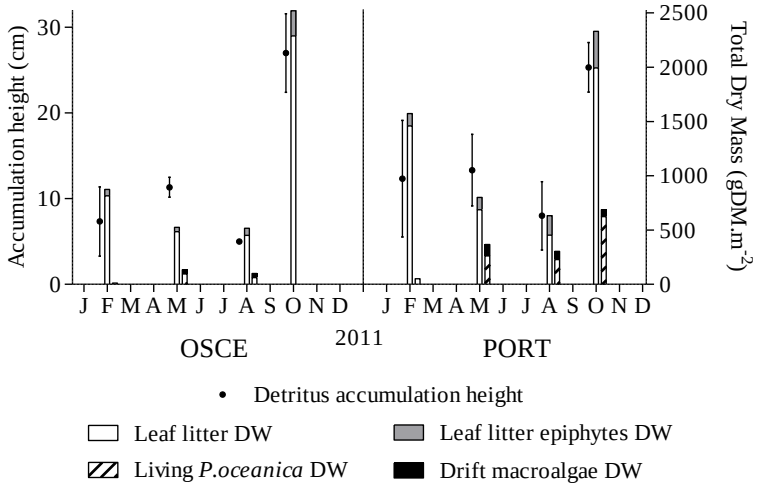


Fig. 3.1 Macrophytodetritus accumulation height represented in cm above the sea floor on the left y-axis (error bars = SD). Dry mass (DM) of Leaf litter, Leaf litter Epiphytes, Living P. *oceanica* and Drift macroalgae are represented on the right y-axis. N = 3

The leaf litter dry mass showed a maximum average dry mass in October of 2287.6 ± 617.9 $gDM.m^{-2}$ for OSCE (representing 90.7% of macrophytodetritus) and 1994.7 ± 860.1 $gDM.m^{-2}$ for PORT (representing 66.1% of macrophytodetritus). The lowest leaf litter dry mass was found in August with 452.1 ± 295.9 $gDM.m^{-2}$ (representing 73.1 % of macrophytodetritus) and 452.5 ± 98.8 $gDM.m^{-2}$ g (representing 48.2 % of macrophytodetritus) for OSCE and PORT, respectively (Fig. 3.1). The factor month showed to be significant especially for October compared to May and August (pair-wise post-hoc test).

The leaf litter epiphyte DM was the highest in October with 233.6 ± 18.2 $gDM.m^{-2}$ for OSCE and 336.9 ± 158.2 $gDM.m^{-2}$ for PORT, representing 9.3% and 11.2% of the total macrophytodetritus. Regarding the macrophytodetritus composition, the highest leaf litter epiphyte DM contribution was found in August for PORT (representing 19.2 % of macrophytodetritus) and for OSCE (representing 10.7 % of macrophytodetritus). Both month and site factors showed a significant effect (Table 3.1). The pair-wise post-hoc test revealed that October differed significantly from the other months. There was a significant difference in site for the month February.

Factors and interaction	Leaf litter DM	Leaf litter epiphytes DM	Drift macroalgae DM	Living *P.oceanica* DM
Month	$F'_{(3,16)}$=12.30 p <0.001 ***	$F'_{(3,16)}$=8.96 p <0.001 ***	$F'_{(3,16)}$=15.14 p< 0.001 ***	$F'_{(3,16)}$=6.21 p= 0.008 **
Site	$F'_{(1,16)}$=0.40 p =0.532	$F'_{(1,16)}$=6.41 p =0.021 *	$F'_{(1,16)}$=28.68 p <0.001 ***	$F'_{(1,16)}$=28.93 p <0.001 ***
Month x Site	$F'_{(3,16)}$=0.80 p= 0.525	$F'_{(3,16)}$=0.39 p =0.783	$F'_{(3,16)}$=3.59 p= 0.043 *	$F'_{(3,16)}$=7.71 p= 0.002 **

Factors and interaction	Detritus accumulation height	Wind gust velocity	Wind gust quantity
Month	$F'_{(3,16)}$=28.30 p <0.001 ***	$F'_{(3,16)}$=372.63 p< 0.001 ***	$F'_{(3,16)}$=435.65 p< 0.001 ***
Site	$F'_{(1,16)}$=1.64 p =0.220	$F'_{(1,16)}$=0.01 p =1.000	$F'_{(1,16)}$=3.90 p =0.068
Month x Site	$F'_{(3,16)}$=0.74 p =0.543	$F'_{(3,16)}$=0.05 p= 0.666	$F'_{(3,16)}$=1.76 p= 0.192

Table 3.1 *Two-way factorial PERMANOVA of environmental variables: detritus and wind descriptors; F' = Pseudo-F value; * = 0.05 < P < 0.01 =significant; ** = 0.01 < P < 0.001 = highly significant; *** = P < 0.001 = very highly significant. DM = Dry Mass*

The living *P. oceanica* DM was the highest in October in site PORT with 623.5 ± 284.9 gDM.m^{-2} and May in site PORT with 262.2 ± 111.8 gDM.m^{-2}, representing respectively 20.6% and 22.4% of the total macrophytodetritus (Fig. 3.1). The lowest biomass was observed in February in site OSCE with 12.5 ± 10.9 gDM.m^{-2} expressing 1.4% of the total macrophytodetritus biomass. No living *P. oceanica* DM was found in October OSCE (Fig. 3.1). All time, site and interaction factors had significant effects. The PERMDISP analysis of the lowest interaction factor was not revealed to be significant.

Drift macroalgae were absent in both sites in February and in the OSCE site in October. The highest drift macroalgae DM was found in May in the PORT

site (106.5 ± 40.5 gDM.m^{-2}) representing 9.1% of the total macrophytodetritus biomass. As for living *P. oceanica* DM, all factors were found to be significant except the lowest interaction factor (Table 3.1).

4.2. Environmental data: Abiotic factors

Median wind gust velocity reached a maximum during February (24.5 m.s^{-1}) and October (22.4 m.s^{-1}) (Fig. 3.2). The median wind gust velocity varied amongst months in decreasing order (OSCE, PORT): February (12.1 m.s^{-1}, 12.4 m.s^{-1}) > October (9.6 m.s^{-1}, 9.6 m.s^{-1}) > May (6.8 m.s^{-1}, 6.5 m.s^{-1}) > August (4.4 m.s^{-1}, 4.4 m.s^{-1}) (Fig. 2). The wind gust quantity varied with the same decreasing trend (OSCE, PORT): February (43.2%, 41.4%) > October (39.2%, 39.4%) > May (23.8%, 23.6%) > August (15.2%, 11.6%) (Fig. 3.2). Both median wind gust velocity and wind gust quantity showed a significant effect with the factor month but not for the site and interaction factors (Table 3.1).

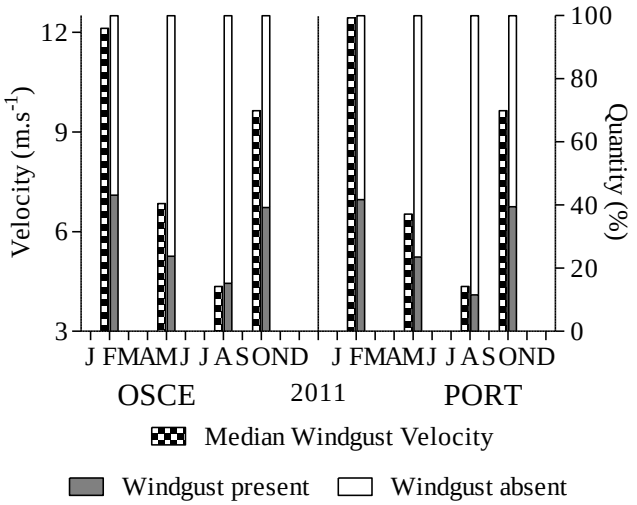


Fig. 3.2 *Wind gusts (selected as winds from North to East, with a velocity > 3.06 m.s-1) represented by their median velocity (during the 4 week's timeframe prior to sampling) on the left y-axis and the quantity of the wind*

gusts compared to all winds measured in percentages on the right y-axis

The oxygen concentration of the water inside the macrophytodetritus (WI) was always lower than the concentration in the water column (WC) and the water just above the macrophytodetritus (WJA). The latter two were always in the same range between 190 and 250 µM (Fig. 3.3A). For the litter values at least one replicate of each sample was always under the hypoxia limit, defined by Middelburg (2009). Some of the replicates of WI May PORT showed a negative value which seems erroneous since concentrations can never have a negative value. As explained in the materials and methods part, the oxygen concentration was obtained through a Winkler titration, implying no direct oxygen measurement but measurement of the corresponding added thiosulfate reagent. Therefore, when thiosulfate was titrated in excess (anoxia), a corresponding negative value of oxygen was calculated. NO_x concentrations showed a high variability (Fig. 3.3B). Nevertheless in February a noticeable increase of the water column NO_x concentration is visible next to a decrease in interstitial water content from May onwards. NH_4 concentrations were at least ten times higher in the interstitial water (IW) then all other positions, exception for the February OSCE sample where a measurement error might have occurred (Fig. 3.3C). The PO_4 concentrations showed no distinct trend apart from the higher concentration in the interstitial water with the exception of the February samples (Fig. 3.3D).

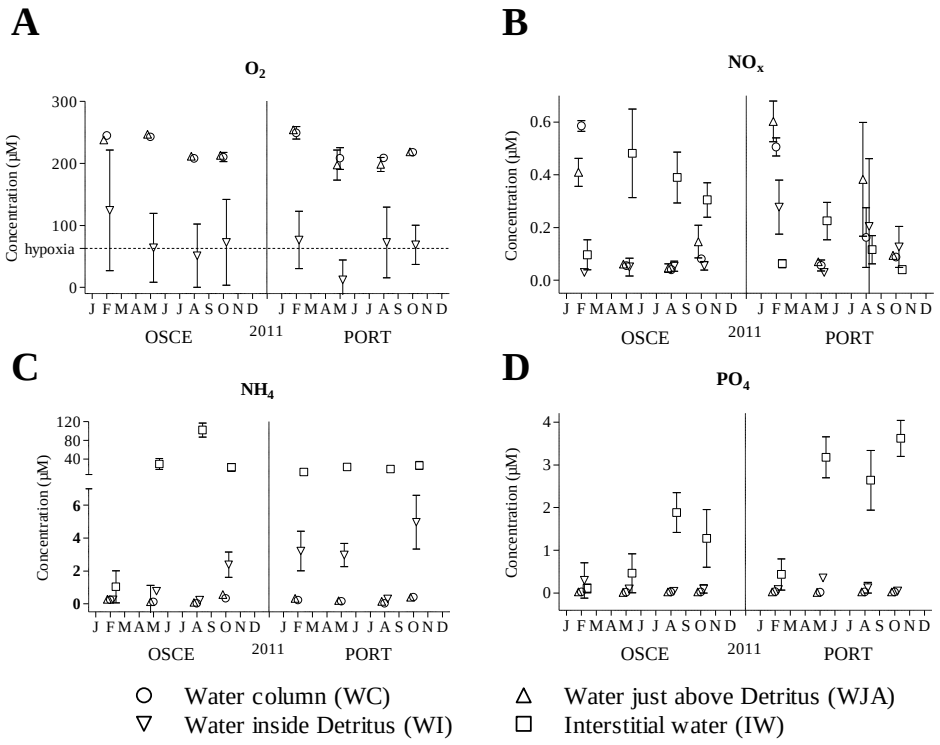


Fig. 3.3 *Oxygen and nutrient concentration measurements in µM on the y-axis, A: Oxygen, B: Nitrates, C: Ammonium and D: Phosphates. N = 6 and error bars represent the standard deviation*

The 3-way PERMANOVA for all nutrient and oxygen concentrations was significant for all factors and interactions except for the Month-Site-Position interaction factor for NO_x, the Site-Position interaction factor for oxygen and NO_x and Month-Position with oxygen (Table 3.2). PERMDISP's for all lowest interaction factors turned out to be significantly different, indicating that the variation within all factors and interactions was due to the dispersion effect and perhaps the location effect as well. All pair-wise correlations between abiotic factors were non-significant, except for wind gust velocity with WI O_2 concentration (r_s = 0.74, N = 24, P = 0.046) and with leaf litter DM (r_s = 0.77, N = 24, P = 0.028).

Factors and interaction	NH_4	NO_x	PO_4	O_2
Month (Mo)	$F'_{(3,160)}$=83.8 P <0.001 *	$F'_{(3,160)}$=38.9 P <0.001 *	$F'_{(3,160)}$=46.9 P <0.001 *	$F'_{(3,120)}$=16.6 P <0.001 *
Site (Si)	$F'_{(1,160)}$=50.5 P <0.001 *	$F'_{(1,160)}$=18.5 P <0.001 *	$F'_{(1,160)}$=120.6 P <0.001 *	$F'_{(1,120)}$=17.4 P <0.001 *
Position (Po)	$F'_{(3,160)}$=584.9 P <0.001 *	$F'_{(3,160)}$=3.5 P <0.015	$F'_{(3,160)}$=572.2 P <0.001 *	$F'_{(2,120)}$=312.3 P <0.001 *
Mo x Si	$F'_{(3,160)}$=89.3 P <0.001 *	$F'_{(3,160)}$=18.5 P <0.001 *	$F'_{(3,160)}$=26.6 P <0.001 *	$F'_{(3,120)}$=15.6 P <0.001 *
Mo x Po	$F'_{(9,160)}$=94.5 P <0.001 *	$F'_{(9,160)}$=3.5 P <0.001 *	$F'_{(9,160)}$=53.6 P <0.001 *	$F'_{(6,120)}$=2.3 P= 0.038
Si x Po	$F'_{(3,160)}$=68.3 P <0.001 *	$F'_{(3,160)}$=1.1 P =0.367	$F'_{(3,160)}$=117.1 P <0.001 *	$F'_{(2,120)}$=2.7 P= 0.074
Mo x Si x Po	$F'_{(9,160)}$=82.7 P <0.001 *	$F'_{(9,160)}$=1.1 P= 0.367	$F'_{(9,160)}$=21.2 P <0.001 *	$F'_{(6,120)}$=4.7 P <0.001 *

Table 3.2 *Three-way factorial PERMANOVA of nutrients and oxygen environmental variables; F' = Pseudo-F value; * = P < 0.001 = significant*

4.3. Meiofauna communities

At a higher taxon level, relative meiofauna composition revealed a clear dominance of Copepoda. Over all months, copepods represented 46.5 ± 14.6% (OSCE) and 49.4 ± 22.2% (PORT) of the meiofauna with a minimum in February and a maximum in August. The second most abundant taxon was Nematoda with 20.3 ± 10.1% in OSCE and 14.8 ± 4.9% in PORT. The lowest relative abundance was present in August and the highest in October for the OSCE site and May for the PORT site. The Copepoda / Nematoda ratio was high in August (8.6) and relatively equal throughout the other seasons: October (2.3), February (2.0) and May (1.8). The remaining taxa encountered in decreasing order, were nauplius larvae (15.7%), Amphipoda (4.9%), Turbellaria (4.5%), copepodites (3.7%),

Polychaeta (< 3%), Ostracoda, Isopoda, Halacaroidea, Tardigrada, Gastropoda, Kinorincha, Leptostraca, Cumacea, Gastrotricha, Oligochaeta, Tanaidacea, Cnidaria, Chaetognatha, Decapoda larvae and Pycnogonida.

The multivariate analysis showed no effect of the site factor or its interaction, and only showed an effect of month (2-way PERMANOVA, $F'_{(3,16)} = 6.7$, $P < 0.001$) on the meiofauna assemblage. The Principal coordinate analysis (PCO) of meiofauna taxa composition showed temporal separation with similarity contours of 75% separating the months(Fig. 3.4A).

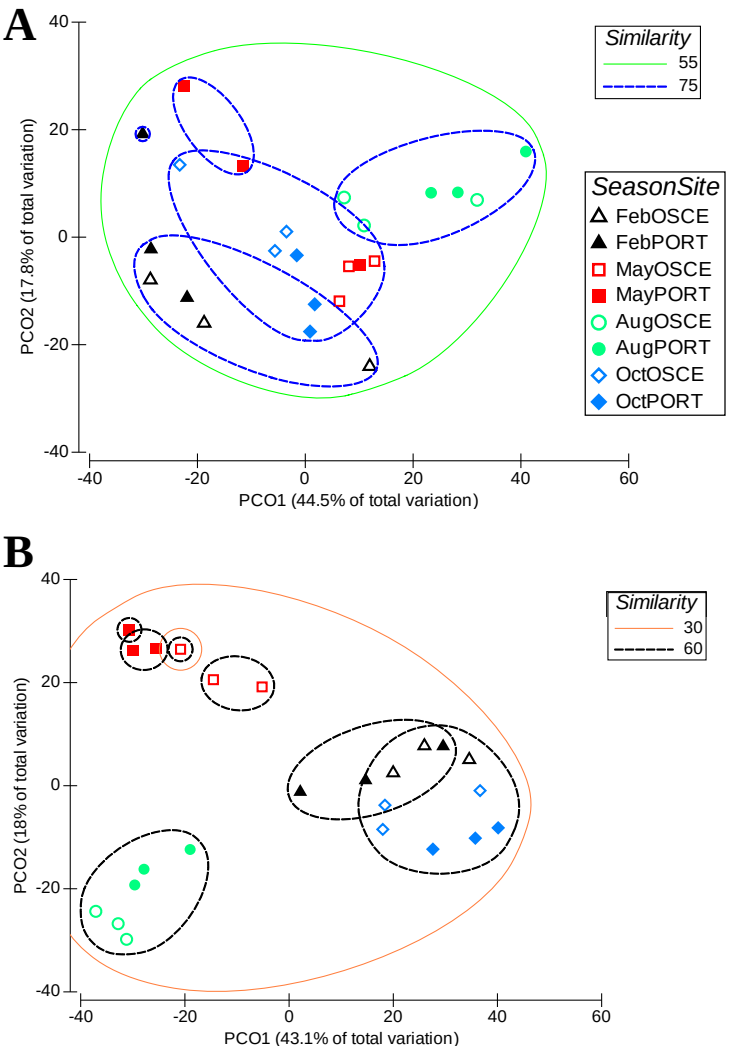


Fig. 3.4 *Principal coordinate analysis (PCO) based on a Bray Curtis similarity resemblance matrix on untransformed data of A: relative meiofauna taxon composition with 55% (green full line) and 75% (blue dashed line) similarities and B: relative harpacticoid copepod species composition with 30% (orange full line) and 60% (black dashed line) similarities. Filled symbols represent the Oscelluccia site (OSCE) and the un-filled symbols the harbour site (PORT). Black triangles: February; red squares: May; green circles: August and blue diamonds: October*

Total number of individuals reached their maximum in May for the OSCE site (60564 indiv. m^{-2}) and in August for the PORT site (78062 indiv. m^{-2}). In October for OSCE (34462 indiv. m^{-2}) and in February for PORT (31025 indiv. m^{-2}) a minimum total meiofauna amount was reached (Fig. 3.5A). Meiofaunal standardization towards gram leaf litter dry mass yielded the same maxima. The month of October returned low numbers of organisms per gram leaf litter in both sites (Fig. 3.5B). The univariate 2-way PERMANOVA on total meiofauna per m^2 displayed no significant differences in either factors (month, site) or interactions. In terms of total meiofaunal abundance per gram dry mass, the factor site had no significant effect, but the factor month (2-way PERMANOVA, $F'_{(3,16)} = 12.1$, $P < 0.001$) and the interaction factor (2-way PERMANOVA, $F'_{(3,16)} = 4.8$, $P < 0.001$) had a highly significant effect. The PERMDISP for the interaction factor was not significant and the pair-wise post-hoc test revealed that only May and August were not significantly different.

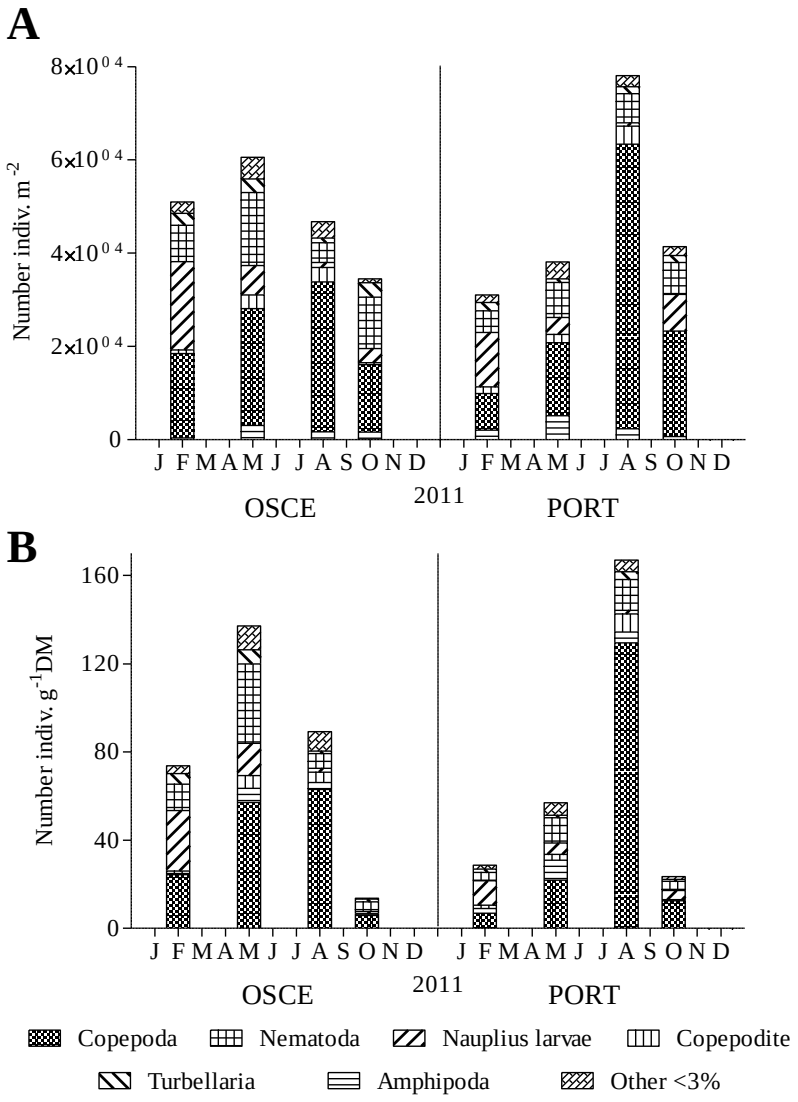


Fig. 3.5 *Main meiofauna taxa densities per sampled month and site with left, Oscelluccia (OSCE) and right, harbour (PORT). A: abundance per m² and B: abundance per g dry mass (DM) leaf litter*

The global multivariate BEST analysis revealed that wind gust velocity was the best explanatory variable (rho = 0.669) for the meiofaunal taxa assemblage, followed by its combination with the WI NO_x concentrations (rho = 0.587). The tertiary best explanatory correlation was the combination of wind gust velocity and WI NH_4 concentration (rho = 0.535). Oxygen concentration (WI O_2) correlation with all taxa abundances gave only one significant outcome (Amphipoda, r_s = -0.809, N = 24, P = 0.015). Correlating wind gust velocity with the different taxa abundances gave no significant Spearman correlation, with the exception of the Copepoda taxa (r_s = -0.74, N = 24, P = 0.046). The copepodite correlation was not significant, nonetheless the p-value was close to the significance threshold level (r_s = -0.69, N = 24, P = 0.069).

4.4. Harpacticoid copepod species composition

In total 44 different species belonging to four copepod orders were identified in the macrophytodetritus accumulations under study (Table 3.3). The majority of species (41) belonged to the order of the Harpacticoida, representing 87.2 ± 10.0%, while three species belonged to the orders Calanoida, Cyclopoida and Syphonostomatoida. Within those three orders, species other than those given in table 3 were found only in a juvenile state and therefore were not included in the species list. The most diverse harpacticoid families were Miraciidae and Tisbidae that were represented by four and five different species, respectively. The family Tisbidae was present in the highest absolute densities.

	February		May		August		October	
	OSCE	PORT	OSCE	PORT	OSCE	PORT	OSCE	PORT
	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Harpacticoida								
Ameiridae								
Ameira longipes	2.2 ± 0.9	8.4 ± 1.8	2.2 ± 1.4	8.5 ± 1.5				0.8 ± 1.3
Ancorabolidae								
Laophontodes bicornis	0.8 ± 0.7		1.8 ± 1.2	0.8 ± 0.7	1.4 ± 1.5	1 ± 0.9		1.2 ± 1.2
Laophontodes typicus	0.8 ± 1.4			0.3 ± 0.6				
Canuelidae								
Canuella furcigera	1.8 ± 0.9	0.4 ± 0.7						
Cletodidae								
Cletodes limicola		0.4 ± 0.7						
Cylindropsyliidae								
Cylindropsyllus laevis							0.4 ± 0.7	
Dactylopusiidae								
Dactylopusia tisboides	2.1 ± 1.4	1.4 ± 2.4	12.9 ± 4.4	3.9 ± 4.3	6.7 ± 5.6	5.9 ± 1.7		
Diarthrodes minutus	2.1 ± 0.6	1.3 ± 0	2.2 ± 1.4	1.5 ± 1.5				
Paradactylopodia brevicorni	2.1 ± 1.4	0.9 ± 0.8	1 ± 0.9	0.8 ± 0.7	1.4 ± 1.2	1.4 ± 1.4		
Ectinosomatidae								
Ectinosoma dentatum	12.8 ± 4.8	10.7 ± 1.7	2.2 ± 0.4	9.9 ± 2.7	12.5 ± 0.5	18.7 ± 5.2	10.2 ± 4	4.6 ± 0.6
Ectionosoma sp.		0.9 ± 0.8		0.5 ± 0.8	0.3 ± 0.6	1 ± 1.8		0.4 ± 0.7
Microsetella norvegica	0.8 ± 1.4	0.4 ± 0.8		0.3 ± 0.6	0.7 ± 0.6	0.5 ± 0.9	1.1 ± 1.9	0.9 ± 1.5
Euterpinidae								
Euterpina acutifrons			0.3 ± 0.6					
Hamondiidae								
Ambunguipes rufocincta			3.1 ± 2.3	0.8 ± 0.7				
Harpacticiidae								
Harpacticus littoralis	3.5 ± 0.5		0.9 ± 1.5	4.6 ± 3.5	5.9 ± 4	3.4 ± 0.9	1.1 ± 2	1.2 ± 2
Laophontidae								
Esola longicauda			0.6 ± 1.1		2.7 ± 2	3 ± 2.6		
Laophonte cornuta		0.9 ± 1.5	2.4 ± 1.2	8.3 ± 1.6	1.4 ± 1.2	1 ± 0.8		
Paralaophonte brevirostris	1.2 ± 2.1		6.9 ± 1.9	3.2 ± 1.9	4.2 ± 2.2	2.9 ± 1.3		2.2 ± 2
Longipediidae								
Longipedia minor			1.4 ± 0.6	1.1 ± 1	0.4 ± 0.6	0.5 ± 0.9		
Metidae								
Metis ignea				0.3 ± 0.6			0.4 ± 0.7	
Miraciidae								
Amphiascoides debilis	2.2 ± 0.9	8.4 ± 1.8	2.2 ± 1.4	8.5 ± 1.5				0.8 ± 1.3
Amphiascus minutus			9.2 ± 5.3	19.3 ± 2.2	3.1 ± 1.7	2.1 ± 3.6		4.1 ± 2.5
Sarsamphiascus tenuiremis			2.3 ± 2	1.5 ± 1.5	13.2 ± 3.2	13.5 ± 2.8		3.8 ± 1.3
Diosaccus tenuicornis	2.5 ± 2.1	5.8 ± 2.9	4.3 ± 0.8	3.9 ± 2.3	3.2 ± 2.9	9.2 ± 2.9	8.4 ± 5	3.8 ± 3.4
Peltiidae								
Alteutha depressa	2.2 ± 0.8		1.3 ± 1.1					
Porcellidiidae								
Porcellidium ovatum	4.4 ± 1.7	5.8 ± 2	7.6 ± 2.2	4.7 ± 2.9	2.4 ± 3.4		0.5 ± 0.9	
Pseudotachidiidae								
Dactylopodella flava					1.8 ± 1.6	1.9 ± 0.7		
Xouthous laticaudatus	1.2 ± 2.1			3.9 ± 4.3	6.7 ± 5.6	5.9 ± 1.7		

Tegastidae								
Tegastes areolatus			0.3 ± 0.5	0.9 ± 0.8	0.7 ± 0.6		0.6 ± 1	1.3 ± 2.2
Tegastes falcatus	1.8 ± 0.9	0.5 ± 0.8	1.4 ± 0.6					
Tegastes satyrus	0.5 ± 0.8		0.7 ± 0.6	2.1 ± 2.1	2.5 ± 2.2	2 ± 1		
Tetragonicepsidae								
Diagoniceps laevis			13.2 ± 18.3				0.6 ± 1	0.4 ± 0.7
Phyllopodopsyllus bradyi			2.2 ± 0.4		2 ± 3.5			0.4 ± 0.7
Thalestridae								
Parathalestris harpactoides	1.2 ± 2.1			0.8 ± 0.7				
Rhynchothalestris helgolandi								1.3 ± 2.2
Thalestris rufoviolascens			1.1 ± 0.2	0.3 ± 0.6				
Tisbidae								
Idyella exigua		11.2 ± 4.8			1.4 ± 1.6		7.1 ± 1.4	12.2 ± 2.6
Tisbe elegantula			0.3 ± 0.5				6.9 ± 6.8	3.8 ± 1.5
Tisbe ensifer	23.9 ± 1	10.6 ± 2.3	2.9 ± 1.6	3.6 ± 3	4.2 ± 1.9	2.4 ± 2.1	18.4 ± 10.1	12.5 ± 8
Tisbe furcata	27 ± 14.9	36.3 ± 6.9	5.1 ± 2.8	13.6 ± 2	10.5 ± 1.4	3.4 ± 0.9	40.3 ± 1.5	37.2 ± 7.8
Sacodiscus littoralis			2.4 ± 1.3					
Calanoida								
Clausocalanidae								
Clausocalanus arcuicornis	0.4 ± 0.7	0.4 ± 0.8		2.8 ± 4.8	0.4 ± 0.6			1.3 ± 1.3
Cyclopoida								
Cyclopinidae spp.	17.2 ± 12.9	9.6 ± 7.8	13.7 ± 15.4	26.7 ± 17.5	15.5 ± 9.5	21.3 ± 9.2	1.3 ± 1.2	4.2 ± 2.2
Siphonostomatoida								
Artotrogidae								
Cribropontius normani	1.7 ± 1.9	0.4 ± 0.7	1.5 ± 1.6	1 ± 1.6			0.6 ± 1	

Table 3.3 *List of relative abundances (%) of Copepoda species based on subsamples of 120 individuals averaged over three replicates (Avg.) ± standard deviation (SD). PORT = Harbour and OSCE = Oscelluccia. Species with bold font are the five most abundant harpacticoids*

Principal coordinate analysis (PCO) of harpacticoid copepod species showed a strong seasonal separation (Fig. 3.4B). In each month, clusters per site could be detected except for February and October. The separation by months was supported by a multivariate PERMANOVA ($P < 0.001$; Table 3.4).

SIMPER results comparing months showed that the one species (*Tisbe furcata*) was always among the top five similarity contributors. *Ectinosoma dentatum* was ranked important in all months except in May. *Diosaccus tenuicornis, Idyella exigua, Tisbe ensifer* and *Ameira longipes* were ranked as important contributors in at least two months (Table 3.4). In May (53.6% similarity) both sites showed the lowest cumulative contribution of the first five contributors (50.7%; Table 3.4). The highest dissimilarity (75.4% dissimilarity) was found between May and October. The two lowest

dissimilarities were found between February and October with a dissimilarity of 38.9%. The multivariate PERMANOVA analysis showed no separation per site (P = 0.014; Table 3.4). Over all months, four of the five most contributing species (SIMPER) were found in both sites. *Ameira longipes*, *Ectinosoma dentatum*, *Tisbe ensifer* and *Tisbe furcata* accounted together for 64.3% in OSCE and 65.0% in PORT (Table 3.4).

The samples from the month of May harboured the highest species richness (S) in terms of harpacticoid copepod species in OSCE (24.7 ± 2.1) and in PORT (20.0 ± 4.6). The lowest S value was noted in October for OSCE (9.0 ± 1.0) and in February for PORT (12.3 ± 0.6). Species richness differed significantly for every factor and interaction (Table 3.5), with a non-significant PERMDISP of the interaction factor (P = 0.324). The variability of N_1 (more sensitive to the number of abundant species) and N_2 (giving more weight to the dominant species) differed significantly for the factor month (PERMANOVA) (Table 3.5).

Across factor *Month* (PERMANOVA: $P < 0.001$)

February (75.9% similarity)

Species	%	cum. %
Tisbe furcata	32.2	32.2
Tisbe ensifer	21.3	53.5
Ectinosoma dentatum	12.7	66.2
Amphiascoides debilis	5.9	72.0
Idyella exigua	5.5	77.5

May (53.6% similarity)

Species	%	cum. %
Amphiascus minutus	15.3	15.3
Tisbe furcata	10.2	25.5
Dactylopusia tisboides	9.9	35.4
Porcellidium ovatum	7.8	43.3
Ameira longipes	7.4	50.7

August (72.5% similarity)

Species	%	cum. %
Ameira longipes	24.3	24.3
Ectinosoma dentatum	18.0	42.3
Sarsamphiascus tenuiremis	14.9	57.1
Tisbe furcata	8.0	65.1
Diosaccus tenuicornis	5.6	70.8

October (72.4% similarity)

Species	%	cum. %
Tisbe furcata	49.6	49.6
Tisbe ensifer	13.6	63.2
Idyella exigua	11.6	74.8
Ectinosoma dentatum	8.2	83.1
Diosaccus tenuicornis	4.7	87.7

Across factor *Site* (PERMANOVA: $P = 0.014$)

Oscelluccia (OSCE, 69.7% similarity)

Species	%	cum. %
Tisbe furcata	24.6	24.6
Tisbe ensifer	14.5	39.2
Ectinosoma dentatum	11.0	50.2
Ameira longipes	8.9	59.1
Dactylopusia tisboides	5.2	64.3

Harbour (PORT, 67.5% similarity)

Species	%	cum. %
Tisbe furcata	27.6	27.6
Ectinosoma dentatum	12.4	40.0
Ameira longipes	11.3	51.3
Amphiascus minutus	7.0	58.3
Tisbe ensifer	6.7	65.0

Table 3.4 *Multivariate PERMANOVA and SIMPER results with factors month and site for harpacticoid copepod species contributions. First five contributing species are shown*

Standardization of harpacticoid copepod abundances towards gram dry mass leaf litter was significantly affected by the factor month and its interaction with the factor site. The interaction had a non-significant PERMDISP (P = 0.360). However, when copepod densities were standardized by square meter, only the factor month showed an effect (Table 3.5). A pair-wise test revealed that only February-May and May-October were not significantly different from other months.

The global multivariate BEST analysis found wind gust velocity to be best explanatory variable for the harpacticoid copepod assemblage (rho =

0.510), followed by wind gust velocity combined with leaf litter DM (rho = 0.425). The tertiary explanatory variable was the combination of the two latter and the drift macroalgae DM (rho = 0.409). Harpacticoid S and environmental factors yielded a significant correlation for leaf litter DM (rs = -0.82, N = 24, P = 0.011).

The five univariate BEST analyses of the five most dominant harpacticoids yielded different best explanatory variables. The *Tisbe furcata* (19.0% of all harpacticoids) test revealed primary variables that were a combination of leaf litter DM, wind velocity and Epi/Lit ratio (rho = 0.503). The *Ectinosoma dentatum* (8.9% of all harpacticoids) test revealed primary variables that were a combination of leaf litter epiphytes DM and living *P. oceanica* DM (rho = 0.149). The *Tisbe ensifer* (8.6% of all harpacticoids) and *Ameira longipes* (8.4% of all harpacticoids) test displayed combinations of drift macroalgae DM and wind velocity as the best explanatory variables (rho = 0.480 and rho = 0.305, respectively). The *Diosaccus tenuicornis* (4.5% of all harpacticoids) test found a combination of leaf litter epiphytes DM, WI O_2 concentration and Epi / Lit ratio (rho = 0.144) as the best explanatory variables. The DISTLM results (see Ch. 7§ 2.2 and Addendum II) revealed oxygen as a predictor variable for the copepod community composition (9% of the variation), corroborating the species-specific effect of oxygen levels to copepod species from the BEST results.

Factors and interaction	S	N_1	N_2	Harpacticoida indiv. m^{-2}	Harpacticoida indiv. g^{-1}DM
Month	$F'_{(3,16)}$=37.8 *P*< 0.001 ***	$F'_{(3,16)}$=18.2 *P* <0.001 ***	$F'_{(3,16)}$=10.7 *P* <0.001 ***	$F'_{(3,16)}$=4.3 *P*= 0.006 **	$F'_{(3,16)}$=15.1 *P* <0.001 ***
Site	$F'_{(1,16)}$=10.5 *P* =0.005 **	$F'_{(1,16)}$=3.6 *P* =0.07	$F'_{(1,16)}$=1.7 P=0.209	$F'_{(1,16)}$=1.3 *P* =0.271	$F'_{(1,16)}$=2.1 *P* =0.106
Month x Site	$F'_{(3,16)}$=7.6 *P* =0.002 **	$F'_{(3,16)}$=2.5 *P*= 0.09	$F'_{(3,16)}$=1.2 *P* =0.331	$F'_{(3,16)}$=2.0 *P*= 0.099	$F'_{(3,16)}$=6.5 *P* <0.001 ***

Table 3.5 *Two factorial PERMANOVA of harpacticoid copepod species diversity indices and abundance standardised per square meter (indiv. m^{-2}) and per gram dry mass leaf litter (indiv. g^{-1}DM). S = species richness, N_1 and N_2*

*= heterogeneity of diversity; * = 0.05 < P < 0.01 = significant; ** = 0.01 < P <0.001 = highly significant; *** = P < 0.001 = very highly significant*

5. Discussion

5.1. Harpacticoid copepod species assemblage in detritus

According to Hicks and Coull (1983), harpacticoid copepods are regularly encountered as the most dominant and diverse meiobenthic taxon in phytal substrata. The comparison between macrophytodetritus accumulations and seagrass canopy revealed similar trends of the harpacticoid copepod community. The macrophytodetritus accumulations harboured the same density in order of magnitude (10^4-10^5 indiv.m^{-2}) as *P. oceanica* meadows (Novak 1982; Mascart *et al.,* 2013). The species richness, around 30 to 50 harpacticoid species, was similar to other phytal ecosystems (Hicks 1977b; Heip *et al.,* 1983; Johnson and Scheibling 1987; Steinarsdóttir *et al.,* 2003; De Troch *et al.,* 2008b). In this study, five abundant harpacticoid species were found belonging to different ecological and morphological groups. Two of them belonged to the phytal-swimmers group (Tisbidae family, genus *Tisbe*), known as very good swimmers. Two belonged to the phytal group *sensu stricto* (*Ameira longipes* and *Diosaccus tenuicornis*) and one was a typical mesopsammic species (Giere 2009) of the Ectinosomatidae family (*Ectinosoma dentatum*). The abundant species found in the macrophytodetritus are cosmopolitan and are recorded in other habitats as well (Bell *et al.,* 1987; Walters 1991; Colangelo *et al.,* 1996; Steinarsdóttir *et al.,* 2003; Giere 2009). During the calmest and lowest accumulation months of May and August, harpacticoid species were abundant and diverse. This rise coincides with the rise in primary production and increase in densities of mostly phytal harpacticoids such as *Ameira longipes, Diosaccus tenuicornis, Sarsamphiascus tenuiremis*, *Dactylopusia tisboides* and *Porcellidium ovatum*. In months with high leaf litter biomass (February and

October), *Tisbe furcata* and *T. ensifer* dominated the community. Both species are phytal-swimmers and seemed more adapted to the higher hydrodynamic disturbance. The distribution of the mesopsammic *Ectinosoma dentatum* on the other hand seemed to be linked, although with little strength (low rho), to the amount of leaf litter epiphytes and living *P. oceanica* present. Henceforth, we could assume that *Ecinosoma dentatum* migrated into the macrophytodetritus accumulation to avoid low oxygen levels in the sediment underneath or to search for more accessible food. Harpacticoids are known to feed on a wide variety of food sources (Lee *et al.,* 1977; Hicks and Coull 1983), displaying species-specific food preferences (Decho and Castenholz 1986; Pace and Carman 1996; Buffan-Dubau and Carman 2000; Wyckmans *et al.,* 2007; De Troch *et al.,* 2012b). Hicks and Coull (1983) stated that the existence of a wide variety of morphologically similar species in one habitat is allowed as a consequence of harpacticoids' selective feeding. This all points to the possibility that the harpacticoid community is mainly associated with the macrophytodetritus for food availability and shelter (Coull and Wells 1983).

Leaf litter has been recognised as a food source for harpacticoid copepods (Meyer and Bell 1989) since detrital forms of organic material were more palatable and more accessible than fresh material for consumers (Harrison and Mann 1975; Enriquez *et al.,* 1993; Edgar *et al.,* 1994). It is thus possible that macrophytodetritus accumulations yield a more readily available food for harpacticoids in contrast to other habitats and this will attract them (Norkko and Bonsdorff 1996). However, laboratory and field studies stated that the meiofaunal detritus-feeders primarily rely on the micro-epiphytes associated with the leaf litter surface (Ustach 1982; Hicks and Coull 1983; Carman and Thistle 1985). At this study site, the leaf litter epiphytes consisted of an abundant community of micro-epiphytic organisms such as bacteria, marine fungi, protozoa, micro- and detrital-algae (Lepoint *et al.*, 2006). This complex community of leaf litter epiphytes created micro-scale variability in resources and shelter for the associated fauna. However, no

difference in terms of epiphytic members was found in the present study and consequently we can state that the leaf litter epiphytes represent a bulk of macro- and micro-epiphytes. In order to obtain more information on the species-specific feeding preference of harpacticoid copepods additional investigations (e.g. food source tracing experiment) are certainly required.

The most abundant species in the macrophytodetritus accumulations were also commonly found in adjacent habitats (Novak 1982; Hicks and Coull 1983; Mascart *et al.,* 2013). Colonization by invertebrates is rapid, however, it is limited in its extent and magnitude (Palmer 1988; Norkko and Bonsdorff 1996). For that reason active migration or passive dispersion towards macrophytodetritus accumulations was not sufficient to explain comparable quantities and diversities. Dimech *et al.* (2006) suggested that small accumulations that persisted during the year in depressions of the seabed, harboured some fauna living permanently in this detritus. This implies that some harpacticoid species (morphologically specialized to a certain habitat) also live permanently in macrophytodetritus accumulations.

5.2. Environmental factors

The macrophytodetritus showed the highest accumulations and leaf litter dry mass in October, which coincided with the annual leaf fall, starting in September (Bay 1984). The peak could be explained by the annual senescence, though other factors presumably play a role in the variation of the litter amount during the rest of the year. Enhanced hydrodynamics related to storms had been put forward to explain the rise in accumulations of dislodged seagrasses and drift algae. The accumulation of dislodged material was shown to enhance habitat function by increasing structural complexity and food availability (Lenanton *et al.,* 1982; Kirkman and Kendrick 1997; Ólafsson *et al.,* 2013). However, during higher

hydrodynamic periods, the relative contribution of drift macroalgae and living *P. oceanica* was low. Subsequently, the meiofauna community assembly was not directly influenced by dislodged material (drift macroalgae and living *P. oceanica*) in the macrophytodetritus accumulation. However, drift macroalgae were a tertiary explanatory variable for the harpacticoid assemblage and a primary variable for the most abundant harpacticoid species, *Tisbe ensifer* and *Ameira longipes*. This result, points at a possible species-specific effect regarding the presence of drift macroalgae and its associated micro epiphytes.

The BEST analysis revealed the median wind gusts as the best explanatory variable for the meiofauna and harpacticoid assemblages in the macrophytodetritus accumulations. Dauby *et al.* (1995) conducted a sediment trap experiment that in the Bay of Calvi that showed northerly winds peak from October to April, with maximum values from mid-January to early March, which would cause a bigger disturbance during those months. Vetter (1995) reported low diversities of macro-invertebrates in disturbed leaf litter patches and attributed it to the disturbance by currents. Hovel *et al.* (2002) stated that hydrodynamic differences between seasons and years could explain the variability in crustacean density by directly influencing larval settlement, feeding rates and / or locomotion of crustaceans. Nonetheless, the yearlong presence of planktonic adults and juveniles in macrophytodetritus accumulations could highlight species–specific adaptations. It is known that copepods have an ability to swim and to emerge from the bottom into the water column and back (Teasdale *et al.,* 2004; Guidi-Guilvard *et al.,* 2009). As a possible consequence some planktonic species could adapt to an epibenthic life (Huys *et al.,* 1992; Giere 2009). Although in general the orders Calanoida, Cyclopoida and Syphonostomatoida have a planktonic life cycle, feeding on suspended fine-organic matter or they are parasitic on fish and invertebrates (Boxshall and Hasley 2004). Since, Thistle (2003) concluded that high hydrodynamic flows suppressed emergence, it was thus highly

probable that the non-harpacticoid adults and juveniles actively sought shelter in the macrophytodetritus from extensive hydrodynamic movements and predation. Consequently, we could conclude that benthic meiofauna and harpacticoid assemblages were negatively correlated with the wind gust induced water movements. Subsequently, planktonic copepods were to a lesser extent affected by the hydrodynamics, but sought shelter or adapted partially to the macrophytodetritus accumulations.

According to the accumulation and compaction of the detritus, a difference in oxygen penetration depth in the detritus accumulation could be expected. An oxygen gradient from the oxic water column and top layer of the detritus to the hypoxic bottom layer was present and directly influenced the vertical distribution and diversity of the meiobenthos (Higgins and Thiel 1988). Highly active fauna, especially crustaceans who are usually highly sensitive to hypoxia will be impacted first (Tietjen 1969). Since harpacticoid copepods are the most sensitive taxon to decreased oxygen (Moodley *et al.*, 2000), they are typically limited to the top layer of the detritus package. Nematodes conversely are more tolerant to low oxygen levels (Murrell and Fleeger 1989; Wetzel *et al.,* 2001). The Copepoda / Nematoda ratio peaked in August, while the nematode abundance remained fairly constant through the year. This sudden rise in copepod abundances and diversity (especially in the harbour) coincided with the calmest wind period and lowest accumulation height and leaf litter dry mass. Thus we might expect a trade-off between harpacticoid copepods and nematode densities according to the oxygen levels present in the accumulation. In our study, no such correlation of oxygen level with the Copepoda/Nematoda ratio was found. This could indicate the patchiness of the oxygen distribution within the accumulation and the high mobility of copepods that could migrate vertically out of the accumulation towards the oxygen-rich water just above the detritus. It could furthermore indicate a species-specific behaviour of harpacticoids, like *Diosaccus tenuicornis*, which was the only dominant harpacticoid species influenced by the

oxygen levels. Hence, no overall effect of oxygen concentrations on higher taxa was present, except for the Amphipoda. These abundances negatively correlated to oxygen levels inside the macrophytodetritus. A possible explanation could be the species-specific behaviour of amphipods towards hypoxia (Gamenick *et al.*, 1996). Another factor to be taken into account is that adults belong to the macrofauna and thus the organisms found are juveniles. These juveniles could possess physiological characteristics which allow them to use the hypoxic accumulation to shelter temporarily from predators that are not adapted to hypoxia.

Next to oxygen, other physico-chemical aspects were altered inside the macrophytodetritus accumulation. Meiofauna are not known to directly assimilate dissolved nutrients (Siebers 1982; Mitwally and Fleeger 2013), but physico-chemical fluctuations influence their potential food sources (Hicks 1977b, 1980; Hall and Bell 1988; Atilla *et al.,* 2005). Therefore, nutrient fluctuations would also presumably impact meiofauna indirectly by altering the habitat structure (Arroyo *et al.*, 2013). The DISTLM results (see Ch. 7§ 2.2 and Addendum II) did not reveal oxygen or nutrient concentrations as a predictor variable. Moreover, nutrients NO_x and PO_4^{2-} showed no significant relation with the multivariate copepod composition. This could indicate that single nutrients don't regulate meiofauna community compositions, however some of the nutrients are correlated to each other through biochemical reactions. Therefore, it can be assumed that the nutrients as a whole will influence meiofauna composition.

The present seasonal study demonstrated that meiofauna and harpacticoid copepod assemblages in macrophytodetritus accumulations reflect a seasonal cycle with a maximum abundance and diversity during spring-summer and a minimum during winter, coinciding with the epiphytic primary production cycle. These results are congruent with Hall and Bell (1988) and Johnson and Scheibling (1987) who showed that dominant motile invertebrates' abundances and diversities are positively correlated

with the habitat complexity as measured by the biomass of seagrass epiphytic algae. As pointed out by several authors, e.g. Weiser (1959), Novak (1982) and Hicks and Coull (1983), abundances and diversity of meiofauna of marine vegetation are positively correlated with habitat complexity and negatively correlated with water movements, which is confirmed by this study.

5.3. Conclusions

Meiofauna was ubiquitously present in the macrophytodetritus accumulations and half is composed of the crustacean subclass Copepoda, of which 87% belonged to the order Harpacticoida. As a consequence of the harpacticoid copepod species-specific selective feeding, a variety of morphologically and ecologically different species were present. The macrophytodetritus played an important role in sheltering, housing and feeding possibilities for meiofauna and harpacticoid copepods. Temporal community dynamics follow the epiphytic primary production cycle. They were positively correlated with habitat complexity and negatively correlated with water movements and leaf litter accumulations that varied throughout the study period. Migration and dispersion from other adjacent habitats seemed to promote faunal communities. However, a permanent population in the macrophytodetritus accumulations should not be excluded.

Three specific questions in this study were addressed in the introduction and answered as follows: (1) The macrophytodetritus accumulation and associated communities were mainly determined by seasonal wind induced hydrodynamics and leaf litter biomass. (2) Meiofauna and harpacticoid copepod assemblages displayed a maximum abundance and diversity during May-August (site specific) and a minimum during February. (3) Several ecological groups of copepods, including planktonic, parasitic,

mesopsammic, phytal and phytal-swimmers copepods were present.

Picture: Grande nacre – *Pinna nobilis*

Chapter 4

Inter-annual variation of the macrophytodetritus and associated communities

1. Abstract

The results shown in this Chapter 4 continue on the findings of previous Chapter 3. The main objective was to asses if differences in communities occurred between two successive years. The environmental factors (detritus composition, wind, oxygen levels) were not significantly different between both years and sites. However, differences were found between August months due to a higher accumulation of macrophytodetritus in 2012 (26.1 cm vs. 11.2 in 2011). The higher accumulation, especially higher detritus dry mass was due to a strong wind (12 $m.s^{-1}$) six days prior to sampling that exported the senescent leaves from inside the meadow to accumulate on the samplings site's sand patches. The meiofauna communities were similar to 65% and were regulated by the dry mass of the detritus. Copepod community composition was very heterogeneous and seemed less impacted by the differences in macrophytodetritus height between August 2011 and 2012. The same five most abundant copepods were found back from the seasonal follow-up study. Thus this interannual study highlighted the seasonality of macrophytodetritus patches and the resilience of copepod communities throughout time and its similarity on a space scale.

2. Introduction

The previous Chapter 3 presented a seasonal study demonstrating that meiofauna and harpacticoid copepod assemblages in macrophytodetritus accumulations reflected a seasonal cycle with a maximum abundance and diversity during spring-summer and a minimum during winter. Since temporal fluctuations were shown, the question rose to whether these fluctuations will reoccur every year at the same season or was the year of sampling an exceptional one. Therefore, the same approach and sampling

strategy was used in the subsequent year to unravel any inter-annual variation. However, the BEST (Chapter 3) and DISTLM (Addendum III) analysis revealed the litter leaf dry mass and the median wind gusts as best explanatory variables for the meiofauna and harpacticoid assemblages in the macrophytodetritus accumulations. Northerly winds, impacting both sites, peak from October to April, with maximum values from mid-January to early March (Dauby *et al.,* 1995). During the months of October and March bigger disturbances could be expected. To be able to compare between two similar seasons of two different years, conditions should be approximatively similar. The spring and summer seasons are relatively stable and calm, hence, fitted for the inter-annual analysis. In contrast, the autumn and winter seasons possess a very unpredictable weather pattern, including storms. The effect of storms on the macrophytodetritus is enormous (see Fig 2.5). Consequently to avoid a storm effect bias and non-comparable seasons, this chapter will focus on the months of May and August of 2011 and 2012 for comparison.

Physico-chemical environmental factors like concentration of oxygen (O_2), nitrogen (NH^{4+} and NO_x) and phosphate (HPO_4^{2-}) inside the accumulations are thought to influence potential food sources of meiofauna (Hicks 1977b, 1980; Hall and Bell 1988; Atilla *et al.,* 2005). However, meiofauna are not known to directly assimilate dissolved nutrients (Siebers 1982; Mitwally and Fleeger 2013). Therefore, nutrient fluctuations would presumably impact meiofauna only indirectly by altering the habitat structure (Arroyo *et al.*, 2013). Chapter 3 demonstrated that no clear pattern was visible in the nutrient concentrations. The BEST and especially the DISTLM output (Addendum III) could not find any of the nutrients as predictor variable for the meiofauna and copepod community composition. Therefore, in this chapter nutrients will not be used as environmental parameters. Oxygen on the other hand, did have a species-specific influence on for instance the *Diosaccus tenuicornis* species.

The abundant copepod species found in the macrophytodetritus in Chapter

3 (Table 3.3), were cosmopolitan and were recorded in other habitats as well (Bell *et al.,* 1987; Walters 1991; Colangelo *et al.,* 1996; Steinarsdóttir *et al.,* 2003; Giere 2009). The five abundant harpacticoid species found belonged to different ecological and morphological groups (See Table I.1 in Addendum I). Two of them belonged to the phytal-swimmers group (genus *Tisbe*), known as very good swimmers. Two belonged to the phytal group *sensu stricto* (*Ameira longipes* and *Diosaccus tenuicornis*) and one was a typical mesopsammic species (*Ectinosoma dentatum*). Presumably, not all five copepod species will be found in similar abundances in 2012. However, their dominant role should be noticed.

3. Materials and methods

Concerning the sampling sites and strategy, the same PORT and OSCE sites are used as in Chapter 3 and are described in Ch. 2 § 1.3. The methodologies for collecting and processing the materials are identical to Chapter 3. As mentioned before we selected the spring and summer seasons, respectively May and August in two successive years to see if there is an inter-annual difference. The data used for the 2011 seasons came from Chapter 3 and new data were acquired for the 2012 samples. On top of the 2012 data, extra replicates taken in 2011 were processed and added to the existing 2011, N=3, bringing the total replicate amount in this chapter for the 2011 and 2012 data to N=6. A fully crossed 3-way fixed factors PERMANOVA should be performed with variables site, month and year. However, the factor month is nested in year and therefore becomes a random variable. A Bray-Curtis and Euclidean distance based resemblance matrix was used for untransformed multivariate and normalised univariate measures, respectively.

4. Results

4.1. Environmental data

Median wind velocity reached 4.47 ± 2.65 m.s^{-1} in 2011 and 3.63 ± 3.64 m.s^{-1} in 2012. No significant difference was thus found between both years. However, this is the median velocity over the four weeks prior to sampling. In August 2012, the week before sampling a wind peak at 12 m.s^{-1} was recorded coming from the North-East.

The month of August 2012 had a high detritus accumulation height of 25.0 ± 7.0 cm for OSCE and 27.3 ± 11.0 cm for PORT (Fig 4.1). No significant differences were found except for the interaction term (Table 4.1). Pair-wise tests revealed a significant differences between August 2011 and 2012 (Pair-Wise, P = 0.649).

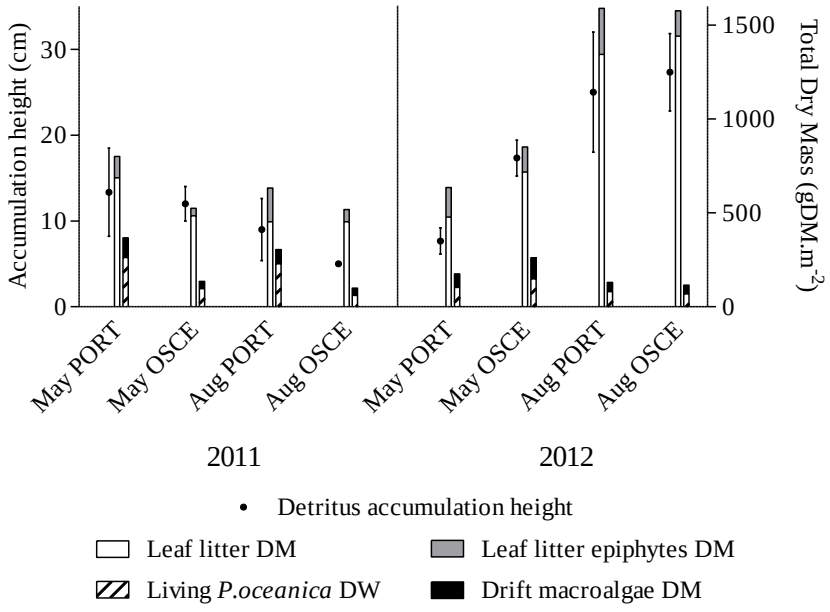


Fig. 4.1 Macrophytodetritus accumulation height represented in cm above the sea floor on the left y-axis (error bars = SD). Dry mass (DM) of Leaf litter, Leaf litter Epiphytes, Living P. *oceanica* and Drift macroalgae are represented on the right y-axis. N = 6

The leaf litter dry mass showed a maximum average dry mass in October August 2012 of 2705.2 ± 332.3 gDM.m^{-2} for OSCE (representing 75.9% of macrophytodetritus) and 1344.1 ± 619.4 gDM.m^{-2} for PORT (representing 72.7% of macrophytodetritus). The lowest leaf litter dry mass was found in August 2011 with 452.1 ± 295.9 gDM.m^{-2} (representing 73.1 % of macrophytodetritus) and 452.5 ± 98.8 gDM.m^{-2} g (representing 48.2 % of macrophytodetritus) for OSCE and PORT, respectively (Fig. 4.1). The PERMANOVA showed to be significant for nested factor month and for the interaction term (Table 4.1)

The leaf litter epiphyte DM was the highest in August 2012 with 254.1 ± 39.3 gDM.m^{-2} for OSCE and 246.6 ± 103.1 gDM.m^{-2} for PORT, representing 7.1% and 13.3% of the total macrophytodetritus. Regarding the macrophytodetritus composition, the highest leaf litter epiphyte DM contribution was found in August 2011 for PORT (representing 19.2 % of macrophytodetritus) and for OSCE (representing 10.7 % of macrophytodetritus). The factor month nested in year showed a significant effect (Table 4.1).

The living *P. oceanica* DM was the highest in August 2011 in site OSCE with 504.2 ± 297.7 gDM.m^{-2} and May 2011 in site PORT with 262.2 ± 111.8 gDM.m^{-2}, representing respectively 14.1% and 22.4% of the total macrophytodetritus (Fig. 4.1). The lowest biomass was observed in August 2011 in site OSCE with 61.3 ± 30.7 gDM.m^{-2} expressing 9.9% of the total macrophytodetritus biomass. The PERMANOVA yielded the same significant factors as the leaf litter DM.

Drift macroalgae were absent in both sites in February and in the OSCE site in October. The highest drift macroalgae DM was found in May 2011 in the PORT site (106.5 ± 40.5 gDM.m^{-2}) and in May 2012 OSCE site (112.1 ± 16.3 gDM.m^{-2}), representing 9.1% and 10.1% of the total macrophytodetritus

biomass, respectively. As for living *P. oceanica* DM, only the nested factor showed to be significant (Table 4.1).

Factors and interaction	Detritus accumulation height	Leaf litter DM	Leaf litter epiphytes DM	Living *P. oceanica* DM	Drift macroalgae DM
Year (Yr)	$F'_{(1,40)}$=23.8 *P*= 0.338	$F'_{(1,40)}$=1.2 *P*= 0.331	$F'_{(1,40)}$=2.9 *P*= 0.321	$F'_{(1,40)}$=0.4 *P*= 0.992	$F'_{(1,40)}$=0.4 *P*= 0.994
Site (Si)	$F'_{(1,40)}$=0.5 *P*= 0.729	$F'_{(1,40)}$=1.5 *P*= 0.322	$F'_{(1,40)}$=15.3 *P*= 0.078	$F'_{(1,40)}$=0.5 *P*= 0.733	$F'_{(1,40)}$=1.5 *P*= 0.348
Nested Month (Mo [Yr])	$F'_{(2,40)}$=0.8 *P*< 0.447	$F'_{(2,40)}$=77.1 *P*< 0.001 *	$F'_{(2,40)}$=1.2 *P*< 0.01 *	$F'_{(2,40)}$=13.6 *P*< 0.001 *	$F'_{(2,40)}$=11.5 *P*< 0.001 *
Yr x Si	$F'_{(1,40)}$=0.2 *P*= 0.673	$F'_{(1,40)}$=2.5 *P*= 0.231	$F'_{(1,40)}$=10.6 *P*= 0.100	$F'_{(1,40)}$=6.8 *P*= 0.114	$F'_{(1,40)}$=1.2 *P*= 0.376
Si x Mo [Yr]	$F'_{(2,40)}$=7.6 *P*< 0.001 *	$F'_{(2,40)}$=12.2 *P*< 0.001 *	$F'_{(2,40)}$=0.6 *P* <0.531	$F'_{(2,40)}$=4.5 *P*< 0.001 *	$F'_{(2,40)}$=7.1 *P*= 0.005

Table 4.1 *Nested-design PERMANOVA of environmental detritus descriptors; F' = Pseudo-F value; * = P < 0.001 = significant. DM = Dry Mass*

The oxygen concentrations of the water inside the macrophytodetritus (WI) were for 2012 always above the hypoxia limit defined by Middelburg (2009). In contrast, for 2011 most of the replicates were under the limit. The oxygen concentrations in the water column (WC) and in the water just above the macrophytodetritus (WJA) were in 2011 and 2012 consistently in the same range between 190 and 250 µM (Fig. 4.2). The 4-way PERMANOVA with nested factor site in position showed no significant differences. Except all the interaction factors which included the nested site in position factor. The subsequent PERMDISP analyses yielded a significant result.

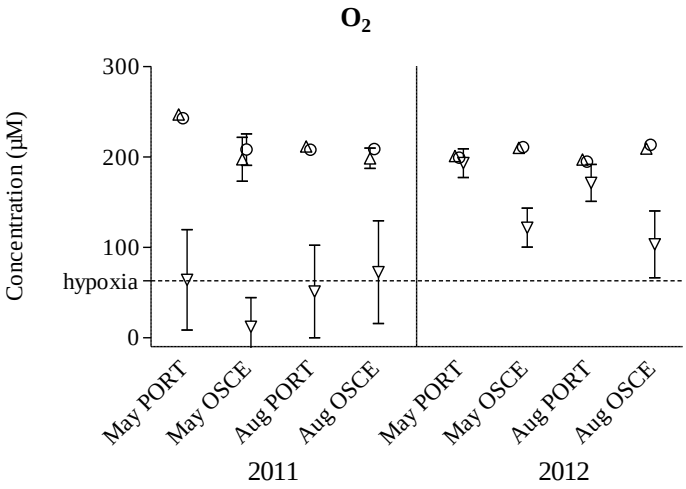


Fig. 4.2 *Mean ± SD of oxygen concentrations at different positions in the water column (circle), just above the detritus (triangle) and inside the litter accumulation (reverse triangle)*

4.2. Meiofauna community composition and diversity

A total of 22 different higher meiofauna taxa were identified. Based on relative composition, the most abundant organisms belonged to two higher taxa: Copepoda and Nematoda (18.0 ± 7.9%). Overall, copepods had the highest relative abundance (51.0 ± 14.7%) with a maximum in the 2011 August PORT (78.2%) and a minimum in 2012 May PORT (37.4%) (Fig 4.4). Nematodes accounted on average for 18.0 ± 7.9% with a maximum in the 2012 May PORT sample (26.6%) and a minimum in the 2011 August PORT (7.9%). Beside the two main taxa, nauplius larvae and copepodites accounted on average for 7.7 ± 5.5% and 7.6 ± 4.0%, respectively. Amphipoda, as adults part of the macrofauna and as juveniles, part of the meiofauna accounted on average for 5.9 ± 4.2%. The last taxon present for more than 3% on average was the Turbellaria with an average of 3.4 ± 1.8% (Fig. 4.4). The remaining 6.4 ± 2.9% were made of 16 taxa, in order of decreasing abundance: Polychaeta, Ostracoda, Isopoda, Halacaroidea,

Tardigrada, Gastropoda, Kinorincha, Leptostraca, Cumacea, Gastrotricha, Oligochaeta, Tanaidacea, Cnidaria, Chaetognatha, Decapoda larvae and Pycnogonida.

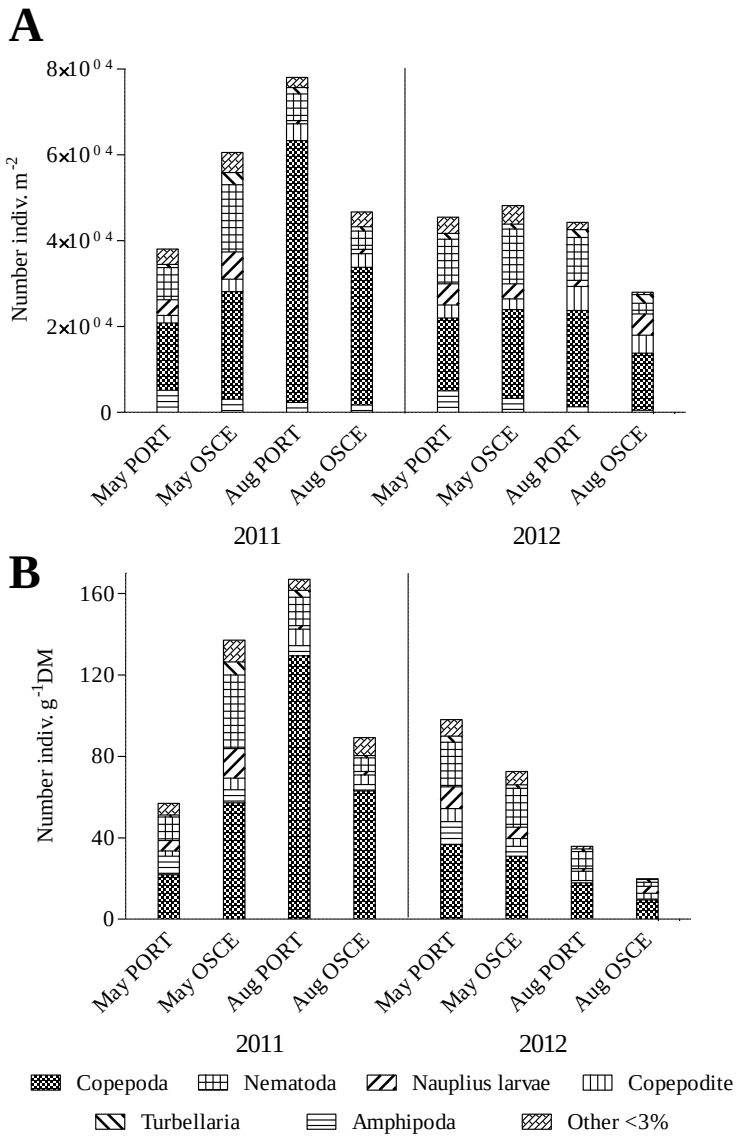


Fig 4.4 *Main meiofauna taxa densities per sampled year, month and site with left the year 2011 and right, the year 2012. A: abundance per m^2 and B: abundance per g dry mass (DM) leaf litter*

No significant differences between both years neither both sites in terms of total meiofauna abundance g^{-1} DM and copepod abundances g^{-1} DM were found (PERMANOVA, all $P > 0.001$). In terms of relative composition, the PERMANOVA statistics with nested design revealed only a significant difference in the nested factor (Table 4.1). A pair-wise comparison of both months within each year yielded a significant difference (2011: Pair-wise, t = 8.5, $P < 0.001$ and 2012: Pair-wise, t = 4.9, $P < 0.001$). The PCO constructed on non-transformed relative meiofauna taxa densities (Fig 4.5 A) displayed the difference between months, especially for the month August (red squares vs. red circles). Both years (blue vs. red) and sites (open vs. filled symbols) are clearly intertwined (Fig 4.5 A).

The DISTLM results yielded as best environmental predictor variable for the meiofauna community assembly the Detritus Dry Mass (31.4% variation, $P = 0.001$). The second best descriptor variable was de Macroalgae dry mass (14.4% variation, $P = 0.001$) followed by de wind velocity (8.0% variation, $P = 0.001$).

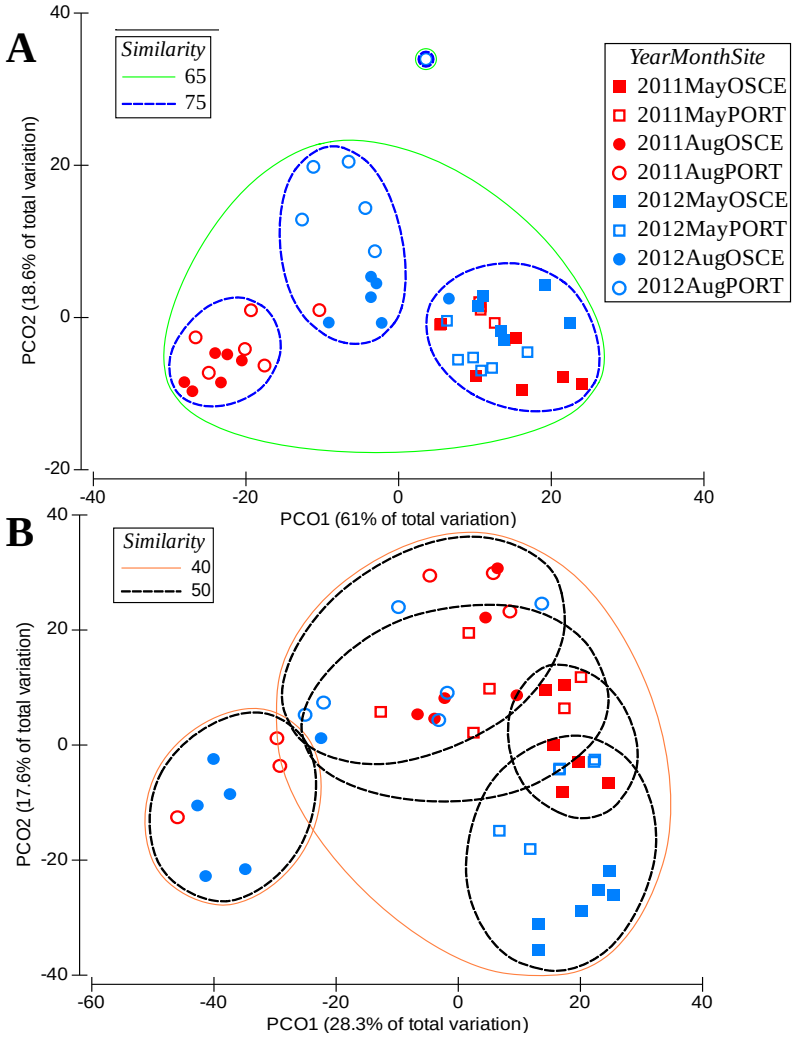


Fig. 4.5 *Principal coordinate analysis (PCO) based on a Bray Curtis similarity resemblance matrix on untransformed data of A: relative meiofauna taxon composition with 65% (green full line) and 75% (blue dashed line) similarities and B: relative harpacticoid copepod species composition with 40% (orange full line) and 50% (black dashed line) similarities. Filled symbols represent the Oscelluccia site (OSCE) and the un-filled symbols the harbour site (PORT). Red represents the year 2011 and blue the year 2012. Squares: May and circles: August.*

4.3. Harpacticoid copepod species composition

In total 44 different species belonging to four copepod orders were identified in the macrophytodetritus accumulations under study (Table 4'.3). The majority of species (41) belonged to the order of the Harpacticoida, while three species belonged to the orders Calanoida, Cyclopoida and Syphonostomatoida. The most diverse harpacticoid families were Tisbidae, Miraciidae and Tegastidae that were represented by five, four and four different species, respectively. The family Tisbidae was present in the highest absolute densities. The most abundant harpacticoid species were in decreasing order of total abundance: *Tisbe furcata* (11.6%), *Ameira longipes* (10.0%), *Ectinosoma dentatum* (7.3%), *Dactylopodia tisboides* (6.7%) and *Diosaccus tenuiremis* (6.2%). The cyclopoid copepod Cyclopinidae sp. was the most abundant non harpacticoid copepod with 8.3% of the total abundance (Table 4.2).

Principal coordinate analysis (PCO) of harpacticoid copepod species showed three main clusters at a similarity of 75% (Fig. 4.4B). The first cluster encompassed the August 2011 sites, the second cluster the August 2011 sites and the third cluster the sites of month May in both years. August 2012 seemed a priori different in terms of meiofauna composition compared to the same month the following year.

The PERMANOVA with nested design of the multivariate meiofauna community composition was only significant in the interaction term (Mo[Yr]xSi, $F'_{(2,40)} = 3.7$, $P < 0.001$) and in the nested term (Mo[Yr], $F'_{(2,40)} = 9.8$, $P < 0.001$). The consequent PERMDISP on the highest interaction term revealed a *P*-value of 0.005

The 2-way crossed SIMPER with factors year and month presented *Tisbe furcata*, *Ectinosoma dentatum* and *Ameira longipes* in every top five contributions. The similarity of 2011 was 52.3% and of 2012 was 56.7% with a cumulative top five composition of 55.8% and 50.2, respectively.

	2011				2012			
	May		August		May		August	
	OSCE	PORT	OSCE	PORT	OSCE	PORT	OSCE	PORT
Harpacticoida								
Ameiridae								
Ameira longipes	2.2 ± 1.4	8.5 ± 1.5			3.6 ± 1.7	10.8 ± 2.2	3.4 ± 1.8	19.1 ± 12.8
Ancorabolidae								
Laophontodes bicornis	1.8 ± 1.2	0.8 ± 0.7	1.4 ± 1.5	1 ± 0.9	1.9 ± 1.4			
Laophontodes typicus		0.3 ± 0.6			0.3 ± 0.5			0.2 ± 0.4
Cletodidae								
Cletodes limicola					0.3 ± 0.5		0.2 ± 0.4	0.4 ± 0.9
Dactylopusiidae								
Dactylopusia tisboides	12.9 ± 4.4	3.9 ± 4.3	6.7 ± 5.6	5.9 ± 1.7	10.6 ± 2.7	5.9 ± 4.5	1.9 ± 1.9	3.5 ± 2
Diarthrodes minutus	2.2 ± 1.4	1.5 ± 1.5			3.7 ± 1.6	1.3 ± 1.2	0.2 ± 0.4	
Paradactylopodia brevicornis	1 ± 0.9	0.8 ± 0.7	1.4 ± 1.2	1.4 ± 1.4	3.9 ± 1.3	6.9 ± 4.3	1.5 ± 1.2	5.2 ± 5.5
Ectinosomatidae								
Ectinosoma dentatum	2.2 ± 0.4	9.9 ± 2.7	12.5 ± 0.5	18.7 ± 5.2	6.5 ± 2	3.4 ± 1.1	6 ± 1.6	8.9 ± 3.3
Euterpinidae								
Euterpina acutifrons	0.3 ± 0.6				0 ± 0	0 ± 0	0.3 ± 0.8	0.6 ± 1.6
Hamondiidae								
Ambunguipes rufocincta	3.1 ± 2.3	0.8 ± 0.7				0.3 ± 0.8		0.4 ± 0.7
Harpacticiidae								
Harpacticus littoralis	0.9 ± 1.5	4.6 ± 3.5	5.9 ± 4	3.4 ± 0.9				2.4 ± 1.3
Laophontidae								
Esola longicauda	0.6 ± 1.1		2.7 ± 2	3 ± 2.6	0.3 ± 0.5		0.5 ± 0.9	2.1 ± 1.3
Laophonte cornuta	2.4 ± 1.2	8.3 ± 1.6	1.4 ± 1.2	1 ± 0.8	1.5 ± 1.6	1.8 ± 0.8	0.2 ± 0.4	0.5 ± 1.1
Paralaophonte brevirostris	6.9 ± 1.9	3.2 ± 1.9	4.2 ± 2.2	2.9 ± 1.3	4.2 ± 2.3	5.3 ± 1.7	0.3 ± 0.5	4.5 ± 3.6
Longipediidae								
Longipedia minor	1.4 ± 0.6	1.1 ± 1	0.4 ± 0.6	0.5 ± 0.9	1.6 ± 0.9	2.5 ± 1.3		
Metidae								
Metis ignea		0.3 ± 0.6						
Miraciidae								
Amphiascoides debilis	2.2 ± 1.4	8.5 ± 1.5			4.3 ± 2.2	13.2 ± 8.1	4.2 ± 1.9	12.8 ± 8.4
Amphiascus minutus	9.2 ± 5.3	19.3 ± 2.2	3.1 ± 1.7	2.1 ± 3.6	2.7 ± 0.6	4.7 ± 2.9	1.4 ± 1.1	2 ± 1.9
Sarsamphiascus tenuiremis	2.3 ± 2	1.5 ± 1.5	13.2 ± 3.2	13.5 ± 2.8	0.7 ± 1	0.3 ± 0.5		
Diosaccus tenuicornis	4.3 ± 0.8	3.9 ± 2.3	3.2 ± 2.9	9.2 ± 2.9	2.8 ± 1.2	2.9 ± 0.9	5.6 ± 6.5	1.8 ± 2.7
Peltiidae								
Alteutha depressa	1.3 ± 1.1				0.2 ± 0.4			
Porcellidiidae								
Porcellidium ovatum	7.6 ± 2.2	4.7 ± 2.9	2.4 ± 3.4		7.8 ± 3.8	1.6 ± 0.4	1 ± 2	
Pseudotachidiidae								
Dactylopodella flava			1.8 ± 1.6	1.9 ± 0.7	2.1 ± 1.7	4.5 ± 0.2	1.7 ± 1.3	1.4 ± 1.3
Xouthous laticaudatus		3.9 ± 4.3			0.8 ± 0.7			

Tegastidae								
Parategastes sphaericus					1.6 ± 1.7		0.4 ± 0.6	
Tegastes areolatus	0.3 ± 0.5	0.9 ± 0.8	0.7 ± 0.6		0.6 ± 0.9			0.6 ± 0.9
Tegastes falcatus	1.4 ± 0.6					1.6 ± 1.1	0.5 ± 0.6	
Tegastes satyrus	0.7 ± 0.6	2.1 ± 2.1	2.5 ± 2.2	2 ± 1		0.5 ± 0.5	3.2 ± 3.2	3.7 ± 2.1
Tetragonicepsidae								
Diagoniceps laevis	13.2 ± 18.3				0.7 ± 1	0.3 ± 0.5		
Phyllopodopsyllus bradyi	2.2 ± 0.4		2 ± 3.5				0.5 ± 0.8	
Thalestridae								
Parathalestris harpactoides		0.8 ± 0.7			0.7 ± 0.7	1.7 ± 1.2		
Rhynchothalestris helgolandica					0.6 ± 0.5	2 ± 2.8		0.8 ± 1.3
Thalestris rufoviolascens	1.1 ± 0.2	0.3 ± 0.6			0.4 ± 0.5			
Tisbidae								
Idyella exigua			1.4 ± 1.6			0.6 ± 1	3.3 ± 2.5	
Tisbe elegantula	0.3 ± 0.5					0.3 ± 0.5		
Tisbe ensifer	2.9 ± 1.6	3.6 ± 3	4.2 ± 1.9	2.4 ± 2.1	4.9 ± 3.2	2.1 ± 2.7	13.9 ± 5.8	7.8 ± 8.1
Tisbe furcata	5.1 ± 2.8	13.6 ± 2	10.5 ± 1.4	3.4 ± 0.9	4.6 ± 2.3	7.9 ± 6.7	35.2 ± 7.6	10.3 ± 5.9
Sacodiscus littoralis	2.4 ± 1.3				12.3 ± 3.4	0.4 ± 0.5		
Calanoida								
Clausocalanidae								
Clausocalanus arcuicornis		2.8 ± 4.8	0.4 ± 0.6		1.6 ± 1.3	1.9 ± 1.5	2.6 ± 1.4	5.7 ± 2.4
Cyclopoida								
Cyclopinidae spp.	13.7 ± 15.4	26.7 ± 17.5	15.5 ± 9.5	21.3 ± 9.2	0.3 ± 0.5	1.5 ± 1.3	10.9 ± 9	6.8 ± 3.1
Oithonidae spp.					0.7 ± 0.6	1 ± 1		
Siphonostomatoida								
Artotrogidae								
Cribropontius normani	1.5 ± 1.6	1 ± 1.6			0.3 ± 0.5	0.5 ± 0.5		

Table 4.2 *List of relative abundances (%) of Copepoda species. Average ± standard deviation.*

Across factor *Month*

2011 (52.3% similarity)

Species	%	cum. %
Ameira longipes	14.1	14.1
Cyclopinidae sp.	12.5	26.6
Ectinosoma dentatum	10.8	37.4
Tisbe furcata	9.9	47.3
Amphiascus minutus	8.5	55.8

2012 (56.7% similarity)

Species	%	cum. %
Tisbe furcata	15.6	15.6
Diosaccus tenuiremis	9.4	25.0
Ameira longipes	8.6	33.5
Amphiascoides debilis	8.4	41.9
Ectinosoma dentatum	8.3	50.2

Across factor *Year*

May (57.2% similarity)

Species	%	cum. %
Diosaccus tenuiremis	12.2	12.2
Ameira longipes	9.3	21.6
Amphiascus minutus	6.9	28.4
Tisbe furcata	6.7	35.1
Ectinosoma dentatum	6.4	41.5

August (51.7% similarity)

Species	%	cum. %
Tisbe furcata	19.6	19.6
Ameira longipes	13.3	33.0
Ectinosoma dentatum	13.0	46.0
Cyclopinidae sp.	12.1	58.0
Tisbe ensifer	9.1	67.1

Table 4.3 *Contribution (%) and cumulative contribution (cum. %) of the copepod species responsible for the similarity for each selected factor (SIMPER)*

The DISTLM yielded as best environmental predictor variables responsible for the variation of the Copepoda community, the environmental factor Detritus DW (21.3% variation, $P = 0.001$). Oxygen concentration came out as second predictor variable with 18.5% variation ($P = 0.001$) and with a variation of 7.7%, the predictor environmental variable wind velocity ($P = 0.001$).

5. Discussion

5.1. Macrophytodetritus composition and variability

Six days prior to sampling in August 2012, a wind peak exported detritus from the meadow to the sand patch. The wind data (velocity and quantity) was calculated on 4 weeks and 4 days prior to sampling (See Ch. 3 § 3 for details). Unfortunately, the hydrodynamic effect happened only once and shortly. Therefore, the environmental wind variables do not represent it since it is lost in the averaging of the data. The wind speeds corresponded to 6 Beaufort and is described as a strong breeze, which forms long waves of 4 metres high (Stewart 2004). Moreover, the sampling in August 2012 happened later in the season than the sampling in 2011 (18 and 21 days, for PORT and OSCE, respectively). The later timing was enough to witness the first strong breeze at the end of the summer.

Detritus accumulation height was for no factor significantly different. Pair-wise testing showed no difference except for August 2012. It could be presumed that sooner sampling, corresponding to the time of sampling the previous year would have avoided any noticeable wind induced hydrodynamics.

The oxygen content revealed no significantly different values for the water column and for the water just above the detritus. However in the water inside the detritus, the samples of 2012, seemed well aerated, especially in the PORT site. No measured factor can explain this difference in site at both months in 2012. It could be due to a disturbance, e.g. scuba divers that mixed the macrophytodetritus prior to the sampling. More realistically, it is due to the small scale patchiness of the oxygen content among the macrophytodetritus patch (Mateo *et al.*, 2006).

5.2. Structural response of meiofauna and copepods

In this study, harpacticoid copepods compromised more than half of the meiofaunal organisms, which is congruent with studies in seagrass beds (Hall and Bell 1993; Danovaro *et al.,* 2002). On the other hand, some studies have proved that they are the second most dominant taxa present after nematodes (Hicks and Coull 1983; De Troch *et al.,* 2001a), except in epiphytic habitats where they can occur in higher densities compared to nematodes (De Troch *et al.,* 2001a; Mascart *et al.,* 2013).

The meiofauna community was similar between both sites and year. Differences in community composition occurred between both sites due to the difference in detritus dry mass revealed by the DISTLM analysis. Apparently, meiofauna is sensitive to changes in detritus accumulations and the composition of the taxa will change after an import of new material.

In contrast, copepod composition seems to be more resilient to changes in detritus dry mass. Dry mass is the primary influencing factor, but the composition seems to be less impacted by the arrival of new material of dew days prior to sampling. Two explanations are possible. The copepod community got disturbed and recovered fast to its prior composition or the composition was not disturbed and it could cope with the slight disturbance. The abundant harpacticoid copepods (excluding the

cyclopoids species) are the same as in the seasonal follow up (Chapter 3). The results show the exact same five species as most abundant and those are responsible for the similarity between samples. Surprisingly, the presumably oxygen sensitive copepod *Diosaccus tenuiremis* was the second most important copepod explaining the similarity of the samples in 2012. In 2012, the oxygen levels were significantly higher than in 2011, demonstrating that species-specific reaction to environmental factor play a role in the community composition.

6. Conclusions

Both year 2011 and 2012 were not different from each other in terms of meiofauna and copepods community composition. Oxygen concentrations inside the detritus were higher in 2012 influencing specific species like *Diosaccus tenuiremis*. Detritus accumulation was subject to wind-induced hydrodynamics importing new detrital material from presumably the meadow to the patch. Under similar detritus conditions meiofauna composition remains similar and when extra detrital material is imported the community composition changes. In contrast copepod communities do not seem to be impacted, showing the resilience of the copepod community.

Picture: Revellata lighthouse seen from the Bay

Chapter 5

Copepod feeding ecology and niche width: insights from SI and FA

Adapted from:

Mascart T., De Troch M., Remy F. & Lepoint G. (in prep). Feeding ecology and niche width of macrophytodetritic copepod species: insights from stable isotopes compositions and fatty acid profiles

1. Abstract

This study investigated the dual ($\delta^{13}C$, $\delta^{15}N$) stable isotope and fatty acid compositions of the four most abundant copepods living in seagrass litter in a N-W Mediterranean *Posidonia oceanica* seagrass beds (Calvi, Corsica). Those selection of the four copepods (Fig 5.2B) was based on macrophytodetritus community structure data from chapter 3 § 4.4, chapter 4 § 4.3 and chapter 6 § 4. Three copepods belonged to the order Harpacticoida and are the most abundant copepods in macrophytodetritus accumulations: *Diosaccus tenuicornis*, *Tisbe furcata* and *Ectinosoma dentatum*. The fourth copepod was a copepod from the order Calanoida: *Calanus arcuicornis*. Each species belonged to a different eco-morphological type with potential different preferential feeding niches (see Table I.1 in Addendum I): *Ectinosoma dentatum* (mesopsammic-type), *Diosaccus tenuiremis* (phytal-type), *Tisbe furcata* (epibenthic-type) and *Clausocalanus arcuicornis* (water-column-type). Those four copepods, by their abundance, characteristic morphology, and dispersal mechanisms can be seen as representatives for less abundant copepods sharing the same eco-morphological group. The present study investigates the realized ecological niche of those different copepods on a spatio-temporal scale. A stable isotope and fatty acid analysis is performed together with a mixing model to unravel their realised feeding niches (relative contribution of food sources to their diet) to infer their ecological niches. Three potential food sources (macrophytodetritus, epiphytes and SOM) present in *Posidonia oceanica* seagrass macrophytodetritus accumulations were investigated over four subsequent seasons and two sites. Our results indicated that all

four copepod species, feed on a combination of those sources in different proportions according to the season of the year, but mostly according to their specific preferences. The first copepod, a water-column bound Calanoid *Clausocalanus arcuicornis*, showed a continuous feeding on mainly SOM with occasionally grazing on diatoms and bacteria. The epibenthic-swimmer *Tisbe furcata* showed an equal continuous feeding on all three sources throughout the year. The phytal-type *Diosaccus tenuiremis* showed a clear preference for the epiphytic biofilm and sporadically feeding on the other sources. The mesopsammic *Ectinosoma dentatum* showed the biggest fluctuation in food preferences of all species displaying a potential opportunistic feeding behaviour. Every representative species of the ecological group's feeding strategy allowed them to comply with their nutritional needs. Due to the different strategies used, the copepods showed a trophic niche specialization.

2. Introduction

Benthic harpacticoid copepods play a major role in the benthic energy flow: on the one hand as consumers of primary and secondary production and on the other hand as food for higher trophic levels. Because of their short life cycle and high turnover rates (Hicks and Coull 1983), harpacticoid copepod communities respond rapidly to organic matter inputs and are closely coupled to primary production inputs (Escaravage *et al.,* 1989). In spite of their potentially significant role in energy transfer, the exact quantity and efficiency of primary production channelled through copepods to higher levels is still under debate (Gibbons 1988; Shaw and Jenkins 1992; Danovaro *et al.,* 2007). One of the reasons for the limited knowledge is the very broad dietary spectrum of harpacticoid copepods. Their main food sources consist of microalgae, mainly diatoms (Buffan-Dubau and Carman 2000; De Troch *et al.,* 2006; De Troch *et al.,* 2012b), but also protozoans, bacteria, fungi and organic detritus have been reported as food for

harpacticoids (for overview see Hicks & Coull 1983). With such a wide array of potential food sources, resource partitioning is made possible to avoid inter- and intraspecific trophic competition (Pace and Carman 1996; De Troch *et al.,* 2005a). Observations on congeneric species document species-specific nutritional exploitation of food sources (Vanden Berghe and Bergmanns 1981; Carman and Thistle 1985). However, as the specific copepod diet (i.e. autotrophic production, primary heterotrophs or detritus) responds also to environmental variation, those fluctuations will have consequences for the copepod's value as food for higher trophic levels (e.g. St. John *et al.* 2001). Therefore, spatio-temporal patterns in food distribution in combination with specific food preferences will stimulate trophic niche specialization (Azovsky *et al.,* 2005). Nevertheless, only few studies in coastal ecosystems tackled the harpacticoid species-specific food selection *in situ* on a spatio-temporal scale (Carman and Fry 2002; Rzeznik-Orignac *et al.,* 2008).

Seagrass beds are a typical example of a highly diverse and dynamic ecosystem. They are one of the most productive coastal ecosystems (Duarte and Chiscano 1999), however, 30-50% is unavailable for direct consumption making seagrasses a carbon sink (Borges *et al.,* 2005; Fourqurean *et al.,* 2012). From the fraction left, one part will be directly consumed through herbivory (Valentine and Heck 1999), while the rest will be locally deposited in adjacent habitats fuelling the detrital pool under the form of leaf litter (Mateo *et al.,* 2006; Heck *et al.,* 2008). The leaf litter and its attached epiphytes are seen as an important trophic subsidy for food webs, since degraded material may be more accessible or of greater nutritional value than fresh material for consumers (Dethier *et al.,* 2014). In Mediterranean *Posidonia oceanica* (L.) Delile meadows, shed leaf litter accumulates as macrophytodetritus on unvegetated sand patches. Primary and secondary consumers such as macrofauna (Dimech *et al.,* 2006; Lepoint *et al.,* 2006; Sturaro *et al.,* 2010) and meiofauna, especially harpacticoid copepods (Danovaro 1996; Mascart *et al.,* 2013), are present in high density and diversity. The copepod community in the

macrophytodetritus accumulations present seasonal fluctuations due to environmental variations (Mascart *et al.,* 2015b). Nonetheless, some abundant species, belonging to different ecological types (Mascart *et al.,* 2015a) are continuously present which could point at an interspecific trophic niche specialization.

Insights in trophic interactions at the basis of marine food webs increased considerably thanks to the use of biomarkers that are applied to trace the specific contributions of food sources and trophic niche specialization of consumers (Dalsgaard *et al.,* 2003; Fry 2006; Kelly and Scheibling 2012). Carbon and nitrogen stable isotopes analyses are a widely used tool in food web studies (e.g. Post 2002). They allow determination of food sources that are actually assimilated in the tissues of consumers over time, in contrast to, for instance gut content analysis (Fry 2006). A common application is to use the stable isotope (SI) composition of consumers and their food sources to estimate the consumer's assimilated diet (Inger and Bearhop 2008; Fry 2013). To convert the stable isotope data to food source contributions, stable isotope mixing models (SIMMs) are used (Phillips and Gregg 2001; Parnell *et al.,* 2013). Recently, several Bayesian mixing models have been developed in order to incorporate uncertainties of amongst others, isotopic values and concentration dependences (Hopkins and Ferguson 2012; Phillips *et al.,* 2014). The consumers' specific resource partitioning will lead to specific trophic niches (Jackson *et al.,* 2011). However, the stable isotope approach and applied mixing models have limitations for data gathered in coastal environments. Isotopic composition overlap of autotrophs can occur (Peterson 1999; Phillips *et al.,* 2005) and trophic enrichment, whereby light isotopes of food sources are lost during the conversion into consumer tissues, needs to be taken into account. To correct for the trophic enrichment, trophic enrichment factors (TEF) need to be incorporated in the models. These are deduced from laboratory studies but unfortunately these studies are not prevalent and therefore not many crustacean taxa TEFs exist, let stand species specific TEFs (France and Peters 1997; Vanderklift and Ponsard 2003; Borgå *et al.,* 2012; Michel

et al., 2014).

A second biomarker, complementing stable isotope analyses, is fatty acid (FA) profiling (e.g. Alfaro *et al.* 2006, Dethier *et al.* 2013). Fatty acids are widely distributed in all living cells and they play a fundamental role in organisms as a source of energy, as structural components of cell membranes and as precursors of biologically active compounds (Arts *et al.,* 2001). Fatty acids provide information on sources of organic matter since different taxonomic groups including bacteria, diatoms, dinoflagellates and plants tend to have different dominant FAs (Dalsgaard *et al.,* 2003; Kelly and Scheibling 2012). Polyunsaturated FAs (PUFA, with two or more double bounds) cannot be synthesised *de novo* by animals (so-called essential FA, EFA) and therefore must be obtained through diet or bioconversion (i.e. the ability to modify FA composition) (Iverson 2009). Therefore, most PUFA in aquatic food webs originate from primary producers (Dalsgaard *et al.,* 2003; De Troch *et al.,* 2012a) or originate through biosynthesis of active enzymes, which is the case for many crustaceans, for instance planktonic copepods (McMeans *et al.,* 2012; Noyon and Froneman 2014). Some harpacticoid copepods are also able to convert dietary PUFA from 18:3ω3 to 20:5ω3 (EPA) and 22:6ω3 (DHA) (Nanton and Castell 1998; Rhodes and Boyd 2005).

The aim of this study was to investigate (1) chemical composition of *P. oceanica* leaf litter macrophytodetritus, (2) potential copepod species-specific food preferences, (3) changes in food preference according to spatio-temporal patterns and (4) trophic niche specialization and resource partitioning among 4 benthic copepod species. Following hypothesises are made regarding the outcome. (1) The elemental and SI composition of MPD will change according to seasonality and perhaps site, (2) eco-morphologically different groups will have a different SI and FA composition, (3) different eco-morphological groups will have different niche width and (4) consequently, interspecific tropic niche specialization will occur.

3. Material and methods

3.1. Study site and sample collection

The investigation was conducted near the STARESO marine research station (University of Liège) in the Revellata Bay (Gulf of Calvi, Corsica), in the north-western Mediterranean Sea (42°35'N, 8°43'E). Sampling dives (SCUBA) were carried out on a seasonal basis, i.e. in the months February, May, August and October of 2011 representing winter, spring, summer and autumn, respectively. Two locations at 10 m depth at about 1 km from each other were selected. Both sites were characterised by sandy patch intermissions in the continuous *P. oceanica* seagrass meadow. The first sampling site was located in front of the STARESO research facility, while the second sampling site faced the Punta Oscellucia peninsula. Both sites were previously investigated in terms of seasonal characteristics (i.e. physico-chemical characteristics and copepod community structure) (Mascart *et al.,* 2015b) and are referred to as PORT and OSCE, respectively. Copepod consumers and food sources (different components of macrophytodetritus and suspended organic matter, SOM) were collected and in order to prevent any loss of material or contamination, sealed under water. SOM was collected about 1 m above the seafloor with a SCUBA hand-held Niskin-bottle (2.5 L). The content was afterwards vacuum-filtered onto precombusted glass fibre filters (47 mm, GF/F Whatman) and stored in darkness at -80°C for FA analysis and in -20°C for SI analysis. The qualitative samples were collected using 30 L plastic bags (Fig 2.9) where the macrophytodetritus material and associated fauna was scooped in by hand. To avoid edge effects, only material from the centre of the macrophytodetritus accumulation (minimum 1 m away from the edge) was collected. Subsequently the collected material was kept alive in a 0.75 m^3 aquaria filled with 38µm filtered seawater from the Bay and air stones at the research facility in order to prevent deterioration for analyses. The

content of the aquaria was, in a first stage, rinsed over a 1 mm mesh size sieve to exclude large animals or food source material (except SOM) and on a 38 µm sieve to retain copepod consumers.

The 38 µm fraction holding the consumers was, in a seconds stage, kept in a 0.002 m^3 aquaria with an air stone in order to collect harpacticoid copepods using positive phototaxis attraction, using a setup similar to Svensson *et al.* (2010). By means of a stereomicroscope, individuals were subsequently separated alive per species using the identification keys and reference books by Boxshall & Hasley (2004) and Lang (1948, 1965). The extracted copepods were rinsed and placed overnight in filtered seawater to empty their gut content. Three of the most abundant harpacticoid copepod species present in the macrophytodetritus habitat (Mascart *et al.,* 2015b) were selected for biomarker analyses (see Chapter 3 and 4). Besides, those three species were the most abundant species in the colonization experiment, each one representing a different ecological type (see Chapter 6). Moreover, next to benthic harpacticoid copepods, pelagic copepods are present in the macrophytodetritus (Mascart *et al.,* 2015b). Therefore, in this study a fourth copepod, representing the water-column ecological group was added. Hence, four different species belonging to four different ecological groups as described in Table I.1 in Addendum I were finally selected for this study: *Ectinosoma dentatum* (mesopsammic-type), *Diosaccus tenuiremis* (phytal-type), *Tisbe furcata* (epibenthic-type) and *Clausocalanus arcuicornis* (water-column-type). The species were pooled per sample in order to have enough biomass for reliable measurements since benthic copepods are small and have a very low biomass (SI: 60-100 indiv.; FA: 120-200 indiv. per replicate). All samples were stored at -80°C for FA and -20°C for SI analyses.

The 1 mm fraction holding all food sources, except SOM, was rinsed using fresh water to remove attached motile organisms prior to composition separation. The sampled macrophytodetritus 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. Leaf litter and attached epiphyte food sources were separated using the technique of Dauby & Poulicek (1995) creating four different potential detritus *sensu lato* food sources. Afterwards triplicates of those food sources were dried and stored in liquid nitrogen at -80°C for FA analysis and in -20°C for SI analysis.

3.2. Stable isotope ratio analysis

Food sources were separately dried at 60°C for 96 h before being ground to a homogenous powder using a ball-mill (Retsch Mixer Mill MM301). The attached epiphytes and drift macroalgae material were subdivided in two parts. One part was acidified by fumigation using 37% fuming HCl overnight to remove inorganic carbonates prior to carbon measurements since dissolved inorganic carbon (DIC) has a different $\delta^{13}C$ composition than dissolved organic carbon (DOC) (Nieuwenhuize *et al.,* 1994). After acidification the samples were dried at 60°C to remove the H_2O formed during the acidification reaction. However, there are some uncertainties about the use of HCl during sample processing for isotopic analyses of nitrogen (Bunn *et al.,* 1995; Vizzini and Mazzola 2003; Vafeiadou *et al.,* 2013). Other authors have reported no significant effects of acidification with HCl on the isotopic compositions of marine animals (Bosley and Wainright 1999). In order to limit the possible effect of acidification, we measured the $\delta^{13}C$ values on acidified material and $\delta^{15}N$ values on non-acidified material. Ground food source samples (2-3 mg) were subsequently loaded into tin capsules for isotopic measurements. Pooled consumer 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 and precisely weighed (± 0.001 mg).

Isotopic ratios and elemental content (C/N) measurements were performed with a mass isotope ratio spectrometer (Isoprime 100,

Isoprime, UK) coupled to a continuous flow C-N-S elemental analyser (Vario microtube, Elementar, Germany) for combustion and automated analysis. Isotopic ratios are conventionally expressed as δ values (‰): $\delta^{13}C$ and $\delta^{15}N = [(R_{sample}/R_{standard})-1]x1000$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ or $\delta^{15}N$, respectively. $R_{standard}$ is the ratio of the international standard Vienna Pee Dee Belemnite (vPDB) for carbon and atmospheric N_2 for nitrogen. Reference materials were IAEA-CH6 ($\delta^{13}C = -10.4 \pm 0.5$‰) and IAEA-N2 ($\delta^{15}N = +20.3 \pm 0.2$‰). The analytical precision of the measurements were 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

Post *et al.* (2007) advised a lipid correction as C/N values of aquatic organisms > 3.5 indicate that lipid concentrations are not uniformly low and extraction or normalization may have an influence on the $\delta^{13}C$ values. In other words, lipids are more depleted in ^{13}C compared to other major biochemical compounds in animal tissues and therefore a bias can be expected. However, typical lipid corrections are mostly valid for vertebrates and should not be applied to aquatic invertebrates (Kiljunen *et al.,* 2006).The four consumers revealed no significant difference in C/N ratios (one-way PERMANOVA, $P = 0.186$) was found and their average C/N ratio (4.45 ± 1.05) is close to the recommended limit of Post *et al.* (2007). In addition, a $\delta^{13}C$ to C/N correlation was performed revealing no trend and yielding a R^2 of 0.01. Consequently, no species-specific lipid content variation is expected, spreading the possible influence uniformly over all consumers and thus minimizing the lipid effect in the reconstruction of their diet. This is congruent to other studies, where marine invertebrate data of homogenized entire organisms show less pronounced changes of $\delta^{13}C$ after lipid removal in contrast to specific tissues, like for instance fish muscles (Logan *et al.,* 2008).

3.3. Lipid extraction and fatty acid analysis

Prior to the lipid extraction, food source and pooled consumer samples were freeze-dried and transferred to test 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.* (2012b) including a lipid hydrolysis and fatty acid methylation achieved by a modified 1-step derivatization method after Abdulkadir and 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 capillary column (J&W HP88, Agilent, USA) coupled to a mass spectrometer (HP 5973, Agilent, USA). The subsequent identification of the FAMEs was based on comparison of the relative retention time and on mass spectral libraries (FAMES, WILEY) by means of the MSD ChemStation software (Agilent Technologies). Quantification of individual FAMEs was accomplished by linear regression of the chromatographic peak areas and corresponding known concentrations of internal standard C19:0 (Fluka 74208, Sigma-Aldrich, USA). Some measurements were under the detection limit because of insufficient biomass. Therefore, no sufficient SOM replicates were obtained and some site replicates were missing as well. Subsequently, both sites were pooled for the FA analysis of epiphyte and macrophytodetritus food sources. Due to its very small size, the samples of copepod *Ectinosoma dentatum* did not always reach the detection limit and in case they did, concerns about the measurement error were raised. Therefore, only the FA results of 3 copepod species are shown in this study: *Calanus arcuicornis* (CA), *Tisbe furcata* (TF) and *Diosaccus tenuiremis* (DT). The FA short hand notation A:BωX was used, where A represents the number of carbon atoms, B gives the number of double bounds and X gives the position of the double bound closest to the terminal methyl group (Guckert *et al.,* 1985). FAs were reported as percentage of the total fatty acids (%TFA ± SD) and grouped as saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA)

FAs.

3.4. Data analyses

The C/N values and stable isotope composition of sources and consumers were compared using a three-way univariate PERMANOVA based on an untransformed Euclidean matrix with fixed factors Month-Source-Site and Month-Species-Site, respectively. The downside of this analysis of variance is the difficulty of distinguishing the source of the variation (due to location or dispersion). Therefore, homogeneity of dispersion was tested with a PERMDISP, using distances amongst centroids calculated on the lowest level (Quinn and Keough 2002).

All data analyses for FA were performed on relative (%) FA concentrations. A Bray-Curtis similarity matrix was constructed using square-root transformed relative FAs. The resultant groupings were visualized using a PCO (Principle coordinate analysis) with an overlayed hierarchical clustering. To identify the FAs primarily providing discrimination between the groups, a SIMPER (Similarity Percentages) analysis was done. A two-way multivariate PERMANOVA with the factors Month and Source or Species was performed to determine whether the food sources and consumer species differed significantly through time, based on their FA profiles. Pair-wise tests type III and Monte-Carlo P-values were used since sometimes the total number of unique permutations did not exceed 100 (Anderson *et al.,* 2008).

All the above mentioned analyses were performed with the Primer 6.1.11 software (Clarke and Gorley 2006) with PERMANOVA add-on software (Anderson *et al.,* 2008). A significance level of $P < 0.05$ was used in all tests. Graphs were constructed in R, MixSIAR GUI, and GraphPad 5.03 for Windows (GraphPad Software, San Diego, USA).

The Bayesian mixing model MixSIAR and associated graphical user

interface (GUI) (Stock and Semmens 2013) in R (R Core Team 2014) based on MixSIR (Moore & Semmens 2008) and SIAR (Parnell *et al.* 2010) were used. Each different species was used as a group in MixSIAR. Regarding our approach and the possibilities of MixSIAR to use covariates (categorical or continuous) a second model was created with site and month as categorical variable. Unfortunately due to the low amount of replicates, the outcome was doubtful and the model diagnostics worse than the first model. Therefore, the second model was not used. A third model was setup, using the FA concentrations (e.g. all FA with a cumulative contribution of 70%). Unfortunately the selection of the appropriate FA had an impact on the model diagnostics. Looking for better fitting combinations of FA was too time consuming and thus not execute here in this PhD research. However, for publication of this chapter, more complex models will be incorporated. The approach of the SIMM allowed to estimate dietary contributions and to consider uncertainties related to isotopic variation in the consumer and in the food sources as well as in the trophic fractionation factors (TEF) (Parnell *et al.,* 2010). The TEF are crucial to correctly estimate the food source proportions, however the variability of these factors is huge. It is habitat, taxon, diet and tissue specific. For example marine organisms will have a lower TEF, due to nitrogen loss during ammonia excretion. Therefore using classical fractionation factors from literature can yield several issues (Vander Zanden and Rasmussen 2001). The typical TEF of ~3.4‰ for nitrogen and ~1.0‰ for carbon are mainly based on average fractionation across an entire food chain. The best alternative would be to calculate specific trophic enrichment values to the system and organism one works with (Caut *et al.,* 2008). Besides the difficulty of setting up tracer food experiments, the duration of the experiment to allow for isotopic equilibrium between predator and prey is often unknown(Caut *et al.,* 2010). As a result, several laboratory studies misinterpreted their data set (Vander Zanden and Rasmussen 2001; Vanderklift and Ponsard 2003). Marine detritivores invertebrates tend to have variable stable isotopes compositions close to their food sources with TEFs close to zero or even

negative (McCutchan *et al.,* 2003). Therefore, for this copepod study, trophic enrichment factors from a grazing experiment of closely related detritivores amphipods (0.2 ± 0.6 ‰ for $\delta^{13}C$ and 1.2 ± 0.5 ‰ $\delta^{15}N$) present in a similar system were used as a basis (Michel 2011). Next to the diet appropriated value, estimating the error variance can be calculated mathematically. However, not having all the required data, try-and-error model runs yielded the best scenario with following values incorporating a similar or wider error then for the detritivores amphipods: 0.6 ± 0.6 ‰ for $\delta^{13}C$ and 0.8 ± 0.8 ‰ $\delta^{15}N$.

In the frame of the SIMMs limitations that isotopic composition of autotrophs can overlap (Phillips *et al.,* 2005), some sources are combined. The combined sources were sources displaying no significant differences in their isotopic compositions. The following potential sources were combined: epiphyte free 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 epilithic macroalgae and scraped epiphytes from the dead leaf litter (PERMANOVA for every month: for $\delta^{13}C$, all $P > 0.097$ and for $\delta^{15}N$, all $P > 0.348$). The remaining combined three significantly different potential food sources (PERMANOVA per month for $\delta^{13}C$ and $\delta^{15}N$ $P = 0.001$) finally used in the SIMM are: (1) macrophytodetritus (MPD), i.e. primarily dead *P. oceanica* seagrass leaves without epiphytes; (2) epiphytes (EPI), i.e. the scraped leaf epiphytes and drift epilithic algae and (3) suspended organic matter (SOM).

A first metric for isotopic niche calculations: convex hulls (Euclidean method), was used to define the isotopic niche space of a species in a community (Layman *et al.,* 2007). However, it is sensitive to small sample sizes (Jackson *et al.,* 2011). Therefore, a second metric (standard ellipse area, SEA) was used. The multivariate ellipse based metrics are unbiased with respect to sample size and their estimation via Bayesian inference allows robust comparison with data sets comprising different sample sizes

(Jackson *et al.,* 2011). However, for small sample sizes, the area of ellipses (SEA) could be underestimated and therefore using the metric SEAc was more correct. The SIBER (Stable Isotope Bayesian Ellipses in R) routine in R (R Core Team 2014) was used to investigate the isotopic niche space. The yielded standard ellipses, containing ~40% of the data (centred on the mean and SDs of the bivariate data as semi-axes), and convex hulls were used to delineate an isotopic niche space per species. The difference in niche area and niche overlap among the ellipses was derived using a Bayesian inference based on 100000 posterior probabilities draws of the SEA_C model (Jackson *et al.,* 2011). Despite variable and relatively small sample sizes, the isotopic niche areas are thus assumed to reflect the niche widths and the level of specialization of the selected copepods species.

4. Results

4.1. Stable isotope composition

Carbon and nitrogen ratio (C/N) calculated from elemental analyses of the food sources tend to be different according to the food sources and the sampled month (Table 5.1). SOM had the lowest C/N ratio on average from al food sources in each month. The highest ratio for SOM was found in August PORT and the lowest was found in May PORT. Macrophytodetritus had the highest C/N values from all food sources with a maximum average ratio in October OSCE and a minimum in February PORT. Epiphytes showed the highest ration in October OSCE and the lowest in February OSCE. All three sources displayed significantly different C/N ratios except in February where the ratio of SOM and epiphytes were not significantly different (Pair-wise, P= 0.231). Both sites were only significantly different for MPD in February and for SOM in October (Pair-wise, both $P < 0.001$).

		February			May		
		$\delta^{13}C$	$\delta^{15}N$	C/N	$\delta^{13}C$	$\delta^{15}N$	C/N
MPD	PORT	-12.6 ± 0.2	3.4 ± 0.2	28.1 ± 3.0	-13.4 ± 0.1	2.8 ± 0.2	44.4 ± 3.9
	OSCE	-13.2 ± 0.2	3.1 ± 0.4	41.2 ± 5.6	-14.7 ± 0.3	2.5 ± 0.1	43.5 ± 3.0
EPI	PORT	-18.6 ± 0.9	3.4 ± 0.4	6.7 ± 1.4	-15.8 ± 2.4	3.3 ± 0.9	7.3 ± 1.2
	OSCE	-16.9 ± 0.4	2.1 ± 0.2	5.5 ± 0.8	-18.9 ± 0.4	3.2 ± 0.2	6.1 ± 0.5
SOM	PORT	-26.5 ± 1.4	1.9 ± 0.2	6.2 ± 3.2	-25.4 ± 0.8	2 ± 0.5	3.8 ± 0.5
	OSCE	-26.7 ± 1	1.7 ± 0.4	5.4 ± 2.3	-25.8 ± 1.1	1.4 ± 0.5	3.8 ± 1.0

		August			October		
		$\delta^{13}C$	$\delta^{15}N$	C/N	$\delta^{13}C$	$\delta^{15}N$	C/N
MPD	PORT	-12.2 ± 0.8	1.3 ± 0.4	54.1 ± 2.3	-12.7 ± 0.3	1.8 ± 0.1	59.1 ± 2.5
	OSCE	-13.6 ± 0.7	0.9 ± 0.4	49.6 ± 6.1	-12.8 ± 0.1	1.2 ± 0.2	61.6 ± 1.2
EPI	PORT	-19.1 ± 0.9	0.9 ± 0.4	11.3 ± 1.0	-14.5 ± 1.3	1.2 ± 0.4	15.2 ± 1.9
	OSCE	-17.6 ± 0.5	1 ± 0.3	12.0 ± 1.1	-15.9 ± 1.3	1 ± 0	15.8 ± 1.3
SOM	PORT	-24.8 ± 0.7	1.7 ± 0.5	7.6 ± 3.0	-23.3 ± 0.9	1.7 ± 0.6	4.4 ± 1.1
	OSCE	-26.2 ± 1	1.5 ± 0.7	7.1 ± 2.2	-24.8 ± 1.1	1.7 ± 0.7	4.9 ± 0.6

Table 5.1 *Stable isotope ($\delta^{13}C$, $\delta^{15}N$) composition and carbon to nitrogen (C/N) ratio of food sources per month and site (PORT, OSCE). All values are means ± standard deviations. N=5. MPD= macrophytodetritus, EPI= epiphytes, SOM= suspended organic matter.*

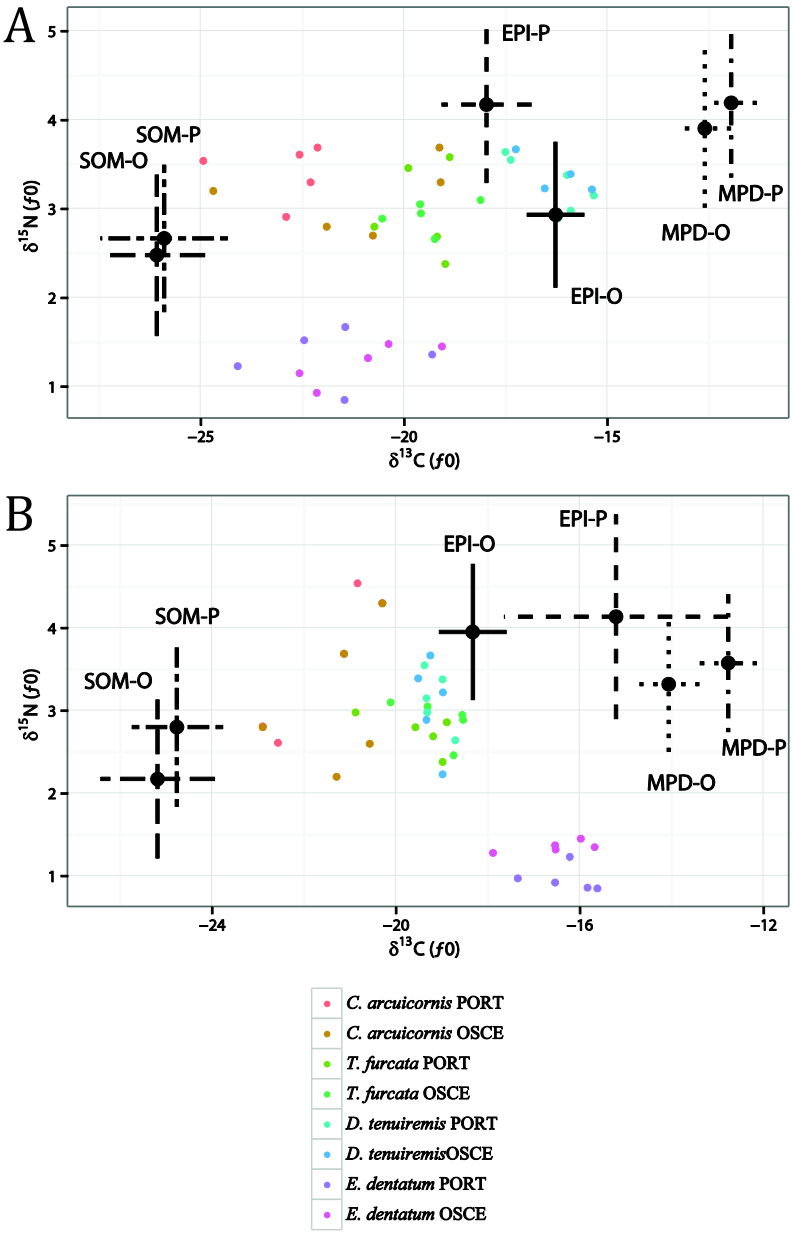
All $\delta^{13}C$ values of food sources ranged from -26.7 (SOM Feb PORT) to -11.3‰ (MPD Aug PORT) (Fig. 5.1). On an annual basis, EPI (average $\delta^{13}C$ value of -17.2 ± 1.9‰) exhibited the largest fluctuations in their $\delta^{13}C$ values. MPD was the food source with the highest average $\delta^{13}C$ value (-13.1 ± 0.8‰) and SOM with the lowest average $\delta^{13}C$ value (-25.4 ± 1.4‰). Regarding the spatial variability, both sites showed similar compositions for most sources in all months, except for the month Aug where all sources were significantly different in each site. Regarding the temporal variability, the month Aug showed the largest spread of values, while Oct exhibited the narrowest spread of $\delta^{13}C$ values. The three-way PERMANOVA showed significant effects of all factors season, source and site for the carbon composition (Table 5.2). The PERMANOVA and the PERMDISP test on the highest interaction were significant (both P = 0.001). All pair-wise tests revealed that all sources were significantly different within a season (Table 5.3). A difference between both sites was present, though the sites seemed to be influenced by the seasonal factor, explaining the significant interaction term with a non-significant PERMDISP test (P = 0.05). The SOM

values were significantly different between months, except between May and August.

Sources			Consumers		
Factors and interaction	$\delta^{13}C$	$\delta^{15}N$	Factors and interaction	$\delta^{13}C$	$\delta^{15}N$
Month (Mo)	$F'_{(3,119)}$=23.3 P= 0.001 *	$F'_{(3,119)}$=80.5 P= 0.001 *	Month (Mo)	$F'_{(3,159)}$=19.2 P= 0.001 *	$F'_{(3,159)}$=3.8 P= 0.01 *
Source (So)	$F'_{(2,119)}$=1806.5 P= 0.001 *	$F'_{(2,119)}$=10.3 P= 0.001 *	Species (Sp)	$F'_{(3,159)}$=118.5 P=0.001 *	$F'_{(3,159)}$=140.1 P= 0.001 *
Site (Si)	$F'_{(1,119)}$=16.8 P= 0.001 *	$F'_{(1,119)}$=20.0 P= 0.001 *	Site (Si)	$F'_{(1,159)}$=3.4 P= 0.064	$F'_{(1,159)}$=0.1 P= 0.777
Mo x So	$F'_{(6,119)}$=7.3 P= 0.001 *	$F'_{(6,119)}$=21.0 P= 0.001 *	Mo x Sp	$F'_{(9,159)}$=23.9 P= 0.001 *	$F'_{(9,15960)}$=7.8 P= 0.001 *
Mo x Si	$F'_{(3,119)}$=5.6 P= 0.0021 *	$F'_{(3,119)}$=0.9 P= 0.416	Mo x Si	$F'_{(3,159)}$=1.1 P= 0.374	$F'_{(3,159)}$=0.3 P= 0.782
So x Si	$F'_{(2,119)}$=1.2 P= 0.288	$F'_{(2,119)}$=0.2 P= 0.805	Sp x Si	$F'_{(3,159)}$=3.9 P=0.01 *	$F'_{(3,159)}$=1.4 P=0.223
Mo x So x Si	$F'_{(6,119)}$=6.3 P= 0.001 *	$F'_{(6,119)}$=2.3 P= 0.038 *	Mo x Sp x Si	$F'_{(9,159)}$=0.9 P= 0.505	$F'_{(9,159)}$=0.5 P= 0.868

Table 5.2 *Three-way factorial PERMANOVA of univariate Stable Isotope data; F' = Pseudo-F value; * = P < 0.05.*

All $\delta^{15}N$ values of food sources ranged from 0.23 ‰ (EPI Aug PORT) to 4.59 ‰ (EPI May PORT). The $\delta^{15}N$ values of EPI showed the largest variation throughout the year and among sites (average $\delta^{15}N$ value of 2.0 ± 1.1‰) (Fig. 5.1). MPD was the food source with the highest average $\delta^{15}N$ value (2.1 ± 0.9‰) and SOM with the lowest average $\delta^{13}C$ value (1.7 ± 0.5) (Table 5.3). The sources EPI and MPD showed similar annual average $\delta^{15}N$ with values of 2.0 ± 1.1‰ and 2.1 ± 0.9‰, respectively. However, these two sources were always different per month except in Aug (Pair-wise, P = 0.467). The three-way PERMANOVA showed significant effects of all factors (Table 5.2). The PERMDISP test on the highest interaction was not significant (P = 0.194).


A
EPI-P
SOM-P
SOM-O
MPD-P
MPD-O
EPI-O
δ15N (‰)
δ13C (‰)
B
EPI-P
EPI-O
SOM-P
SOM-O
MPD-P
MPD-O
δ15N (‰)
δ13C (‰)
C. arcuicornis PORT
C. arcuicornis OSCE
T. furcata PORT
T. furcata OSCE
D. tenuiremis PORT
D. tenuiremisOSCE
E. dentatum PORT
E. dentatum OSCE

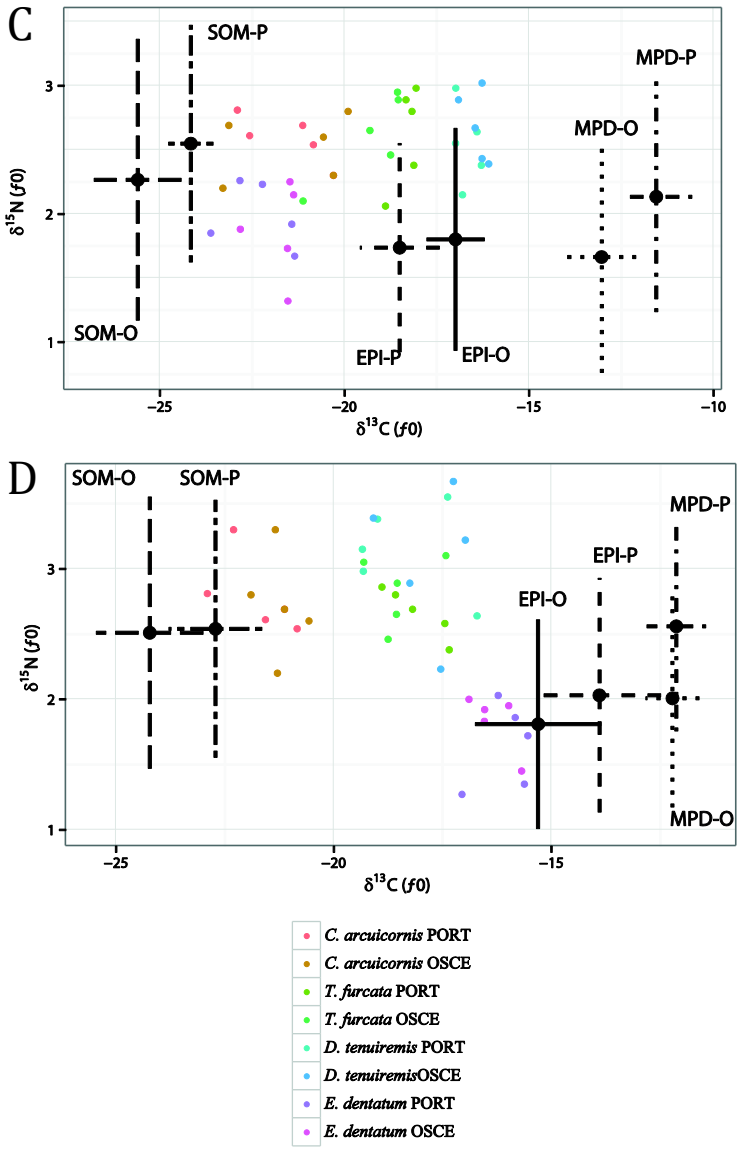


Fig. 5.1 *Stable Isotope analysis biplot ($\delta^{13}C$, $\delta^{15}N$). Consumer data are per species per site (colour) and source data are labelled per site PORT and OSCE, respectively P and O. Error bars indicate combined source and discrimination uncertainty ± 1 SD. Following months are represented: (A) February; (B) May; (C) August; (D) October. Note: beware of different scaling*

All $\delta^{13}C$ values of consumers ranged from -24.9 (*C. arcuicornis* Feb PORT) to -15.3‰ (*D. tenuiremis* Feb PORT) (Fig. 5.1, Table 5.3). The factors species and month were significantly different in contrast to the factor site, where no significant effect was found (Table 5.2). On an annual basis *C. arcuicornis* was the most $\delta^{13}C$-depleted species and showed no significantly different $\delta^{13}C$ values between months (annual mean = 21.4 ± 1.4‰) except between Aug and Oct (Table 5.3). The three other most abundant species did not show significant difference in their annual mean $\delta^{13}C$. Temporal $\delta^{13}C$ fluctuations were particularly clear for *D. tenuiremis* and *E. dentatum* (Table 5.3). The months Aug and Oct were similar for both species. *D. tenuiremis* reached its most depleted $\delta^{13}C$ values in February and its least depleted in May. In contrast, *E. dentatum* reached its most depleted ^{13}C-value in February (Fig. 5.1, Table 5.3).

	$\delta^{13}C$			
	Annual mean (‰)		Pair-Wise tests	
	PORT	OSCE	Comparisons	*P* -value(s)
Sources				
MPD	-12.7 ± 0.3	-12.8 ± 0.1	Feb ≠ May; May ≠ Aug; May ≠ Oct	0.001; 0.001; 0.001
EPI	-14.5 ± 1.3	-15.9 ± 1.3	Feb ≠ Oct; May ≠ Oct; Aug ≠ Oct	0.001; 0.007; 0.001
SOM	-23.3 ± 0.9	-24.8 ±1.1	May = Aug	0.823
Consumers				
Clausocalanus arcuicornis	-21.9 ± 1.2	-20.9 ± 1.4	Aug ≠ Oct	0.043
Tisbe furcata	-18.9 ± 0.9	-19.1 ± 0.9	Feb ≠ Oct; Oct ≠ May	0.003; 0.007
Diosaccus tenuiremis	-18.2 ± 1.3	-17.9 ± 1.2	Aug = Oct	0.189
Ectinosoma dentatum	-18.0 ± 2.5	-18.1 ± 2.0	Aug = Oct	0.439
	$\delta^{15}N$			
	Annual mean (‰)		Pair-Wise tests	
	PORT	OSCE	Comparisons	*P* -value(s)
Sources				
MPD	1.8 ± 0.1	1.2 ± 0.2	≠	< 0.05
EPI	1.2 ± 0.4	1.0 ± 0.0	Aug = Oct	0.341
SOM	1.7 ± 0.6	1.7 ± 0.7	=	> 0.05
Consumers				
Clausocalanus arcuicornis	3.1 ± 0.6	2.9 ± 0.6	Feb = May; Aug = Oct	0.786; 0.141
Tisbe furcata	2.8 ± 0.4	2.8 ± 0.3	=	> 0.05
Diosaccus tenuiremis	3.0 ± 0.4	3.0 ± 0.5	Feb ≠ Aug; May ≠ Aug; Aug ≠ Oct	0.001; 0.012; 0.013
Ectinosoma dentatum	1.5 ± 0.5	1.6 ± 0.4	Feb = May; Aug = Oct	0.168; 0.168

Table 5.3 *Annual mean of stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) of food sources and consumers (‰, mean ±SD). Summary of pair-wise comparisons of means*

between seasons and associated P-values. N=5. MPD = Macrophytodetritus; EPI = Epiphytes; SOM = Suspended organic matter

All $\delta^{15}N$ values of consumers ranged from 0.9 (*C. arcuicornis* May PORT) to 4.5 (*E. dentatum* May PORT) (Fig. 1). As for the carbon, the factor site seemed to have no significant effect (Table 5.2). *T. furcata* showed the most constant $\delta^{15}N$ value over the year, in contrast to *C. arcuicornis* and *E. dentatum* (Table 5.3). Both species showed their most depleted $\delta^{15}N$-values in May and their least depleted $\delta^{15}N$-values in August.

4.2. Stable isotopic mixing model

The results from the first MixSIAR isotopic mixing model indicated an overall high variability of species-specific diet contribution depending on the site and season (Table 5.4). The model indicated a higher reliance of *C. arcuicornis* on SOM compared to the other two sources. *T. furcata* showed no clear preference between the three sources, except in Feb OSCE and Oct PORT (Table 5.4). *D. tenuiremis* appeared less dependent on SOM than *T. furcata* and relied more on MPD than the latter. *E. dentatum* exhibited the strongest diet fluctuation through seasons and sites. For instance, in February and August at the PORT site, its diet was more similar to *C. arcuicornis*. In May and October, *E. dentatum* mainly relied on MPD, while this was not the case in February and August (Table 5.4). On an average annual basis different species-specific diet compositions are visible (Fig 5.3).

	PORT						OSCE					
	MPD		EPI		SPOM		MPD		EPI		SPOM	
February	Mode	95% C.I.	Mode	95% C.I.	Mode	95% C.I.	Mode	95% C.I.	Mode	95% C.I.	Mode	95% C.I.
Clausocalanus arcuicornis	4.6	(0-20)	4.2	(0-26.8)	81.4	(64.2-92.9)	36.7	(0.8-61.4)	4.3	(0-52.2)	45.4	(13.4-65.2)
Tisbe furcata	21.0	(1.8-44.2)	34.9	(0.2-53.8)	49.6	(36.9-59.4)	48.0	(14.3-61.7)	3.9	(0-48.8)	40.4	(25.6-50)
Diosaccus tenuiremis	39.8	(11.7-69.5)	39.8	(0.6-74.1)	20.7	(6.2-35.3)	72.3	(31.1-81.6)	4.5	(0-60.3)	15.7	(2.6-24.7)
Ectinosoma dentatum	3.2	(0-38.3)	31.4	(0.5-51.5)	60.1	(32.1-78.1)	3.3	(0-37)	43.3	(4-60.5)	48.3	(33-64.1)
May												
Clausocalanus arcuicornis	2.7	(0-32.2)	33.3	(1.1-49.8)	60.8	(41.1-73.6)	17.1	(0-38.3)	43.4	(5.2-71.1)	40.3	(22.5-59.2)
Tisbe furcata	28.2	(7.6-48)	27.8	(4.1-49.9)	44.9	(33.8-53.3)	15.2	(0.5-39.8)	75.2	(35.6-93.9)	11.2	(1.4-26.2)
Diosaccus tenuiremis	41.6	(10.5-52.8)	10.6	(0-51.6)	45.2	(31.8-50.4)	26.0	(0.3-44.6)	55.5	(23.3-95.6)	17.4	(3.3-33)
Ectinosoma dentatum	50.3	(28.3-76.3)	30.0	(0-54.2)	20.8	(8.6-29.5)	75.8	(43.2-87.2)	3.6	(0-46.6)	13.0	(0-25.8)
August												
Clausocalanus arcuicornis	16.4	(0-28.7)	5.3	(0-54)	64.0	(37.3-78.7)	9.3	(0-40.4)	39.4	(0.4-54.5)	49.3	(32.7-68.9)
Tisbe furcata	40.8	(27-52.5)	28.7	(0-50.4)	34.5	(19.7-50.4)	34.9	(6.6-55.7)	32.7	(0-65.4)	35.5	(19.3-49.6)
Diosaccus tenuiremis	55.8	(38-65.1)	6.1	(0-53.6)	29.5	(5.1-37.5)	46.3	(29.4-73.8)	45.9	(6.7-69.4)	7.6	(0.1-19.7)
Ectinosoma dentatum	15.6	(0.3-23.4)	3.3	(0-41)	75.9	(51.7-86.6)	3.7	(0-33.3)	41.6	(1.5-51.7)	54.2	(44.7-66.6)
October												
Clausocalanus arcuicornis	5.8	(0-18.8)	4.0	(0-21.2)	82.1	(70.5-92.7)	12.0	(0.3-30.1)	6.0	(0-39.9)	65.8	(53.8-74.1)
Tisbe furcata	43.3	(5-51.2)	4.2	(0-50.5)	47.5	(36.5-55)	33.4	(8.8-53.6)	32.3	(0-55.6)	40.8	(29.6-50.5)
Diosaccus tenuiremis	31.7	(1.3-49.4)	17.0	(0-52.2)	47.2	(32.4-60.8)	34.0	(8.6-58)	34.2	(0.3-62.6)	34.0	(21.4-45.7)
Ectinosoma dentatum	38.9	(18.4-66.5)	35.3	(1.8-57.9)	26.3	(17.3-35.2)	46.2	(28.4-72)	34.1	(0-53.9)	22.8	(12.4-32.1)

Table 5.4 *MixSIAR output data on the diet composition of the consumers. Mode + (Min-Max) 95% confidence interval (C.I.). MPD = Macrophytodetritus; EPI = Epiphytes; SOM = Suspended organic matter.*

4.3. Isotopic niche space and niche area

Distinct isotopic realized niche space was found between all four copepod species. The probability of niche overlap between the niches of *C. arcuicornis* or *E. dentatum* was not supported by the ellipses (Fig. 5.3A). However, the realized niche of *T. furcata* and *D. tenuicornis* overlapped significantly (0.21‰^{2}) (Fig 5.3A). The isotopic niche areas (‰^{2}) of the SEA_c delineated ellipses were different. *E. dentatum* occupied the largest niche area (3.7‰^{2}), while *D. tenuicornis* showed the smallest isotopic niche area (0.9‰^{2}) (Fig 5.3C).

Fluctuations of isotopic niche area throughout the seasons were visible. The month February exhibited the largest niche area (7.0‰^{2}) and the month August showed the smallest niche area (2.0‰^{2}) (Fig 5.3B). The largest niche overlap occurred between August and October where 94.6% overlapped (3.0‰^{2}) and the smallest overlap was found between February and August (1.4‰^{2})(Fig 5.3D).

Both sampling sites showed no distinct isotopic niche space. The overlap between the communities of both sites was considerable (98.8%) revealing a shared isotopic niche space and no different isotopic baseline.

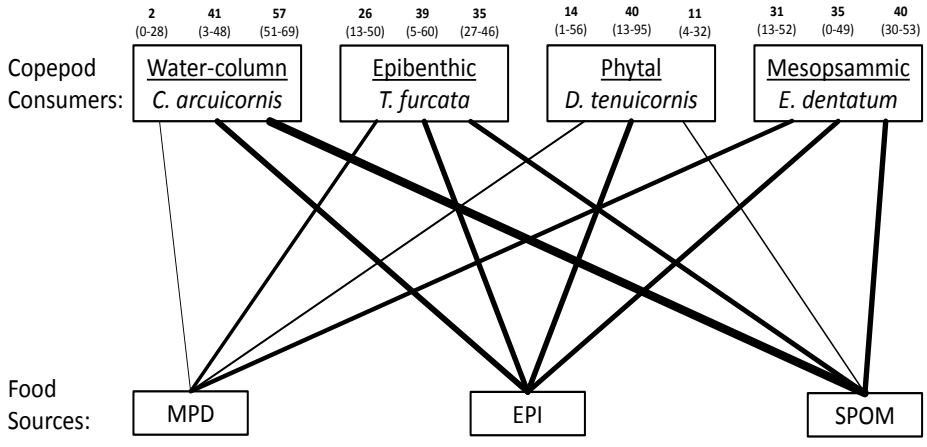


Fig. 5.2 *Bayesian mixing model output MixSIAR for the three sources and four consumers averaged over all seasons and sites. Numbers represent average mode and 95% contingency interval (min-max) of the contribution of each source to its consumer. The ecological type per consumer copepod species is underlined. The thickness of the lines is proportional to the source's contribution. MPD = Macrophytodetritus; EPI = Epiphytes; SOM = Suspended organic matter.*

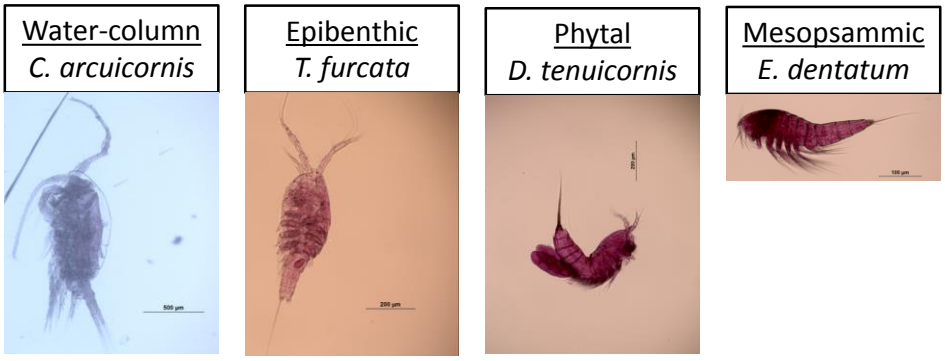


Fig 5.2B *Microscopic pictures of the 4 used species. Natural colour of copepods is gone due to Roze Bengal staining*

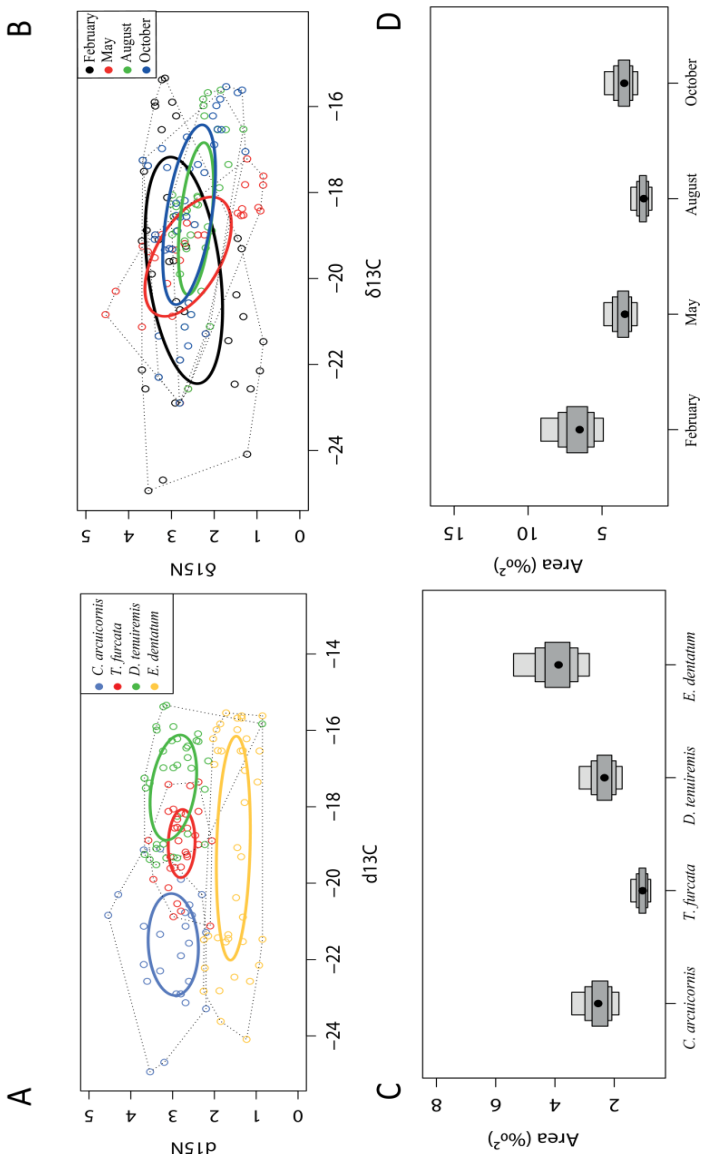


Fig. 5.3. *(A) Variation in δ¹³C and δ¹⁵N combined per species through all seasons and sites. Thick coloured lines and thin grey lines: ~40% CI bivariate ellipses and convex hulls respectively, demonstrating the significant isotopic niche partitioning among the 4 copepod species. (B) Variation in δ¹³C and δ¹⁵N combined per season through all species and sites. Thick coloured lines and thin grey lines: 95% CI bivariate ellipses and convex hulls respectively, demonstrating the significant copepods isotopic niche partitioning among the 4 seasons. (C) Surface area measurements per species. Measures of*

uncertainty and central tendency (black circles = mode) of standard ellipse areas (SEA_C). Boxes show 95, 75 and 50% credibility intervals from light to dark grey respectively. (D) Surface area measurements per season. Measures of uncertainty and central tendency (black circles = mode) of standard ellipse areas (SEA_C). Boxes show 95, 75 and 50% credibility intervals from light to dark grey respectively.

4.4. Fatty acid profiling

A total of 17 FAs were identified in the sources, with a percentage contribution to total FA's above 0.5% in at least one of the samples (Table 5.4). The relative FA contribution of the sources showed a similar composition with relatively high amounts of SAFAs, especially in the macrophytodetritus compared to the epiphyte samples (72.4 ± 9.3% and 55.4 ± 5.1%, respectively). The most abundant SAFA was 16:0 (palmitic acid) with 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 were present in similar percentages (12.1 ± 4.0% and 14.0 ± 5.0%, respectively).

Fatty Acid	February		May		August		October	
	MPD	EPI	MPD	EPI	MPD	EPI	MPD	EPI
14 :0	4.3 ± 0.63	4.21 ± 0.74	3.58 ± 0.02	5.24 ± 2.05	2.48 ± 1.02	4.91 ± 0.63	5.48 ± 0.55	4.29 ± 0.55
15 :0	0.41 ± 0.07	2 ± 0.2	0.12 ± 0.03	1.07 ± 0.17	0.32 ± 0.1	1.4 ± 0.17	0.37 ± 0.07	1.26 ± 0.47
16 :0	31.2 ± 3.35	37.83 ± 2.54	32.8 ± 5.29	33.8 ± 2.9	31.44 ± 2.62	32.21 ± 2.06	28.02 ± 2.17	40.22 ± 5.15
16:1ω7	4.23 ± 0.97	6.7 ± 1.64	6.03 ± 2.83	4.3 ± 3.42	7.18 ± 0.57	9.62 ± 0.97	1.4 ± 0.16	3.99 ± 0.48
16:1ω5	n.d.	1.23 ± 0.26	n.d.	2.54 ± 0.88	n.d.	0.52 ± 0.29	n.d.	1.12 ± 0.41
17 :0	0.29 ± 0.2	1.23 ± 0.14	0.57 ± 0.21	0.94 ± 0.21	0.43 ± 0.06	0.95 ± 0.14	0.4 ± 0.08	1.31 ± 0.1
18 :0	6.87 ± 1.55	6.06 ± 0.16	6.98 ± 3.39	3.75 ± 0.22	8.49 ± 0.8	4.85 ± 0.92	3.22 ± 2.39	5.45 ± 0.45
18:1ω9	2.36 ± 0.54	6.45 ± 0.65	3.5 ± 1.19	7.64 ± 5.26	3.57 ± 1.03	4.74 ± 1.22	1.9 ± 1.01	6.97 ± 2.38
18:1ω7	4.63 ± 1.65	8.74 ± 3.09	5.65 ± 2.28	7.41 ± 4.16	4.64 ± 0.11	2.82 ± 1.17	3.51 ± 0.86	3.97 ± 0.67
18:2ω6	9.05 ± 1.9	3.54 ± 0.99	5.28 ± 1	3.39 ± 0.53	2.22 ± 0.58	6.78 ± 0.72	3.42 ± 0.79	5.49 ± 3.1
18:3ω3	2.25 ± 0.38	3.41 ± 1.89	2.6 ± 1.09	3.96 ± 1.26	4.49 ± 0.81	6.51 ± 0.9	5.33 ± 1.03	5.02 ± 2.29
20:4ω6	0.87 ± 1.41	7.6 ± 1.32	2.36 ± 2.56	5.89 ± 2.64	6.07 ± 1.33	12.44 ± 1.08	0.65 ± 0.57	11.64 ± 0.93
20:5ω3	0.15 ± 0.13	13.79 ± 2.86	2.37 ± 2.91	3.58 ± 0.98	8.43 ± 2.69	9.02 ± 1.43	0.32 ± 0.45	7.75 ± 7.01
22 :0	0.3 ± 0.26	n.d.	n.d.	0.11 ± 0.33	0.34 ± 0.3	0.28 ± 0.08	1.83 ± 0.34	1.31 ± 2.06
24 :0	3.75 ± 1.12	0.58 ± 0.62	3.25 ± 1.38	1.84 ± 0.14	2.32 ± 0.47	1.23 ± 0.42	7.93 ± 0.45	0.61 ± 0.37
26 :0	10.47 ± 3.16	0.63 ± 0.57	9.72 ± 2.24	4.67 ± 0.42	7.88 ± 0.79	1.09 ± 0.13	19.6 ± 3.1	1.23 ± 0.34
28 :0	10.54 ± 2.09	2.14 ± 0.56	17.31 ± 7.83	9.51 ± 1.4	8.83 ± 0.55	1.8 ± 0.91	17.62 ± 1.78	1.65 ± 0.63
Σ SAFA	68.13 ± 3.89	54.67 ± 3.53	74.32 ± 11.54	60.94 ± 1.13	62.55 ± 3.25	48.72 ± 2	84.47 ± 1.47	57.33 ± 3.66
Σ MUFA	11.22 ± 0.3	23.11 ± 5.15	15.18 ± 6.28	21.88 ± 4.53	15.39 ± 0.68	17.71 ± 3.64	6.82 ± 0.97	16.04 ± 2.59
Σ PUFA	12.33 ± 3.53	28.34 ± 2.99	12.61 ± 5.13	16.82 ± 1.14	21.21 ± 4.06	34.76 ± 1.17	9.71 ± 1.11	29.9 ± 12.79

Table 5.4 *Relative FA composition (%) of food sources. All values are mean ±*

standard deviation. N= 3. Only FAs contributing more than 0.5% are listed. MPD = macrophytodetritus and EPI = epiphytes

In contrast, in the epiphytes, PUFAs were more abundant than the MUFAs (27.5 ± 7.5% and 19.7 ± 3.4%, respectively). The three most abundant MUFAs were 16:1ω7 (Palmitoleic acid), 18:1ω7 and 18:1ω9 (Table 5.5). The most abundant PUFAs in the epiphyte source were 20:4ω6 (ARA) and 20:5ω3 (EPA) (9.4 ± 3.2% and 8.5 ± 4.2%, respectively). The most abundant PUFAs in the macrophytodetritus were 18:2ω6 (5.0 ± 2.9%) and 18:3ω3 (3.7 ± 1.5%). The PCO clearly represents a separation between both sources (PERMANOVA, P <0.001). However a less pronounced discrimination between months was visible (PERMANOVA, , P <0.001) (Fig 4). The SIMPER analysis showed dissimilarity between both sources of 26.6%. The main FAs responsible (48.6 cum. %) 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, the month May separated most clearly from the other months, with the largest dissimilarity between May and August (15.9%).

Fatty Acid	February			May			August			October		
	CA	TF	DT	CA	TF	DT	CA	TF	DT	CA	TF	DT
14 :0	5.35 ± 0.58	2.67 ± 0.55	4.12 ± 0.16	7.54 ± 0.87	3.5 ± 0.79	4.55 ± 0.47	4.69 ± 0.67	4.53 ± 0.17	3.56 ± 0.28	5.28 ± 1.05	2.26 ± 0.69	3.64 ± 1.1
α15 :0	3.31 ± 0.97	5.74 ± 1.32	1.64 ± 0.09	2.68 ± 0.13	2.98 ± 1.84	2.22 ± 0.07	3.32 ± 0.62	2.83 ± 0.42	2.48 ± 1.02	3.2 ± 0.18	1.1 ± 0.07	0.82 ± 0.18
15 :0	3.2 ± 0.12	1.55 ± 0.36	1.41 ± 0.1	2.74 ± 0.16	3.93 ± 1.95	3.28 ± 1.72	3.36 ± 0.59	3.39 ± 0.68	2.94 ± 0.56	3.13 ± 0.3	1.08 ± 0.38	0.94 ± 0.28
16 :0	54.11 ± 9.84	21.06 ± 3.93	35.02 ± 0.94	36.99 ± 5.75	23.66 ± 3.75	47.25 ± 2.94	59.46 ± 3.98	41.21 ± 1.24	41.33 ± 6	52.2 ± 3.79	22.79 ± 1.52	40.92 ± 5.52
16:1ω7	3.47 ± 0.69	11.83 ± 0.08	7.93 ± 0.41	3.47 ± 1.34	12.87 ± 3.07	5.84 ± 1.04	3.64 ± 0.05	7.43 ± 1.33	6.13 ± 1.58	3.47 ± 0.22	7.94 ± 3.54	8.55 ± 3.34
17 :0	2.56 ± 0.67	4.47 ± 0.74	4.18 ± 0.35	9.35 ± 0.28	4.35 ± 0.7	3.97 ± 0.7	2.57 ± 0.05	4.42 ± 1.66	3.92 ± 0.58	5.15 ± 0.96	2.7 ± 0.46	1.84 ± 0.27
18 :0	27.38 ± 5.2	13.33 ± 2.67	18.8 ± 0.8	24.59 ± 2.31	24.31 ± 5.88	21.58 ± 2.64	21.38 ± 3.58	22.99 ± 2.94	25.75 ± 3.64	22.28 ± 1.73	14.28 ± 3.19	17.06 ± 2.6
18:1ω9	n.d.	4.52 ± 0.45	2.28 ± 0.23	10.01 ± 2.79	1.63 ± 1.47	2.61 ± 1.85	0.25 ± 0.25	2.56 ± 2.22	1.72 ± 0.59	3.6 ± 1.27	3.88 ± 2.01	2.45 ± 0.67
18:1ω7	0.64 ± 0.32	7.36 ± 0.75	2.55 ± 0.17	6.23 ± 1.53	3.01 ± 3.19	2.93 ± 1.63	2 ± 1.35	3.16 ± 1.88	3.47 ± 2.05	2.82 ± 0.49	3.75 ± 1.48	2.2 ± 0.6
18:2ω6	n.d.	1.88 ± 0.38	3.62 ± 1.08	1.34 ± 0.45	n.d.	1.57 ± 1.09	0.35 ± 0.35	0.74 ± 0.45	0.32 ± 0.56	0.67 ± 0.14	2.33 ± 0.49	1.7 ± 0.95
18:3ω3	n.d.	0.5 ± 0.86	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.19 ± 0.32	n.d.
18:4ω3	n.d.	1.33 ± 0.34	n.d.	n.d.	2.34 ± 3.61	0.26 ± 0.45	n.d.	0.87 ± 1.13	1.3 ± 1.69	n.d.	0.97 ± 0.4	0.75 ± 0.12
20 :0	n.d.	0.29 ± 0.51	0.56 ± 0.49	0.57 ± 0.58	n.d.	n.d.	0.14 ± 0.14	0.09 ± 0.09	0.18 ± 0.2	1.18 ± 1.61	0.26 ± 0.1	0.57 ± 0.14
20:4ω6	n.d.	2.09 ± 0.59	3.07 ± 0.44	2.09 ± 3.06	1.01 ± 0.08	2 ± 0.38	n.d.	1.63 ± 1.14	0.73 ± 0.37	0.12 ± 0.05	1.89 ± 0.51	1.44 ± 1.26
20:5ω3	n.d.	1.22 ± 0.37	0.39 ± 0.03	0.07 ± 0.05	0.92 ± 0.18	0.19 ± 0.19	n.d.	0.38 ± 0.05	0.32 ± 0.2	n.d.	1.47 ± 0.23	0.56 ± 0.17
22 :0	n.d.	0.65 ± 0.58	1.31 ± 0.35	0.26 ± 0.23	1.16 ± 0.03	1.51 ± 0.63	0.06 ± 0.06	0.94 ± 0.19	1.06 ± 0.43	0.09 ± 0.02	1.65 ± 0.43	3.48 ± 1.6
22:6ω3	n.d.	18.41 ± 7.38	8.39 ± 0.99	0 ± 0	14.99 ± 1.78	n.d.	n.d.	5 ± 0.59	5.07 ± 4.48	n.d.	28.77 ± 3	9.41 ± 8.17
23 :0	n.d.	0.47 ± 0.41	0.81 ± 0.7	0 ± 0	1.84 ± 2.05	1.9 ± 2.87	n.d.	1.24 ± 0.81	0.22 ± 0.19	n.d.	0.76 ± 0.16	0.78 ± 0.38
24 :0	n.d.	0.66 ± 0.57	3.92 ± 0.54	0 ± 0	1.65 ± 0.82	1.01 ± 0.8	n.d.	0.88 ± 0.29	1.3 ± 0.15	n.d.	1.95 ± 0.55	2.89 ± 2.08
Σ SAFA	95.91 ± 4.16	50.87 ± 10.2	71.76 ± 2.89	84.73 ± 3.56	67.36 ± 4.67	87.26 ± 7.43	94.99 ± 0.58	82.53 ± 2.26	82.73 ± 3.73	92.5 ± 0.74	48.82 ± 5	72.95 ± 5.94
Σ MUFA	4.11 ± 1	23.7 ± 1.13	12.76 ± 0.73	19.71 ± 5.63	17.52 ± 2.05	11.39 ± 4.51	5.9 ± 1.55	13.15 ± 3.51	11.32 ± 1.01	9.89 ± 1.82	15.56 ± 6.98	13.2 ± 4.54
Σ PUFA	n.d.	25.43 ± 9.1	15.48 ± 2.23	3.5 ± 3.01	19.27 ± 5.34	4.03 ± 1.83	0.35 ± 0.35	8.62 ± 1.17	7.75 ± 5.05	0.79 ± 0.16	35.62 ± 2.24	13.85 ± 10.08

Table 5.5 *Relative FA composition (%) of copepods. All values are means ± standard deviation (N=3). Only FAs contributing for more than 0.5% are listed. CA = Clausocalanus arcuicornis, TF = Tisbe furcata and DT = Diosaccus tenuicornis.*

In the four consumer copepod species, a total of 19 FAs were identified above the detection limit (Table 5.5). 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, 20:0, 22:6ω3 and 23:0. The SAFAs were the most important class, accounting for 92.0 ± 5.1% in *C. arcuicornis*, 78.7 ± 7.5% in *D. tenuiremis* and 62.0 ± 55.8% in *T. furcata.* The predominant groups of SAFAs were 16:0 and 18:0, on average over all species 39.7 ± 12.8% and 21.1 ± 5.1%, respectively. The MUFAs were more abundant in *T. furcata* were they accounted for 17.5 ± 4.5% of the total FA composition. Palmitoleic acid (16:1ω7) was the most abundant MUFA with 10.0 ± 2.7%, as well as for the MUFAs composition of *D. tenuiremis* (7.1 ± 1.33%).

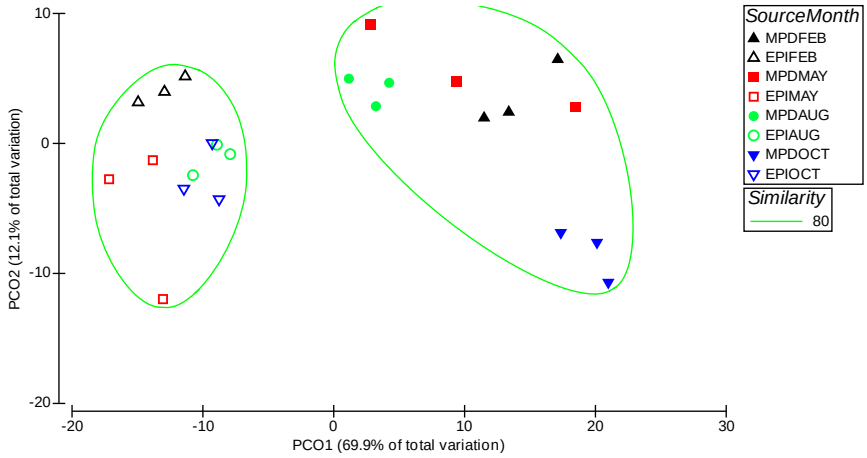


Fig 5.4 *PCO of the total FA composition of the sources with an overlayed cluster (dashed line) at a similarity of 80%. Open symbols represent the epiphyte source (EPI) and closed symbols the macrophytodetritus source (MPD. Black triangles = February (FEB), red squares= May (MAY), green circles = august (AUG), blue reverse triangles = October (OCT).*

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*, contrarily to *T. furcata* and *D. tenuiremis* where it was the most important PUFA with 16.8 ± 9.8% and 5.7 ± 4.2%, respectively. The PERMANOVA revealed a difference between months (P <0.001), in addition a stronger difference between species was visible (PERMANOVA, P <0.001). The PCO with overlayed cluster visualization clearly separates *C. arcuicornis* from the two other species (Fig 5). The SIMPER revealed mainly PUFAs, for instance ARA, DHA and oleic acid as typical for this cluster and separating it from the 2 other clusters. Both species' FA compositions (*T. furcata* and *D. tenuiremis*) were never different from each other except in the month May and February (PERMANOVA, pair-wise, $P_{(MC)}$ = 0.018 and 0.016, respectively). The two species had an average dissimilarity of 17.39% with a maximum value of 25.8% in May.

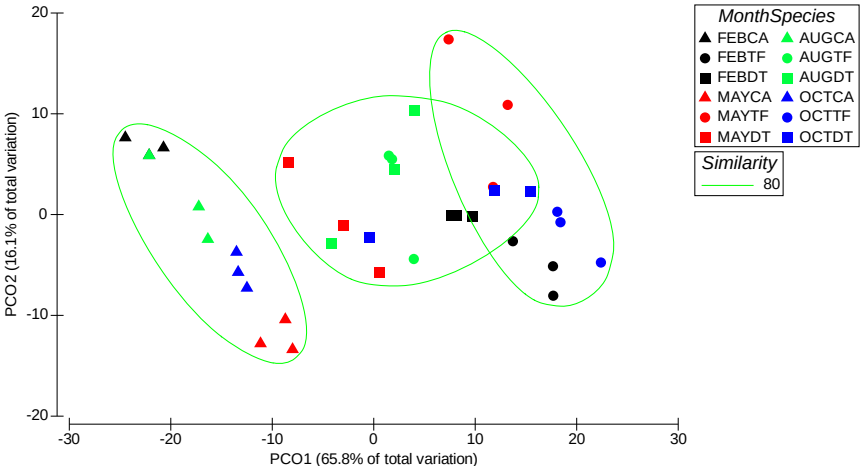


Fig 5.5 *PCO of the total FA composition of the consumers with an overlayed cluster (dashed line) at a similarity of 80%. Black = February (FEB), red = May (MAY), green = august (AUG) and blue = October (OCT). Triangles = Clausocalanus arcuicornis (CA), squares = Tisbe furcata (TF) and circles = Diosaccus tenuicornis (DT).*

5. Discussion

5.1. Food source characterization and the role of macrophytodetritus

The isotopic composition of macrophytodetritus of *P. oceanica* seagrass was the most ^{13}C enriched food source in the collected samples. Isotopic ratios of macrophytodetritus were slightly more depleted than living *P. oceanica* leaves at the same site (Lepoint *et al.,* 2000) or in other parts of the Mediterranean (Vizzini *et al.,* 2002). This slight difference is due to the N-recycling before the abscission of a leaf (Lepoint *et al.,* 2002a) and the aging of the degrading material (Dethier *et al.,* 2014). Values of the macrophytodetritus were similar to studies at the same site (Sturaro *et al.,* 2010; Michel *et al.,* 2014). The second least depleted food source, epiphytes, yielded similar values as previous studies in the Revellata Bay (Lepoint *et al.,* 2006; Mascart *et al.,* 2013). The isotopic variability in carbon and nitrogen was due to the composition of the pooled source. Since in this study, epiphytes encompass epiflora, epifauna and drift epilithic algae a variability could be expected, especially in the months February and May when the proportion of epifauna with lower C/N ratio are more abundant. The most depleted composition was found for SOM matching previous research in the Gulf of Calvi (Dauby 1989; Michel *et al.,* 2014).

The macrophytodetritus FA profiles were mainly composed of saturated compounds (SAFAs) 14:0, 16:0, 18:0, as shown in a previous study (Michel *et al.,* 2014). However the total SAFA contribution was higher in this study. Those ubiquitous SAFAs are omnipresent in living organisms, including the ones in the aquatic ecosystems (Arts *et al.,* 2009). Odd-branched saturated FA like 15:0 and 17:0 were present, though in a small proportion. These branched FA are used as biomarkers for bacteria in a variety of coastal ecosystems (for a review see Kelly & Scheibling 2012). The epiphyte source had a higher abundance of branched FA indicating a greater quantity of

bacteria in the epiphytic biofilm (Belicka *et al.,* 2012). Other FA such as 18:1ω9 and 18:2ω6 are tracers for cyanobacteria (Caramujo *et al.,* 2008). Another compound, more specifically 18:1ω7, was found in higher proportions than the odd-branched FA and is, next to being a diatom tracer, also characteristic for bacteria (Nichols *et al.,* 1985). This proves that bacteria colonized dead litter leaves in order to decompose the material. The other abundant MUFA, 16:1ω7 is present in both sources and is a marker for diatoms (Jaschinski *et al.,* 2011). The most abundant PUFAs of epiphytes were ARA and EPA, known to be abundant in red and brown algae (Graeve *et al.,* 2002). Other less abundant PUFAs like 18:2ω6 and 18:3ω3 are typical seagrass FA indicators (Viso *et al.,* 1993; Graeve *et al.,* 2002). As in the study of Kharlamenko *et al.* (2001) where low proportions (10 times lower than living seagrass) of those FAs were found in decomposing *Zostera marina*, similarly low proportions were found in *P. oceanica* macrophytodetritus (Michel *et al.,* 2014). Other typical vascular plant long-chained saturated FA were present in high numbers in macrophytodetritus, for instance 24:0, 26:0 and 28:00 (Viso *et al.,* 1993).

Most of the components characterising microorganisms (i.e. bacteria, fungi, etc.) are present in both sources, although in lower abundances in the macrophytodetritus source. Microorganisms are an inherent part of the epiphyte biofilm, therefore since the macrophytodetritus leaves are stripped from their epiphytic biofilm (Dauby and Poulicek 1995), one would expect to find no more microorganism FA, especially photophilic diatoms. The presence of microorganisms FA could indicate a caveat for the scraping technique when used on fragile and brittle litter fragments. Sosik & Simenstad (2013) tested the validity of the scraping method for microbes on *Saccharina* kelps and yielding a reduction from 49 to 78% after scraping. Since the thallus of *P. oceanica* is probably even more brittle in a more decomposed state, it is suggested that the efficiency of the technique on detritus is not as high as on living leaves. Therefore, the proportion of microorganisms in the macrophytodetritus source could be slightly overestimated. The same is not only valid for fatty acid composition, but

also for isotopic signatures. When scraping off the epiphytic film, surface cells of the macrophytodetritus leaf get damaged and contaminate the epiphyte sample. Oppositely, when removing epiphytes, some remnants stay behind. Therefore, epiphyte and macrophytodetritus isotopic composition could be pulled towards each other.

To conclude, all three sources had a different SI composition. Although, the close vicinity of MPD to EPI raises questions about the used techniques and potential food sources.. Both food sources in terms of FA composition were different. Microorganism tracers were present in both sources and MPD had typical long chained SUFAs, characteristic for seagrasses.

5.2. Consumer's composition and specific preferences

Bulk SI and FA analyses are used to complement each other and to cross-validate results especially when one tracer is insufficient to clearly separate among two sources. The copepod *C. arcuicornis* displays a clear SOM preferred diet, in both FA and SIMM results. FA profiles revealed a high amount of short-chained SAFAs, typical for SOM (Lebreton *et al.,* 2011; Michel *et al.,* 2014). Epiphytes could also contribute to their diet, especially diatoms and bacteria.

For other species, the epiphyte source was the most assimilated source throughout the seasons and species. The presence of diatom tracer FA 16:1ω7, 18:1ω7 and EPA FA is congruent with the SIMM results. Other tracers such as red algae and fungi ARA are omnipresent in the copepod's composition and EPI source (Kharlamenko *et al.,* 2001; Kelly and Scheibling 2012). Epiphytes can thus be considered as the copepods' main food source, with the exception of *C. arcuicornis*. Variability between the species and between the seasons composition demonstrates that every species uses the available food sources in slightly different proportions. An exact quantification of every component of the epiphytes that contributes to each species' specific composition is difficult to do with natural samples.

The complex composition of the epiphytes confounds the trophic diversity among copepod species. In order to tackle that question, food specific laboratory tracer experiments are useful (De Troch *et al.,* 2012b; Rzeznik-Orignac and Fichet 2012). Levels of seagrass FA 18:2ω6 and 18:3ω3 were found in very small amounts in the copepod species. The first tracer was not present in *C. arcuicornis* and for *T. furcata* and *D. tenuiremis* it only showed some proportions in October and February. The second tracer was mostly not detected. Of the other seagrass tracers, being saturated long chained FAs (>22:0), only 24:0 is found in low amounts in *T. furcata* and *D. tenuiremis*. Compared to the proportions present in macrophytodetritus, it can thus be considered that macrophytodetritus is an unlikely food source, especially for *C. arcuicornis*. However, the unsaturated seagrass FAs are also precursors for PUFA synthesis, so FA alone cannot be conclusive on the importance of macrophytodetritus in the copepods diet (Kharlamenko *et al.,* 2001; Alfaro *et al.,* 2006). Moreover, the SIMM results present macrophytodetritus as a yearlong food source up to 50% for *T. furcata* and *D. tenuiremis.* This is doubtful since copepods don't have the appropriate enzymes to digest cellulose, like seagrass leaves (Knotz *et al.,* 2006). Two explanations are possible for the overestimation of the SIMM: (1) the separation of epiphytes from the leaf surface was not perfect, demonstrated for example by the presence of typical epiphytic FA tracers in the macrophytodetritus profile. Consequently, Macrophytodetritus signature is contaminated by non-separated epiphytes. (2) A potential food source was missed, like for instance macrophytobenthos. The missed food source will down weigh the proportions of MPD and give a more realistic MPD estimation.. Consequently, macrophytodetritus should not be seen as a primary food source, but only a substitute at times when preferred food source is scarce. Second, the obtained signal of macrophytodetritus transfer does not necessarily imply direct grazing. Bacteria and fungi degrading the seagrass conserve the stable isotope composition of the degraded substrate. Therefore, copepods assimilate microbial biomass which may conserve a stable carbon isotopes composition close to the

seagrass one but a fatty acid composition partly determined by assimilated microbes (Bouillon *et al.,* 2004a). Bacteria odd-branched FAs were present in the copepod profiles. The α15:0 FA bacteria tracer was only found in copepods. Consequently, this marker has reached the copepods through another pathway than tested in this study. On the other hand, a potential second indirect way to assimilate both seagrass and bacterial carbon could be faecal pellets ingestion. These pellets are colonized by bacteria (De Troch *et al.,* 2010) and the bacteria present on the surface of the copepod's faecal pellets are known to be grazed upon and farmed by harpacticoid copepods (De Troch *et al.,* 2009; Frangoulis *et al.,* 2011).

This study is partially consistent with the results of Danovaro (1996) and Tenore (1983) which demonstrated that nematode deposit-feeders incorporate more nitrogen from associated microbes and bacteria than from the detritus itself. Warwick (1987) reported that bacteria may represent the most suitable food source for nematodes in detrital systems while Montagna (1984) found that for diatoms were preferred. Furthermore, it confirms that the seasonal changes in trophic structure of meiofauna assemblages are dependent upon the food sources (Danovaro and Gambi 2002). Regardless of the way of its assimilation (direct vs. indirect), our results indicate that copepod fauna are presumably a very important actor in fuelling detritus carbon and nitrogen from seagrass meadows to higher trophic level (Coull 1990), although higher trophic levels were not studied in this research.

The four copepod species (Fig 5.2.B) showed different feeding strategies. The water-column-type calanoid *C. arcuicornis* primarily feeds on SOM in the water column but migrates to the macrophytodetritus accumulations to secondarily feed on the epiphytic biofilm, mainly during spring. Subsequently, *C. arcuicornis* has a moderate niche width, mainly focussed on SOM. The epibenthic-swimmer-type *T. furcata* keeps a constant composition throughout the year and therefore slightly changes the proportion of each food source according to the moment of the year. The

trophic niche width is the narrowest of all four species, revealing a high specific feeding strategy of *T. furcata*. The phytal-type *D. tenuicornis* has a fixed diet, mainly epiphyte based. Since the baseline of the diatoms fluctuates slightly throughout the year, the overall niche width of *D. tenuiremis* is moderate. The mesopsammic-type *E. dentatum* displayed the largest niche width. Consequently, it seems to feed on a variety of food sources of different signatures at different seasons.

To conclude, all four species revealed specific diet preferences and thus niche specialization. It seems that there is a link between the niche width, the realised niche and the type of eco-morphological copepod. It can be hypothesized that copepods with good swimming abilities will actively find their preferred food, e.g. *C. arcuicornis* and SOM or their preferred combination of food: e.g. *T. furcata*. *D. tenuicornis* on the other hand is highly specialized in phytal grazing and therefore can allow targeting epiphytes, mainly diatoms. For the mesopsammic species it is more difficult, since no FA data can confirm what it assimilated. However it seems to have a wide diet range potentially linked to his position in time, since it disperse infaunaly, it should not be rejected that some of the feeding happened in the sediments.

5.3. Implications for the energy flow in the seagrass ecosystem

The realized niche of the copepods shifts throughout the seasons according to the baseline of the primary production. In this research, both sites had the same baseline and similar copepods from different sites did not show different SI compositions. Therefore we can assume that the baseline on a spatial scale of 1km is similar.

High overlap is present in the realized niches showing the constant availability of food in macrophytodetritus accumulations. Notwithstanding, macrophytodetritus seems to have a strong seasonal variability and dynamic character (Mascart *et al.,* 2015b), the available food sources seem

to be present all year long. It attracts numerous copepods from the surrounding habitats (e.g. *C. arcuicornis* from the water-column, see Chapter 6). The copepods exhibit resource partitioning and graze on different fractions of mainly the epiphytic biofilm in different proportions. The four copepods investigated in this study belong to four ecological types of copepods. Due to the resource partitioning, all ecological types are able to share this macrophytodetritus environment.

As suggested by Belicka (2012), more studies on stable isotope and fatty acid analysis of degraded seagrass material and benthic invertebrates are necessary to allow further evaluation of detrital energy pathways and to present more comprehensive food web analyses. Our study showed the strong dependence of primary meiofaunal consumers on mainly epiphytes. Nonetheless, every eco-morphological type seems to cope in different ways with spatio-temporal fluctuations of food sources. Independent of the conditions of the environment their strategies will allow them to comply with their nutritional needs. This illustrates the high resilience of the copepod community present in macrophytodetritus accumulations.

To conclude, the aims and hypothesis previously stated of this study are answered: (1) the chemical composition of *P. oceanica* macrophytodetritus shows to be a poor food source and fluctuates seasonally and to a lesser extent spatially. However, extensively colonized by microorganisms they directly serve as food source or leaching DOM. (2) Species-specific food preferences for copepods are suspected, resulting in trophic niche partitioning and different SI compositions (3) Not completely, the specific food preferences are stronger than the spatio-seasonal fluctuations. (4) Trophic niche specialization and resource partitioning is present.

Picture: Installation of the experimental setup

Chapter 6

How do harpacticoid copepods colonize detrital seagrass leaves?

Adapted from:

Mascart T., Agusto L., Lepoint G., Remy F. & De Troch M. (2015). How do harpacticoid copepods colonize detrital seagrass leaves? *Marine Biology* 162(5), 929-943

1. Abstract

An experiment was carried out to investigate the colonization ability and specific pattern of copepods towards a dynamic benthic habitat. Since copepods are known to disperse passively and actively, the experiment aimed to investigate the pool of colonizers of macrophytodetritus and the species-specific active colonization pathways. The experiment was performed in a Mediterranean seagrass *Posidonia oceanica* meadow on defaunated macrophytodetritus accumulations (mainly dead seagrass leaves) for two time intervals (24 h and 96 h). Active colonization by copepods from adjacent source pool habitat (bare sandy sediments, *P. oceanica* canopy, water column and macrophytodetritus) occurred within 24 h. Natural densities (as in the control treatments) were only reached by active colonization through the water column. Neither diversities nor species composition on natural macrophytodetritus were ever reached by one single migratory pathway. Therefore only a combination of interstitial migration and water column migration can explain the species occurrence under natural condition. Moreover, every potential adjacent source pool habitat contributed species to the newly colonized macrophytodetritus. Nevertheless, the main colonizers were mostly species with good swimming capabilities. The diverse pool of species present in the newly colonized macrophytodetritus underlines the complex communities and dispersion capabilities of copepods. Hence, macrophytodetritus possesses the ability to be a colonizer source pool for every adjacent habitat and thus behaves as a copepod hub for the entire seagrass ecosystem.

2. Introduction

Dispersion and colonization of new habitats by meiofauna (i.e. the benthic fauna belonging to size class 38 µm - 1mm) are highly variable in space and time. This variability is caused by complex interactions between habitat structures, species-specific biological traits, hydrodynamics, resource availability, predation pressure and environmental deterioration (Armonies 1994; Commito and Tita 2002; Bostrom *et al.,* 2010). Since meiofauna lack planktonic larvae (Hagerman and Rieger 1981; Huys and Boxshall 1991) the dispersion mode of adults is crucial for population dispersion. On small scales (up to a meter), early studies revealed horizontal migration through the sediments interstices as primary mean for meiofauna colonization (i.e. infaunal migration) (McIntyre 1969). However, most of the meiofauna lives on the sediment-water interface being capable of active swimming and infaunal migration on short distances during low water flow (Fleeger *et al.,* 1995). These are thus susceptible to passive erosion and are therefore classified as passive dispersers on larger scales (Palmer 1988; Armonies 1994; Sun and Fleeger 1994; Fleeger *et al.,* 1995). Similar trends for passive dispersal are seen in lotic freshwater ecosystems (Palmer and Gust 1985) and soft-bottom coastal ecosystems with regular tidal currents and strong hydrodynamic forces (Sedlacek and Thistle 2006). Nevertheless, in hydrodynamic calm environments (low flow or with biogenic structures reducing the flow), meiobenthic organisms were found suspended in the near-bottom waters revealing active emergence, especially on a diurnal cycle at the onset of dusk (Fleeger *et al.,* 1984; Hicks 1986; Walters 1991; Teasdale *et al.,* 2004). Morphological characteristics were put forward to endorse the emergence availability of phytal and epibenthic meiofauna (Bell *et al.,* 1987; Thistle and Sedlacek 2004; Sedlacek and Thistle 2006). Lower taxonomic classification seems thus to be irrelevant in predicting the habitat utilization of copepods (Sedlacek and Thistle 2006). Noodt (1971), see Fig. 1.5, attempted a provisional classification of Copepoda based on the variety

of evolved ecological forms (Remane 1952). He highlighted different trends in specialization of eco-morphological characteristics, distinguishing various types of copepod adapted to certain conditions of various habitats (e.g. sediment-living, phytal-living, pelagic). Subsequently, a preferred habitat could be deduced from eco-morphological characteristics. However, a classification of specific copepod colonization abilities is still missing, conversely to nematodes (Bongers 1990). Nowadays, phytal and epibenthic copepods are mainly classified as active dispersers and sedimentary copepods as passive dispersers (Hicks 1986; Kurdziel and Bell 1992).

Though, in case of colonization of new habitat or in the habitat connectivity context, the exact habitat source pool of the colonizers is often unknown. Several studies documented copepods' colonization ability within relatively stable habitats, such as coral fragments (Gheerardyn *et al.,* 2009; Callens *et al.,* 2012), hard substrates (Thomsen *et al.,* 2011), coastal soft sediments (Thielemans and Heip 1984; Scheef and Marcus 2010) and deep-sea sediments (Thistle 1978; Guidi-Guilvard *et al.,* 2009). However, few studies tackled the colonization of provisional habitats such as temporary ponds (Frisch and Green 2007), marine snow aggregates (Kiorboe 2000; Koski *et al.,* 2005) or floating vegetal material (Faust and Gulledge 1996; Ólafsson *et al.,* 2001). The present *in situ* experiment investigated the colonization of dynamic and patchy dead seagrass detritus, hereafter referred to as macrophytodetritus. The majority of the macrophytodetritus accumulates on bare sand patches close to seagrass meadow and is decomposed within a few days to several months depending on the chemical composition and biotic and abiotic fragmentation speed (Romero *et al.,* 1992; Mateo and Romero 1997). These accumulations thereby support high values of secondary production in the receiving communities (Vetter 1995; Mateo and Romero 1997). The structurally complex macrophytodetritus accumulations seem to facilitate the development of meiofaunal communities in coastal marshes (Sanmarti and Menendez 2007), mangrove forests (Torres-Pratts and Schizas 2007) and seagrass beds (Hicks 1980;

Coull and Wells 1983; Mascart *et al.,* 2013). In term of copepod community, connectivity between these accumulation and other adjacent habitats (i.e. seagrass meadow, water column and sediment) is still unstudied, as well as the colonizing process and specific pattern of this colonization.

A novel field experiment was deployed in order to understand the mode of copepod's colonization of Neptune grass *Posidonia oceanica* macrophytodetritus. The objectives here were threefold: (1) to assess the species composition, densities and diversity of the colonist's source pool (i.e. the sediment, the water column, the *P. oceanica* canopy or other macrophytodetritus patches); (2) to investigate the rate of active colonization of defaunated seagrass macrophytodetritus from adjacent habitats, and (3) to contribute to our knowledge of species-specific colonization characteristics.

3. Materials and methods

3.1. Experimental design and sampling site

The experimental site was located in the Gulf of Calvi, Corsica, northwest Mediterranean (42°35'N, 8°43'E) near the oceanographic station of STARESO (Station de Recherches Sous-marines et Océanographiques, University of Liège). The site consisted of a sand patch (about 100-200 m^2) at a depth of 12 m, inside a *P. oceanica* seagrass meadows, partly covered with macrophytodetritus accumulation (MPD). Macrophytodetritus accumulation (10-20 cm thick) was mostly composed of dead *P. oceanica* leaves and living uprooted seagrass shoots, typical for the Bay area and the time of the year (Mascart *et al.,* 2015b).

The *in situ* experiment, comprised of cylindrical experimental PVC units

(inner diameter of 10 cm) was set up by scuba divers on 26th of October 2012 for 24h of incubation and on 2nd of November 2012 for 96 h of incubation. During the experiments, the site was characterized by a constant salinity of 38, calm weather conditions and weak currents (4-5 $cm.s^{-1}$). Temperatures varied between 18°C to 21°C and light intensities were highest (1200 lux to 4000 lux) between 11 and 14 'o clock (HOBO ® Onset Computer Corporation).

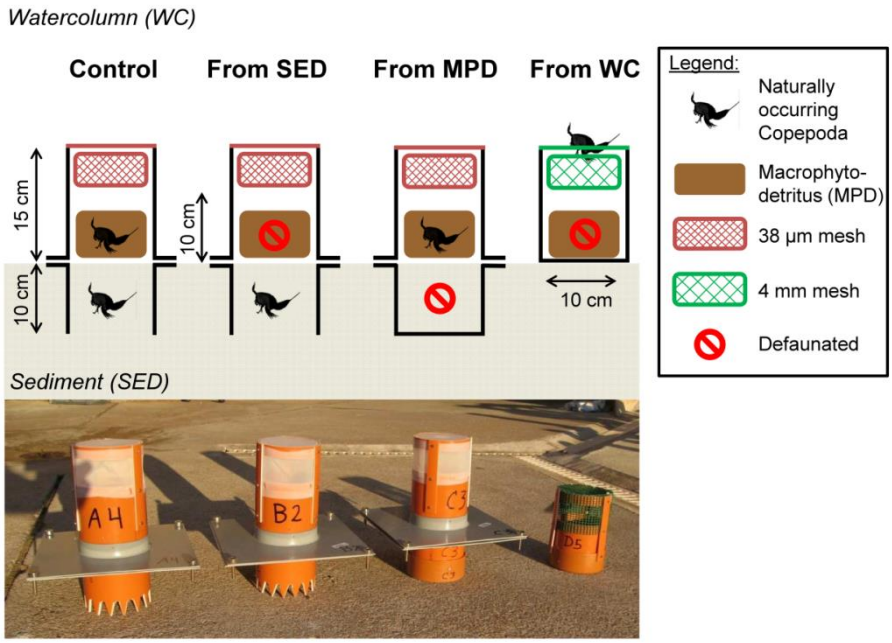


Fig 6.1 *Upper part: experimental design representing the four treatments, showing the dimensions, the two mesh sizes used, the defaunated habitats and the two compartments: upper (on top of the sediments) and lower (inside the sediments). Lower part: picture of the assembled corresponding treatment units on the dock prior to setup.*

The cylindrical experimental PVC units were divided into two parts: (1) an upper compartment containing on average 12 ± 6 gDM fresh macrophytodetritus (i.e. slightly degraded dead seagrass leaves) (MPD) and (2) a lower compartment containing sandy sediment (SED) (Fig. 6.1).

The upper compartment had a height of 15 cm, as used in emergence traps (Walters and Bell 1986) in order to exclude water flow driven effects and random contamination of mesopsammic copepods. At 10 cm height a circumference window was made and together with the open top of the tube, both were covered with a 38 µm mesh, in order to exclude any contamination and predation by macrofauna but to allow water and oxygen exchange. The lower compartment was inserted 10 cm deep into the sediment. This depth was chosen since vertical penetration of copepods happens in the surficial layer (Danovaro and Fraschetti 2002; Kotwicki *et al.,* 2005; Giere 2009), rarely exceeding 5-10 cm depth and therefore contamination from the surrounding sediments is excluded. The bottom of the tube remained open. Both compartments were placed on top of each other and fitted to stabilizing plates at the water-sediment interface providing support. The stabilizing plates offered guidance for the insertion of the splitter plate used to collect upper or lower compartment at the end of the incubation (Fig. 5.1).

The experiment was conducted twice. A first short-term incubation of 24 h (T_{24}) lasting a full diurnal cycle was carried out to rule out any pattern of vertical migration due to the change in light intensity (Walters and Bell 1986). A second mid-term incubation of 96 h (T_{96}) was performed to give a chance to the colonizing community to stabilize, however not too long to avoid potential inter-specific competition and thus a colonization-competition trade-off (Chandler and Fleeger 1983; De Troch *et al.,* 2005b). Both experiments started at midday, but were not set up simultaneously to avoid disturbance during collection of an experiment. Each experiment was preceded by a reference sampling (T_0) in order to define the natural species composition and variability in the adjacent, potential source pool, namely the natural bare sandy sediments (T_0 SED), the natural macrophytodetritus (T_0 MPD), the adjacent *P. oceanica* canopy (T_0 POS) and the water column 1 m above the site (T_0 WC). These four habitats were sampled with respectively sediment meiocores (De Troch *et al.,* 2001b),

detritus-cores (Mascart *et al.,* 2015b), plastic bags (Lepoint *et al.,* 2006; Mascart *et al.,* 2013) and 50 µm mesh hand towed horizontal plankton nets (Hamner and Carleton 1979), respectively.

The experimental design consisted of four treatments in quadruplicates (Fig. 5.1). The first treatment 'Control' was set to test for possible effects of the deployed units on meiofauna MPD and sediment natural community. The upper and lower compartments were filled with natural macrophytodetritus and sediments, respectively. The second treatment 'From SED' tested the colonization of copepods from natural bare sediments towards defaunated macrophytodetritus positioned above it. The upper and lower compartments were filled with defaunated macrophytodetritus and natural sediments, respectively. The third treatment 'From MPD' tested the opposite colonization direction from natural macrophytodetritus towards defaunated sediments. The upper compartment contained natural macrophytodetritus, while the lower compartment contained defaunated sediments. The lower compartment was closed to supress colonization by the sediment. The fourth treatment 'From WC' only consisted of an upper compartment filled with defaunated macrophytodetritus and was closed with a 4 mm mesh to allow larger planktonic copepods to enter the system, while excluding macrofaunal predators (e.g. juvenile fishes, amphipod crustaceans and shrimps). This last treatment tested for copepod colonization towards the macrophytodetritus from the surrounding water column.

Defaunation of macrophytodetritus and sediment was performed prior to the experiment. For this purpose, additional natural samples of macrophytodetritus were taken and were rinsed thoroughly with fresh water and an 8% $MgCl_2$-solution (Hulings and Gray 1971) on a 1mm sieve in order to stun and remove attached organisms while keeping the loss of epiphytes living on the dead leaves surface as minimal as possible. Additional sediment samples were collected and defaunated by, a gentle defaunization technique (in contrast to the destructive methods applied by

Chandler & Fleeger, 1983 and Chertoprud *et al.* 2005) to prevent loss of attractiveness for the potential colonizing copepods. The sediments were bathed in freshwater for several minutes (to detach copepods), mixed by hand and decanted. A control subsample was taken and analysed under a stereomicroscope to confirm the successful defaunation. The process was repeated on average five times until no copepods remained

3.2. Sample collection and treatment

At the end of the incubation, prior to sampling, both compartments were isolated by inserting a splitter plate in between the stabilizing plates. Each compartment together with its content was subsequently transferred to a closed plastic zip bag to avoid contamination and any loss of material. The upper compartments containing macrophytodetritus were afterwards rinsed with an 8% $MgCl_2$-solution and fresh water in order to separate meiofauna from the macrophytodetritus. The rinsed samples were sieved over a 1 mm and 38 µm mesh sieve to exclude macrophytodetritus and retain copepods, respectively. The copepods were preserved in a 4% formaldehyde seawater solution. The separated macrophytodetritus was dried at 60°C for four days (Mascart *et al.,* 2015b) to measure dry mass for further standardization of copepod densities. The lower compartments comprising sediments were subsampled with meiocores and afterwards preserved in a 4% formaldehyde seawater solution. The copepods present in the sediment were subsequently extracted by centrifugation with Ludox HS40 (specific density of 1.18 $g.dm^{-3}$). All the copepods were stained with Rose Bengal, counted, picked out and mounted on slides for microscopic identification to species level based on identification keys of Lang (1948) and Boxshall & Halsey (2004). Densities of copepods in the macrophytodetritus were standardized towards the dry mass of macrophytodetritus (indiv. g^{-1}DM) and towards the sediment surface in the case of benthic samples (indiv. 10 cm^{-2}).

3.3. Data analysis

To analyse the structure of the community, five species diversity metrics were used reflecting the different elements of biodiversity (see Magurran (2004) and Magurran and McGill (2011) for an overview): S = number of species observed (species number); d = Margalef's corrected number of species for N number of individuals (species richness); H′ = the Shannon's diversity index based on natural logarithm (species diversity); E_H = Heip's evenness index sensitive to rare species (species evenness) and N_1 = number of species that would have been found in the sample when all species would be equally common (dominance metrics).

In order to identify the most typifying copepod species primarily providing the discrimination between and within factors, a SIMPER (similarity percentages) routine was used after an ANOSIM (analysis of similarity) difference test. To visualise the community structure a principal coordinate analysis (PCO) based on the zero-adjusted Bray-Curtis similarity resemblance matrix of the log-transformed relative multivariate data of copepod species abundances was performed.

The analysis of variance in univariate or multivariate data were examined using a 2-way PERMANOVA routine and post-hoc pair-wise comparisons with fixed factors Treatment (Control, From SED, From MPD and From WC), Habitat (MPD, SED) or Time (T_0, T_{24} and T_{96}). PERMANOVA allows us to perform an ANOVA with *P*-values obtained by permutation (Anderson *et al.,* 2008), thus avoiding the assumption of normality. Prior to run 2-way PERMANOVA's, assumption of homogeneity of dispersion was tested with a PERMDISP and distances amongst centroids calculated on the interaction level (Quinn and Keough 2002). Euclidean distance and Bray-Curtis based resemblance matrices were used, respectively, for univariate and multivariate measures. Pair-wise tests type III and Monte-Carlo *P*-values were used since sometimes the total number of unique permutations did not exceed a hundred, whereas 4999 unique permutation is favourable

(Anderson *et al.,* 2008).

The calculated resemblance matrixes were based on a zero adjusted log transformed data with the addition of one dummy variable, for the reason that defaunated compartments contained no species at the start of the incubation. Prior to the analysis, both experiments' reference samples (T_0 samples of T_{24} and T_{96}) were checked for significant differences in species composition for the factors Habitat and Time. No differences were found between both time references sample for each habitat ($P = 0.471$). In both experiments all four reference habitats were significantly different in species composition ($P < 0.001$). Therefore both incubations reference samples were pooled into one T_0 reference per habitat in order to have a higher replication and thus higher statistical power. Due to different standardization methods within each unit compartment (habitat MPD per gram dry mass vs. SED per surface area) differences in total absolute copepod densities were examined per compartment using factors Time and Treatment. Variance in species compositions between the start (T_0) and the end of incubation (T_{24} or T_{96}) were investigated using the fixed factors Treatment and Habitat.

All the above mentioned analyses were performed with the Primer 6.1.11 software (Clarke and Gorley 2006) with PERMANOVA add-on software (Anderson *et al.,* 2008). A significance level of $P < 0.05$ was used in all tests. Graphs were constructed in GraphPad 5.03 for Windows (GraphPad Software, San Diego California USA).

4. Results

Within 24 h and 96 h of incubation all defaunated habitats were colonized by copepods, therefore no repulsive effect of the experimental set-up was found (all pair-wise $P > 0.1$) (Fig. 6.2).

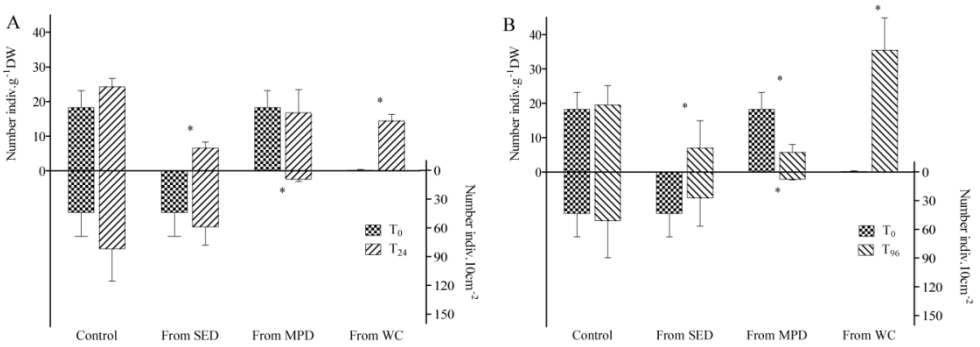


Fig 6.2 *Mean Copepoda densities per treatment for the 24h incubation experiment (A) and the 96h incubation experiment (B). Upper part represents the upper compartment with macrophytodetritus (MPD) standardized per indiv. g^{-1}DM (Dry Weight) on the left y-axis. Lower part represents the lower compartment with the sediments (SED) standardized per indiv. 10 cm^{-2} on the right y-axis. Error bars represent the standard deviation. N = 4 per treatment and WC = water column. * shows a significant difference (P < 0.05) in total densities between start and end incubation.*

Over all samples, a total of 58 different species were identified belonging to three Copepoda orders. The majority of the species (50) belonged to the order Harpacticoida, representing 83.8 ± 2.1 % (average ± standard deviation, henceforth used as notation) of the encountered species. Five species belonged to the order Cyclopoida and three species to the Calanoida order (Table 6.1). Noodt (1971) attempted a provisional classification of Copepoda based on the variety of morphological forms which are adapted to special conditions in various habitats. All eco-morphological types except parasitic-types were present in this study: (M) Mesopsammic-types, primarily sediment living; (P) Phytal-types, clinging to phytal structures; (E) Epibenthic-types, epibenthic-swimmers; (W) Water-column-types, pelagic free-swimmers. A complete classification per species was made here (Table 6.1) in accordance with former studies and eco-morphological traits (Lang 1948; Noodt 1971; Bell *et al.,* 1987; Higgins and Thiel 1988; Bodin and Leguellec 1992; Thistle and Sedlacek 2004). Following the obtained results, a classification in terms of active

colonization was added per species: (I) Infaunal colonizers, interstitial dispersal pathway; (S) Suspension-colonizers, water-bound dispersal pathway; (-) Non-active colonizer, persists in its initial habitat (Table 6.1). In order to keep a comprehensive overview, the results are presented per factor Time and succinctly by factor Treatment.

		Time:	T_0				T_{24}							T_{96}						
Active	Morpho-	Treatment:					Control		From SED		From MPD		From WC	Control		From SED		From MPD		From WC
Colonizer	Eco. Type	Species	Habitat:	SED	MPD	POS	WC	MPD	SED	MPD	SED	MPD	SED	MPD	MPD	SED	MPD	SED	MPD	SED	MPD
		Harpacticoida																			
		Ameiridae																			
I/S	P	*Ameira longipes*			X	X	X	X	X	X		X	X	X	X	X	X		X		X
S	P	*Ameiropsis nobilis*		X	X			X			X	X		X	X				X		X
		Ancorabolidae																			
I/S	P	*Laophontodes bicornis*			X	X		X						X	X		X		X		X
-	P	*Probosciphontodes stellata*			X			X							X				X		
		Canuelidae																			
-	M	*Canuella furcigera*		X					X		X					X		X			
		Cletodidae																			
I/S	M	*Cletodes limicola*		X	X						X		X	X							
		Dactylopusiidae																			
S	P	*Dactylopusia tisboides*			X	X		X				X		X	X				X		X
-	P	*Diarthrodes minutus*			X	X		X				X			X				X		
-	P	*Paradactylopodia brevicornis*			X	X		X				X			X						
		Ectinosomatidae																			
I/S	M	*Ectinosoma dentatum*		X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X
-	M	*Arenosetella tenuissima*		X							X							X			
-	M	*Pseudobradya hirsuta*		X							X							X			
S	W	*Microsetella norvegica*					X						X								X
		Hamondiidae																			
-	P	*Ambunguipes rufocincta*			X			X				X			X				X		
		Harpacticiidae																			
S	P	*Harpacticus littoralis*			X	X		X				X									X
		Laophontidae																			
I	M	*Asellopsis duboscqui*		X	X					X	X	X	X				X			X	
S	P	*Esola longicauda*											X								X
I	M	*Laophonte cornuta*		X				X		X		X			X				X	X	X
I/S	M	*Laophonte elongata elongata*		X	X			X		X				X	X		X		X		X
I	M	*Laophontina posidoniae*		X					X	X	X					X		X	X		
I/S	P	*Paralaophonte brevirostris*			X	X		X		X		X	X	X	X		X		X		X
		Leptastacidae																			
I	M	*Leptastacus laticaudatus*		X					X		X		X			X		X	X	X	
		Leptopontiidae																			
I	M	*Leptopontia curvicauda*		X	X				X	X	X		X			X		X	X	X	
		Longipediidae																			
S	P	*Longipedia minor*			X	X		X				X		X	X		X				X
		Metidae																			
I	P	*Metis ignea*			X	X		X	X	X		X	X		X						
		Miraciidae																			
I/S	M	*Amphiascoides debilis*		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
I/S	P	*Diosaccus tenuicornis*		X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X
I/S	M	*Amphiascus minutus*		X	X	X		X	X	X		X	X	X	X	X	X	X	X	X	X
I/S	M	*Sarsamphiascus tenuiremis*		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
I/S	M	*Delavalia normani*		X	X			X	X	X	X	X	X	X		X				X	

		Rhizotrichidae																				
I	M	*Rhizothrix curvatum*	X	X				X		X		X				X		X		X		
		Paramesochridae																				
I	M	*Wellsopsyllus (Scott.) robert.*	X	X				X		X	X	X				X		X	X	X		
I	M	*Wellsopsyllus (Inter.) interm.*	X					X				X				X				X		
		Peltiidae																				
S	P	*Alteutha depressa*		X	X		X				X		X		X				X		X	
		Porcellidiidae																				
S	P	*Porcellidium ovatum*		X	X		X				X		X		X				X		X	
S	P	*Porcellidium fimbriatum*		X	X		X				X				X				X		X	
		Pseudotachidiidae																				
I/S	P	*Dactylopodella flava*		X	X		X	X	X	X	X	X	X		X		X		X		X	
S	P	*Xouthous laticaudatus*		X											X		X		X		X	
		Tegastidae																				
S	P	*Parategastes sphaericus*			X						X		X						X		X	
S	P	*Syngastes cornalinus*		X			X				X		X									
-	P	*Tegastes calcaratus*		X							X											
S	P	*Tegastes satyrus*		X	X		X				X		X		X		X		X		X	
		Tetragonicepsidae																				
I/S	P	*Diagoniceps laevis*			X		X	X					X			X					X	
I/S	M	*Phyllopodopsyllus bradyi*	X	X						X	X	X	X		X	X		X	X	X	X	
I	M	*Tetragoniceps scotti*	X					X				X				X			X	X		
		Thalestridae																				
S	P	*Rhynchothalestris helgolandica*		X							X				X				X		X	
		Tisbidae																				
S	E	*Tisbe elegantula*		X	X		X				X		X		X						X	
S	E	*Tisbe ensifer*		X	X		X						X								X	
S	E	*Tisbe furcata*		X	X		X	X	X		X		X		X		X	X	X		X	
-	P	*Sacodiscus littoralis*			X																	
		Calanoida																				
		Clausocalanidae																				
S	W	*Clausocalanus arcuicornis*		X	X	X	X	X	X	X	X	X	X		X				X		X	
		Lucicutiidae																				
S	W	*Lucicutia magna*			X	X	X	X					X		X		X				X	
		Paracalanidae																				
-	W	*Paracalanus parvus parvus*				X																
		Cyclopoida																				
		Cyclopinidae																				
S	W	species 1	X	X	X	X	X	X	X		X		X		X	X					X	
S	W	species 2				X							X				X				X	
-	W	species 3				X																
		Oithonidae																				
S	W	*Oithona nana*			X	X									X		X	X	X	X	X	
S	W	*Oithona similis*			X	X							X		X		X		X		X	

Table 6.1 *Cumulative presence list of Copepoda species sorted per order and per family based on four replicates. X = presence. Blank cells = absence. The active colonization pathway in the outer left column are I = Infaunal colonizers, interstitial dispersal pathway; S = Suspension-colonizers, water-bound dispersal pathway; - = Non active colonizer, persists in its initial habitat. The ecological types presented in the second column are M = Mesopsammic-types, primarily sediment living; P = Phytal-types, clinging to phytal structures; E = Epibenthic-types, epibenthic-swimmers; W = Water-column-types, pelagic free-swimmers.*

4.1. Reference samples T0

All T_0 reference samples in sediment, water column, *P. oceanica* canopy and macrophytodetritus were significantly different from each other in terms of species composition (ANOSIM, global R = 0.994, $P < 0.001$). The top four

species typifying a reference habitat were always distinct with the exception of *Sarsamphiascus tenuiremis* (Miraciidae family) which was omnipresent in all reference habitats, except for the water column (Table 6.2). Since a high similarity between replicates was found in each habitat, the dissimilarity between pairs of habitats is as expected high (> 81 %), except between the macrophytodetritus and *P. oceanica* canopy where the dissimilarity is reduced to 46.2 %. The average evenness E_H was 0.27 ± 0.04 for all samples. The highest number of species and species richness was accounted for macrophytodetritus with an S of 25.3 ± 1.0 and d = 5.1 ± 0.2, which is quite similar to the number of species in the *P. oceanica* canopy with S= 23.5 ± 1.7 and d = 4.9 ± 0.3. The sediments had a lower number of species S = 14.3 ± 0.6 and richness d = 3.6 ± 0.3. The lowest species number and richness was found in the water column with S = 8.0 ± 1.4 and d = 1.6 ± 0.3. The evenness was similar in all four samples. The total copepod density was 42.7 ± 24.5 indiv. 10 cm^{-2} for the bare sediments (T_0 SED), 18.3 ± 4.9 indiv. g^{-1} DM for the natural macrophytodetritus (T_0 MPD), 8.1 ± 2.4 indiv. g^{-1} DM for the *P. oceanica* canopy (T_0 POS) and 120.2 ± 4.6 indiv. m^{-3} for the water column (T_0 WC).

All the species found in the seagrass meadow were found in macrophytodetritus, with the exception of *Sacodiscus littoralis* (family Tisbidae). Which was the only non-colonizing species exclusively found in the *P. oceanica* canopy. The species *Ambunguipes rufocincta*, *Probosciphontodes stellate*, *Syngastes cornalinus*, *Tegastes calcaratus*, *Rhynchothalestris helgolandica* and *Xouthous laticaudatus* were only present in the macrophytodetritus habitat, while the following species were exclusively present in the sediment: *Arenosetella tenuissima*, *Canuella furciger*, *Pseudobradya hirsuta* and *Wellsopsyllus (Intermediopsyllus) intermedius*. All the water column habitat species were present in the newly colonized macrophytodetritus, except for Cyclopinidae sp. 3 and *Paracalanus parvus parvus* (Table 6.1).

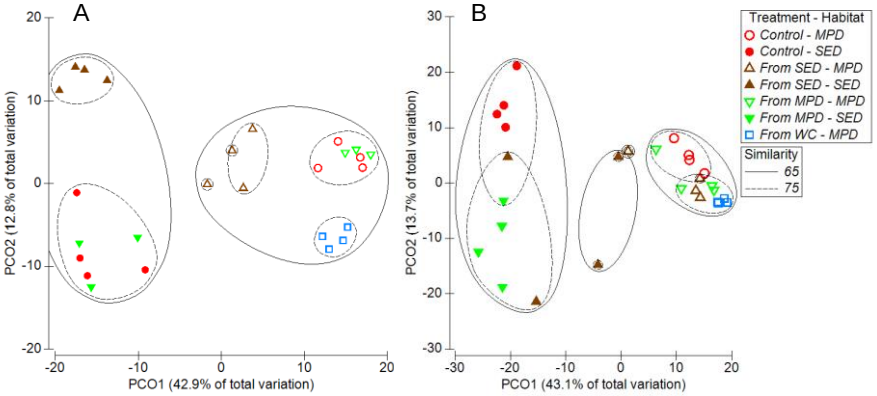


Fig. 6.3 *Principal coordinate analysis (PCO) based on a Bray Curtis similarity resemblance matrix on log transformed data of species abundance of A: 24h incubation experiment and B: 96h incubation experiment. Filled symbols represent the Sediments (SED) habitat and the un-filled symbols the Macrophytodetritus (MPD) habitat. Different treatments are represented by symbols: red circles = Control; brown triangles = From SED; green reverse triangles = From MPD; and blue squares = From WC (water column). Full line represents 65 % similarity and dashed line represents 75 % similarity.*

4.2. The 24h experiment

In the first experiment after an incubation of 24 hours, all defaunated habitats were colonized (Fig. 6.2). The multivariate 2-way PERMANOVA was significant for both factors (Habitat and Treatment) and interaction factor (Table 6.3). PERMDISP's for the interaction factor turned out to be significantly different, indicating that the variation within all factors and interactions was due to the dispersion and location effect, probably because of the large number of zeros present. Pair-wise comparisons (Habitat x Treatment model) revealed no significant difference between the T_{24} 'Control' habitats and the T_0 reference samples (Table 6.3). In comparison to other treatments, the 'Control' treatment, showed only significant differences for the treatment 'From SED' SED habitat (Pairwise: t = 2.39, $P_{(MC)}$ = 0.013) and for 'From WC' MPD habitat (Pairwise: t = 2.94, $P_{(MC)}$ = 0.003) (Table 6.3).

In the 'From SED' treatment, the defaunated MPD compartment was colonized by sediment copepods reaching a density of 6.6 ± 1.7 indiv. g^{-1}DM (Fig. 6.2A). A dissimilarity of 65.0 % (SIMPER) was found between the 'From SED' and 'Control' treatment MPD habitat, although species composition was not significantly different (Table 6.3). In the 'From SED' treatment, the PCO clearly separates SED from MPD habitats, each were however similar at 65 % (Fig. 6.3A). Diversity in this treatment is lower than the 'Control' sample (Table 6.5) due to the high level of dominance (> 56%) of *S. tenuiremis* (Table 6.4). The source pool community for this colonization, the SED habitat of the 'From SED' treatment had a lower diversity, evenness (Table 6.5) and a distinct species assemblage (Fig. 6.3A). Even though *Sarsamphiascus tenuiremis* remained the dominant species (Table 6.4).

The reverse treatment ('From MPD') tested a possible active migration from the natural macrophytodetritus to the defaunated sediments. The defaunated SED habitat was colonized with a density of 8.6 ± 2.4 indiv. $10cm^{-2}$ and reached a diversity comparable to the 'Control' SED habitat. The PCO displayed an assemblage almost identical to the 'Control'. No significant difference in composition was found and four species of the top five most contributing species to the similarity were identical. *S. tenuiremis* was the dominant species (Table 6.4). The species assemblage had a low dissimilarity of 37.2 % and *Dactylopodella flava* remained the dominant species (Table 6.4). The emigration to the sediments from the MPD compartment showed no significant impact on the latter's initial density (Fig. 6.2A) and diversity (Table 6.5), displaying the high variability present within the source pool.

Colonization from the surrounding water column was unambiguous since the density raised from zero to 14.4 ± 1.9 indiv. g^{-1}DM (Fig. 6.2A). The species composition was dominated by a cyclopoid species from the Cyclopinidae family and the calanoid, *Clausocalanus arcuicornis* (Table 6.4). The diversity metrics were in the same order of magnitude as in the

'Control' MPD habitat, however the diversity and evenness were very low (Table 6.5). In comparison to treatment 'From SED', where the sediments exclusively serve as source pool a higher influx of individuals and species occurred. The species composition present after MPD colonization through the water column was significantly different from all four adjacent reference habitat samples (Table 6.3).

Species typifying reference habitats (PERMANOVA P < 0.001)

T_0 SED (54.4% Similarity)

Species	%	cum. %
Sarsamphiascus tenuiremis	43.0	43.0
Leptastacus laticaudatus	11.6	54.6
Wellsopsyllus (Scottopsyllus) robert.	8.1	62.7
Amphiascoides debilis	8.1	70.8
Ectinosoma dentatum	8.0	78.7

T_0 MPD (74.4% Similarity)

Species	%	cum. %
Dactylopodella flava	22.9	22.9
Ameira longipes	12.3	35.2
Dactylopusia tisboides	9.0	44.2
Sarsamphiascus tenuiremis	8.0	52.2
Amphiascus minutus	6.7	58.9

T_0 POS (73.1% Similarity)

Species	%	cum. %
Harpacticus littoralis	14.3	14.3
Diosaccus tenuicornis	12.2	26.5
Sarsamphiascus tenuiremis	9.58	36.1
Amphiascus minutus	7.56	43.6
Porcellidium ovatum	6.38	50.0

T_0 WC (90.0% Similarity)

Species	%	cum. %
Paracalanus parvus parvus	36.3	36.3
Clausocalanus arcuicornis	27.2	63.5
Oithona similis	13.3	76.8
Cyclopinidae sp. 1	9.72	86.5
Microsetella norvegica	7.82	94.3

Table 6.2 *SIMPER results representing the typifying species of the reference samples T_0. First five contributing species are shown. SED = Sediments. MPD = Macrophytodetritus. POS = Posidonia oceanica canopy. WC = Water column.*

4.3. The 96h experiment

The copepod density in both 'Control' compartments of T_{96} was not significantly different from the respective T_0 reference samples habitat. A density of 19.5 ± 5.7 indiv. g^{-1}DM and 50.3 ± 38.4 indiv.10 cm^{-2} were respectively found for the 'Control' MPD and SED compartment (Fig. 6.2B). The species richness (S) was significantly lower (Table 6.5) in both 'Control' compartments after 96 h of incubation (Pairwise: t = 2.92, $P_{(MC)}$ = 0.025 for MPD and t = 2.68, $P_{(MC)}$ = 0.031 for SED). In terms of species composition, pair-wise comparisons revealed significant differences

between the T_{96} 'Control' habitats and the T_0 reference samples (Table 6.3). The dissimilarity between the T_0 and the T_{96} 'Control' reached 53.5 % for the MPD and 52.9 % for the SED habitats (SIMPER).

Colonization of defaunated macrophytodetritus from the sediments (treatment 'From SED') took place. After 96 h, 7.2 ± 7.0 indiv. g^{-1}DM were found, however a large variability amongst the replicates was present (Fig. 6.2B). The species composition present in the newly colonized MPD habitat showed no significant difference (Table 6.3) compared to the 'Control', nonetheless a dissimilarity of 68.6 % (SIMPER) was present. The newly colonized habitat was dominated by *Ectinosoma dentatum* (Ectinosomatidae family) (Table 6.4). In terms of species number, diversity and richness, lower values were noted compared to the 'Control'. However a larger evenness was found (Table 6.5). In the natural sediment, the colonisers' source compartment, two species dominated after 96 h: the *E. dentatum* and *Leptastacus laticaudatus* (Table 6.4). Again a low species number, diversity and richness were combined with a high evenness (Table 6.5).

Colonization of the defaunated sediments by copepods from the natural macrophytodetritus was effective within 96 h. The copepod densities of the colonized habitat in the treatment 'From MPD' increase to 7.4 ± 0.8 indiv.10 cm^{-2} with species diversity metrics showing no significant differences with the 'Control' (Fig. 6.2B). However, the species composition was significantly different (Table 6.3). Two species were most abundant in the SED compartment: *L. laticaudatus* and *Rhizotrix curvatum* (Table 6.4). The colonisers' source MPD compartment showed, in spite of a decrease in density towards 5.8 ± 2.3 indiv. g^{-1}DM, a similar diversity and species composition as the 'Control' MPD habitat. Worth noticing are the two non-harpacticoid species *Oithona nana* and *Clausocalanus arcuicornis* present in the top five contributors of the similarity (Table 6.4).

The colonization of defaunated macrophytodetritus from the water column, treatment 'From WC', displayed a strong increase in copepods

density from zero to 35.4 ± 9.4 indiv.g^{-1}DM, which is more than double of the densities after 24 h (Fig. 6.2B). After 96 h incubation similar species numbers and richness were found, however with a higher evenness and diversity compared to the 24 h incubation. The species composition changed and another Cyclopoid copepod, *O. nana*, became dominant closely followed by *E. dentatum* (Table 6.4).

Factors and interaction:	T_{24}	T_{96}
Habitat (Ha)	$F'_{(1,2)} = 12.9$; $P = 0.001$ **	$F'_{(1,2)} = 18.4$; $P = 0.001$ **
Treatment (Tr)	$F'_{(3,2)} = 4.1$; $P = 0.001$ **	$F'_{(3,2)} = 3.2$; $P = 0.001$ **
Ha x Tr	$F'_{(3,2)} = 2.5$; $P = 0.011$ *	$F'_{(3,2)} = 2.8$; $P = 0.003$ **

Pair-Wise comparisons:	T_{24}		T_{96}	
	SED	MPD	SED	MPD
T_0 vs. Control	t = 1.39 ; $P_{(MC)} = 0.137$	t = 1.30 ; $P_{(MC)} = 0.166$	t = 1.97 ; $P_{(MC)} = 0.035$ *	t = 2.09 ; $P_{(MC)} = 0.012$*
From SED vs. Control	t = 2.39 ; $P_{(MC)} = 0.013$ *	t = 1.69 ; $P_{(MC)} = 0.065$	t = 1.86 ; $P_{(MC)} = 0.039$ *	t = 1.61 ; $P_{(MC)} = 0.063$
From MPD vs. Control	t = 0.98 ; $P_{(MC)} = 0.431$	t = 1.11 ; $P_{(MC)} = 0.293$	t = 2.98 ; $P_{(MC)} = 0.004$ **	t = 1.30 ; $P_{(MC)} = 0.169$
From WC vs. Control	-	t = 2.94 ; $P_{(MC)} = 0.003$ **	-	t = 2.01 ; $P_{(MC)} = 0.011$ *
T_0 SED vs. From WC	-	t = 4.06 ; $P = 0.038$ *	-	t = 5.13 ; $P = 0.032$ *
T_0 MPD vs. From WC	-	t = 3.48 ; $P = 0.031$ *	-	t = 5.60 ; $P = 0.035$ *
T_0 POS vs. From WC	-	t = 3.55 ; $P = 0.029$ *	-	t = 5.36 ; $P = 0.032$ *
T_0 WC vs. From WC	-	t = 6.60 ; $P = 0.020$ *	-	t = 8.77 ; $P = 0.033$ *

Table 6.3 *Two-way multivariate PERMANOVA of species composition and post-hoc Pair-Wise tests. F' = Pseudo-F value, $P_{(MC)}$: Monte Carlo P-value, * = 0.05 > P > 0.01 =significant; ** = 0.01 > P > 0.001 = highly significant. On the left side the 24h incubation experiment and on the right the 96h incubation experiment. SED = Sediments. MPD = Macrophytodetritus. POS = Posidonia oceanica canopy. WC = Water column.*

5. Discussion

5.1. Macrophytodetritus colonization

Our results showed colonization of all defaunated habitats by species originating from adjacent habitats. These adjacent habitats were composed of very specific communities. The densities and diversities encountered during the experiment were congruent with previous studies in the area (Dauby 1980; Heip *et al.,* 1983; Mascart *et al.,* 2013; Mascart *et al.,* 2015b).

Colonization occurred from adjacent habitats within the first 24 h, which is corresponding to recovery times found after a physical disturbance (Sun and Fleeger 1994; Fleeger *et al.,* 1995). Meiobenthic copepods exert colonization and active habitat selection via two pathways: infaunal dispersion through the interstitial spaces and dispersion through suspension in the water column. The majority of adjacent habitat specific typifying species were present in the macrophytodetritus, therefore showing traits of dispersion and making the macrophytodetritus a diverse copepod hub. Moreover, some species seemed to be exclusively associated to macrophytodetritus. It could thus be hypothesised that species have more than one generation in these dynamic accumulations, highlighting the ecological role of macrophytodetritus to the overall seagrass system.

When defaunated macrophytodetritus covered natural bare sediments, an almost immediate interstitial colonization through the boundary layer from the sediment community occurred. Nonetheless, the formed assemblage did not fully resemble the possible macrophytodetritus assemblage as found in the control. Similar patterns were found in the colonization from the water column towards defaunated macrophytodetritus. On the contrary, during colonization from the natural macrophytodetritus towards defaunated sediments, the formed assemblage in the sediment resembled the control as shown in the PCO (Fig. 6.3A). It can therefore be

concluded, firstly, that species defining the sedimentary assemblage were present in the natural macrophytodetritus and crossed the boundary layer downwards. Secondly, that infaunal colonization through the interstitial spaces played a major role, in the occurrence of two contiguous habitats. In case two habitats were not contiguous, recruitment occurred through dispersal through the water column. Thirdly, assemblages in macrophytodetritus were a mixture of the surrounding habitat assemblages. Subsequently, not only habitat-specific species actively migrate as suggested by Hicks (1986). This study concluded copepods, members of different eco-morphologically groups originating from diverse habitats were conspicuous dispersers and actively migrated towards defaunated habitats using their species-specific preferred dispersal pathways.

24h incubation experiment

	Control			From SED			From MPD			From WC		
Upper compartment (habitat MPD)	Species (65.7% Similarity)	%	cum. %	Species (43.7% Similarity)	%	cum. %	Species (72.8% Similarity)	%	cum. %	Species (73.8% Similarity)	%	cum. %
	Dactylopodella flava	23.7	23.7	*Sarsamphiascus tenuiremis*	56.6	56.6	*Dactylopodella flava*	14.7	14.7	Cyclopinidae sp. 1	17.3	17.3
	Sarsamphiascus tenuiremis	17.2	41.0	*Diosaccus tenuicornis*	9.6	66.2	*Dactylopusia tisboides*	13.0	27.7	*Clausocalanus arcuicornis*	16.8	34.2
	Ameira longipes	13.4	54.3	*Ectinosoma dentatum*	9.6	75.8	*Sarsamphiascus tenuiremis*	12.9	40.6	*Sarsamphiascus tenuiremis*	12.3	46.5
	Amphiascus minutus	11.1	65.4	*Ameira longipes*	9.6	85.3	*Tegastes satyrus*	7.8	48.4	*Tisbe furcata*	10.3	56.8
	Diosaccus tenuicornis	6.6	72.0	*Amphiascus minutus*	4.8	90.2	*Paradactylopodia brevicornis*	7.2	55.6	*Amphiascus minutus*	7.4	64.2
Lower compartment (habitat SED)	Species (59.8% Similarity)	%	cum. %	Species (61.1% Similarity)	%	cum. %	Species (61.7% Similarity)	%	cum. %			
	Sarsamphiascus tenuiremis	38.5	38.5	*Sarsamphiascus tenuiremis*	33.1	33.1	*Sarsamphiascus tenuiremis*	23.9	23.9			
	Rhizothrix curvatum	14.1	52.6	*Leptopontia curvicauda*	24.5	57.6	*Wellsopsyllus (Inter.) intermedius*	16.4	40.3			
	Leptopontia curvicauda	10.0	62.6	*Canuella furcigera*	18.0	75.6	*Rhizothrix curvatum*	16.4	56.7			
	Amphiascoides debilis	10.0	72.6	*Delavalia normani*	16.0	91.6	*Leptopontia curvicauda*	11.0	67.7			
	Wellsopsyllus (Inter.) inter.	9.1	81.6	*Phyllopodopsyllus bradyi*	2.0	93.6	*Ectinosoma dentatum*	9.3	77.0			

96h incubation experiment

	Control			From SED			From MPD			From WC		
Upper compartment (habitat MPD)	Species (50.4% Similarity)	%	cum. %	Species (34.4% Similarity)	%	cum. %	Species (56.1% Similarity)	%	cum. %	Species (73.3% Similarity)	%	cum. %
	Ameira longipes	17.0	17.0	*Ectinosoma dentatum*	47.9	47.9	*Ectinosoma dentatum*	37.6	37.6	*Oithona nana*	31.4	31.4
	Ectinosoma dentatum	13.2	30.2	*Amphiascus minutus*	17.2	65.2	*Oithona nana*	15.8	53.4	*Ectinosoma dentatum*	25.4	56.7
	Tisbe furcata	13.2	43.4	*Ameira longipes*	13.5	78.6	*Clausocalanus arcuicornis*	15.7	69.1	*Clausocalanus arcuicornis*	12.4	69.1
	Dactylopodella flava	10.3	53.7	*Tisbe furcata*	11.6	90.2	*Dactylopodella flava*	6.1	75.2	*Tegastes satyrus*	10.8	79.9
	Diosaccus tenuicornis	9.0	62.7	*Sarsamphiascus tenuiremis*	3.8	94.1	*Ameira longipes*	4.8	80.0	*Tisbe furcata*	10.0	89.9
Lower compartment (habitat SED)	Species (66.9% Similarity)	%	cum. %	Species (24.0% Similarity)	%	cum. %	Species (59.8% Similarity)	%	cum. %			
	Leptopontia curvicauda	28.7	28.7	*Ectinosoma dentatum*	30.0	30.04	*Leptastacus laticaudatus*	36.1	36.1			
	Sarsamphiascus tenuiremis	28.6	57.3	*Leptastacus laticaudatus*	25.4	55.4	*Rhizothrix curvatum*	30.8	66.9			
	Amphiascoides debilis	11.7	69.0	*Canuella furcigera*	17.1	72.47	*Wellsopsyllus (Scott.) robert.*	20.3	87.2			
	Rhizothrix curvatum	9.6	78.7	*Leptopontia curvicauda*	11.8	84.27	*Leptopontia curvicauda*	6.2	93.4			
	Tetragoniceps scotti	6.7	85.3	*Laophontina posidoniae*	6.2	90.46	*Sarsamphiascus tenuiremis*	2.6	96.0			

Table 6.4 *Similarity percentages (SIMPER) for both experiments with factors treatment and habitat for copepod species contributions. First five contributing species are shown in percentage (%) and cumulative percentage (cum. %).*

5.2. Dispersion and colonization drivers

In our study, hydrodynamic flow effects were excluded, due to the experimental set-up shielding the macrophytodetritus. Nonetheless in natural environments, the recruitment or settlement of water bound benthic organisms is defined by landscape attributes and hydrodynamic processes (Armonies 1994; Commito and Tita 2002). Palmer (1984) states that meiofauna inhabiting unvegetated habitats avoid the benthic boundary layer during high flow disturbances and frequent the sediment surface during reduced flow. Since above ground structures locally reduce the hydrodynamic disturbance and diminish the predation risk associated to freely swimming in the water column (Coull and Wells 1983; Palmer and Gust 1985), macrophytodetritus stabilizes the hydrodynamic flow and serves as refuge from predators. However, macrophytodetritus is only deposited on the seafloor and is not rooted. Therefore, during low hydrodynamic flow it indeed provides shelter from flow and predation, in contrast, during high hydrodynamic flow or storms, the macrophytodetritus and the upper layer of sediment passively (re)suspends in the water column together with their associated organisms (Thistle *et al.,* 1995). Subsequently, the suspended material resettles randomly on different sand patches, driven by hydrodynamic flows. Hence, rafting or drifting on macrophytodetritus triggered by storms should be considered as a dispersal method at larger spatial scales (Bonsdorff 1992; Norkko and Bonsdorff 1996; Ólafsson *et al.,* 2001).

High disturbances have an adverse effect on densities, however intermediate disturbances could have an opposing effect, corresponding to the intermediate disturbance hypothesis (Connell 1978; Cadotte 2007). It

states that high diversity is a consequence of continually changing conditions. As a result disturbance is put forward as the explanation for coexistence of species and often high disturbance resets the local succession pathway (Connell 1978). Under sheltered conditions macrophytodetritus interstitial water slowly changes from water-column-like chemical conditions to oxygen-poor conditions, due to bacterial respiration and reduced compound advection from sediment (Mascart *et al.,* 2015b). Community structure significantly changed between the T_0 reference samples and the T_{96} 'Control' samples, without showing a drop in densities. For macrofauna, Gallmetzer (2005) and Remy (unpublished data) found a dominance shift towards low-oxygen tolerant species, reducing the overall diversity in the macrophytodetritus accumulation under stable conditions. Therefore the experimental units are not expected to have a negative impact on a short term interval. However, on the long term a plausible oxygen drop could occur and perturb the initial community structure. Hence, the T_{96} incubations displayed the variability of the community structure and its sensibility to a potential drop in oxygen levels.

The habitat selectivity of the copepod resettlement (following a passive erosion or active emergence) depends on the chemical and microbiological signals perceived at small distances (Hicks 1977b; Fleeger *et al.,* 1995). Decho and Fleeger (1988) stated that copepods exhibit a preference for food enriched microhabitats over their initial habitat food availability. Macrophytodetritus are abundantly colonized by microepiphytes (diatoms, bacteria, fungi, protist) (Lepoint *et al.*, 2006) degrading detritus material or using remineralized nutrients. Macrophytodetritus could support a higher microbial biomass and production than living material, since, it may have reduced levels of polyphenolic compounds, which in living leaves deter both bacterial colonization and herbivory (Dethier *et al.,* 2014). Degrading seagrass material could be also a minor source of food for few species (Mascart *et al.*, 2013).

In conclusion, our study is congruent with Boström and Bondsdorff (2000), who stated that structurally complex plant assemblages (like macrophytodetritus) may trap or attract organisms more efficiently than structurally simple leaf canopies or bare sediments. Structural complexity and dispersion drivers, such as hydrodynamics (i.e. disturbances), habitat complexity (i.e. shelter effect) and food availability control the colonization ability of a copepod population.

24h incubation experiment

		T_0 Reference	T_{24} Control	T_{24} From SED	T_{24} From MPD	T_{24} From WC
Upper compartment (habitat MPD)	S =	25.25 ± 0.96	23.75 ± 0.96	8 ± 0.82	23.67 ± 2.08	21 ± 1.83
	d =	5.07 ± 0.23	4.32 ± 0.18	2.43 ± 0.09	4.37 ± 0.33	4.39 ± 0.42
	H' =	2.82 ± 0.07	2.87 ± 0.06	2.37 ± 0.26	2.9 ± 0.06	1.64 ± 0.1
	E_H =	0.25 ± 0.01	0.29 ± 0.02	0.58 ± 0.15	0.3 ± 0.03	0.1 ± 0.01
	N_1 =	16.78 ± 1.13	17.74 ± 1.08	10.94 ± 3.06	18.27 ± 1.15	5.18 ± 0.49
Lower compartment (habitat SED)	S =	14.33 ± 0.58	12.64 ± 5.1	10.25 ± 0.5	14 ± 1.73	
	d =	3.6 ± 0.29	3.03 ± 0.92	2.31 ± 0.19	3.12 ± 0.35	
	H' =	2.24 ± 0.13	2.14 ± 0.46	1.9 ± 0.06	2.76 ± 0.07	
	E_H =	0.26 ± 0.04	0.32 ± 0.11	0.27 ± 0.03	0.45 ± 0.05	
	N_1 =	9.49 ± 1.21	9.19 ± 4.09	6.73 ± 0.39	15.79 ± 1.1	

96h incubation experiment

		T_0 Reference	T_{96} Control	T_{96} From SED	T_{96} From MPD	T_{96} From WC
Upper compartment (habitat MPD)	S =	25.25 ± 0.96	17 ± 4.4	8 ± 2.83	17 ± 3.92	19 ± 2.71
	d =	5.07 ± 0.23	4.38 ± 0.65	2.53 ± 0.81	3.78 ± 0.55	3.59 ± 0.45
	H' =	2.82 ± 0.07	2.62 ± 0.24	1.84 ± 0.34	2.37 ± 0.31	2.16 ± 0.21
	E_H =	0.25 ± 0.01	0.32 ± 0.02	0.37 ± 0.09	0.26 ± 0.05	0.18 ± 0.02
	N_1 =	16.78 ± 1.13	13.99 ± 3.34	6.57 ± 1.99	11.12 ± 3.85	8.82 ± 1.83
Lower compartment (habitat SED)	S =	14.33 ± 0.58	10.75 ± 0.96	6 ± 2	9.25 ± 2.22	
	d =	3.6 ± 0.29	2.74 ± 0.25	1.92 ± 0.25	2.08 ± 0.6	
	H' =	2.24 ± 0.13	2.06 ± 0.13	1.6 ± 0.16	1.65 ± 0.23	
	E_H =	0.26 ± 0.04	0.3 ± 0.06	0.39 ± 0.08	0.24 ± 0.04	
	N_1 =	9.49 ± 1.21	7.91 ± 0.98	4.87 ± 0.69	5.33 ± 1.22	

Table 6.5 *Diversity metrics (average ± standard deviation) based on N = 4 per treatment per compartment for both incubation experiments. S = number of species observed; d = Margalev's species richness; H′ = Shannon species diversity; J′ = Shannon species evenness and N_1 = abundance of the most dominant species. MPD = macrophytodetritus; SED = sediments and WC =*

water column.

5.3. Species-specific behaviour and ecological types

This study rigorously tried to quantify and qualify the species-specific active colonization of copepods from adjacent habitats towards defaunated habitats. Our results showed that the dominant *Sarsamphiascus tenuiremis* (Miraciidae family) rapidly colonized macrophytodetritus, using two different dispersal pathways, one via the water column and another via the sediment and macrophytodetritus interstitial spaces. In order to be able to use the former pathway, one needs to actively emerge from the substrate's surface (e.g. seagrass leafs or sediment) and possess well-developed swimming abilities to than disperse (Thistle and Sedlacek 2004). Other genera, for instance *Ambunguipes*, *Ameira*, *Amphiascoides*, *Amphiascus*, *Dactylopodella*, *Diosaccus* and *Ectinosoma* seemed to have a similar behaviour in our study. On the other hand, the genera *Arenosetella*, *Canuella*, *Leptastacus, Leptopontia*, *Rhizotrix*, *Tetragoniceps* and *Wellsopsyllus* did not seem to behave in an emergent way and preferably stayed in the sediment where they can interstitially migrate between bordering substrata. This is congruent with the study of Hockin and Ollason (1981) whom differentiated two sets of species, one with superior and one with inferior dispersal capabilities. Indeed several of the above mentioned species were exclusively present in the sediments, showing no tendency to migrate and therefore can be tagged as species with inferior dispersal abilities. Regarding the species with superior dispersion abilities Kurdziel and Bell (1992) defined seven true phytal-dwelling families on *Thalassia testudinum* in accordance with previous research by Hicks and Coull (1983). Although studying a temperate water seagrass meadow, our results were analogous as those families were all present in the *P. oceanica* canopy and both studies share the same dominant genus, *Harpacticus*. The *P. oceanica* phytal-dwelling species contributed for 50 % to the species

composition of naturally occurring macrophytodetritus. The prominent role of seagrass species can partially be explained by the origin of macrophytodetritus. Phytal copepods have prehensile legs, allowing them to cling to a substrate (Hicks and Coull 1983), therefore it can be assumed that some species are passively transported attached on the falling dead leaves.

Besides, Kurdziel and Bell (1992) found free-swimming cyclopoids in the *T. testudinum* canopies. Our study found similar copepods in the macrophytodetritus together with calanoids. Hence, free-swimming species can play an important role and represent up to 30-40 % in the densities of the macrophytodetritus assemblage. It is known that these copepods migrate vertically to the bottom for shelter, reproduction and feeding purposes (Teasdale *et al.,* 2004). Seen their high abundance in the 'From WC' treatment, especially with time, it should not be excluded that macrophytodetritus has an important ecological role for free-swimming copepods from coastal areas.

Consequently, macrophytodetritus species assemblages are composed of passive leaf clingers, active infaunal dispersers, active suspended dispersers and free-swimming copepods. All those different dispersion mode of adult copepods seem to confirm their importance to population dispersion. Chandler and Fleeger (1983) concluded in their colonization experiment, that suspended water column dispersion is more important than interstitial infaunal transport. However, the latter should not be neglected. Every species with good swimming capacities and capability of emergence (e.g. Armonies, 1994; Kurdziel and Bell 1992; Thistle and Sedlacek, 2004) colonized or showed a predisposition to migrate to new or provisional and dynamic habitats. Therefore, It would be preferable to refine the provisional classification of Noodt (1971) with a more ecological and mobility-based grouping. Subsequently, in Table 1 the active colonization capabilities of some species were incorporated. Hicks (1986) predicted the epibenthic capabilities of many phytal species, however he

agreed upon Palmer and Gust (1985) ideas of passive erosion being the only pathway to suspend benthic copepods from unvegetated habitats. The presented results prove that active emergence and migration occur from sedimentary habitats, water column habitats and phytal habitats. Therefore it would be desirable to correct the idea that exclusively phytal components actively contribute members to the water column, as the results proved that not only phytal-dwelling copepods possess well-developed swimming abilities.

The formation of the communities during low disturbance conditions seems to distinguish two stages. The initial stage is characterised by colonizer species and subsequently after a few days competitor species arrive on site, impacting the subsequent settlement (Sun and Fleeger 1994; Jacobi and Langevin 1996). Colonization experiments over time (e.g. Chertoprud *et al*., 2005; De Troch *et al*., 2005) generally observed a stage shift after more than four days, the duration of the second incubation experiment. However, an initiation of a stage shift seemed visible in our 96h experiment. *Ectinosoma dentatum* became the most dominant species in the newly colonized macrophytodetritus after 96 h. Hence a colonization-competition trade-off seemed to be present between *S. tenuiremis* and *E. dentatum.* However, in the reference samples *S. tenuiremis* is more abundant than *E. dentatum*. During a seasonal characterization study in the same bay (Mascart *et al.,* 2015b), effects of oxygen gradients were measured for *Diosaccus tenuicornis*, a species from the same Miraciidae family as *S. tenuiremis* . In contrast *E. dentatum* seemed not to be impacted by the oxygen level present in the macrophytodetritus. De Troch (2005b) confirmed the definition of Hicks and Coull (1983) of Ectinosomatidae as “jacks of all habitats”, so called r-strategist. The experiment potentially revealed a sensibility of *S. tenuiremis* towards a possible drop in oxygen gradient induced by the experimental setup after 96 h. This could explain the difference between the control and the reference samples after 96 h. Therefore it can be concluded that the

experiment duration was not long enough to see a stage shift, but the potential effect of drop in oxygen could be present. Nonetheless, it confirms the important role and flexibility of *E. dentatum* within the macrophytodetritus accumulations and the main colonizer role for *S. tenuiremis*. Both, *E. dentatum* and *S. tenuiremis* used the infaunal and suspension pathway for active colonization.

6. Conclusion

In conclusion, free-living harpacticoid copepods actively colonize adjacent defaunated macrophytodetritus and sediment within 24 h. Eco-morphologically different copepods originating from diverse habitats (sediment, phytal and pelagic) were conspicuous dispersers and actively migrated towards defaunated habitats using their species-specific preferred dispersal pathways. Two pathways occur for active colonization: infaunal migration through the boundary layer and migration suspended via the water column. Eco-morphological characteristics can be used to predict the potential preferred habitat of copepods. However, copepods are not obligate residents of their preferred habitat since phytal and sedimentary components actively contribute to the water column. Therefore, copepods are opportunistic and are capable of assembling dynamic communities in highly variable habitats. Macrophytodetritus offers a structurally complex substitute habitat offering shelter and food supply, therefore serving as a hub for all copepods of the surrounding habitats. Due to the high diversity of associated fauna, the macrophytodetritus assemblage has a certain resilience which allows it to cope with and restore swiftly from disturbances. Swimming abilities, structural complexity and dispersion drivers, such as hydrodynamics, habitat complexity and food availability, control the colonization ability of a copepod population.

Picture: School of *Sarpa salpa* in a *P. oceanica* meadow

Chapter 7

General discussion, novel findings and future considerations

The overall aim of this PhD thesis was to investigate the main structuring factors and the functional traits (mainly feeding ecology and colonisation traits) of the meiofauna communities, especially copepods in macrophytodetritus accumulations in a Mediterranean coastal ecosystem. The discussion is structured in three main parts. The first part, dealing with the macrophytodetritus habitat, will discuss the factors influencing it and its composition. The second part will focus on the copepods' diversity within macrophytodetritus in comparison to other habitats and the migration dynamics of the small invertebrates. The third part will tackle the attractiveness of macrophytodetritus as food sources and the trophic ecology of selected copepod species. Finally, future perspectives for research on the role of meiobenthos in detrital ecosystem functioning are formulated in addition to some methodological considerations.

1. Macrophytodetritus as feeding grounds

1.1. Macrophytodetritus on unvegetated sand patches

Seagrass meadows rank among the most productive ecosystems on Earth (Duarte and Chiscano 1999). Seagrasses themselves together with the epiphytes and macroalgae support a gross primary production higher than their respiration rate (Hemminga and Duarte 2000). As a result, seagrass ecosystems are generally regarded as net autotrophic communities (Duarte *et al.,* 2010; Kennedy *et al.,* 2010; Champenois and Borges 2012).

In contrast to the vegetated ecosystem (e.g. seagrasses, saltmarshes, kelps), unvegetated benthic ecosystems are usually net heterotrophic, with an annual respiration and mineralization often exceeding photosynthesis (Hemminga and Duarte 2000). In addition, significantly lower faunal and floral biomass and diversity are generally observed in those unvegetated systems than on vegetated ones (e.g. Connelly 1997; Edgar *et al.*, 1994;

Apostolaki *et al.,* 2010). Hence, heterotrophic unvegetated systems depend on the import of allochtonous organic matter. Moreover, the imported material will strongly modify the habitat function of the unvegetated habitat and presumably other adjacent habitats (Lenanton *et al.,* 1982; Hyndes and Lavery 2005; Hyndes *et al.,* 2014).

Exported detritus from the living meadow thus accumulates on unvegetated sand patches. Such exported leaf litter mixes with drift epilithic macroalgae, uprooted living seagrass shoots with rhizomes, other seagrass litter, seeds, dead macrofauna and fine sediment to form a heterogeneous detritus accumulation (Hyndes *et al.,* 2014). In addition to the heterogeneous nature, some of those elements like leaf litter will become overgrown by sessile (micro & macro) epiphytes, which are mainly composed of erected and crustose algae, diatoms, bacteria and fungi (Lepoint *et al.*, 2006; Fenchel 1970; Gatune *et al.*, 2012).

In *Posidonia oceanica* meadow systems, exported detritus, mainly under the form of shed leaf litter, undergoes a seasonal cyclic pattern. Autumn is the season with the highest export rate of primary production and consequently, the period is characterised by the highest accumulation of detrital material in unvegetated habitats (Fig 3.1) (Cebrian *et al.,* 1997; Mateo and Romero 1997). However, several authors including Champenois & Borges (2012) and Lepoint *et al.*, (2006) and this study argue that in Mediterranean Neptune grass dominated systems, a yearlong input of shed leafs exists. Our results showed an accumulation at every season of the sampled years (Fig 3.1 & 4.1). Nevertheless, temporal fluctuations are present and the main factors affecting the export of detrital material from the seagrass to unvegetated habitats is mainly phenology of leaf decay within the meadow, hydrodynamics and to a lesser extent geomorphology which allow macrophytodetritus to concentrate in certain area (see Hyndes 2014). Consequently, the unvegetated sand patch will not always be subject to the presence of an accumulation (e.g. Fig 2.5, right side), in contrast to the several sampling campaigns where a continuous presence of

macrophytodetritus was recorded. Moreover, the sampling campaigns were random and took place during a very short time interval. Therefore, the chance is high that the sampling campaigns missed the moment unvegetated sand patches were empty. However, Michel (pers. obs.) and Remy (unpubl. data) followed up on a daily basis integrated over several weeks to months the OSCE site and to a lesser extent the PORT site. Both concluded that both sites had a continuous amount of macrophytodetritus except during and just after storm event (Fig 2.5). This is congruent to Champenois & Borges (2012) and Lepoint *et al.*, (2006) stating that a continuous yearlong flow of macrophytodetritus originates from the meadows with peaks at certain moments of the year.

As mentioned, 'storm effects' are short moments in time, like pulses that mixes every non-attached material with the water column (pers. obs). Once suspended, away from the hydrodynamic shade of an obstacle (meadow, rock, etc.) the aggregated mixture will be transported away to a new unvegetated sand patch (submerged or terrestrial). On the way to a new destination, the suspended aggregate, including passively transported organisms will encounter new habitats where exchange of species can happen. To conclude by a metaphor, macrophytodetritus should not only be seen as a "carpet" passively lying on unvegetated sand patches. It also serves, under certain conditions, as a "flying carpet" transporting meiofauna and macrofauna over vast distances.

1.2. Factors influencing macrophytodetritus decay rate

As mentioned before, the exported material will accumulate on the unvegetated patches were it will continue to decay and support a diverse detritivore community. Detritus decay normally follows a decreasing exponential pattern that comprises leaching, decomposition and slow breakdown of refractory phases (Mateo *et al.,* 2006). The rate of the decay will directly be influenced by: (1) the quality of the litter, (2) the physico-

chemical condition inside the accumulations and (3) the composition of the detritivores community (Graca 2001).

The nutritional quality of the seagrass litter is of course related to the prior decomposition rate and the initial recycling or retranslocation before senescence (Pergent *et al.,* 1994; Alcoverro *et al.,* 2001; Perez *et al.,* 2001; Lepoint *et al.,* 2002a). Our results showed temporal and spatial differences in the elemental composition of macrophytodetritus (Table 5.1). Fresh, barely degraded autumn litter displayed a higher carbon content than more degraded (more fragmented, finer and lighter colour) February leaf litter (pers. obs.). This difference can be caused by three factors: (1) age, (2) location and (3) fragmentation. First, the age of the shed leaf will have a different composition due to a change in photosynthetic parameters (Alcoverro *et al.,* 1998). Leaf litter, right after being shed, undergoes a rapid initial decomposition and the older the litter becomes, the more depleted it becomes (Mateo and Romero 1996). Secondly, depending on the location and depth, the seagrass' photosynthetic processes will differ, yielding a different elemental composition of leaves (Lepoint *et al.,* 2003; Vizzini *et al.,* 2003). Thirdly, the more degraded litter endured leaching and fragmentation (biological or physical) making the litter more accessible for heterotrophic microorganisms such as bacteria and fungi that will assimilate the refractory carbon (Rice and Tenore 1981). The presence of those decomposing microorganisms was shown through FA composition of the macrophytodetritus. Bacterial markers such as 15:0 and 18:1ω7 (Nichols *et al.,* 1985) were present in the macrophytodetritus food source (Table 5.5). These fatty acids, also present in epiphytes, are likely due to the bacterial colonization of macrophytodetritus.

Our results established that physico-chemical conditions in the macrophytodetritus accumulation are different from those in the water column and that subsequently litter acts as a barrier between sediment and water (Fig 3.3). The physico-chemical conditions inside the accumulations are dependent on one hand of internal process within the accumulation

(respiration, nutrient efflux from sediment, nutrient recycling from degrading biomass, etc.), and, on the other hand, of the refreshing of the interstitial water by water column mixing. In the absence of a hydrodynamic event slowing down the refreshing of interstitial water, the top layer of the accumulation will present a transition from oxic to hypoxic interstitial litter water. This depletion in oxygen can have an important impact on the present faunal community (Tietjen 1969). Our research showed a species specific impact on the *Diosaccus tenuiremis* copepod species (Ch. 3 § 5.2). In the colonization experiment, during the 96h experimental set up, a potential impact on species composition due to oxygen decrease was present (Ch. 6 § 5.3). Other nutrients concentrations will alter the efficiency of degrading detritivores micro-organisms in regulating nitrification and denitrification, since those nutrients are at the basis of biochemical reactions (Wafar *et al.,* 1997; Pedersen *et al.,* 1999; Holmer and Olsen 2002). Consequently, the seasonality and intensity of hydrodynamics seems to affect directly and indirectly the fragmentation and degradation rates of macrophytodetritus (Wittman *et al.*, 1981).

The detritivore community is made of several functional groups (e.g. decomposers, shredders, bioturbators, etc.). Decomposer micro-organisms (mainly bacteria and fungi) at work on a plant substrate use several strategies to efficiently degrade the solid structure (e.g. penetrating the plant cell wall or diffusing of cellulose digestive enzymes) (Newell 1996). Consequently, since the cellulosic walls are damaged, the content of the cell will be released, resulting in an elemental decrease of the detritus. It can thus be assumed that most of the nitrogen present in the degraded litter leaves originates from degrading micro-organism biomass, making their degrading activity a driving factor in carbon flow from vascular plants (Odum *et al.,* 1979; Peduzzi and Herndl 1991). In addition, with time more nitrogen-containing micro-organisms are present (Fenchel 1970; Holmer and Olsen 2002). The degrading micro-organism community assimilates carbon from other origins than the decaying vascular plant (Raymond and Bauer 2001). As mentioned by Vizzini (2009) and tackled by Holmer and

her team (Holmer *et al.,* 2001; Holmer and Olsen 2002) seagrasses leach and exudate dissolved organic matter (DOM) contributing to an increase in bacteria and microbial loop. Not only living or dead vascular material leaches organic matter, also the associated epiphytes contribute. Higher excretion rates were found for Z. marina and associated epiphytes compared to epiphyte free leaves (Penhale and Smith 1977). The exact leaching quantities vary depending, for instance, on material composition, season and light regime (Barrón *et al.,* 2006; Barrón and Duarte 2009; Apostolaki *et al.,* 2010a). Next to DOM, DOC (dissolved organic carbon) has been recognized as a potentially important component of carbon transfer (Camilleri and Ribi 1986). The DOC (dissolved organic carbon) is mainly originating from the mineralization in the sediment (Dubois *et al.,* 2012). Other minor sources of DOC like leaching and excretion are supporting the microbial food web and consequently the carbon flow (Velimirov 1986; Barron *et al.,* 2014).

Besides micro-organisms, macro-organisms such as invertebrate crustaceans (e.g. amphipod and isopods) correspondingly degrade vascular plant tissues by mechanical breakdown. It can be by physical shredding, cutting it to smaller pieces or by ingesting it, without necessarily assimilating the detritus (e.g. Gallmetzer *et al*., 2005; Michel *et al*., 2014, Sturaro *et al*., 2010). However, assimilation should not be rejected, since some species seem to have developed a unique symbiosis with refractory compound digesting bacteria (Michel 2011). Mechanical breakdown increases fragmentation favouring microorganism colonization. The higher microorganism activity enhances overall decomposition and associated nutrient recycling. Finally, psammivorous megafauna seem to play a role in degrading the macrophytodetritus. These holothurians feed on surface sediments and ingest and assimilate copious amounts of fine detritus (Vizzini and Mazzola 2004; Vizzini 2009). The detritivore fauna will on its turn serve as food source for higher trophic levels and so transfer the detrital seagrass material to higher trophic levels. To conclude, several

factors play an important role in degrading macrophytodetritus. All these factors are interlinked and with time, the detrital matter, product of the seagrass primary production will through several pathways (mainly microbial loop) find its way to higher trophic levels of the seagrass meadow food web.

2. Meiofauna communities in macrophytodetritus

2.1. A continuous presence

Our results showed a ubiquitous presence of meiofauna in macrophytodetrital accumulations. Since crustacean macrofauna are known to be present in macrophytodetritus accumulation (e.g. Gallmetzer *et al*., 2005; Michel *et al*., 2014, Sturaro *et al*., 2010, Lepoint *et al.*, 2000), the presence of their nauplius larvae, juveniles and small-size species is to be expected. The abundant presence of nauplius larvae, especially in some month (Ch. 3 § 4.3) can only indicate the importance as shelter of the macrophytodetritus. However, since nauplius larvae are found in several Crustacean taxa (e.g. Branchiopoda, Cephalocarida, Malacostraca, Decapoda and Copepoda) it is difficult to formulate any conclusion. However, copepods possess six naupliar stages, followed by six copepodite stages (Higgins and Thiel 1988). Copepodites were present in lower abundances, yet on average for more than 3% (Ch. 3 § 4.3) and gravid female copepods were present in all samples (pers. obs.). Consequently, it could be hypothesised that copepods have more than one generation in the dynamic macrophytodetritus habitats.

Nematodes are the second most abundant taxon in macrophytodetritus, which is congruent with Heip *et al.* (1985), stating that nematodes and harpacticoid copepods are generally the most abundant meiofauna. As stated by Hicks (1986) and Heip *et al.* (1985) nematodes will dominate in

fine-grain sediment, while going towards coarse-grain sediment and phytal habitat, harpacticoid copepods will dominate. This was expected since macrophytodetritus, is made of phytal material and the sand present under the accumulations is relatively coarse (> 500µm, Gobert *et al*. 2003). Even though, the presence of nematodes in macrophytodetritus is not surprising (Gwyther 2003). The food availability for those organisms is sufficient to maintain a community and they are omnipresent in all the adjacent habitats (Danovaro and Gambi 2002). To a lesser extent than copepods, nematodes can actively colonize, however, passive dispersion seems the most common dispersal mode (e.g.. Fleeger *et al.*, 1984; Boeckner *et al.* 2009).

2.2. Factors influencing the detrital copepod community

Before starting this discussion, two statistical corrections or updates, depending on how one sees it, should be highlighted. In order to compare the nutrient and oxygen environmental variables in Chapter 3 a 3-way PERMANOVA was used (See Table 3.2). In the results (Ch. 3§ 4.2), the last paragraph described the PERMANOVA and PERMDISP results as being significantly different on many levels making the conclusion less straightforward. Therefore, a more adequate design of PERMANOVA is brought forward: factor site should be seen as a random variable and not as a fixed variable, and thus factor site can be nested in factor position. Since Chapter 3 is already published as such, the new version of table 3.2 can be found in annexe IV. Further, it was suggested to include the BEST-analysis. The BEST algorithm is based on ranked similarities and is used to find the best explanatory environmental variable. The same ranked similarities are used for an ANOSIM or an MDS representation. In Chapter 3 we decided not to use ranked similarities since it gives a skewed and forced two dimensional view of the multivariate space we are working in. Therefore we opted for PERMANOVA and PCO representation. To be congruent we

should have opted for a DISTLM based on the actual similarities. The DISTLM approach actually fits a linear model of the predictor environmental variable to the species data cloud (Anderson *et al.,* 2008). Subsequently, the BEST/BIOENV analysis gives more information on the useful environmental variables to explain the species community, while DISTLM gives a 'number' on the variance of that predictor environmental variable. Since Chapter 3 is already published, the results of the DISTLM model can be found in Addendum III.

Copepods, especially benthic harpacticoids are known to have a seasonal life cycle (Hicks 1979). At higher latitude, reproduction is generally confined to 6 months or less (Moore 1972). However, at lower latitudes breeding occurs through the year (Hall and Bell 1993). Hicks (1979) suggested that phytal harpacticoids will not show a clear seasonal pattern since their food resources are supposed to be unlimited. Nevertheless, Rudnick (1989) documented a clear seasonality with harpacticoid copepods reaching a maximum abundance in the warmer summer months as development and reproduction rates correlate positively with temperature (Heip and Smol 1976; Hicks and Coull 1983). Consequently, meiofauna densities seem to follow a seasonal cycle. Our data confirmed the seasonal relation with high densities of meiofauna and copepods during August and low densities in February (Chapter 3 § 4.3). A continuous presence of benthic copepods was found. The macrophytodetritus accumulations harboured the same density in order of magnitude (10^4-10^5 indiv.m^{-2}, Addendum IV) as *P. oceanica* meadows i.e. the epiphytic canopy community on the living plants (Novak 1982; Mascart *et al.,* 2013). The diversity, in the range of 30 to 50 harpacticoid species, was similar to other phytal ecosystems (Hicks 1977a; Heip *et al.,* 1983; Johnson and Scheibling 1987; Steinarsdóttir *et al.,* 2003; De Troch *et al.,* 2008b). As a result, our study showed fluctuations in density and diversity following a seasonal pattern, strongly related to wind-induced hydrodynamic events (Raffaelli and Mason 1981).

The most stringent environmental condition for benthic copepods is probably oxygen concentrations, since they are usually very sensitive to hypoxia (Wetzel *et al.,* 2001; De Troch *et al.,* 2013). However, species-specific differences in oxygen-tolerance are reported (Grego *et al.,* 2013). Our results suspect, the creation of a potential vertical oxygen during hydrodynamic calm periods. Consequently, the highly mobile copepods will follow this gradient with the most tolerant species closer to the detritus-sediment interface and less tolerant species closer to the detritus-water column interface, will follow. Hence, the density, diversity and composition (oxygen-depletion tolerant vs. intolerant) of fauna inside the macrophytodetritus accumulations will be influenced (Hovel *et al.,* 2002).

Next to the environmental constrains, food availability plays an important role in the distribution of meiobenthic communities (Danovaro *et al.* 1996). In sediments, copepods are closely related to sedimentary phytopigments and carbohydrates, suggesting the role of food availability. Consequently, bottom-up control seems to be predominant for benthic meiofauna and macrofauna (Lee *et al.,* 1977; Decho and Fleeger 1988; Albertelli *et al.,* 1999; Covazzi Harriague *et al.,* 2007).

2.3. A community at the crossing of communities

In addition to the seasonally patterned density and diversity fluctuations, some abundant copepods representing different ecological groups (Noodt 1971; Giere 2009) were present (Chapter 6). Based on the obtained results, we suggest using ecological grouping instead of taxonomic composition to estimate habitat utilization (Sedlacek and Thistle 2006). In this study the classification of Copepoda by Noodt (1971), see Fig. 1.5, based on the variety of evolved ecological forms (Remane 1952) was revised (Table 6.1). Different trends in specialization of eco-morphological characteristics, distinguishing several types of copepod adapted to certain conditions of different habitats (e.g. mesopsammic-living, phytal-living, epibenthic-living

and water-column-living). Subsequently, following the eco-morphological classification, the four types present in the macrophytodetritus show habitat-specific characteristics. Hence, an origin or preferred habitat composed of very specific communities and species compositions could be deduced from eco-morphological characteristics (e.g. the water column, the seagrass canopy and the bare sediment). For this reason, a mesocosm experiment was set up in the habitat connectivity context (Chapter 6) to trace back the exact habitat source pool of the macrophytodetritus colonizers. However, those adjacent habitats are not situated on a scale of a few centimetres from each other, but more on a scale of one to several metres.

Harpacticoid copepods are known to swim actively (Palmer 1988) and the migratory power of harpacticoid copepods is well-established since they display daily vertical migration (Walters 1991; Sanchez-Jerez *et al.,* 1999a), temporary emergence (Armonies 1988; Thistle 2003) and possible dispersal over large distances (Hicks 1977b; Hicks and Coull 1983; Ólafsson *et al.,* 2001). On the other hand at small scales (within a few meters), dispersion is driven by food selectivity and thus local heterogeneous densities occur (Decho and Castenholz 1986; Decho and Fleeger 1988; Sun and Fleeger 1991). We can thus expect the copepods to colonise the macrophytodetritus from their origin habitats either carried passively by a detached senescent leave from the seagrass meadow or from another litter accumulation, either by active migration (Palmer 1988; Ingolfsson 1995). Our mesocosm outcome revealed that active colonization occurs from all three above mentioned origin habitats. This shows the potential resilience of copepod communities, for instance to recover after a physical disturbance (Sun and Fleeger 1991; Fleeger *et al.,* 1995). A second aspect shown here is the attraction of the macrophytodetritus. It has previously been hypothesised that some species have more than one generation in these dynamic and variable accumulations, highlighting the ecological role of macrophytodetritus to the overall seagrass system. Moreover, since copepods originating from adjacent habitats were present

in the macrophytodetritus, it can be concluded that macrophytodetritus, at the crossing of different habitats, acts as a copepod hub.

In order to clarify "hub" (draaischijf, nl/plaque tournante, fr) on a non-scientific way, one must see it as a train station where copepods come from adjacent habitats to the macrophytodetritus "station" as a final destination to stay and feed and find shelter or during transfer to another adjacent habitat.

3. Ecosystem functioning and trophic interactions in the macrophytodetritus habitat

3.1. The role of macrophytodetritus accumulations

As mentioned, accumulated macrophytodetritus offer an alternative, notwithstanding highly dynamic habitat, to organisms present in seagrass meadow ecosystems. Boström and Bondsdorff (2000), stated that structurally complex plant assemblages may trap or attract organisms more efficiently than structurally simple leaf canopies or bare sediments. This is certainly valid in the bare sediments, however *P. oceanica* should not be seen as structurally simple (Borowitzka *et al.,* 2006). The abundant epiphytic growth creates a complex habitat with different available niches. Hence, the enhanced structural complexity created by intricate levels of epiphytes seems to enhance the meiofaunal density and diversity. This is congruent with observations of Weiser (1959), Novak (1982), Hicks and Coull (1983) and this study (Chapter 3&4). Abundances and diversity of meiofauna of marine vegetation are thus positively correlated to habitat complexity. The heterogeneous nature of the macrophytodetritus seemed to create a habitable habitat wider or more complex than any of the surrounding adjacent habitats. Hall and Bell (1988) and Johnson and

Scheibling (1987) demonstrated that dominant motile invertebrates' abundances and diversities were positively correlated with the habitat complexity as measured by the biomass of seagrass epiphytic algae.

Besides the link between habitat complexity and abundances, the choice of a habitat will be mainly determined by what the animal expects from it, for instance (1) shelter, (2) access to mates and (3) food availability (Coull and Wells 1983; Boström and Mattila 1999; Chemello and Milazzo 2002). Since copepods are food for higher trophic levels (Coull 1990), sheltering for their predators will definitely be advantageous (Hicks 1980). The shelter effect will be present, however taking into account the very dynamic role of the accumulations this should be nuanced. Macrophytodetritus accumulations are subject to hydrodynamic flows, being small or big. During low flow macrophytodetritus will play the role of a connection habitat between adjacent habitats, with serious advantages for the migrating organisms in contrast to an open unvegetated sand patch (Atilla *et al.,* 2005). During bigger hydrodynamic events (e.g. storms) most of the material gets resuspended in the water column, as has been observed on the Oscelluccia sand patch during a storm (Fig 2.5). Subsequently, there is no more accumulation and no shelter effect. Concerning the requirement of finding mates, our results showed a constant presence of all life stages of copepods. Consequently, if the accumulations persist long enough, several generations are possible (Coull and Fleeger 1977). Our data demonstrated that copepods directly feed in macrophytodetritus accumulations (but see the next paragraph). Therefore, taking into account trophic specialization and habitat complexity, meiofauna and especially harpacticoid copepods play a significant role in various benthic habitats, including macrophytodetritus accumulations (Coull 1990; Pinckney *et al.,* 2003; Van Gaever *et al.,* 2009).

3.2. Trophic diversity and trophic link of macrophytodetrital copepods

In marine detrital systems, meiofauna are known as being opportunistic and able to utilize a wide variety of food sources and sustain rapid turn-over rate (Warwick 1987). Meiofauna showed increasing densities on decaying mangrove leaves (Gee and Somerfield 1997; Torres-Pratts and Schizas 2007) where it mainly fed on biofilm (Faust and Gulledge 1996; Gwyther 2003). Litter studies highlighted the ecological and trophic importance of plant derived litter for near shore unvegetated ecosystems and the role of motile macro- and meiofauna in the decomposition and shredding of it (Vetter 1995; Mancinelli and Rossi 2002; Hyndes and Lavery 2005; Lillebo *et al.*, 2007). Although, these meiofauna are not supposed to shred the leaf litter since they are too small. They tend to behave more like grazer-scrapers which utilise the present biofilm (Danovaro 1996; Lemke *et al.*, 2007; Mascart *et al.*, 2013). These are primary consumers, secondary consumers or detritivores feeders and consequently play a key role in the transfer of organic matter in the food chain.

Harpacticoid copepods are known to feed on a variety of food sources (Hicks and Coull 1983), ranging from microbes (Carman and Thistle 1985) and protozoa (Klein Breteler *et al.,* 1999) to detrital algae (Lemke *et al.,* 2007). The detrital pool appeared to be a potential food source that is often overlooked, but is not less important (Mateo and Romero 1997; Bouillon *et al.,* 2004b; Lepoint *et al.,* 2006; Vizzini 2009). The macrophytodetritus, by its three-dimensional configuration, and chemical features (DOM) could be a better substrate for detritivore microorganisms. Since more degraded material may be more accessible or of greater nutritional value than fresh material for consumers, subsequently, the decaying vegetal material and its attached epiphytes are seen as an important trophic subsidy for food webs (Dethier *et al.,* 2014).

The conceptual model, presented in Fig. 7.1, attempts to incorporate the potential trophic pathways and energy flow inside a macrophytodetritus accumulation. Based on the trophic biomarker analyses (Chapter 5), we concluded that the general copepod community predominantly fed on epiphytes (being mainly fungi, bacteria, and diatoms) associated to the surface of macrophytodetritus leaves. In addition different ecological groups of copepods use (or don't use) each compartment of the potential food sources differently (Fig 7.2). As such, macrophytodetritus sustains complex food webs in coastal ecosystems (Hyndes *et al.,* 2014).

Starting at the bottom of the Fig 7.1, macrophytodetritus can be assimilated by macrofauna. Several species of amphipods are adapted (gut flora) to digest the highly refractory material (Michel *et al*., 2014). Copepods showed through intermediate of the mixing model to assimilate macrophytodetritus. However, long chained SUFAs (26:0, 28:00) were missing from their diet. Those are typical SUFA's for higher plants (Kharlamenko *et al.*, 2004). The second argument is that the signature of epiphytes ran in the model might have been too depleted in ^{13}C. Consequently, the model overestimated the proportion of macrophytodetritus in the diet of copepod consumers. A third argument to minimize the real proportion of macrophytodetritus in copepod's diet is the copepods themselves. The copepod mandibular system is not developed to break long lignin and cellulose walls (Boxshall and Hasley, 2004), to the contrary of macrofauna. Therefore, it can be hypothesized that copepods do not or at least on a low scale assimilate directly macrophytodetritus.

During breakdown, macrophytodetritus and macroepiphytes will leach DOM that will be taken up by detritivore microorganisms (e.g. bacteria) and microepiphytes (e.g. diatoms). Both form the main food source of harpacticoid copepods (Hicks and Coull, 1983) and these will form the basis for the indirect uptake of macrophytodetritus material by meiofauna.

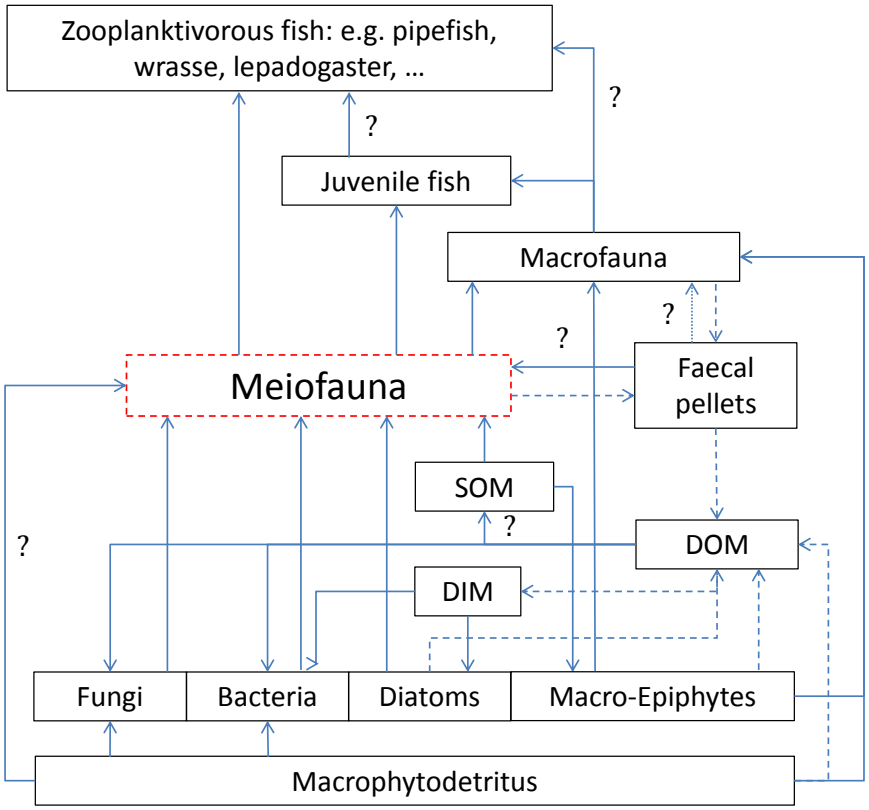


Fig 7.1 *Summary of major trophic linkages between meiofauna and food sources in macrophytodetritus accumulations. Arrows represent interactions: ingestion/assimilation (full line) or leaching/excreting/degrading (dotted line). SOM= Suspended organic matter, DOM= Dissolved organic matter, DIM= Dissolved inorganic matter.*

The meiofauna, represented in Fig 7.1 is detailed in Fig 7.2. Nematodes feed on a variety of food sources. Tenore (1983) showed nematode preferences for bacteria, while Montagna (1984) showed nematode preferences for diatoms. Nematodes feeding preferences are however easier to predict than copepods, since nematodes are divided in functional feeding modes (Heip *et al.* 1985). Since no differentiation is done is this study, no specific conclusion can be made except that if they would be analysed per feeding

guild clear resource partitioning should be visible. The four groups of copepods (Fig 5.2B) were investigated in this study and the four respective representative species showed through the intermediate of biomarkers (Chapter 5) slightly different feeding preferences. The water-column-type calanoid *Clausocalanus arcuicornis* primarily feeds on SOM in the water column but migrates to the macrophytodetritus accumulations to secondarily feed on the epiphytic biofilm, mainly during spring. The epibenthic-swimmer-type *Tisbe furcata* keeps a constant composition throughout the year and therefore slightly changes the proportion of each food source according to the moment of the year. The phytal-type *Diosaccus tenuicornis* has a fixed diet, mainly epiphyte based. Its composition will therefore fluctuate according to the availability of the food source. The mesopsammic-type *Ectinosoma dentatum* seems to change his diet drastically depending on the accessibility of food sources.

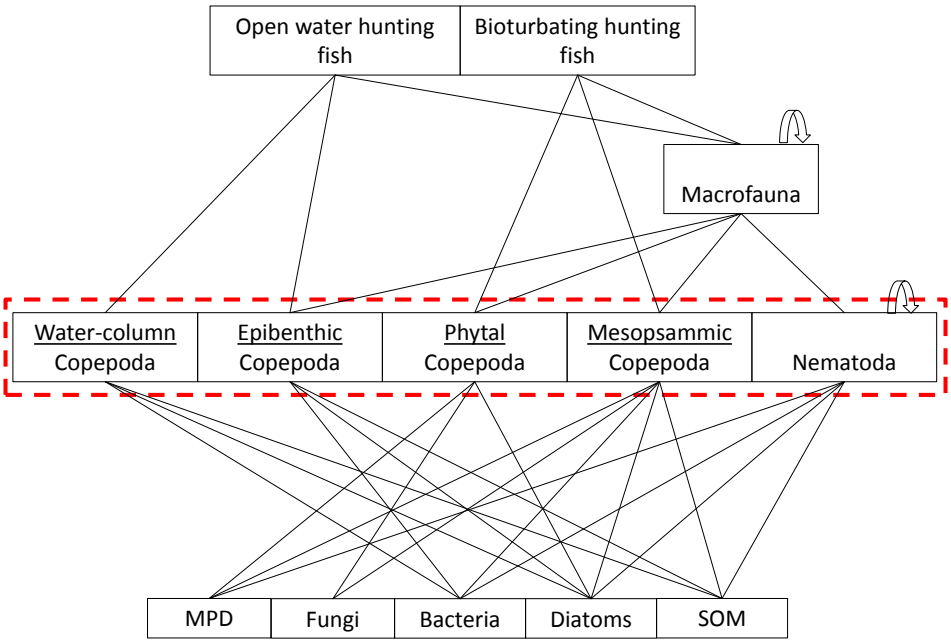


Fig 7.2 *Summary of major trophic linkages zoomed in on meiofauna of Fig 7.1 revealing the four copepod eco-morphological types and nematodes. Lines represent interactions. Arrow represents cannibalism. MPD= macrophytodetritus, SOM= Suspended organic matter.*

Several factors could be at the base of these differences: (1) morphological differences, (2) dietary requirements and (3) intraspecific resource partitioning. Each eco-morphological group possesses its morphological characteristics (see Chapter 2). Not per se in buccal organs, however the calanoid copepod compared to harpacticoid copepods will be different, since the first one is more a filter feeder. However, epiphytes are found back in the diet of the pelagic copepod, so even with slight morphologically different mouth parts it did not deter its ability to graze on micro-epiphytes. The morphological difference will out in their dispersing capacities (Fig 5.2B, Chapter 6) (e.g. Bell *et al.*. 1987).

The phytal and epibenthic copepod have similar body morphology and the highest niche overlap (Chapter 5), although the epibenthic type is a better swimmer. By having a higher swimming capacity it could allow it to travel easier and to be more selective for food sources. Purely phytal copepods on the contrary swim less well and are thus more constrained to graze upon the food it encounters (Chapter 6). This could explain its narrower niche, since it stayed in one specific preferred place.

The mesopsammic species, revealed a very fluctuating diet. Although it is known to exhibit swimming capabilities its isotopic composition was more depleted in February and August, displaying a higher proportion of SOM (Chapter 5). During those two months, the lowest accumulations of macrophytodetritus were recorded. It can be hypothesised that the macrophytodetritus accumulation was reduced, concentrating the copepod community (Dimech 2006). The mesopsammic copepod got outcompeted by phytal harpacticoids and as a result it shifted its diet to sinking SOM.

The water-column calanoid with well-developed swimming abilities primarily fed on SOM in the water-column. However, tracers of epiphytic diet were found back, revealing the opportunistic character of this copepod that will into the macrophytodetritus accumulation to feed on more nutritional diatoms or bacteria (Brugnano *et al.,* 2012).

In general concerning the copepod assemblage, small differences among species and small temporal variations in carbon isotopic compositions suggested selective feeding by copepod species or dietary shifts among similar food sources. Selective feeding by harpacticoids on different food sources was shown here (Chapter 5), confirming the fine-scaled resource partitioning of copepods illustrated by e.g. De Troch *et al.* (2006, 2012b), Arroyo *et al.* (2007) and Rieper (1982).

Meiofauna are also preyed upon. In Fig 7.2, a difference is made between predating fish techniques. Fishes like wrasses will target a copepod resting on a surface, while pipefishes and lepadogaster will wait until a copepod swims by, to create a draft with the movement of the proboscis. Consequently, both techniques target different copepods. The first technique will target crawling copepods while the second will target swimming copepods. Macrofauna, (e.g. amphipods, and decapods) will mainly target copepods on the surface of the substrate to avoid being visible for their predators

Finally, as mentioned, harpacticoid copepods can be seen as scraper-grazers. However it seems that the majority of the harpacticoid copepods belong to the two main eco-morphological groups: mesopsammic and phytal (Table I.1, Addendum I). The interactions between copepods within one eco-morphological group could be double. On the one hand it could be negative, driven by for example competition for food (Fig 7.2) or habitat. On the other hand, it could be positive as an increasing in diversity reduces the top-down control (Duffy *et al.,* 2001). The redundancy between species within one eco-morphological group could explain the high diversity within

macrophytodetritus copepod communities.

4. General conclusion

The general objective of this PhD thesis was to investigate the importance of meiofauna, more specifically copepods' species diversity, dynamics and trophic ecology in macrophytodetritus accumulations. This was achieved by means of a field study on the communities' seasonal variation on species-specific diversity, density and variation in the diet of different ecological groups of copepods demonstrating their link with the primary production. A field experiment provided insight in the colonization mode, source pool habitat and attractiveness of the macrophytodetritus.

In the introduction, five specific objectives were put forward:

I. Identifying the factors controlling the dynamic and variability of macrophytodetritus accumulation at different spatio-temporal scales.

Variability in composition of macrophytodetritus accumulations are influenced by the origin of the heterogeneous material composing the macrophytodetritus, for instance the origin of the shed leaf (depth, age and moment of shed). Local hydrodynamism, from small waves and currents to heavy storms influence the compaction and the persistence time of the accumulation.

II. Characterizing the associated meiofauna taxa and copepods species communities at different spatio-temporal scales, and unravelling the colonization potential of the copepods and their origin.

The meiofauna community will primarily be attracted to the accumulation for its shelter, food and nursing capacities. Like the accumulation it will firstly be subject to hydrodynamic forcing and secondly niche partitioning will regulate the community over time. The community will differ spatially according to the local physico-chemical characteristics and inter-specific competition for niche space. A colonizer-competitor trade-off is expected and every adjacent habitat can be a source pool for colonizers.

III. Assessing the trophic ecology of the copepod communities present in the macrophytodetritus at the specific level and eco-morphological level and placing them in a macrophytodetritus food web conceptual model.

Four different ecological groups were discussed and each one reveals a specific diet ranging from mainly SOM for water-column-type copepods to almost exclusively diatom based phytal-types. Resource partitioning has thus a major role in moderating intra-specific competition. The fate of macrophytodetritus carbon is multiple. Nonetheless a main pathway through bacteria and diatoms to copepods to higher trophic levels is present. This confirms the importance of the detrital pool for coastal ecosystems.

5. Novel findings

Throughout this PhD study, several novel findings were put forward. Amongst others:

- It is the first study to our knowledge that inventories copepod communities in Corsica on a spatio-temporal scale (Chapter 3 & 4). So far, only one short-term study was conducted on *Cystoseira* at the Punta Revellata (Heip *et al.*, 1983).
- It is to our knowledge the first study to characterise copepod communities in *P. oceanica* meadow macrophytodetritus accumulations at the species level.
- A new mesocosm design allowed to conclude that free-living copepods actively colonize new adjacent habitats within 24 h. Colonizer species originate from different habitats using a species-specific dispersion pathway.
- This PhD research provides the first overview of colonization abilities of harpacticoid copepods (Table 6.1), as reported before for nematodes (Bongers 1990).
- The ecological classification of harpacticoid copepods based on the variety of evolved morphological forms (Noodt 1971) was revised by adding information on trophic ecology. In contrast to Noodt (1971) using genus-level information, species-specific information was used in this PhD research (Table I.1).

6. Future Considerations

Based on the outcome of this PhD research, some recommendations for further research are made and new research questions are launched.

- Trophic niche ecology has been proven a useful tool. Including the trophic data in a larger food web of the macrophytodetritus accumulations is the next step forward. Incorporating different levels, for instance macrofauna detritivores and planktivorous fishes as well as more food sources would greatly enhance our knowledge on the complex food web at display in this dynamic environment.
- One of the limiting factors of the trophic model used is the food sources. Other potential food sources like e.g. microphytobenthos should be added to the model to refine the food source contributions.
- Very few studies on macrophytodetritus accumulations are done in marine coastal ecosystems. With this study, information on the *P. oceanica* detrital pathways is gathered. However extrapolating these results is not so evident. Therefore, different seagrass species, yielding different types of detritus should be investigated in view of the blue carbon solution.
- Copepods are known to be very sensitive to oxygen levels. Physico-chemical characteristics displayed here were all measured in the upper 10 cm layer due to technical constraints. No measurements were performed in the lower layers. It can be expected that the copepod community is different at the surface in comparison to the bottom of the accumulation. Copepods will presumably migrate according to the vertical oxygen profile and generate a trade-off between more and less tolerant species. Hence, copepod sampling and oxygen concentration measurements should be performed at different depths of the accumulation.

- Copepods were omnipresent (~>50%), but so where nematodes (~20%). Very little is known on their role in seagrass macrophytodetritus. Further research should be done in order to come forward with stronger and more complete information on the role of meiofauna in macrophytodetritus food webs.
- Distance of copepod dispersion is mostly defined as 'within a few metres'. Flow laboratory experiments could be useful to measure the migration radius of colonizer species in the frame of seagrass recovery.
- Moreover, maze feeding experiments with colonizing copepods using, one side natural macrophytodetritus, and on the other side, clean macrophytodetritus cleared from associated food sources (e.g. epiphytic algae) could give an insight if the copepods are actively attracted towards macrophytodetritus or to the associated food source.
- Macrophytodetritus accumulations and their associated communities can be transported to deeper abyssal plains during winter storms. Later onwards, these accumulations can come back to more shallow areas. What happens to the "coastal" communities present inside the macrophytodetritus at depth? Do they all die and a "sterile" macrophytodetritus comes back or are they also adapted to survive in his environment?
- Subsequently, is this macrophytodetritus transport the elusive link between some of the coastal and deep-sea environment shared species?

Picture: Alga beach covered in macrophytodetritus “wrack”

Addendum I

Alphabetic list of Copepoda species encountered in the macrophytodetritus habitat per sampling site and encountered month throughout the PhD time frame. A tentative eco-morphological classification has been added in the first column (Table I.1).

Morph-Eco. Type	Species	PORT				OSCE				BANANE
		Feb	May	Aug	Oct	Feb	May	Aug	Oct	Oct
P	*Alteutha depressa*					X	X			X
P	*Ambunguipes rufocincta*		X	X			X			X
P	*Ameira longipes*	X	X	X	X	X	X	X		X
P	*Ameiropsis nobilis*									X
M	*Amphiascoides debilis*	X	X	X	X	X	X	X		X
M	*Amphiascus minutus*		X	X	X		X	X		X
M	*Asellopsis duboscqui*									X
M	*Canuella furcigera*	X				X				
W	*Clausocalanus arcuicornis*	X	X	X	X	X	X	X		X
M	*Cletodes limicola*	X		X			X	X		X
Pa	*Cribropontius normani*	X	X			X	X		X	
W	Cyclopinidae spp.	X	X	X	X	X	X	X	X	X
M	*Cylindropsyllus laevis*								X	
P	*Dactylopodella flava*		X	X			X	X		X
P	*Dactylopusia tisboides*	X	X	X		X	X	X		X
M	*Delavalia normani*									X
P	*Diagoniceps laevis*		X		X		X		X	X
P	*Diarthrodes minutus*	X	X			X	X	X		X
P	*Diosaccus tenuicornis*	X	X	X	X	X	X	X	X	X
M	*Ectinosoma dentatum*	X	X	X	X	X	X	X	X	X
P	*Esola longicauda*			X			X	X		X
W	*Euterpina acutifrons*			X			X	X		
P	*Harpacticus littoralis*		X	X	X	X	X	X	X	X
E	*Idyella exigua*	X		X	X		X		X	
M	*Laophonte cornuta*	X	X	X			X	X		X
M	*Laophonte elongata elongata*									X
M	*Laophontina posidoniae*									X
P	*Laophontodes bicornis*		X	X	X	X	X	X		X
M	*Laophontodes typicus*			X		X		X		

Continuation of Table I.1

M	*Leptastacus laticaudatus*									X
M	*Leptopontia curvicauda*									X
P	*Longipedia minor*		X	X			X	X		X
W	*Lucicutia magna*									X
P	*Metis ignea*		X						X	X
W	*Microsetella norvegica*	X			X		X		X	X
W	Oithonidae spp.		X				X			X
P	*Paradactylopodia brevicornis*	X	X	X		X	X	X		X
P	*Paralaophonte brevirostris*		X	X	X	X	X	X		X
P	*Parategastes sphaericus*						X	X		X
P	*Parathalestris harpactoides*		X			X	X			
M	*Phyllopodopsyllus bradyi*				X		X	X		X
P	*Porcellidium fimbriatum*		X				X	X		X
P	*Porcellidium ovatum*	X	X	X		X	X	X	X	X
M	*Probosciphontodes stellata*									X
M	*Rhizothrix curvatum*									X
P	*Rhynchothalestris helgolandica*		X	X	X		X			X
P	*Sacodiscus littoralis*		X				X			
M	*Sarsamphiascus tenuiremis*		X	X	X		X	X		X
P	*Syngastes cornalinus*									X
P	*Tegastes areolatus*		X	X	X		X	X	X	
P	*Tegastes calcaratus*									X
P	*Tegastes falcatus*	X	X			X	X	X		
P	*Tegastes satyrus*		X	X		X	X	X		X
M	*Tetragoniceps scotti*									X
P	*Thalestris rufoviolascens*		X				X			
E	*Tisbe elegantula*		X		X		X		X	X
E	*Tisbe ensifer*	X	X	X	X	X	X	X	X	X
E	*Tisbe furcata*	X	X	X	X	X	X	X	X	X
M	*Wellsopsyllus (Scott.) robertsoni*									X
M	*Wellsopsyllus (Inter.) intermedius*									X
P	*Xouthous laticaudatus*		X			X	X			X

Table I.1 *Alphabetic species list of all encountered copepod species per site and month. X = presence. Blank cells = absence. The tentative eco-morphological types presented in the first column are E = Epibenthic-types, epibenthic-swimmers; M = Mesopsammic-types, primarily sediment living; P = Phytal-types, clinging to phytal structures; Pa = Parasitic-types, mainly parasitic on vertebrate fish and W = Water-column-types, pelagic free-swimmers.*

Addendum II

Classification based on identification keys of Lang (1948) and Boxshall and Halsey (2004) to species level of all encountered Copepoda during this PhD research independently of the location, time and habitat. All species were checked by the World register of Marine species (WORMS) database (Boxshall *et al.,* 2015).

PHYLUM Arthropoda
SUBPHYLUM Crustacea
SUBCLASS Copepoda
INFRACLASS Neocopepoda Huys & Boxshall, 1991

Superorder Podoplea Giesbrecht, 1882
Order Harpacticoida Sars, 1903

Family Ameiridae Boeck, 1865		
	Ameira longipes	Boeck, 1865
	Ameiropsis nobilis	Sars G.O., 1911
Family Ancorabolidae Sars G.O., 1909		
	Laophontodes bicornis	Scott A., 1896
	Laophontodes typicus	Scott T., 1894
	Probosciphontodes stellata	Fiers, 1988
Family Canuelidae Lang, 1944		
	Canuella furcigera	Sars G.O., 1903
Family Cletodidae Scott T., 1904		
	Cletodes limicola	Brady, 1872
Family Cylindropsyliidae Sars G.O., 1909		
	Cylindropsyllus laevis	Brady, 1880
Family Dactylopusiidae Lang, 1936		
	Dactylopusia tisboides	Claus, 1863
	Diarthrodes minutus	Claus, 1863
	Paradactylopodia brevicornis	Claus, 1866
Family Ectinosomatidae Sars G.O., 1903		
	Ectinosoma dentatum	Steuer, 1940
	Arenosetella tenuissima	Klie, 1929
	Pseudobradya hirsuta	Scott T. & A., 1896
	Microsetella norvegica	Boeck, 1865
Family Euterpinidae Brian 1921		

Euterpina acutifrons Dana, 1847
Family Hamondiidae Huys, 1990
Ambunguipes rufocincta Norman, 1880
Family Harpacticiidae Dana, 1846
Harpacticus littoralis Sars G.O., 1910
Family Laophontidae Scott T., 1904
Asellopsis duboscqui Monard, 1926
Esola longicauda Edwards, 1891
Laophonte cornuta Philippi, 1840
Laophonte elongata elongata Boeck, 1873
Laophontina posidoniae Fiers, 1986
Paralaophonte brevirostris Claus, 1863
Family Leptastacidae Lang, 1948
Leptastacus laticaudatus Nicholls, 1935
Family Leptopontiidae Lang, 1948
Leptopontia curvicauda Scott, 1902
Family Longipediidae Boeck, 1865
Longipedia minor Scott T. & A., 1893
Family Metidae Boeck, 1873
Metis ignea Philippi, 1843
Family Miraciidae Dana, 1846
Amphiascoides debilis Giesbrecht, 1881
Diosaccus tenuicornis Claus, 1863
Amphiascus minutus Claus, 1863
Sarsamphiascus tenuiremis Brady, 1880
Delavalia normani Scott T., 1905
Family Rhizotrichidae Por, 1986
Rhizothrix curvatum Brady, 1880
Family Paramesochridae Lang, 1944
Wellsopsyllus (Scott.) robertsoni Scott T. & A., 1895
Wellsopsyllus (Inter.) intermedius Scott T. & A., 1895
Family Peltiidae Claus, 1860
Alteutha depressa Claus, 1863
Family Porcellidiidae Boeck, 1865
Porcellidium ovatum Haller, 1879
Porcellidium fimbriatum Claus, 1863
Family Pseudotachidiidae Lang, 1936
Dactylopodella flava Claus, 1866
Xouthous laticaudatus Thompson I.C., 1903
Family Tegastidae Sars G.O., 1904

Parategastes sphaericus Claus, 1863
Syngastes cornalinus Monard, 1924
Tegastes areolatus Monard, 1935
Tegastes calcaratus Sars G.O., 1910
Tegastes falcatus Norman, 1869
Tegastes satyrus Claus, 1860

Family Tetragonicepsidae Lang, 1944
Diagoniceps laevis Willey, 1930
Phyllopodopsyllus bradyi Scott T., 1892
Tetragoniceps scotti Sars G.O., 1911

Family Thalestridae Sars G.O., 1905
Parathalestris harpactoides Claus, 1863
Rhynchothalestris helgolandica Claus, 1863
Thalestris rufoviolascens Claus, 1866

Family Tisbidae Stebbing, 1910
Idyella exigua Sars G.O., 1905
Tisbe elegantula Sars G.O., 1905
Tisbe ensifer Fischer, 1860
Tisbe furcata Baird, 1837
Sacodiscus littoralis Sars G.O., 1904

Order Cyclopoida Burmeister, 1835

Family Cyclopinidae Sars G.O. 1913

Family Oithonidae Dana, 1853
Oithona nana Giesbrecht, 1893
Oithona similis Claus, 1866

Order Siphonostomatoida Thorell, 1859

Family Artotrogidae Brady, 1880
Cribropontius normani Brady, 1876

Superorder Gymnoplea Giesbrecht, 1882
Order Calanoida Sars, 1903

Family Clausocalanidae Giesbrecht, 1893
Clausocalanus arcuicornis Dana, 1849

Family Lucicutiidae Sars G.O. 1902
Lucicutia magna Wolfenden, 1903

Family Paracalanidae Giesbrecht, 1893
Paracalanus parvus Claus, 1863

Addendum III

III.1 Alternative 3-way PERMANOVA output of the oxygen and nutrient concentrations with random factor Site nested in Position

Factors and interaction	NH_4	NO_x	PO_4	O_2
Month (Mo)	$F'_{(3,160)}$=0.99 P <0.408	$F'_{(3,160)}$=7.1 P= 0.006 *	$F'_{(3,160)}$=2.1 P= 0.167	$F'_{(3,120)}$=16.6 P <0.001 *
Position (Po)	$F'_{(1,160)}$=9.2 P <0.011 *	$F'_{(1,160)}$=0.6 P= 0.667	$F'_{(1,160)}$=4.9 P <0.031 *	$F'_{(1,120)}$=17.4 P <0.001 *
Nested Site (Si [Po])	$F'_{(3,160)}$=63.8 P= 0.001 *	$F'_{(3,160)}$=5.4 P< 0.01 *	$F'_{(3,160)}$=117.9 P <0.001 *	$F'_{(2,120)}$=312.3 P <0.001 *
Mo x Po	$F'_{(3,160)}$=1.1 P <0.421	$F'_{(3,160)}$=0.6 P= 0.760 *	$F'_{(3,160)}$=2.4 P <0.065	$F'_{(3,120)}$=15.6 P <0.001 *
Mo x Si [Po]	$F'_{(9,160)}$=84.3 P= 0.001 *	$F'_{(9,160)}$=5.4 P <0.001 *	$F'_{(9,160)}$=22.5 P <0.001 *	$F'_{(6,120)}$=2.3 P= 0.038

III.2 Results of the extra DISTLM analysis in parallel to the BEST analysis of Chapter 3

Environmental predictor variables responsible for the variation of the meiofauna community:

Detritus DM: responsible for 24.3% of the variation (P-value = 0.001)

Epiphyte DM: responsible for 13.0% of the variation (P-value = 0.001)

Macroalgae DM: responsible for 9.4 % of the variation (P-value = 0.043)

Environmental predictor variables responsible for the variation of the Copepoda community:

Detritus DM: responsible for 22.7% of the variation (*P*-value = 0.001)

Wind velocity: responsible for 21.5% of the variation (*P*-value = 0.001)

0_2 concentration: responsible for 9.0 % of the variation (*P*-value = 0.043)

Variables presenting no significant relation with the multivariate copepod species composition: PO_4 and NO_x concentrations (*P*-values > 0.064)

Addendum IV

Part of the MSc thesis:

Mascart T. (2010) The role of meiofauna in the energy transfer in a Mediterranean seagrass bed (Calvi, Corsica), MSc dissertation, Ghent University, Ghent.

Published as:

Mascart T., Lepoint G., De Troch M. (2013). Meiofauna and harpacticoid copepods in different habitats of a Mediterranean seagrass meadow, *Journal of Marine Biological association of the UK*, 93 (6), 1557-1566

1. Abstract

This study investigated whether associated meiobenthic communities, especially harpacticoid copepods differed, amongst habitats. Five pre-defined habitats within and next to the *Posidonia oceanica* seagrass meadow were sampled: living seagrass canopy leaves (LL), small (SMF) and large (LMF) macrophytodetritus fragments accumulations and sand, bare (BS) and covered (CS). The highest meiofauna abundances were recorded in the BS for the core sampled habitats (BS, CS, SMF and LMF) and in the LMF for seagrass material habitats (SMF, LMF and LL). Harpacticoid copepods were the most abundant taxon in all habitats. The assemblage composition at copepod family level showed two distinct habitats clusters: a leaf (LMF and LL) and a sediment cluster (BS, CS and SMF). Subsequently, stable isotope analyses were conducted to analyse the relationship between copepods and their potential food sources in seagrass material habitats. Based on $\delta^{13}C$ isotopic analyses and SIAR mixing model,

harpacticoid copepods relied for 70% on epiphytes and for 30% on *P. oceanica* leaf material in the LMF and LL habitats.

2. Keywords

Meiofauna, harpacticoid copepods, seagrass, detritus, *Posidonia oceanica*, stable isotopes

3. Introduction

The endemic *Posidonia oceanica* (L.) Delile, the dominant seagrass in the Mediterranean Sea, is a long-living organism displaying considerable autumnal leaf fall (Pergent *et al.*, 1997). The macrophytodetritus is scattered by currents and waves, and accumulates *in situ* or is exported to adjacent habitats where it degrades and often becomes predominant (Mateo *et al.,* 2003; Cardona *et al.,* 2007). Accumulation of macrophytodetritus on sand patches adjacent to the seagrass meadow can be substantial, i.e. several hundred cubic meters, especially in relative enclosed systems (Cebrian and Duarte 2001). These patches are ephemeral environments which remain for a few days to several months depending on the degradation rate and hydrodynamics of the area (Mateo and Romero 1997). The accumulated materials are heterogeneous, mainly composed of phytodetritus (variable in size and degradation status), but also of living seagrass shoots and macroalgae. Macrophytodetritus nutritional quality is low due to its high lignocellulose content and its nutritional depletion by N-resorption and recycling before abscission (Lepoint *et al.,* 2002a). Nevertheless, macrophytodetritus is typically colonized by epiphytic organisms, i.e. all organisms associated to phytal habitats (leaves) as e.g. heterotrophic microbial communities (bacteria and fungi), autotrophes (e.g. epiphytic diatoms), protists, meiofauna and macrofauna (Danovaro 1996; Gallmetzer *et al.,* 2005). The latter two referring to metazoans

passing through a 1 mm sieve retained on a 38 μm screen and retained on a 1 mm sieve, respectively. A possible reason for the high colonization is that macrophytodetritus create the conditions for the development of a high structural diversity of associated communities. This condition is generated on one hand by physical fragmentation, due to hydrodynamism or biological fragmentation through foraging behaviour. On the other hand by the higher nutritional quality of the epiphytic organisms compared to macrophytodetritus and also the accessibility of those epiphytic organisms that serve as food sources (hereafter referred to as epiphytes) for associated consumers. These consumers are likely to be crucial for the degradation and transport of organic matter to higher trophic levels. For example, macrofauna (juvenile fish prey) is known to ingest detritus with their associated epiphytes and microbial communities (Romero *et al.,* 1992; Vizzini *et al.,* 2002; Mateo *et al.,* 2003; Lepoint *et al.,* 2006; Sturaro *et al.,* 2010). As such, seagrass ecosystems hold a significant fraction of autotrophic biomass (Duarte *et al.*, 2005) that is passed indirectly to higher trophic levels. It can thus be assumed that these ecosystems are macrophytodetritus based (Romero *et al.,* 1992; Mateo and Romero 1997; Pergent *et al.,* 1997).

Little is known on the exact role of meiofauna in the macrophytodetrital accumulations. *P. oceanica* beds are hotspots for meiofaunal production ranging between 7.5 and 13.2 g C m^{-2} yr^{-1} (Danovaro *et al.*, 2002). These values are comparable to the ones reported for seagrass systems in general, but are higher than the meiofaunal production in the Atlantic, the North Sea or the Baltic Sea. Meiofauna organisms occur in high densities, have a high turnover rate and most probably spend their entire lifecycle on or around the same substrate (Giere 2009). They are known to feed on a wide variety of food sources, including epiphytic biofilm (Hicks and Coull 1983; Caramujo *et al.,* 2008; De Troch *et al.,* 2008a; 2009).

Meiofaunal studies mainly focus on patterns of occurrence in sediment

habitats (Danovaro *et al.*, 2000; Mirto *et al.*, 2010) or in the epiphytal canopy environment (Hall and Bell 1993). Studies combining both habitats are very limited (Bell *et al.,* 1984; De Troch *et al.,* 2001b) and hardly focus on the third non-negligible habitat in this system, namely the macrophytodetritus accumulation on sand patches (Dimech *et al.*, 2006). The present study aimed to characterise the meiofaunal community and diversity in different habitat types, namely seagrass canopy, adjacent sand and macrophytodetritus accumulations. In addition to the structural diversity of meiofauna, a more functional approach concentrating on harpacticoid copepods is included. Hence, natural stable isotope compositions ($\delta^{13}C$) are used to trace food sources in consumers (Fry *et al.*, 1987; Lepoint *et al.*, 2000; Vizzini *et al.*, 2002). Potential food sources and harpacticoid copepods from the different habitats were analysed to infer their diet and to study what are the main food sources for harpacticoid copepods at the basis of the food web in the seagrass ecosystem. As the trophic position of copepods in the sediments has been already documented (Carlier *et al.*, 2007; De Troch *et al.*, 2007), the present research will clarify which part of the seagrass ecosystem is consumed by harpacticoid copepods, i.e. the macrophytodetrital matter, the epiphytic biofilm or the living leaves.

4. Materials and methods

4.1. Sampling site and strategy

Samples were collected in the Revellata Bay (Gulf of Calvi, Corsica, northwest Mediterranean) at the Punta Oscellucia site (42°35'N, 8°43'E) (Figure 1). The sampling site was located close to a sandy patch and in a *P. oceanica* meadow facing the Oscellucia peninsula (Punta Oscellucia). At the study site, *P. oceanica* seagrass meadows cover about 50% of the total bay

surface down to a depth of 38 m (Bay 1984) and are ranked among the most productive *P. oceanica* beds in the northwest Mediterranean (Pergent-Martini *et al.*, 1994). Samples were taken in August 2009, which correlates with the start of the autumnal leaf fall cycle. Sampling was carried out at a depth of 10 m by scuba divers during day time. The salinity, around 38, and the water temperature, around 26°C, remained stable during the entire sampling campaign.

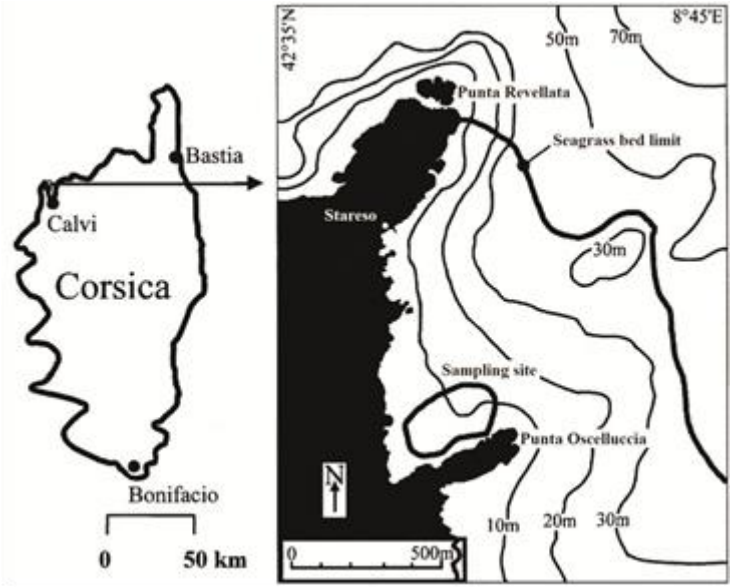


Fig. 1. *Location of the Revellata Bay within the sampling site and the P. oceanica meadow isobaths with lower depth distribution limit. Adapted from Gobert et al. (2003)*

Five potential habitats for meiofauna were collected in triplicate. The meadow leaves stratum, living leaves of the *P. oceanica* canopy (abbreviation: LL) represented a first potential habitat. Two different types of *P. oceanica* macrophytodetritus accumulated on adjacent sand patches were included as other habitat options: large macrophytodetritus fragments (abbreviation: LMF) comprising leaf lengths from 0 to 30 cm of *P. oceanica* and small macrophytodetritus fragments (abbreviation: SMF)

comprising leaf length restricted from 0 to 3 cm of *P. oceanica*. Finally, two sand habitats of adjacent sand patches were sampled: bare sand (abbreviation: BS) and sand covered by macrophytodetrital material of *P. oceanica* (abbreviation: CS).

Living *P. oceanica* shoots representing the living leaves stratum (LL) were cut off at the sediment-water interface and put in 2 L plastic jars. The large macrophytodetritus fragments (LMF) and small macrophytodetritus fragments (SMF) were sampled using a 25 cm diameter tube randomly placed on the macrophytodetritus patch. All macrophytodetritus present inside the tube were scooped off from the seafloor by hand and collected in 2 L plastic jars. For the sand samples (BS and CS), 20 cm^2 surface area and 5 cm deep cores of bare sand were collected. In order to clear the covered sand sample present underneath the macrophytodetrital material, the latter was very carefully moved aside to avoid fine sediment being brought in suspension and to get access to the bare sand. All jars were closed under water to ensure no loss of material or contamination of the samples. Standardization over all samples was not possible due to the different sampling techniques. Therefore two standardizations were made: one to compare all cored samples (BS, CS, SMF and LMF) per unit of surface (10 cm^{-2}) and one to compare all seagrass material (SMF, LMF, LL) per unit of dry mass (g^{-1} DM).

4.2. Community characterization

In order to extract the attached meiofauna, a 8% $MgCl_2$-solution was added to stun the organisms (Hulings and Gray 1971). Samples were afterwards rinsed twice over a 1 mm mesh sieve to exclude macrofauna and on a 38 µm mesh sieve to retain the meiofauna, prior to preservation with a 4% formaldehyde seawater solution. In the lab, the 38 µm-1 mm fraction of each replicate was centrifuged three times with Ludox HS40 (specific density of 1.18 g/dm^3). Meiofauna was stained with Rose Bengal before

being sorted and enumerated at a higher taxon level based on Higgins & Thiel (1988) using a Wild M5 binocular. One hundred harpacticoid copepods were randomly picked and stored in 75% ethanol. Copepods were mounted on glycerine slides for further identification to copepod family level using the identification keys and reference books by Boxshall & Hasley (2004) and Lang (1948, 1965).

4.3. Environmental data

The samples for the community characterization were also used to assess the total organic carbon content (TOC). Subsamples were weighed after being dried at 60°C for 72 h. Total organic carbon content (TOC) was measured after acidification with HCl and pulverization for 30 minutes of the dried material. TOC analysis were carried out with a ThermoFinnigan Flash1112 elemental analyser using the method of Niewenhuize *et al.* (1994).

4.4. Trophic biomarkers

For stable isotope analysis separate qualitative samples were taken from the seagrass material habitats. The two macrophytodetritus habitats (SMF & LMF) were sampled using 30 L bags where the material was scooped in by hand. The living leaves habitat was sampled by cutting 5 shoots at the sediment interface. All samples were afterwards kept in aquaria in order to collect harpacticoid copepods using positive phototaxis attraction and a pipette. The extracted copepods were rinsed and placed overnight in filtered seawater to empty their gut content. Two potential food sources including leaves (living or as macrophytodetritus) without and with associated epiphytes were collected according to the technique used by

Dauby & Poulicek (1995). The leaves without epiphytes, the epiphytes and the copepod samples (60 ind. sample^{-1} in order to have a sufficient amount of carbon for reliable measurements) were stored directly after collection in liquid nitrogen at -80°C. All the samples were dried afterwards for 24 h at 60°C and loaded into tin capsules for isotopic measurements with a C-N-S elemental analyser (Carlo Erba, Italy) coupled to a mass spectrometer (VG Optima, Micromass, UK). Prior to the encapsulation all seagrass material potential food sources were ground, except for harpacticoid copepods. The isotopic data were expressed as δ value (‰) relative to the VPDB (Vienna Peedee Belemnite) carbon standard. Reference material used to calibrate was IAEA (International Atomic Energy Agency) CH-6 ($\delta^{13}C$=-10.4±0.2‰). The standard deviation of repeated $\delta^{13}C$ measurements of the internal standard was ±0.2‰.

Contribution of potential food sources to the carbon pool of harpacticoid copepods was estimated by a SIAR mixing model developed in R (Parnell *et al.*, 2010). The model was run for 500000 iterations and the first 50000 iterations were discarded. Isotopic ratios of copepods and food sources were compared considering a trophic enrichment of 0.2 ± 0.6‰ for $\delta^{13}C$ (adapted from Vander Zanden (2001)). In this study only the mode and lowest and highest 0.95% confidence interval (CI) were detailed. The value used in the model was the overall mean of epiphytes from the two other habitats (-20.0±1.0‰), due to the lack of data on epiphytes from small macrophytodetritus fragments. To reduce the number of food sources and to avoid bias, the model was run with two sources (epiphytes and leaves without attached epiphytes), except for the small macrophytodetritus fragments where no separation of epiphytes was possible, leaves with epiphytes were used. The living leaves and macrophytodetritus with associated epiphytes were not considered in the mixing models since this source was a combination of two other potential food sources and therefore biasing the mixing model outcome.

4.5. Data analysis

Diversity indices were calculated using S= number of meiofauna taxa or copepod families; N_{inf}= dominance index, the reciprocal of the proportional abundance of the most common taxon or family (reciprocal of the Berger-Parker index); J'= Pielou evenness index and H'= Shannon-Wiener diversity index based on the natural logarithm (ln). A resemblance matrix based on Bray–Curtis similarities was constructed from the log transformed abundances for meiofaunal taxa and harpacticoid copepod families. A cluster analysis based on the Bray–Curtis similarity matrix was performed to explore community structure similarities among the different habitats using the group average linkage method. Prior to the cluster analysis an ANOSIM (Analysis of Similarity) was carried out to test whether the defined communities (based on habitats) were significantly different. A SIMPER (Similarity Percentages) analysis based on a Bray–Curtis similarity matrix of the harpacticoid copepod composition was done to reveal which families characterise and discriminate each habitat. All the above mentioned analysis were performed with the Primer 6.0 software (Clarke and Gorley 2006). Differences in composition among all habitats were tested by means of a one-way analysis of variance (ANOVA) done on relative data. *A posteriori* comparisons were carried out with the Tukey test using 95% confidence limits. Significant differences among two habitats were tested by means of a t-test. The ANOVA's, Tukey-tests, t-tests and graphs were made using GraphPad 5.04 for Windows (GraphPad Software, La Jolla, California BSA).

5. Results

5.1. Meiofauna communities

For meiofauna abundances the standardization against surface area yielded a decreasing trend: 217.5±92.8 ind.10 cm^{-2} (BS), 146.2±63.6 ind.10 cm^{-2} (LMF), 72.1±26.7 ind.10 cm^{-2} (CS) and 57.8±20.2 ind.10 cm^{-2} (SMF) (Figure 2, A). In terms of meiofaunal densities, all four cored habitats were significantly different (one-way ANOVA, $F(3,8)=4.75$, $p=0.0035$). The Tukey test comparison revealed that only BS and SMF were significantly different from each other (Tukey, $p<0.05$). Standardization against dry mass generated the following decreasing trend: 39.4±4.1 ind.g^{-1} DM (LMF), 14.9±3.0 ind.g^{-1} DM (SMF) and 13.9±2.0 ind.g^{-1} DM (LL) (Figure 2, B). Regarding meiofaunal densities, all three seagrass material habitats were significantly different (one-way ANOVA, $F(2,6)=54.42$, $p<0.0001$). The Tukey test comparison revealed that only SMF and LL were not significantly different from each other (Tukey, $p>0.05$).

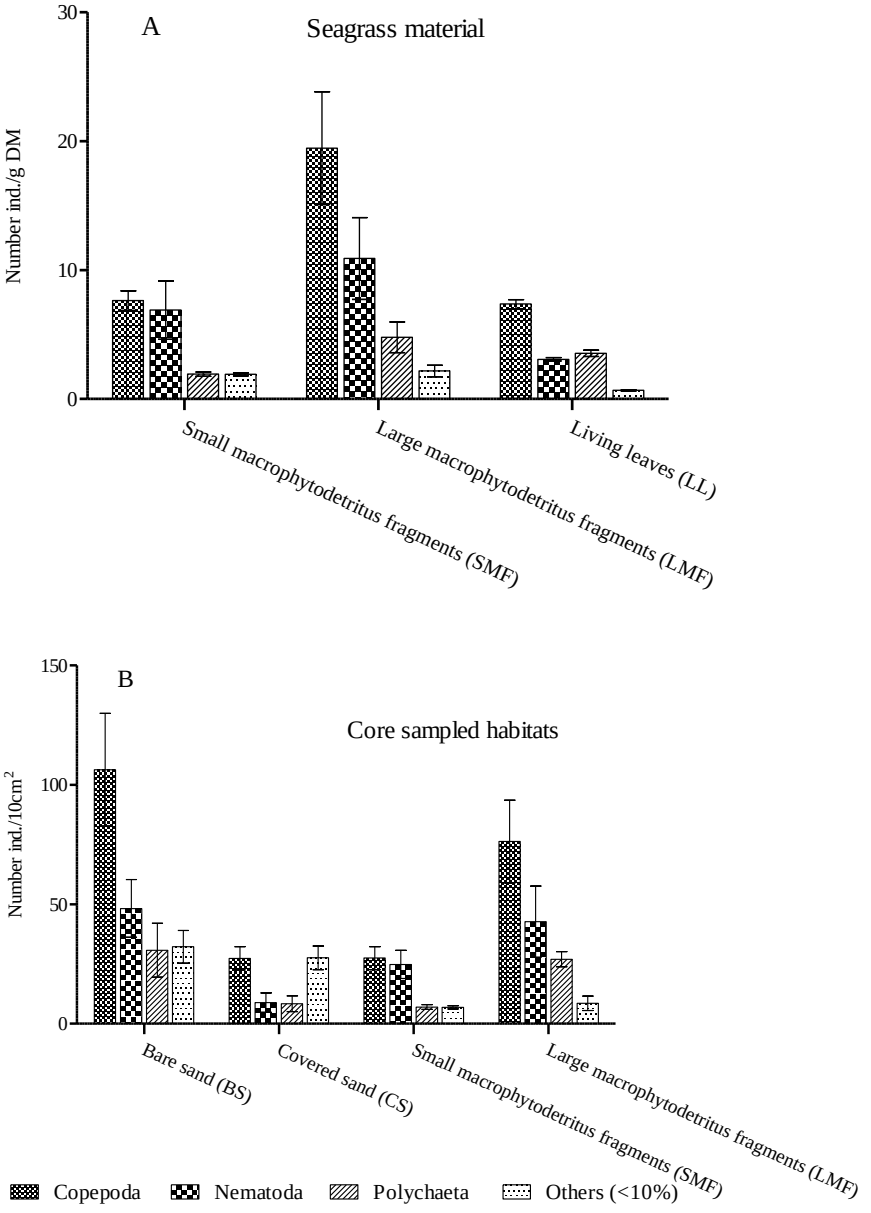


Fig. 2 *Mean meiofauna densities per habitat type. A: Mean densities in gDM-1, B: Mean densities in 10cm-2 surface area. Error bars represent the standard*

deviation (n=3).

In relation to relative composition, 88% of all organisms belonged to three taxa*:* copepods, nematodes and polychaetes. Copepods represented the highest average relative abundance of 49.4±3.4% with a maximum of 54.1±4.8% in LFM. Nematodes accounted on average for 23.3±6.8% with a maximal density of 32.3±6.4% in SFM. Polychaetes accounted on average for 15.0±5.1% with a maximum of 24.1±2.2% in the LL. The remaining 12% consisted of, in order of decreasing abundance: juvenile Amphipoda, nauplii, Ostracoda, Tanaidacea, Isopoda, Halacarida, Turbellaria, Leptostraca, Oligochaeta, Tardigrada, Decapoda, Chaetognatha, Cnidaria, Pycnogonida, Cumacea and Paguroidea.

The meiofaunal community did not show any significant difference among the habitats in terms of assemblage structure (ANOSIM, R=0.37, *p*=0.001), with an overall similarity of 84% (SIMPER). The diversity indices showed no significant differences among all five habitats (t-test, p-values >0.05) (Table 1).

	S	N_{inf}	J'	H'
Meiofauna				
BS	12.00 ± 0.00	2.02 ± 0.11	0.60 ± 0.02	1.49 ± 0.04
CS	11.33 ± 0.58	2.13 ± 0.42	0.61 ± 0.04	1.47 ± 0.04
SMF	13.33 ± 2.52	2.02 ± 0.21	0.53 ± 0.07	1.37 ± 0.11
LMF	13.67 ± 3.06	2.02 ± 0.21	0.43 ± 0.08	1.36 ± 0.13
LL	15.00 ± 2.65	1.89 ± 0.20	0.51 ± 0.04	1.22 ± 0.02

	S	N_{inf}	J'	H'		S	N_{inf}	J'	H'
Copepoda									
BS	10.00 ± 0.00	2.68 ± 0.41	0.73 ± 0.05	1.68 ± 0.10					
CS	9.00 ± 1.00	2.20 ± 0.22	0.70 ± 0.01	1.53 ± 0.10	Sediment (Cluster 1)	8.00 ±2.40	2.07 ± 0.64	0.71 ± 0.03	1.35 ± 0.40
SMF	5.00 ± 1.00	1.32 ± 0.05	0.53 ± 0.06	0.84 ± 0.08					
LMF	8.67 ± 1.53	2.57 ± 0.56	0.79 ± 0.14	1.70 ± 0.24	Leaf (Cluster 2)	9.50 ±1.52	2.52 ± 0.45	0.69 ± 0.13	1.72 ± 0.45
LL	10.33 ± 1.15	2.47 ± 0.43	0.74 ± 0.05	1.73 ± 0.13					

Table 1. *Meiofauna and harpacticoid copepods diversity indices (S = number of harpacticoid families, Ninf = dominance index, J' = Pielou evenness index, H' Shannon-Wiener index). Sediment and leaf regrouping were based on the harpacticoid copepod cluster analysis (Figure 3). Values are Mean ± standard deviation. BS: Bare sand; CS: Covered sand; SMF: Small macrophytodetritus fragments; LMF: large macrophytodetritus fragments and LL: living leaves.*

5.2. Copepoda family composition

For harpacticoid copepod abundances, standardization against surface area yielded the same decreasing trend as for meiofauna: 10.6±4.1 ind.10 cm^{-2} (BS), 7.6±3.0 ind.10 cm^{-2} (LMF), 2.7±0.8 ind.10 cm^{-2} (CS) and 2.7±0.8 ind.10 cm^{-2} (SMF) (Figure 2, A). In terms of copepod densities, all four cored habitats were significantly different (one-way ANOVA, $F(3,8)=6.7$, $p=0.0141$). The Tukey test comparison revealed that only BS vs. CS and BS vs. SMF were significantly different from each other (Tukey, $p<0.05$). Standardization against dry mass generated the following decreasing trend: 19.5±1.3 ind.g^{-1} DM (LMF), 7.0±0.70 ind.g^{-1} DM (LL) and 6.2±1.9 ind.g^{-1} DM (SMF) (Figure 2, B). In terms of copepod densities, all three seagrass material habitats were significantly different (one-way ANOVA, $F(2,6)=87.95$, $p<0.0001$). As observed for meiofauna, the Tukey test revealed that only SMF and LL were not significantly different (Tukey, $p>0.05$).

In total, 4 copepod orders and 16 harpacticoid families were found in 1500 identified copepod specimens. The majority of the copepods (90.6±3.0%) belonged to the order Harpacticoida. The remaining copepods were copepodites (2.9±2.8%), representatives of the Cyclopoida order (6.1±2.8%) or belonged to the orders Calanoida and Misophrioida (0.4±0.3% together). Since only a minority (few %) of the collected copepods did not belong to the Harpacticoida order, further analysis focussed only on the latter. The harpacticoid families found, in decreasing order according to their overall relative abundance, were, Tisbidae, Thalestridae, Miraciidae, Laophontidae, Ameiridae, Longipediidae, Ectinosomatidae, Tetragonicipitidae, Harpacticidae, Porcellidiidae Ancorabolidae, Peltidiidae, Canuellidae, Tegastidae, Cletodidae and Cylindropsyllidae.

The harpacticoid copepods showed a significant difference among the

habitats in terms of assemblage structure (ANOSIM, R = 0.92, p=0.001), with an overall dissimilarity of 53% (SIMPER). ANOSIM habitat type pairwise comparisons test separated well all groups (R>0.75) except for BS&CS and LMF&LL showing overlap but still yielding a difference of R=0.70 and R=0.63 respectively. Cluster analysis (Figure 3) revealed two major clusters: cluster 1 with an overall similarity of 62.6% and cluster 2 with an overall similarity of 52.9%. In the first cluster (cluster 1) a clear distinction was found among two habitats: the large macrophytodetritus fragments (cluster 1A, 71.7% similarity) and the living leaves (cluster 1B, 68.7% similarity). The second cluster (cluster 2) consisted of: two replicates of covered sand (cluster 2A, 81.0% similarity) and a cluster (2B, 62.6% similarity) of small macrophytodetritus fragments, bare sand and a single replicate of covered sand.

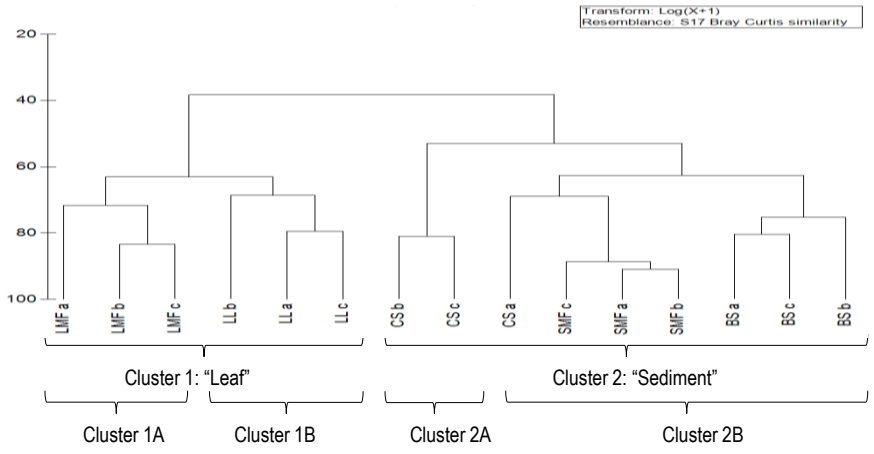


Fig. 3 *Cluster analysis of relative harpacticoid copepod densities. LL: Living leaves; LMF: Large macrophytodetritus fragments; SMF: Small macrophytodetritus fragments; CS: Covered sediments; BS: Bare sand. Similarity coefficient in % on y-axis and a, b, c refer to the replicates.*

The two main clusters were significantly different (ANOSIM, R=90, p=0.001), showing an average dissimilarity of 61.7% (SIMPER). Cluster 1 had an overall average similarity percentage of 67.4% and four harpacticoid families were abundant with a cumulative contribution to the similarity of 86.8%. According to SIMPER analysis, the families primarily

responsible for the similarity were, in decreasing order: Thalestridae (48.2%), Laophontidae (20.4%), Miraciidae (8.5%) and Tisbidae (8.7%). Cluster 2 had an average overall similarity percentage of 63.3% and was mainly characterised by three families with a cumulative contribution of 89.4% (SIMPER). A different decreasing contribution order was seen in comparison to cluster 1: Tisbidae (57.1%), Miraciidae (21.9%) and Thalestridae (10.4%) (Figure 4).

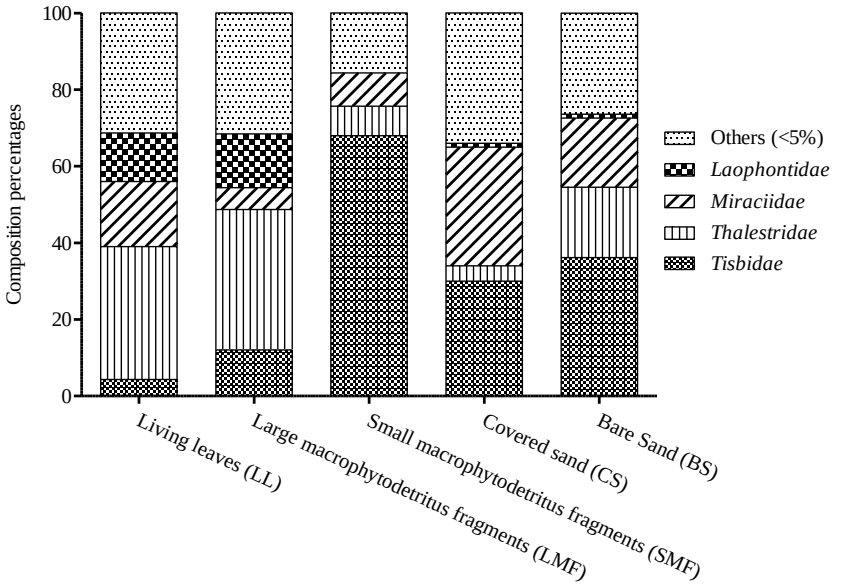


Fig. 4 *Mean relative composition (%) of the harpacticoid copepod families. Families shown represent a total contribution of more than 5% over all samples.*

No significant differences were found in number of families (t-test, $t(13)=1.4$, $p=0.199$) between cluster 1 (S=9.5±1.5) and cluster 2 (S=8.0±2.4). In contrast to the significant difference among all habitats (one-way ANOVA, $F(4,10)=12.0$, $p=0.0008$), there was no significant

difference among both clusters in terms of dominance index and Shannon-Wiener index (t-test, $t(19)=1.0$, $p=0.333$ and $t(13)=2.1$, $p=0.056$, respectively). The Pielou's evenness index showed a significant difference between the two clusters (t-test, $t(13)=2.6$, $p=0.023$) (Table 1).

5.3. Environmental data

TOC values showed significant differences among the habitats (one-way ANOVA, $F(2,3)=179.7$, $p=0.0008$) except for SMF and LMF comparison (Tukey, $p>0.05$). Following TOC content were found in decreasing order: LL (31.0±0.5%), LFM (20.6±0.3%), SFM (19.7±1.0%) and the sand habitats (<0.1%).

5.4. Trophic interactions by means of stable isotopes

Harpacticoid copepods from the different seagrass material habitats showed slightly different, but analogous $\delta^{13}C$ values: -16.6±1.1‰ (LL), -18.5±1.3‰ (LMF) and -16.8±1.1‰ (SMF) (Figure 5). Leaves with epiphytes were the most enriched ^{13}C sources: $\delta^{13}C$=-9.1±1.5‰ (LL) and $\delta^{13}C$=-14.5±1.5‰ (LMF), however collection of epiphytes from SMF could not be achieved due to the high fragmentation and fragility of the macrophytodetrital material. Therefore only SMF with associated epiphytes could be analysed ($\delta^{13}C$=-13.7±1.1‰), which ranged within the values of LMF without epiphytes. The epiphytes present on the LL and on the LMF material (Figure 5) were by far the most depleted ($\delta^{13}C$=-19.5±2.2‰ and -20.0±1.0‰ respectively) in the three seagrass material habitats. The combination of living leaves and macrophytodetritus with their attached epiphytes ranged between the values of the two latter separately with $\delta^{13}C$=-11.4±1.8‰ and -16.9±1.2‰ for the LL and the LMF, respectively (Figure 5).

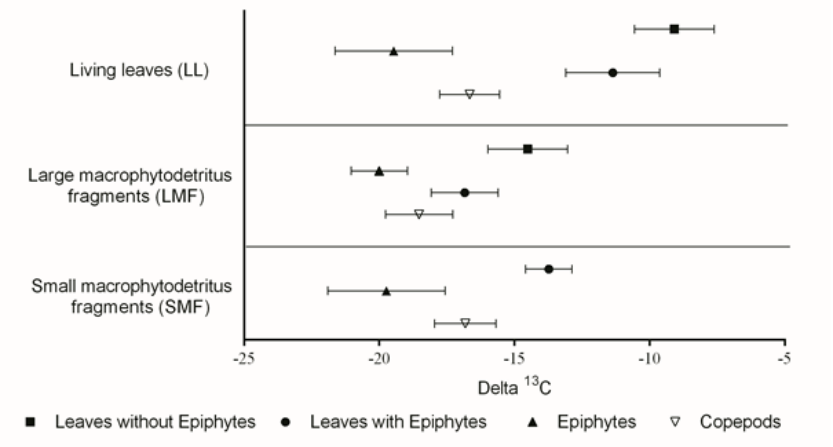


Fig. 5 *$\delta^{13}C$ values (‰) of plant material as potential food sources and copepods (mean ± SD, n=3). The full symbols are potential sources: leaves (living or macrophytodetritus) without epiphytes (squares), the removed epiphytes (triangles) and the leaves (living or macrophytodetritus) with their epiphytes (rounds). The open symbols are the harpacticoid copepods, primary consumers (reversed triangles).*

Mixing model computation showed higher contribution of epiphytes than leaf material as carbon source for copepods in LL and LMF. For the LL habitat, epiphytes 0.95 confidence interval (CI) ranged from 42% to 97% (mode 70%) and LL without epiphytes 0.95 CI was 2-57% (mode 30%). For LMF, epiphytes 0.95 CI ranged from 37% to 99% (mode 72%) and for LMF without epiphytes 0.95 CI was 0-63% (mode 28%). For SMF habitat, epiphytes 0.95 CI ranged from 23% to 81% (mode 51%) and SMF with epiphytes 0.95 CI ranged from 18% to 76% (mode 49%) (Figure 6).

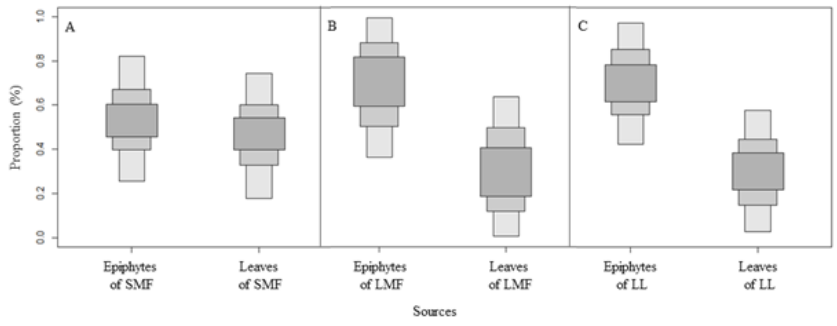


Fig. 6 *SIAR boxplots show the proportional contribution (%) of different sources to the diet of harpacticoid copepods in (A) the small macrophytodetritus fragments habitat (SMF), (B) the large macrophytodetritus fragments habitat (LMF) and (C) the living leaves habitat (LL) with 95% (darkest grey), 75% and 25% (lightest grey) credibility intervals.*

6. Discussion

In the present study, it was remarkable to see that harpacticoid copepods numerically dominated the meiofauna samples in all habitats. Half of the meiofaunal organisms were harpacticoid copepods and only a quarter were nematodes. This is in contrast with most studies on meiofauna in seagrass meadows sediments (e.g. Fonseca *et al.*, 2011; Losi *et al.*, 2012) where nematodes dominate numerically. Nevertheless, dominance of copepods is often documented for seagrass meadow canopy leaves (Hall and Bell 1993; De Troch *et al.,* 2001b; Hooper and Davenport 2006).

The analysis of meiofauna at taxon level did not show any significant differences among the different habitats either in terms of assemblage composition or diversity. Indeed, the five habitats presented a high similarity, i.e. more than 84% (SIMPER). The LMF had the highest meiofaunal abundance from the seagrass material habitats which could indicate that the potential food quality/quantity or protection from predation was the highest in these habitats. A potential increase in complexity can be a reason for this, however concrete measurements (e.g. compactibility of the accumulation) to prove the possible rise in complexity were not part of this study. It could also be caused by an increase of biofilm quantity or quality as dead leaves are colonised by degrading microbes and generally lie on the sediment, exposed to sun light, in contrast to living leaves which are vertically disposed and often over-shaded.

Within the core sampled habitats, a significant difference in abundance between bare sand and covered sand was observed. This could be explained by the possible anoxia created by the layer of

macrophytodetritus above the covered sand. We expect that the oxygen does not penetrate deep enough through the macrophytodetritus to reach the sand and thus oxygen levels can become limiting. As a result, meiofauna could migrate upwards from the sand towards the macrophytodetritus. In the present study, no measurements of oxygen were available, but it could be definitely interesting to include them in future research.

In terms of harpacticoid copepod density, the same higher abundance trend for bare sand and large macrophytodetritus fragments compared to covered sand and small macrophytodetritus fragments was found, similar to the meiofauna results. We could assume that bare sand includes most of the copepods and when covered by macrophytodetritus these then migrate/ emerge into large macrophytodetritus fragments. Nevertheless, in terms of harpacticoid copepod family composition, two main significantly different clusters with a similarity of approximately 38.3% were displayed. The low similarity indicates that the small macrophytodetritus fragments habitat behaves like a sediment habitat (CS and BS) and that the large macrophytodetritus fragments habitat was similar to the living leaves habitat for copepod family composition. Therefore, cluster 1 (LL and LMF) and cluster 2 (SMF, CS and BS) will further be referred to as 'leaf" and 'sediment', respectively. Thus we can hypothesise that bare sand and large macrophytodetritus fragments attract harpacticoid copepods adapted to different habitat. Harpacticoid copepods are morphologically diverse and well-adapted group occurring in numerous environments (Hicks and Coull 1983; Huys and Boxshall 1991; Boxshall and Hasley 2004). The phytal living leaves environment is exposed to waves and currents. Organisms living in that habitat developed attachment capabilities such as prehensile grasping legs (e.g. copepod families Thalestridae and Laophontidae) or body flattening (Porcellididae and Peltitidae). Macrophytodetritus accumulations on sandy patches are temporary, what implies that all meiofauna could be transported passively on the senescent leaves or immigrate actively from the surrounding habitat. High vagility of the phytal

meiofauna was demonstrated by Bell & Hicks (1991) and the active emergence and colonization abilities of copepods to richer or easier accessible food resources was proven by Armonies (1988) and Thistle & Sedlacek (2004). Experiments with artificial substrates (Mirto and Danovaro 2004) emphasise the good swimming and colonization abilities of copepods, which partially explains the higher abundance of copepods in the large macrophytodetritus fragment. As such, the good colonization abilities of harpacticoid copepods could explain the high resemblance in terms of copepod assemblage structure between large macrophytodetritus fragments and living leaves on the one hand and between small macrophytodetritus fragments, bare sand and covered sand on the other hand. Novak (1984) stated that epiphytic cover increases from young to mature leaves. Large macrophytodetritus fragments which are senescent mature leaves exhibit a lower total organic carbon content then living leaves. Nonetheless, large macrophytodetritus fragments displays by far the highest number of individuals per gram dry mass. Therefore we can hypothesise that the content of the potential food sources does not seem to be the determining factor, but rather, the accessibility of the food source. It has been demonstrated that harpacticoid copepods occur primarily in function of resource availability (Webb 1990), followed by hydrodynamic exposure, surface area colonisable by epiphytes (De Troch *et al.,* 2005b) and food accessibility. Well-developed and accessible epiphytic biofilms could thus enhance the species richness and density of the meiofauna (Hall and Bell 1993; Peachey and Bell 1997). This was congruent with some morphological adaptations found among the meiofaunal taxa. Only a small fraction of the phytal meiofauna (some nematodes, tardigrads and halacarids) can feed directly from the plant tissue, whereas the majority of meiofauna graze on the organic biofilm present on the leaves (e.g. Giere, 2009). Macrophytodetrital accumulations function thus as refuge from predators and provide ample food supply (Bonsdorff 1992; Norkko *et al.,* 2000).

Stable isotope analysis was used to identify trophic interactions and

dietary relationships between the harpacticoid copepods and their potential food sources. The recorded stable isotope data of *P. oceanica* leaves showed a range in $\delta^{13}C$ values between –13‰ and –8‰, however most delta values varied around –12‰ (Hemminga and Mateo 1996). These relatively high $\delta^{13}C$ values compared to other potential source materials, like epiphytes ($\delta^{13}C$ value around –20‰) make it possible to trace seagrass material in fauna diet. In our present study harpacticoid copepods were more ^{13}C depleted than the *P. oceanica* leaves or macrophytodetritus and more ^{13}C enriched than the attached epiphytic biofilm. The analogous harpacticoid copepod compositions in all three seagrass material habitats suggest that they feed on similar sources, independent of the habitat where they occur. The SIAR mixing model revealed that epiphytes contribute more to the carbon resource for harpacticoid copepods than plant material, living or macrophytodetrital, do. This was congruent with the conclusion of Fry *et al.* (1987) on the basis that epiphytic algae can have an equal or greater nutritional importance than seagrasses for consumers in seagrass meadows. Since the outcome of the mixing model for SMF was biased due to the non-separation of the epiphytes from the leaves, no clear conclusion can be drawn for this particular habitat.

Harpacticoid copepods are known to feed on a variety of food sources (Hicks and Coull 1983) and they can colonise plastic seagrass mimics (De Troch *et al.,* 2005b). It was known that the epiphytic biofilm consists of different food sources ranging from cyanobacteria to diatoms and fungi and consequently represents a very variable food quality (Novak 1984; Lepoint *et al.,* 2006). The macrophytodetritus epiphytic biofilm compared to the seagrass epiphytic biofilm was composed of a higher number of detrital organisms, such as fungi and bacteria (Lepoint *et al.*, 2006). Therefore we can hypothesise that the main reason why epiphytes contribute primarily to the food supply of harpacticoid copepods was due to the richer organic content in comparison to the poor macrophytodetrital material.

Nevertheless, the isotopic composition did not exclude the occurrence of seagrass and macrophytodetritus in the diet of copepods. The question arose as to whether harpacticoid copepods actively graze on plant material or accidently ingest and assimilate it during epiphytic biofilm grazing. The isotopic compositions of *P. oceanica* macrophytodetritus (LMF and SMF) differ from living leaves (LL) by 3.9±1.1‰. This can be explained by the fractionation during decomposition. Nutrients are resorbed in senescing leaves before abscission and labile nutrients are quickly mobilized by bacterial degradation (Mateo and Romero 1997). Next to the decomposition fractionation, the origin of the macrophytodetrital leaves could play a major role. Macrophytodetritus found on the studied sand patch does not necessarily come from the adjacent meadow but may be from deeper meadows which exhibit a lower isotopic composition due to the light constraint on photosynthesis rate (Lepoint *et al.,* 2003).

7. Conclusions

Meiofauna community assemblages were similar in the five habitats analysed in the present study. Meiofauna densities were the highest in the bare sand, followed by large macrophytodetritus fragments compared to the other core sampled habitats. Harpacticoid copepods were found to be the most abundant taxa and had the highest density in large macrophytodetritus fragments compared to the other seagrass material habitats. On the basis of harpacticoid copepod assemblage structure, the five different habitats were divided in two main clusters: a sediment and a leaf habitat group. The small macrophytodetritus fragments showed a higher similarity with both sand habitats in harpacticoid copepod composition and therefore can thus be considered to be more similar to sediment habitats than to phytal habitats. Copepod stable isotope compositions were similar, which indicates they feed on analogous food sources in the different habitats. As epiphytes contribute for 70% to the

copepods' carbon composition in living leaves and large macrophytodetritus fragments, they appear to be the preferential carbon source for harpacticoid copepods (SIAR mixing model).

8. Acknowledgments

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Publication list

A1 – Peer reviewed Articles

Mascart T., De Troch M., Remy F. & Lepoint G. (in prep). Feeding ecology and niche width of macrophytodetritic copepod species: insights from stable isotopes compositions and fatty acid profiles.

Mascart T., Agusto L., Lepoint G., Remy F. & De Troch M. (2015) How do harpacticoid copepods colonize detrital seagrass leaves? *Marine Biology* 162 (5), 929-943.

Mascart T., Lepoint G., Deschoemaeker S., Binard M., Remy F., & De Troch M. (2015). Seasonal variability of meiofauna, especially harpacticoid copepods, in *Posidonia oceanica* macrophytodetritus accumulations. *Journal of Sea Research* 95, 149-160.

Mascart T., Lepoint G., & De Troch M. (2013). Meiofauna and harpacticoid copepods in different habitats of a Mediterranean seagrass meadow. *Journal of the Marine Biological Association of the United Kingdom* 93(6), 1557-1566.

Oral presentations

Mascart T., Lepoint G., Biondo R., Remy F., Agusto L., & De Troch M. (2014, December 12th). Colonization of a new habitat by copepods: An *in situ* experiment. Oral presentation at Zoology 2014 (21th Benelux congress of Zoology), Liège, Belgium.

Mascart T., De Troch M., Remy F., & Lepoint G. (2014, August 4th). Feeding ecology of harpacticoid copepod species: Insights from stable isotopes analysis and fatty acid profiling. Oral presentation at the 9th International Conference on the Applications of Stable Isotope Techniques to Ecological Studies (IsoEcol 9), Perth, Australia.

Mascart T., De Troch & Lepoint G. (2013, February 21st) Corsican seagrass detritus: an opportune shelter or a copepod Eldorado? Oral presentation at DuodeceMBSS, Gent, Belgium

Mascart T., De Troch & Lepoint G. (2011, April 1st). Functional diversity of meiofauna in detritus accumulations in Mediterranean *Posidonia* beds. Oral presentation at NoveMBSS, Gent, Belgium

Poster presentations

Sturaro N., Borges A., Das K., Dauby P., Gobert S., Mascart T., Michel L., Remy F. and Lepoint G. (2015, March 26th). Applications of stable isotopes in environmental studies at the University of Liège. Poster presented at the BASIS symposium 2015, Utrecht, The Netherlands.

Remy F., Mascart T., Dauby P., Gobert S., & Lepoint G. (2014, December 12th). Seasonal sampling and stable isotopes use to delineate seagrass phytodetritus macrofauna trophic ecology: baseline variation or actual diet change? Poster presented at Zoology 2014 (21th Benelux Congress of Zoology), Liège, Belgium.

Mascart T., De Troch M., Gobert S., Biondo R., Remy F., & Lepoint G. (2014, May 5th). Hypoxia in macrophytodetritus accumulation: Species specific harpacticoid copepod adaptation? Poster presented at the 46th International GHER Colloquium, Liège, Belgium.

Remy F., Mascart T., Dauby P., Gobert S., & Lepoint G. (2014, May 5th). Has oxygen depletion an impact on nutrients and macrofauna in a highly dynamic macrophytodetritus accumulation? Poster presented at the 46th GHER Liège International Colloquium 2014, Liège, Belgium.

Mascart T., Lepoint G., Remy F., Gobert S., Dauby P., & De Troch M. (2014, March 7th). Corsican seagrass detritus: An opportune shelter or a copepod Eldorado? Poster presented at the 14th VLIZ Young Scientist day, Brugge, Belgium

Mascart T., De Troch M., Remy F., & Lepoint G. (2012, August 21th). Trophic and specific diversity of harpacticoid copepods associated to *Posidonia oceanica* macrophytodetritus. Poster presented at the International Conference on Applications of Stable Isotope Techniques to Ecological Studies (ISOECOL 8), Brest, France.

Remy F., Mascart T., Dauby P., & Lepoint G. (2012, August 20th). Leaf fall impact on diversity and trophic ecology of vagile macrofauna associated with exported *P. oceanica* litter. Poster presented at the 8th International Conference on Applications of Stable Isotope Techniques to Ecological Studies (ISOECOL 8), Brest, France.

Lepoint G., Borges A., Darchambeau F., Dauby P., Mascart T., Remy F., & Champenois W. (2012, November 25th). A descriptive study of

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