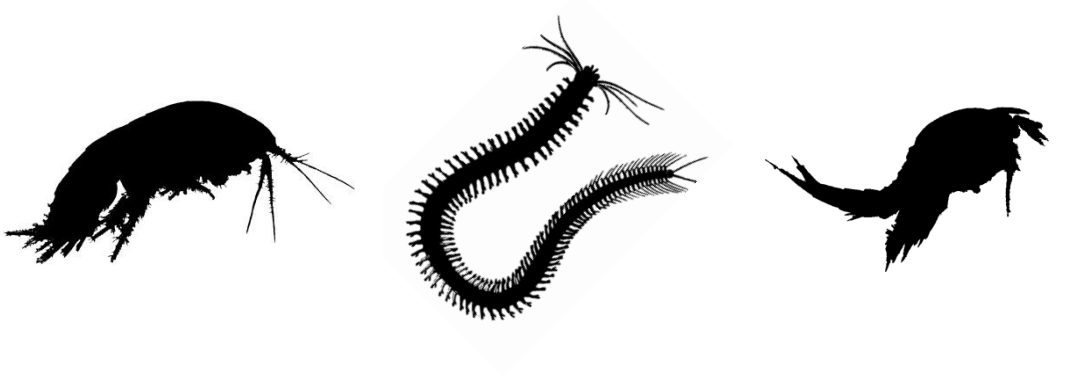
Océanologie biologique

Characterization, dynamics and trophic ecology of macrofauna associated to seagrass macrophytodetritus accumulations (Calvi Bay, Mediterranean Sea)



a PhD thesis dissertation of

François REMY

2016

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Stable Isotopes in Environmental Sciences and Trophic Ecology

Université de Liège

Characterization, dynamics and trophic ecology of macrofauna associated to seagrass macrophytodetritus accumulation (Calvi Bay, Mediterranean Sea)

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Academic year 2015-2016

Publically defended on 12/05/2016

To refer to this thesis, please cite as:
Remy F., 2016. Characterization, dynamics and trophic ecology of macrofauna associated to seagrass macrophytodetritus accumulation (Calvi Bay, Mediterranean Sea). University of Liège, 285 pp.

Front cover: Stereomicroscopic pictures of *Gammarus aequicauda*, *Nebalia strausi* and *Platynereis dumerilii*.

Back cover: Underwater photography of the *Posidonia oceanica* meadow close to macrophytodetritus accumulation near STARESO, Calvi, Corsica.

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Remerciements / Acknowledgements

Cette thèse n'aurait pas été possible sans l'aide, les conseils et les remarques d'un grand nombre de personnes. Voici venu le moment très agréable de les remercier car chacun a participé de près ou de loin à ces 4 années de recherches. Je tiens avant tout à m'excuser auprès de ceux que j'oublierais, n'y voyez pas une attaque personnelle ou une préférence pour les personnes citées, simplement de la distraction.

Tout d'abord, je voudrais remercier mon promoteur, Gilles Lepoint. Gilles, merci de ton accueil depuis mon mémoire jusqu'à la fin de cette thèse. Merci d'avoir toujours été disponible quand un conseil était nécessaire. Merci d'avoir été présent lors de nombreuses campagnes sur le terrain. Sans toi, on se serait probablement moins perdu dans le fond de la Baie de Calvi, et Sylvain aurait probablement plus de cheveux, mais ça n'aurait pas été pareil. « Faire et défaire c'est toujours travailler », voilà une chose que tu m'auras apprise durant cette thèse, car le chemin qu'un travail de recherche prend, est rarement celui qu'on s'imaginait au départ, et ce qui en ressort n'en est que plus intéressant. Merci pour tout ça, Dr LepointG, ces 4 années ont vraiment été un plaisir.

J'ai eu la chance de travailler dans le laboratoire d'Océanologie, qui a connu un passage de témoin entre deux chefs. Merci tout d'abord à Jean-Marie Bouquegneau de m'avoir accueilli, puis à Sylvie d'avoir pris la relève en endossant avec brio la bien nommée « Veste du Chef ». Madame Chef, merci pour tes conseils lors de nos discussions sur le terrain et au labo. Tes conseils ont probablement fait évoluer cette thèse dans le bon sens. Merci pour ton accueil.

Cette thèse n'aurait pas été possible sans l'aide reçue lors de la réalisation des expériences, l'identification de toutes les petites bestioles qui composent ce travail. Merci à Celia et Aurélie qui ont fait grandement avancer cette thèse durant leur mémoire.

Cette thèse n'aurait pas non plus été la même sans l'ensemble des membres de l'équipe de la STARESO. Merci à Pierre, Aurélia, Alex, MiniMatt, Michoune, Antho, Adèle, Annick, Nico, sans oublier Lola et Wistiti. Merci à Sylvain pour son aide lors des très nombreuses plongées. Merci à Coco pour son aide lors de la détermination de certains vers récalcitrants et sa bonne humeur durant mes longues campagnes de terrain. Merci à Richard et Nilu sans qui la station ne serait pas la même et merci à Marine « cheville d'acier » Chevreau pour les bons moments passés.

Ensuite, merci à la « team filou », Nono et Chouchoune pour les bons moments passés à la station mais aussi et surtout en dehors. Nos randos par 35° et sans la moindre bouteille d'eau resteront longtemps dans ma mémoire. Chouchoune, merci d'avoir été là à chaque campagne, de m'avoir accueilli dans ton château alors que ça t'empêchait de dormir, et d'avoir bu un verre de champagne à 2500m par -5° au Lac Blanc. Ces moments font qu'une thèse passe bien trop vite, une fwè.

Merci à Renzo, le MacGyver de l'océano, pour ses conseils lors de la réalisation des designs expérimentaux et lors des mesures au SKALAR. Merci, sans toi, ces expériences auraient probablement fini sur le front Liguro-Provençal, arrachées par la première vague venue.

Merci à tous les membres temporaires, passés et présents du laboratoire pour leurs conseils et tous ces autres moments passés ensemble, principalement lors des biens nommés FRIDAY-DRINKS. Merci à Doro, France, Marie-Jeanne, Mariana, Alice, Bob, Aurélie, Christelle, Jojo, Amandine, J-H, Anne, Krishna, Emi, Pam. Merci tout particulier à Loïc « Pitbull » Michel et Nico « Marco » Sturaro pour leurs conseils lors de nos très nombreuses discussions, souvent autour d'un verre (voir deux…), mais qui ont énormément compté pour réaliser et finaliser cette thèse. Nico, cette thèse est 100% gluten-free, n'hésite pas. Loïc, « Elle avance cette thèse ? », « T'as pas une thèse à finir toi ? », « Je veux le manuscrit pour lire dans l'avion »…à part ça, nos discussions ont fait énormément évoluer mon travail, merci à toi!

Merci également à Pat, pour son avis et ses conseils lors des comités de thèses tout au long de ce travail.

Merci à Amandine « Licorne » Gillet pour sa présence durant la seconde moitié de cette thèse. Merci pour nos déconnades au sujet de l'inutilité totale de la baleinologie, la stupidité des marsouins et des calamars. Merci pour tes conseils et ta présence.

Et parce que sans financement, pas de recherche, je remercie profondément le FRS-FNRS (bourse FRIA F3/5/5-MCF/ROI/BC FC 91092) ainsi que l'ULg pour le financement de ces 4 années de thèse.

Merci à tous les gens totalement extérieurs au monde de la biologie marine mais qui ont été là tous les jours et évitent de perdre la boule pendant 4 ans. Merci à vous. J'évite ici l'énumération, car je suis certain d'oublier involontairement l'une ou l'autre personne. Si vous n'avez pas encore été cité, c'est dans cette catégorie que vous vous trouvez. Merci particulier à Perrine pour la relecture de mon anglais lors de la finalisation de ce travail.

Enfin, merci à Thibaut « Patulacci » Mascart pour ces 4 années de travail en tandem ! Merci pour toutes ces plongées faites lors cette thèse, mais aussi lors d'autres occasions de l'autre côté du monde. Merci pour toutes ces bières de décompression, les tempêtes d'Halloween, les réparations approximatives de l'IRMS qui semblait nous en vouloir personnellement. Merci pour tous les échanges qu'on a eu au sujet de notre boulot et au sujet de tout le reste ! Me faire un ami, flamand qui plus est (on ne pourra pas me qualifier d'intolérant après ça), est probablement le meilleur résultat de ces 4 ans de thèse.

Pour terminer, je remercie mes parents, ma famille et surtout mon chat (principalement pour ces précieux conseils), de m'avoir encouragé et supporté durant toutes ces années d'études, mais aussi ces 4 années de thèse. Merci de m'avoir soutenu toutes ces années de recherche, et j'espère que ça ne fait que commencer !

Résumé

Les herbiers de *Posidonia oceanica* sont un écosystème marin très important en zone côtière Méditerranéenne. Bien que très productive, cette plante à fleur marine endémique de Méditerranée n'est que peu consommée à l'état vivant par les organismes herbivores. Lors de la sénescence automnale des feuilles, une grande partie (jusqu'à 90%) de la production primaire foliaire de cette plante termine ses jours dans le « compartiment détritique ». Ces feuilles mortes, aussi appelées macrophytodétritus, commencent immédiatement à se dégrader dans l'herbier, mais une grande partie sera rapidement exportée hors de l'herbier, vers des zones d'accumulations sans végétation, souvent des zones sableuses. Associés à des macroalgues détritiques, des pousses vivantes détachées de Posidonies, des micro-organismes et du sédiment fin, ces macrophytodetritus forment ce que nous appelons, la litière exportée de Posidonies.

Cette litière constitue un habitat extrêmement dynamique pour toute communauté d'invertébrés marins : la meiofaune (38µm < taille < 500µm) et la macrofaune (taille ≥ 500µm) sur laquelle nous nous sommes concentrés. Ce caractère dynamique pourrait jouer un rôle structurant majeur au niveau de l'abondance, la diversité et l'écologie trophique de cette communauté de macro-invertébrés tant au niveau saisonnier, annuel, ou spatial, mais également lors de perturbations aléatoires, très brèves et de grande ampleur : les pulses de ressources.

Dans ce contexte, cette thèse de Doctorat avait 7 objectifs principaux :

i. Caractériser pour la première fois de façon exhaustive la communauté d'invertébrés vagiles de la litière de Posidonies.
ii. Evaluer les variations spatio-temporelles subies par la litère et ces macro-invertébrés à 2 échelles de temps différentes.
iii. Mettre en relation ces variations avec des paramètres environnementaux mesurés.
iv. Démontrer qu'une stratification des conditions environnementales apparait rapidement au sein de la litière et que cette stratification influence la présence et la répartition des macro-invertébrés.
v. Démontrer pour la première fois l'impact potentiel d'un pulse de ressources sur la litière de Posidonies et sa communauté de macro-invertébrés.

vi. Décrire pour la première fois le réseau trophique de cette communauté de macro-invertébrés vagiles au moyen d'examens de contenus stomacaux et d'analyses d'isotopes stables (C et N).

vii. Evaluer les variations spatiotemporelles de ce réseau trophique et évaluer si ces variations sont dues à de réels changements de régimes alimentaires, ou seulement à une modification de la ligne de base isotopique des sources de nourriture.

Cette thèse de Doctorat a démontré que la litière de Posidonies est majoritairement (70-80%) composée de feuilles mortes de Posidonies. Cette litière suit le cycle annuel de la Posidonie et présente globalement un maximum d'abondance en automne, juste après la senescence des feuilles. Les paramètres environnementaux mesurés montrent également une grande variabilité, liée à différents facteurs, tels que la force du vent et sa direction, l'abondance de litière et potentiellement la température. La présence continue de macro-invertébrés au sein de la litière de Posidonies a également été démontrée. Cette communauté, composée de 115 espèces, est largement dominée par les arthropodes (77%) suivis de loin par les annélides (12%) et les mollusques (7%), les autres taxa étant plus anecdotiques. Bien que la diversité soit assez importante, seules quelques espèces dominent très largement. En effet, 19 espèces représentent à elles seules plus de 90% de l'abondance globale rencontrée. Une espèce en particulier est à retenir: l'amphipode *Gammarella fucicola*, car il est l'espèce typique la plus abondante et dominante de cette communauté, représentant 40-50% de l'abondance globale rencontrée.

En plus de cette organisation générale, la communauté de macro-invertébrés de la litière de Posidonies présente des variations importantes, principalement saisonnières et interannuelles. Ces variations peuvent être reliées pour certaines espèces à différents paramètres environnementaux mesurés, mais force est de constater que beaucoup d'espèces semblent ne dépendre d'aucun paramètre mesuré lors de cette thèse de Doctorat. La concentration d'oxygène présente dans litière fut toutefois le paramètre le plus intéressant, permettant d'expliquer les variations observées chez 7 des 19 espèces les plus dominantes de la communauté. La stratification des conditions environnementales démontrée expérimentalement lors de la partie expérimentale de cette thèse de Doctorat est intimement liée à ce paramètre oxygène. Nous avons démontré que la répartition de certaines espèces au sein des différentes couches de litière suit cette concentration en oxygène, mais également en nutriments (principalement NH_4).

En plus de ces variations saisonnières, une approche à courte échelle de temps nous a permis d'identifier plusieurs évènements pouvant être considérés comme des pulses de ressources. Ces pulses de ressources ont été identifiés comme pouvant jouer un rôle important dans la structure de la communauté, favorisant grandement les espèces détritivores ainsi que les organismes tolérants à l'hypoxie. Nous avons aussi démontré que ces évènements pouvaient induire temporairement une plus grande consommation de feuilles mortes de Posidonies par les invertébrés vagiles associés, favorisant potentiellement la décomposition de la litière.

Le réseau trophique décrit dans cette thèse de Doctorat est composé de plusieurs niveaux trophiques, allant du consommateur primaire détritivore/herbivore jusqu'au prédateur de second ordre. Différentes préférences alimentaires ont été mises en évidence, mais une information majeure est que les feuilles mortes de Posidonies sont ingérées par une grande majorité des espèces rencontrées (85%). De plus, les analyses d'isotopes stables confirment que la litière de Posidonies est assimilée par la majorité des consommateurs primaires, et que ce « signal » détritique peut être retrouvé jusqu'aux niveaux trophiques supérieurs, ce qui est un argument en faveur de l'importance la macrofaune vagile comme décomposeurs majeurs de la litière de Posidonies et au transfert de matière organique depuis l'herbier, vers les chaines trophiques côtières. Une certaine variabilité saisonnière des niches trophiques a été observée. D'après le modèle de mélange SIAR, cette variabilité correspond dans certains cas à un réel changement saisonnier de régime alimentaire, probablement liée à la disponibilité variable des sources de nourriture.

Cette thèse de Doctorat associant un échantillonnage standardisé à 2 échelles temporelles différentes, analyses du réseau trophique (contenus stomacaux et isotopes stables) et expérimentations originales, nous a permis de décrire une communauté diversifiée et abondante de macro-invertébrés vagiles associés aux litières de Posidonies exportées, ses variations temporelles ainsi que le lien existant entre certaines espèces et certains paramètres environnementaux mesurés. Cette thèse de Doctorat a également décrit le réseau trophique de cette communauté et démontré l'importance des feuilles mortes de Posidonies en tant que source de nourriture pour de nombreux invertébrés. Ces derniers semblent donc jouer un rôle clé dans la décomposition de la litière et donc dans le flux de matière organique entre l'herbier de Posidonies lui-même et les réseaux trophiques côtiers Méditerranéens.

Summary

Posidonia oceanica meadows are a major coastal Mediterranean ecosystem. Although highly productive this Mediterranean marine flower plant is not much consumed by herbivore organisms. During autumnal senescence, most (up to 90%) of the foliar primary production of *P. oceanica* ends in the "detrital compartment". These dead leaves, also called "macrophytodetritus", begin to degrade immediately inside the meadow, but a large amount will be rapidly exported to adjacent unvegetated accumulation zones, such as bare sand patches. Associated to drift macroalgae, living detached *P. oceanica* shoots, micro-organisms and fine sediment, these macrophytoderitus form what we call "exported *P. oceanica* litter".

This exported litter is a highly dynamic habitat for a whole community of invertebrates: meiofauna (38µm < size < 500µm) and macrofauna (size ≥ 500µm) on which we focused on. This dynamic nature of exported litter could play a major structuring role in terms of abundance, diversity and trophic ecology of this vagile macrofauna community at a seasonal, annual or spatial scale, but also during stochastic, brief and very strong perturbations: resource pulses.

In this context, this PhD Thesis had 7 main objectives:

i. Characterize for the first time exhaustively the macrofauna community.
ii. Evaluate the spatiotemporal changes occurring at two different time scales in the detritus themselves and in the macrofauna community.
iii. Relating these variations with measured environmental parameters.
iv. Experimentally demonstrate the stratification occurring in a stable *P. oceanica* litter accumulation and the impact of this stratification on environmental conditions and on the macrofauna.
v. Experimentally demonstrate the impact of resource pulses on the exported *P. oceanica* litter macrofauna community.
vi. Unravel for the first time the global *P. oceanica* litter macrofauna food web using gut contents examinations and stable isotopes (C and N).
vii. Evaluate the spatiotemporal changes of diet preferences of this community and determine if the observed changes are really synonym of true diet changes.

This PhD Thesis demonstrated that exported *P. oceanica* litter was mainly composed of dead *P. oceanica* leaves (70-80%). It followed the natural annual cycle of *P. oceanica* and presented a maximum abundance in autumn just after leaves senescence. Measured environmental parameters also showed important variations linked to different factors such as force and direction of the wind, litter abundance and probably temperature. The continuous presence of the vagile macrofauna community throughout the year was demonstrated as well. This community was composed of 115 species and largely dominated by arthropods (77%), followed by annelids (12%) and mollusks (7%), while other taxa were much more anecdotal. Even if diversity is quite important, only a few species dominate largely the community. Indeed, 19 species represent more than 90% of the total abundance. One species to keep in mind: *Gammarella fucicola*, the most typical dominant and abundant amphipod species, representing 40-50% of the total abundance.

In addition to this general pattern, litter vagile macrofauna presented important seasonal and annual variations. In the case of several species, these variations could be linked to some measured environmental parameters, but we had to recognize that most species did not seem to be influenced by environmental parameters measured during this PhD. However, oxygen concentration was the most important environmental parameter, potentially influencing 7 of the 19 most dominant and abundant species. The experimentally demonstrated physico-chemical stratification occurring inside litter accumulations was strongly related to this oxygen parameter. Indeed we demonstrated that several species were distributed in the different layers of a litter accumulation according to oxygen concentration and to a lesser extent, to nutrients concentration (mostly NH_4).

Besides, smaller time scale sampling allowed the identification of several stormy events corresponding to the definition of resource pulses. These pulses were demonstrated to play a potentially important role on the structure of the macrofauna community, favoring importantly the detritivore species and hypoxia tolerant species. It was also demonstrated that resource pulses could induce diet switching increasing the consumption of dead *P. oceanica* leaves just after the events, potentially increasing the litter decomposition by the macrofauna.

The trophic web described in this PhD Thesis was composed of several trophic levels, from the primary herbivore/detritivore consumer, to second order carnivore predators. Different dietary preferences were highlighted, but major information was that dead *P. oceanica* leaves were ingested by a majority (85%) of the sampled species. Moreover, stable isotope analysis confirmed that *P. oceanica* litter was assimilated by most primary consumers and this "detrital signal" could be identified to the upper trophic levels, which is an argument in favor of the importance of macrofauna as major dead *P. oceanica* leaves decomposers. This also highlighted their potential role in terms of organic matter transfer from the *P. oceanica* meadow itself to the Mediterranean coastal food webs. Seasonal variations were observed in terms of trophic niches, and SIAR mixing model confirmed that this variability was sometimes caused by real diet modifications, potentially linked to the variable availability of food sources.

This PhD Thesis, combining standardized sampling at two different time scales, trophic web analysis (gut contents and stable isotopes) and original experimentation allowed us to describe a diverse and abundant macrofauna community associated to *P. oceanica* exported litter, its temporal variations, potential responses to resource pulses as well as the link existing between some species and measured environmental parameters. This PhD also described the food web of this community and demonstrated the importance of dead *P. oceanica* leaves as food source for many invertebrates composing this community. These invertebrates thus seemed to play an important role in both litter decomposition and organic matter flux from the P. oceanica meadow to the Mediterranean coastal food webs.

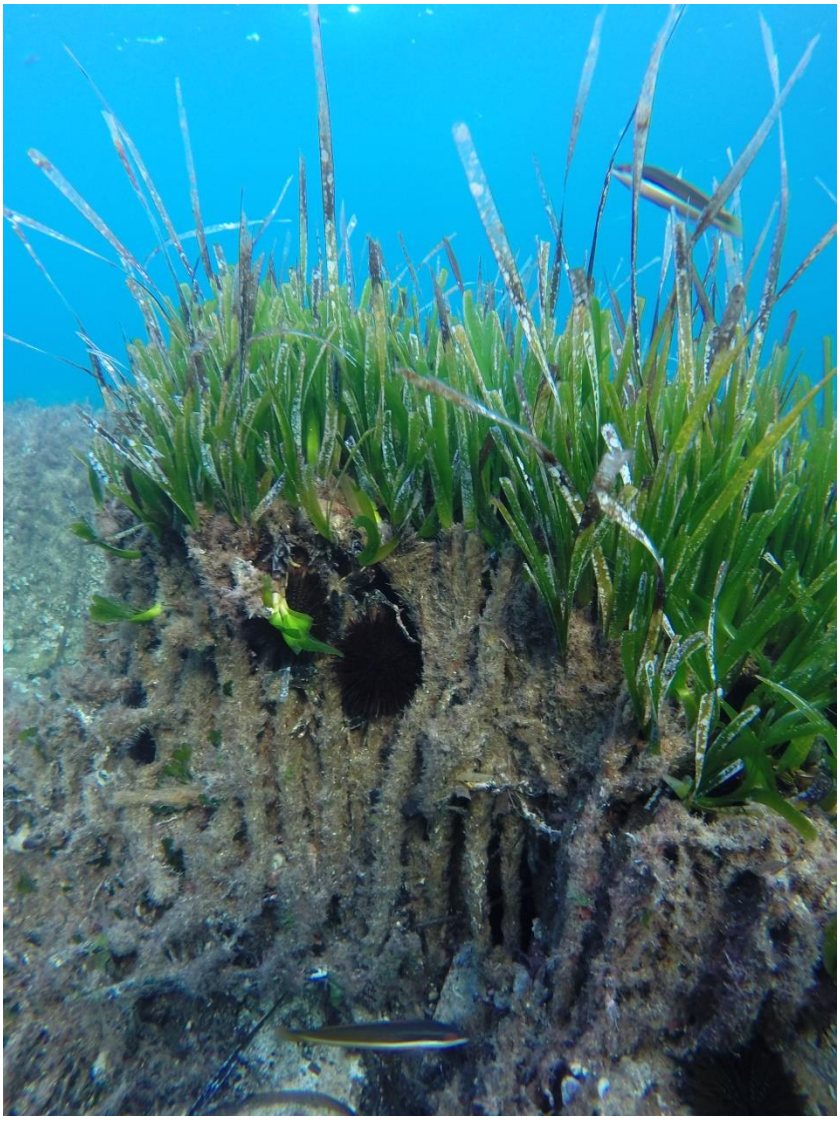
List of abbreviations

1-λ': Simpson evenness index
Ac: *Apanthura corsica*
An: *Athanas nitescens*
Br: *Bittium reticulatum*
C: harpacticoid copepods
DL: dead *P. oceanica* leaves
DM: dry mass (in table and figures)
DR: dead *P. oceanica* rhizomes
E: epiphytes (in Chapter 3 and 4), epiphytes/macroalgae (in Chapter 5)
EMA(s): exported macrophytodetritus accumulation(s)
Ga: *Gammarus aequicauda*
Gf: *Gammarella fucicola*
GFMH: pool of *Gammarella fucicola* and *Melita hergensis*
Gi: *Galathea intermedia*
Gspp: *Gobius* spp.
H': Shannon-Wiener diversity index
HARBOR-site: sampling site in the STARESO harbor
Hl: *Hippolyte leptocerus*
Ib: *Idotea balthica*
IW: interstitial water
Lh: *Liocarcinus holsatus*
LL: living *P. oceanica* leaves
Ln: *Liocarcinus navigator*
MA: drift epilithic macroalgae
Mh: *Melita hergensis*
Ml: *Macropodia linaresi*
Ngu: *Nototropis guttatus*
Ns: *Nebalia strausi*
OSCE-site: sampling site at the Punta Oscelluccia
P: pool of "intermediate" organisms
Pe: *Processa edulis*
PF: pool of *Palaemon xiphias* and *Processa edulis*
Pspp: Polycheata spp.
Px: *Palaemon xiphias*
RMA: drift red macroalgae
S: species richness
SD: standard deviation
SEAc: standard ellipse area
SIA: stable isotope analysis
SIAR: stable isotope analysis in R
SIBER: stable isotope Bayesian ellipses in R
Sl: *Stenosoma lancifer*
SPOM: suspended particulate organic matter

T-defaun.: treatment composed only of “defaunated” dead *P. oceanica* leaves without macrofauna
T-fauna: “natural” litter treatment composed of both dead *P. oceanica* leaves and the macrofauna
$_{Total}$DM: total litter dry mass
WC: water column
WI: water inside *P. oceanica* litter
WJA: water just above *P. oceanica* litter

Chapter 1

General introduction



1. Seagrass ecosystems

1.1. General definition

Seagrasses are defined as Magnoliophyta confined to the marine environment, growing and reproducing in the photic zone. All seagrasses satisfy **five** major criteria: (1) ability to grow while wholly submerged; (2) toleration towards salinity; (3) developing efficient anchoring roots and rhizomes; (4) capacity for hygrophilous pollination; and (5) capacity to disperse in the marine environment (Arber, 1920; denHartog, 1970). Seagrasses all belong to the Monocotyledonae group (Monocots), but constitute more a **paraphyletic ecological group** than an actual taxonomic group. Indeed, seagrasses are composed of various families, not necessarily phylogenetically closely related (denHartog and Kuo, 2006). Seagrasses often form extensive beds called "meadows" which can be constituted of a single species (monospecific meadows, often in the temperate areas) or of an assemblage of different species (polyspecific meadows, often in the tropical areas).

1.2. Systematics and biogeography

Seagrasses represent only 0.02% of the total Alismatiflorae (Monocots) and the 66 known species are classified in 6 families and 14 genera. Families are : (1) **Zosteraceae**, comprising genera *Zostera*, *Heterozostera* and *Phyllospadix*; (2) **Cymodoceaceae**, comprising genera *Cymodocea*, *Halodule*, *Thalassodendron*, *Amphibolis* and *Syringodium*; (3) **Posidoniaceae**, comprising only genus *Posidonia*; (4) **Hydrocharitaceae**, comprising genera *Enhalus*, *Thalassia* and *Halophila*; (5) **Ruppiaceae**, comprising genus *Ruppia*; (6) **Zannichelliaceae**, comprising genus *Lepilaena*. Out of these 14 genera, 12 are exclusively marine. To these 12 genera, the genus *Ruppia* and the genus *Lepilaena* are added comprising two marine seagrass species and other aquatic plants. The status of "true" seagrass is still debated for *Ruppia aff. Tuberosa* and *Lepiaena marina* because these plants are found in the marine environment, but not restricted to it (denHartog and Kuo, 2006).

Seagrasses are present **worldwide** (Table 1.1). Genera *Thalassia*, *Cymodocea*, *Syrindodium*, *Halodule*, *Halophila*, *Thalassodendron* and *Enhalus* are mainly distributed along the world's tropical areas. Genera *Zostera*, *Posidonia*, *Pyllospadix*, *Heterozostera* and *Amphibolis* are more concentrated along the world's temperate shores. Genus *Zostera* crosses the Arctic Circle in Europe and Northern Pacific. No seagrass is found in Antarctica.

Table 1.1: summary table of world repartition of seagrass genus (adapted from Papenbrock, 2012 and DenHartog and Kuo, 2006).

Family	Genus	Distribution
Zosteraceae	*Zostera*	1,3,4,5,6
	Heterozostera	6
	Phyllospadix	4
Cymodoceaceae	*Cymodocea*	1,3,5
	Halodule	1,2,5
	Thalassodendron	5,6
	Amphibolis	6
	Syringodium	2,5
Posidoniaceae	*Posidonia*	3,6
Hydrocharitaceae	*Enhalus*	5
	Thalassia	2,5
	Halophila	2,3,4,5,6
Ruppiaceae*	*Ruppia*	1,3,6
Zannichelliaceae*	*Lepilaena*	6

1 Temperate North Atlantic; 2 Tropical Atlantic; 3 Mediterranean; 4 Temperate North Pacific; 5 Tropical Indo-Pacific; 6 Temperate Southern Oceans

* true seagrass status still debated

1.3. Mediterranean Sea: a particular place

Only **five species** can be found in the coastal zones of the Mediterranean Sea. (1) *Zostera noltii* can be found from the intertidal zone to a depth of a few meters on sandy and muddy substrates; (2) *Zostera marina*, considered as a relict species, forms meadows from the intertidal zone to a depth of a few meters on sandy and muddy substrates but also in lagoons; (3) *Cymodocea nodosa* mostly grows in shallow waters but can rarely be found to 35-40 meters on sandy bottoms; (4) *Halophila stipulacea*, a recently introduced species from the Red Sea, is found in the eastern Mediterranean basin from the intertidal zone to a depth of 25 meters on muddy and sandy bottoms but also colonizing *P. oceanica* "dead matte" (see § 2.1); (5) *Posidonia oceanica*, the

biggest and **most abundant** seagrass of the area, forms extensive meadows from the surface to a maximum depth of 40-45 meters on sandy and rocky substrates (denHartog, 1970; Bay, 1984; Lipkin *et al*., 2003; Procaccini *et al*., 2003; Gambi *et al.* 2009). *Posidonia oceanica* will be discussed in detail in the next section.

Reports of *Ruppia spp*. along Mediterranean coasts exist, but little is known and due to the unclear status of these species as "true" seagrass, they will not be considered further.

2. The case of *Posidonia oceanica*

2.1. General morphology, biogeography and biology

Genus *Posidonia* contains **8 species** and is considered to be the only seagrass genus to be part of the earliest marine Magnoliophyta (denHartog, 1970, Aires *et al*., 2011). Seven species are found in southwestern, south and southeastern Australia, and one, *Posidonia oceanica*, is only found in the Mediterranean Sea. *P. oceanica* has been separated from the two Australian clades for at least 65.5 million years (Aires *et al*., 2011).

Neptune grass grows in **wide** temperature intervals, ranging from 9 to 29°C (Boudouresque and Meinez, 1982; Gobert et al. 2006), does not grow in salinity lower than 33 (but see Meinez, 2009) and is not tolerant to water turbidity or desiccation. *P. oceanica* is found from Almeria-Oran front in the western Mediterranean (it does not grow in the Atlantic surface waters near Gibraltar), and in all the eastern Mediterranean apart from Syria, Israel and Lebanon coasts (Fig. 1.1). Except in these two zones, *P. oceanica* forms continuous meadows from the surface to a maximum depth of 40-45 meters except in areas influenced by large estuaries (mostly Rhone, Po and Nile estuaries) (Gobert et al. 2006). *P. oceanica* occupies from 25 $.10^3$ km^2 to 45 $.10^3$ km^2, which represents 1-2% of the Mediterranean (Pasqualini *et al*., 1998).

As mentioned in § 1.3, *Posidonia oceanica* is the most abundant and biggest seagrass of the Mediterranean Sea. It is considered to be endemic to the Mediterranean even if small and long isolated populations have been found in the Dardanelles strait and Marmara Sea, questioning its endemic status (Meinez, 2009).

Figure 1.1: Geographical distribution (solid green line) of Posidonia oceanica in the Mediterranean Sea. R = Rhone estuary, P = Po estuary, N = Nile estuary. Using data from Lipkin et al., 2003; Procaccini et al., 2003; Gobert et al., 2006 ; Meinesz et al., 2009 and Boudouresque et al., 2012; Vacchi et al., 2016.

The Neptune grass, *P. oceanica* is composed of a belowground part, comprising roots and rhizomes, and an aboveground part comprising the leaves. Leaves are **long** (75 cm on average, but up to 130 cm) (Gobert, 2002; Pérez-Lloréns *et al*., 2013), ribbon-shaped and flexible (Cinelli *et al*. 1995; Hemminga and Duarte, 2000; Boudouresque *et al*. 2006), which enhances the sediment and particular matter **deposition** in the meadows (Gacia and Duarte, 2001; Gobert *et al*., 2006) (Figure 1.2). Leaves of *P. oceanica* are very dense (up to 1000 shoots.m^{-2} in very dense meadows), which increases drastically (each square meter corresponds to 6-29 m² of available surface) the available surface of the meadows for **epiphyte** colonization (Buia *et al*., 2000). Groups of 4 to 8 leaves are attached to rhizomes by strong and fibrous lignified petioles (also called "**scales**" after leaves abscission) forming a "shoot". Rhizomes are of two types: (1) "**Plagiotropic**" rhizomes, growing horizontally to colonize new adjacent favorable habitats; (2) "**Orthotropic**" rhizomes, growing vertically for the sun and avoiding burial by particular material deposition.

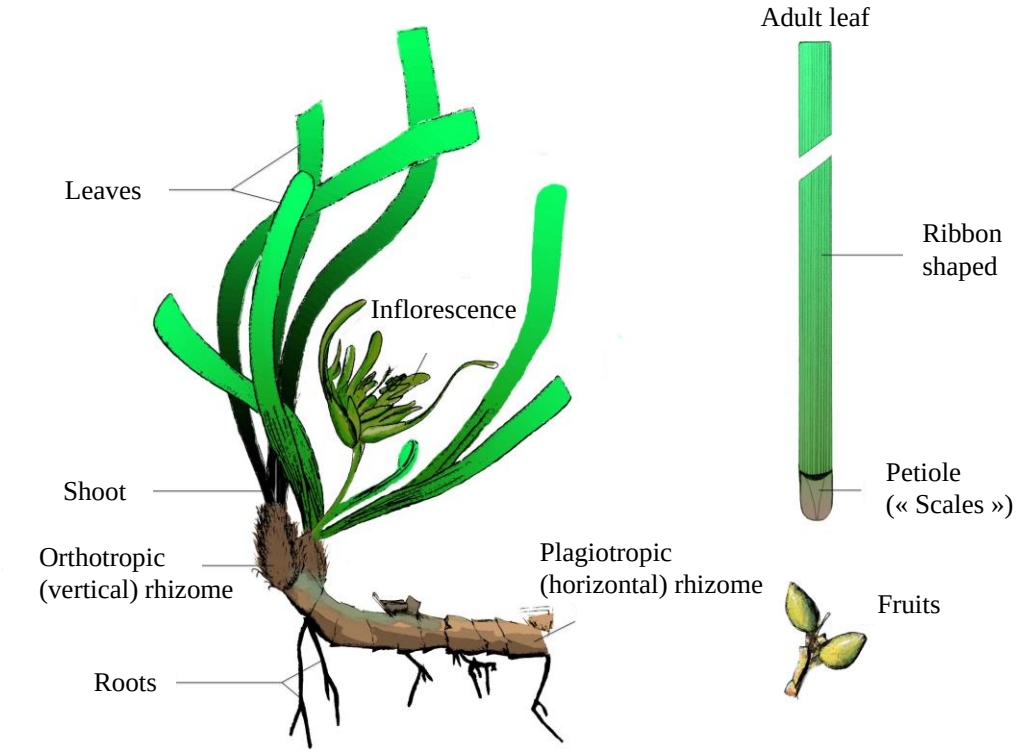


Figure 1.2: General morphology of a flowering Posidonia oceanica shoot as well as two mature fruits (right). Adapted from pictures in Boudouresque et al., 2012.

This rhizome growth associated with roots, huge amounts of deposited sediment and dead lignified scales forming a typically flat and terraced structure is called the "**matte**" (Boudouresque and Meinez, 1982, Boudouresque *et al*., 2006, Gobert *et al*., 2006). Rhizomes are growing slowly (Buia *et al*., 2000), and the matte can accumulate dead refractory material for long periods of time. Residence time of deposited material in the matte has been estimated between 2800 and 12500 year (Mateo *et al*., 1997; Mateo *et al*., 2002). The matte may eventually become "**dead matte**" if *P. oceanica* dies, leaving the interlaced dead rhizomes, scales and sediment devoid of life.

2.2. Various roles of *P. oceanica*

Foundation species are defined as "single species that define much of the structure of a community by creating locally stable conditions for other species, and by modulating and stabilizing fundamental ecosystem processes" (Dayton, 1975). Due to its major importance in ecosystems and the important impact of its depletion or disappearance, *P. oceanica*, like all seagrasses, kelp or mangroves, is a "foundation species" (Valentine and Duffy, 2006).

P. oceanica drastically influences the coastal Mediterranean Sea by physical and chemical processes, making it a true **ecosystem engineer** (Jones *et al*., 1994). *P. oceanica* meadows reduce hydrodynamism (Gacia and Duarte, 2001; Gobert *et al*., 2006) and thus enhance particular matter deposition (causing matte accumulation, see § 2.1). They also reduce the impact of coastal erosion by waves and storms, thus playing a major role in coastal stabilization (Hemminga and Duarte, 2000; Boudouresque *et al*. 2006). This stabilization role may take place far from the meadow itself by the means of exported dead leaves and rhizomes. This dead material, exported on the beach forms large beach wrack accumulations called "**banquettes**" (Boudouresque and Meinez, 1982).

Figure 1.3: Huge dead P. oceanica leaves "banquette" accumulation in June 2015 on Junquidou beach near Ile-Rousse, Corsica.

They can reach up to 2.2 meters (Boudouresque *et al*. 2006, personal observation, Figure 1.3) and play an important role in erosion mitigation (Mateo *et al.*, 2003), on sediment chemistry due to nutrient leakage (Orr *et al.* 2005; Cardona and Garcia, 2008) but also as habitat and food provider (Cardona and Garcia, 2008).

P. oceanica also plays a role in various biogeochemical processes (Figure 1.4): oxygen production (up to 14 $L.m^{-2}$); nutrients recycling, organic matter mineralization (through bacterial activity) and subsequent importance for C/N/P/S cycles (Bay, 1984; Marbà *et al*., 2006).

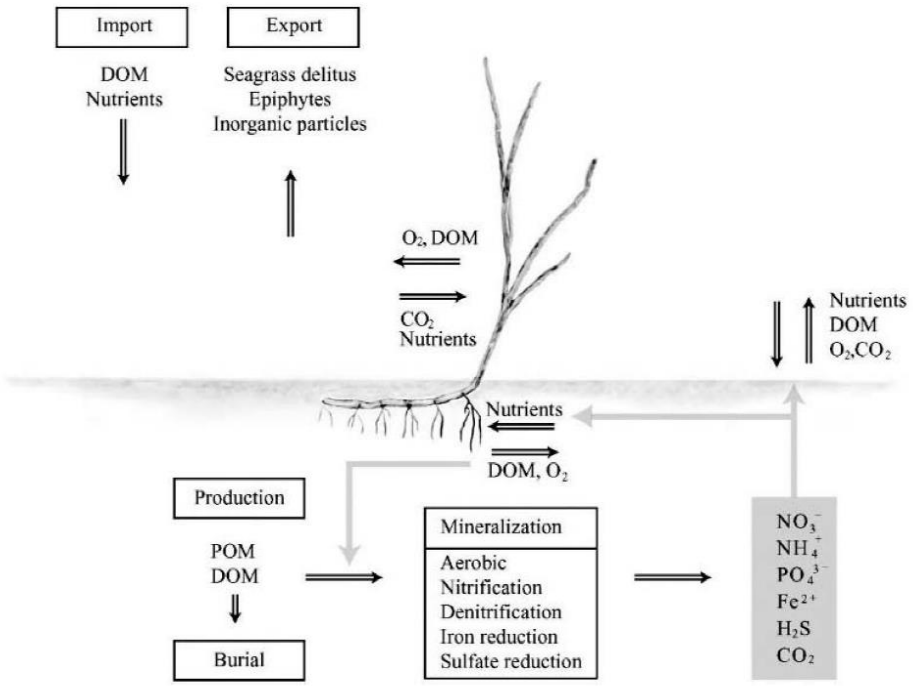


Figure 1.4: Biogeochemical role of Posidonia oceanica and exchanges between the plant, the sediment and water column. From Marbà et al., 2006

Posidonia oceanica is also of major importance as a **supplier of habitat** and food source for a large amount of species. The long life span and complex structure of the aboveground and belowground parts of the meadow provide drastically different microhabitats for various organisms (more than 1000 species), epiphytes or not, like bacteria, fungi, protozoans, algae, invertebrates and fishes, displaying various ecological preferences (Hemminga and Duarte,

2000; Boudouresque *et al*., 2006). Neptune grass is not only a habitat for organisms, but also a food source. Direct **herbivory** is generally considered to be rather **limited** in most of the Mediterranean Sea (Pergent *et al*., 1994; Mateo and Romero, 1997; Cebrian and Duarte, 2001; Walker *et al*., 2001; Moore *et al*., 2004; Cardona *et al*., 2007) and it has been estimated that only 10% of *P. oceanica* organic matter enters under its living form in the Mediterranean coastal food webs (Cebrian *et al*., 2006). It has however recently been demonstrated that herbivory impacts on seagrasses, shows important spatio-temporal variations, and has been vastly underestimated in some particular places. It can represent up to 70% of consumed *P. oceanica* organic matter (Thomas *et al*., 2005; Heck and valentine, 2006; Prado *et al*., 2007). Grazers are mainly the fish *Sarpa salpa* and the urchin *Pracentrotus lividus*, contributing in some places to the consumption of 40% and 17% of leaf production, respectively (Prado *et al*. 2007). In some places, on the other hand, herbivory only represents 2% of leaf production consumption (Thomas *et al*., 2005) and high consumption must be taken with care as herbivores sometimes **assimilate very ineffectively** *P. oceanica* tissues. Some fishes, like *Sarpa salpa* assimilate only 20% of the ingested living *P. oceanica* leaves (Vélimirov, 1984; Havelange *et al*., 1997). Reasons for this often low consumption are the low quality and digestibility, but also the presence of phenolic herbivore **deterrent** substances in particular taniferous cells inside *P. oceanica* leaf blades (Zapata and McMillan, 1979; Duarte, 1990; Vergès *et al*., 2007; Vizzini, 2009). In many places of the Mediterranean Sea, the detrital pathway is thus a very important way for incorporation of *P. oceanica* organic matter in the coastal food webs as a large amount of the foliar primary production ends in the **detrital compartment** (see §2.3, §3.1 and §3.2) (Romero *et al*., 1992; Valentine and Heck, 1999).

Epiphytes also constitute an important source of organic matter for various consumers. They are particularly abundant on *P. oceanica* leaves (up to 40% of foliar biomass) thanks to their **long life span** (up to 12 months) and may be of different types : (1) microepiphytes mainly consisting of cyanobacteria and diatoms; (2) epiflora, mainly represented by crustose Rhodophyta and Phaeophyta; (3) epifauna, mainly constituted by bryozoans (e.g.: *Electra posidoniae*), hydrozoans, foraminiferans, polychaetes and sponges (Novak, 1983; Mazzella *et al*., 1989; Buia *et al*., 2000; Hemminga and Duarte, 2000). Due to this diversity, their availability and better nutritional quality, epiphytes are a food source for many vagile invertebrates, but also part

of the diet of true *P. oceanica* consumers like *Sarpa salpa* and *Pracentrotus lividus* (Thomas *et al*., 2005; Prado *et al*., 2007).

2.3. Fate of *P. oceanica* primary production

Seagrasses in general are **important primary producers** for coastal areas even if covering a relatively small surface (up to 0.6 $.10^{-6}$.km^{-2} in Mateo *et al*., 2006) and are generally compared to tropical forests in terms of total primary production (Ferguson *et al*., 1980; Pergent *et al*., 1997). In the marine environment, only mangroves show higher primary production levels (Mateo *et al*., 2006), but primary production values present high variability according to the considered species. **Epiphytes** are very important contributors to the seagrasses primary production and biomass. They can represent 50% of the total primary production and 30% of the foliar biomass (Lepoint *et al*., 1999; Borowitzka *et al*., 2006; Mateo *et al*., 2006).

Compared to high turnover species like *Zostera noltii*, *P. oceanica* usually presents quite low values of primary production (Pérez-Lorénz *et al*., 2013), 875 gC.m^{-2}.year^{-1} and 392 gC.m^{-2}.year^{-1} respectively (but see Ott, 1980 mentioning a total production of more than 1000 gC.m^{-2}.year^{-1}). Foliar primary production of *P. oceanica* is very variable depending on season, depth, location and ranges from 126 gDM.m^{-2}.year^{-1} to 1230 gDM.m^{-2}.year^{-1} (roughly from 50 gC.m^{-2}.year^{-1} to 450 gC.m^{-2}.year^{-1}) but values of more than 3000 gDM.m^{-2}.year^{-1} have been mentioned (Ott, 1980; Pergent and Pergent-Martini, 1991; Pergent *et al*., 1994, Pergent-Martini *et al*., 1994; Buia *et al*., 2000, Gobert, 2002, Gobert *et al*., 2006). Rhizome primary production is much lower and ranges from 4 gDM.m^{-2}.year^{-1} to 85 gDM.m^{-2}.year^{-1} (roughly from 1 gC.m^{-2}.year^{-1} to 30 gC.m^{-2}.year^{-1}) and does not present the seasonal pattern observed for leaves (Bay, 1984; Pergent *et al*., 1994, Pergent-Martini *et al*., 1994; Buia *et al*., 2000, Gobert, 2002). For Calvi Bay at 10 m, conditions corresponding to our study site, foliar and rhizome primary production have been recorded as being 603 gDM.m^{-2}.year^{-1} and 34 gDM.m^{-2}.year^{-1} respectively (Bay, 1984). In terms of total biomass, the belowground compartment is much more important than the aboveground compartment. Foliar biomass of *P. oceanica* ranges from 175 gDM.m^{-2} to 900 gDM.m^{-2} (Duarte and Chiscano, 1999; Buia *et al*., 2000) and belowground (rhizomes+scales+roots) biomass ranges from 1610 gDM.m^{-2} to 6526 gDM.m^{-2}, representing approximately up to 10 times the foliar biomass (Duarte and Chiscano, 1999; Buia *et al*., 2000; Champenois, 2009).

As it was mentioned before (§ 2.2) herbivory importance can be high in particular places, but is most of the time quite low in most of the Mediterranean Sea. Most of the foliar primary production of *P. oceanica* (up to 90%) often ends in the "**detrital pool**" after shedding (see § 2.2 about herbivory) and degrades inside the meadow itself or is exported to other areas due to hydrodynamism (Pergent *et al.*, 1994; Cebrian and Duarte, 2001). These exported accumulations of dead leaves form what is called "exported litter", which is a very important link (Figure 1.5) between the meadow, other adjacent areas and deeper places, in terms of habitat and of food source (litter is described in detail in the next section in §3.1 and §3.2). Dead leaves not only accumulate underwater, they can also be exported in large amounts (Figure 1.5) to the beaches to form "**banquettes**" (see § 2.2) which also constitute a habitat and food source for many terrestrial invertebrates. These banquettes can also eventually break during storm events and return to the sea, entering the pool of exported litter again. As mentioned earlier, leaf scales, rhizomes and roots form the "matte", which is a long-term sink for organic matter which can represent an important part of *P. oceanica* primary production and biomass (Figure 1.5).

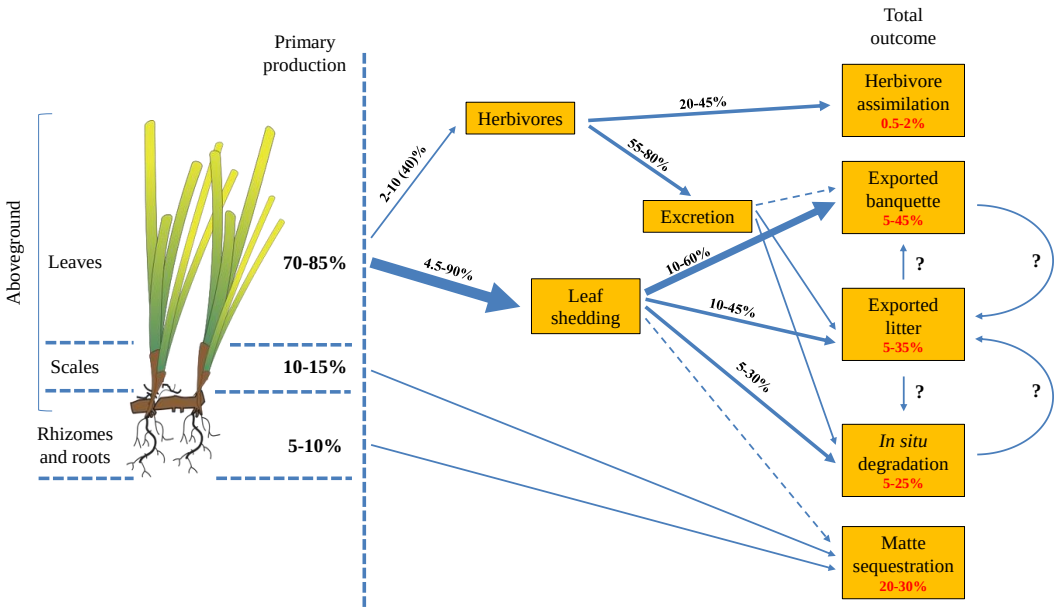


Figure 1.5: Fate of Posidonia oceanica primary production. Modified after Luque del Villar and Templado, 2004. Completed with data from Whitman et al., 1981; Romero et al., 1992 ; Pergent et al., 1994; Pergent et al., 1997; Cebrian and Duarte, 2001; Mateo et al., 2003, Mateo et al. 2006; Heck et al., 2008; Pérez-Lorénz et al., 2013.

3. Macrophytodetritus compartment

3.1. Litter general definition and the case of *P. oceanica* detritus

In all aquatic or terrestrial ecosystems based on macrophytes, detritus are present, and may constitute important and structuring components for the ecosystems themselves but also for other adjacent ecosystems. **Detritus** accumulations can be found in ecosystems driven by terrestrial trees, mangrove trees, algae, or seagrasses (Pergent *et al*., 1994; Tzetlin *et al*., 1997; Cebrian and Duarte, 2001; Mancinelli and Rossi, 2002; Vähätalo and Søndergaard., 2002; Lemke *et al*., 2007; Komiyama *et al*., 2008; Kristensen *et al*., 2011; Nagelkerken *et al*., 2008; Malhi *et al*., 2011; Mellbrand *et al*., 2011; Chiu *et al*., 2013; Boudouresque *et al*., 2015).

Forests are generally highly productive ecosystems where leaves shedding produces **detritus**. A very variable part of the total primary production ends in the detritus. Up to 40-50% of this total primary production is constituted by foliar production, the remaining 50-60% being sequestrated in wood and root production (Malhi *et al*., 2011; Malhi, 2012). About 90% of the foliar primary production forms detritus, supporting detritus based food webs composed of microorganisms like bacteria or fungi, and invertebrates like amphipods, springtails, worms or small mollusks (Gessner *et al*., 2010; Berg, 2014).

Mangroves are positioned at the **interface between land and sea** in the subtropical zones, constituting important ecosystems above and underwater (Bouillon et al., 2008; Kristensen *et al*., 2008; Komiyama *et al*., 2008; Nagelkerken *et al*., 2008). Litter fall represents only 13-41% of the total mangrove primary production, the remaining 60-85% being consumed by herbivore organisms (5-10%) or sequestrated in wood and roots (Alongi *et al*., 2005; Kristensen *et al*., 2008). Mangrove trees tend to show higher below ground biomass (roots) than terrestrial trees, partly due to the presence of a

large number of pneumatophores (respiratory roots) (Komiyama *et al*., 2008). Even if algae are present, numerous invertebrates, including meiofauna, herbivore insects and crabs consume and degrade the leaves present in the detrital pool. Consumption of detritus by crab species can sometimes be very important (up to 80%) (Bouillon *et al*., 2008). But mangroves also play a role in organic matter provider for adjacent ecosystems through exportation of organic matter from the detrital pool. Exportation can correspond to 50% of total litter production, making mangroves important food providers for other ecosystems (Kristensen *et al.*, 2008).

Ecosystems supported by **macroalgae**, such as kelp, are highly productive and also present an important detrital pool (Dayton, 1985). Litter production may account for more than 25% and up to 80-85% of total algal production, and is regulated mostly by hydrodynamic forces during stormy events (Dayton, 1985; Krumhansl and Scheibling, 2012; Orr *et al*., 2014). This detrital pool may be exported to beaches where it provides shelter and food for diverse invertebrate communities (Bustamante and Branch, 1996; Tzetlin *et al*., 1997; Orr et al., 2014). But detached kelp detritus can also be exported to adjacent or deeper underwater ecosystems and serve as habitat and food source (up to 65 of present organic matter) for more than 50 species of macroinvertebrates (Bustamante and Branch, 1996; Tzetlin *et al*., 1997). Deep sea urchins and brittle-stars, various suspension feeders, amphipods or leptostraceans are known to feed mostly on detached kelp detritus in a large variety of marine ecosystems, making **kelp detrital pool** a very important source of organic matter for other ecosystems (Duggins and Eckman, 1997; Tzetlin *et al*., 1997; Miller and Page, 2012; Filbee-Dexter and Scheibling, 2014; Orr et al., 2014).

Seagrass ecosystems are very productive but few herbivores are able to ingest and assimilate living leaves (see § 2.2 in the previous section) (Cebrian and Duarte, 2001; Cardona *et al*., 2007). Detritus thus generally play a key pathway of organic matter transfer from seagrasses to ecosystems and food webs (Mann, 1988; Pérez *et al*., 2001). For seagrasses like Halophila ovalis, Cymodocea nodosa, Thalassi hemprichii or Zostera marina, **detritus** show a clear seasonal abundance pattern and can represent up to **80%** of foliar primary production. Detritus then decompose underwater or can be consumed by a variety of bacteria and invertebrates, be exported (up to 30-35% of foliar production) or buried in the sediment. In the sediment, buried fine fractions of leaves may also constitute a food source for deposit feeders like sea cucumbers

or urchins (Hillman *et al*., 1995; Pérez *et al*., 2001; Anesio *et al*., 2002; Mancinelli and Rossi, 2002; Vähätalo and Søndergaard., 2002; Liu *et al*., 2013; Chiu *et al*., 2013).

P. oceanica meadows are no exception and **dead leaves** are present most of the year inside or outside the meadow, forming important and structuring macrophytodetritus accumulations (Moore *et al*., 2004). As mentioned in § 2.2 of the previous section, *P. oceanica* leaves shed all around the year with a massive shedding event in autumn (Velimirov, 1987; Cebrian *et al*., 1997; Mateo and Romero, 1997; Gobert *et al*., 2006). Dead leaves remain and decay in the meadow or, for most of them, are exported and form the "**detrital pool**" (Romero *et al*., 1992; Pergent *et al*., 1994; Heck *et al*., 2008; Vizzini, 2009; Pérez-Lorénz *et al.*, 2013). This detrital pool represents an important part of the plant foliar primary production (§2.3 from the previous section) and may end on beaches, forming beach wracks accumulations, or remain underwater where it constitutes a habitat and food source for various organisms (Gallmetzer *et al*., 2005, Lepoint *et al*., 2006, Michel, 2011; Mascart *et al*., 2014). Underwater, dead leaves associated with bacteria, fungi, microalgae, macroalgae, living leaves, uprooted rhizomes, dead organisms and fine sediment form what is called "**exported macrophytodetritus accumulations**", EMAs (Anesio *et al*., 2003; Boudouresque *et al*., 2006; Lepoint *et al*., 2006; Lemke *et al*., 2007; Mascart *et al*., 2014). These EMAs should not be confused with "*in situ*" litter accumulations, litter deposition inside the *P. oceanica* meadow, as they are completely different compartments in terms of dynamic and physicochemical conditions (Michel, 2011). This detrital pool is a very important key compartment in terms of organic matter flux from the meadow itself, to the adjacent ecosystems and the whole coastal environment in general (Romero et al., 1992 ; Pergent *et al*., 1994; Mateo *et al*. 2006; Heck *et al*., 2008; Pérez-Lorénz *et al.*, 2013).

3.2. Stocks, dynamics and decomposition

As mentioned earlier, exported dead leaves may **constitute from 10 to 80-90% of annual foliar primary production**, representing from 5 $gC.m^{-2}.year^{-1}$ to 682 $gC.m^{-2}.year^{-1}$ (Ott, 1980; Cebrian and Duarte, 2001; Heck *et al*., 2008, Boudouresque *et al*., 2015). From these exported leaves, litter constituting "banquettes" may account for up to 60% (Mateo *et al*., 2003;

Mateo *et al*., 2006; Heck *et al.*, 2008) and up to 35-40% (more than 230 $gC.m^{-2}.year^{-1}$) of the exported leaves thus form the EMAs. But precise EMAs stocks are particularly difficult to estimate due to the complex dynamic relationship between EMAs and other compartments. EMAs stocks are linked to stocks of exported leaves forming "banquettes", stocks of litter accumulated inside the meadow itself and stocks of litter exported to deeper areas. During **winter storm** events, there's a constant dead leaves importation-exportation balance and exchange between the 3 compartments and the EMAs ("detrital pool") making it very difficult to estimate the real stock of litter constituting EMAs.

As mentioned in §2.3 from the previous section, litter stocks and deposition show important seasonal variations, directly related to *P. oceanica* foliar production and hydrodynamism (Pergent *et al*., 1994, Buia *et al*., 2000, Gobert, 2002). Litter accumulation occurs in spring and summer when foliar production is high, but EMAs importance is maximal after early autumn massive shedding event. Litter accumulates inside the meadow where degradation and fragmentation begin (Romero *et al*., 1992; Pergent *et al*., 1994). A large amount of this litter is then exported during winter and this litter is transported to beaches to form "**banquettes**", deeper exportation sites and back to the meadow or to the shallow sand patches (EMAs), constituting a dynamic "detrital pool" (Figure x). Without being replaced due to the very low foliar production during winter and early spring, litter cover is much lower at the end of winter (Gallmetzer *et al*., 2005; Mateo *et al*. 2006; Michel, 2011). Accumulation increases again in late spring with the increase of foliar primary production.

From a purely chemical angle, *P. oceanica* leaves decomposition and degradation begin shortly before autumnal shedding with the **remobilization** of nutrients. Indeed it has been estimated that up to 30% P and 11% N could be removed from leaves just before senescence in autumn. The mechanism of these losses is unclear but leaching and reallocation inside the plant itself could occur together (Romero *et al*., 1992; Lepoint *et al.*, 2002). After the shedding event, degradation continues and after 100-120 days, additional depletion of 37% N and 35% P is measured in decaying dead *P. oceanica* leaves (Romero *et al*., 1992). Decay rate is quite slow and variable according to depth for *P. oceanica* leaves. Indeed, it has been demonstrated that after a 6-month experiment, a loss of 64% of dry mass at 20 m and 44% at 5 m was measurable (Pergent *et al*., 1994). Moreover, young litter decays faster than old dead leaves due to the presence of more refractory compounds in old litter (Mateo and

Romero, 1996). **Hydrodynamism** and transportation of these already chemically degraded dead leaves might also play a role in purely **mechanical fragmentation** during exportation throughout the whole year and especially in winter storm events by scraping leaves on the sea floor. Moreover, leaves deposited on beaches can be decomposed by erosion, microorganisms and small invertebrates like talitrid amphipods (Ince *et al*., 2007, Cardona and Garcia, 2008) and then return to the "detrital pool" and EMAs.

Once in the EMAs, dead leaves are also submitted to a very different degradation: degradation by living organisms inhabiting the exported litter. These organisms are comprised in two main categories: (1) **epiphytes**, (2) **vagile organisms**. Epiphytes are from different taxa: microorganisms like diatoms, bacteria and fungi, but also sessile macroinvertebrates like hydrozoans, bryozoans and sedentary polychaetes (Gambi *et al*., 1992; Lepoint *et al*., 1999; Kurilenko *et al*., 2001; Borowitzka *et al*., 2006; Lepoint *et al*., 2014). Most of these epiphytes are already present on living leaves inside the meadow before shedding, but showing a less diverse community pattern in the EMAs (Lepoint *et al*., 1999; Lepoint *et al*., 2014). Microepiphytes are known to degrade and live on refractory material such as highly lignified cells of the dead leaves and thus play a role in organic matter transfer from the EMAs to the coastal food webs (Romero *et al*., 1992; Romani *et al*., 2006; Mancinelli *et al*., 2009; Vizzini, 2009, Panno *et al*., 2013). Vagile organisms are composed of **meiofauna** (38µm < size < 1mm) and **macrofauna** (size > 0.5-1mm). Meiofauna is composed of species from the "permanent meiofauna" but also from the "temporary meiofauna", in other words, species which only spend a limited time in the meiofauna (often, only their larval stages) and spend their adult mature stage in the macrofauna category (Mascart, 2015). Meiofauna of the EMAs is mainly composed of copepods (up to 50% and largely dominated by harpacticoid copepods) and nematods (up to 20%) but also of less abundant taxa such as amphipods, flat worms, polychaetes and Nauplius larvae of diverse invertebrates (Mascart *et al*., 2015a). This quite diverse meiofauna community shows important spatiotemporal variations and contributes indirectly (through bacteria and biofilm consumption) to litter degradation and organic matter transfer from *P. oceanica* to higher trophic level organisms (Mascart, 2015). Macrofauna is discussed in detail in the next section.

3.3. Vagile macrofauna of EMAs

Oppositely to sessile macrofauna, vagile macrofauna is capable of free movements inside the EMAs. To our knowledge, **vagile macrofauna** of the EMAs in general has not been very much studied in the literature (See Gallmetzer *et al*., 2005; Dimech *et al*., 2006; Como *et al*., 2008 for preliminary community level studies and Lepoint *et al*., 2006; Sturaro *et al*., 2010 for particular species and taxa studies), making information about this community quite scarce. EMAs are known to serve as a shelter for a variety of vagile macroinvertebrates (Gallmetzer *et al*., 2005, Dimech *et al*., 2006). EMAs vagile macrofauna is composed of up to 80-100 species, largely **dominated by arthropods**, but significant abundances of **mollusks** and **annelids** are also reported. Arthropods are dominated by crustacean amphipods, representing from 80 to 97% of the total abundance, from which *Gammarella fucicola* represents up to 89% (Gallmetzer *et al*., 2005) followed by *Gammarus aequicauda*. Decapods are also well represented with *Athanas nitescens*, *Pisa tetraodon* or *Galathea intermedia*. Isopods are present in a lower but non-negligible abundance with *Idotea spp*. and *Stenosoma lancifer*. Among small arthropods, leptostracean *Nebalia sp.* is quite abundant (Gallmetzer *et al*., 2005). Mollusks seem to be much less abundant (up to 25% of total abundance) except for the cerithiid *Bittium reticulatum*. Annelids are the third main taxa in terms of abundance (up to 7% of total abundance), represented by polychaetes, dominated by the nereid *Platynereis dumerilii*. Echinoderms and juvenile fishes are also reported in very low abundances (Gallmetzer *et al*., 2005; Remy, 2010). This community is composed by organisms also found in other compartments or ecosystems (*P. oceanica* meadow canopy or *C. nodosa* beds), but in very **different assemblage** diversity and composition (Gambi *et al*., 1992; Como *et al*., 2008, Remy, 2010; Bedini *et al*., 2011; Michel *et al*., 2014) making EMAs a particular compartment in terms of the community present. EMAs vagile macrofauna seems to be influenced by the decomposition of the dead leaves, the resulting greater structural complexity of the habitat and possible oxygen stratification (Gallmetzer *et al*., 2005, Remy, 2010) but these hypotheses have not yet been tested. Case studies of particular species of amphipods and isopods demonstrate that most species ingest dead fragments of *P. oceanica* leaves, thus playing a potentially important role in mechanical fragmentation of the litter (Lepoint *et al*., 2006; Remy, 2010; Sturaro *et al*., 2010). It also appeared that several species assimilate a non-negligible part of

their organic matter from dead fragments of *P. oceanica* leaves (*e.g.*: dead leaves represent up to 20% of 3 idoteids species average diet in Sturaro *et al*., 2010 and up to 35-45% of *Gammarus aequicauda* average diet in Lepoint *et al*., 2006; Michel, 2011), thus also playing a role in organic matter transfer from the *P. oceanica* meadow, to the coastal food webs through the "detrital pathway".

4. Pulses: structuring events?

4.1. Definition and general framework

In terrestrial, estuarine and marine ecology, **disturbance** is regarded as playing a central structuring role of ecosystems (Giller, 1996; Lake, 2000). Disturbances, defined as potentially damaging forces to a habitat space occupied by a population/community, can be classified in 3 different classes, characterized by their temporal patterns: (1) **pulse**, (2) press and (3) ramp (Lake, 2000). Ramps are a steady increase of the disturbance over time (and often space), presses are a sharp increase of the disturbance that maintains a constant level afterwards and pulses are short term and sharp disturbances (perturbation, or resource). **Resource pulses** have recently been more precisely defined as "rare, brief and intense episodes of increased resource availability in space and time" (Figure 1.6) (Ostfeld and Keesing, 2000; Yang *et al*., 2008) and can take place in many different ecosystems (*e.g.*: massive floods in arid ecosystems or floodplains, dead leaves input in mangroves or forests, massive emergence of insects, seed mast events or storm-driven nutrients runoffs). Pulses can be caused by different factors: (1) climatic or environmental causes, (2) temporal accumulation and release, (3) spatial accumulation and release, (4) outbreak population dynamics (Yang *et al*., 2008).

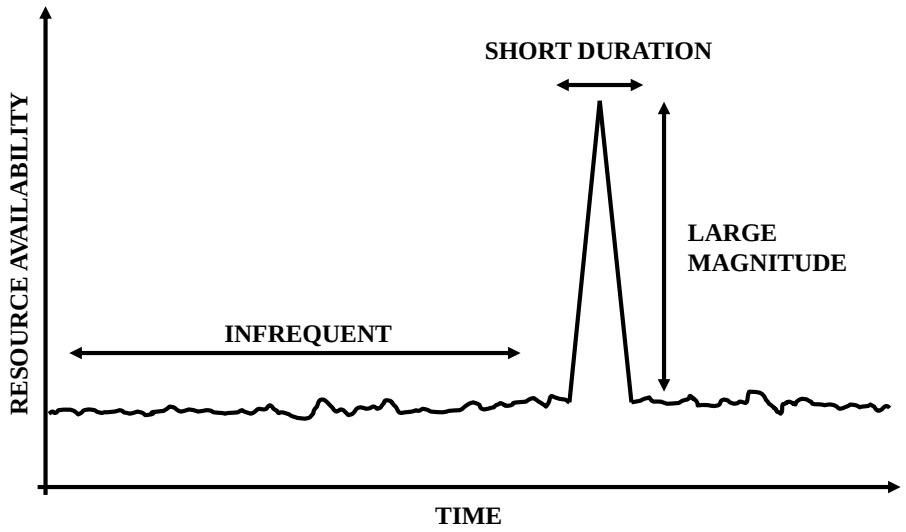


Figure 1.6: Resource pulses main characteristics. Low frequency, huge magnitude and short duration. Adapted from Yang et al., 2008.

Pulses are regarded as **single**, **brief** and **strong** events, but in nature, many pulsed events happen to recur over some time scales, like El Niño events, leaves "regular" inputs in forests, mangroves or streams, seasonal inundation of floodplains. Regarding the dynamic characteristics of EMAs and the link between litter availability with *P. oceanica* foliar production, with leaves shedding in autumn and with random storm events of winter, EMAs can be considered to be submitted all year long to the influence of pulsed perturbations in terms of resource availability and habitat conditions stability.

4.2. Impact on ecosystems

Pulses can play **major roles in structuring** ecosystems and regulating interactions between ecosystems, communities, populations or organisms within populations (Yang *et al*., 2008; Yang *et al.*, 2010) and their effects are strongly linked to their own duration, magnitude and frequency (Holt, 2008). Resource pulses can impact ecosystems at different levels: (1) individual level, making generalist species modify their diet but showing small effect on mobile specialists or opportunistic species; (2) population level, presenting evident bottom-up effects, favoring aggregation of individuals and/or increasing reproduction success and increasing the importance of detritivore species in

ecosystems; (3) community level, mostly increasing of diversity and indirect bottom-up effects through trophic levels followed by lagged top-down effect through trophic cascade. Pulses may also increase coexistence of different species with relatively similar ecological niches if recruitment increases sufficiently. If these effects alter drastically the present communities, effects of pulses can be permanent and the community deeply modified (Ostfeld and Keesing, 2000; Chesson et al., 2004; Yang, 2006; Holt, 2008; Nowlin *et al*., 2008; Yang *et al*., 2008; Shaner and Macko, 2011; Yee and Juliano, 2012).

Marine/aquatic and terrestrial ecosystems show very different responses to pulsed events mainly because of the characteristics of primary organic matter sources and of different trophic webs present (Nowlin *et al.*, 2008). Although clearly part of the marine ecosystems, EMAs, composed mainly of dead leaves of *P. oceanica*, present common characteristics with marine and terrestrial ecosystems driven by pulses. Like most marine/aquatic pulsed driven ecosystems, EMAs experience pulses of allochtonous resources, EMAs support a diverse community of invertebrates (meiofauna and macrofauna) showing for most of them low body mass, fast turnover and low generation time, inducing potentially shorter duration of pulses, shorter lag of response between trophic levels and lower persistence of pulsed events (Yang, 2006; Holt, 2008; Nowlin *et al*., 2008; Yee and Juliano, 2012). But EMAs, like forests, present a detritus based food web, and this detrital pool is composed of detritus less labile and digestible than other aquatic detritus, inducing potentially more persistent effects of pulses, potentially slower propagation of responses through the food webs, and a potentially higher impact of pulses on detritivore organisms and lower impact on herbivores (Anderson *et al*., 2008; Holt, 2008; Nowlin *et al*., 2008;).

Regarding these mixed characteristics, EMAs may constitute an intermediate compartment, presenting characteristics from both marine and terrestrial pulsed driven ecosystems and thus potentially showing intermediate responses to pulsed events.

5. Trophic ecology: gut content examination and stable isotope analysis

Trophic ecology has been a major topic in ecology for a long time and this field of study still receives much attention nowadays, mainly due to the discovery of a new and efficient technique a few decades ago: stable isotope analysis, **SIA** (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981; Dauby, 1989; Vanderklift and Ponsard, 2003; McCutchan *et al*., 2003; Fry 2006; Lepoint *et al*., 2006; Boecklen *et al*., 2011; Michel *et al*., 2015). Along with classical techniques like direct observation or gut content observations, stable isotopes have now proved their reliability to help understanding better animal trophic relationships.

5.1. Different approaches to delineate animal diets

5.1.1. A classical technique : gut content examination

One of the once most widespread, but nowadays too often neglected techniques to assess animal feeding habits is the **gut content examination**. This is usually done by dissection of the animal. Note that in specific cases, emptying the gut of live animals to avoid dissection, or direct observation of the gut after body wall discoloration is possible (Michel *et al*., 2014 after a method described in Guerra-Garcia and Tierna de Figueroa, 2009). Qualitative, semi-quantitative or quantitative gut content examination and identification is now possible. But this technique, even if potentially very informative, suffers **from several major imperfections**.

First, examination of digestive tracts can only give a very short-term "**snapshot**" idea of the animal diet at a given moment and at a given place. This can be a major problem as it is known that animal diets and food sources experience important variations through time and space (Dalsgaard *et al.*, 2003; Witteveen *et al*., 2009; Mascart, 2015; Michel *et al*., 2015).

The other major problem is that gut content examination only gives information about the ingested items, but ingestion does not always mean **assimilation**, especially in a detritus-dominated food web, such as the food web present in the EMAs, where items of very contrasted digestibility and palatability can cause a non-negligible bias by overestimating the real

consumption of too highly digestible or poorly digestible refractory items (Latyshev *et al*., 2004; Lepoint *et al.* 2006; Michel *et al.* 2014).

These limitations led to a clear conclusion: “classical” techniques are not sufficient to get a complete and clear overview of animal diets. This led to the discovery of other techniques and the development of trophic markers that give indirect information about the dietary habits of a given animal. The **perfect trophic marker** was defined by Dalsgaard *et al*. in 2003 as "a compound whose origin can be uniquely and easily identified, that is inert and non-harmful to the organisms, that is not selectively processed during food uptake and incorporation, and that is metabolically stable and hence transferred from one trophic level to the next in both a qualitative and quantitative manner".

This hypothetical perfect marker has not been found yet, and studies therefore rely on less perfect markers such as **stable isotopes**.

5.1.2. Stable isotopes

5.1.2.1. Definitions and general aspects

Atoms of a given element, that have the same number of electrons and protons (hence, the same atomic number) but that differ in the number of neutrons in the nucleus, and therefore differ in their atomic mass, are called **isotopes**. Isotopes can be **radioactive** or **stable**. Radioactive isotopes are unstable and show a tendency to progressively disintegrate into smaller and more stable nuclei, while stable isotopes, on the other hand, show no tendency to disintegration (Fry, 2006). Scientists suspect the existence of around 120 elements on the planet and around 3100 different corresponding isotopic forms (or nuclides). Among these 3100, most are radioactive, and only 283 (< 10%) are stable (Fry, 2006).

Except phosphorus (P), all major elemental constituents of organic matter (C, H, O, N, S) have at least two naturally occurring stable isotopes. Natural abundances of these isotopes can be found in Table 1.2.

Table 1.2: Average natural abundances of stable isotopes of major organic matter component elements (modified after Fry, 2006 and Tcherkez, 2010)

Element	Stable isotope	Mean natural relative abundance (%)	International standard
Carbon	^{13}C	1.11	Pee-Dee Belemnite (PDB)
	^{12}C	98.89	
Hydrogen	^{2}H	0.02	Standard Mean oceanic Water (SMOW)
	^{1}H	99.98	
Oxygen	^{18}O	0.2	Standard Mean oceanic Water (SMOW)
	^{17}O	0.04	
	^{16}O	99.76	
Nitrogen	^{15}N	0.36	Atmospheric Air
	^{14}N	99.64	
Sulfur	^{36}S	0.01	Canyon Diablo Troilite (CDT)
	^{34}S	4.20	
	^{33}S	0.75	
	^{32}S	95.04	

Trophic ecology often concentrates on C and N, but S has recently been increasingly used to delineate trophic relationships (Kharlamenko *et al*., 2001; Connolly *et al*., 2004; Dethier *et al*., 2013; Hobson *et al*., 2015). Table 1.2 gives the natural relative abundances, which are in fact rarely really informative. Indeed, the most useful parameter is the isotopic ratio (hereafter noted **R**) between abundances of the heavy isotope on the light one of a given element (Eq. 1). In the case of the most important biogenic elements (Table 1.2) these ratios are often very low and not very intuitive for further interpretation. A relative notation was thus developed to allow the use of very easily usable values. This notation, called "**delta notation**", or "**δ**", has the major advantage to position the obtained values on a common scale by comparing them to international standards.

The delta value is indeed a measure of the deviation of the analyzed sample from the value of the standard. This relative delta notation is expressed in per mil (‰) and is calculated according to Eq. 2:

$$R = \frac{[{}^{*}X]}{[X]} \qquad (1)$$

$$\delta\ {}^{*}X = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \times 10^{3} \qquad (2)$$

where R is the isotopic ratio, $^{*}X$ is the heavier isotope of a given element, X is the lighter isotope of a given element.

C, H, O, N and S stable isotopes are recognized as useful tools in ecological and biogeochemical applications (see the major reviews of Fry, 2006; Tcherkez, 2010 and Boecklen *et al*., 2011). In this thesis, a bidimentional isotopic approach was chosen, using the coupling of C and N stable isotope analysis to delineate the trophic relationships between the invertebrates living in the EMAs.

5.1.2.2. Use of C and N stable isotopes in trophic ecology

"You are what you eat (plus a few per mil)…" is the well-known sentence from DeNiro and Epstein in their founding paper of 1976 that summarized the main principle underlying the use of stable isotopes in trophic ecology. *"You are what you eat"* means that for a given element, the isotopic composition of a consumer is a **proportional mixture** of the isotopic composition of its food sources. The added *"few per mil"* are what is called the isotopic fractionation. **Isotopic fractionation** occurs for all elements and is controlled by biological, physical and chemical parameters. The different isotopes of a given element will show the same chemical behavior (same electronic structure) and thus be involved in the same reactions. However the different number of neutrons and the resulting difference of atomic mass influence their physical behavior and more particularly the reaction rate. Heavier isotopes react a little more slowly than the lighter ones, resulting in a difference of isotopic composition before and after each reaction.

The net result of all the fractionations taking place in an organism usually leads to a slight enrichment towards the heaviest isotope and is called **trophic enrichment**, or Trophic Enrichment Factor, **TEF** (Fry, 2006) and is calculated according to Eq. 3:

$$\Delta\ {}^{*}X = \delta\ {}^{*}X_C - \delta\ {}^{*}X_{FS} \quad (3)$$

where ${}^{*}X$ is a given element, $\delta^{*}X_C$ is the isotopic composition of a consumer and $\delta^{*}X_{FS}$ is the isotopic composition of its food source.

Carbon and Nitrogen behave very differently in terms of fractionation (Fry, 2006; Tcherkez, 2010).

Carbon fractionation occurs mainly during the assimilation of inorganic carbon assimilation and photosynthesis by primary producers at the base of the food web (Tcherkez, 2010). Due to the different reactions and enzymes involved in the different plant metabolisms (C3, C4, CAM) to fix CO_2, $\delta^{13}C$ experiences large variations among the different primary producers. It is even more complicated with aquatic plants that are able to use HCO_3^- or CO^2 dissolved in water. As HCO_3^- shows a much less negative $\delta^{13}C$ than CO_2, it has an important impact on the $\delta^{13}C$ of aquatic primary producers (Lepoint *et al*., 2004; Raven *et al*., 2002). The impact of initial carbon fixation and photosynthesis process by primary producers in terms of fractionation is far greater than all the other reactions involving carbon in the primary producers or in their consumers. This leads to one of the major properties of carbon stable isotope use in trophic ecology: $\Delta^{13}C$ between a food source and the consumer is often low and around 0-1‰, which mainly corresponds to the effect of the enzymes involved in respiration of the consumer (Vander Zanden and Rasmussen, 2001; Post, 2002; McCutchan *et al*., 2003; Fry, 2006). This means that the $\delta^{13}C$ is quite conservative in a trophic web and that $\delta^{13}C$ can often be used as an **organic carbon tracker**, to identify the different potential food sources of a consumer (DeNiro and Epstein, 1978; Post, 2002).

Nitrogen fractionation at the base of the food web is more variable and unclear than the fractionation observed for carbon (Vanderklift and Ponsard, 2003; Schmidt *et al*., 2004; Fry, 2006). It depends on the many nitrogen pools used by the consumer and on the various intake mechanisms. Unlike carbon, excretion of nitrogenous wastes and protein metabolism of the consumers induce a non-negligible fractionation. $\Delta^{15}N$ is thus generally higher and much

more variable than, $\Delta^{13}C$ (McCutchan *et al*., 2003; Vanderklift and Ponsard, 2003), around 1-4‰ between a food source and its consumer. This induces the major property of nitrogen stable isotope use in trophic ecology: $\delta^{15}N$ increases between every trophic level of a food web and can thus be used as a good **estimator of the trophic level** of an organism (DeNiro and Epstein, 1981; Hobson *et al*., 1995; Fry, 2006).

Note that these very general statements about $\delta^{13}C$ and $\delta^{15}N$ are in no way absolute rules and that TEF values can be very different (see Vanderklif and Ponsard, 2003; Devries *et al*., 2015).

These general statements are summarized in Figure 1.6, which is, of course, a trivial highly simplified version of a real trophic web. Consumers have most of the time more than one food source, TEFs are highly variable from one species to another and sometimes from one food source to another mainly because of consumer age, food composition and "quality" (see Box 3) or nitrogen excretion processes (Adams and Sterner, 2000; Vander Zanden and Rasmussen, 2001; McCutchan et al., 2003; Vanderklift and Ponsard, 2003; Caut et al., 2010). Figure 1.6 depicts a highly simplified trophic web, comprising a hypothetical single primary producer (*P. oceanica*), a single primary consumer (amphipod) and a single predator (shrimp).

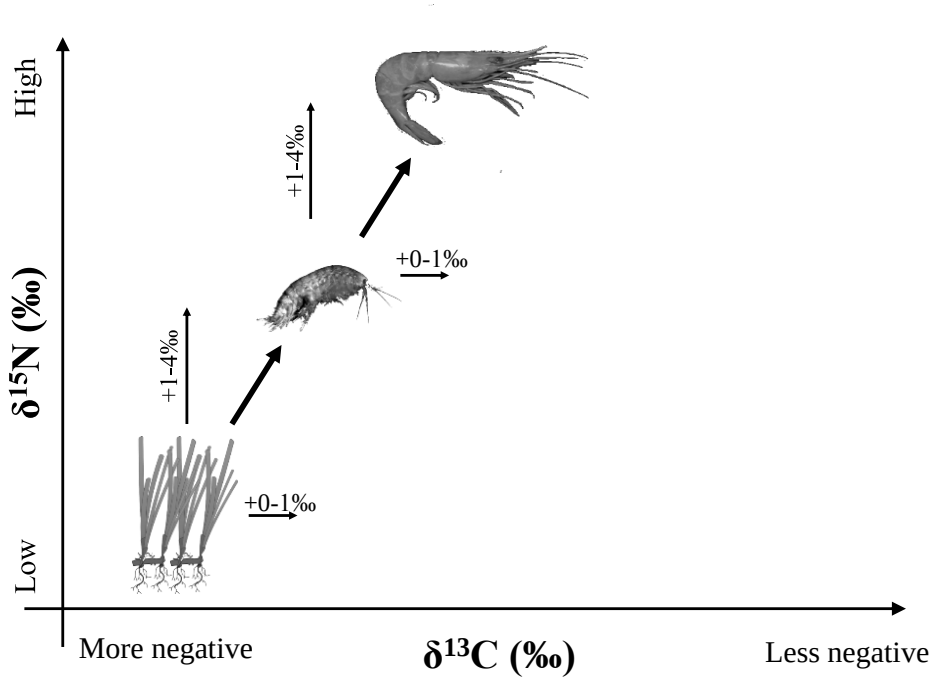


Figure 1.6: $\delta^{13}C$ vs. $\delta^{15}N$ biplot of a simplified hypothetical 3 level food web (amphipod and shrimp pictures are from François REMY, seagrass picture is taken and modified from Luque del Villar and Templado, 2004).

Compared to gut content analysis, stable isotopes present three major advantages. First, they only reflect the assimilated fraction of the diet. Secondly, as they do not disintegrate, they integrate animal diet information for a longer time (but isotopic turnover time is very organ-dependent, see Schmidt *et al*., 2004). Finally, isotopic composition of a given consumer is the proportional mixture of the isotopic compositions of its food sources, and this thus allows an important quantitative aspect (more details about mixing models in § 1.2.3).

This could seem to promote stable isotopes to the rank of "perfect trophic markers". However, stable isotopes are far from being perfect. Indeed, the use of stable isotopes as trophic markers depends on one important feature: the fact that every food source is isotopically distinguishable. It is often not the case and, for example, two very different food sources can have exactly the same isotopic composition, making discrimination and quantification of each food source contribution to the consumer diet impossible. Fractionation variability and isotopic routing makes it even more complicated to use and interpret (Gannes et al., 1997).

This is why trophic markers like stable isotopes are much more powerful when used in combination with other techniques such as gut contents examinations. This was the combination we chose to use during this thesis.

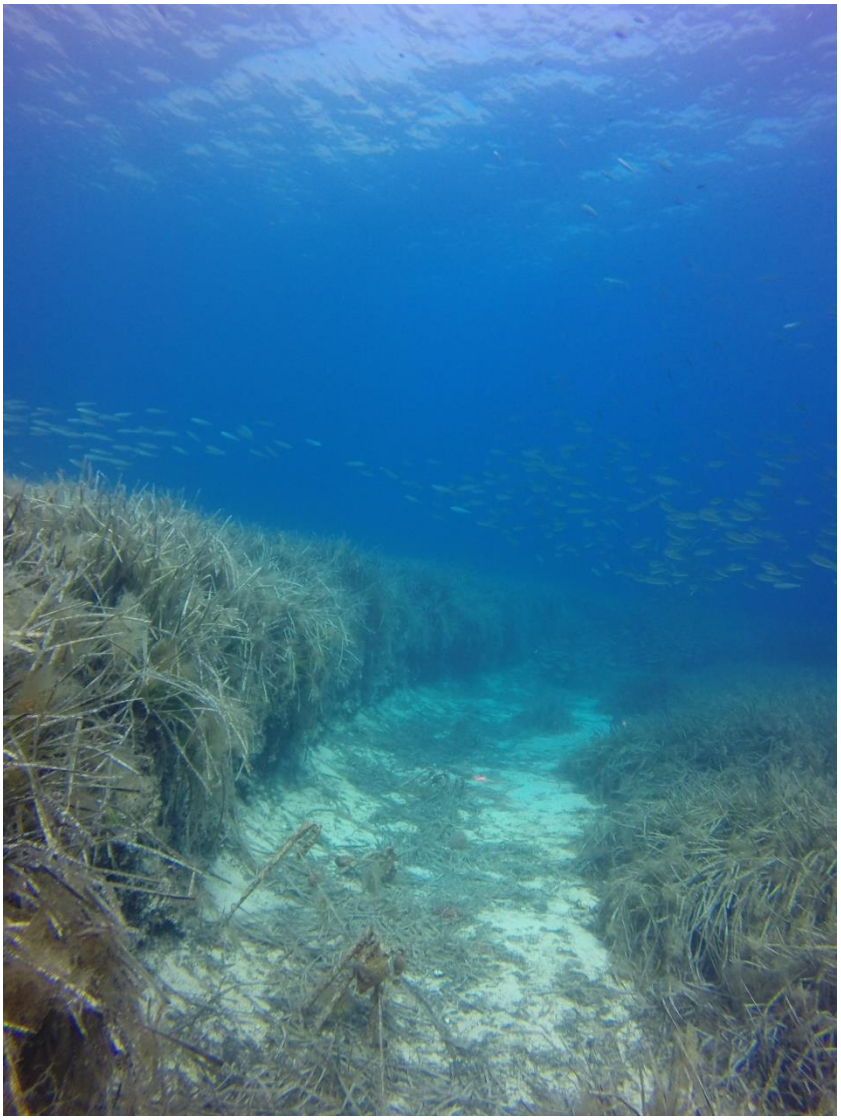
6. PhD specific objectives

The general objective of this PhD was to characterize the exported *P. oceanica* litter macrofauna community and to assess its dynamics and trophic ecology in Calvi Bay, Corsica. To achieve this goal, we tried to fulfill the following specific objectives:

i. Characterize for the first time the macrofauna community using a multi-year, multi-season and multi-site sampling (Chapter 3).
ii. Using this global baseline, evaluate the spatiotemporal changes occurring at two different time scales in the detritus themselves and in the macrofauna community (Chapter 3).
iii. Trying to evaluate the relationships between environmental parameters and the variations we observed at the community and the specific level (Chapter 3).
iv. Experimentally demonstrate the stratification occurring in a stable *P. oceanica* litter accumulation, the impact of this stratification on environmental conditions and on the macrofauna (Chapter 4).
v. Experimentally demonstrate the impact of resource pulses on the exported *P. oceanica* litter macrofauna community (Chapter 5).
vi. Unravel for the first time the global *P. oceanica* litter macrofauna food web using a multi-season and multi-site sampling (Chapter 5).
vii. Evaluate the spatiotemporal changes of diet preferences of 5 very abundant species and determine if the observed changes are really synonym of true diet changes (Chapter 5).

Chapter 2

General Material and Methods



1. Sampling site description

1.1. Calvi Bay

Calvi Bay lies on the northwestern coast of Corsica, in the western Mediterranean (42°35'N; 8°43'E, see Figure 2.1). The Punta di Revellata is the western limit or this 22 km² bay which is divided in two distinct areas: the Gulf of Calvi (east) where the city of Calvi lies, and the Revellata Bay (west) where the **STARESO** (STAtion de REcherches Sous marines et Océanographiques) oceanographic research station (University of Liège) is located.

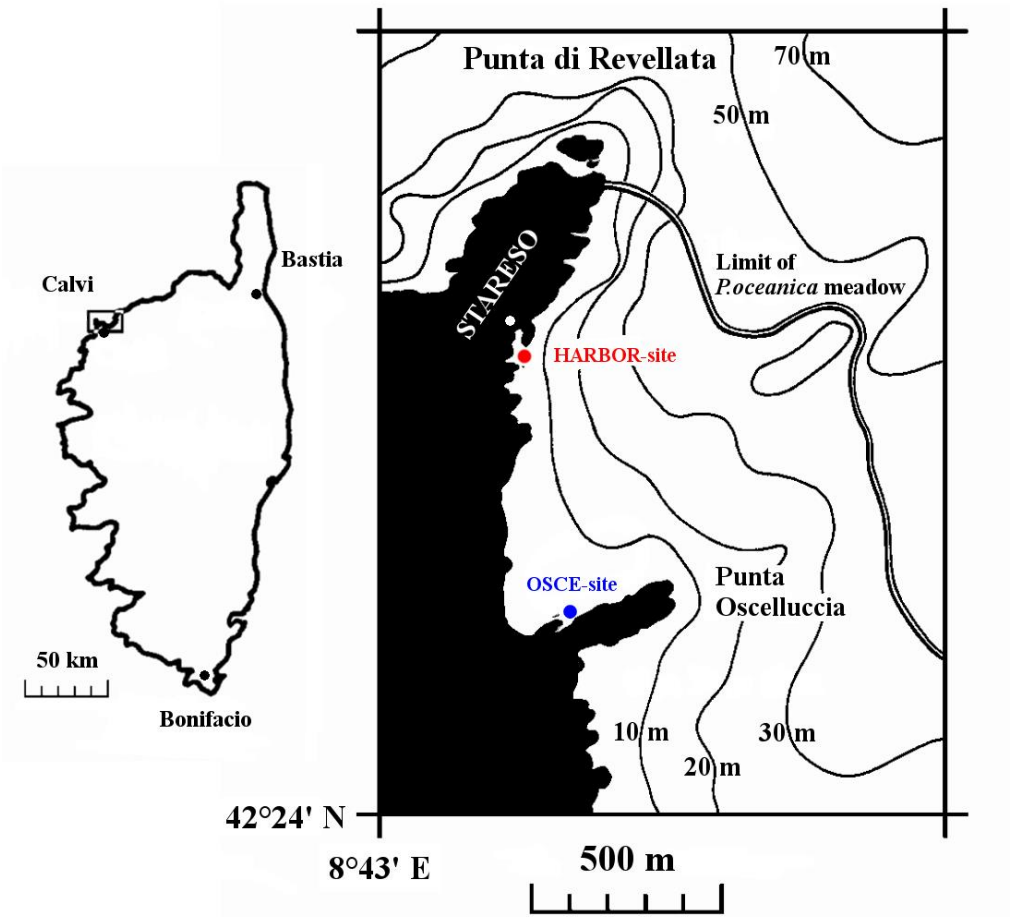


Figure 2.1: Left: general map of the location of Calvi Bay in Corsica. Right: precise location of the STARESO research station on the Punta di Revellata showing the two sampling sites, the lower limit of the P. oceanica meadow near STARESO and the 10, 20, 30, 50, 70m isobaths (modified after Gobert, 2002).

Tidal amplitude is rather weak (less than 10 cm) and the salinity is about 38 and also rather stable throughout the year. Surface water (3 m) temperature varies between about 26°C in August and about 12°C in February, with a marked thermocline at 25-30 m from May to October. Nutrients (N & P) concentrations in the water column show typical low values of oligotrophic areas (Gobert, 2002).

P. oceanica meadow covers 4.94km² in Calvi Bay (Figure 2.2), representing about **50% of the area of the Bay**, but shows a constant regression pattern since the 1990s (Abadie, 2012). *P. oceanica* can be found from 3 m to 38 m near the STARESO station, generally forming a continuous meadow (local "intermattes" may occur) mostly on soft substrate. Foliar biomass and primary production of Calvi Bay meadow are reported to be important (Bay, 1984; Gobert, 2002).

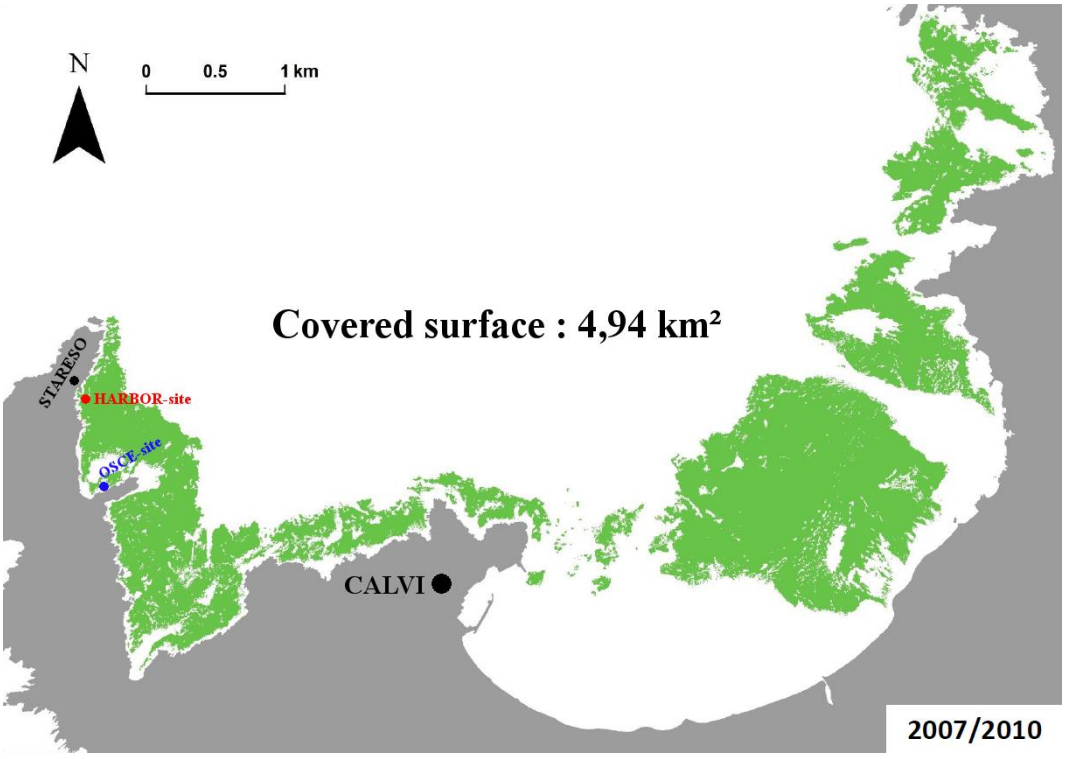


Figure 2.2: Map showing P. oceanica meadow extension and situation in Calvi Bay in 2007-2010. The position of both sampling sites is shown (red and blue dots) as well as the location of STARESO research station and the city of Calvi (modified after Abadie, 2012)

1.2. Sampling sites in details

The sampling sites were chosen for their relative proximity to the STARESO research facility, the regular observation of exported litter on them, and were located on the eastern side of the Punta di Revellata. **Two sites** of almost similar total area were defined for this PhD: the first one directly inside the harbor of the station: hereafter "**HARBOR-site**" (in red on Figure 2.1 and 2.2), and the second one 750 m away, right next to the northern side of the Punta Oscelluccia: hereafter "**OSCE-site**" (in blue on Figure 2.1 and 2.2). Both sites are shallow (8-10 m) sandy patches.

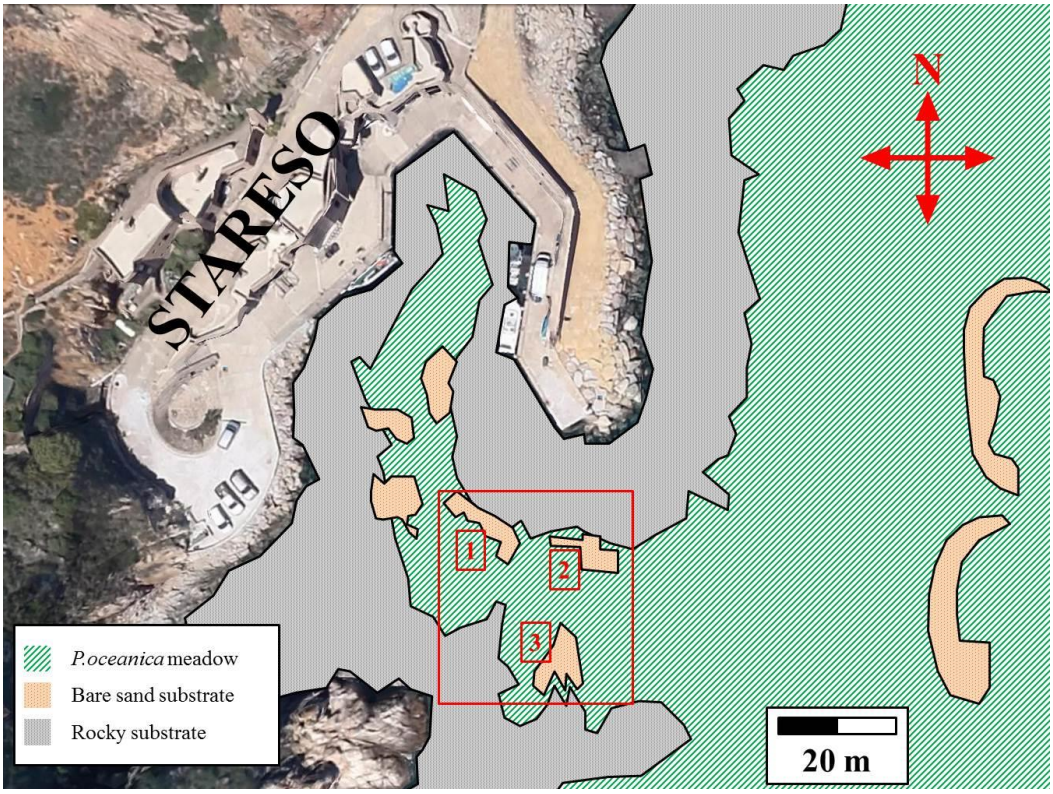


Figure 2.3: Simplified general map of the HARBOR-site (large red square) showing the different components of the sea bottom in the area. In lined-green: P. oceanica meadow; in dotted-brown : bare sand patches; in dotted-grey : rocky areas. Sandy patches marked 1, 2 and 3 are composing the HARBOR-site. Map based on Google earth image.

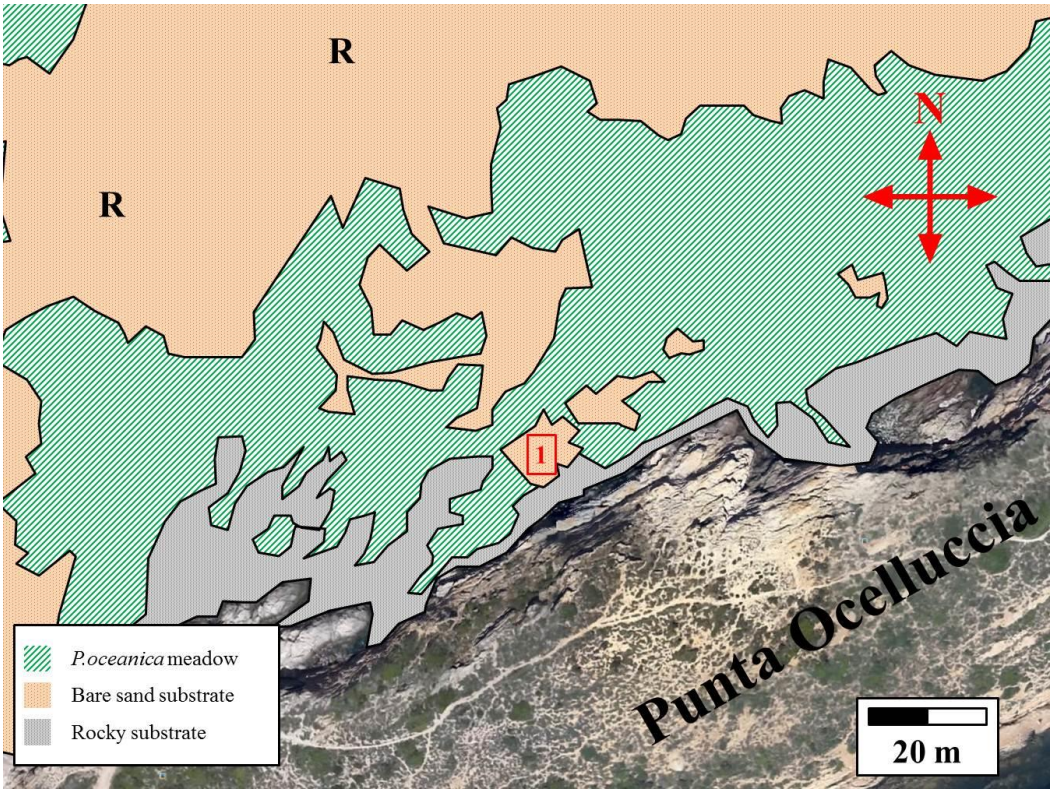


Figure 2.4: Simplified general map of the OSCE-site showing the different components of the sea bottom in the area. In lined-green: P. oceanica meadow; in dotted-brown: bare sand patches; in dotted-grey : rocky areas. R: central "rivière de retour"; 1: OSCE-site patch. Map based on Google earth image.

1.2.1. HARBOR-site:

HARBOR-site was chosen for its immediate proximity to the STARESO research station and its relative protected situation by the STARESO jetty. The HARBOR-site has an area of about 111 m² and is composed of 3 smaller sandy patches separated from each other by only a couple of meters of *P. oceanica* meadow and presenting similar characteristics (Fig 2.3).
All situated at **8 m depth**, the 3 sand patches are relatively well protected from waves by the jetty and are known to be covered most of the year by exported *P. oceanica* detritus. All 3 sub-sites were precisely measured (Fig 2.5).

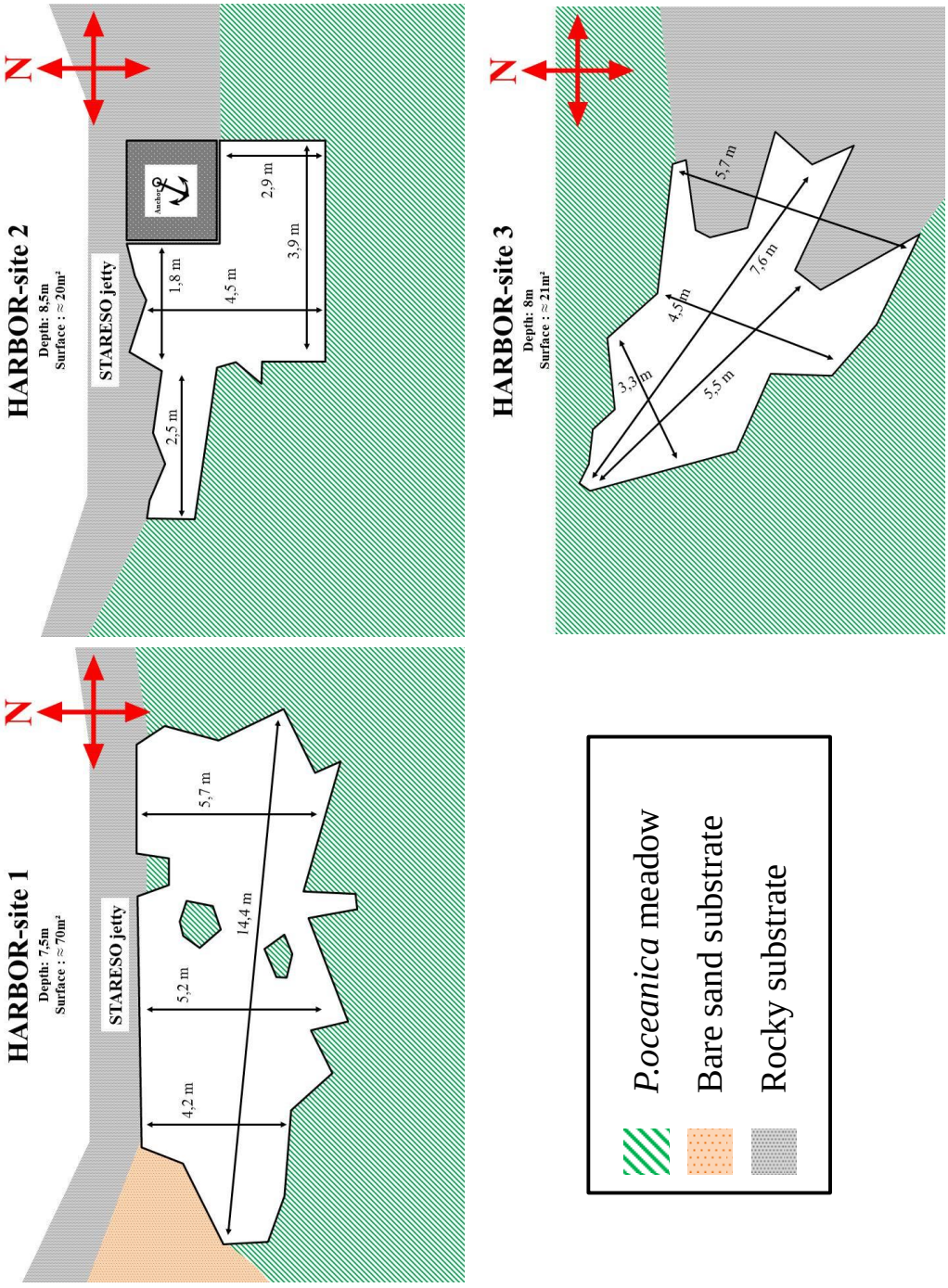


Figure 2.5: Detailed plan of the 3 sub-sites composing the HARBOR-site. In lined-green: P. oceanica meadow; in dotted-brown: bare sand patches; in dotted-grey: rocky areas.

1.2.2. OSCE-site:

OSCE-site was chosen due to the good knowledge we had about this area from previous studies. This site has an area of about 115 m² and is situated at a **10 m depth**, just next to the northern part of Punta Osceluccia. It is surrounded at its northeastern side by a *P. oceanica* meadow and by a rocky cliff at its southwestern side (Figure 2.4 and 2.6). Due to its location, this site is relatively protected from the influence of the strong bottom returning currents occurring in the middle ("rivière de retour") of the small peninsula formed by the "Anse de l'Oscelluccia" (Blanc & Jeudy de Grissac, 1984).

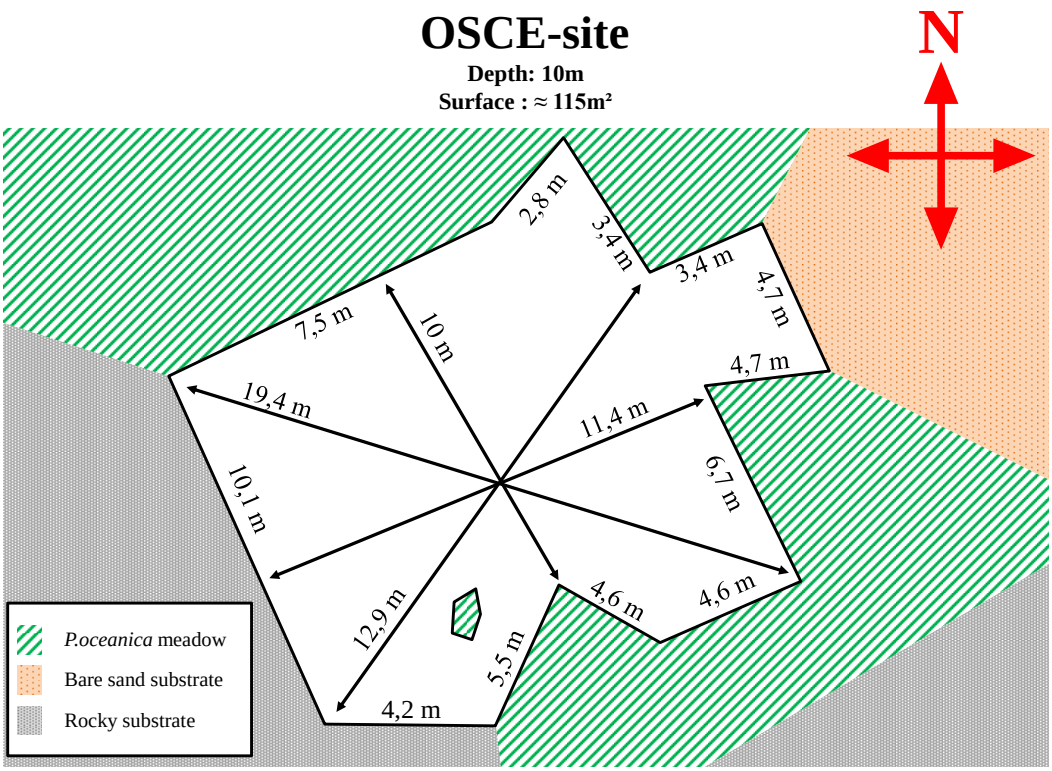


Figure 2.6: Detailed plan of the OSCE-site. In lined-green: P. oceanica meadow; in dotted-brown: bare sand patches; in dotted-grey: rocky areas.

This site was known to be covered quite constantly by *P. oceanica* detritus throughout the year even if coverage and thickness of the accumulation revealed to be drastically variable at a seasonal scale and even at a more daily scale (see Chapter 3).

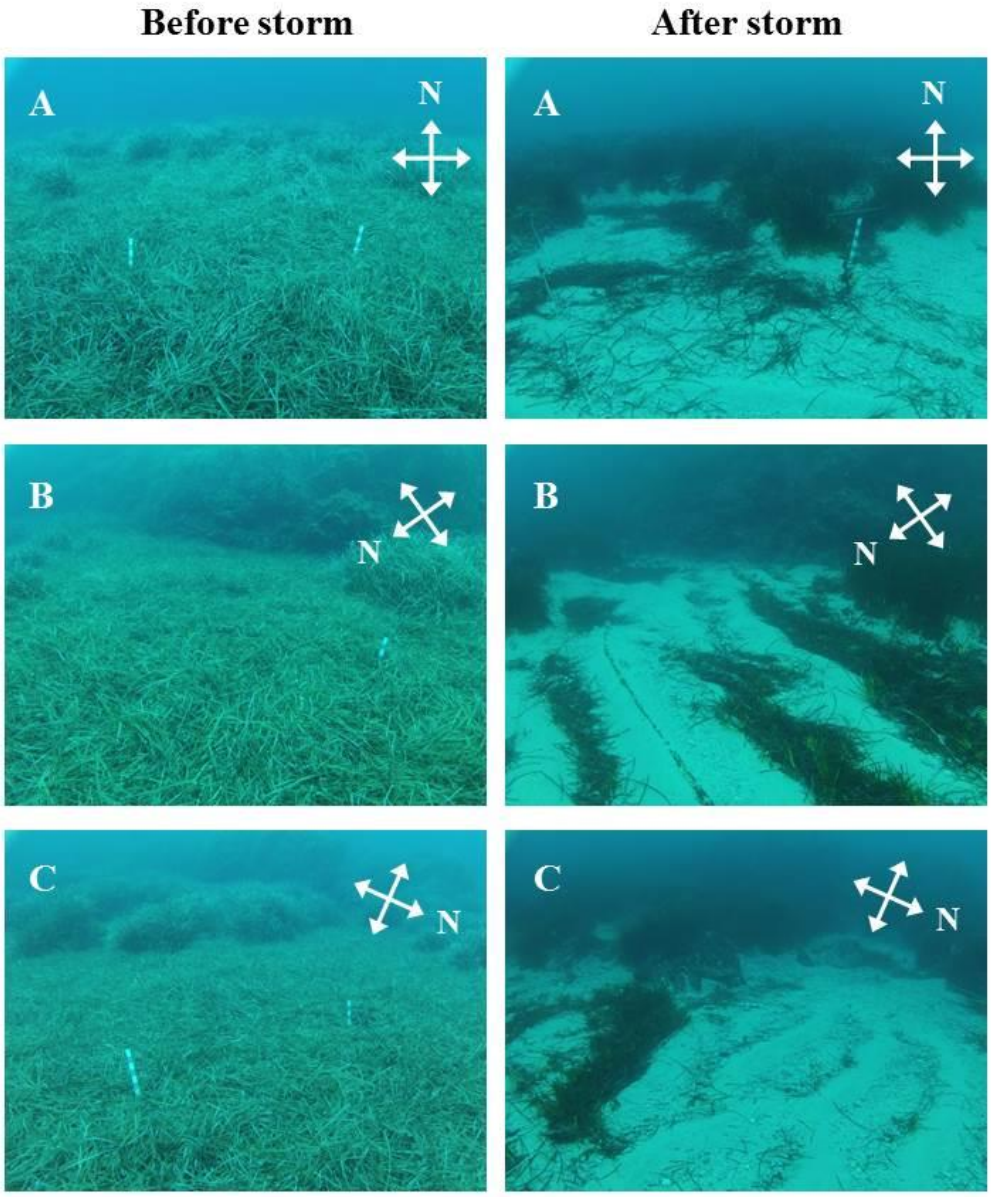


Figure 2.7: 360° GoPro pictures of the OSCE-site from a 2.5 m steel pool in the middle of the sand patch. A, B and C representing pictures taken respectively at 0°, 120° and 240° orientation. All pictures have been taken at 12:00 am before and after an autumnal storm during November 1st night. The OSCE-site is surrounded by P. oceanica meadow (A and C) and by rocks from the Punta Oscelluccia (B).

2. Sampling dates

A total of 9 sample campaigns were conducted for this PhD for a total of **239 days of fieldwork**. Table 2.1 summarizes the dates and main purposes of all the field campaigns conducted during this study.

Table 2.1: Dates and aims of the field sampling campaigns conducted for this PhD.

Dates	Aim of the campain
August 2011	Spatiotemporal community characterization 1 + Spatiotemporal trophic ecology assessment 1
October 2011	Spatiotemporal community characterization 2 + Spatiotemporal trophic ecology assessment 2
March 2012	Spatiotemporal community characterization 3 + Spatiotemporal trophic ecology assessment 3
May 2012	Spatiotemporal community characterization 4 + Spatiotemporal trophic ecology assessment 4
September 2012	Setup and start of long term and high frequency temporal dynamics of community assessment + Long term and high frequency yrophic ecology temporal assessment
October 2012 to January 2013	Long term and high frequency temporal dynamics of community assessment 1 + Long term and high frequency yrophic ecology temporal assessment 1
April 2013 to June 2013	Long term and high frequency temporal dynamics of community assessment 2 + Long term and high frequency yrophic ecology temporal assessment 2
October 2014	Pulse impact experiment + Oxygen stratification impact experiment

3. General sampling techniques

3.1. Standardized « community » fauna sampling

For all community and biodiversity assessment sampling, a quantitative, standardized and handy sampling protocol was designed to guarantee that the same surface was always sampled. A team of 2 divers was necessary to apply this protocol properly. A weighted **490 cm²** PVC "litter core" was applied against the litter until bare sand was reached, not to be moved for all the duration of the sampling procedure (Figure 2.8). While maintaining the cylinder firmly, the whole litter contained inside the 490 cm² area was carefully and gently **manually** sampled and put into plastic jars, **sealed** until the separation processing in the lab, always resulting in an exhaustive sampling of the same surface. Prior to that, litter height was measured using a ruler stick pushed inside the litter just next to the core.

Figure 2.8: Standardized sampling. A: 25 cm diameter PVC "litter core" used for standardized sampling. B: A team of two divers applying the protocol, one holding the sealed plastic tanks, the other using the PVC cylinder for manual sampling.

To our current knowledge, no published information is available on patchiness or **edge effect** on vagile macrofauna associated with seagrass macrophytodetritus. Literature however indicates that for macrofauna from seagrass habitats, species-specific responses to edge effect or patchiness could be observed (Boström *et al*., 2006). Factors like landscape patchiness, habitat configuration or fragmentation, and proximity to other habitats may influence those responses (Eggleston *et al*., 1999; Bell *et al*., 2001; Hovel, 2003; Healey and Hovel, 2004). In order to limit the influence of such patchiness or edge effect of *P. oceanica* or rocky substrate proximity, all samples were taken at a **minimum of 1 m** from the nearest adjacent rocky substrate or *P. oceanica* meadow.

This sampling technique by hand could be considered less efficient than some other techniques such as compressed air "airlift" techniques (Bussers *et al*., 1983). However, species accumulations curves estimators (Chao2) for global abundance data for all species created with 999 permutations in Primer v6.1.13 (Clarke and Gorley, 2006) concluded that the maximum theoretical number of species present in the exported litter accumulations was 138 (Figure 2.9, A). With 115 species encountered in our samples (seasonal axis), representing around **85%** of the theoretical number of species, it was estimated that our sampling was sufficiently exhaustive to have quite a complete view of what was present inside the exported litter accumulations. Moreover, the same analysis performed on the 19 species contributing to 90% of the total abundance (Figure 2.9, B) showed that after 3 to 6 samples, 81-92% of these dominant species were sampled, meaning that 3 to 6 samples are satisfying in order to estimate the spatiotemporal abundance variations of these species.

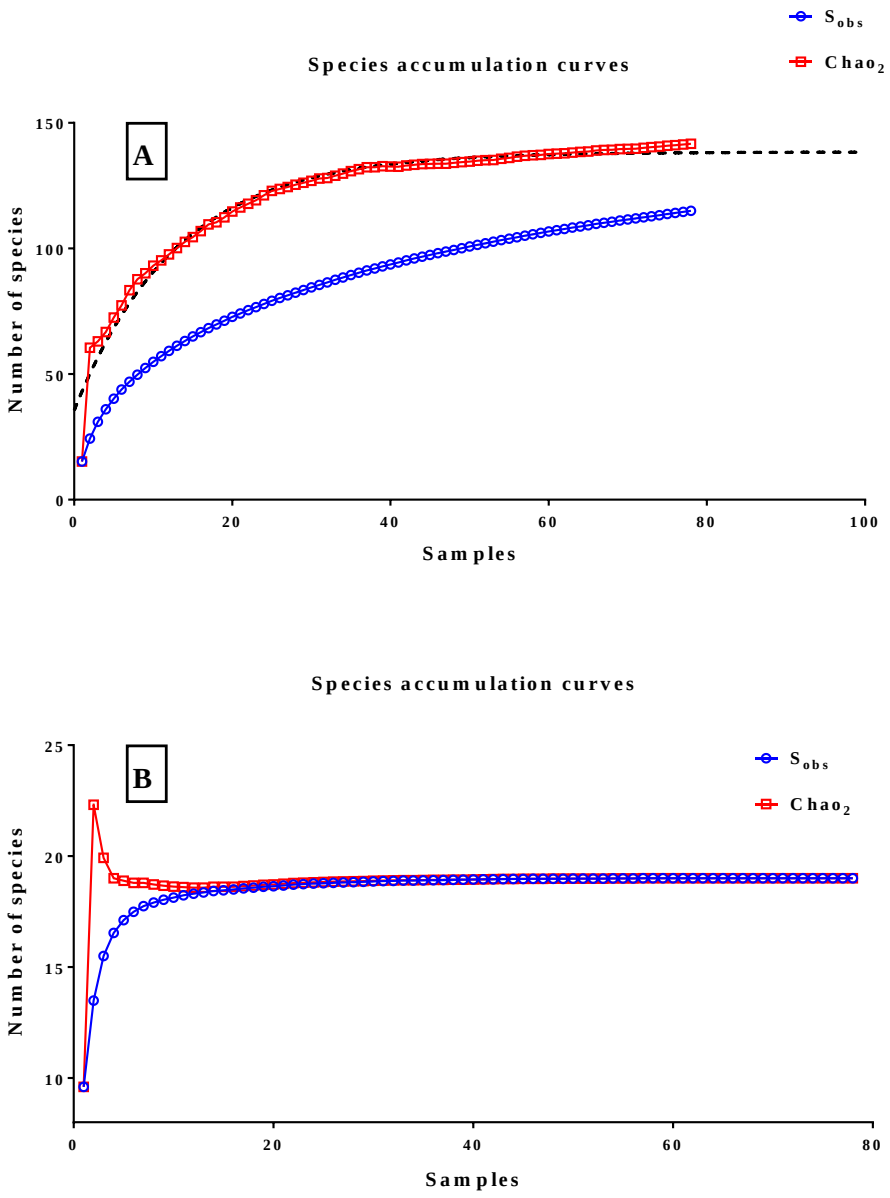


Figure 2.9: Species accumulation curves plot with S_{obs} (blue circles) and $Chao_2$ estimator (red squares) for global abundance of every species (A) and for the 19 dominant species (B). Black dotted line corresponds to significant non-linear regression fitted on Chao2 estimator.

3.2. Non-standardized « trophic » fauna sampling

For all the trophic ecology sampling, only a sufficient number of individuals was necessary and standardization was thus not compulsory. Qualitative sampling for trophic ecology spatiotemporal and high frequency temporal assessment was performed using large **50 L plastic bags**. The litter was **manually** put inside the bag until it was full. Plastic bags were then sealed using plastic rings until separation processing in the lab.

Suspended particulate organic matter, SPOM, was collected at the same time, 1-2 meters above seafloor, using hand-held Niskin-Bottle (2.5 L). Sampled water was then vacuum-filtered using precombusted (450°C) glass fiber filters (47 mm, Whatman GF/F) and filters were afterwards frozen at -20°C until stable isotope analysis.

3.3. Water sampling

Along with every standardized sampling described above, water was always sampled for further nutrients (HPO_4^{2-}, NH^{4+} and NO_2+NO_3, hereafter NO_x), and dissolved oxygen concentration measurements.

Before every sampling event, the whole sampling equipment was carefully cleaned with a 2-3% chlorhydric acid/ mili-Q water solution. Water was sampled in the water column (hereafter called "**WC**"), in the water just above the litter (hereafter called "**WJA**") and in the water inside the litter (hereafter called "**WI**"). For nutrients only, interstitial water was also sampled inside the sediment (hereafter called "**IW**").

Water was sampled with **60 mL syringes** equipped with "3-ways tap" and filters (Gobert *et al.*, 2006), each of them rinsed 3 times underwater with seawater. To improve homogeneity of sampling, a steel "trident" was used for sampling for WC, WJA and WI (see Figuer x , B). For the same purpose a steel "quadrident" was used for sampling for WS.

60 mL were sampled each time, 30-40 mL for oxygen concentration measurement and 20-25 mL for nutrients. For WS, smaller 20 mL syringes were used.

Oxygen concentrations were measured using the modified Winkler technique with 13 mL BOD (Biological Oxygen Demand) bottles, and titration of iodine with thiosulfate solution adapted for small water volumes (Carpenter, 1965; Strickland and Parsons, 1968). Nutrients concentrations were measured using an **autoanalyser** (SKALAR San+ Continuous Flow Analyser) following the method of Grasshoff *et al.* 2007 adapted of oligotrophic seawater (detection limits: 0.05, 0.04 and 0.1 µM for HPO_4^{2-}, NH^{4+} and NO_x respectively).

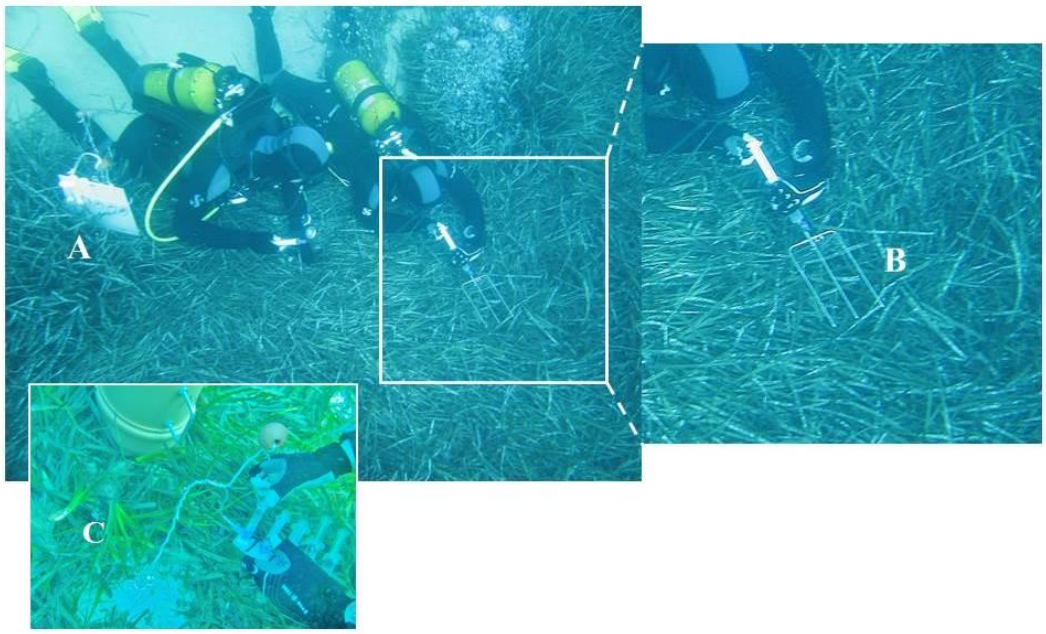


Figure 2.10: A team of two divers sampling water for oxygen and nutrients concentration measurements. A: handy and weighted syringe holder for easy and quick sampling. B: detail of the steel "trident" for homogenous WC, WJA and WI sampling. C: detail of the steel "quadrident" for IW sampling.

3.4. Samples processing and lab treatment

3.4.1. Community sampling

Sampling pre-processing in STARESO was the same for all the samples. The samples were rinsed with freshwater on 10 mm and 500 µm sieves for optimal and handy separation. The 500 µm fraction was preserved in a 4% formaldehyde seawater solution and kept until further analysis. Back in Liège, the 4% formaldehyde seawater solution was replaced by distilled water for final sorting, specific identification under stereomicroscope (Zeiss Stemi 2000-C), counting and then stored in 99.8% ethanol.

The remaining defaunated detritic fraction (Figure 2.11) was classified into 4 different classes of material: (1) dead *P. oceanica* leaves (hereafter "**DL**"), (2) living shoots, leaves and rhizomes of *P. oceanica* (hereafter "**LL**"), (3) drift epilithic macro algae (hereafter "**MA**") and (4) for a single season when they were very abundant, dead rhizomes (hereafter "**DR**"). Each class was dry weighed and 100 dead leaves were measured for each sample to investigate degradation state throughout seasons and years.

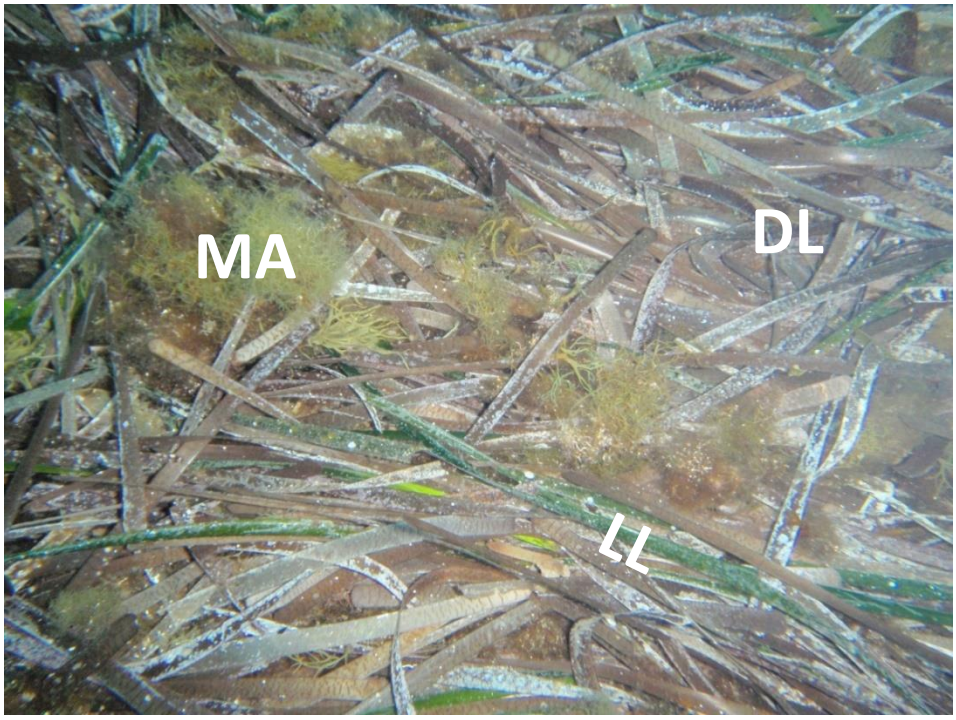


Figure 2.11: Detailed view of the general aspect of an exported detritus accumulation. MA: drift macroalgae. DL: deal P. oceanica leaves. LL: living leaves, shoots and rhizomes.

3.4.2. Trophic sampling

Sampling pre-processing in STARESO was the same for all the trophic samples. The samples were kept alive in 750 L storage tanks and then rinsed with freshwater on **10 mm and 500 µm sieves** for optimal and handy separation. The 500 µm fraction was preserved in a smaller 2 L tank filled with oxygenated seawater. The organisms were then put individually in 4 mL vials and frozen (-20°C) until further analysis. Back in Liège, the frozen organisms were identified to the specific level under stereomicroscope (Zeiss Stemi 2000-C), and their digestive tracts removed and spread on microscopic slides in glycerin for further gut content analysis.

Gut content analyses were performed using the **semi-quantitative** technique described by Wilson and Bellwood in 1997, but adapted for this study, for the very small gut contents of invertebrates. A 4 cm² grid composed of **100 squares** of 4 mm² was used. **25 squares** were **randomly chosen** and market out of the 100 and in each square only the dominant food item was taken into account (Wilson and Bellwood, 1997). Dominant food items for this study were classified in 5 categories: (1) dead *P. oceanica* leaves, (2) living *P. oceanica* leaves, (3) other vegetal material, (4) animal material and (5) unknown material. Once the 25 squares were examined and the most dominant item noted for each of them, the relative abundance (%) of each category was calculated. Organisms presenting empty gut or less than 10 squares containing one of the determined items were excluded from further analysis.

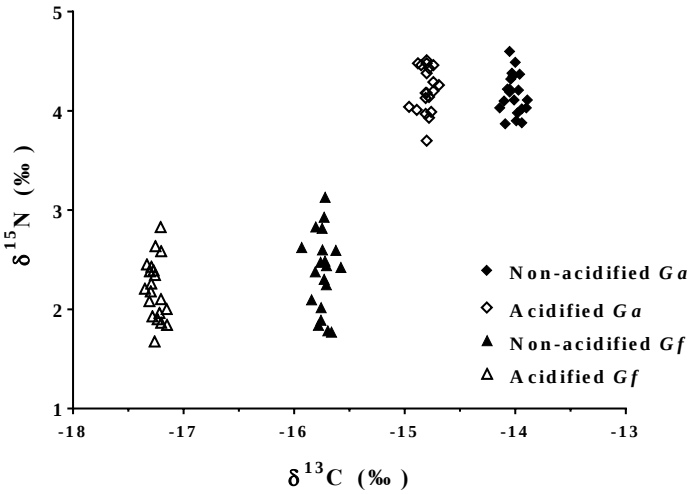
After gut removal, all sampled individuals were dried for at least 96h (60°C), then ground to a homogenous powder manually or, for big individuals, using a ball-mill (Retch Mixer Mill MM301). After grinding, all crustaceans were acidified using 37%HCl fumigation protocol for 12 hours, removing the inorganic carbon of the cuticle ($Ca\underline{\mathbf{C}}O_3$) prior to any stable isotope (**SI**) analysis since inorganic carbon ("diet-independent" carbon) has a different $\delta^{13}C$ composition than organic carbon ("diet-dependant" carbon) (Nieuwenhuize *et al.*, 1994; Soreide *et al.* 2006). But literature remains uncertain on the impact of HCl acidification protocol on nitrogen SI measurements (Vizzini and Mazola, 2003; Kolasinski, 2008; Vafeiadou *et al.*

2013). However, our 12 hours 37% HCl fumigation protocol does not seem to show a significant impact on $\delta^{15}N$ measurements, apparently (see Box 1) on our small invertebrates. This result is coherent with Bosley and Wainright, 1999. Samples were thus all acidified prior to C and N stable isotope analysis. Only 2 big species with thick cuticles containing more $CaCO_3$ were analyzed one first time for $\delta^{15}N$ analysis on non-acidified material, and a second time for $\delta^{13}C$ analysis on acidified material. After acidification, samples were dried a second time for 24h (60°C) to remove the remaining moisture formed during acidification process and then precisely weighed (0,01 mg on a Mettler Toledo AX-105 DeltaRange) for analysis. The same protocol was applied for the potential food sources.

All stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) and C:N measurements were performed in Liège with an Isotope Ratio Mass Spectrometer (Isoprime 100®, Isoprime, UK) coupled in a continuous flow to an elemental analyzer (Vario microcube®, Elementar, Germany) used for automated analysis routine and combustion. Stable isotope ratios are expressed as δvalues (‰) relative to their respective international standards Vienna PeeDee Belemnite and atmospheric N_2. Pure gasses of CO_2 and NO_2 were used and calibrated against certified reference materials, i.e. sucrose (IAEA-C6; $\delta^{13}C$= −10.5±0.5‰), ammonium sulfate (IAEA-N2; $\delta^{15}N$= 20.3±0.3‰), obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria). The analytical precision was assessed by procedural blanks, duplicate samples and internal (i.e. glycine and an in-house crustacean and seagrass standards) and certified reference standards (i.e. IAEA-C6, IAEA-N2 and IAEA-S1). Analytical precision on measurements presented hereafter were 0.12‰ for $\delta^{13}C$, 0.24‰ for $\delta^{15}N$.

Neither chemical lipid extractions nor *a posteriori* lipid corrections have been performed for SIA on the sampled organisms for four main reasons. (1) Our organisms showed an average C:N value of 4.58 ± 0.60, close to the recommended threshold value of 3.5 (Post *et al.*, 2007). (2) *A posteriori* lipid corrections have been shown to be of questionable use in the case of aquatic invertebrates (Kiljunen *et al.*, 2006) containing high proportions of chitin in addition to lipids and proteins (Logan *et al.*, 2008). (3) The C:N values of the analyzed macrofauna were quite constant (between 3.26 and 7.01) and moreover, no significant linear trend ($R^2 = 7.03\ 10^{-5}$) was found between C:N ratio and $\delta^{13}C$ (Figure 2.12). Lipid bias was thus not species-specific and hypothesized to influence quite uniformly all the consumers of the food web. (4) We think that lipids are nonetheless part of the invertebrates' diet and that

BOX 1



Removing diet-independent carbon from crustacean samples before stable isotope analysis has always been a major concern for trophic ecologists. Indeed, inorganic carbon of the cuticle (Ca**C**O3) has a different $\delta^{13}C$ composition than organic carbon (Nieuwenhuize *et al*., 1994; Soreide *et al*. 2006). The presence of this non-dietary carbon, more enriched in ^{13}C than organic carbon can thus be an important bias for stable isotope analysis and this is a problem for stable isotope analysis, meant to reflex only the diet of organisms. Acidification using HCl is a method used to remove carbonates from the crustacean cuticle but this method has already been sometimes reported to modify not only $\delta^{13}C$, but also $\delta^{15}N$ in a variable and random way (Vizzini and Mazola, 2003; Kolasinski, 2008; Vafeiadou *et al.* 2013).

Our purpose was to perform individual stable isotope analysis on the sampled organisms, which would often require performing the analysis on entire organisms due to mass requirement for the analysis. We thus tested the impact of our acidification method (12h HCl fumigation) on individual organisms the two most abundant species of amphipods sampled: *Gammarella fucicola* (*Gf*) and *Gammarus aequicauda* (*Ga*). Stable isotope analysis was performed on one half of each organism without acidification, and on the other half after acidification. Results indicated that $\delta^{13}C$ was modified for both species, shifted to the more negative side of the isotopic space after acidification. This indicated that our method efficiently removed the inorganic carbon of the cuticle since its $\delta^{13}C$ displays more positive values than organic carbon. Another interesting result is that $\delta^{15}N$ did not display significant changes between acidified and non-acidified samples, indicating that for our organism, 12h HCl fumigation impact on $\delta^{15}N$ measurements was negligible.

lipid content and composition of a given organism are linked to its feeding habits. Variations in lipid stocking and/or metabolism could thus be considered as mechanisms involved in the expression of a certain trophic diversity. For these reasons, lipids were not removed from the analyzed tissues or corrected *a posteriori*.

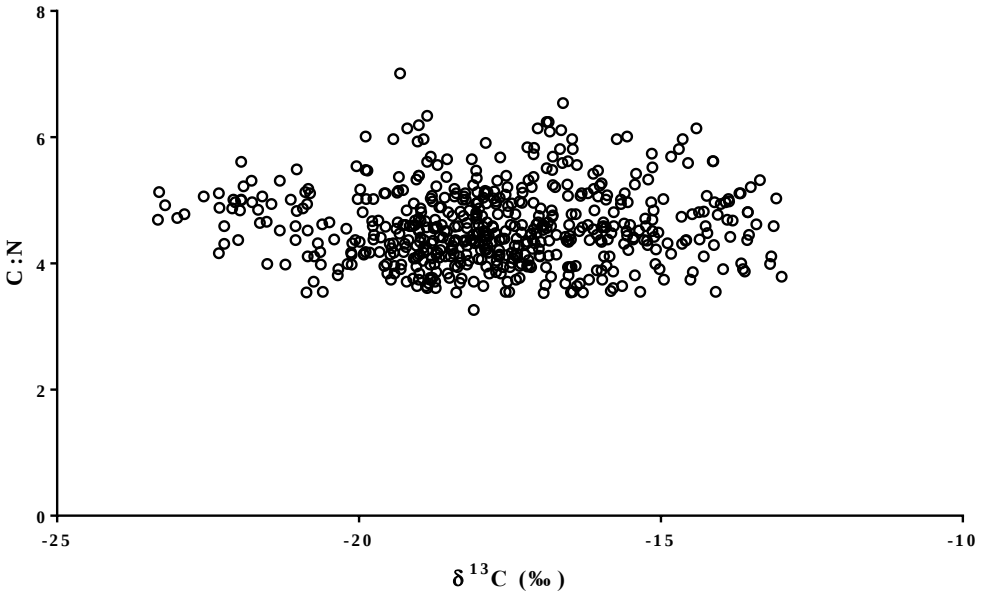


Figure 2.12: biplot of C:N ratio vs. $\delta^{13}C$ of all the sampled organisms.

3.4.3. Data analysis

3.4.3.1. Distance-based modelling

The relationships between macrofauna assemblages and environmental variables were analyzed using a distance-based linear model analysis (DistLM; Legendre and Anderson, 1999; McArdle and Anderson, 2001). DistLM performs variation partitioning for sets of explanatory variables (here, my environmental data), and allows significance testing of explanatory variables for a multivariate response variable in the form of a resemblance matrix (Anderson *et al.*, 2008). Prior to DistLM analysis, collinearity among environmental variables was tested using "draftsman plot". The analysis was based on a Bray–Curtis distance matrix after square-root transforming the

weighed abundance data. The “best” selection procedure, with AIC (Akaikes's information criterion) as the selection criterion based on 9,999 permutations was used to test environmental variables. DistLM analysis was repeated using only significant variables ($p \leq 0.01$; exception for PO_4 with $p = 0.038$). A distance-based redundancy analysis graphical representation (dbRDA) was performed to visualize the fitted model in 2-D space. Unlike nMDS, this analysis is constrained, meaning that the resulting ordination depends on both species composition and habitat variables.

3.4.3.2. Hierarchical clustering

The purpose of clustering techniques is to investigate relationships between items (here, samples) by grouping them in “natural” clusters. The key concept is that samples grouped in one given cluster are closer to each other than they are to samples of other clusters.

The input data for this multivariate exploratory technique is the Euclidean distance matrix associated to the data of interest. The Ward classification method then fuses the items into groups (that’s why the technique is called “agglomerative”). The Ward grouping method is distinct from all other classical grouping methods because it uses an analysis of variance approach to evaluate the distances between clusters. In short, this method attempts to minimize the intra-group distance while maximizing the inter-group distance at each step (Ward, 1963). Often used in ecology, this method is regarded as quite efficient. The resulting clustering tree displays bootstrap probabilities (BP) and approximately unbiased values (AU, computed by 10000 bootstrap resampling) that give an idea of the “significance” of the formed clusters. The higher the value, the more trust worthy the cluster.

The data used to perform clustering trees were square root-transformed weighed abundances. This very common transformation was useful to reduce the “weight” of very abundant species when grouping the samples and allow the least dominant ones to be taken into account.

3.4.3.3. SIMPER analysis

The purpose of one-way SIMPER (SIMilarity PERcentage) analyses is to highlight the variables (here, species) best explaining the dissimilarity between two groups of items (here, samples), or best explaining the similarity between samples forming a single group. A classical one-way SIMPER analysis has two parts. The first one is a breakdown of the total inter-group dissimilarity. The program calculates the Bray-Curtis dissimilarity between each pair of inter-group items, and then computes the mean inter-group dissimilarity. This mean value is then broken down into relative dissimilarity contributions for each variable (here, species) and the associated standard deviation.

In parallel to this evaluation of dissimilarity contributions for each variable, the software preforms an evaluation of the intra-group similarity. The procedure is equivalent to the one described above, but is based on the average similarity between all pairs of items of a group.

SIMPER analysis is thus useful to identify the most typical species of each group, and the species contributing the most to differentiate two groups.

3.4.3.4. Diversity indexes

Diversity and specific composition of a species assemblage is a complex phenomenon, driven by many different factors and thus multivariate by nature. Diversity indexes are a way to reduce such parameters in several univariate indexes, which allow an easier evaluation of diversity in samples. Indexes are generally classified in 3 main categories (Jørgensen *et al*., 2005):

- Species richness indexes: this type of index only consists in standardized measures of the number of species present in a sample.
- Diversity indexes *stricto sensu*: this type of index takes into account the number of species present in a sample but also the number of individuals per species.
- Evenness indexes: this type of index takes into account the number of species, the number of individuals per species but also the way in which the individuals are distributed among the different species present.

Shannon-Wiener index (H'):

This index belongs to the second category of indexes and is calculated using:

$$H' = \sum_i p_i . ln(p_i)$$

where p_i is the proportion of the total effective belonging to the *i* species (i.e., the number of specimens of the *i* species divided by the total number of specimens of the sample). The value of this index is minimal when the sample only contains one species. Its maximal value is theoretically infinite, because it increases with the number of species present in the sample.

Jørgensen *et al* (2005) proposed, for the "Water" framework directive (EU directive 2000/60), a general scale supposed universally applicable (Table 2.2).

Table 2.2: H' class values as accepted in the 2000/60 EU directive (from Jørgensen *et al*., 2005)

Lower limit	Upper limit	H' class
0	0.69	VERY LOW
0.69	1.39	LOW
1.39	2.08	MODERATE
2.08	2.77	HIGH
2.77	ln(S)	VERY HIGH

Simpson index (1-λ'):

This index belongs to the third category of indexes and is calculated using:

$$\mathbf{1 - \lambda' = 1 - \left(\sum_{i} \frac{n_i(n_i - 1)}{N(N-1)} \right)}$$

where n_i is the number of individuals of the *i* species, and *N* the total effective of the sample. This index does not give more information than the Pielou (J') evenness index, but is much less sensitive to sample size. This was a major advantage since our samples were, by nature, very variable.

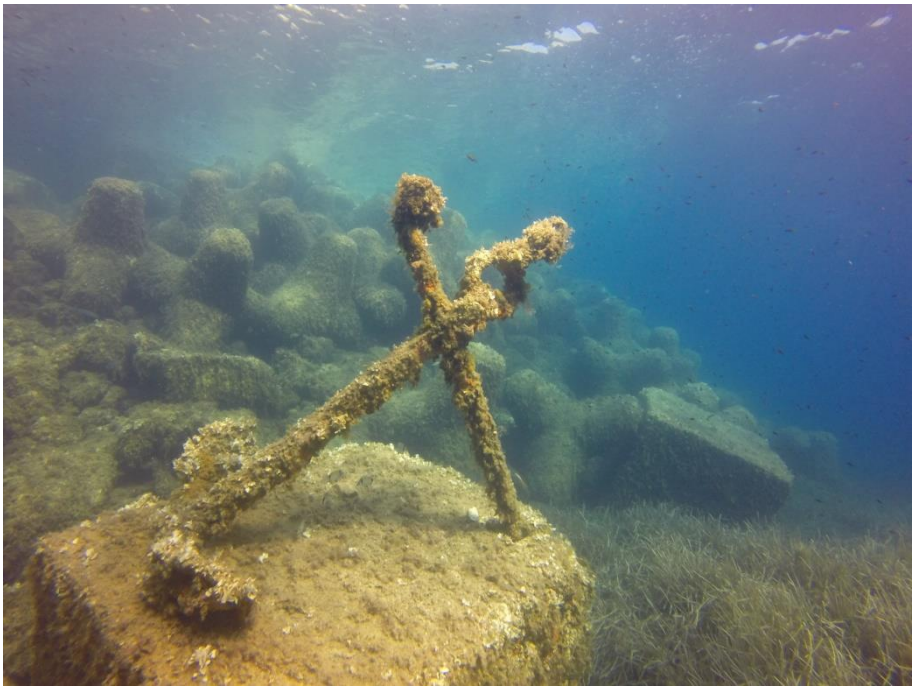
3.4.3.5. Other Statistical analyses and softwares

Classical statistical analysis (factorial ANOVA, factorial MANOVA and Hierarchical Ward Dendrogam) were performed using R and the dedicated "Rcmdrv2.2-3" and "pvclustv2.0-0" packages. Diversity indexes, SIMPER analysis, DistLM and dbRDA graphical ordinations were performed using PRIMER 6.1.13 (Clarke and Gorley, 2006) with PERMANOVA additional software (Anderson *et al*., 2008). A significance level of $p < 0.01$ was always used in all tests.

Graphs were built with R, PRIMER 6.1.13 and GraphPad PRISM 6.01 software for Windows (GraphPad Software, San Diego, USA).

Chapter 3

Spatio-temporal variability of *Posidonia oceanica* exported phytodetritus compartment and characterization of the associated macrofauna community.



1. Introduction

At the end of summer, the Mediterranean endemic seagrass *Posidonia oceanica* loses most of its foliar biomass after autumnal senescence. The fate of this important foliar necromass varies a lot (Pergent *et al*., 1997; Boudouresque *et al*., 2015). A part of it decays and is buried inside the meadow, but up to 55% of the annual foliar primary production is exported out of the *P.oceanica* meadow, to other adjacent terrestrial (beach wracks called "banquettes") or coastal habitats where it constitutes a major allochthonous organic matter input (Romero *et al*., 1992; Pergent *et al*., 1994; Cebrian and Duarte, 2001; Boudouresque *et al*., 2015). Exported dead leaves mix with drift macroalgae, fine sediment, living and dead *P.oceanica* shoots or rhizomes to form "exported macrophytodetritus accumulations", **EMA**. EMAs are dense and highly variable accumulations (Wittmann *et al*., 1981; Mascart *et al*., 2015) forming preferentially on sand patches directly adjacent to the meadow itself, depending on local hydrodynamics and patch morphology (Vetter and Dayton, 1999).

Like other macrophytes detritus litter (Norkko *et al*., 2000), EMAs are a habitat and a food source for a diverse community of animals. As food webs from seagrass ecosystems are considered to be mainly detrital (Pergent *et al*., 1994; Mateo and Romero, 1997; Cardona *et al*., 2007), all these animals potentially act in the fragmentation process of the dead leaves and organic matter transfer from the seagrass to the coastal food webs. These organisms are bacteria, fungi, microalgae and sessile or vagile invertebrates. Vagile organisms, capable of moving freely inside the EMAs, are composed of meiofauna (38µm < size < 500µm) and macrofauna (size ≥ 500µm). Meiofauna of EMAs is highly dominated by the crustacean subclass Copepoda, of which up to 87% belongs to the order Harpacticoida. This community shows important spatiotemporal variations and seems to be impacted by physico-chemical conditions inside the EMAs and the complexity of EMAs themselves (Mascart *et al*., 2015).

While the animal communities of seagrass meadows have been well investigated (Heck and Orth, 1980; Gambi *et al*., 1992; Sanchez-Jerez *et al*., 1999), vagile macrofauna community present in EMAs has not been much studied in its entirety in literature (Gallmetzer *et al*., 2005; Dimech *et al*., 2006; Como *et al*., 2008; Remy, 2010). Although "snapshots" of small periods of time, these studies provide first insights of the macrofauna community

associated with EMAs. These studies revealed that EMAs macrofauna is composed of approximately 45-80 species from various taxa. Arthropods are highly dominant but significant abundances of mollusks and annelids are also reported. Arthropods are dominated by crustacean amphipods, representing from 80 to 97% of the total abundance, from which *Gammarella fucicola* represents up to 89% (Gallmetzer *et al*., 2005) followed by *Gammarus aequicauda*. Decapods are also well represented with *Athanas nitescens*, *Pisa tetraodon* or *Galathea intermedia*. Isopods are present in a lower but non-negligible abundance with *Idotea spp*. and *Stenosoma lancifer*. Among small arthropods, leptostracean *Nebalia sp.* is quite abundant (Gallmetzer *et al*., 2005). Mollusks seem to be much less abundant (up to 25% of total abundance in Remy, 2010) except for the cerithiid *Bittium reticulatum*. Annelids are the third main taxa in terms of abundance (up to 7% of total abundance in Remy, 2010), represented by polychaetes, dominated by the nereid *Platynereis dumerilii*. Echinoderms and juvenile fishes are also reported in very low abundances (Gallmetzer *et al*., 2005; Remy, 2010).

To our current knowledge, no study has ever assessed the vagile macrofauna community of EMAs on long-term duration from the perspective of spatio-temporal variation and characterization of this community and parameters that influence it. Our general aim was to characterize as exhaustively as possible the macrofauna community associated to EMAs and evaluate the importance of spatio-temporal variation of that community but also of the EMA habitat itself. To complete that general objective, specific questions were addressed: (1) What are the macrofauna dominant species and taxa inside EMAs? (2) Are these dominant species dependent on EMAs physicochemical spatio-temporal variations? (3) Do these potential variations occur at large and smaller time scale? (4) Which parameter influences most this community?

2. Material and Methods

2.1. Site description and general sampling strategy

All samples were collected near the STARESO (STAtion de REcherches Sous-marines et Océanographiques) research station in Calvi Bay (42°35'N; 8°43'E) in Corsica. *P. oceanica* meadow covers 4.94km² in Calvi Bay, representing about 50% of the area of the Bay. Continuous meadow can be found from 3 m to 38 m (Bay, 1984) near STARESO (local "intermattes" may occur) mostly on soft substrate. Tidal amplitude is rather weak in Calvi Bay (less than 10 cm). Salinity is about 38 and is also rather stable throughout the year. Surface water (3 m) temperature varies between about 26°C in August and about 12°C in February, with a marked thermocline at 25-30 m from May to October. Nutrients (N & P) concentrations in the water column show typical low values of oligotrophic areas (Gobert, 2002).

This study is divided into two main axes: (1) A two year-long seasonal sampling at two different sites. (2) An 18-week-long weekly sampling at one site. For the first axis, samples ($_{TOTAL}N=78$) were collected seasonally for two years, between spring 2010 and spring 2012.

Two different sampling sites (Figure 3.1), at about 750 m from each other, were chosen for their contrasting characteristics in terms of location, hydrodynamics, EMA shape and location, but also for their proximity to the STARESO station. The two sites are sand patches situated between 8 and 10 meters deep and approximately equivalent in terms of total areas (111-115 m²), one directly at the entrance of STARESO harbor, hereafter called "HARBOR-site", and the second one 750m away, right next to the northern side of the Punta Oscelluccia: hereafter "OSCE-site". These two sites are described in details in Chapter 2 (§1.2). For the second axis, samples ($_{TOTAL}N=54$) were collected weekly during two long-term field campaigns for a total of 267 days. Sampling was conducted between September 2012 and January 2013 and from April 2013 and June 2013 only at the OSCE-site.

For this axis, 3 replicates were collected at both sampling sites for each season in 2010-2011 and 6 replicates were collected at both sampling sites for each season in 2011-2012 ($_{TOTAL}N=78$) (see Chapter 2, §3.1). For the second axis, 3 replicates were taken for each of the 18 sampling dates, only at OSCE-site ($_{TOTAL}N=54$).

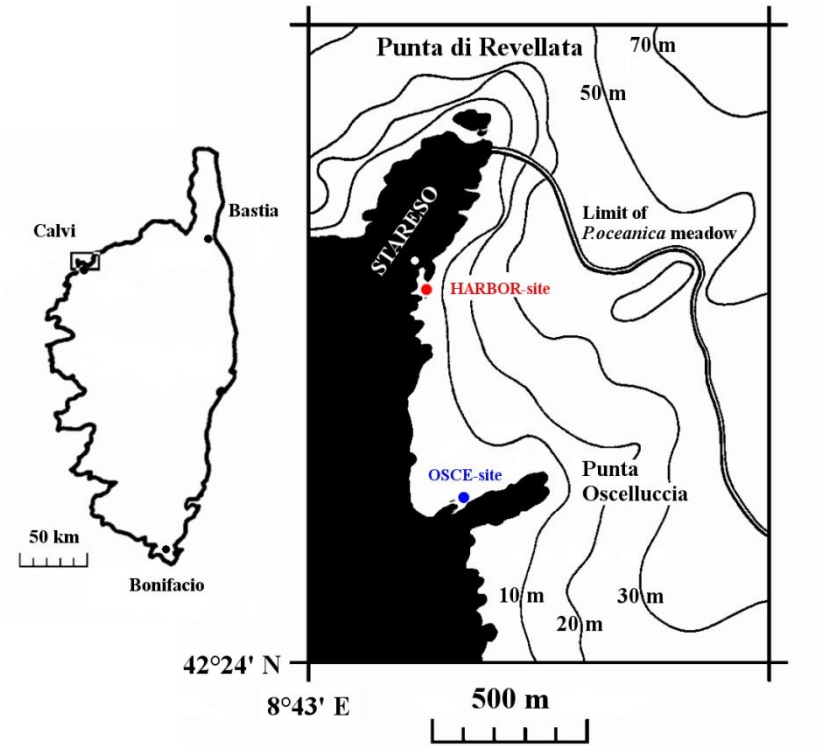


Figure 3.1: Left: location of Corsica in the western Mediterranean. Center: general map of the location of Calvi Bay in Corsica. Right: precise location of the STARESO research station on the Punta di Revellata showing the HARBOR-site (red) and the OSCE-site (blue), the lower limit of the P.oceanica meadow near STARESO and the 10, 20, 30, 50, 70m isobaths (modified after Gobert, 2002).

Detritus and fauna samples were collected for both first and second axes using the same technique. A PVC-core (diameter =25 cm, surface = 0.0490 m²) was randomly pushed into the EMAs until the sediment was reached. Detritus contained inside the PVC-core were manually removed and put inside 6 L sealed PVC tanks until further process in the lab. Tanks were closed and sealed underwater to prevent fauna to escape and avoid any loss of detritic material. Sediment was never taken with the detritus to prevent contamination by infauna. Collected samples were rinsed several times on a 10 mm sieve stacked on a 500 µm nylon-mesh sieve in order to separate the macrofauna from the detritic material. The defaunated >10 mm detrital fraction was frozen (-18°C) and kept until further analysis in lab. Macrofauna was fixated with 4% fomaldehyde-seawater solution replaced after 48h by 99.8% denaturated ethanol and kept until further analysis.

2.2. Detritus characterization and abiotic factors

Some EMAs parameters have been measured during the whole sampling procedure. EMAs height (detritus thickness) was measured using a ruler stick pushed to the sediment through the accumulation next to the PVC-core used for sampling also allowing compaction (Dry mass/height) measurement. In lab, defaunated detritic material from each sample was characterized and classified into 4 different classes of material: (1) dead *P. oceanica* leaves (hereafter "**DL**"), (2) living shoots, leaves and rhizomes of *P. oceanica* (hereafter "**LL**"), (3) drift epilithic macro algae (hereafter "**MA**") and (4) dead shoots and rhizomes (hereafter "**DR**"). Prior to drying, 100 dead leaves from each sample were precisely measured to investigate fragmentation state through time. These 100 leaves measurements were used to determine a fragmentation index, ***F***, stating the availability of leaves from different size classes:

$$F = {}^{1}/_{\sqrt{x}} \qquad (1)$$

where F is the fragmentation index and x the standard deviation calculated on the 100 leaves measurements. F close to 1 represents samples with high variability of fragment sizes and many long leaves, while F close to 0 represents sample with low variability of fragment sizes and few long leaves.

Epiphytes (hereafter "**E**") were scraped from the first 25 fragments according to the method from Dauby and Poulicek (1995), thus removing the weight bias on dead leaves dry mass caused by epiphytes. Scraped epiphytes, as long as detrital material from the 4 categories were then dried at 60°C for 96h to allow dry mass (DM) calculation. Epiphytes dry mass from the 25 fragments was used to calculate a ratio between epiphyte DM and dead leaves DM (hereafter Epi/Lit), used to extrapolate the real DL dry mass.

Abiotic factors were also measured in parallel with faunal sampling (details in Chapter 2 § 3.3). Water was sampled for further nutrients (HPO_4^{2-}, NH^{4+} and NO_2+NO_3, hereafter NO_x), and dissolved oxygen concentration measurements. Water was sampled with 60 mL syringes equipped with "3-ways tap", using the method described by Gobert *et al.* (2006). Samples were collected at different positions: (1) in the water column (hereafter called "**WC**"), (2) in the water just above the litter (hereafter called "**WJA**") and (3) in the water inside the litter (hereafter called "**WI**"). For nutrients only,

interstitial water was also sampled (4) inside de sediment (hereafter called "**IW**"). Oxygen concentrations were measured using the modified Winkler technique with 13 mL BOD (Biological Oxygen Demand) bottles, and titration of iodine with thiosulfate solution adapted for small water volumes (Carpenter, 1965; Strickland and Parsons, 1968). Oxygen values below 2 $mgO_2.mL^{-1}$ were defined as hypoxic (Levin *et al.*, 2009). Nutrients concentrations were measured using an autoanalyser (SKALAR San+ Continuous Flow Analyser) following the method of Grasshoff et al. 2007 adapted of oligotrophic seawater (detection limits: 0.05, 0.04 and 0.1 µM for phosphates, ammonium and NO_x respectively). Meteorological data were recorded between autumn 2010 and spring 2012 in order to monitor the effect of hydrodynamics on the other abiotic parameters. Surface wind is generally considered as a good proxy of near bottom current of shallow places (Cushman-Roisin and Beckers, 2011; and references therein) due to the direct influence of the surface wind on shear turbulence and friction which dominate in shallower regions (typically with a 10m depth). Due to the orientation and location of Calvi Bay, the sampling sites were sheltered from east-southerly to western winds. Only wind from the first quadrant (0-90°) had thus a potential impact. Following the protocol from Mascart *et al.*, 2015 only wind gusts, **WG**, (maximum wind speed over a two-second period at any time each 20 minutes) from first quadrant and higher than 3.06 $m.s^{-1}$ (3 Beaufort) were taken into account. Due to the fast occurring compaction and stabilization of the EMAs and associated water, the selected time scales were 48 hours and 24 hours before sampling (see Chapter 4).

2.3. Photographic sampling

Along with the faunal and physicochemical measurements described above, an automated underwater photographic sampling protocol was developed to constantly follow detritus cover and height at the OSCE-site. A marine quality steel stake topped with a steel support for 3 GoProHD2 cameras was designed (See Fig 3.2). This steel support was composed of a telescopic main stake buried in about 1.5 m of sediment held in place by a stabilizing steel "cross" placed on the sediment with an attachment steel bar at each end. The support for the GoPros was securely locked at a height of 2.5m above the sediment and was left in place between September 2012 and June 2013 underwater. GoPros were equipped with a Cam-do ® programmable "time-lapse intervalometer" external board, programmed to take one picture every hour and then shutting

all cameras down, giving 6 to 8 weeks of autonomy to the system. A Gopro pointed north (0°), another southeast (120°) and the third one southwest (240°), covering almost 360°.

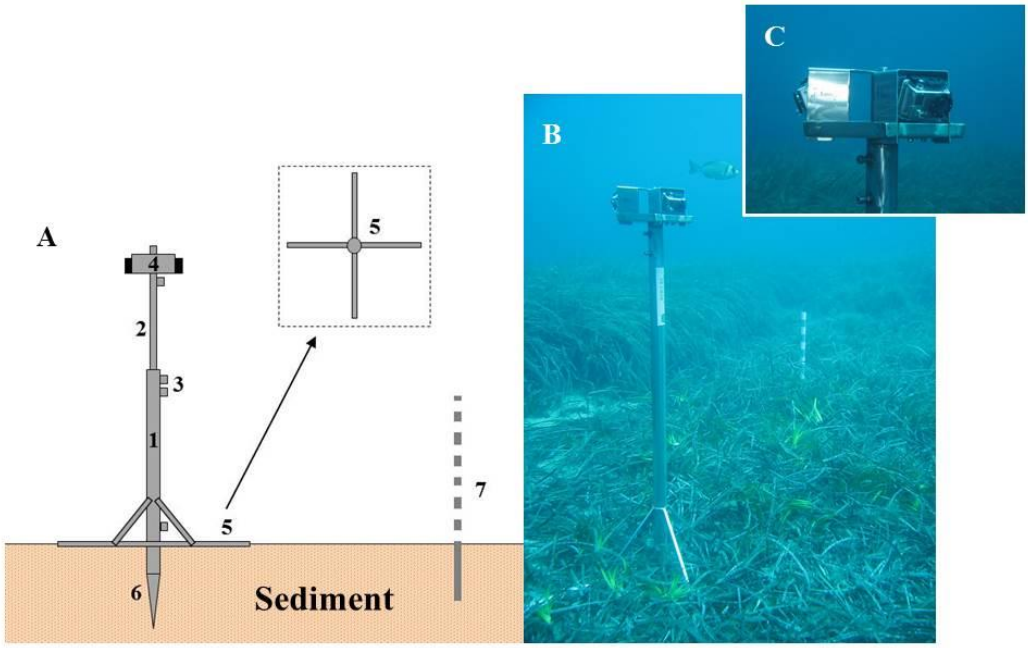


Figure 3.2: Detail of photographic sampling support design. A: schematic view of the design with the main steel stake (1), the telescopic top part of the steel stake (2), the steel locks (3), the GoPro steel support (4), the stabilizing steel "cross" (5), the steel pointy end of the main stake buried in 1,5 m of sediment (6), and the 65 cm PVC height makers (7). B: the whole design placed on the litter accumulation with a PVC height marker visible in the distance. C: detail on the top GoPro camera steel support.

Images taken (more than 5600) during the sampling period were used to estimate the thickness of the EMA (litter height) using between 5 to 8 (depending on the weather) 65 cm long fixed PVC markers as references. They were also used to estimate the litter cover proportion of the patch through time (*i.e.* the percentage of the patch covered by litter). Using Tucsen-TS View 7 software, images showing a complete cover of the sampling site were used as a 100% reference area. Areas were then calculated daily for 3 images (covering

all angles) taken at 02:00 pm, and the difference measured between this area and the reference area was used to estimate the cover proportion.

2.4. Data analysis

Hierarchical clustering construction, DistLM analysis, dbRDA representation, SIMPER analysis and diversity indexes calculations are detailed in Chapter 2 (§ 3.4.3).

Classical statistical analysis (factorial ANOVA, factorial MANOVA and Hierarchical Ward Dendrogam) were performed using R and the dedicated "Rcmdrv2.2-3" and "pvclustv2.0-0" packages. Diversity indexes calculations, SIMPER analysis, DistLM and dbRDA graphical ordinations were performed using PRIMER 6.1.13 (Clarke and Gorley, 2006) with PERMANOVA additional software (Anderson *et al*., 2008). A significance level of $p < 0.01$ was always used in all tests.

Graphs were built with R, PRIMER 6.1.13 and GraphPad PRISM 6.01 software for Windows (GraphPad Software, San Diego, USA).

3. Results

3.1. Seasonal sampling

3.1.1. Exported Macrophytodetritus characterization

Total litter dry mass (hereafter: "$_{Total}$**DM**") sampled showed important variations throughout the 2010-2012 period (Figure x, A). $_{Total}$DM showed a maximum of 2309.8 ± 936.2 gDM.m^{-2} in autumn 2011 at the HARBOR-site and of 2235 ± 578.2 gDM.m^{-2} in autumn 2010 at the OSCE-site. $_{Total}$DM showed a minimum of 696.9 ± 694.7 gDM.m^{-2} in spring 2012 at the HARBOR-site and of 462.7 ± 97.7 gDM.m^{-2} in summer 2010 at the OSCE-site. Autumn was generally the season showing the maximum $_{Total}$DM whatever the site or year, except for 2010 at the HARBOR-site.

Litter composition also showed drastic variations throughout the 2010-2012 period (Figure x, B). On average for the 2010-2012 period, dead *P. oceanica* leaves, **DL**, was by far the most abundant component of EMAs (793.1 ± 689.9 gDM.m^{-2}) followed by living *P. oceanica* leaves, **LL**, (126.4 ± 183.7 gDM.m^{-2}), dead *P. oceanica* rhizomes, **DR**, (98.1 ± 217.2 gDM.m^{-2}), epiphytes, **E**, (89.5 ± 76.3 gDM.m^{-2}) and drift macroalgae, **MA**, (28.1 ± 45.3 gDM.m^{-2}).

DL showed significant variations as well throughout the 2010-2012 period (Table x). DL showed a maximum mass of 1991.8 ± 596.7 gDM.m^{-2} in autumn 2011 at the HARBOR-site (representing 85.7% of $_{Total}$DM) and of 2057.6 ± 669.2 gDM.m^{-2} in autumn 2010 at the OSCE-site (representing 90.9 % of $_{Total}$DM). DL showed a minimum mass of 303.7 ± 330.7 gDM.m^{-2} in spring 2012 at the HARBOR-site (representing 42.4% of $_{Total}$DM) and of 280.8 ± 75.3 gDM.m^{-2} in spring 2010 at the OSCE-site (representing 58.4% of $_{Total}$DM).

DR showed marked variations throughout the 2010-2012 period (Table x). DR showed a maximum mass of 739.5 ± 582.1 gDM.m^{-2} in summer 2010 at the HARBOR-site (representing 39.1% of $_{Total}$DM) and of 124.8 ± 167.5 gDM.m^{-2} in spring 2012 at the OSCE-site (representing 9.0% of $_{Total}$DM). DR showed a minimum mass of 8.5 ± 29.5 gDM.m^{-2} in winter 2012 at the HARBOR-site (representing 0.6% of $_{Total}$DM) and was totally absent from EMAs in autumn 2010, autumn 2011 and winter 2012 at the OSCE-site.

LL showed no variation throughout the 2010-2012 period (Table 3.1). LL showed a maximum mass of 251.4 ± 465.5 $gDM.m^{-2}$ in summer 2011 at the HARBOR-site (representing 18.2% of $_{Total}$DM) and of 281.6 ± 194.6 $gDM.m^{-2}$ in summer 2011 at the OSCE-site (representing 29.5% of $_{Total}$DM). LL was totally absent from EMAs in autumn 2010 and winter 2011 at the HARBOR-site and was totally absent from EMAs in autumn 2010 at the OSCE-site.

E also showed variations throughout the 2010-2012 period (Table 3.1). E showed a maximum mass of 216.6 ± 101.4 $gDM.m^{-2}$ in autumn 2011 at the HARBOR-site (representing 9.9% of $_{Total}$DM) and of 205.2 ± 49.4 $gDM.m^{-2}$ in autumn 2011 at the OSCE-site (representing 22.1% of $_{Total}$DM). MA showed a minimum mass of 33.6 ± 27.0 $gDM.m^{-2}$ in winter 2012 at the HARBOR-site (representing 7.3% of $_{Total}$DM) and of 34.3 ± 23.9 $gDM.m^{-2}$ in winter 2012 at the OSCE-site (representing 6.3% of $_{Total}$DM).

MA also showed variations throughout the 2010-2012 period (Table 1). MA showed a maximum mass of 140.7 ± 125.2 $gDM.m^{-2}$ in spring 2010 at the HARBOR-site (representing 19% of $_{Total}$DM) and of 76.3 ± 29.9 $gDM.m^{-2}$ in spring 2010 at the OSCE-site (representing 16.7% of $_{Total}$DM). MA showed a minimum mass of 4.8 ± 4.5 $gDM.m^{-2}$ in winter 2011 at the HARBOR-site (representing 0.3% of $_{Total}$DM) and was totally absent from EMAs in autumn 2010 at the OSCE-site.

Throughout the 2010-2012 period, spring and summer were the seasons showing the most diversity of items on EMAs for both sites.

Litter height was highly variable as well throughout the 2010-2012 period (Table 1). Height showed a maximum of 17.0 ± 4.05 cm in autumn 2011 at the HARBOR-site and of 19.0 ± 5.58 cm in autumn 2010 at the OSCE-site. Height showed a minimum of 4.33 ± 2.06 cm in winter 2012 at the HARBOR-site and of 4.33 ± 3.05 cm in winter 2011 at the OSCE-site.

Compaction did not show significant variation throughout the 2010-2012 period (Table 3.1).

Multivariate analysis for all the environmental parameters was significant for temporal and spatial factors. Significant seasonal and/or annual variations were observed for TotalDM, Litter height, DM, LL, MA and E. Significant spatial variation was only observed for DR

Litter fragmentation also experienced drastic variations throughout the 2010-2012 period (Figure x). Fragmentation index, *F*, showed a maximum value (minimum fragmentation) of 0.82 in winter 2011 at the HARBOR-site and of 0.62 in spring 2011 at the OSCE-site. *F* showed a minimum value (maximum fragmentation) of 0.30 in spring 2010 at the HARBOR-site and of 0.37 in autumn 2010 at the OSCE-site.

Table 3.1: Summary Table of 3-way MANOVA on measured environmental parameters for the annual, seasonal and spatial factors and interactions.

3-way MANOVA

Factors and interactions	Leaf Litter $_{Total}$DM			Leaf Litter Height			Leaf Litter Compaction		
	F	df	p	F	df	p	F	df	p
Year	4.87	2	ns	6.93	2	*	0.50	2	ns
Season	12.68	3	***	20.43	3	***	0.52	3	ns
Site	2.93	1	ns	1.69	1	ns	1.25	1	ns
Year x Season	0.27	3	ns	0.75	3	ns	0.35	3	ns
Year x Site	2.81	2	ns	7.52	2	*	1.02	2	ns
Season x Site	0.57	3	ns	0.43	3	ns	0.84	3	ns
Year x Season x Site	2.12	3	ns	1.09	3	ns	2.87	3	ns

Factors and interactions	Dead Leaves DM			Dead Rhizomes DM			Living Leaves DM		
	F	df	p	F	df	p	F	df	p
Year	7.48	2	*	6.02	2	*	3.04	2	ns
Season	24.83	3	***	1.70	3	ns	4.33	3	**
Site	0.34	1	ns	11.04	1	*	0.04	1	ns
Year x Season	0.29	3	ns	1.81	3	ns	0.44	3	ns
Year x Site	4.68	2	ns	8.57	2	**	0.53	2	ns
Season x Site	0.82	3	ns	1.06	3	ns	0.14	3	ns
Year x Season x Site	2.11	3	ns	1.38	3	ns	0.16	3	ns

Factors and interactions	Drift Macroalgae DM			Epiphytes DM		
	F	df	p	F	df	p
Year	3.70	2	ns	20.18	2	***
Season	9.55	3	***	37.44	3	***
Site	3.53	1	ns	2.13	1	ns
Year x Season	7.76	3	**	0.15	3	ns
Year x Site	3.08	2	ns	1.16	2	ns
Season x Site	2.16	3	ns	0.17	3	ns
Year x Season x Site	2.02	3	ns	0.18	3	ns

* = 0.01 < P < 0.001 = significant
** = 0.001 < P < 0.0001 = highly significant
*** = P < 0.0001 = very highly significant
ns = not significant

3.1.2. Abiotic factors

Wind gust (WG) velocity varied a lot throughout 2011-2012. In HARBOR-site, mean wind gust (WG) velocity was maximum in winter 2011 (8.68 ± 2.98 $m.s^{-1}$) and minimum in spring 2012 (0.00 ± 0.00 $m.s^{-1}$). In OSCE-site, mean WG velocity was maximum in winter 2012 (14.93 ± 2.89 $m.s^{-1}$) and minimum in winter 201 and spring 2012 (0.00 ± 0.00 $m.s^{-1}$ for both periods).

Multiple regression based on environmental variables (DL, DR, LL, MA, E, O_2, NO_x, NH_4 and PO_4) revealed that only O_2 concentration showed significant positive influence of WG velocity and that only the WG velocity 24h before sampling was significant (Multiple Regression, Partial Correlation = 0.70, $p = 0.007$).

Oxygen concentration was always lower and much more variable in water inside the litter, WI, than in the water column, WC, or in the water just above the litter, WJA, (Figure 3.3). The latter two layers showed concentrations always between 6.21 and 8.80 $mgO_2.L^{-1}$during the 2010-2012 period while 32.04% of WI samples showed concentrations below 2 $mgO_2.L^{-1}$, the hypoxia limit as defined by Levin (2009). Oxygen concentration of WI were very highly significantly different (TukeyHSD, $p < 0.0001$) from oxygen concentrations in WC and WJA, which were not different from each other, making EMAs very particular places in terms of oxygen concentration and variations. WI was thus the only data further discussed. It must be noticed that 20.38% of WI O_2 concentrations showed negative values, which seems incoherent since concentrations cannot be negative. As mentioned in §2.2 of this chapter, oxygen concentration was evaluated using Winkler reverse titration technique, implying no direct measure of oxygen concentration but the measurement of the corresponding added thiosulfate reagent. Therefore, "negative concentrations" correspond to thiosulfate titrated in excess, corresponding to null O_2 concentrations in addition to other reducing compounds present within WI (*e.g.*: sulphides). These negative values were treated as concentrations of 0 $mgO_2.L^{-1}$. The 4-way ANOVA for O_2 concentration was significant for all factors and interactions (Table 3.2). Within the WI layer, sampling sites showed very highly significant differences (TukeyHSD, $p < 0.0001$) and only summer appears to be highly significantly different from other seasons (TukeyHSD, $p < 0.001$). Years also showed highly significant differences between them (TukeyHSD, $p < 0.001$).

Table 3.2: Summary Table of 4-way ANOVAs on physico-chemical parameters for annual, seasonal, spatial and layer treatments and interactions.

4-way ANOVAs

	NO_x			NH_4		
Factors and interactions	F	df	p	F	df	p
Year	44.71	2	***	120.33	2	***
Season	40.25	3	***	40.64	3	***
Site	10.96	1	*	41.92	1	***
Layer	11.10	3	***	351.67	3	***
Year x Season	43.48	3	***	29.15	3	***
Year x Site	50.58	2	***	13.41	2	***
Season x Site	1.70	3	ns	24.60	3	***
Year x Layer	17.13	6	***	75.83	6	***
Season x Layer	24.83	9	***	30.66	9	***
Site x Layer	28.54	3	***	44.21	3	***
Year x Season x Site	2.58	3	ns	33.48	3	***
Year x Season x Layer	9.52	9	***	34.31	9	***
Year x Site x Layer	10.78	6	***	7.52	6	***
Season x Site x Layer	7.09	9	***	29.57	9	***
Year x Season x Site x Layer	10.17	8	***	22.48	8	***

	PO_4			O_2		
Factors and interactions	F	df	p	F	df	p
Year	95.88	2	***	170.74	2	***
Season	46.82	3	***	27.77	3	***
Site	106.97	1	***	24.93	1	***
Layer	520.75	3	***	445.34	2	***
Year x Season	6.80	3	***	8.44	3	***
Year x Site	31.56	2	***	22.82	2	***
Season x Site	15.99	3	***	4.34	3	*
Year x Layer	118.22	6	***	75.38	4	***
Season x Layer	24.18	9	***	5.51	6	***
Site x Layer	61.18	3	***	10.70	2	***
Year x Season x Site	10.68	3	***	10.45	3	***
Year x Season x Layer	19.67	9	***	4.58	6	**
Year x Site x Layer	52.62	6	***	10.98	4	***
Season x Site x Layer	6.42	9	***	3.24	6	*
Year x Season x Site x Layer	13.97	8	***	3.14	6	*

* = 0.01 < P < 0.001 = significant
** = 0.001 < P < 0.0001 = highly significant
*** = P < 0.0001 = very highly significant
ns = not significant

For nutrients, NO_x is quite constant in all layers, and showed values between 0.04 and 2.82 µM. NH_4 is quite constant in WC and WJA layers (Figure 3.4), showing values between 0.07 and 1.60 µM. In WI, NH_4 is much more variable and showed values between 0.13 and 27.00 µM. Variability is even higher in IW (Figure 3.5), where NH_4 showed values between 0.10 and 101.66 µM. PO_4 is quite constant in WC, WJA and WI showing values between 0.03 and 1.07 µM, but much more variable in IW showing values between 0.03 to 4.11 µM.

4-way ANOVAs for all nutrients were significant for all factors and interactions except for the "Season x Site" and the "Year x Season x Site" interactions for NO_x. For NO_x and NH_4, WI and IW were significantly different from each other but also from WC and WJA (TukeyHSD, $p < 0.001$) which showed very similar concentrations. For PO_4, WI was significantly different (TukeyHSD, $p < 0.0001$) from WC, WJA and IW which were not significantly different from each other.

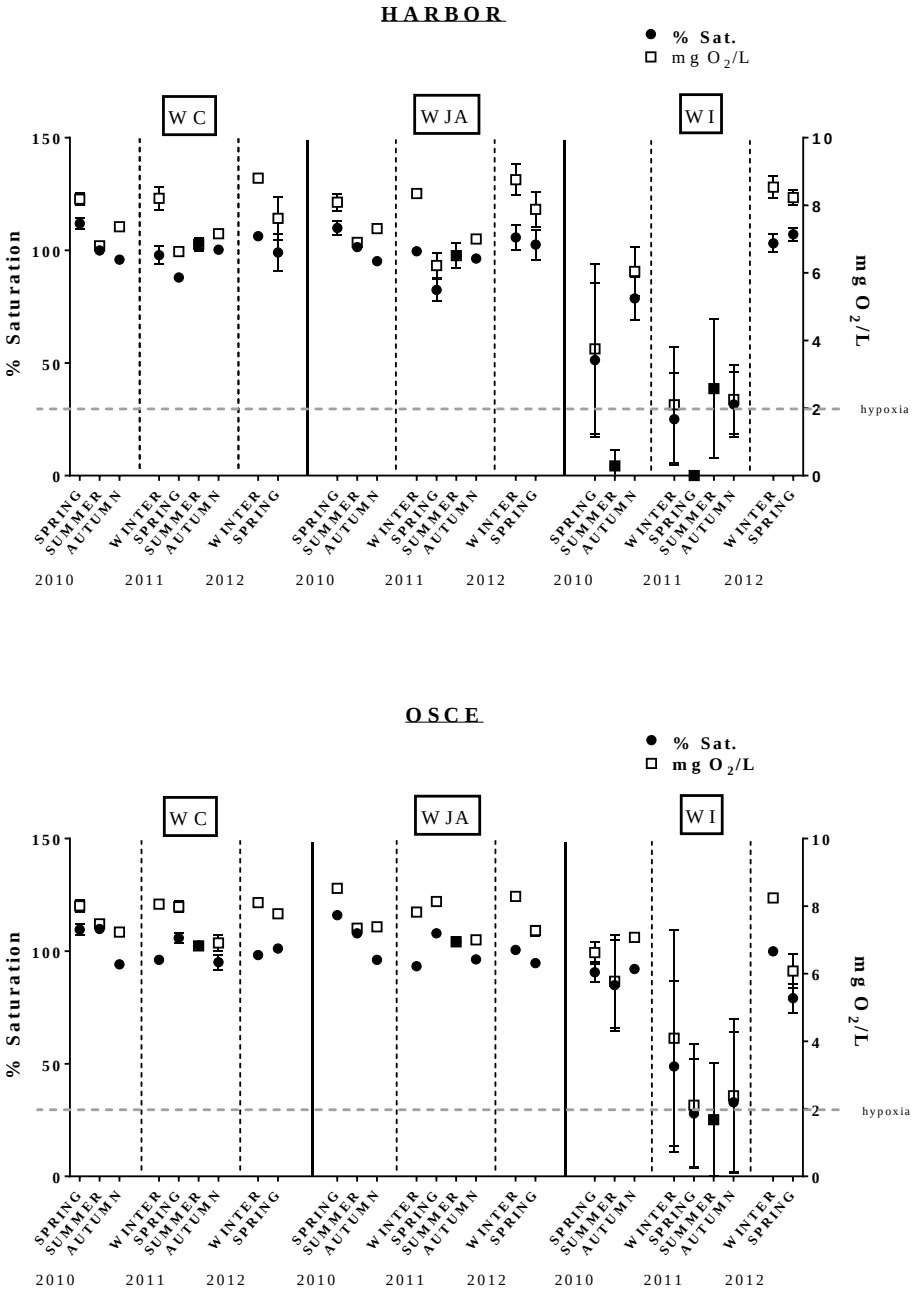


Figure 3.3: Plot of mean O_2 concentrations ($mg.L^{-1}$) and saturation (%) in 2010-2012, for every season, at the two sites and for water column (WC), water just above litter (WJA) and water inside litter (WI). The light grey dotted horizontal line represents the hypoxia threshold of 2 mg $O_2.L^{-1}$ (Levin, 2009). Error bars are standard deviations.

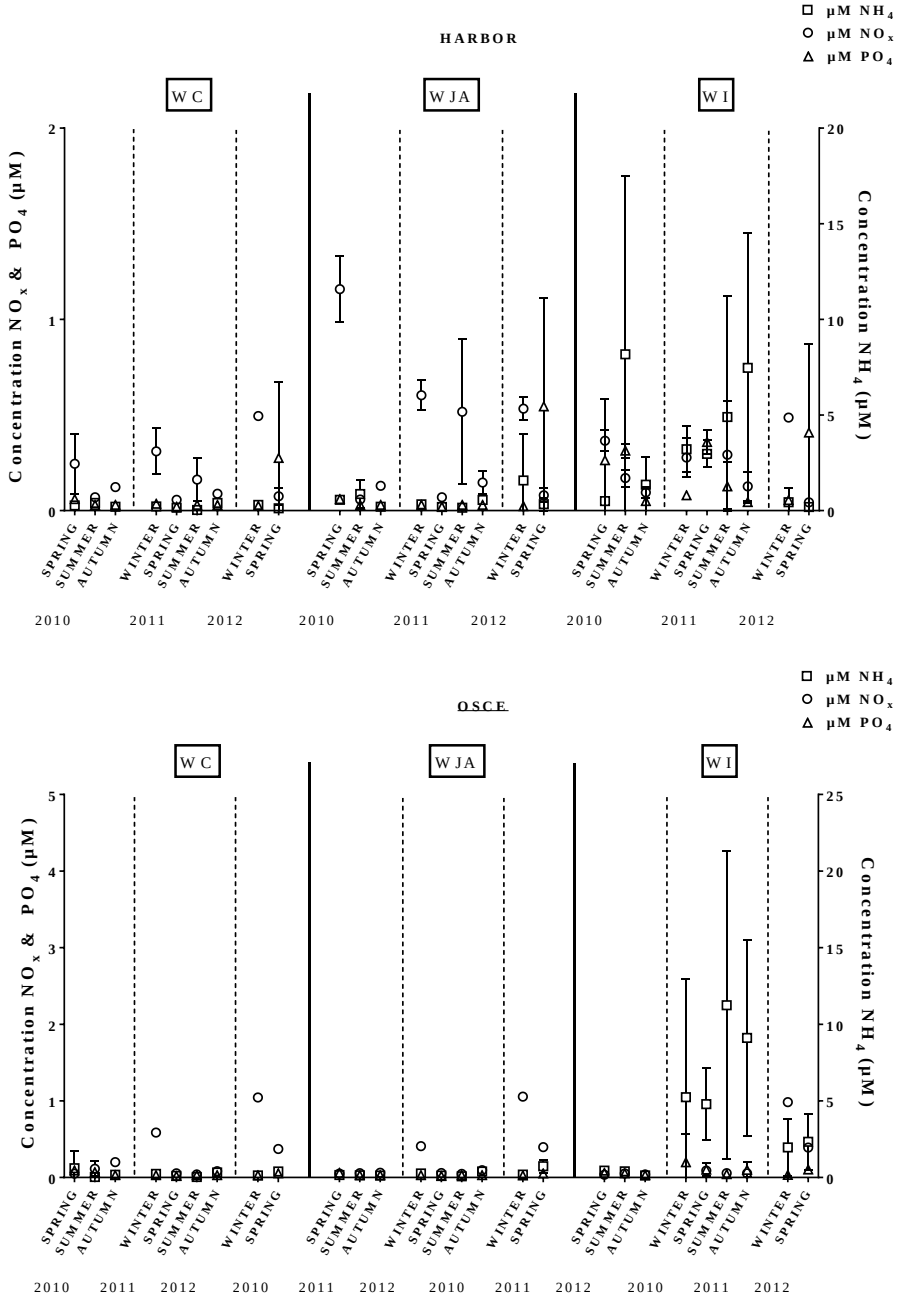


Figure 3.4: Plot of NH_4, NO_X and PO_4 mean concentrations (µM) in 2010-2012, for every season, at the HARBOR-site and the OSCE-site, for water column (WC), water just above litter (WJA) and water inside litter (WI). Error bars are standard deviations.

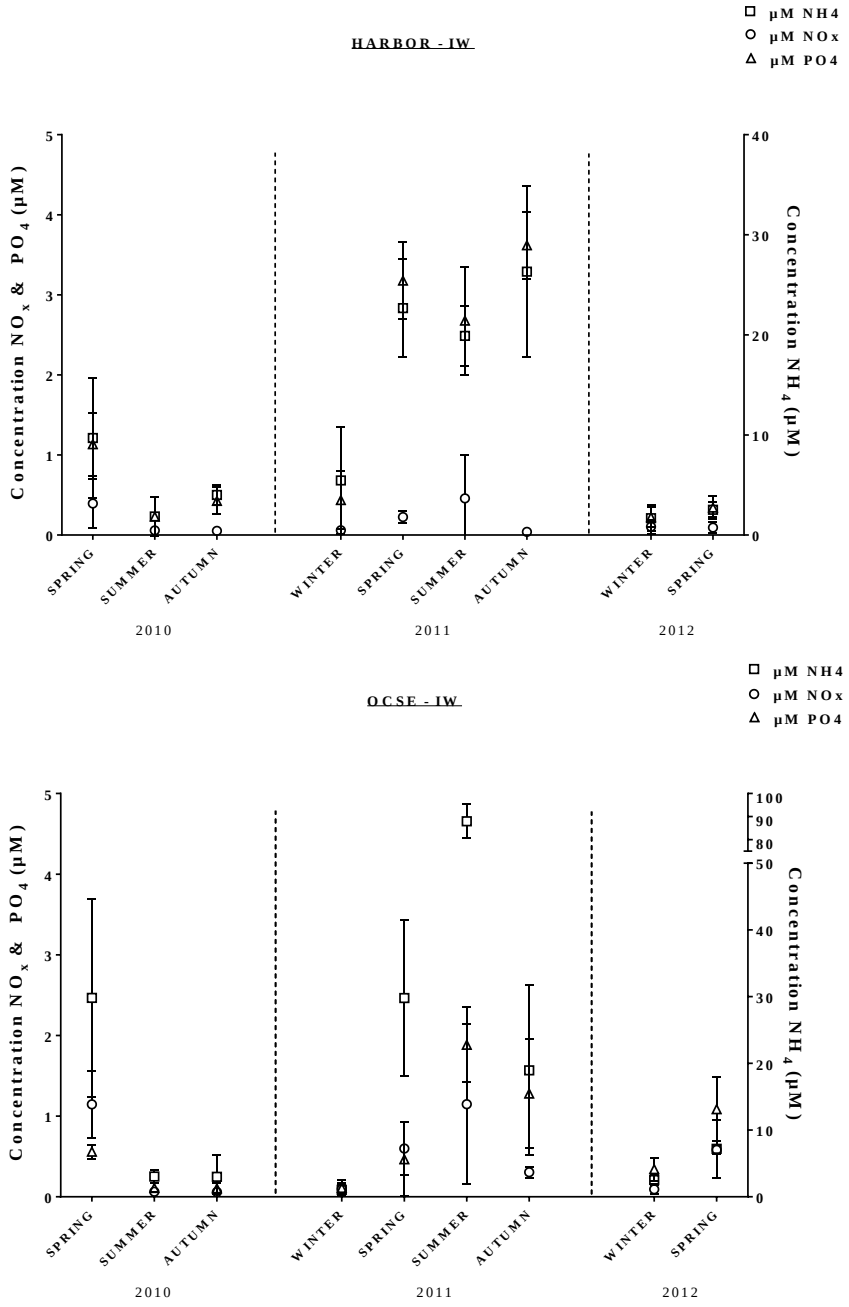


Figure 3.5: Plot of NH_4, NO_X and PO_4 mean concentrations (µM) in 2010-2012, for every season, at the HARBOR-site and the OSCE-site, for interstitial water (IW). Error bars are standard deviations.

3.1.3. Macrofauna community

3.1.3.1. Litter Mass bias

To minimize the bias of sampled mass in further analysis and to reflect the fact that a variable volume was sampled despite the constant sampling surface, abundance was weighed against the Total litter dry mass, and every further analysis or discussion will be performed on this weighed abundance (ind. gDM^{-1}) and not on surface abundance (ind. m^{-2}). Nevertheless, potential links between macrofauna and litter biomass will also be discussed further. Moreover total macrofauna abundance showed a very significant positive linear relationship with litter dry mass sampled ($R^2 = 0.18$, $p < 0.0001$) which supported this choice.

3.1.3.2. High taxonomic level:

For the 2010-2012 period, this study identified 9435 individuals from 115 species belonging to macrofauna for a mean global weighed abundance of 3.30 ± 2.67 ind.gDM^{-1}.

At the level of the phylum, EMAs were highly dominated by arthropods, representing more than 75% of the sampled macrofauna for a weighed abundance of 2.03 ± 1.81 ind.gDM^{-1}. Arthropods represent 76.00 ± 15.56% and 77.75 ± 14.37% of the macrofauna at the HARBOR-site and the OSCE-site respectively, with a maximum respectively in winter 2011 and summer 2010. The second most abundant phylum was annelids, representing more than 10% of the sampled macrofauna for a weighed abundance of 0.37 ± 0.53 ind.gDM^{-1}. Annelids represented 13.97 ± 12.13% and 11.87 ± 8.78% of the macrofauna at the HARBOR-site and the OSCE-site respectively, with a maximum respectively in summer 2010 and winter 2011. The third most abundant group was mollusks for a weighed abundance of 0.18 ± 0.24 ind.gDM^{-1}, representing more than 7% of the sampled macrofauna. Mollusks represented 7.19 ± 7.51% and 8.17 ± 13.22% at the HARBOR-site and the OSCE-site respectively, with a maximum in autumn 2011 for both sites. The other phyla were far less abundant and were nemerteans (0.9%), echinoderms (0.7%), chordates (0.3%) and platyhelminths (0.2%).

Highest global weighed abundance of macrofauna was observed in spring and the lowest abundance was observed in autumn, except for the HARBOR-

site in 2011. Multivariate analysis on weighed abundances (ind.gDM^{-1}) showed a significant effect of the seasonal factor for arthropods, annelids, echinoderms and nemerteans. An effect of the spatial factor was found only for nemerteans (3-way MANOVA, $p < 0.001$). However, hierarchical clustering analysis (Ward method) based on the square root –transformed weighed abundance data from phyla only partly showed this "seasonal" pattern, forming one well-supported cluster corresponding to group formed by autumn and winter samples. Two other clusters corresponding to mixed samples from spring and summer were also well supported but were not forming a cluster together (Figure 3.6). No annual and spatial effects were observable here.

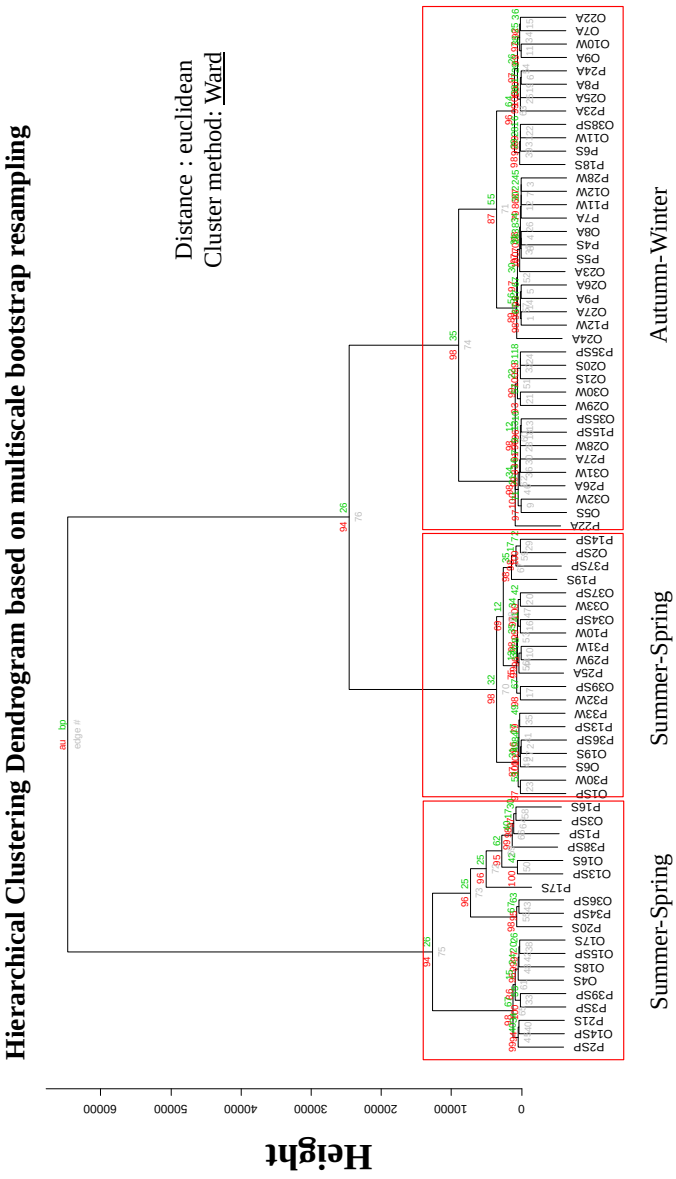


Figure 3.6 : Hierarchical clustering dendrogram using Euclidean distances and Ward grouping method. Based on square-root transformed weighed abundances of all the high-level taxa. Each sample is represented by a code where O or P is the sampling site (O for OSCE-site and P for HARBOR-site), the sample number and S, A, W ad SP, the seasons (S for summer, A for autumn, W for winter and SP for spring). The Y axis represents the Euclidean distance between the samples. Red numbers can be interpreted as the probability a cluster has been formed during the 10000 iterations of the bootstrap resampling process (values above 75 are considered as "high"). Green numbers are the bootstrap value.

3.1.3.3. Lower taxonomic level

Within arthropods, composed of 49 species, the most dominant order was amphipods, representing 77.81 ± 21.60% of the arthropods during the 2010-2012 period. Amphipods were followed by isopods (8.76 ± 9.99%), decapods (7.48 ± 12.33%) and leptostraceans (5.67 ± 9.23%). The remaining arthropods were much more anecdotic and represented less than 0.2%. Amphipods were strongly dominated by only two species: *Gammarella fucicola*, representing 48.23 ± 26.43% (1.11 ± 1.26 ind. gDM^{-1}) and, to a lesser extent, *Gammarus aequicauda*, representing 8.69 ± 15.62% (0.21 ± 0.74 ind. gDM^{-1}). It must be noted that *Gammarella fucicola* was the most dominant species in the EMAs macrofauna and represented 37.98 ± 22.84% of the total sampled individuals for the 2010-2012 period.

Within annelids, representing 35 species, only polychaetes were identified. They were dominated by three species: *Platynereis dumerilii* representing 27.85 ± 28.25%, *Hesiospina aurantiaca* representing 25.46 ± 29.64%and *Polyophtalmus pictus*, representing 15.35 ± 19.83%.

Mollusks composed exclusively of gastropods (>98%), represented 25 species. They were largely dominated by two species: *Bittium reticulatum*, representing 73.14 ± 29.38% and *Tricolia tenuis*, representing 10.49 ± 18.58%.

From the 115 identified species in the 2010-2012 sampling, 19 represented 90% of the global weighed abundance and only these 19 species (Table 3.3) were integrated to further analyses. To guarantee the maximal consistency among the different chapters of this thesis, only species from these 19 most abundant ones will be included in the “low taxonomy level” part of every chapter.

Table 3.3 Summary Table of the mean global abundances and relative abundances of the 19 species representing 90% of the total sampled macrofauna.

Order	Species	Mean global weighted abundance (indiv.gDM^{-1})	Relative abundance (%)	Cumulated relative abundance (%)
Amphipoda	*Gammarella fucicola*	1.14	37.98	37.98
Amphipoda	*Gammarus aequicauda*	1.25	6.99	44.97
Amphipoda	*Nototropis guttatus*	1.23	5.02	49.99
Gastropoda	*Bittium reticulatum*	1.21	4.9	54.89
Leptostraca	*Nebalia strausi*	1.15	4.38	59.27
Amphipoda	*Melita hergensis*	3.01	4.32	63.59
Isopoda	*Jaera (Jaera) nordmanni*	1.18	4.24	67.83
Phylodocida	*Platynereis dumerilii*	1.23	3.84	71.68
Phylodocida	*Hesiospina aurantiaca*	1.21	3.01	74.68
Amphipoda	*Microdeutopus chelifer*	1.23	3.01	77.69
insertae sedis	*Polyophthalmus pictus*	1.25	2.78	80.47
Decapoda	*Athanas nitescens*	2.05	2.39	82.86
Decapoda	*Galathea intermedia*	1.47	1.36	84.22
Amphipoda	*Lysianassa costae*	1.31	1.21	85.43
Phylodocida	*Chrysopetalum debile*	1.11	1.19	86.62
-	*Nemertea spp.*	1.12	1.18	87.8
Gastropoda	*Tricolia tenuis*	1.12	0.69	88.49
Amphipoda	*Dexamine spinosa*	1.22	0.66	89.15
Amphipoda	*Apherusa chiereghinii*	1.14	0.67	89.82

3-way MANOVA based on weighed abundances of the 19 species showed a very highly significant ($p < 0.0001$) effect of the seasonal and annual factor as well as their interactions. A significant effect ($p < 0.001$) of the annual factor was found for *Melita hergensis*, *Dexamine spinosa*, *Nototropis guttatus*, *Microdeutopus chelifer*, *Galathea intermedia*, *Athanas nitescens*, *Hesiospina aurentiaca* and *Nemertea* spp.. A significant effect of the seasonal ($p < 0.001$) factor was observed for *Gammarella fucicola*, *Dexamine spinosa*, *Lysianassa costae*, *Nebalia strausi*, *Galathea intermedia*, *Jaera (Jaera) nordmanni*, *Platynereis dumerilii* and *Nemertea* spp.. No significant effects of any factor or interaction were found for *Gammarus aequicauda*, *Apherusa chiereghinii*, *Chrysopetalum debile*, *Polyophtalmus pictus* and *Bittium reticulatum*.

SIMPER analysis (Table 3.4) for seasonal and annual factor based on square root-transformed weighed abundances of the 115 species showed that *Gammarella fucicola* was always the strongest contributor to similarity. It must also be noted that for both factors, top five contributors belonged to the 19 most abundant species representing 90% of total relative abundance inside EMAs.

Table 3.4: Summary Table of the SIMPER analysis showing the total similarity and specific contribution to similarity for seasonal and annual factors based on the square root-transformed weighed abundances of the 115 sampled species.

Seasonal factor

SPRING (51.06% similarity)

Species	%	Cum.%
Gammarella fucicola	31.44	31.44
Gammarus aequicauda	10.43	41.87
Platynereis dumerilii	10.14	52.01
Nebalia strausi	6.7	58.7
Bittium reticulatum	6.54	65.25

SUMMER (44.40% similarity)

Species	%	Cum.%
Gammarella fucicola	28.72	28.72
Nebalia strausi	9.82	38.54
Jaera (Jaera) nordmanni	8.83	47.37
Platynereis dumerilii	7.94	55.3
Athanas nitescens	6.06	61.36

AUTUMN (37.18% similarity)

Species	%	Cum.%
Gammarella fucicola	57.73	57.73
Bittium reticulatum	6.89	64.61
Melita hergensis	5.85	70.47
Hesiospina similis	5.47	75.94
Gammarus aequicauda	4.73	80.67

WINTER (45.55% similarity)

Species	%	Cum.%
Gammarella fucicola	36.11	36.11
Nototropis guttatus	13.72	49.82
Melita hergensis	13.57	63.39
Bittium reticulatum	8.22	71.6
Hesiospina similis	7.59	79.19

Annual factor

2010 (34.24% similarity)

Species	%	Cum.%
Gammarella fucicola	36.24	36.24
Platynereis dumerilii	7.66	43.9
Nebalia strausi	6.3	50.2
Nototropis guttatus	5.44	55.63
Microdeutopus chelifer	4.78	60.41

2011 (39.12% similarity)

Species	%	Cum.%
Gammarella fucicola	44.33	44.33
Bittium reticulatum	10.09	54.42
Nebalia strausi	8.67	63.09
Melita hergensis	5.2	68.3
Platynereis dumerilii	4.89	73.19

2012 (49.84% similarity)

Species	%	Cum.%
Gammarella fucicola	28.68	28.68
Melita hergensis	11.95	40.62
Nototropis guttatus	11.82	52.45
Hesiospina similis	8.26	60.71
Bittium reticulatum	7.77	68.48

Dissimilarity between seasons appeared to be high (58.65 - 70.27%) and *Gammarella fucicola* was always the strongest contributor for dissimilarity between seasons. Dissimilarity between spring and summer (58.65%) was lower than dissimilarity between spring/summer and winter (69.73% - 70.21%) or spring/summer and autumn (70.06% -70.27%). The same observation was made for the dissimilarity between autumn and winter. Dissimilarity between years was also high (63.31 – 66.27%) and *Gammarella fucicola* was always the strongest contributor for dissimilarity between years.

Species richness (**S**) in terms of total macrofauna was maximum at the HARBOR-site in summer 2011 (28.67 ± 4.13) and minimum at the HARBOR-site in winter 2011 (5.67 ± 1.15).

Average Shannon-Wiener diversity index (**H'**) is 2.39 ± 0.50, which corresponds to a "high diversity" ecosystem according to the universally applicable chart accepted by the "Water Framework Directive" (EU directive 2000/60, form Jørgensen *et al*., 2005). H' was maximum at the HARBOR-site in summer 2011 (3.07 ± 0.16; "very high diversity") and minimum at the HARBOR-site in winter 2011 (1.57 ± 0.16; "moderate diversity").

Simpson evenness index (**1-λ'**) was maximum at the HARBOR-site in summer 2011 (0.93 ± 0.01) and minimum at the HARBOR-site in winter 2011 (0.78 ± 0.32).

Multivariate analysis on these indexes showed a very highly significant seasonal effect and the interaction between seasonal and annual factor (3-way MANOVA, $p < 0.0001$). A very highly significant effect for the seasonal factor was observed for **S**, **H'** and **1-λ'**. A very highly significant effect for the interaction between seasonal and annual factors was observed for **S** (Table 3.5).

Table 3.5: Summary Table of 3-way MANOVA for S, H' and 1-λ' indexes, for annual, seasonal and spatial factors and interactions.

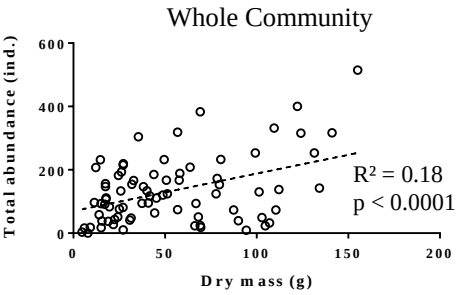
Summary Table: 3-way MANOVA									
	S			**H'**			**1-λ'**		
Factors and interactions	F	df	p	F	df	p	F	df	p
Year	0.92	2	ns	1.23	2	ns	0.89	2	ns
Site	3.22	1	ns	0.06	1	ns	0.48	1	ns
Season	33.28	3	***	23.31	3	***	8.64	3	***
Year x Site	1.44	2	ns	1.14	2	ns	1.40	2	ns
Year x Season	9.85	3	***	3.62	3	ns	0.43	3	ns
Site x Season	1.16	3	ns	1.70	3	ns	1.96	3	ns
Year x Site x Season	1.59	3	ns	0.31	3	ns	0.04	3	ns

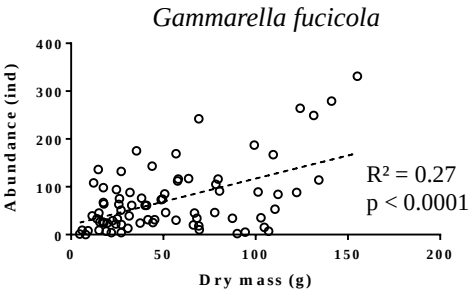
* = 0.01 < P < 0.001 = significant
** = 0.001 < P < 0.0001 = highly significant
*** = P < 0.0001 = very highly significant
ns = not significant

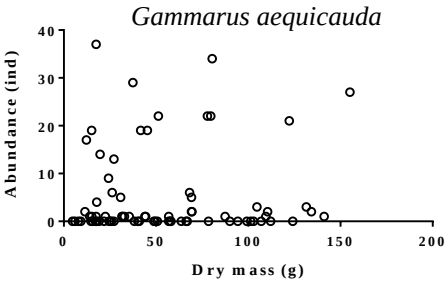
3.1.4. Dry mass impact

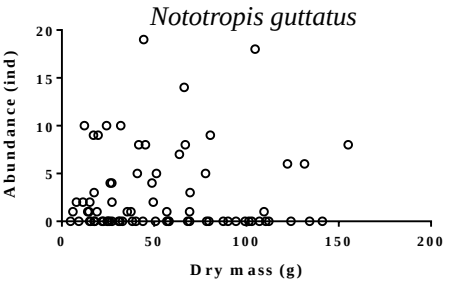
Prior to further investigation of the potential impact of other environmental parameters using weighed abundances, it was decided to assess the potential impact of the most visually variable parameter of the litter which was litter dry mass itself. The potential impact of litter dry mass was tested on the global community and on 7 species belonging to every taxon present in the 19 most abundant species: *Gammarella fucicola, Gammarus aequicauda, Nototropis guttatus, Nebalia strausi, Athanas nitescens, Platynereis dumerilii* and *Bittium reticulatum*. The abundances used were the "raw" abundances data from every species.

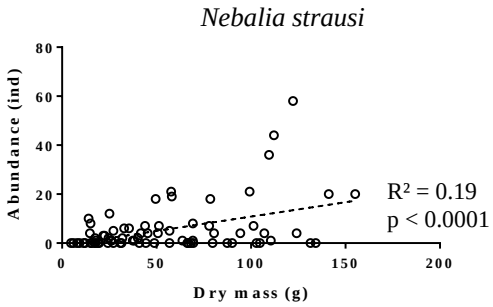
The modest but significant link between litter dry mass and the global community was already demonstrated in paragraph 3.1.3.1 (Figure 3.7). *Gammarella fucicola,* the most abundant invertebrate in our samples, showed a modest but significant positive link with litter dry mass, similar to what was observed for the total community. The leptostracean *Nebalia strausi* showed an even more modest, yet still significant positive link with litter dry mass (Figure 3.7). All the other species presented no relationship at all with litter dry mass.

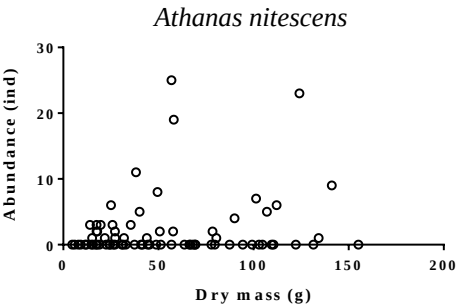

Whole Community
Total abundance (ind.)
Dry mass (g)
R² = 0.18
p < 0.0001

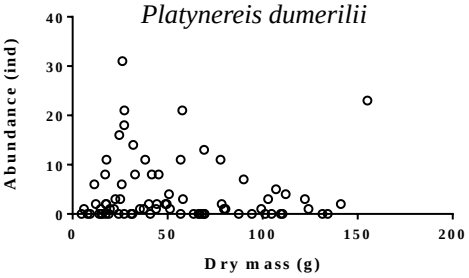

Gammarella fucicola
Abundance (ind)
Dry mass (g)
R² = 0.27
p < 0.0001


Gammarus aequicauda
Abundance (ind)
Dry mass (g)


Nototropis guttatus
Abundance (ind)
Dry mass (g)


Nebalia strausi
Abundance (ind)
Dry mass (g)
R² = 0.19
p < 0.0001


Athanas nitescens
Abundance (ind)
Dry mass (g)


Platynereis dumerilii
Abundance (ind)
Dry mass (g)

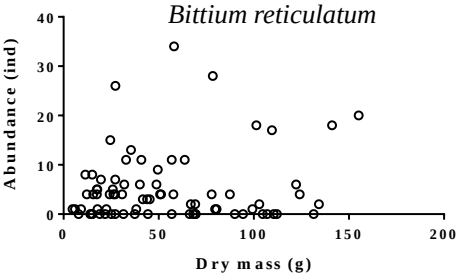

Bittium reticulatum
Abundance (ind)
Dry mass (g)

Figure 3.7: graphical representations of the abundances of: Gammarella fucicola, Gammarus aequicauda, Nototropis guttatus Nebalia strausi, Athanas nitescens, Platynereis dumerilii and Bittium reticulatum. vs. litter dry mass. Dotted lines represent the significant linear relationships.

It must also be noted that no clear relationship between litter O_2 concentration and litter dry mass mount could be observed, meaning that hypoxia could happen at any moment of the year.

3.1.5. Impact of environmental parameters on EMAs macrofauna

To investigate the relationships between macrofauna weighed abundance variation pattern observed and each environmental variable (LL, DR, A, E, O_2, NO_x, NH_4, PO_4), Multiple Regression Analysis, DistLM and dbRDA graphical representation were performed. These tests were performed on the global weighed abundance and individually on the 14 species showing significant abundance variations according to the "year factor" and the "season factor" (See §3.1.3.2).

Multiple regression showed that out of the 8 environmental parameters measured, only O_2 concentration and, to a lesser extent, NH_4 concentrations were the parameters explaining the best the abundance variations observed for these species. Out of the 14 species, only 6 showed significant relationships with O_2 and/or NH_4 (Table 6). The oxygen concentration parameter was always the most significant one, and for two species, NH_4 was also significant. The 3 amphipods species (*Melita hergensis*, *Nototropis guttatus* and *Microdeutopus chelifer*) showed significant positive link to oxygen concentration while the Leptostracean species (*Nebalia strausi*) and the 2 decapods species (*Athanas nitescens* and *Galathea intermedia*) showed a significant negative relationship (Table 3.6). Only 2 species showed a significant impact of NO_X. *Melita hergensis* showed a significant positive link with NH_4, while *Nebalia strausi* showed a significant negative link. The impact of oxygen and NH_4 thus seemed to be very species specific.

Table 3.6: Summary table of the multiple regression, showing the values of partial regression and corresponding p-values for O_2 and NH_4 concentrations, for species showing significance for at least one environmental factor and for Gammarella fucicola and Gammarus aequicauda, the two most abundant species of amphipods.

		Environmental variable			
		O_2 ($mg.L^{-1}$)		NH_4 (µM)	
		PC	p	PC	p
	Gammarella fucicola	-	-	-	-
	Gammarus aequicauda	-	-	-	-
Amphipod	*Melita hergensis*	0,7768	0,0082	0,7976	0,0097
	Nototropis guttatus	0,6695	0,0034	-	-
	Microdeutopus chelifer	0,5886	0,0085	-	-
Leptostracean	*Nebalia strausi*	-0,9133	0,0002	-0,6780	0,0042
Decapod	*Galathea intermedia*	-0,7988	0,0056	-	-
	Athanas nitescens	-0,7353	0,0054	-	-

Another major result of this regression test is that the most dominant species, the two Amphipods *Gammarella fucicola* and *Gammarus aequicauda* showed no relationship at all with any of the measured environmental variables

The distance-based linear regression model (DistLM) indicated an interesting link between the macrofauna assemblage and the environmental parameters measured (Figure 3.8). The model explained 41.8% of the total observed variability, showed no significant collinearity among environmental variables, and contained only 3 parameters. (1) A highly significant one, O_2 (pseudo-F = 5.84, p = 0.001), which is accounting for 26.7% of the total observed variability. (2) A just non-significant one, NH_4 (pseudo-F = 2.28, p = 0.051), which is accounting for 12.5% of the total observed variability. (3) A very non-significant one, PO_4 (pseudo-F = 0.218, p = 0.28), which is accounting for less than 5% of the total observed variability. The first dbRDA axis accounted for 30.58% of the total observed variability in the macrofauna assemblage and discriminated samples based essentially on O_2 concentration (multiple partial correlation = -0.893). The second dbRDA axis accounted for 9.28% of the total observed variability in the macrofauna assemblage and discriminated samples based essentially on NH_4 concentration (multiple partial correlation = -0.890).

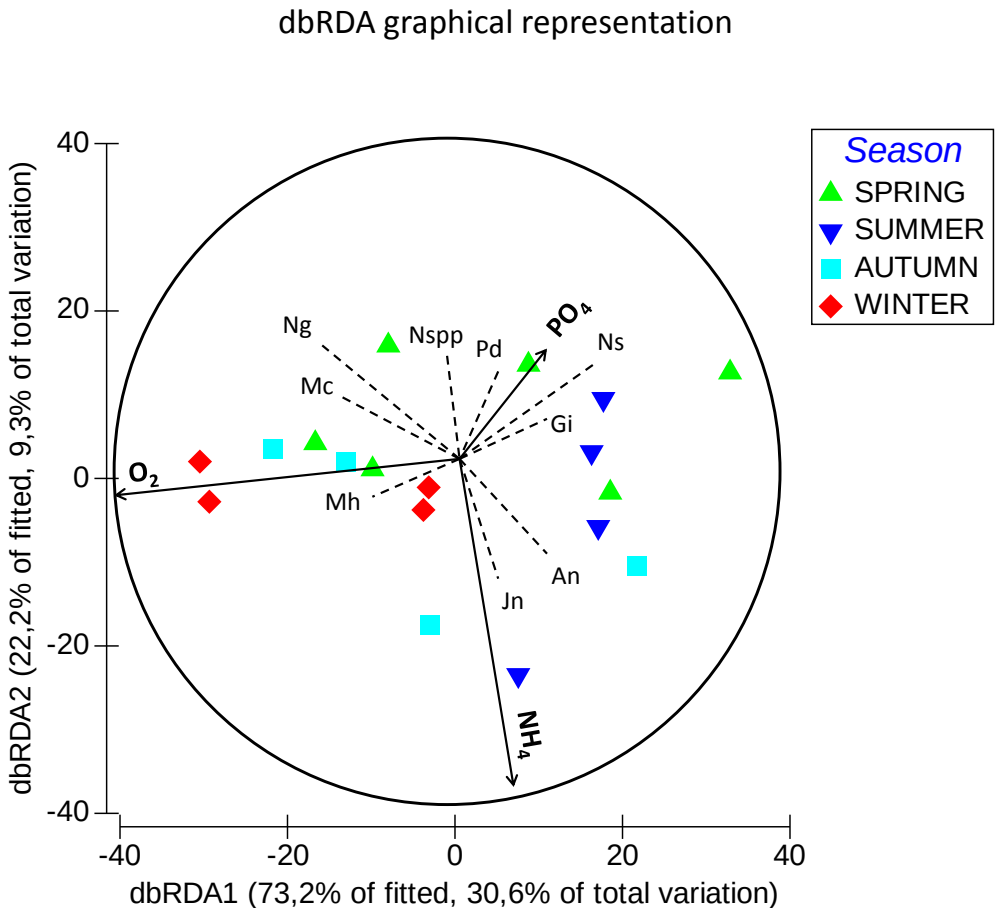
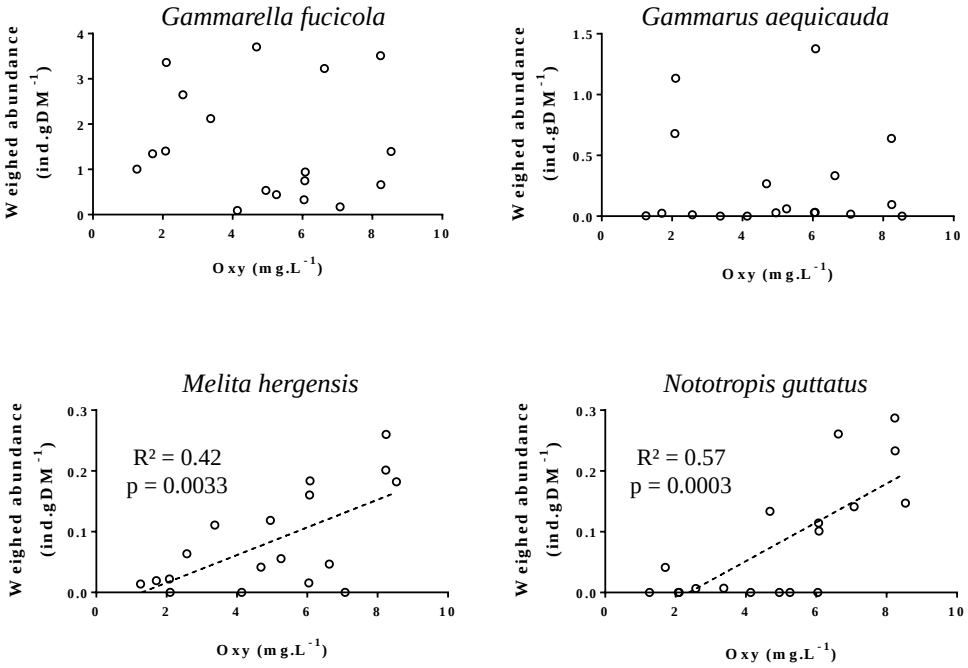


Figure 3.8: Distance-based redundancy ordination (bdRDA) representing the DistLM modelling for the 14 most abundant species and environmental variables. Full vectors represent the direction of increasing values of the environmental variables from the model (O_2, NH_4 and PO_4). Dotted lines represent macrofauna species with correlations ≥ 0.25 to the ordination axes. Vector and lines length represents the partial correlation strength with the dbRDA axes; the circle is a unit circle (radius = 1) whose relative size and position is arbitrary with respect to the underlying plot. Triangles and squares represent the samples, color coded by layer. Species abbreviations: Mh = Melita hergensis; Mc = Micodeutopus chelifer; An = Athanas nitescens; Gi = Galathea intermedia; Jn = Jaera nordmanii; Ns = Nebalia strausi; Pd = Platynereis dumerilii; Pp = Polyophtalmus pictus; Nspp = Nemertea spp.

Among the species presenting correlation ≥ 0.25 of the ordination, 3 amphipods species (*Melita hergensis*, *Nototropis guttatus* and *Microdeutopus chelifer*) had negative values for the first axis (negatively correlated with O_2), supposing a positive link with O_2 concentration (Figure3.8). On the contrary, the leptostracean species (*Nebalia strausi*) and the 2 decapods species (*Athanas nitescens* and *Galathea intermedia*) had positive values for the first axis, supposing a negative relationship with O_2 concentration. Most species presented positive values for the second axis (negatively correlated with NH_4), supposing a negative link with NH_4 concentration and only 2 species had negative values, indicating a positive relationship with NH_4 concentration. All winter samples (red squares) had negative values for the first axis while all summer samples (blue triangles) showed positive values. Spring and autumn samples presented both positive and negative values.



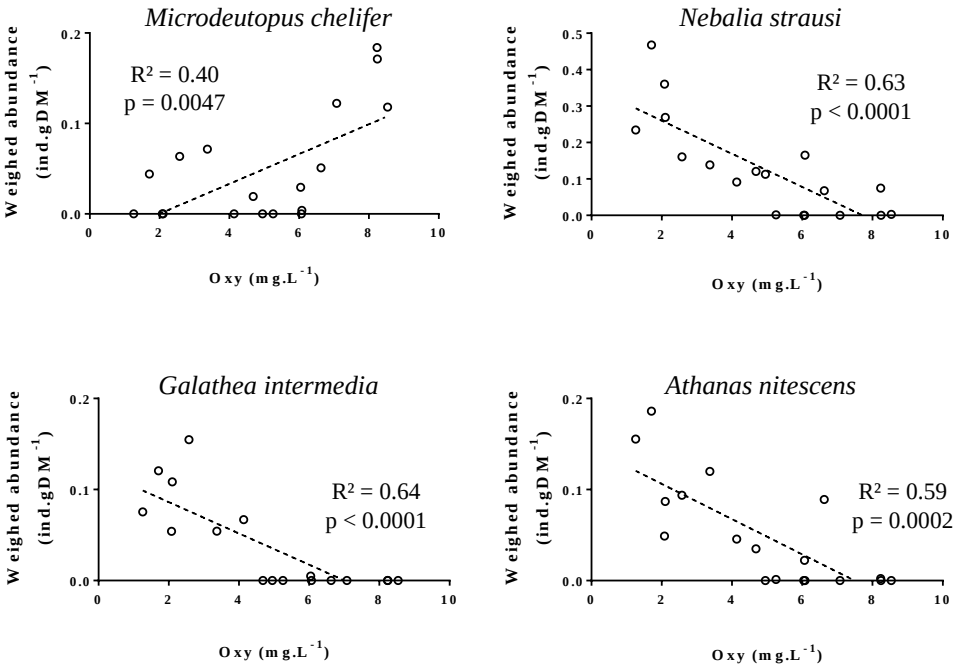


Figure 3.9: Graphical representations of the weighed abundances of: Gammarella fucicola, Gammarus aequicauda, Nototropis guttatus Nebalia strausi, Athanas nitescens, Platynereis dumerilii and Bittium reticulatum. vs. litter O_2 concentration. Dotted lines represent the significant linear relationships.

Coming back to "raw" weighed abundances data also highlighted (Figure 3.9) the obvious significant positive or negative linear links existing between the 6 species and litter O_2 concentration.

3.2. Weekly sampling

3.2.1. Exported Macrophytodetritus time evolution

EMAs showed high variability from week to week between September 2012 and June 2013. $_{Total}$DM and litter height showed an important decline from September 2012 to June 2013 and two important events of $_{Total}$DM and litter height increases (Figure 3.10, A&B) were identified, the first one on November 11th and the second one on May 13th. $_{Total}$DM was maximum of 2759.45 ± 196.28 gDM.m^{-2} on September 15th and a minimum of 250.34 ± 125.01 gDM.m^{-2}. Litter height showed a maximum of 50.83 ± 5.85 cm on November 14th just after the first event and a minimum of 1.17 ± 1.17 cm on May 2nd just before the second event. Litter composition of this weekly sampling showed the same characteristics as the seasonal sampling. DL was the most abundant component (1074.47 ± 863.33 gDM.m^{-2}) followed by LL (182.97 ± 243.98 gDM.m^{-2}) and A (9.67 ± 18.15 gDM.m^{-2}). DR were totally absent of the sampled litter during the whole sampling. Litter composition was also highly variable and only DL and E showed the same pattern as $_{Total}$DM. The other components didn't show a clear pattern of variability.

Litter fragmentation index showed a maximum value (minimum fragmentation) of 0.85 on November 11th and a minimum value (maximum fragmentation) of 0.33 on May 7th. Two important events of drastic fragmentation variation were identified as well (Figure 3.10, C), on November 11th and on May 13th.

Litter cover ratio was highly variable throughout the sampling period, showing a maximum cover of 100% in autumn 2012 and a constant decrease in winter to reach a minimum of 10% in spring 2013. Litter cover showed two important events of extreme variation, one in November and the other in May (Figure 3.10, D).

Both events, associated with strong stormy conditions and important north-eastern winds, were not identical. The first one from November 2012 was composed of two distinct phases. During the first phase, on November 1st, a massive litter departure occurred (Figure 3.11, 1), with litter $_{Total}$DM, litter cover ratio and litter height drastic decrease. This departure event left the OSCE-site accumulation with very low amounts of litter for 1 week. After that, a second phase consisting of a massive litter return (Figure 3.11, 2) occurred mainly on November 11th, with litter $_{Total}$DM, litter cover ratio and litter height drastic increase. The May 13th event only comprised a massive litter addition on the litter accumulation that lasted 6 days. After 6 days, a progressive litter $_{Total}$DM, litter cover ratio and litter height decrease was observed.

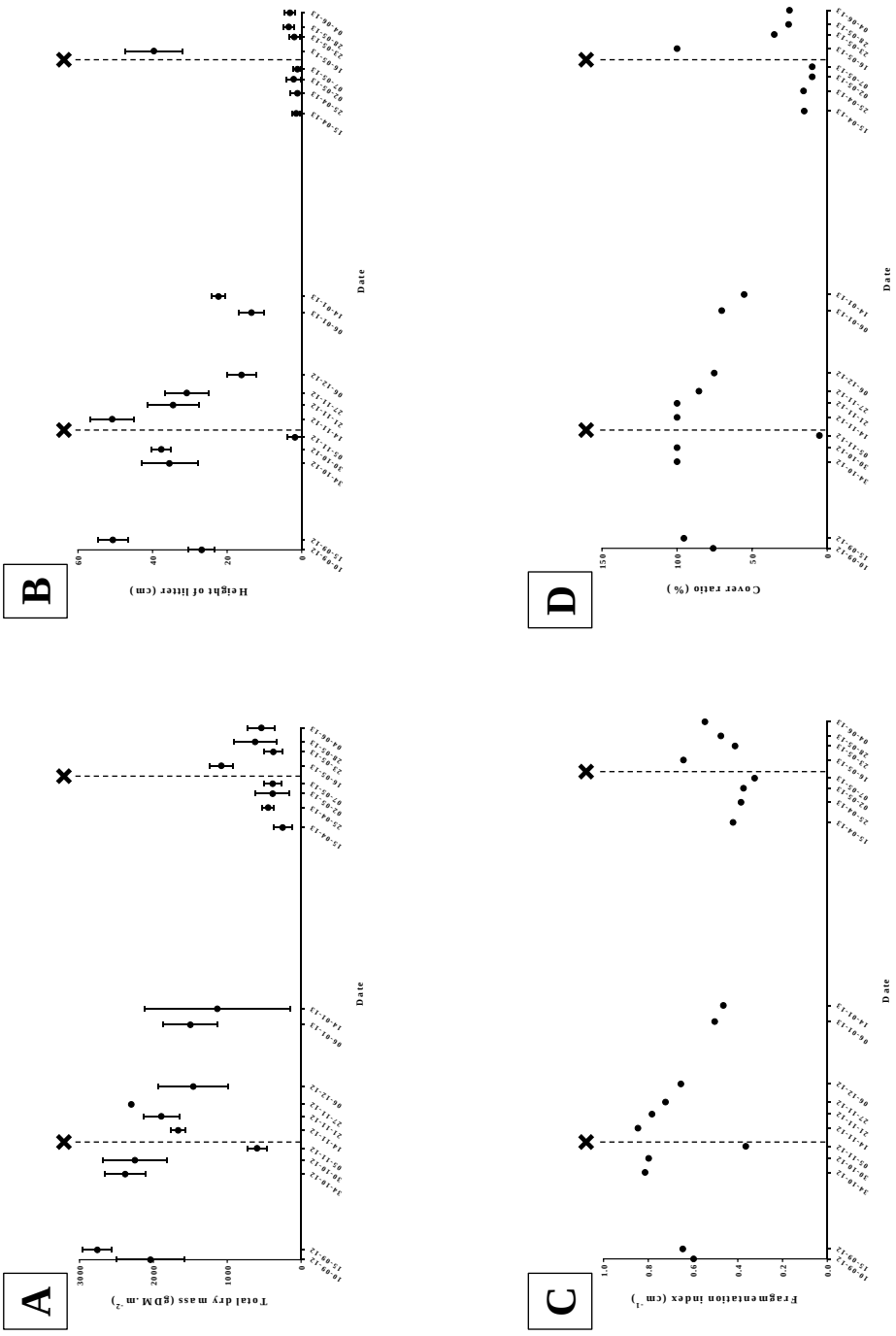
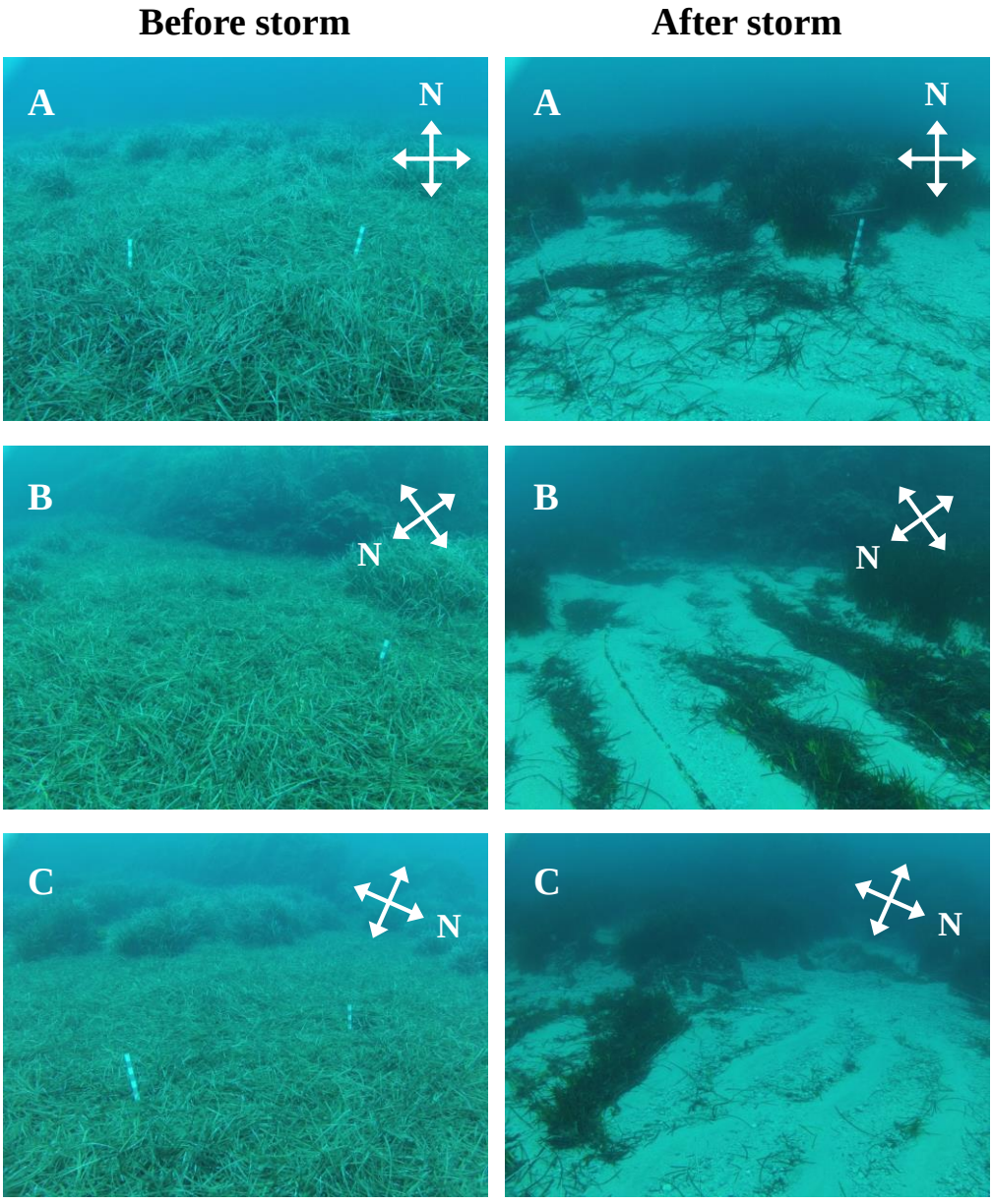


Figure 3.10: Temporal evolution of mean litter dry mass (A), litter height (B), fragmentations (C) and cover ratio (D) between September 2012 and June 2013. Light grey dotted lines and black crosses represent exact dates of the events of November 11th and May 13th. Error bars are standard deviations.


Before storm
After storm
A
N
A
N
B
N
B
N
C
N
C
N

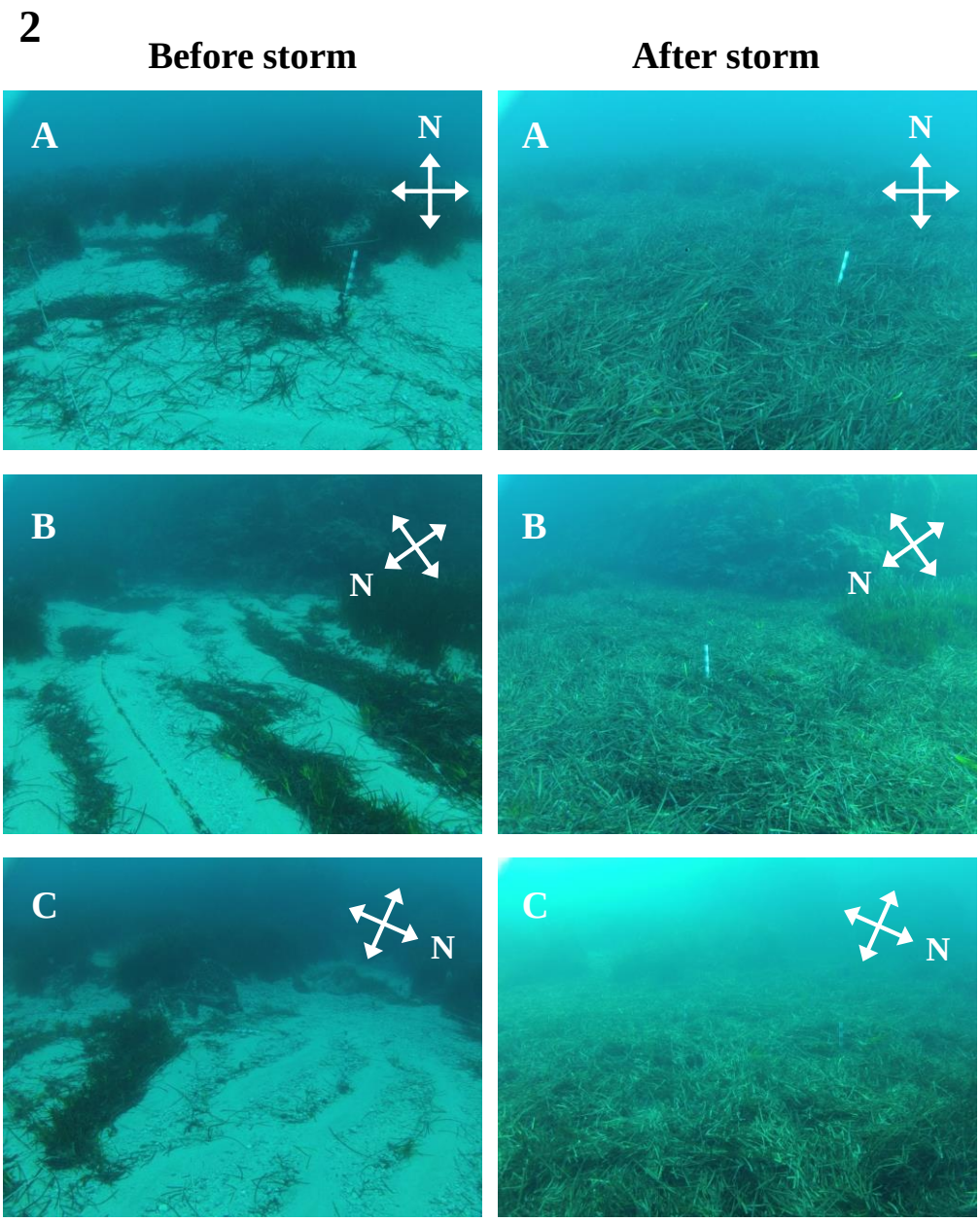


Figure 3.11: Evolution of OSCE-site litter accumulation during the two phases (1 and 2) of the November stormy event. A, B and C represent 3 different cameras capturing different angles of view of the accumulation. N represents the orientation of the north. All pictures were taken at 02:00 pm.

3.2.2. Abiotic factor time evolution

Oxygen concentration in WC and WJA showed a slight and continuous increase from September 2012 to June 2013, and showed values ranging from 6.67 $mgO_2.L^{-1}$ to 8.94 $mgO_2.L^{-1}$. Oxygen concentration was much more variable in WI and showed values ranging from 3.23 $mgO_2.L^{-1}$ to 9.19 $mgO_2.L^{-1}$. In WI, oxygen concentration was minimum at the beginning of the sampling period (September 2012), showing a value of 3.83 ± 0.42 $mgO_2.L^{-1}$on September 9th. Oxygen concentration then increased slightly during winter and spring to reach a maximum value of 8.93 ± 0.30 $mgO_2.L^{-1}$ on June 4th. Two events of rapid decrease of oxygen concentration were observed on November 11th and on May 13th (see §3.2.1 of this chapter). Between T4 and T5, WI oxygen decreased by 29.56%, and between T14 and T15, WI oxygen decreased by 24.38%.

In opposition to what was observed during the seasonal study, a non-negligible and significant link existed between litter O_2 concentration and litter dry mass present on the OSCE-site accumulation ($R^2 = 0.53$, $p = 0.0004$).

NO_x, NH_4 and PO_4 were constant during all the sampling period in WC and WJA. Concentrations of NO_x, NH_4 and PO_4 in WC were 0.44 ± 0.08 µM, 0.43 ± 0.15 µM and 0.05 ± 0.01 µM respectively. Concentrations of NO_x, NH_4 and PO_4 in WJA were 0.43 ± 0.07 µM, 0.41 ± 0.16 µM and 0.05 ± 0.01 µM respectively. For WI, concentrations were constant except during two events of rapid concentration increase observed on November 11th and on May 13th. NOx showed a 40% increase, NH4 showed a 47 fold (4761%) increase, and PO4 showed a 7.4 fold (740%) increase during the two events in WI. Concentrations of NO_x, NH_4 and PO_4 were constantly higher in IW than in the three other layers and also showed two events of drastic concentration increase observed on November 11th and on May 13th. NOx showed a 2.1 fold (210%) increase, NH4 showed a 4.4 fold (438%) increase, and PO4 showed a 3.2 fold (321%) increase during the two events in IW.

3.2.3. Macrofauna time evolution

3.2.3.1. Litter Mass bias

To remain coherent with the seasonal study presented in this chapter (§3.1), minimize the bias of sampled mass in further analysis and to reflect the fact that a variable volume was sampled despite the constant sampling surface, abundance was weighed against the Total litter dry mass, and every further analysis or discussion will be performed on this weighed abundance (ind. gDM^{-1}) and not on surface abundance (ind. m^{-2}). Moreover, similarly to what was observed during the seasonal sampling, a weak but significant linear relationship was observed between global community abundance and litter dry mass ($R^2 = 0.13$, $p = 0.006$). Nevertheless, potential links between macrofauna and litter dry mass will also be discussed further.

3.2.3.2. High taxonomic level

5966 individuals from 60 species were sampled between September 2012 and June 2013, representing a global weighed abundance of 1.85 ± 1.21 $ind.gDM^{-1}$. Global weighed abundance showed no clear evolution pattern during the sampling period (Figure 3.12). Species richness declined from September 2012 to November 2012 (5.00 ± 1.73 sp.), and then increased gradually from January 2013 to June 2013 (16 ± 4.58 sp.). Species richness showed a 33.3% decrease between T3 and T4, corresponding to a stormy event, part of the November event. Arthropods were the most abundant taxon, representing 90.82 ± 6.93 % (1.71 ± 1.17 $ind.gDM^{-1}$), followed by Annelids, representing 5.81 ± 5.53 % (0.10 ± 0.10 $ind.gDM^{-1}$) and Mollusks, representing 2.97 ± 4.35 % (0.04 ± 0.04 $ind.gDM^{-1}$). Echinoderms, Nemertea, Platyhelminths and Chordata were far less abundant, corresponding together to less than 0.4%.

None of the taxa presented clear evolution during all the sampling period, and no important variations were observed on November 11th and on May 13th.

3.2.3.3. Low taxonomic level

Within arthropods, composed of 40 species, the most dominant order was amphipods, representing 88.70 ± 10.78% of the arthropods during the sampling period. Amphipods were followed by isopods (4.42 ± 5.46%), leptostraceans (3.93 ± 7.71%) and decapods (2.14 ± 4.08%). The remaining arthropods were much more anecdotic and represented less than 0.9%. Amphipods were strongly dominated by only two species: *Gammarella fucicola*, representing 67.36 ± 20.09% (1.07 ± 0.93 ind.gDM^{-1}) and, to a lesser extent, *Gammarus aequicauda*, representing 12.72 ± 15.78% (0.14 ± 0.18 ind.gDM^{-1}). It must be noted that *Gammarella fucicola* was the most dominant species in the EMAs macrofauna and represented 54.08 ± 18.01% of the total sampled individuals.

Within annelids, representing 7 species, only polychaetes were identified. They were dominated by three species: *Platynereis dumerilii* representing 63.46± 40.99% (0.07 ± 0.09 ind.gDM^{-1}), *Nereis caudata* representing 9.21 ± 25.47% and C*hrysopetalum debile*, representing 7.70 ± 16.86%.

Mollusks, composed almost exclusively of gastropods, represented 8 species. They were largely dominated by two species: *Bittium reticulatum*, representing 57.84 ± 38.69%, and *Tricolia tenuis*, representing 18.19 ± 31.17%.

Only 3 species seemed to show quite a coherent pattern with the one observed for the environmental parameters (Figure 3.12). For these 3 species, 2 extreme events were identified in November 2012 and May 2013. Indeed, *Gammarella fucicola, Gammarus aequicauda* and *Nebalia strausi* showed very important variations of weighed abundance during these two events (Figure 3.13). It must be noted that *Gammarella fucicola* and *Gammarus aequicauda*, the 2 most dominant species, didn't seem to be really impacted by any environmental factor all along this thesis (See this Chapter §3.1.3.3 and § 3.2.3.2, but also in Chapter 4). However, *Gammarella fucicola* and *Gammarus aequicauda* still present important and fast modifications of their weighed abundance during these two events, as well as *Nebalia strausi*.

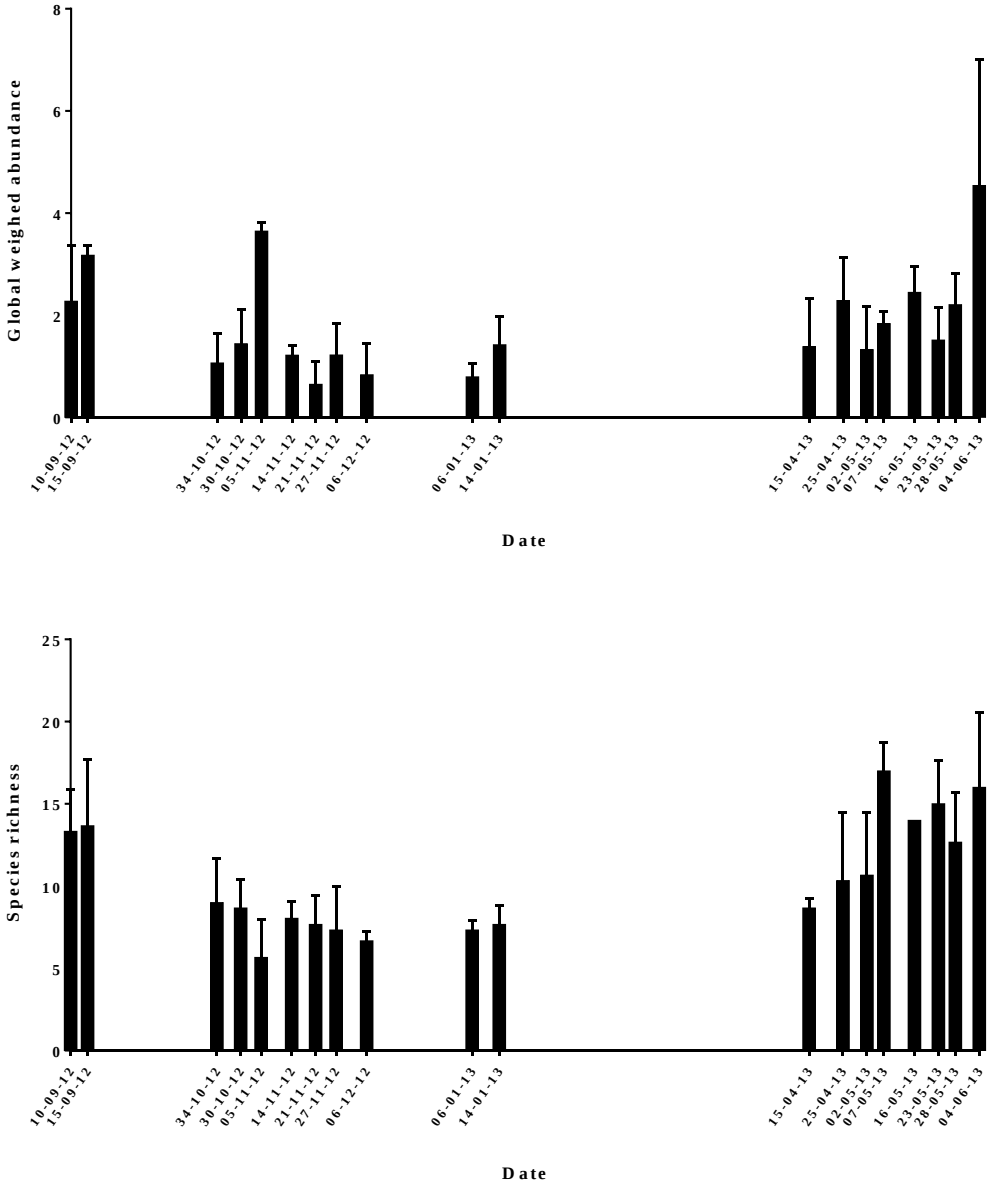
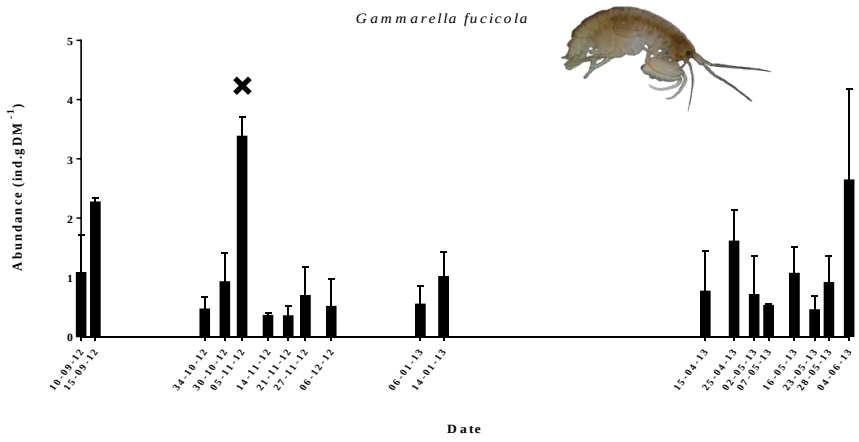
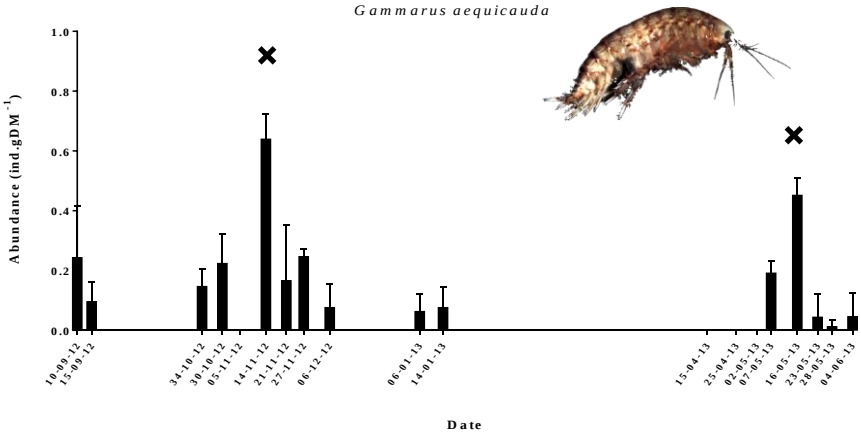


Figure 3.12: Temporal evolution of mean global weighed abundance and species richness between September 2012 and June 2013. Error bars are standard deviations.


Gammarella fucicola
Abundance (ind.gDM-1)
0
1
2
3
4
5
10-09-12
15-09-12
34-10-12
30-10-12
05-11-12
14-11-12
21-11-12
27-11-12
06-12-12
06-01-13
14-01-13
15-04-13
25-04-13
02-05-13
07-05-13
16-05-13
23-05-13
28-05-13
04-06-13
Date


Gammarus aequicauda
Abundance (ind.gDM-1)
0.0
0.2
0.4
0.6
0.8
1.0
10-09-12
15-09-12
34-10-12
30-10-12
05-11-12
14-11-12
21-11-12
27-11-12
06-12-12
06-01-13
14-01-13
15-04-13
25-04-13
02-05-13
07-05-13
16-05-13
23-05-13
28-05-13
04-06-13
Date

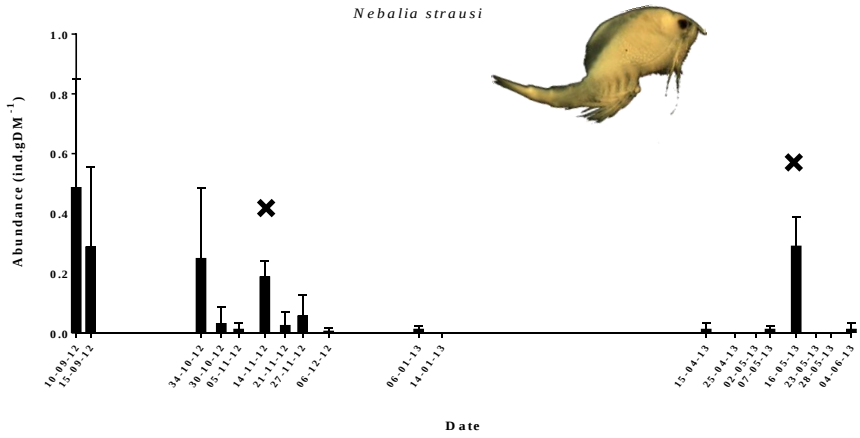

Nebalia strausi
Abundance (ind.gDM-1)
0.0
0.2
0.4
0.6
0.8
1.0
10-09-12
15-09-12
34-10-12
30-10-12
05-11-12
14-11-12
21-11-12
27-11-12
06-12-12
06-01-13
14-01-13
15-04-13
25-04-13
02-05-13
07-05-13
16-05-13
23-05-13
28-05-13
04-06-13
Date

Figure 3.13: Temporal evolution of mean Gammarella fucicola, Gammarus aequicauda and Nebalia strausi mean weighed abundance between September 2012 and June 2013. Crosses represent extreme abundance variations during events of November 11th and May 13th. Error bars are standard deviations.

3.2.4. Impact of environmental parameters on EMAs macrofauna

Among the 19 most abundant species presented in this chapter in §3.1.3.3, 18 were present in the most dominant species from this weekly sampling. To investigate the relationships between macrofauna weighed abundance variations observed and each environmental variable (LL, DR, MA, E, O_2, NO_x, NH_4, PO_4), Multiple Regression Analysis, DistLM and dbRDA graphical representation were performed. The tests were performed on the global weighed abundance of these 18 abundant species.

Multiple regression showed that out of the 11 environmental parameters measured, only O_2 concentration and, to a lesser extent, Living leaves (LL) and Algae (A) quantity were the parameters explaining the best the abundance variations observed. Out of the 18 species, only 5 showed significant relationships with O_2 and/or LL and/or A (Table 3.7).

Table 3.7: Summary Table of the multiple regression, showing the values of partial regression and corresponding p-values for O_2 concentration, living leaves and algae biomass, for species showing significance for at least one parameter and for Gammarella fucicola and Gammarus aequicauda, the two most abundant species of amphipods.

		Environmental variable					
		O_2 (mg.L^{-1})		Living Leaves (gDM)		Algae (gDM)	
		PC	p	PC	p	PC	p
Amphipod	*Gammarella fucicola*	-	-	-	-	-	-
	Gammarus aequicauda	-	-	-	-	-	-
	Melita hergensis	0.79	0.01	-0.73	0.01	-	-
	Microdeutopus chelifer	0.78	0.01	-	-	-	-
Leptostracean	*Nebalia strausi*	-0.92	0	-	-	0.88	0
Decapod	*Athanas nitescens*	-0.93	0	-	-	0.88	0
Nemertean	*Nemertea spp.*	0.74	0	-	-	-	-

The 2 amphipods species (*Melita hergensis* and *Microdeutopus chelifer*) as well as the nemerteans showed a significant positive link with O_2 concentration. The leptostracean species (*Nebalia strausi*) and the decapods species (*Athanas nitescens*) showed a significant negative link with O2 concentration. *Melita hergensis* was the only species showing a significant negative relationship with Living *P. oceanica* Leaves (LL) quantity present in the EMAs. *Nebalia strausi* and *Athanas nitescens* showed a significant positive relationship with Algae (MA) quantity present in the EMAs.

Once again, the two most dominant amphipod species *Gammarella fucicola* and *Gammarus aequicauda* did not show any significant relationship with the 11 measured environmental variables.

The distance-based linear regression model (DistLM) indicated an interesting link between the macrofauna assemblage and the measured environmental parameters (Figure 3.14). Since the weighed abundance was used, DL was not included to the model. The model showed high collinearity (> 0.85) between DL, Cover and Height, and Height and Cover were thus not included in the analysis either. The model explained 30.87% of the total observed variability and contained only 4 parameters. (1) A highly significant one, O_2 (pseudo-F = 4.30, p = 0.0015), which is accounting for 17.2% of the total observed variability. (2) Two other significant parameters, NH_4 (pseudo-F = 2.82, p = 0.02) which is accounting for 9.7% of the total observed variability and (3) NO_X (pseudo-F = 2.51, p = 0.04) which is accounting for 2.6% of the total observed variability. (4) A non-significant one, epiphytes dry mass, E, (pseudo-F = 0.33, p = 0.85) which is accounting for 2.21% of the total observed variability. The first dbRDA axis accounted for 21.1% of the total observed variability in the macrofauna assemblage and discriminated samples based essentially on O_2 concentration and NH_4 concentration (multiple partial correlation = -0.795 and 0.595 respectively). The second dbRDA axis accounted for 6.5% of the total observed variability in the macrofauna assemblage and discriminated samples based essentially on NO_X concentration and E dry mass quantity (multiple partial correlation = -0.642 and -0.414, respectively).

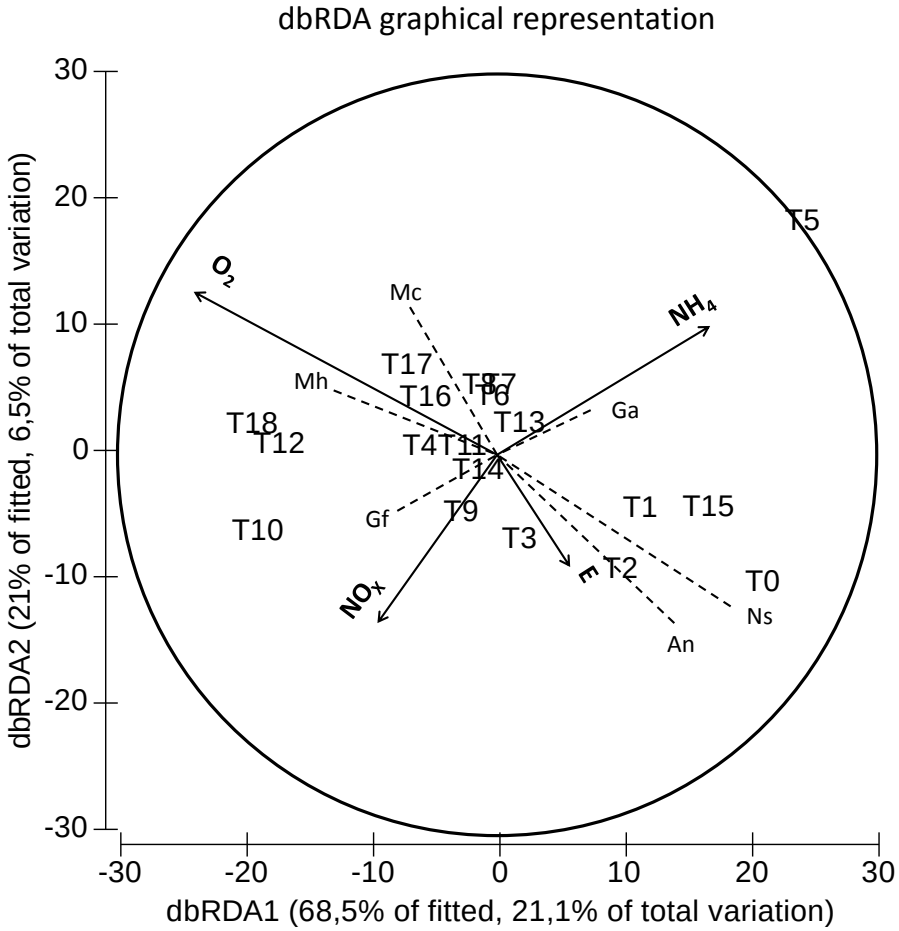


Figure 3.14: Distance-based redundancy ordination (bdRDA) representing the DistLM modelling for the 14 most abundant species and environmental variables. Full vectors represent the direction of increasing values of the environmental variables from the model (O_2, NH_4, NO_X and epiphytes, E). The dotted lines represent macrofauna species with correlations ≥ 0.25 to the ordination axis. Vector and lines length represent the partial correlation strength with the dbRDA axes; the circle is a unit circle (radius = 1) whose relative size and position is arbitrary with respect to the underlying plot. Samples are represented by T1-18, corresponding to the 18 samples taken between September 2012 and June 2013. Species abbreviations: Gf = Gammarella fucicola; Mh = Melita hergensis; Ga = Gammarus aequicauda; Mc = Microdeutopus chelifer; An = Athanas nitescens; Ns = Nebalia strausi.

Among the species presenting correlation ≥ 0.25 of the ordination, 4 amphipod species (*Gammarella fucicola, Gammarus aequicauda, Melita hergensis* and *Microdeutopus chelifer*) presented significant correlations. *Gammarella fucicola*, *Melita hergensis* and *Microdeutopus chelifer* had negative values for the first axis (negatively correlated with O_2 and positively with NH_4 concentrations), supposing a positive link with O_2 concentration and a negative relationship with NH_4 concentrations present in the EMA (Figure 3.14). On the contrary, the amphipod *Gammarus aequicauda*, the leptostracean species (*Nebalia strausi*) and the decapod species (*Athanas nitescens*) had positive values for the first axis, indicating a negative relationship with O_2 concentration and a positive relationship with NH_4 concentrations.

Another important result is that *Gammarella fucicola* and *Gammarus aequicauda* seemed to be linked to O_2 concentration and NH_4 concentrations which was in contradiction with the Multiple regression results.

4. Discussion

4.1. Seasonal sampling

4.1.1. Exported litter accumulations are a dynamic habitat

Our study demonstrated the dynamic nature of the exported *P.oceanica* litter accumulations and of the physico-chemical conditions encountered inside. Litter global height and biomass showed important seasonal variations, with a maximum in autumn and a minimum in winter-spring, which can easily be linked to the autumnal leaves shedding occurring every year in autumn (Bay, 1984). Litter composition was also highly seasonally variable, showing a maximum diversity in spring-summer, with dead leaves mixed with high biomass of living leaves or rhizomes and abundant drift macroalgae and a minimum in autumn, with mostly epiphyte covered leaves. Moreover, physico-chemical parameters measured inside the litter accumulations were also highly variable according to the site, season and year. A general pattern could be identified: a minimum oxygen concentration was observed in spring and summer, associated with a maximum concentration in nutrients (NO_x, NH_4, and PO_4). This is a very general statement, since hypoxic conditions were also randomly encountered in winter or autumn periods.

Our study also demonstrated that the vagile macrofauna community associated with exported *P. oceanica* litter experienced drastic changes all along the year, which will be discussed in detail in the next paragraphs, as well as their links with the highly variable environmental parameters mentioned above.

4.1.2. Global community

According to preliminary studies on the *P. oceanica* detrital compartment (Gallmetzer *et al*., 2005; Dimech *et al*., 2006; Como *et al*., 2008), macrofauna community associated with exported litter is composed of 45-80 species and dominated, by far, by arthropods, followed by annelids, mollusks, and other more anecdotal taxa. Our samples revealed a 145 to 255% more diverse community, composed of 115 species. This very important difference may be due to the different sampling strategies and the fact that these studies considered organisms sampled at only one moment of the year, while this study considered organisms from 78 samples taken at two different sampling sites and at every season between 2010 and 2012, inducing a more exhaustive view of the community. Despite this difference, our results seem congruent with another aspect of these studies, showing a community highly dominated by arthropods (~76%), and especially amphipods and decapods, along with non-negligible densities of annelids, comprising only polychaetes (~14%) and mollusks, comprising mainly gastropods (~7%) as well.

Gammarella fucicola was found to be the most abundant species in our samples (1.11 ± 1.21 ind.gDM^{-1}, representing 48.23 ± 26.43% of the total amphipods or 37.98 ± 22.84% of the global community, which was in accordance with Gallmetzer *et al*. (2005) who found that *Gammarella fucicola* was highly abundant in exported *P. oceanica litter* accumulations, but in opposition to Dimech *et al*. (2006), who found no *Gammarella fucicola* in exported *P. oceanica* litter samples from the northern part of Malta.

Compared to the community present in *P. oceanica* meadow, the exported litter community that was described in the chapter presented major differences in terms of diversity, dominant species and abundances (Gambi *et al*., 1992; Michel, 2011; Zakhama-Sraieb *et al*., 2011; Sturaro, 2012). Indeed, the macrofauna diversity found by Gambi *et al*. (1992) and Covazzi Harriague *et al.* (2006) was up to 3 times more important with 184-312 species composing the sampled community. Dominant taxa found in the *P. oceanica* meadow were mollusks (~51%), followed by arthropods (~47%) and finally polychaetes (~2%). In terms of global abundance, Harriage *et al*. (2011) found between 100 ± 37.8 ind. m^{-2} in spring and 2870 ± 2029.4 ind. m^{-2} in summer, which is much less than what was found in this study, with global abundances ranging from 374.15 ± 51.36 ind. m^{-2} in winter 2011 to 5115.65 ± 2420.27 ind. m^{-2} in summer 2010. Michel (2011) and Sturaro (2012) described in details the

amphipods assemblage found in the *P. ocenanica* meadow and one clear observation is that very abundant and dominant species in the meadow (e.g. *Apherusa chiereghinii*) are replaced by a whole other group of species in the exported litter accumulations. In the litter, *Apherusa chiereghinii* is an anecdotic species sampled only 28 times in the 78 samples processed for this study (on a total of 9435 sampled individuals), and the most abundant species, *Gammarella fucicola*, was only a rare species in the samples from Michel (2011) and Sturaro (2012), which was also congruent with results of Gambi *et al*. (1992). Exported accumulations of *P.oceanica* litter thus seemed to house a diverse, very abundant and peculiar macrofauna community, composed of species commonly found in other coastal habitats (Gambi *et al*., 1992; Michel, 2011; Sturaro, 2012), but presenting very different abundance and diversity patterns compared to what is found in the directly adjacent *P. oceanica* meadow.

While literature about invertebrates inhabiting detached algae detritus accumulations is scarce, it could be found that such algal detritus accumulations present some common features with exported *P. oceanica* litter accumulations in terms of dynamics, temporary availability and potential habitat for various invertebrates (Tzetlin *et al.*, 1997; Alfaro *et al*., 2009; Krumhansl and Scheibling, 2012). Diversity encountered in kelp detritus accumulations was reported to be much less than half the diversity (49 macroinvertebrates species) encountered in this study for exported *P. oceanica* litter. In kelp detritus accumulations, most species encountered are a mix of species found in other adjacent habitats such as living kelp forests or other organic-rich habitats (Tzetlin *et al.*, 1997), which is a common characteristic with macrofauna community living in exported *P. oceanica* litter accumulations. Deep detached algae accumulations are highly dominated by various echinoderm species, which is very different from the invertebrate community described in this study, where echinoderms were mostly anecdotal components. The shallow drift algae mats seem to be less highly dominated by sea urchins, and present a much more diverse community. Alfaro *et al.* (2009) found up to 110 species in drift *Gracilaria* mats, which is almost equivalent to what was found in this study. However, average densities were much more modest, with only up to 175.6 ± 24.2 ind.m^{-2}. Arthropods (~96 ind.m^{-2}) and mollusks (~64 ind.m^{-2}) constituted most of the sampled invertebrates. Another striking difference with exported *P. oceanica* litter accumulations was the very low density on annelids found in these algal mats (~8 ind.m^{-2}).

Vagile macrofauna associated with exported *P.oceanica* litter accumulation thus presented unique abundance and diversity patterns compared to other adjacent or similar detrital ecosystems.

4.1.3. Impact of environmental factors on abundant species

In this study, 19 very dominant and highly abundant species were identified, representing 90% of the global macrofauna abundance sampled and belonging to various benthic taxa such as amphipods, decapods, isopods, leptostraceans, polychaetes, gastropods and nemerteans.

Prior to any other analysis, the impact of the most obvious varying parameter, litter dry mass, was tested for the global community and 7 species belonging to every taxon: the amphipods *Gammarella fucicola, Gammarus aequicauda* and *Nototropis guttatus,* the leptostracean *Nebalia strausi,* the decapod *Athanas nitescens,* the annelid *Platynereis dumerilii* and the mollusk *Bittium reticulatum*. The whole community, *Gammarella fucicola* and *Nebalia strausi* presented weak but significant links with litter dry mass. Due to its status of most abundant and dominant species, *Gammarella fucicola* potentially highly influenced the global link between the whole community and the litter dry mass. Since the links between abundance and litter dry mass were weak and very species-specific, it was decided to focus on the impact of the other parameters. Litter dry mass and the related parameters (*e.g.* height, cover ratio) were thus not included in the following analyses.

Out of these 19 dominant species, 14 presented very important temporal variations during the 2010-2012 sampling period. In spring and summer, the highest diversity, dominance, global abundance and specific abundances were observed for most of these 14 species, while diversity, dominance, global abundance and specific abundances for most of these 14 species were minimum in autumn and winter. Since the measured environmental parameters also varied a lot between the seasons, it was hypothesized that some of the environmental parameters could play a role in these variations of abundances and diversity of the vagile macrofauna all along the year. Since multiple regression and DitLM analysis highlighted the fact that O_2 concentration, and in a much more modest way, NH_4 and PO_4 concentrations were the parameters contributing the most to the variations observed for the weighed abundances of 6 of these 14 species, it was assumed that the seasonal variations observed were caused partly by variations of these important structuring parameters,

especially O_2 concentration. However, this litter O_2 concentration impact, positive or negative, seemed to be very species-specific, which had already been experimentally demonstrated for the Baltic Sea macrofauna (Gamenick *et al.*, 1996).

A striking result is that despite the important seasonal variations experienced by *Gammarella fucicola* and *Gammarus aequicauda*, two very dominant species up to 50% of the global macrofauna abundance in the dead *P. oceanica* litter, these species showed no significant link with O_2 concentration variations. Our hypothesis concerning these two very dominant species is that they present a certain tolerance to hypoxia, and that they are highly adapted to extremely dynamic and different detrital/phytal habitats. Indeed, *Gammarella fucicola* and *Gammarus aequicauda* are found in exported *P. oceanica* litter, in *in situ P. oceanica* litter (non-exported litter that decays inside the meadow, see Michel, 2011), in the *P. oceanica* foliar stratum, (Gambi *et al*., 1992), in maerl beds (Carvalho *et al.*, 2009) or in detrital algae accumulations (Vàsquez-Luis *et al*., 2008). These studies showed how much these two species are found in various coastal detrital habitats and how ubiquitous they are. This lead us to hypothesize that *Gammarella fucicola* and *Gammarus aequicauda* present a certain level of hypoxia tolerance and do not prefer hypoxic conditions or oxic conditions. It was also hypothesized that the observed seasonal variations of these two species were more due to the natural life cycle or other parameters that were not measured during this study, rather than to direct influences of O_2 concentration. Indeed, Prato and Biandolino (2003) observed a peak of abundance between May and September for *Gammarus aequicauda*, and also found no correlation between O_2 concentration and the observed abundances. The absence of litter O_2 concentration impact on *Gammarella fucicola* associated to the weak but significant link between *Gammarella fucicola* abundance and litter dry mass could be another sign that some species, such as *Gammarella fucicola*, could be driven by the availability and complexity of their habitat (and/or food source? See Chapter 5 and 6). As mentioned earlier, *Gammarella fucicola* appeared to be a really adapted species of the exported litter habitat, and this could explain that the only parameter impacting his abundance was the availability and complexity of litter.

The Amphipods *Melita hergensis, Nototropis guttatus, Microdeutopus chelifer* and *Dexamine spinosa* were positively linked to litter O_2 concentrations, meaning that they were found in larger abundances in well-oxygenated samples. This result is congruent with Haselmair *et al*. (2010), who

found that most crustaceans presented important mortality during hypoxia periods. Moreover, behavioral avoidance of hypoxic zones is known for many invertebrates' species (Riedel *et al*., 2014), which could happen here. It was hypothesized that during hypoxic periods, many species that are also known to be found in other adjacent ecosystems could simply avoid litter accumulations until more favorable moments. Since litter O_2 concentration was highly linked to weather (see §3.1.2), and also potentially to *in situ* respiration rate (see Champenois and Borges, 2012) which is influenced by organic matter degradation and micro-organisms activity, these species seemed to avoid litter and decrease drastically in abundance when the weather is particularly calm and favorable to hypoxia and high microbial respiration rates, mainly in spring and summer.

3 species, the Leptostracean *Nebalia strausi* and the two Decapods *Athanas nitescens* and *Galathea intermedia* showed opposite abundance patterns. These species were negatively linked to O_2 concentration, meaning that they are found in larger abundances in hypoxic samples, which is surprising as most Arthropods react first to hypoxia, followed by Annelids and mollusks with decreasing O_2 concentration (Diaz and Rosenberg, 1995; Gambi *et al*., 2009; Levin *et al*., 2009; Hernàndez-Miranda *et al*., 2012; Veas *et al*., 2012).

Hypoxic conditions were not a problem for these three species, and this could be explained by several hypotheses. First, they could be more tolerant than others to low O_2 conditions. This was already briefly mentioned by Gallmetzer *et al*. (2005), who found a species of *Nebalia* in much more important densities at the bottom of very thick *P. oceanica* litter accumulations, suggesting a high tolerance to hypoxic conditions. Another hypothesis is that *Nebalia strausi* hypoxia tolerance could allow this species to avoid competition and predation by moving litter accumulations when hypoxic periods occur. This could lead to the development of a low O_2 tolerance and a behavioral avoidance of litter accumulations when competition/predation is high during more oxic periods. Such O_2 tolerance and competition avoidance is known for *Nebalia hessleri* inhabiting *Macrocystis* detritus accumulations (Okey, 2003) which seem to prefer hypoxic layers of the *Macrocystis* mats when competing amphipods are abundant. This behavioral response associated to hypoxia (and reducing compounds like H_2S) tolerance allows *Nebalia hessleri* to live and spread deeper in the mat while amphipods and other crustaceans are easily hunted by fishes and shrimps in the upper layers of the

mat. For this seasonal assessment, our hypothesis was that *Nebalia strausi* abundance was strongly driven by litter O_2 and reducing compound concentrations, allowing the species to complete its life cycle and spread only during hypoxic periods when it could avoid most of the predation and competition pressure. The fact that *Nebalia strausi* was never found in oxygenated samples (also see Chapter 4) seemed to confirm the distinctive oxic periods avoidance strategy of this species. Since O_2 concentrations were higher in water just above the litter and much lower inside the litter accumulations during litter hypoxic periods, it was also hypothesized that what was observed for *Nebalia hessleri* in *Macrocystis* detritus accumulations (Okey, 2003) could potentially occur in *P.oceanica* litter accumulations for *Nebalia strausi*. It could indeed be possible that living conditions vary a lot between the top layer and the bottom layer of an accumulation, resulting in the creation of different micro-habitats presenting high O_2 concentrations at the top of the litter accumulation and hypoxic conditions deeper in the litter, closer to the sediment. It could potentially allow a spatial segregation of the different macrofauna species observed in the litter, allowing a more important diversity. This hypothesis was supported by the higher global biodiversity encountered during spring and summer, which are seasons characterized by a more frequent and important hypoxia period. In such "layered" habitat, *Nebalia strausi* could occupy only the bottom layers of the litter accumulation, spatially avoiding predation and competition in these "hypoxic refuges". This layer hypothesis elaborated for *Nebalia strausi* particular case could also be coherent with the general community variations observed in this study. Indeed, stratification would be faster and more important during the calm and warmer periods, such as spring and summer. At that moment of the year, stratification could occur in the litter accumulations, creating different micro-habitats from the top to the bottom of the accumulation, allowing the higher abundance and diversity observed. Moreover, this general hypothesis could be in accordance with the weak but significant link observed between *Nebalia strausi* and litter dry mass. Indeed, mainly in autumn and winter, the presence or absence of *Nebalia strausi* seemed to be conditioned by the quantity of litter encountered. In summer and spring, calm weather, bacterial activity and higher temperatures could be enough to induce hypoxia even if litter dry mass is low on the accumulation. In winter and autumn, important quantity of litter could be "required" for litter stratification and hypoxia to occur, since lower temperature and bacterial activity could not be sufficient (see § 4.2.3). This hypothesis of

litter dry mass temporary impact could also potentially explain the absence of link between litter O_2 concentration and litter dry mass on the accumulations, since hypoxic conditions occurred preferentially in spring-summer whatever the litter biomass, but also in autumn-winter, only when litter biomass was important.

Hypotheses were a bit different for *Athanas nitescens*, for the predation avoidance strategy was less plausible due the much more carnivorous diet of *Athanas nitescens*. Data are scarce about *Athanas nitescens* oxygen preferences, but its tolerance to hypoxia has nonetheless already been indirectly observed since this species is known to present much lower oxygen consumption than most amphipods and other arthropods (Bishop *et al.*, 2009). Since *Athanas nitescens* appeared to be hypoxia-tolerant, our hypothesis was that this species could tolerate hypoxic periods, when other carnivorous and less hypoxia-tolerant competitors could possibly not survive, to prey easier on species present during these hypoxic moments. Since it was demonstrated by this study that highly abundant Amphipod species (*Gammarella fucicola* and *Gammarus aequicauda*) were not impacted by litter O_2 concentration variations, and were present also during hypoxic periods, *Athanas nitescens* tolerance to hypoxia could allow an efficient behavioral avoidance of competitors during calm hypoxic moments, making the predation on abundant species easier at these moments. The "layering and spatial niche-segregation" hypothesis exposed for the case of *Nebalia strausi* could also be applicable for *Athanas nitescens*.

To our knowledge, no study exists on oxygen preferences of *Galathea intermedia*. Our hypotheses are thus to be taken with care since no literature was able to support them or not. In this study, *Galathea intermedia* showed a preference/tolerance for hypoxic periods, which is in complete contradiction with what Bridges and Brand (1980), the only available study mentioning consequences of hypoxia on another *Galathea* species: *Galathea strigosa*, found. *Galathea strigosa* was found to be the least hypoxia-tolerant species among the 5 crustacean species studied by Bridges and Brand (1980). One of our hypotheses is that hypoxia tolerance is potentially highly species-specific in the case of the *Galathea* genus, inducing very variable patterns of tolerance to hypoxia depending on the species. But another hypothesis could explain why the hypoxia tolerance previous hypothesis could be a misinterpretation in the particular case of *Galathea intermedia*. The already detailed "layering hypothesis" developed for *Nebalia strausi* and potentially also applicable in the

case of *Athanas nitescens* could possibly explain why *Galathea intermedia* could be mistaken for a hypoxia-tolerant species by our analyses. This hypothesis could allow *Galathea intermedia* to be present in relatively important amounts in samples we considered as hypoxic, if the top layers of the litter accumulation were well oxygenated while lower layers present much lower O_2 concentrations, as reported in our results. *Galathea intermedia* could take advantage of this theoretical stratification, and present behavioral avoidance of hypoxic lower layer, while living normally in the top and well-oxygenated layer. This hypothesis would explain how a potentially hypoxia intolerant species could be found in non-negligible amounts in samples that our sampling protocol made us characterize as hypoxic.

In conclusion, this study characterized for the first time the vagile macrofauna community on a multi-year, multi-season and multi-site base. We highlighted the overwhelming dominance of Amphipods, especially *Gammarella fucicola* and the presence of up to 115 species in the dead *P. oceanica* exported litter accumulations. It was confirmed that even if many encountered species were also already observed in other adjacent or similar ecosystems, the vagile macrofauna community of exported litter accumulations presented quite unique abundance and dominance patterns. Furthermore, we highlighted that even if biodiversity and global abundance are non-negligible, only 19 species represented more than 90% of the global abundance encountered, sign of a high dominance of only a few detritus-adapted species. Moreover, we demonstrated the highly dynamic and inconstant nature of the litter accumulations, corresponding to year-round variable environmental parameters and living conditions. The general effect of litter dry mass was not clear, potentially strongly influenced by the weak but significant link existing between *Gammarella fucicola* and litter dry mass. Since many species did not show clear seasonal or environment-dependent variations, one of our hypotheses is that many species depend on parameters we did not measure. Several parameters such as litter availability, complexity and temperature might potentially be strong drivers at precise moments of the year, strongly influencing species life cycles. However, some of the measured environmental parameters were proven to be important structuring parameters for the community in general, but also for some of the most abundant species, especially litter O_2 concentration. Litter O_2 concentration had no impact on most species, such as the very abundant *Gammarella fucicola* and *Gammarus*

aequicauda but impacted positively or negatively 6 other abundant vagile macrofauna species. This potential impact of litter O_2 concentration variations, resulting from weather condition variations, highlighted different species-specific potential strategies to cope with hypoxic periods. Several species avoided litter accumulations when litter O_2 concentration is too low, while others seemed to avoid oxic periods of the year. One of our main supplementary hypotheses to this statement is that exported litter accumulations could present a sort of layering in terms of environmental parameters. Top layers of litter could be well oxygenated even during calm periods of summer, with low nutrients concentrations, low reducing compounds (H_2S), low organic matter degradation and bacterial respiration (see what Champenois and Borges, 2012 found for the *P.oceanica* meadow). On the other hand, the same litter accumulation at the same moment of the year could also experience hypoxic conditions deeper in the litter, in the bottom layers. Environmental parameters could thus present high spatial variability inside the same litter accumulation, depending on the layer. This possible stratification could thus induce the creation of a succession of different micro-habitats from the top to the bottom of the litter. These very different micro-habitats could accommodate a more important biodiversity during these "hypoxic moments", mainly (but not only) in spring and summer, which was potentially confirmed by the higher diversity found in the summer samples.

4.2. Weekly sampling

4.2.1. Exported litter accumulations: weekly dynamic habitats

In the previous paragraph we demonstrated the highly seasonally dynamic nature of exported litter accumulations and the impact of such drastic variations on the associated vagile macrofauna community. This study demonstrated a short-term type of dynamics, with environmental parameters varying from one week to another. Litter biomass, thickness, and cover ratio showed a clear decreasing general pattern from the beginning of the sampling period, in late summer 2012 to the end of the sampling period in late spring 2013. Fragmentation showed a clear opposite pattern with a marked increase of fragmentation from the beginning of the sampling period, in late summer 2012 to the end of the sampling period in late spring 2013. Litter O_2 and nutrients (NO_x, NH_4, PO_4) concentrations also showed similar general variation patterns. When looking closely to the observed environmental variation patterns, the reader's attention must be drawn to two very drastic changes occurring in contradiction with the general patterns, one between T4 and T5 on November 11th, 2012, the other between T14 and T15, on May 13th, 2013. These two dramatic events, caused by strong north-eastern stormy episodes, showed a very important increase of litter biomass, thickness, and cover ratio, while fragmentation experienced a drastic decrease. Litter O_2 and nutrients concentrations also experienced important changes during these two events, with a 25-30% decrease of O_2 concentration after the events, coupled with important nutrients concentrations increase.

Our study also demonstrated that the vagile macrofauna community associated with exported *P.oceanica* litter experienced drastic changes all along the sampling period as well as during the two events mentioned above, which will be discussed in detail in the next paragraphs, as well as their potential links with the highly variable environmental parameters mentioned previously.

4.2.2. Global community

This study identified 60 species presenting a global weighed abundance of 1.85 ± 1.21 ind.gDM^{-1}, which is less than half of what was found during the seasonal sampling (see § 4.2.2). This huge difference could be explained by many factors. First would be the fact that all samples were taken between September 2012 and June 2013. Spring, and primarily summer, were the seasons presenting the most important diversity and abundances during the seasonal sampling. Since none of our samples was taken in summer, it could be assumed that some of the species were missed. Another hypothesis is simply the least important amount of sampled organisms. Only 5966 individuals were sampled for this weekly study, while more than 9400 were sampled for the seasonal study, which could result in an important underestimation and undersampling of rare species. Another potential explanation could come from the fact that samples were only taken at the OSCE-site, and even if the seasonal study showed a limited spatial effect on the community, it is possible that some species were missed. Important inter-annual variations could also occur, leading to very different biodiversity from one year to another.

Despite these important global diversity and global abundance differences, the general dominance and relative abundance patterns are in accordance with the seasonal study and preliminary studies on the *P. oceanica* associated macrofauna community (Gallmetzer *et al*., 2005; Dimech *et al*., 2006; Como *et al.*, 2008). Indeed, Arthropods were highly dominating the community, representing up to 90% of the sampled macrofauna. In terms of relative abundance, Arthropods were followed by Annelids (~6%) and Mollusks (~3%). *Gammarella fucicola* was once again highly dominant, representing up to 55% of the macrofauna community. Similarities and differences with other adjacent or analogous ecosystems developed in this chapter (§ 4.1.2) remain valid for this weekly study.

4.2.3. Impact of environmental factors on abundant species

It's important to note that despite the differences between the seasonal study and this weekly study, most of the very abundant species were almost identically common in both studies. Indeed, 18 out the 19 species representing 90% of the global macrofauna abundance sampled during the seasonal sampling were also found among the most dominant species identified in this weekly study.

Multiple regression associated with DistLM analysis performed on the 18 species and the environmental parameters highlighted once again that litter O_2 concentration (Gamenick *et al*., 1996), but also nutrients concentrations, and in a much more modest way, epiphytes biomass, contributed to the variations observed for the weighed abundances mainly of 5 of these 18 species. Since multiple regression identified litter O_2 concentration as the main contributor, and DistLM analysis identified O_2, NH_4, NO_X concentration and epiphytes biomass as the main contributors to the observed abundance variability, it was considered that both O_2 and nutrients parameters were playing an important structuring role for a few abundant species of the vagile macrofauna community associated with the *P. oceanica* exported litter. Identically to what was observed for the seasonal study, this important role of litter O_2 concentration and nutrients is only observed for a few species. Once again, it could be reasonably interpreted that during this weekly sampling, most species variations were driven by environmental parameters we did not measure. However, contrarily to what was observed in the seasonal study, the O_2 concentration impact could be linked to the litter biomass present on the OSCE-accumulation between September 2012 and June 2013. This different link between O_2 and litter biomass observed during these two different studies could be one of our major results. Indeed, litter biomass present on the exported litter accumulation seemed to play a different role on the environmental parameters at different moments of the year. We hypothesized that litter biomass is not a driver of environmental conditions inside the accumulation during spring and summer. At that moment of the year, calm weather conditions, high temperature and a potentially high microbial activity (Sarmento *et al*., 2010; Champenois and Borges, 2012) could be sufficient to induce hypoxic conditions inside the litter accumulation, no matter the litter biomass. In autumn and winter, rough weather conditions occurred more often inducing a much mixed litter. In addition to low temperature and a potentially

low microbial activity, this would potentially make litter accumulations less subject to hypoxia. At that moment of the year, litter biomass and thickness could be the main driver of hypoxic conditions since litter could act as a barrier between the mixed water column and the bottom layers of litter. A thick and abundant litter could keep bottom layers stabler, favoring the development of hypoxic conditions. We thus hypothesized that litter biomass could be one major driver impacting directly the environmental conditions inside the exported *P. oceanica* litter accumulations, but not all the time. During summer, other parameters such as calm weather, high temperature and the resulting increased microbial activity could potentially prevail.

Four out of the five species potentially impacted and litter O_2 and nutrients concentration were present among the 6 species also impacted by seasonal variations of these environmental parameters (§ 4.1.3). This result highlighted one of the main discoveries of this study: litter O2 concentration and more modestly nutrients concentration impacted several abundant species of the litter macrofauna community in an almost similar way at two very different time scales. Indeed, *Melita hergensis* and *Microdeutopus chelifer* were positively linked to litter O_2 concentration for both time scales, while *Nebalia strausi* and *Athanas nitescens* presented a strong negative link with litter O_2 concentration for both time scales. Another striking result was the weak but significant impact of both O_2 and nutrients concentration highlighted by the DistLM / dbRDA analysis (but not by the multiple regression) for *Gammarella fucicola* and *Gammarus aequicauda*. Both species seemed more strongly related to the "NH_4-NO_X" axis of the ordination. After careful analysis, this correlation seemed to be caused primarily by samples taken at T4, T5 and T15. These samples were associated to the two stormy events mentioned earlier in this chapter. This result will thus be extensively discussed in the next paragraph. Even if it seemed that many of the most abundant vagile macrofauna species were not directly impacted by the environmental parameters we measured, it is important to emphasize that for some of them probably showing narrower ecological preferences, environmental parameters, and more particularly litter O_2 concentration, linked to weather conditions and litter biomass present on the accumulation, are very important and potentially play a major role on their abundance patterns and on their life cycle at that moment of the year.

4.2.4. November and May resource pulses impact

As mentioned before in this chapter (§ 4.2.1), in addition to the "classic" physico-chemical and faunal patterns observed during this weekly study, two important events were identified, one in November 2012 and one other in May 2013. These events, related to important windy and stormy conditions, were identified precisely in time, using the photographic sampling protocol. Due to the very short duration of the events, the large amount of "new" litter brought to the existing accumulation and the random nature of such stormy conditions, these events were considered as resource pulses *sensu* Ostfeld and Keesing (2000).

As mentioned earlier, the two events differed a bit. The November event was indeed composed of two distinct phases. The first one consisted in a drastic and fast (12h) litter biomass and thickness decrease during a major storm on November 1st, 2012. This litter "departure" from the sampling site induced the formation of a very fragmented litter accumulation and the rapid concentration of the macrofauna inside the remaining small litter patches. This global abundance increase was mainly caused by a dramatic increase of the abundance of *Gammarella fucicola*. This result is congruent with studies concerning impact of habitat fragmentation and habitat loss on communities (Eggleston *et al*., 1999; Hovel, 2003; Farhig, 2003). These authors demonstrated that in most temperate ecosystems, habitat fragmentation *per se* had modest effects on communities but that habitat loss could impact negatively and importantly the biodiversity encountered in a given ecosystem. However, the same authors also found that small and mobile invertebrates such as decapods, isopods or amphipods might present highly species-specific increase of abundance and diversity inside decreased and fragmented habitats, and this constituted a sort of "refuge effect" for specialized species (Eggleston *et al*., 1999). This "refuge effect" could be what we observed in T4, with a community experiencing biodiversity decrease and a strong concentration in the remaining small litter patches of *Gammarella fucicola*, the most abundant and typical amphipods of the exported *P. oceanica* litter accumulations. This "refuge effect" could be one more sign that, in addition to being the most abundant macrofauna species of the exported *P. oceanica* litter, *Gammarella fucicola* was potentially one of the most litter-dependent species encountered. Indeed, this concentration strategy in the few remaining litter patches instead of migrating to another adjacent habitat when litter was scarce, seemed to indicate

that *Gammarella fucicola* is really preferentially associated with *P. oceanica* detritus (see (Gallmetzer *et al*., 2005). Since species richness decreased by about 33% between T3 and T4, it could be hypothesized that several species displayed another strategy and simply left the litter accumulation. This "should I stay or should I go" hypothesis would be coherent with the fact that some globally modestly abundant species could potentially not compete with other well adapted and abundant species when litter biomass was very low, in terms of predation, competition for food, or competition for habitat. This would once again (see §4.1.3) be coherent with another potential role of litter biomass during autumn and winter in terms of habitat availability. Litter biomass is increased by autumnal leaves shedding (Bay, 1984), potentially allowing a certain biodiversity in terms of available space, even if other environmental factors (*e.g.* temperature, sunshine duration, and litter complexity) are less favorable in autumn. The dramatic litter departure on T4 induced an important habitat loss, potentially resulting in the departure or exclusion of several species, while other abundance species displayed no changes, or marked increase of abundance. The second phase of this November event thus occurred on this fragmented litter accumulation, which might have potentially biased its impact on the vagile macrofauna. This second phase corresponded to a massive input of litter (+278% of biomass) and potentially some associated organisms. The increase of species richness after this second phase could potentially partly be explained by litter fast recolonization (see Mascart *et al*., 2015b) or by the presence of organisms in the newly arrived litter on the accumulation. For the May event, *Gammarella fucicola* showed no apparent response. Just after this massive departure of litter, *Gammarus aequicauda* was completely absent of the samples. This could be explained by the fact that *Gammarus aequicauda* could be less strictly dependent on litter accumulations as a habitat than *Gammarella fucicola*. This would be coherent with studies of Gambi *et al*. (1992) and Michel (2011). These authors indeed found *Gammarus aequicauda* in non-negligible amounts inside the *P. oceanica* meadow canopy, which was not the case of *Gammarella fucicola*, present much less abundantly, and only in the dead *P. oceanica* leaves situated inside the meadow itself. This could explain the departure of *Gammarus aequicauda* from the litter during this event to avoid an increased competition for habitat and food, while *Gammarella fucicola* concentrated inside the small remaining litter patches.

Considering both events, 2 other species showed simultaneously important variations potentially caused by the resource pulses: *Gammarus*

aequicauda and *Nebalia strausi*. *Gammarus aequicauda* showed no response to the first phase of the November event, displaying no concentration ("refuge effect") in the remaining litter. This highlighted a possible simultaneous departure of *Gammarus aequicauda* and *P. oceanica* litter. Since *Gammarus aequicauda* is also found in the *P. oceanica* meadow (Gambi *et al*., 1992; Michel *et al*., 2015), it could also be possible that a part of the *Gammarus aequicauda* population actively migrated from the *P. oceanica* litter accumulation to another adjacent habitat such as the *P. oceanica* meadow, to cope with an important decrease of habitat and food source availability. Another important result is that *Gammarus aequicauda* seemed to be highly impacted by the second phase of the November pulse event as well as the May pulse events. The reader's attention is drawn to the fact that *Gammarus aequicauda* is known to feed, not exclusively, but preferentially on dead *P. oceanica* fragments (see Lepoint *et al*., 2006; Michel *et al*., 2015; Chapter 5 for a more detailed view), making it one of the most important true detritivore and quite specialized species of the litter macrofauna community. An interesting thing is that mobile specialist and detritivore organisms' density augmentation in response to resource pulses has already been well demonstrated for many terrestrial ecosystems (Yang, 2006; Yang *et al*., 2008; Yee and Juliano, 2012), which is quite surprising since exported litter accumulations are a marine compartment. However, the "common" features shared by exported *P. oceanica* litter accumulations and terrestrial ecosystem (*e.g.*: deciduous temperate forests) such as the autumnal leaves shedding, the "detritus-based" food webs and the fact that *Posidonia oceanica* is a magnoliophyte (Nowlin *et al*., 2008; Yang *et al*., 2008) could potentially explain the "terrestrial response" observed during the two resource pulses with the detritivore species stimulation.

In addition to the general pattern observed (gradual abundance decrease during the sampling period) *Nebalia strausi* showed important responses to the November and May events too, with a 16.36-fold and 22.3-fold abundance increase, respectively. Since oxygen showed an important decrease during November and May event, and since *Nebalia strausi* is suspected to present hypoxic tolerance mechanisms and behavioral avoidance of oxygenated litter (see in § 4.1.3 of this chapter for more details), it could be hypothesized that the litter biomass increase caused by the resource pulses favored the O_2 concentration decrease and favored the settlement of *Nebalia strausi*. Indeed, the calm conditions immediately following the two events until the sampling

associated to the "new" litter input could potentially favor the quick decrease of litter O_2 concentration measured. However, the important litter O_2 concentration decrease observed just after these two resource pulses might be linked to the "layer hypothesis" (see § 4.1.3 of this chapter for a detailed description of this hypothesis).

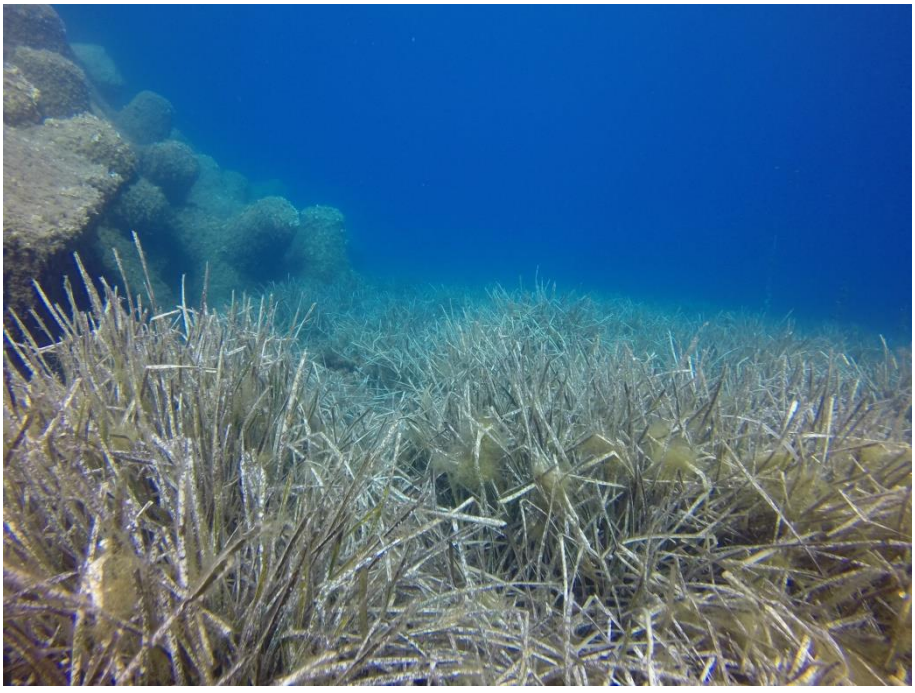
The reader's attention is drawn to the fact that, as mentioned earlier, the variations of abundances observed for *Gammarella fucicola* and *Gammarus aequicauda* were potentially weakly but significantly correlated with both O_2 and nutrients concentration (see DistLM analysis, §4.2.3). This was in opposition with what we found during the seasonal study. Indeed, *Gammarella fucicola* and *Gammarus aequicauda* showed significant seasonal variations but these variations were not linked to any environmental parameter we measured. The two resource pulses observed in this weekly study could potentially explain the link between both species abundances and O_2 / nutrients concentration. Indeed, apart from these two events, their abundances displayed quite constant values during the sampling period (in accordance with what Kevrekidis and Koukouras, 1989 and Prato and Biodolino, 2003 found for *Gammarus aequicauda* at that moment of the year). The responses of *Gammarella fucicola* and *Gammarus aequicauda* during the two events might mislead the DistLM analysis, since both species could consequently appear linked to litter O_2 concentration. In our opinion, the influence of litter O_2 concentration highlighted only during these two events seemed quite unlikely. However, since O_2 and litter dry mass showed a significant linear relationship during this weekly study, the correlations found for *Gammarella fucicola* and *Gammarus aequicauda* could potentially reflect in fact the role of litter dry mass on these very adapted detrital species in terms of habitat availability and/or dietary resource availability. *Gammarella fucicola* would concentrate inside the litter in case of litter departure due to its quite strict dependence on the litter as a habitat, while *Gammarus aequicauda* would take advantage of the new litter input in terms of available food source (see Chapter 5). These two very different responses to litter biomass fluctuations could potentially explain the opposite correlation pattern observed in the DistLM analysis.

In conclusion, this weekly study of an exported *P. oceanica* litter accumulation and the associated macrofauna community demonstrated that litter accumulations are a very dynamic habitat at a much smaller time scale than what was demonstrated earlier in this chapter (§ 4.1). It was also demonstrated that this weekly variability of the environmental parameters impacted several abundant macrofauna species, but not really the community as a whole. We thus hypothesized that many abundant species displayed variations linked to parameters we did not measure and potentially regulating their life cycles. However, a nuance must be added to this general statement. Indeed, due to the significant relationship between litter O_2 concentration and litter biomass during this weekly study, we hypothesized a temporally variable role of litter biomass availability on the environmental conditions encountered inside the litter, and consequently on the macrofauna. The role of litter biomass as a driver for environmental conditions encountered inside the litter would be limited to the "cold" period of autumn and winter, while this role would be much more modest during the "hot" period of spring and summer. Concerning the impact of the measured environmental factors, the two highly dominant species, *Gammarella fucicola* and *Gammarus aequicauda* seemed once again only modestly related to them. However, litter O_2 and nutrients concentration were shown to play a potentially important structuring role at the week time scale for several other abundant species. Tolerance to hypoxia, behavioral avoidance of litter during oxic and hypoxic periods (or zones, see the "layer hypothesis" developed in §4.1.3 of this chapter) were highlighted among these species even at such small time scale. In addition to these weekly variations, two random, brief and important litter biomass variation events were identified and considered as "resource pulses". These resource pulses corresponding to important stormy events of litter departure or litter input potentially triggered different responses at the community and specific scale. These resource pulses impacted the community in general and also 3 very abundant species: *Gammarella fucicola, Gammarus aequicauda* and *Nebalia strausi*. The community showed a strong diversity decrease ("should I stay or should I go" hypothesis) during the first phase of the November pulse (departure event) while *Gammarella fucicola* seemed to simultaneously concentrate ("concentration hypothesis") inside the remaining litter. The "should I stay or should I go" hypothesis could be linked to the role of exported litter as a habitat provider for many generalist species that could simply migrate to other habitats when conditions inside litter accumulations become unfavorable. The

"concentration hypothesis" could be linked to the strict dependence of *Gammarella fucicola* on exported litter as a habitat and its inability to migrate from it, or deal with another adjacent habitat efficiently. On the other hand, *Gammarus aequicauda* and *Nebalia strausi* both showed a dramatic increase of their abundances. The density increase of *Gammarus aequicauda* led us to the hypothesis that exported *P. oceanica* litter accumulations share common features with terrestrial magnoliophyte-based ecosystems, inducing, at least for a part, typical terrestrial responses to resource pulses, such as the detritivore species important stimulation. The density increase of *Nebalia strausi* led to the hypothesis that a litter pulse associated with immediately following calm weather conditions might favor the apparition of hypoxic conditions (see the "layer hypothesis" mentioned above) just after pulses, independently of the season.

Chapter 4

Experimental assessment of oxygen stratification and pulsed events impact on exported litter macrofauna



1. Introduction

Chapter 3 showed that environmental parameters, especially O_2 concentration inside the litter accumulation itself, have a drastic impact on EMAs macrofauna. It also showed that important and brief pulsed events occur randomly inside EMAs, resulting in drastic modifications of oxygen concentrations inside the EMAs, of nutrients inside EMAs or sediment but also on some key invertebrate species. Two in situ experiments were thus conducted in October 2014 to assess and confirm the role of O_2 concentration and pulsed events on the exported litter macrofauna.

1.1. Oxygen impact on EMAs macrofauna

Oxygen is a key element in the metabolism of invertebrates (Diaz and Rosenberg, 1995) and nowadays, natural or human-induced coastal hypoxia has become a global key structuring parameter for marine ecosystems (Riedel *et al*., 2014). The potential effects of hypoxia depend on the frequency, duration and intensity of oxygen concentration decrease (Haselmair *et al*., 2010) and these effects have been quite extensively studied for sedimentary and epibenthic invertebrate communities. Possible effects are at a community level with modification of productivity, community structure and/or function, decrease of biomass, changes of diversity (dominance of only a few tolerant species), but also at an individual level with physiological (*e.g.*: growth, mortality, reproduction,…) or behavioral responses (e.g.: avoidance, migration, decreased activity,…) (Diaz and Rosenberg, 1995; Gray *et al*., 2002; Gambi *et al*., 2009; Haselmair *et al*., 2010; Hernàndez-Miranda *et al*., 2012; Riedel *et al*., 2014).

Oxygen is known to penetrate by diffusion only a few millimeters in most fine sediment types (Diaz and Rosenberg, 1995; Pfeffer *et al*., 2012) inducing a marked stratification of oxygen concentration in these first millimeters and anoxic conditions below. Nutrients are influenced by this stratification thus leading to the availability of micro-habitats of invertebrates in terms of both oxygen levels and nutrients concentrations (Knieb, 1984; Strommer and Smock, 1989). Chapter 3 of this thesis (§3.1.2) showed that, like the sediment, EMAs present, at different moments of the year, hypoxic or anoxic conditions. Mild hypoxic levels (2 $mgO_2.L^{-1}$) are common in the EMAs but sometimes oxygen level reaches severe hypoxia (0.5-0.01 $mgO_2.L^{-1}$) or

even anoxia. Moreover it was shown in Chapter 3 that nutrients (NO_x, NH_4 and PO_4) inside EMAs were present in higher concentrations and more variable than in the water column, but slightly less abundant and variable than inside the sediment. EMAs may thus constitute a transition compartment between the water column and the sediment. EMAs may also act as a barrier and buffer for sediment to water column pulses (nutrients, reducing compounds).

Regarding these results, it was hypothesized that the EMAs could in a certain way show characteristics close to those of the sediment in terms of structure (dead leaves constituting a quite compact 3D habitat for invertebrates), of physicochemical conditions (oxygen and nutrients important stratification) and of resulting availability of micro-habitats for the associated macrofauna. These observations lead us to the following specific questions: (1) Does oxygen stratification occur inside EMAs? (2) If present, how long does it take to observe this stratification? (3) Is the macrofauna impacted and do the dominant species occupy defined positions inside the different micro-habitats?

1.2. Pulsed events on EMAs macrofauna

Chapter 3 showed that EMAs are very inconstant places experiencing dead leaves shedding in autumn and strong random events during winter and early spring storms.

Many terrestrial and aquatic ecosystems experience similar events, also called "resource pulses" or "pulsed perturbations". These pulsed events were defined by Ostfeld and Keesing (2000) as: events that share the characteristic of being rare, brief and intense. Such events take place in a variety of ecosystems (*e.g.*: massive floods in arid ecosystems or floodplains, dead leaves input in mangroves or forests, massive emergence of insects, seed mast events or storm-driven nutrients runoffs) and can be caused by different factors: (1) climatic or environmental causes, (2) temporal accumulation and release, (3) spatial accumulation and release, (4) outbreak population dynamics (Yang *et al*., 2008). Pulses can play major roles in structuring ecosystems and regulating interactions between ecosystems, communities, populations or organisms within populations (Yang *et al*., 2008; Yang *et al.*, 2010) and their effects are strongly linked to their own duration, magnitude and frequency (Holt, 2008). Resource pulses can impact ecosystems at different levels: (1) individual level (*e.g.*: diet modification, survival), (2) population level (*e.g.*: bottom-up, top

down) and (3) community level (*e.g.*: diversity). EMAs are very dynamic places potentially subject to such resource pulses and pulsed perturbations.

In Chapter 3 it was shown that strong and very short windy events occurred in winter and spring, disturbing strongly the physicochemical conditions inside the EMAs, and bringing "fresh" dead leaf material on the EMAs. This could be considered as a pulsed event *sensu* Ostfeld and Keesing (2000). But due to sampling strategy, faunal responses to these disturbances, if present, were not easy to observe. Therefore, to assess the effect of such pulses on the macrofauna community, an *in situ* experimentation was conducted to answer the following specific questions: (1) Can a clear response of the macrofauna to litter pulses be identified? (2) Do pulsed events characteristics influence this potential response? (3) Are pulsed events important drivers to maintain diversity inside EMAs?

2. Material and Methods

2.1. Site description

All samples were collected in October 2014 near the STARESO (STAtion de REcherches Sous-marines et Océanographiques) research station in Calvi Bay (42°35'N; 8°43'E) in Corsica. Weather was very calm during the experimentation period, with a constant salinity of 38 and a temperature between 23 and 22.8°C. Samples for the first experiment were collected at the HARBOR-site on a 20m² EMA on bare fine sand, while samples from the second experiment were collected at the OSCE-site (see more details below) on a 200 m² EMA on bare coarse sand (see Chapter 2, §1.2).

2.2. Oxygen impact

For the oxygen axis, an original experimental design was developed. It consisted of units (N=8) composed of 4 distinct boxes, hereafter referred as "**layers**" (dimensions 30 x 40 cm) each one 5 cm thick. Each box had a 10 mm mesh bottom to allow free water and fauna circulation and movements during the duration of the experiment. Each box was pierced on one side with a 10 cm plastic tube to allow water sampling for oxygen and nutrients analysis without

disturbing the system. Each construction of 4 stacked boxes was put underwater (Figure 4.1) and filled with litter. Each layer contained two mobile PVC panels (Figure 4.2) allowing the reliable closing and separation of each layer at the end of the experiment. Each layer was filled with the litter corresponding to this layer inside the EMA in natural condition to keep consistent fragmentation and size pattern in each layer. The constructions were put at a depth of 8 m inside the EMA of the HARBOR-site for 48h and firmly maintained on the sediment with marine steel poles and strong lines to prevent any movement. Each construction was left in "open configuration" (Figure 4.2, A) for the complete duration of the experiment.

After 48h, water was sampled for further nutrients (HPO_4^{2-}, NH^{4+} and NO_2+NO_3, hereafter NO_x), and dissolved oxygen concentration measurements. Water was sampled with 60 mL syringes in the water column (**WC**) and using the 10 cm plastic tube piercing each layer (**L5**, **L10**, **L15** and **L20**, from bottom to top, see Figure 4.2). Oxygen concentrations were measured using the modified Winkler technique with 13 mL BOD (Biological Oxygen Demand) bottles, and titration of iodine with thiosulfate solution adapted for small water volumes (Carpenter, 1965; Strickland and Parsons, 1968). Oxygen values below 2 $mgO_2.mL^{-1}$ were defined as hypoxic (Levin *et al*., 2009). Nutrients concentrations were measured using an autoanalyser (SKALAR San+ Continuous Flow Analyser) following the method of Grasshoff et al. (2007) adapted of oligotrophic seawater (detection limits: 0.05, 0.04 and 0.1 µM for HPO_4^{2-}, NH^{4+} and NO_x respectively). After careful water sampling, the system was closed (Figure 4.2, B) using PVC plates to "seal" each layer to prevent any exchange during transportation between them, before further sample processing in laboratory.

Each layer was emptied from its litter and macrofauna was separated from the dead *P.oceanica* leaves using freshwater on 10 mm and 500 µm sieves for optimal and handy separation. The 500 µm fraction was preserved in a 4% formaldehyde seawater solution and kept until further analysis. Back in Liège, the 4% formaldehyde seawater solution was replaced by distilled water for final sorting, specific identification under stereomicroscope (Zeiss Stemi 2000-C), counting and then stored in 99.8% ethanol. The remaining defaunated detrital fraction was dried at 60°C for 5 days and then weighed.

Figure 4.1: Design of the experiment, showing the 4-layers construction inside the HARBOR-site EMA. This figure shows the design in "open configuration" at the beginning of the experiment with the 10 mm sieve on the top layer. The figure shows the steel poles and lines used to prevent any movement during the 48h experiment.

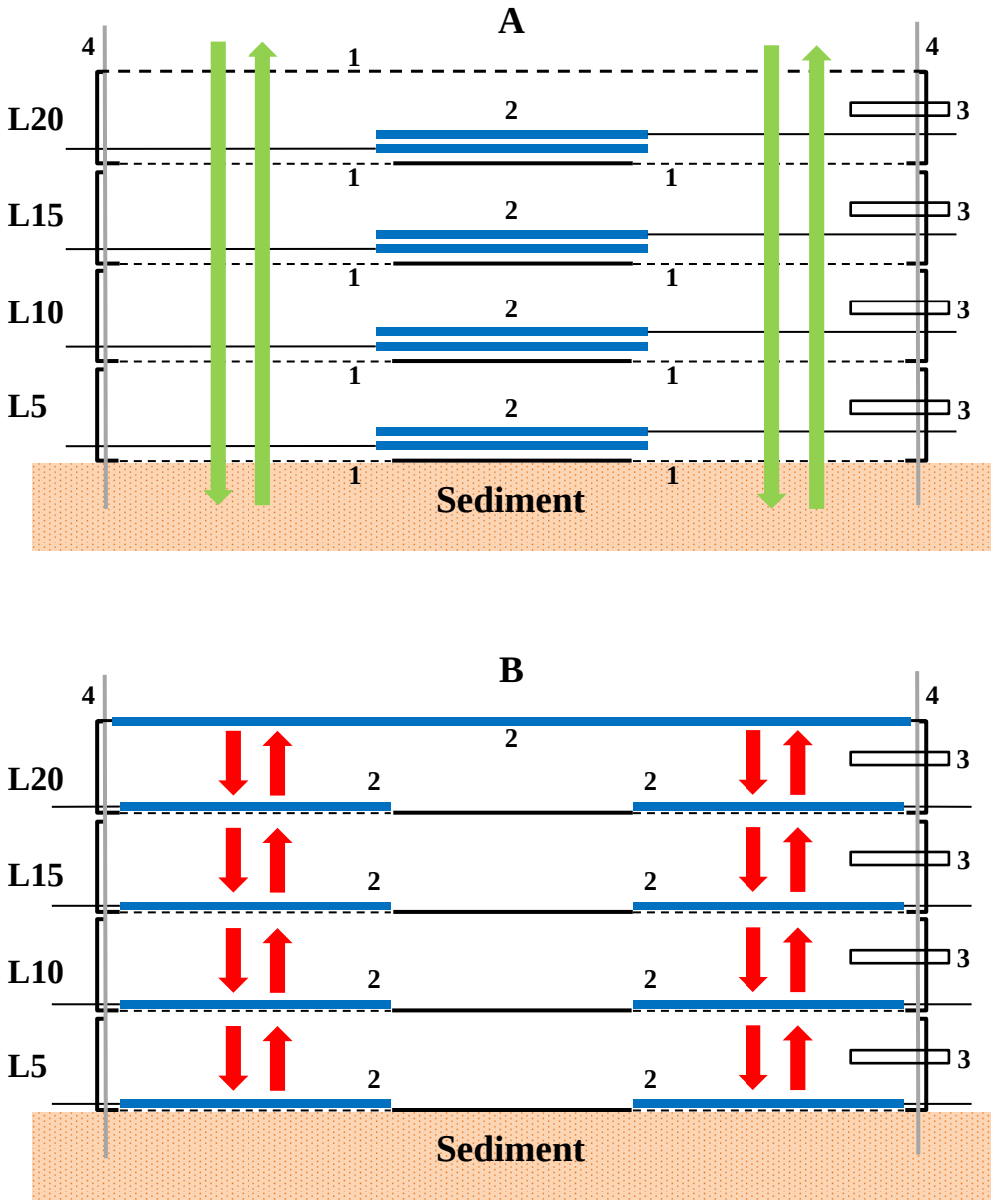


Figure 4.2: Schematic experimental design in "open-configuration" (A) and in "closed-configuration (B). 1: 10 mm sieve, 2: mobile PVC plates, 3: 100 mm plastic tube, 4: steel anchoring poles. L5-20: layers of the experimental design.

2.3. Pulse impact

For the pulse experiment, a mesocosm experimental design was developed. Two treatments and one control (N=6 per treatment) have been decided since Chapter 3 showed us that the main changes occurring after the two pulsed events of 2012-2013 were physicochemical parameters (treated in this chapter in the first experiment), but also the litter quantity available for the macrofauna. This experiment thus planned to assess the impact of litter supply to this community. The control consisted only in PVC mesocosms (described in details later in this §) placed on the EMA. The first treatment, hereafter referred as "**T-defaun**", was composed of PVC mesocosms containing a given additional supply of "defaunated litter", to assess the potential impact of purely vegetal detrital supply. The second treatment, hereafter referred as "**T-fauna**", was composed of PVC mesocosms each containing a given additional supply of "natural litter", comprising the vegetal detrital material and the associated fauna present inside. To insure that the control treatment impact was limited on the macrofauna community, a $\mathbf{T_{final}}$ sample (N=6) was taken at the end of the experiment. This $\mathbf{T_{final}}$ consisted only in sampling the "natural" community present in the EMA outside of the mesocosms to compare it to the control to assess the importance of the "mesocosm effect". The litter intended for being added to the two treatments was sampled 24h before the beginning of the experiment, half of it was defaunated and the other half was kept alive in 750 L storage. Defaunation was achieved by rinsing the sampled litter on a 10 mm sieve stacked on a 500 µm nylon-mesh sieve in order to separate the detrital material from the macrofauna. This method was considered efficient since it was the same method used in Chapter 3 (see Chapter 3 § 2.1) to sample macrofauna.

Each mesocosm was constituted of a PVC box (dimensions: 20 x 30 x 35 cm, 21000 cm^3) from which two sides were replaced by 38 µm nylon mesh to prevent organisms movements and colonization, but allow free water exchange and prevent complete anoxia inside. The 18 mesocosms were placed at a depth of 9 m at the OSCE-site and on an adjacent EMA situated directly North-East (Figure 4.3). Treatments and controls were placed randomly to prevent any bias from a potential position effect. Just before starting the experiment, 205.08 ± 3.29g (wet mass) of litter were put inside each mesocosm for both treatments. This amount of litter represented visually a doubling (+100%) of the amount of litter naturally present at the OSCE-site at the beginning of the

experiment. This corresponded to a moderate resource pulse (see chapter 3). Controls thus contained only the litter already present on site, while T-defaun contained the litter already present on site plus "defaunated litter" and T-fauna contained the litter already present on site plus "natural litter". Mesocosms were anchored on the sediment with marine steel pools and weights to prevent any movement of the setup and prevent dead leaves movements, in or out, at the mesocosm-sediment interface. 14 days after the beginning of the experiment, only 1 replicate from each treatment was detached from the anchoring system and not included in the data analysis (N=5). All mesocosms were sealed underwater and brought back to the lab for further processing. Each mesocosm was emptied from its litter and macrofauna was separated from the dead *P.oceanica* leaves using freshwater on 10 mm and 500 µm sieves for optimal and handy separation. The 500 µm fraction was preserved in a 4% formaldehyde seawater solution and kept until further analysis. After 48h of formaldehyde fixation, the 4% formaldehyde seawater solution was replaced by distilled water for final sorting, specific identification under stereomicroscope (Zeiss Stemi 2000-C), counting and then stored in 99.8% ethanol. The remaining defaunated detrital fraction was dried at 60°C for 5 days and then weighed.

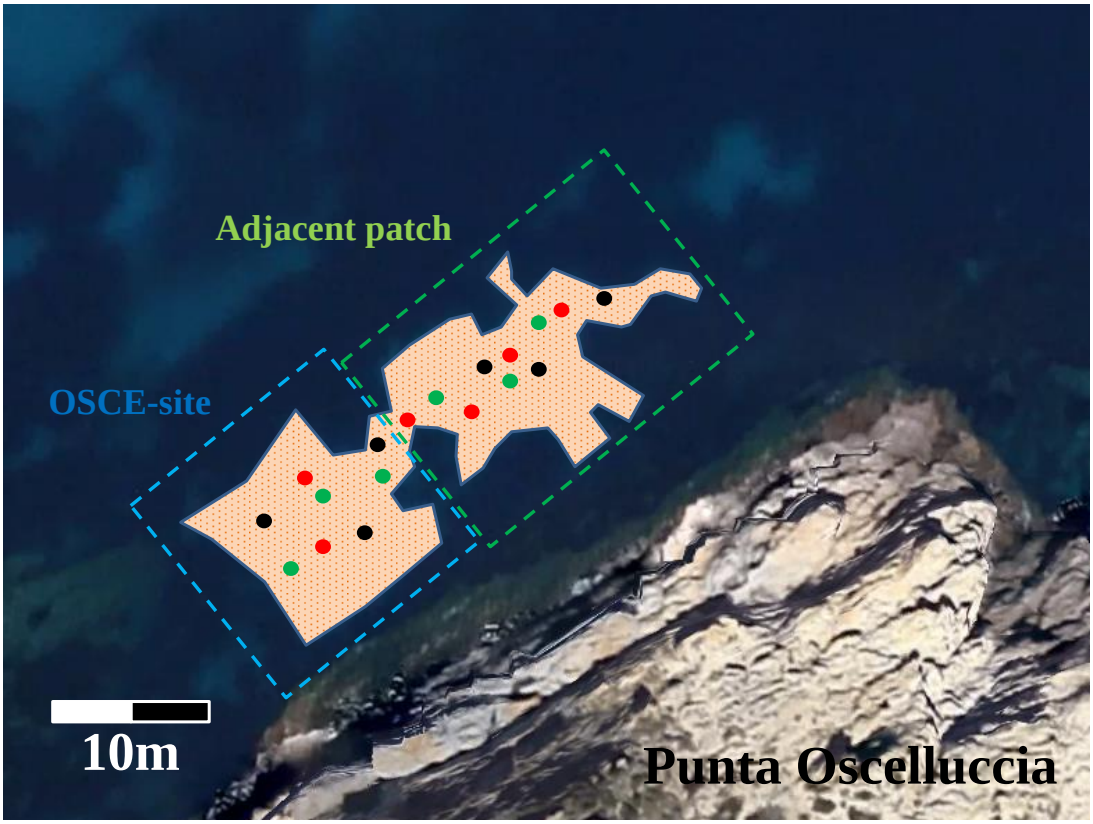


Figure 4.3: Schematic location and disposition of the pulse experiment at the Punta Oscelluccia northern side. Black dots: controls, Red dots: T-defaun, Green dots: T-fauna.

2.4. Data analysis:

Hierarchical clustering construction, DistLM analysis, dbRDA representation, SIMPER analysis and diversity indexes calculations are detailed in Chapter 2 (§ 3.4.3).

Classical statistical analysis (factorial ANOVA, factorial MANOVA and Hierarchical Ward Dendrogam) were performed using R and the dedicated "Rcmdrv2.2-3" and "pvclustv2.0-0" packages. Diversity indexes calculations, SIMPER analysis, DistLM and dbRDA graphical ordinations were performed using PRIMER 6.1.13 (Clarke and Gorley, 2006) with PERMANOVA additional software (Anderson *et al*., 2008). A significance level of $p < 0.01$ was always used in all tests.

Graphs were built with R, PRIMER 6.1.13 and GraphPad PRISM 6.01 software for Windows (GraphPad Software, San Diego, USA).

3. Results

3.1. Oxygen impact experiment

3.1.1. Abiotic factors

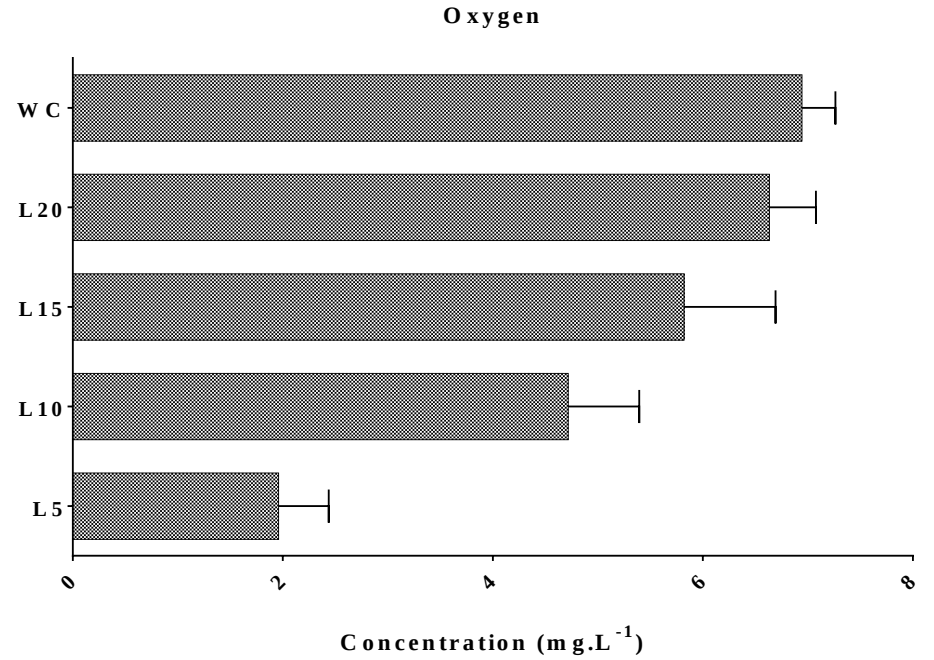
After 48h, oxygen (Figure 4.4) showed a maximum of concentration in WC (6.94 ± 0.31 $mgO_2.L^{-1}$) and concentration slightly decreased in **L20**, **L15** and **L10**, to reach a minimum concentration in **L5** (1.96 ± 0.48 $mgO_2.L^{-1}$) below the hypoxia threshold of 2 $mgO_2.L^{-1}$.

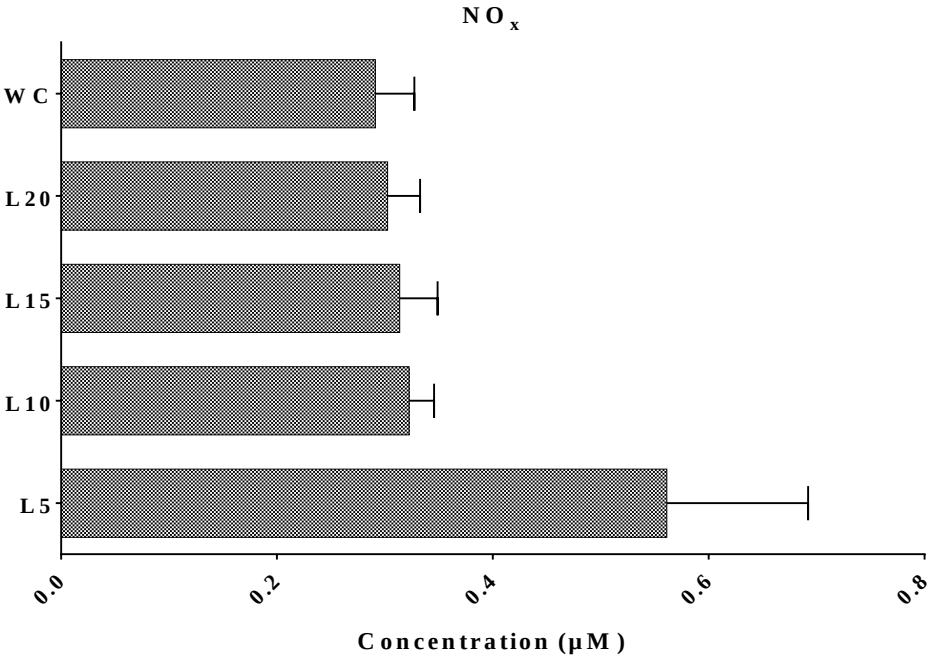
NOx (Figure 4.4) showed an opposite pattern with a minimum concentration in WC (0.29 ± 0.04 µM) and concentration showed relatively stable values in **L20**, **L15** and **L10**, to reach a maximum concentration in **L5** (0.56 ± 0.13 µM).

NH4 (Figure 4.4) showed a minimum concentration in WC (3.60 ± 2.60 µM). Concentration gradually increased in **L20**, **L15** and **L10**, to reach a maximum concentration in **L5** (19.13 ± 3.01 µM).

PO4 did not show any pattern but was much more variable in **L5** than in any other layer.

Multivariate analysis on oxygen and nutrients data showed that they differ significantly depending on the layer of the experimental design (MANOVA, $p < 0.0001$). Further analysis showed that this significant difference was observed for Oxygen, NOx and NH4 concentrations (ANOVA, $p < 0.0001$).


Oxygen
WC
L20
L15
L10
L5
0
2
4
6
8
Concentration (mg.L-1)


NOx
WC
L20
L15
L10
L5
0.0
0.2
0.4
0.6
0.8
Concentration (µM)

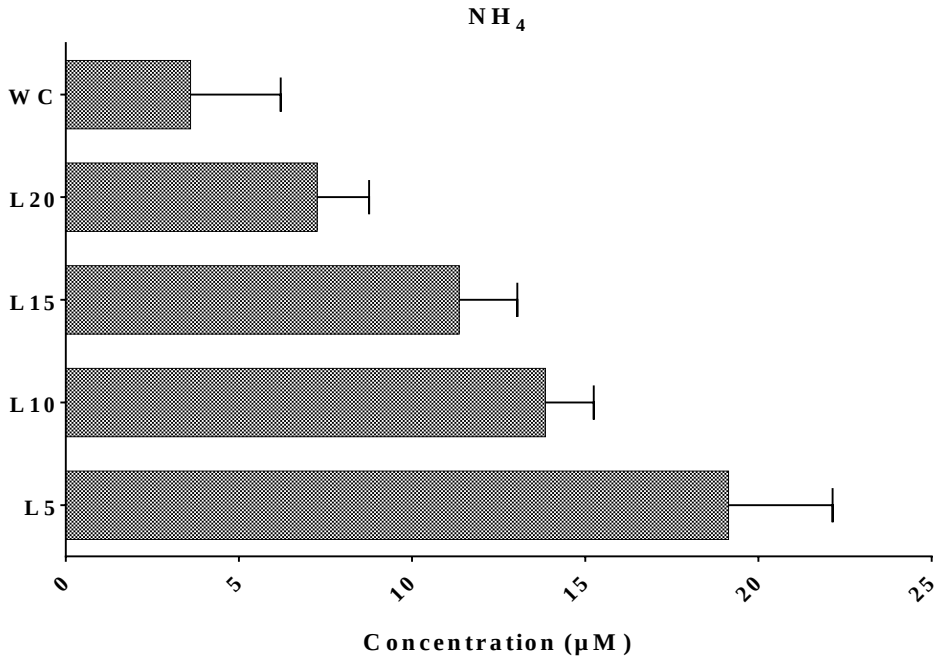


Figure 4.4 Comparison of the 4 experimental layers and the water column for O_2, NO_x and NH_4 concentrations after the 48h experiment. Data are means and SD (N=8 replicates per layer).

3.1.2. Macrofauna

Since total abundance has been shown to be directly linked to litter biomass (see Chapter 3), it was decided to express results in "weighed abundance". Weighed abundance refers to the abundance of a taxon divided by the total litter dry mass found for a sample (algae + epiphytes + living leaves + rhizomes + dead leaves) and will be expressed in $g.DM^{-1}$.

3.1.2.1. High taxonomic level

4331 individuals from 40 species were found during this experiment, representing a mean global weighed abundance of 3.53 ± 2.15 ind.gDM^{-1}. This showed global values in accordance to what was found in Chapter 3 (§3.1.3.1) with a clear dominance of Arthropods (26 species) representing 83.26 ± 15.19% (3.09 ± 1.19 ind. gDM^{-1}), followed by Annelids (4 species) representing 9.77 ± 8.11% (0.46 ± 0.63 ind. gDM^{-1}), Mollusks (6 species) representing 6.11 ± 7.16% (0.28 ± 0.39 ind. gDM^{-1}) and then the other less abundant phyla with Echinoderms (0.54 ± 0.81%) and Chordates (0.32 ± 0.58%). Nemerteans and Platyhelminths were absent of our experimental samples.

Relative abundance of Arthropods increased gradually between **L20** and **L5**, while relative abundance of Annelids, Mollusks, Echinoderms and Chordates decreased drastically between the top layer and the bottom layer.

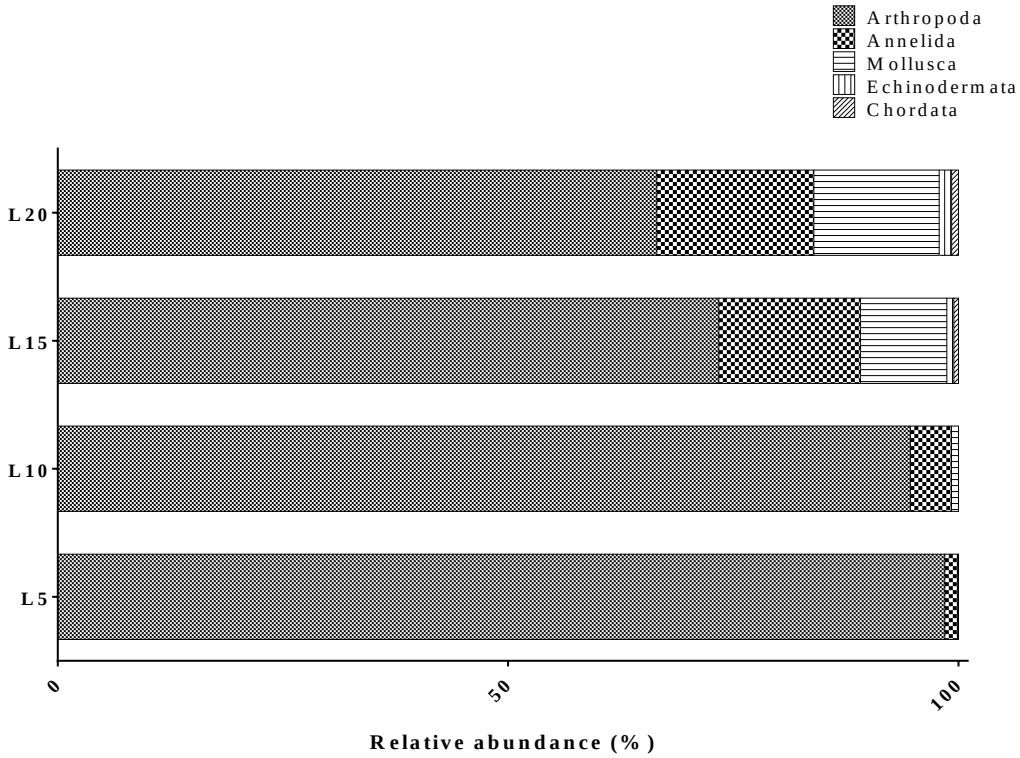


Figure 4.5 Comparison of the 4 experimental layers for the relative abundance (%) of each high level taxon after the 48h experiment. Data are means (N=8 replicates per layer).

Weighed abundance (ind.gDM^{-1}) showed a clear dominance of Arthropods, followed by Annelids, Mollusks, Echinoderms and Chordates (Figure x). However, it told a different story when analyzing the differences between layers (Figure 4.6). Indeed, Arthropods didn't show the constant decrease of relative proportion observed from **L5** to **L20** (Figure 4.5). Arthropod weighed abundance showed no clear evolution from **L20** to **L5**. On the contrary, abundances of Annelids, Mollusks, Echinoderms and Chordates showed the same pattern of constant decrease from (Figure 4.6) **L20** to **L5**.

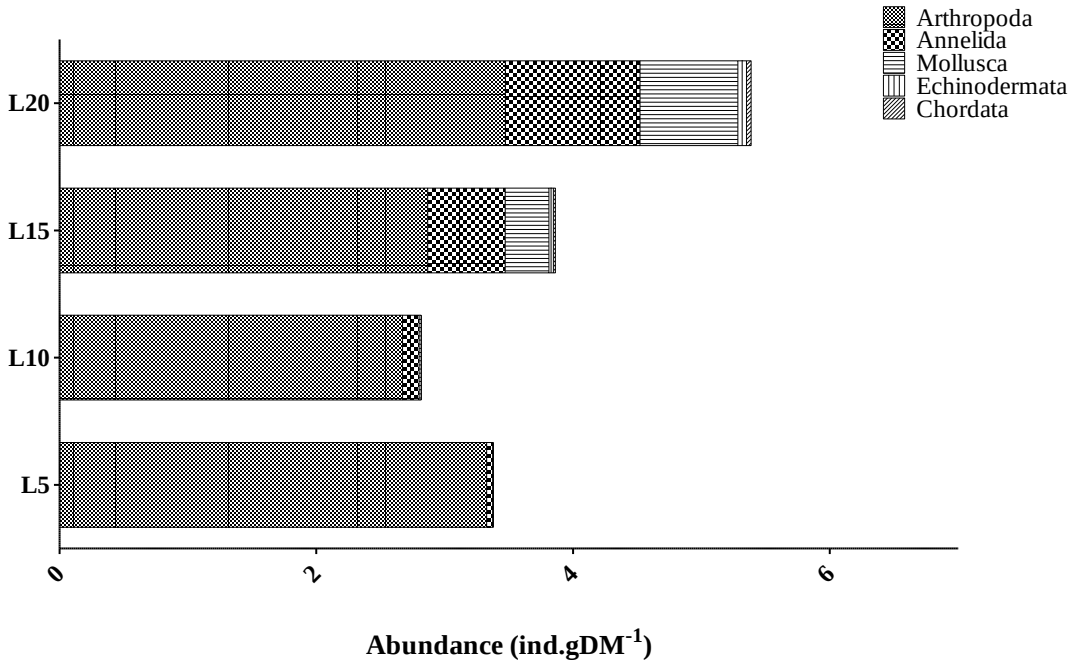


Figure 4.6 Comparison of the 4 experimental layers for the weighed abundance (ind.gDM^{-1}) of each high-level taxon after the 48h experiment. Data are means (N=8 replicates per layer).

Univariate analysis showed that global abundance was variable among the layers. **L20** presented significantly more abundance than the other layers (ANOVA, $p < 0.0001$), while the 3 other layers did not present significantly different global abundances.

Multivariate analysis (MANOVA, $p < 0.0001$) on the weighed abundance of these high-level taxa showed a significant influence of the layer of the experimental setup. Further analysis showed that the significant influence (ANOVA, $p < 0.001$) of the layer is observed for Annelids, Mollusks, Echinoderms and Chordates, but not for Arthropods.

3.1.2.2. Low taxonomic level

Species assemblage found during this experiment was coherent with results developed in Chapter 3.

Within Arthropods (composed of 26 species) the most dominant order was Amphipods representing 78.08% of the sampled Arthropods. Amphipods were followed by Decapods (13.65%), Leptostraceans (4.44%) and Isopods (3.79%). Amphipods were again dominated by *Gammarella fucicola* representing 87.63% (1.64 ± 0.78 ind. gDM^{-1}) of the Amphipods and more than 54% of the total macrofauna sampled for this experiment.

Within Annelids (composed of 4 species), only Polychetes were identified. Two species largely dominated within Polychetes. *Platynereis dumerilii* represented 71.27% and *Polyophtalmus pictus* represented 25.17%.

Mollusks (composed of 6 species), were almost exclusively constituted of Gastropods, representing 99.14%. Polypacophorans and Cephalopods were also present but represented only 0.43% each. Within Gastropods, *Bittium reticulatum* represented 76.51% and *Tricolia tennuis* represented 19.78%.

From the 19 species identified in Chapter 3 (§3.1.3.2) and representing 90% of the total abundance of the sampled macrofauna 2010-2012, 14 were identified in the 40 species sampled during this experiment, representing 92.08 ± 9.39% of the total community. To maintain coherence between Chapters 3 and 4, only these 14 species were considered for further analysis.

Multivariate analysis on weighed abundance data from these 14 species showed that the layer had a significant impact (MANOVA, $p < 0.0001$) on these species. Further analysis showed that a significant effect could be observed (ANOVA, $p < 0.01$) for 9 species out of 14. A significant effect of the layer was found for two Amphipods (*Microdeutopus chelifer* and *Nototropis*

guttatus), two Decapods (*Athanas nitescens* and *Galathea intermedia*), a Leptostracean (*Nebalia strausi*), two Polychetes (*Platynereis dumerilii* and *Polyophtalmus pictus*) and two Gastropods (*Bittium reticulatum* and *Tricolia tennuis*). No significant effect was found for the Amphipods species *Gammarella fucicola*, *Melita hergensis*, *Lysianassa costae*, *Apherusa chiereghinii* and the Isopod species *Jaera nordmanni*. Hierarchical clustering analysis (Ward method) based on the squared weighed abundance data of the 40 species showed quite well this "layer pattern", forming 2 clusters, one of them being "Cluster 5-10", and the other one being "Cluster 15-20" (Figure 4.7).

SIMPER analysis for layer factor based on squared relative abundances data of the 40 species showed that *Gammarella fucicola* was always the strongest contributor to similarity (Table 4.1). From layers 5 to 20, it appeared that the analysis included more and more species to reach the threshold of 90% Cum. Similarity was always quite high and it must also be noted that layers 5 and 10 presented a much lower dissimilarity (24.62%) than layers 15 and 20 (41.82%).

Diversity and equitability indexes also showed important variations. Shannon diversity index (**H'**) and Simpson evenness index (**1-λ'**) were highly variable from one layer to the other. Shannon index showed a minimum value of 0.70 ± 0.08 in **L5** and a maximum value of 2.26 ± 0.20 in **L20**, indicating a much lower diversity in **L5**. Simpson index showed a minimum value of 0.46 ± 0.09 in **L5** and a maximum value of 0.80 ± 0.05 in **L20**, indicating much more dominance of a few species in **L5**.

Multivariate analysis showed (MANOVA, $p < 0.0001$) that indexes were significantly influenced by the layer. Further analysis (ANOVA, $p < 0.01$) showed that both Shannon and Simpson indexes were significantly influenced.

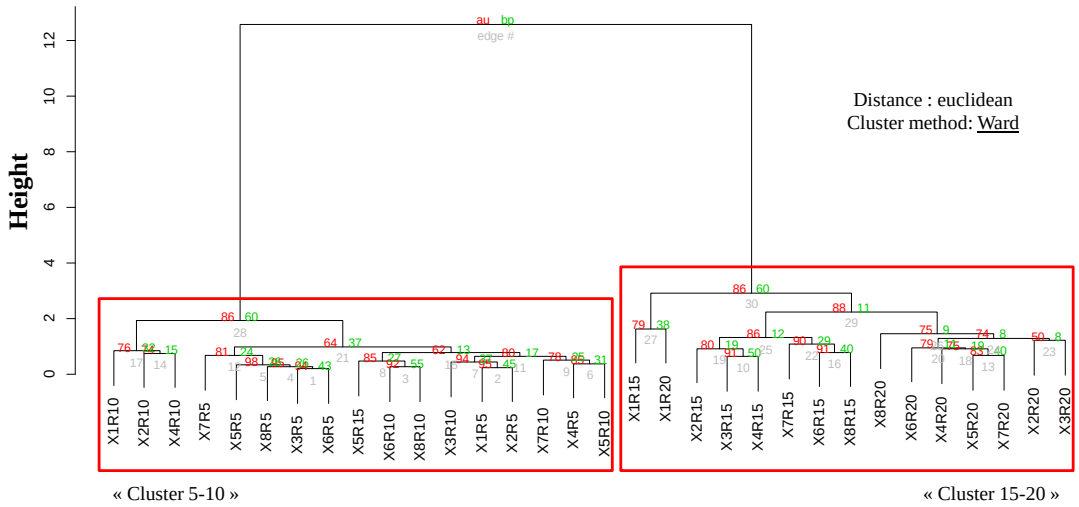


*Figure 4.7 Hierarchical clustering dendrogram using Euclidean distances and Ward grouping method. Based on square-root transformed weighed abundances of all the sampled species. Each sample is represented by a code: **X**A**R**B, where A is the replicate number (from 1 to 8) and B is the number of the layer (from 5 to 20, from bottom to top). The Y axis represents the Euclidean distance between the samples. Red numbers can be interpreted as the probability a cluster has been formed during the 10000 iteration of the bootstrap resampling process (values above 75 are considered as "high"). Green numbers are the bootstrap value.*

Table 4.1 SIMPER result with factor "layer" for macrofauna species contribution to similarity between samples. Species contributing to minimum 90% of the similarity are represented.

Layer factor

LAYER 5 (84.88% similarity)

Species	%	% Cum.
Gammarella fucicola	58.73	58.73
Nebalia strausi	18.10	76.83
Athanas nitescens	17.45	94.27

LAYER 10 (70.86% similarity)

Species	%	% Cum.
Gammarella fucicola	60.21	60.21
Athanas nitescens	18.82	79.03
Platynereis dumerilii	9.04	88.07
Nebalia strausi	6.39	94.45

LAYER 15 (61.27% similarity)

Species	%	% Cum.
Gammarella fucicola	36.41	36.41
Platynereis dumerilii	15.70	52.12
Bittium reticulatum	10.29	62.41
Microdeutopus chelifer	9.97	72.37
Polyophthalmus pictus	6.79	79.17
Hippolyte leptocerus	5.67	84.83
Nototropis guttatus	4.23	89.06
Athanas nitescens	3.32	92.38

LAYER 20 (68.84% similarity)

Species	%	% Cum.
Gammarella fucicola	20.34	20.34
Microdeutopus chelifer	11.30	31.64
Bittium reticulatum	10.68	42.32
Platynereis dumerilii	9.88	52.2
Polyophthalmus pictus	7.26	59.46
Tricolia tenuis	6.20	65.65
Hippolyte leptocerus	5.73	71.38
Galathea intermedia	4.19	75.57
Anapagurus chiroacanthus	4.15	79.72
Cymodoce truncata	4.10	83.82
Nototropis guttatus	3.90	87.72
Palaemon xiphias	3.71	91.42

3.1.3. Impact of abiotic factors on macrofauna

Chapter 3 showed that variability observed for weighed abundances of some of the most dominant macrofauna species of the EMAs, was mainly driven by O_2 and to a lesser extent, by NH_4. These drastic potential effects of O_2 and NH_4 were very species-specific.

The 14 same species were chosen to perform Multiple Regression, DistLM and dbRDA graphical representation. Draftsman plot on the environmental variables (O_2, NH_4, NO_x and PO_4) showed that O_2 and NH_4 were very highly negatively linked (R = -0.867) and NH_4 was thus removed from further analysis.

Multiple regression analysis showed that only O_2 concentration was linked significantly to 8 of the 14 chosen species. Two Amphipod species (*Microdeutopus chelifer* and *Nototropis guttatus*), one Decapod (*Galathea intermedia*), two Polychetes (*Platynereis dumerilii* and *Polyophtalmus pictus*) and a Gastropod (*Bittium reticulatum*) showed highly significant positive relationship with O_2 concentration (Table 4.2). Only one Leptostracean species (*Nebalia strausi*) and one Decapod species (*Athanas nitescens*) showed a significant negative relationship with O_2 concentration.

Out of these 8 species, 5 are common with those showing relationship with O_2 in natural conditions in chapter 3 (§ 3.1.4) and, as in chapter 3, it must be noted that the most abundant species, *Gammarella fucicola* , did not show any relationship to O_2 or any other environmental parameter.

DistLM test indicated an important relationship between the macrofauna assemblage observed during this experiment and the environmental parameters. The model explained 58.54% of the observed variability and contained 3 parameters: O_2, NO_x and PO_4. (1) A highly significant one, O_2 (pseudo-F = 39.38, $p < 0.001$), which is accounting for 56.8% of the total observed variability. (2) Another highly significant one, NO_X (pseudo-F = 13.14, p = 0.001), which is accounting for 30.4% of the observed variability. (3) A just non-significant one, PO_4 (pseudo-F = 2.596, p = 0.038), which is accounting for less than 5% of the total observed variability. The first dbRDA axis accounted for 57.79% of the total observed variability in the macrofauna assemblage and discriminated samples based essentially on O_2 concentration (multiple partial correlation = -0.963). The second dbRDA axis accounted for 0.24% of the total observed variability in the macrofauna assemblage and discriminated samples based essentially on NO_x concentration (multiple partial correlation = 0.962).

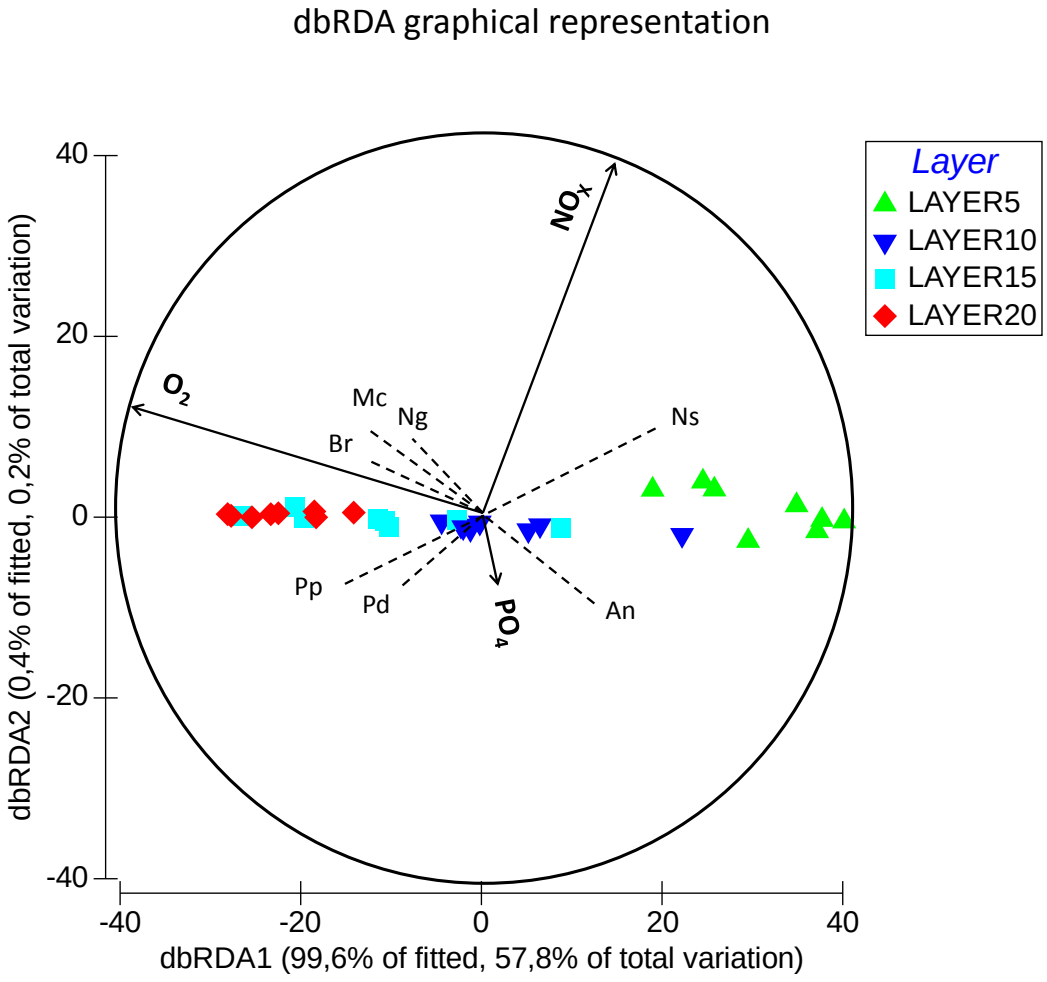


Figure 4.8 Distance-based redundancy ordination (bdRDA) representing the DistLM modelling for the 14 most abundant species and environmental variables. Full vectors represent the direction of increasing values of the environmental variables from the model. Dotted lines represent macrofauna species with correlations ≥ 0.25 to the ordination axes. Vector and lines length represent the partial correlation strength with the dbRDA axes; the circle is a unit circle (radius = 1) whose relative size and position are arbitrary with respect to the underlying plot. Triangles and squares represent the samples, color coded by layer. Species abbreviations: Mc = Micodeutopus chelifer; Ng = Nototropis guttatus; An = Athanas nitescens; Ns = Nebalia strausi; Pd = Platynereis dumerilii; Pp = Polyophtalmus pictus; Br = Bittium reticulatum.

Among the species presenting correlation ≥ 0.25 of the ordination, 5 species (*Nototropis guttatus*, *Microdeutopus chelifer*, *Platynereis dumerlilii*, *Polyophtalmus pictus* and *Bittium reticulatum*) had negative values for the first axis (negatively correlated with O_2), supposing a positive link with O_2 concentration (Figure 4.8). On the contrary, the Leptostracean species (*Nebalia strausi*) and the Decapod species (*Athanas nitescens*) had positive values for the first axis, supposing a negative relationship with O_2 concentration. All samples were ordinated horizontally following the first axis with poorly oxygenated layers on the right, showing positive values (Layers 5 and 10) and well-oxygenated layers on the left, showing negative values (Layers 15 and 20).

3.2. Pulse impact experiment

3.2.1. High taxonomic level

A total of 3503 individuals from 36 species were sampled at the end of this 14 days *in situ* experiment, representing a mean global abundance of 4.37 ± 1.38 ind. gDM^{-1}. 26 species were sampled in controls (hereafter referred as "**C**"), 35 species in defaunated litter treatment (**T-defaun.**), 36 species in unmodified litter treatment (**T-fauna**) and 25 species were sampled in the $\mathbf{T_{final}}$. These 9 species represent 2.21% of the total abundance in T-defaun. and 2.55% in T-fauna. It must be noted that *Nebalia strausi* accounted for more than half of these values in both treatments.

As in Chapter 3, Arthropods (24 species) were globally extremely dominant, representing on average 85.63 ± 2.77% (3.54 ± 1.15 ind. gDM^{-1}) of the total weighed abundance. Arthropods were followed by Annelids (6 species) representing 8.19 ± 2.20% (0.35 ± 0.17 ind. gDM^{-1}), Mollusks (4 species) representing 4.15 ± 1.72% (0.18 ± 0.11 ind. gDM^{-1}) and Echinoderms (2 species) representing 2.03 ± 1.33% (0.08 ± 0.04 ind. gDM^{-1}).

Multivariate analysis based on global weighed abundance (ind.gDM^{-1}) and weighed abundance of high-level taxa showed that global weighed abundance showed no significant effect of treatments (Figure 4.10), and that only Echinoderms were showing significant differences according to the treatment (1-way MANOVA, $p < 0.001$).

Table 4.2: Summary table of mean weighed abundances of the high-level taxa and 7 invertebrate species in the T_{final}, Control, T-defaun. and T-fauna. The 7 species presented significant abundance variations between the control and the two treatments. Values are mean ± standard deviation.

	Weighed abundance (ind. gDM^{-1})			
	T_{final}	Control	T-defaun.	T-fauna
Arthropoda	4.36 ± 0.83	4.47 ± 1.64	2.97 ± 0.53	3.18 ± 0.2
Annelida	0.4 ± 0.13	0.45 ± 0.24	0.25 ± 0.09	0.34 ± 0.12
Mollusca	0.18 ± 0.13	0.21 ± 0.18	0.16 ± 0.06	0.16 ± 0.08
Echinodermata	0.04 ± 0.03	0.03 ± 0.04	0.09 ± 0.03	0.1 ± 0.03
Gammarella fucicola	3.28 ± 0.63	3.5 ± 1	1.14 ± 0.19	1.08 ± 0.19
Gammarus aequicauda	0.13 ± 0.02	0.11 ± 0.06	0.7 ± 0.18	0.72 ± 0.12
Nototropis guttatus	0.05 ± 0.03	0.06 ± 0.04	0.03 ± 0.02	0.09 ± 0.04
Athanas nitescens	0.02 ± 0.03	0.03 ± 0.04	0.13 ± 0.02	0.15 ± 0.06
Palaemon xiphias	0.04 ± 0.02	0.05 ± 0.04	0.08 ± 0.01	0.18 ± 0.07
Galathea intermedia	0.02 ± 0.03	0.01 ± 0.02	0.01 ± 0.02	0.07 ± 0.03
Nebalia strausi	0 ± 0	0 ± 0	0.19 ± 0.08	0.24 ± 0.06

3.2.2. Lower taxonomic level

Within Arthropods, Amphipods were the globally most dominant taxa, representing 78.18 ± 12.45%, followed by Decapods representing 9.64 ± 6.06%, Isopods representing 7.29 ± 3.33% and Leptostraceans representing 4.69 ± 3.79%. The Amphipod *Gammarella fucicola* was typically the most dominant species representing alone 62.86 ± 17.61% of the Amphipods and 44.03 ± 20.22% (1.90 ± 1.29 ind. gDM^{-1}) of the total macrofauna. The Amphipod *Gammarus aequicauda* was also quite abundant, representing 13.78 ± 8.96% (0.51 ± 0.32 ind. gDM^{-1}) of the amphipods. Annelids were composed of 100% of Polychetes (from which *Platynereis dumerilii* represented 46.52 ± 16.28%) and Mollusks were composed almost exclusively of Gastropods (from which *Bittium reticulatus* represented 75.75 ± 15.40%).

Multivariate analysis based on weighed abundance (ind.gDM^{-1}) of low-level taxa showed that for weighed abundance, Amphipods, Decapods and Leptostraceans were showing highly significant differences according to the treatment (1-way MANOVA, $p < 0.0001$). Control and T_{final} were systematically different from the two treatments. Hierarchical clustering analysis (Ward method) based on the squared weighed abundance data of the 36 species showed clearly this pattern, forming 2 main clusters, one of them being "Control cluster" composed of Control replicates and T_{final} replicates, and the other one being "Pulse cluster" composed of T-defaun. and T-fauna replicates (Figure 4.9). Within "Pulse cluster", no clear separation between T-defaun. and T-fauna was observed.

Diversity and equitability indexes also showed important variations. Shannon diversity index (**H'**) and Simpson evenness index (**1-λ'**) were variable from one treatment to the other. Shannon index showed a minimum value of 2.33 ± 0.21 and 2.42 ± 0.17 in T_{final} and C, respectively and values of 2.94 ± 0.16 and 3.01 ± 0.15 in T-defaun. and T-fauna respectively, indicating a lower diversity in C. Simpson index showed a minimum value of 0.82 ± 0.09 and 0.86 ± 0.03 in T_{final} and C, respectively and a maximum value of 0.94 ± 0.01 in both T-defaun. and T-fauna, indicating more dominance of a few species in T-defaun. and T-fauna.

Multivariate analysis showed (MANOVA, $p < 0.0001$) that indexes were significantly influenced by the treatment. Further analysis (ANOVA, $p < 0.0001$) showed that both Shannon and Simpson indexes were significantly influenced.

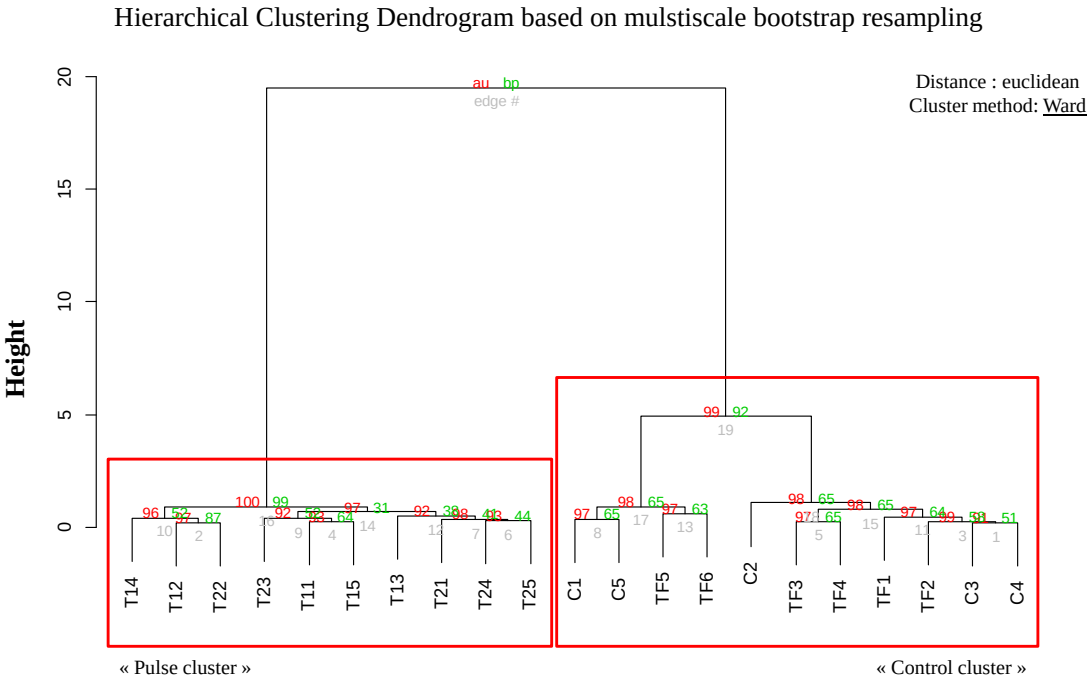


*Figure 4.9 Hierarchical clustering dendrogram using Euclidean distances and Ward grouping method. Based on square-root transformed weighed abundances of all the sampled species. Each sample is represented by a code: **C** 1-5 are the five replicates of the control; **T1** 1-5 are the five replicates of "T-defaun"; **T2** 1-5 are the five replicates of "T-fauna"; **TF** 1-6 are the 6 replicates of T_{final}. The Y axis represents the Euclidean distance between the samples. Red numbers can be interpreted as the time proportion a cluster has been formed during the 1000 iterations of the bootstrap resampling process (values above 75 are considered as "high"). Green numbers are the bootstrap value.*

From the 19 most abundant species found in Chapter 3, 15 were present in the most abundant species from this experiment. Although representing a relatively limited contribution, *Palaemon xiphias*, the only big predatory Decapod found in EMAs was added to the analysis to monitor the potential impact of a pulsed event on a species from a predator trophic level. Multivariate analysis based on weighed abundance of these 16 species showed a significant effect of the treatment for 7 of them (1-way MANOVA, $p < 0.001$). Two Amphipod species (*Gammarella fucicola* and *Gammarus aequicauda*) and the Leptostracean species (*Nebalia strausi*) showed very highly significant effects of the treatment (ANOVA, $p < 0.00001$). The 3 Decapod species (*Athanas nitescens*, *Palaemon xiphias* and *Galathea*

intermedia) showed significant effects of the treatment (ANOVA, $p < 0.01$). The remaining Amphipod species (*Nototropis guttatus*) showed no significant effect concerning the weighed abundance. For these 7 species, weighed abundances showed drastic variations between C or Tfinal and the treatments (Figure 4.10). *Gammarella fucicola* showed a much lower weighed and relative abundance in C or T_{final} than in T-defaun. or T-fauna. The 6 other species showed an opposite pattern with a higher weighed and relative abundance in C and T_{final}.

Gammarus aequicauda experienced by far the most drastic differences, going from a weighed abundance value of 0.11 ± 0.06 ind. gDM^{-1} in C, to 0.71 ± 0.13 ind. gDM^{-1} in both treatments. Relative abundance of *Gammarus aequicauda* also varied a lot, going from a value of 2.09 ± 0.95% in C, to 19.48 ± 2.94% in both pulsed treatments.

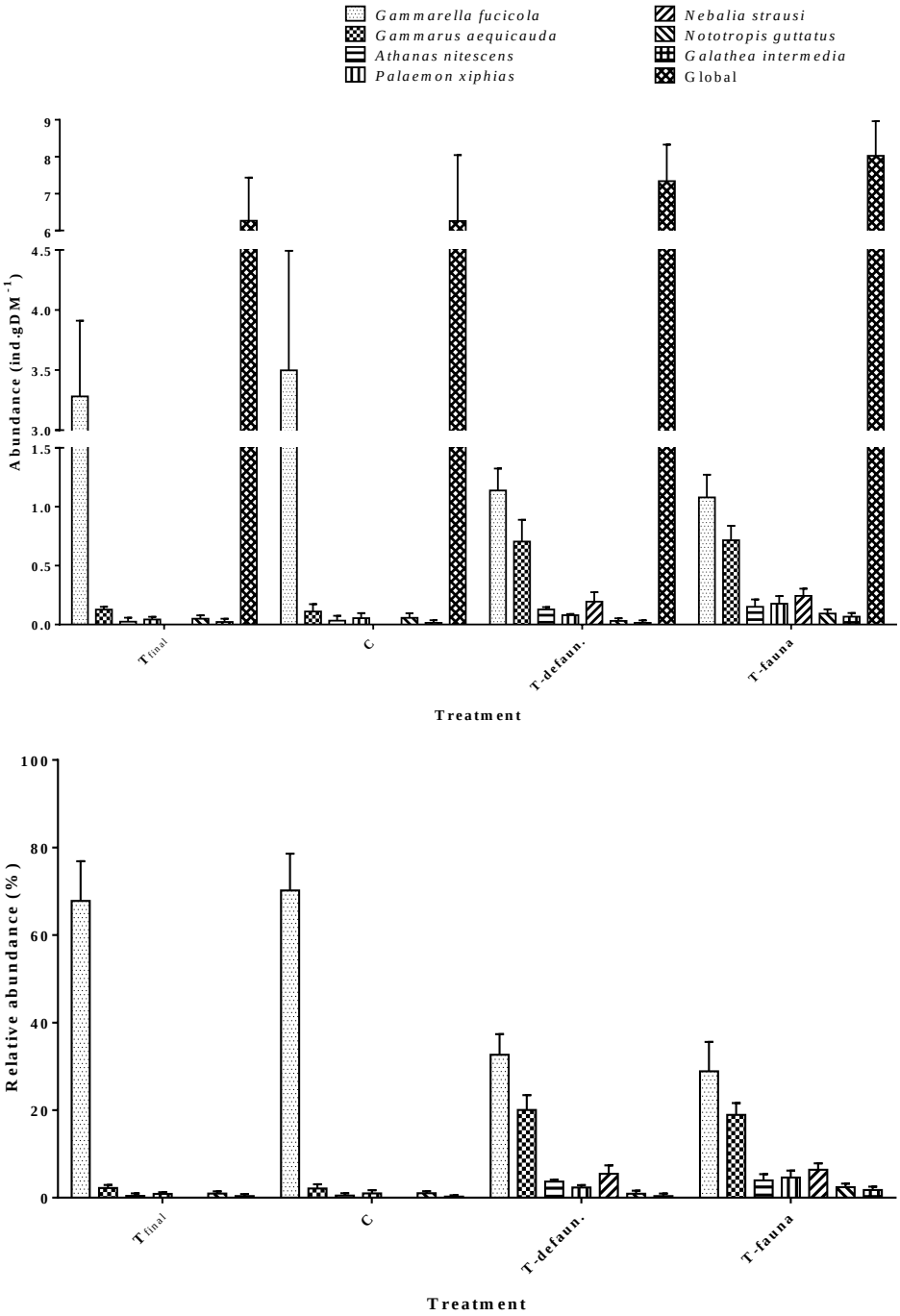


Figure 4.10 Representation of the global weighed abundance as well as the weighed and relative abundances of the 7 species showing a significant effect of the treatment factor. A: weighed abundance (ind. gDM^{-1}); B: relative abundance (%).

SIMPER analysis for treatment factor based on weighed abundances data of the 36 species showed that *Gammarella fucicola* was always the strongest contributor to similarity (Table 4.3). Similarity was high for all treatments ranging from 71.35% to 78.56%. Specific contributions showed a same pattern in the control and in T_{final} and that pattern was very different from the one observed in T-defaun. or T-fauna treatments. A striking result was that in the control or T_{final}, *Gammarella fucicola* was by far the species contributing the most to the similarity, while in T-defaun. or T-fauna, *Gammarella fucicola* was still the strongest contributor, followed closely by *Gammarus aequicauda*, which was only a minor contributor in the control or T_{final}.

It must be noted that *Gammarus aequicauda* contributed much more to the dissimilarity between T-defaun. and control, or T-fauna and control than between T.defaun. and T-fauna.

Table 4.3: Summary table of the SIMPER result with factor "treatment" for macrofauna species contribution to similarity between samples. Species contributing to minimum 90% of the similarity are represented.

Treatment factor

T_{final} (76.91% similarity)

Species	%	% Cum.
Gammarella fucicola	37.07	37.07
Platynereis dumerilii	8.53	45.6
Polyophthalmus pictus	7.7	53.3
Microdeutopus chelifer	7.15	60.45
Melita hergensis	6.35	66.8
Bittium reticulatum	6.19	72.99
Jaera nordmanni	6.17	79.16
Gammarus aequicauda	5.9	85.06
Nototropis guttatus	3.53	88.59
Palaemon xiphias	3.51	92.09

Control (71.35% similarity)

Species	%	% Cum.
Gammarella fucicola	38,54	38,54
Platynereis dumerilii	9,25	47,79
Polyophthalmus pictus	8,09	55,88
Jaera nordmanni	7,07	62,95
Bittium reticulatum	6,44	69,39
Microdeutopus chelifer	6,39	75,77
Gammarus aequicauda	6,21	81,98
Melita hergensis	5,46	87,44
Nototropis guttatus	3,78	91,23

T-defaun. (75.99% similarity)

Species	%	% Cum.
Gammarella fucicola	18.19	18.19
Gammarus aequicauda	14.02	32.21
Jaera nordmanni	7.5	39.71
Nebalia strausi	6.71	46.42
Athanas nitescens	6.12	52.54
Bittium reticulatum	4.99	57.53
Melita hergensis	4.95	62.48
Palaemon xiphias	4.91	67.39
Microdeutopus chelifer	4.6	71.99
Platynereis dumerilii	4.12	76.11
Polyophthalmus pictus	3.7	79.81
Ampipholis squamata	3.36	83.17
Anapagurus chiroacanthus	3.22	86.39
Hesiospina autantiaca	2.05	88.44
Nototropis guttatus	1.79	90.23

T-fauna (78.56% similarity)

Species	%	% Cum.
Gammarella fucicola	15.54	15.54
Gammarus aequicauda	12.66	28.2
Jaera nordmanni	7.34	35.54
Nebalia strausi	7.2	42.73
Palaemon xiphias	5.71	48.44
Platynereis dumerilii	5.64	54.08
Athanas nitescens	5.24	59.32
Melita hergensis	4.64	63.96
Bittium reticulatum	4.47	68.43
Ampipholis squamata	4.17	72.6
Nototropis guttatus	4.07	76.67
Anapagurus chiroacanthus	3.64	80.31
Galathea intermedia	3.4	83.71
Polyophthalmus pictus	3.25	86.96
Hesiospina autantiaca	2.56	89.52
Chrysopetalum debile	1.92	91.44

4. Discussion

4.1. Oxygen impact experiment

Our study demonstrated the strong stratification of physico-chemical conditions occurring in exported *P.oceanica* macrophytodetritus accumulations, and the high species-specific influence of oxygen as one of the main structuring factors for some abundant species of the associated vagile macrofauna community. The global densities and diversities sampled during this experiment were in accordance with previous general studies of this coastal ecosystem (Gallmetzer *et al*., 2005; Dimech *et al*., 2006; see Chapter 3 for a more detailed view of the litter community).

Gradual stratification of O_2 and NH_4 occurred very quickly, within the first 48h, which is congruent with the fast (< 24h) O_2 concentration decrease that Lepoint *et al.* (2012) found for calm days at the bottom of a 40 cm thick exported litter accumulation by direct Optode measurement. NH_4 concentration followed an inverted pattern compared to O_2 concentration. The gradual NH_4 increase within the deep layers of our experimental design could be partly explained by a potentially increased detrital animal and vegetal material degradation and resulting increased nitrogen remineralization in the deep layers, but also partly by a potentially increased anaerobic bacterial nitrogen fixation in the hypoxic layers of litter or diffusion from sediment (Gruber, 2004). Another potential hypothesis could be that NR-SOB bacteria were present in the hypoxic layer, reducing nitrates to ammonium in sediments (Hubert and Voordouw, 2007; Bonaglia *et al*., 2014). This hypothesis was supported by the fact that this process is strongly linked to H_2S oxidation in SO_4^{2-} . Since H_2S is known to be produced in hypoxic zones such as litter accumulations, it was thus possible that NR-SOB bacteria played a role in the increasing level of NH_4 in the less oxygenated layers.

Global abundance and diversity decreased highly and quickly (<48h), from the oxygenated top layer (L20) to the hypoxic bottom layer (L5) of our experimental design. While global abundance and diversity decreased, dominance increased drastically, which is a common phenomenon in places driven by frequent temporary low oxygen conditions (Veas *et al*., 2012). Gambi *et al*. (2009) monitored experimentally similar fast and drastic responses for a Mediterranean benthic community, resulting in the dominance

of only a few hypoxia-tolerant taxa at the end of the experiment. Global abundances of Mollusks, Annelids, Echinoderms and Chordates decrease drastically and almost completely disappear from the bottom hypoxic layer, L5. This pattern is found in many benthic ecosystems dealing with hypoxic conditions (Diaz and Rosenberg, 1995; Gambi *et al*., 2009; Levin *et al*., 2009; Hernàndez-Miranda *et al*., 2012; Veas *et al*., 2012) where Arthropods and Echinoderms react first to hypoxia, followed by Annelids and Mollusks with decreasing O_2 concentration. However, in this study, Arthropods did not show any global abundance pattern among the layers, which was not what could be expected according to literature. But as it was already mentioned in the previous chapter, the key concept of such studies is to consider organisms at the specific level. Indeed, while the global Arthropods abundance seemed constant among the layers, observations made for the dominance and diversity indexes, confirmed by the SIMPER analysis, were clear: the most important and typical species of each layer were very different, which indicated that even if global abundance did not change, the species composing the Arthropod taxa were not the same in the top oxygenated layer as in the bottom hypoxic one. Arthropod community thus experienced important modifications within this 48h experiment.

Since review studies on benthic ecosystems showed that the hypoxia threshold (≤ 2 $mgO_2.L^{-1}$) induced fast behavioral avoidance of hypoxic zones within the first 24h of an hypoxic event by many macrofauna species (Levin *et al*., 2009; Haselmair *et al*., 2010; Riedel *et al*., 2014), similar behaviors were expected for most of our species. Surprisingly, the most dominant amphipod species encountered, and representing up to 50% of the global macrofauna abundance, *Gammarella fucicola*, experienced no significant density changes among the layers, even if most benthic Arthopods are known to be quite hypoxia intolerant (Diaz and Rosenberg, 1995). Our hypothesis concerning this very dominant species is that it present a certain tolerance/adaptation to hypoxia, and that it is really adapted to extremely dynamic and different detrital/phytal habitats. Indeed, *Gammarella fucicola* is found in exported *P.oceanica* litter, in *in situ P.oceanica* litter (non-exported litter that decays inside the meadow, see Michel, 2011), in the *P.oceanica* foliar stratum (Gambi *et al*., 1992), in maerl beds (Carvalho *et al*., 2009) or in detrital algae accumulations (Vàsquez-Luis *et al*., 2008). These studies showed how much this species are found in various coastal detrital habitats and how ubiquitous they are. This leads us to hypothesize that *Gammarella fucicola* present a

certain level of hypoxia tolerance/adaptation, does not prefer hypoxic zones or oxygenated zones inside the litter accumulations, but that it is able to colonize all the layers no matter the O_2 concentration because it is really adapted to this type of habitat. Since *Gammarus aequicauda* was not sampled during this *in situ* experiment, no conclusions were drawn for this other abundant species.

However, 7 of the other most abundant species that were sampled showed a decrease or increase of abundance depending on the layer. Most Amphipods, Decapods, Isopods, Polychetes and Gastropods composing these 7 species were present in much higher densities in the top oxygenated layer than in the bottom hypoxic one, showing a positive link between O_2 concentration and their densities. This concentration of species in a more oxic zone, avoiding hypoxia and H_2S, is frequent for macrofauna-dominated ecosystems (Gray *et al*., 2002) and is probably more due to the active migration and behavioral response of the very mobile sensitive species encountered than to a mostly improbable increased mortality (Riedel *et al*., 2014). The particular case of two Arthropods species, *Nebalia strausi* and *Athanas nitescens*, was interesting, because these two species were completely absent of the oxygenated top layer, and showed an important density increase in the bottom hypoxic layer, becoming quite dominant species in this layer. This indicated a strong negative link between O_2 concentration and their abundances. Hypoxic conditions were not a problem for these two species, and this could be explained by several hypotheses. First, they could be more tolerant than others to low oxygen conditions. This was already briefly mentioned by Gallmetzer *et al*. (2005), who found a species of *Nebalia* in much more important densities at the bottom of very thick *P.oceanica* litter accumulations, suggesting a high tolerance to hypoxic conditions. Another hypothesis is that *Nebalia strausi* hypoxia tolerance could allow this species to avoid competition and predation by moving to hypoxic zones. This could lead to the development of a low O_2 tolerance and a behavioral avoidance of zones where competition/predation is high by using hypoxic zones as "refuges". Such O_2 tolerance and competition avoidance is known for *Nebalia hessleri* inhabiting *Macrocystis* detritus accumulations (Okey, 2003), which seem to prefer hypoxic parts of the *Macrocystis* mats when competing amphipods are abundant. This behavioral response associated to hypoxia (and reducing compounds like H_2S) tolerance allows *Nebalia hessleri* to live and spread deeper in the mat while amphipods are easily hunted by fishes and shrimps in the upper layers of the mat. For this experimental study, our hypothesis was that *Nebalia strausi* abundance was

strongly driven by O_2 and reducing compound concentrations, allowing the species to complete its life cycle and spread only in hypoxic zones where it avoided most of the predation and competition. The fact that *Nebalia strausi* was never found in oxygenated layers or oxygenated samples (also see Chapter 3) seemed to confirm the distinctive oxic zones avoidance strategy of this species.

Hypotheses were a bit different for *Athanas nitescens*, for the predation avoidance strategy was less plausible due to the much more carnivorous diet of *Athanas nitescens*. Data are scarce about *Athanas nitescens* oxygen preferences, but its tolerance to hypoxia has nonetheless already been indirectly observed since this species is known to present much lower oxygen consumption than most Amphipods and other Arthropods (Bishop and Iliffe, 2009). Since *Athanas nitescens* appeared to be hypoxia-tolerant, our hypothesis was that this species could migrate to hypoxic zones, where other carnivorous and less hypoxia-tolerant competitors could possibly not survive, to prey easier on species present in such hypoxic places. Since it was demonstrated by this study that highly abundant Amphipod species were not impacted by O_2 concentration variations, and were present in every layer, *Athanas nitescens* tolerance to hypoxia could allow an efficient behavioral avoidance of competitors and spatial ecological niche segregation, to find preys in low oxygen concentration zones of the exported *P.oceanica* accumulations.

These results concerning fast stratification and the resulting specific responses are of major importance for the exported dead *P.oceanica* litter accumulations, which are basically a very dynamic place. Since oxygenation of the litter accumulation is influenced drastically by parameters such as hydrodynamics, litter accumulation thickness or litter fragmentation, it can thus be hypothesized that the frequent alternation of calm and stormy conditions in the litter accumulation plays an important structuring role for the vagile associated macrofauna community. The calm periods allow the creation of a layering inside the litter accumulations, favoring the creation of micro-habitats with various physico-chemical conditions. These micro-habitats allow various species to aggregate in the different layers, momentarily enhancing the global biodiversity present inside the litter accumulations. The generally brief stormy periods allow a full oxygenation, mixing and potential partial renewing of both vegetal matter and animals present in the litter accumulation, preventing the creation of completely anoxic local “dead zones” inside the litter that could lead to the loss of most species encountered. This alternation of stratified and

mixed conditions could thus maintain the diverse and abundant detrital vagile macrofauna community present in these key habitats.

In conclusion, this experimental study demonstrated for the first time how fast the physiochemical conditions inside exported dead *P.oceanica* litter accumulations could experience an important stratification during a very calm period. It took only 48h for O_2 concentration to present a dramatic decrease, from the top to the bottom of the litter accumulation, even reaching the hypoxia threshold (≤ 2 $mgO_2.L^{-1}$) in the bottom layer. This important stratification thus allowed the creation of different micro-habitats, which was confirmed by the very different faunal assemblages occupying the different layers. Vagile macrofauna associated to dead *P.oceanica* litter presented a slightly different reaction to hypoxia compared to other well-studied benthic communities. Very abundant organisms like *Gammarella fucicola* did not seem to be impacted by O_2 concentration, indicating its adaptation to both oxic and hypoxic conditions in this detrital habitat. Most abundant sampled organisms showed a clear active migration to the most oxygenated layer, while two species, *Nebalia strausi* and *Athanas nitescens*, showed an opposite pattern, with a clear preference of the hypoxic layer and a clear behavioral avoidance of the oxic layers. This indicated high hypoxic tolerance and particular competition/predation-avoidance behaviors. This study thus also demonstrated the various living strategies inside exported dead *P.oceanica* litter accumulations as well as importance of working at the specific level to highlight these different strategies.

4.2. Pulse impact experiment

This *in situ* preliminary experimental study demonstrated the potential impact of resource pulses on the exported dead *P.oceanica* litter accumulation compartment.

The global abundance, diversity and dominance patterns observed in the control during this experiment corresponded completely to previous general studies of this coastal ecosystem (See Chapter 3 for a more detailed view of the litter community and also Gallmetzer *et al*., 2005; Dimech *et al*., 2006).

First of all, the two different treatments were significantly different from the control and the T_{final}, but did not show significant differences between them, and it was thus decided for this discussion to refer to both of them as a single treatment, hereafter: "the treatments". Control and T_{final} showed no significant difference between each other either. This is a first very interesting result, showing that the effect of the experimental design was quite negligible compared to the effect of the treatments.

This experiment also showed that global diversity and dominance were influenced by both treatments, and that diversity increased significantly with the addition of dead leaves with or without the associated macrofauna. The dominance also significantly increased, indicating that while the number of species encountered increased, a limited number of species seemed literally boosted by the addition of dead leaves. Such important density variations associated to the relatively short duration of the experiment (14 days) lead to the conclusion that the density responses observed could not only be caused by increased/decreased fitness or increased/decreased reproductive success and larval recruitment. Colonization and migration of macro-invertebrates have more than probably occurred (see Mascart *et al*., 2015b) during the whole experiment, potentially explaining the large magnitude of some of the observed responses, such as the drastic changes of the observed densities. Since controls and T_{final} replicates showed globally very similar weighed abundances and relative abundances for all the species, it could be assumed that colonization and migration of macrofauna only occurred in the treatments. Such behavioral aggregation of consumers is a common fast response to resource pulses (Anderson *et al*., 2008; Holt, 2008; Yang *et al*., 2008; Yang *et al*., 2010; Mascart *et al.*, 2015b) and the linked density variations observed in both treatments could be considered as a community level first response to a dead leaves pulse.

Another striking result was the absence of effect of the addition of organisms in the treatment T-fauna on the global abundance of the observed community. A more important "dilution" could be reasonably expected in T-defaun. than in T-fauna, which was not observed. Moreover, T-fauna presented no significant global abundance differences compared to the control or T_{final}, which is also surprising since the amount of added "higher quality" animal organic matter was high in this treatment (Yee and Juliano, 2012). Different hypothesis could explain this result. First, the experiment was carried out for 14 days, which could be too long to witness such effects on an animal pulse. Indeed, after 14 days we observed the final result of what happened during the 2 weeks of experiment. During these two weeks, added organisms density might have been leveled by predation and/or by behavioral migration resulting from the potentially increased competition for space and resource inside the mesocosms. Secondly, since it was very difficult to really estimate the invertebrate's density inside the added litter without disturbing too much the community, it could be hypothesized that density of added macrofauna was too low, or too high, inducing damped or extreme responses within the first days of the experiment. Too high densities could result in immediate extreme increase of competition inside the T-fauna mesocosms, potentially resulting in organisms mortality or more probably in active migration of the macrofauna out of the mesocosms within the first days of the experiment, since behavioral migration could occur very quickly (Holt, 2008). Densities could then stabilize after a few days and after 14 days present a final response, which could not be differentiated from the dead leaves addition of T-defaun. treatment. Too low densities could simply induce a very low global first response, practically not visible anymore 14 days later, after the occurring community changes and active migrations, once again presenting ultimately a final response which could not be differentiated from the dead leaves addition of T-defaun. treatment.

Except for *Gammarella fucicola*, all the other species experienced a significant increase of their densities. The most striking observation is the 7-fold increase of density of the detritivore amphipod *Gammarus aequicauda*, going from < 0.15 ind. gDM^{-1} (congruent with the 0.10 ± 0.13 ind. gDM^{-1} expected at this moment of the year, see Chapter 3) in the control, to > 0.70 ind. gDM^{-1} in the treatments. It must be noted that the SIMPER analysis coupled to the clustering dendrogram confirmed that the increased/reduced densities of these 7 species allow the strong differentiation of the control/T_{final}

and the two treatments into two very distinct groups, one potentially reacting to a resource pulse, the other being what could be expected from a "natural" community at that moment of the year.

This striking density increase of the most important truly detritivore species found in the exported dead *P.oceanica* litter accumulations leads to one important observation: such responses to resource pulses are mostly found in terrestrial ecosystems (Nowlin *et al*., 2008; Yang *et al*., 2008). Indeed, it has been demonstrated that terrestrial and aquatic ecosystems differ deeply in their responses to resource pulses, mainly because of the different characteristics of the organisms composing the terrestrial and aquatic communities, but also because of the different pathways followed by aquatic and terrestrial pulses (Nowlin *et al*., 2008). The thing is, exported dead *P.oceanica* litter accumulations present important similarities with terrestrial forest ecosystems in the pulse point of view. Like most terrestrial plants, *Posidonia oceanica* is a flower plant which sheds its leaves in autumn. Like most terrestrial macrophyte-driven ecosystems, these leaves form an important detrital pool with associated well-developed "detrital" food webs. These characteristics lead to one typical terrestrial response to pulses: detritivore organisms could take much more advantage of resource pulses (Yang, 2006) than herbivores, which is what was observed during this experimental study with the important increase of *Gammarus aequicauda* abundance in the treatments associated to a non-negligible decrease of *Gammarella fucicola* density. This important density increase is potentially a mixed result of active colonization of the "treatments mesocosms" by new *Gammarus aequicauda*, of increased larva recruitment. Moreover, *Gammarus aequicauda* is known to feed, not exclusively, but preferentially on dead *P.oceanica* fragments (see Lepoint *et al*., 2006; Michel *et al*., 2015; Chapter 5 for a more detailed view) which makes it quite a specialized organism. The important response of *Gammarus aequicauda* to the treatments was also congruent with Yang *et al*. (2008) and Yee and Juliano (2012), who stated that mobile specialists could take much more advantage of resource pulses than other organisms and that detritus feeders could benefit more than any other feeding group of such important pulses.

Such bottom-up responses are not the only ones to pulses. Top-down responses of predators always follow with a certain lag time (Ostfeld and Keesing, 2000; Chesson *et al*., 2008; Yang *et al*., 2010) depending on the generation time of the organisms composing the impacted community. This

study was not primarily designed to witness such longer term responses but the observed significant abundance increase of carnivore species such as *Palaemon xiphias* or *Athanas nitescens* could be linked to the primary responses developed earlier in this paragraph. This secondary response was fast and occurred in less than 14 days, which probably also implied an active colonization of the "treatment mesocosms" by these two species. This response could be congruent with Holt (2008), who stated that a very intense but very brief resource pulse could result in a drastic primary consumer density increase within the first day after the event and a moderate but fast predator density increase within the first 10 days after the event. This is also in accordance with Nowlin et al. (2008), who stated that invertebrate communities composed of organisms with short generation times could respond very fast to resource pulses. The increase of *Palaemon xiphias* or *Athanas nitescens* densities could thus be a result of the important increase of one of their potential prey, *Gammarus aequicauda*. This predatory response could also induce cascading effects on other preys, like the very abundant *Gammarella fucicola*. The important decrease of *Gammarella fucicola* density could be the result of the potential reduced fitness of the species, of the increased competitiveness of *Gammarus aequicauda* but also of the increased predatory stress due to *Palaemon xiphias* or *Athanas nitescens*.

Another interesting result was the non-negligible presence of *Nebalia strausi* in both treatments and its absence from the control and T_{final}. Considering the very small size of *Nebalia strausi*, active migration inside the "treatment-mesocosms" is more than probable. As demonstrated earlier in this chapter (see also Chapter 3), *Nebalia strausi* is highly tolerant to hypoxia, indicating that the addition of dead leaves in the treatments mesocosms potentially induced a certain level of hypoxia. It can thus also be hypothesized that resource pulses in exported dead *P.oceanica* litter accumulations could logically induce hypoxia in the bottom layer of the accumulation if the resource pulse is immediately followed by a calm weather period.

In conclusion, this preliminary experimental study gave a first insight of the impact the impact of a resource pulse on an exported dead *P.oceanica* litter accumulation and on the associated vagile macrofauna community. We demonstrated that exported dead *P.oceanica* litter accumulations presented similarities with terrestrial ecosystems in terms of pulse material origin (macrophyte-driven ecosystem) and "detrital pathway" of this material. These common characteristics induced a similar primary response which was the marked increase of *Gammarus aequicauda* density, a detritivore species. It was also proved that exported dead *P.oceanica* litter accumulation also presented characteristics of aquatic ecosystems, such as the presence of an invertebrate-based community, which induced a fast response of primary consumers and potentially of their predators to the resource pulse. We thus hypothesize that dead seagrass litter accumulations present combined characteristics from both terrestrial and aquatic ecosystems, and we predicted the following potential responses of this ecosystem to resource pulses: 1) very fast increase of mobile detritivore species density; 2) fast but moderate responses of predators; 3) persistent effect of the resource pulse on the community; 4) increased dominance of detritivore species and decrease of herbivore competitiveness; 5) diet modification of generalist invertebrates; 6) increased chances of hypoxia and creation of micro-habitats due to the resulting O_2/nutrients stratification; 7) longer term increase of the total biodiversity.

Chapter 5

Macrofauna feeding ecology and niche width: insights from SIA and gut content observations

1. Introduction

*Posidonia oceanic*a is a highly productive (Pergent *et al*., 1994; Gobert *et al*., 2006) Mediterranean seagrass that forms extensive meadows that cover vast areas (between $2.5.10^4$ and $4.5.10^4$ km^{-2}) of the coastal Mediterranean Sea (Pasqualini *et al*., 1998).

Like in most seagrass-based ecosystems, direct consumption of this important foliar production by herbivores is quite limited (but see Heck and valentine, 2006; Prado *et al*., 2007) and often represents less than 10% of the total foliar annual primary production (Pergent *et al*., 1994; Mateo and Romero, 1997; Boudouresque *et al*., 2015). This unconsumed important foliar primary production thus enters the "detrital pathway" after leaf fall in autumn and remains inside the meadow or, for most of it, is exported to adjacent unvegetated sand patched to form what is called "Exported *P. oceanica* Macrophytodetritus Accumulations", or **EMAs**. It has been estimated that up to 80-90% of the total foliar production could end in these EMAs, making them a potentially very important link between *Posidonia oceanica* and the coastal Mediterranean food webs (Cebrian and Duarte, 2001; Heck *et al*., 2008; Boudouresque *et al*., 2015).

These EMAs are composed of dead *P. oceanica* leaves, associated with their epiphytes, drift macroalgae, living *P. oceanica* leaves and rhizomes and micro-organisms (Anesio *et al*., 2003; Boudouresque *et al*., 2015). These litter accumulations serve as a habitat for quite a diverse and abundant community of invertebrates, composed of vagile meiofauna (Mascart *et al*., 2013; Mascart *et al*., 2015a) and vagile macrofauna.

Vagile invertebrates (size $\geq$ 500µm) are capable of free movements inside the EMAs and are largely dominated by arthropods, representing more than 75%, followed by annelids, representing more than 10%, and mollusks, representing more than 7% of the total abundance. (See Chapter 3 and 4 for more details, and Gallmetzer *et al*., 2005). Trophic ecology studies focused on specific taxa showed that several species ingest dead fragments of *P. oceanica* leaves and were also significantly assimilating *P. oceanica* dead leaves organic matter. Sturaro *et al*. (2010) found that dead leaves represented up to 20% of 3 idoteids species diet and Lepoint *et al*. (2006) showed that dead leaves represented 35-45% of *Gammarus aequicauda* average diet. These studies suggest the important potential role of the vagile macrofauna food web in terms

of organic matter transfer from the *P. oceanica* meadow, to the coastal food webs through the "detrital pathway".

Trophic relationships between organisms and food web delineation have been a major topic in field ecology for a long time. To achieve this goal, different methods have been developed, such as gut contents examination and stable isotope analysis, **SIA**.

Gut content examination is a long-known classical and once widespread method to get insights of feeding habits of animals. Too often neglected nowadays, this classical technique can still be very informative about animal diets (see Caut *et al*., 2008; Michel *et al*., 2015), allowing sometimes to study diets at a specific level. However, it presents a couple of major limitations. First, the examination of digestive tracts can only give a very short term "**snapshot**" idea of the animal diet at a given moment and place but gives no information about the temporal and spatial variations experienced by food sources and consumers (Dalsgaard *et al.*, 2003; Witteveen *et al*., 2009; Mascart, 2015; Michel *et al*., 2015). The other major problem is that gut content examination only gives information about the ingested items, but ingestion does not always mean **assimilation**, especially in a detritus-dominated food web, such as the food web present in the EMAs, where items of very contrasted digestibility and palatability can cause a non-negligible bias by overestimating the real consumption of too highly digestible or poorly digestible refractory items (Latyshev *et al*., 2004; Lepoint *et al.* 2006; Michel *et al.* 2015). At last, undetermined items may constitute a non-negligible part of the gut contents of invertebrates (Michel *et al*., 2015). This undetermined material might potentially be linked to food processing or ingestion of already highly degraded detritus.

To cope with these limitations, other powerful techniques were developed, especially the use of trophic markers such as the use of carbon (C) and nitrogen (N) stable isotopes ratios (i.e., $^{13}C/^{12}C$ and $^{15}N/^{14}N$, expressed as $\delta^{13}C$ and $\delta^{15}N$ respectively). Stable isotope analyses, SIA, are now widely used in trophic ecology studies (e.g. Post, 2002; Fry, 2006; Boecklen *et al*., 2011). Far from being perfect biomarkers (see Chapter 1, § 5.1.2.2), SIA can provide interesting information about the isotopic composition of the consumer and its food sources to estimate the assimilation proportion of each source, but also information on the trophic position occupied by each member of the food web (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981; Post, 2002). $^{13}C/^{12}C$ ratio is quite conservative between a food source and its consumer, and

consequently often used as a food source tracer inside a food web. $^{15}N/^{14}N$ ratio is more variable and often experiences a non-negligible increase between a food source and its consumer, making it an interesting trophic level estimator.

This trophic ecology study focused on the macrofauna community associated to *P. oceanica* macrodetritus accumulations to delineate this poorly studied food web and understand more about the potential role of this community in the transfer of *P. oceanica* organic matter to coastal food webs. To reach this general objective, a two-axes study was carried out and tried to answer the following specific questions: (1) Do the dead leaves represent a significant proportion of assimilated diet for the most abundant macrofauna species? (2) Do the food sources and consumers experience significant isotopic composition variations at a spatial and temporal level and do these differences reflect actual diet modifications, or only food sources baseline shifts? (3) Can these isotopic variations occur quickly in response to natural pulsed events?

2. Materials and methods

2.1. General aspects, sampling sites and sample collection

This study was composed of two distinct but related axes: (1) the first one consisted in a seasonal and spatial sampling in 2011-2012 to assess the potential spatiotemporal variations of isotopic composition of the whole EMAs macrofauna community along with all the potential food sources. This sampling was distinct, but related (taken at the same time) to the seasonal biodiversity sampling from Chapter 3. (2) The second one consisted in a regular and much higher frequency sampling to see how fast the impact of natural pulsed events propagated through this food web. This sampling was distinct, but related (taken at the same time) to the weekly biodiversity sampling from Chapter 3.

A total of 566 individuals were collected at two different sites for the first axis (HARBOR-site and OSCE-site), and a total of 220 individuals were sampled at one site (OSCE-site) for the second axis of this study (see Chapter 2 for detailed site descriptions). Sampling sites were chosen for their relative proximity to the STARESO oceanographic research facility (Université de

Liège) in Clavi Bay (42°35'N; 8°43'E, Corsica), the regular observation of exported litter on them, and were located on the eastern side of the Punta di Revellata. The two sites showed almost similar total areas: the first one directly inside the harbor of the station (hereafter "HARBOR-site"), and the second one 750 m away, right next to the northern side of the Punta Oscelluccia: hereafter "OSCE-site". Both sites are shallow (8-10 m) sandy patches.

Samples were taken seasonally in August 2011, November 2011, March 2012 and May 2012 at the two sampling sites for the first axis, and every two weeks between September 2012 and May 2013 only at OSCE-site for the second axis (see Chapter 2, Table 2.1).

For this trophic ecology sampling, only a sufficient number of individuals was necessary and standardization or exhaustive sampling was thus not compulsory. Qualitative sampling for trophic ecology spatiotemporal and high frequency temporal assessment was performed using large 30 L plastic bags. The litter and associated fauna were manually put inside the bag until it was full. Plastic bags were then sealed using plastic rings until separation processing in the lab. Sample processing back in STARESO was the same for all the trophic samples from both axes. The samples were kept alive in 750 L storage tanks and then, rinsed with freshwater on 10 mm and 500 µm sieves for optimal and handy separation. The 500 µm fraction was preserved in a smaller 2 L tank filled with oxygenated seawater. The organisms were then put individually in 4 mL vials and frozen (-20°C) until further analysis. At the same time, potential food sources (dead leaves, living leaves, drift macroalgae, epiphytes, suspended particulate organic matter (SPOM)) were also sampled and frozen (-20°C) until further analysis (see details in Chapter 2, §3.2).

2.2. Gut content examination

Back in Liège, the frozen organisms were identified to the specific level under stereomicroscope (Zeiss Stemi 2000-C), and their digestive tracts removed and spread on microscopic slides in glycerin for further gut content analysis. Gut contents analyses were performed using the semi-quantitative technique described by Wilson and Bellwood in 1997, but adapted for this study, for the very small gut contents of invertebrates. A 4 cm^2 grid composed of 100 squares of 4 mm^2 was used. 25 squares were randomly chosen and market out of the 100 and in each square only the dominant food item was taken into account (Wilson and Bellwood, 1997). Dominant food items for this study were classified in 5 categories: (1) dead *P. oceanica* leaves, (2) living *P. oceanica* leaves, (3) other vegetal material, (4) animal material and (5) unknown material. Once the 25 squares were examined and the most dominant item noted for each of them, the relative abundance (%) of each category was calculated. Organisms presenting empty gut or less than 10 squares containing one of the determined items were excluded from analysis.

2.3. Elemental and stable isotope analysis

After gut removal, all sampled individuals were dried for at least 96h (60°C), then ground to a homogenous powder manually or, for big individuals, using a ball-mill (Retch Mixer Mill MM301). After grinding, all crustaceans were acidified using 37% HCl fumigation protocol for 12 hours, removing the inorganic carbon of the cuticle ($CaCO_3$) prior to any stable isotope (SI) analysis since inorganic carbon ("diet-independent" carbon) has a different $\delta^{13}C$ value than organic carbon ("diet-defendant" carbon) (Nieuwenhuize *et al.*, 1994; Soreide et al. 2006). As our 12 hours 37% HCl fumigation protocol did not seem to show a significant impact on $\delta^{15}N$ measurements (see BOX 1 and also Bosley and Wainright, 1999) on our small invertebrates, samples were all acidified prior to C and N stable isotope analysis. Only two big decapod species with thick cuticles containing more $CaCO_3$ were analyzed one first time for $\delta^{15}N$ analysis on non-acidified material, and a second time for $\delta^{13}C$ analysis on acidified material. After acidification, samples were dried a second time for 24h to remove the remaining moisture and hydrated compounds formed during acidification process and then precisely weighed (Mettler

Toledo AX-105 DeltaRange, 0.01 mg precision) for analysis. The same protocol was applied for all the potential food sources which were: dead *P. oceanica* leaves (DL), living *P. oceanica* leaves (LL), a pool of epiphytes and brown photophilous macroalgae (E), red macroalgae (RMA) and suspended organic matter (SPOM). It must be noted that brow photophilous macroalgae and epiphytes were supposed to be separated food sources. But since they presented very similar isotopic compositions at every season, these two sources were pooled together and considered as one unique food source.

The stable isotope ratios as well as the elemental composition were determined **individually** using isotopic ratio mass spectrometer (Isoprime 100™, Isoprime, UK) interfaced in continuous flow with an elemental analyzer (vario MICRO cube™, Elementar). C and N elemental composition were reported in % of the dry mass of the sample, C:N ratio and isotope ratios were reported conventionally in per mil (‰) using standard delta (δ) notation relative to their respective international standards, Vienna-Pee Dee Belemnite (V-PDB) and atmospheric N2, respectively for C and N:

$$\delta\ {}^{*}X = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \times 10^{3} \qquad (1)$$

where X = ^{13}C or ^{15}N, R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and standard = Vienna-Pee Dee Belemnite (V-PDB) and atmospheric N_2 respectively for carbon and nitrogen. Pure gases of CO_2 and N_2 were used and calibrated against certified reference materials, i.e. sucrose (IAEA-C6; $\delta^{13}C$= −10.8±0.3‰), ammonium sulfate (IAEA-N2; $\delta^{15}N$= 20.3±0.3‰), obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria). The analytical precision was assessed by procedural blanks and internal replicates (i.e. glycine and an in-house crustacean and seagrass standards) and isotopic certified reference standards (i.e. IAEA-C6 and IAEA-N2). Standard deviation on replicated measurements presented hereafter were 0.4% for N elemental composition, 0.7% for C elemental composition, 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. Neither chemical lipid extractions nor a posteriori lipid corrections (see in Chapter 2, §3.4.2) were applied.

2.4. Data analysis

2.4.1. Mixing model and niche estimator

Numerical estimation of the contribution of each potential food source of a given organism is a key data in trophic ecology. To achieve this goal, the SIAR (Stable Isotope Analysis in R) mixing model is used (Parnell *et al*., 2010). This model based on Bayesian inference techniques offers a major advantage: it allows the incorporation of uncertainty into the input parameters, such as the end members (consumers), sources and Trophic Enrichment Factors (TEFs or Δ).

$\delta^{13}C$, $\delta^{15}N$ and C and N relative elemental concentrations of invertebrates and potential food sources were used as input data of the model. Every sample was thus analyzed individually and $\delta^{13}C$, $\delta^{15}N$ as well as elemental data are real individual measurements.

Some sources presented very close isotopic compositions and since they were not really isotopically distinguishable from each other, it was decided to pool them.

TEFs (or Δ) are in a simple way the difference calculated between the isotopic composition of a given consumer and the isotopic composition of its food source. $\Delta^{13}C$ and $\Delta^{15}N$ are thus parameters of major importance that influence drastically mixing models outputs and reliability (Bond and Diamond, 2011; Phillips *et al*., 2014). Since existing TEF data were scarce concerning our invertebrates (see Michel, 2015 and Michel *et al*., 2015), a controlled feeding experiment was conducted on *Gammarus aequicauda*, a very abundant species suspected to show a diet depending a lot on dead leaves organic matter (Lepoint *et al*., 2006; Michel *et al*, 2015). The main result is that TEF is not constant for a given organism for all the food sources. An animal food source induced typical TEFs found for carnivorous species while a detrital food source induced typical TEFs found for detritivorous species (see BOX 3 in this Chapter). It is thus important to use TEFs according to the food source type, more than according to the general suspected physiology of the organism of interest. Used TEFs are detailed in Table 5.1 for each source and organism.

Table 5.1: Summary table of all TEFs used for every potential food source for the mixing model aspect of this study, along with the corresponding acronyms and literature they originate from. Values are means ± standard deviations.

Food Source	Acronym		TEF values $\Delta^{13}C$	TEF values $\Delta^{15}N$	
Dead *P. oceanica* leaves	DL		1.00 ± 0.40	0.90 ± 0.70	Remy *et al*., 2016 (submitted)
Living *P. oceanica* leaves	LL		1.00 ± 0.40	0.90 ± 0.70	Remy *et al*., 2016 (submitted)
Epiphytes/macroalgae	E		0.20 ± 0.60	1.20 ± 0.50	Michel *et al*., 2015
Drift red macroalgae	RMA		0.20 ± 0.60	1.20 ± 0.50	Michel *et al*., 2015
Suspended particulate organic matter	SPOM		0.20 ± 0.60	1.20 ± 0.50	Michel *et al*., 2015
Pool of harpacticoid copepods	C	basal species	0.90 ± 0.70	2.90 ± 0.60	Remy *et al*., 2016 (submitted)
		higher species	0.30 ± 0.14	2.30 ± 0.18	VanderZanden and Rasmussen, 2001
Pool of *Gammarella fucicola* and *Melita hergensis*	GFMH		0.30 ± 0.14	2.30 ± 0.18	VanderZanden and Rasmussen, 2001
Gammarus aequicauda	GA		0.30 ± 0.14	2.30 ± 0.18	VanderZanden and Rasmussen, 2001
Pool of "intermediate" invertebrates	P		0.30 ± 0.14	2.30 ± 0.18	VanderZanden and Rasmussen, 2001
Pool of *Palaemon xiphias* and *Processa edulis*	PX		0.30 ± 0.14	2.30 ± 0.18	VanderZanden and Rasmussen, 2001
Gobius spp.	GSPP		0.30 ± 0.14	2.30 ± 0.18	VanderZanden and Rasmussen, 2001

More recently, Bearhop *et al*. (2004) have proposed that the variability of isotopic composition of a population or a species (*i.e*. its isotopic niche) may be used as a proxy to assess the trophic niche of this population or species and/or the degree of individual specialisation in the population. This isotopic niche concept has been developed considerably through diverse numerical methods (Matthews and Mazumder, 2004; Layman *et al*., 2007; Newsome *et al*., 2007; Jackson *et al*., 2011; Newsome *et al*., 2012). A set of metrics was developed by Layman *et al*. (2007) but this method presented a major problem: it was very sensitive to small sample size and to sample size in general (Jackson *et al*., 2011). Therefore, since our dataset was composed of various species comprising very different and often quite small sample sizes, another type of metrics was used, based on Bayesian inference techniques. The SIBER (Stable Isotope Bayesian Ellipses in R) package was used to investigate the isotopic niche areas and overlap between different niches. SIBER standard ellipse area metric (SEA), containing ~40% of the data, is insensitive to sample

size, but potentially underestimates the ellipse area for very small sample size (< 10 replicates). A corrected standard ellipse area (SEAc) is used in that case to cope with that possible bias. The computation of niche area and overlap among ellipses was derived using Bayesian inference based on 100000 posterior probabilities draws of the SEAc model (Jackson *et al*., 2011).

2.4.2. Non-metric multidimensional scaling (NM-MDS)

Since gut content examination data are compositional and did not allow the use of classical tests, it was decided to use NM-MDS and ANOSIM analysis to distinguish potential temporal patterns. One of the most reliable and used ordination techniques is the non-metric multidimensional scaling (NM-MDS). It is based on an iterative procedure. For a given number of dimensions, many iterations are computer-generated. Each iteration corresponds to a possible ordination. A "stress" value is calculated for each attempt, which is in fact a way to express the error associated to the ordination procedure, *i.e.* the mismatch between theoretical inter-sample similarities and real distances between those samples, measured on the ordination. The iteration that shows the lowest stress value is then considered as being the best way to map the samples. In this study, we performed a 2D NM-MDS using the "MDS" routine of PRIMER v6.1.13 for Windows. We used relative proportion data from gut content examination. The resemblance matrix was built by calculating Bray-Curtis similarity. The number of iterations was set to 99, and the minimum stress level at 0.01.

ANOSIM analysis is widely used in ecology and has some analogies with ANOVA-like analysis; however, it is used to evaluate directly a dissimilarity matrix rather than raw data. Together with the complementary NM-MDS, this analysis is adapted to differentiate 2 or more groups for multivariate non-parametric data. ANOSIM analysis was performed relative proportion data using PRIMER v6.1.13 for Windows.

2.4.3. Other statistical analysis

Classical statistical analyses (factorial ANOVAs) were performed using R and the dedicated "Rcmdrv2.2-3" package. The agglomerative clustering tree presented in the discussion was built using R and "pvclustv2.0-0" package. A significance level of $p < 0.01$ was always used in all tests.

Graphs were built with R, PRIMER 6.1.13 and GraphPad PRISM 6.01 software for Windows (GraphPad Software, San Diego, USA).

3. Results

3.1. Seasonal characterization of the food web

3.1.1. Gut contents examination

Out of the 566 organisms sampled between August 2011 and May 2012, 24.39% had empty guts or presented too few material in it for useful observation (see § 2.2 of this Chapter). Guts from 428 individuals from 14 species were thus examined (Figure 5.1).

Among the 14 species, 12 of them ingested non-negligible amounts of dead *P. oceanica* leaves (between 2.1 ± 2.7% and 73.4± 19.1%).

Amphipods, that dominate the community (Chapter 3) presented variable ingestion patterns. 3 species, *Gammarella fucicola*, *Melita hergensis* and *Nototropis guttatus* ingested mostly algal material (between 56.3 ± 10.2 and 68.9 ± 15.4%), while another species, *Gammarus aequicauda*, mainly ingested dead *P. oceanica* fragments (73.4± 19.1%).

The 2 isopod species encountered presented very different patterns. *Stenosoma lancifer* ingested almost exclusively algal material (84.4 ± 9.8%) while *Idotea balthica* ingested mainly dead *P. oceanica* fragments (58.2 ± 7.8%).

Decapods showed more mixed diets, with 5 species ingesting various amounts of dead *P. oceanica* fragments, algal material and animal material. Another species, *Palaemon xiphias*, presented a carnivorous ingestion pattern, ingesting mostly animal material (65.4 ± 27.6%). The reader's attention is drawn to *Liocarcinus navigator*, the only species sometimes presenting non-

negligible amounts of ingested living leaves of *P. oceanica,* since 3 out of the 19 sampled individuals revealed up to 45% of ingested living leaves.

The only leptostracean species, *Nebalia strausi*, ingested mainly algal material (63.1 ± 8.8%).

The juvenile fishes found in the samples belonged to the *Gobius* genus and, even if specific identification was not possible, they were considered as a single taxon. They presented a strictly carnivorous ingestion pattern, ingesting 91.4 ± 9.0% of animal material.

Almost 30% of the examined organisms contained plastic-like artificial fibers in their gut contents. These small fibers (< 5mm) have been observed in every species, no matter the season or the site. Raman spectroscopy showed that these were indeed composed of viscose, artificial man-made cellulose, and two different types of dyes (Remy, Collard *et al*., 2015). These results are detailed in BOX 2.

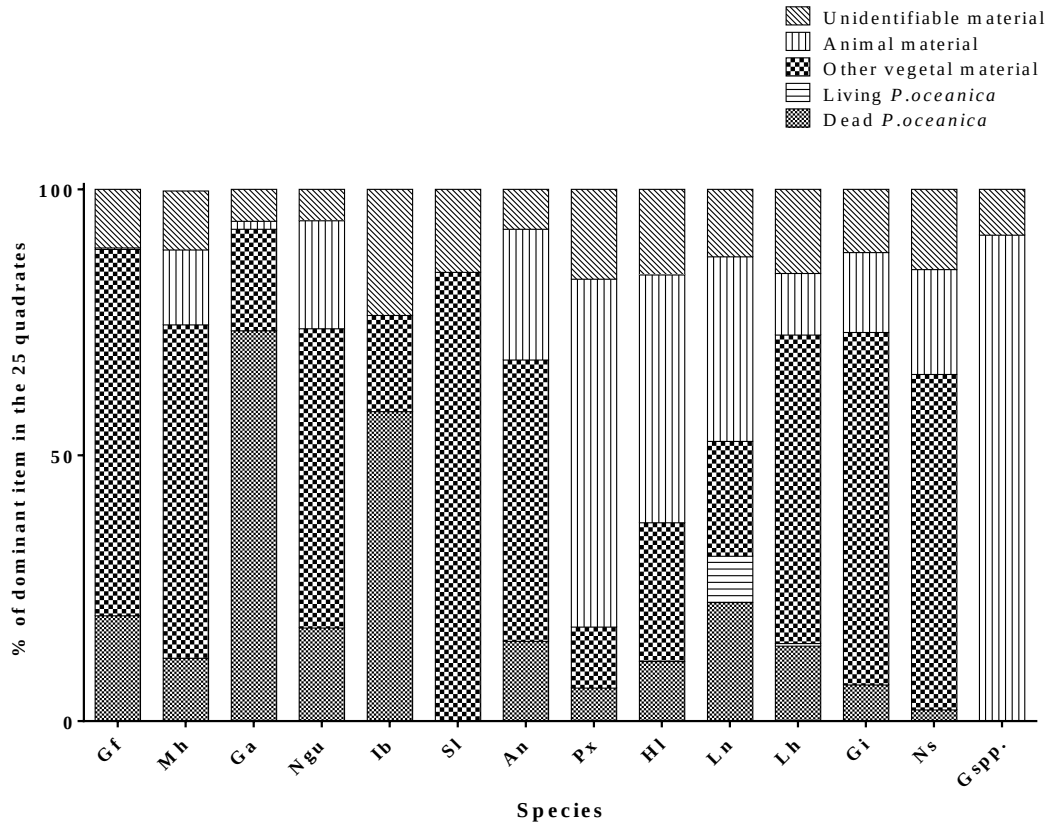
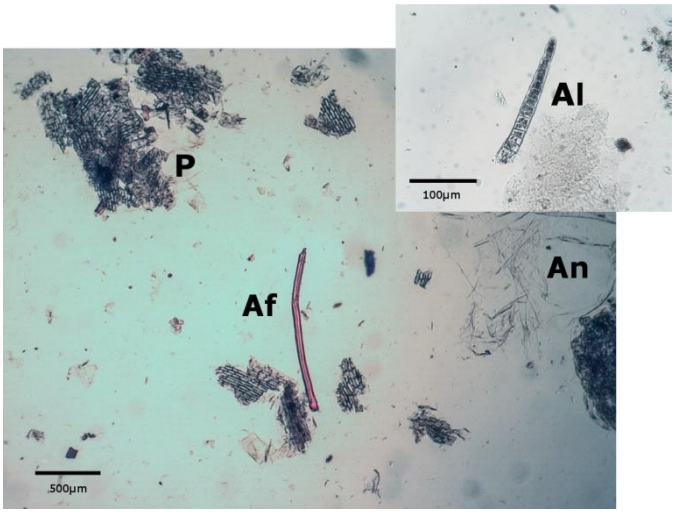


Figure 5.1: Proportion (mean %) of dominant items observed in the 25 random quadrates for the 14 most dominant vagile invertebrate species.

BOX 2

Adapted from Remy F., Collard F., *et al*., 2015 "When Microplastic Is Not Plastic: The Ingestion of Artificial Cellulose Fibers by Macrofauna Living in Seagrass Macrophytodetritus". *Environ. Sci. Technol.*, 2015, 49 (18), pp 11158–11166.



About a third of the examined gut contents contained various "classical" items (*e.g.* dead *P. oceanica* fragments, **P**; algal fragments, **Al**; and animal fragments, **An**) but also undefined colored fibers, **Af**, first suspected to be microplastics. These suspected plastics were present in gut contents of most species and we showed no significant differences between species, between seasons of between sampling sites, indicating a widespread ingestion of these small fibers.

Surprisingly, RAMAN spectroscopy analysis showed that these fibers were not composed of plastic, but composed of artificial cellulose, also called "viscose". These man-made textile fibers constitute about 6% of the total worldwide fiber production (4.5 million tons) and are increasingly used due to the recent interest for "natural" or "wood made" clothes.

Even if cellulose can in itself be considered as harmless for the *P. oceanica* litter macrofauna, the same does not apply for the dyes also found in the fibers. Indeed, fibers were discovered to be constituted of an artificial cellulose matrix, but containing a non-negligible amount of dyes. Two of these were identified: Direct Red 28 and Direct Blue 22. Direct blue 22 is not known to be toxic, but Direct Red 28 is classified as a carcinogenic, mutagenic, or toxic to reproduction coloring agent. Its negative effect on marine invertebrates remains uncertain but is clearly proven in the case of mammals and fishes. Since some invertebrates present in the exported *P. oceanica* litter community are known to be able to digest cellulose, the presence of such potentially toxic dyes associated to the ability to digest cellulose rapidly might constitute a major problem for this community.

One last major result is that from a global point of view, unidentifiable material was not as abundant as we expected, representing only from 5.9% to 23.7% of the ingested material (see Michel *et al*., 2015).

In order to assess the relationship between the 14 species according to their ingested food, bidimensional ordination (NM-MDS) and ANOSIM analyses were performed (Figure 5.2). The stress value of the ordination was very satisfying (0.05). The NM-MDS coupled to the result of the ANOSIM showed that 5 distinct groups were distinguishable. One group was composed of primary consumer species *Gammarus aequicauda* and *Idotea balthica*, mainly ingesting dead *P. oceanica* fragments. Another was composed of *Gammarella fucicola*, *Stenosoma lancifer* and *Licocarcinus holsatus*, mostly ingesting algal material. A third group was composed of *Anthanas nitescens*, *Nototropis guttatus*, *Galathea intermedia* and *Nebalia strausi*, ingesting important amounts of algal material but also non-negligible amounts of animal material. The fourth group was composed of *Liocarcinus navigator* and *Hippolyte leptocerus*, ingesting mainly animal material but also non-negligible amounts of algal or seagrass material. The last group was composed of *Palaemon xiphias* and *Gobius spp*. ingesting mostly animal material.

The case of *Melita hergensis* is less clear as this amphipod presented a wider range of ingestion preferences and was part of two different groups.

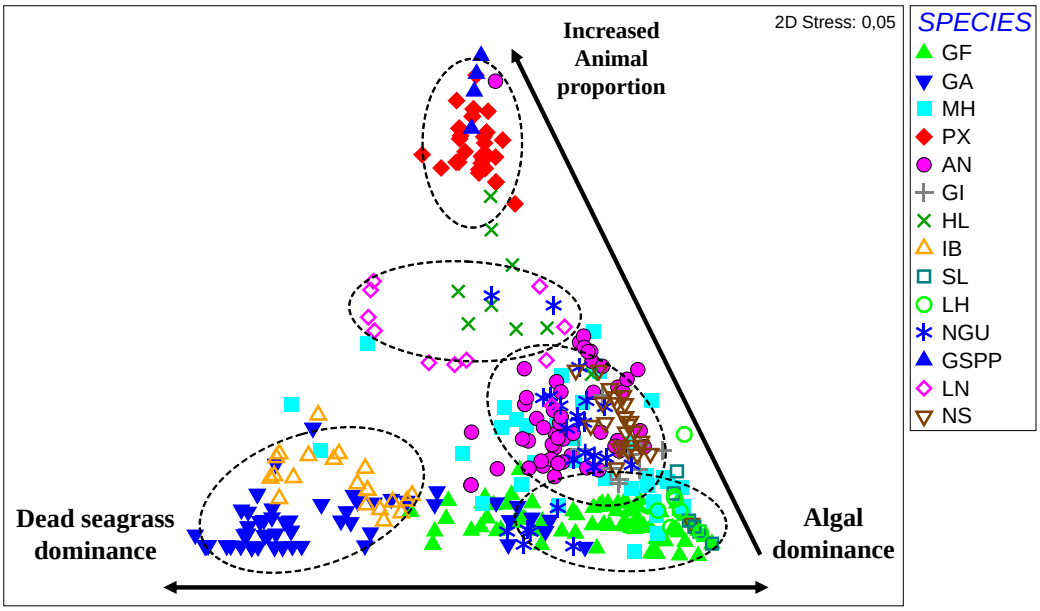


Figure 5.2: 2D-ordination and superposed ANOSIM groups of ingestion pattern of the 14 sampled species in 2011-2012. Black dotted ellipses represent the 5 groups formed by species not significantly different ($p > 0.05$) according to the ANOSIM analysis. Species code: GF= Gammarella fucicola; GA= Gammarus aequicauda; MH= Melita hergensis; NGU= Nototropis guttatus; PX= Palaemon xiphias; AN= Athanas nitescens; HL= Hippolyte leptocerus; LN= Liocarcinus navigator; LH= Liocarcinus holsatus; GI= Galathea intermedia; IB= Idotea balthica; SL= Stenosoma lancifer; NS= Nebalia strausi; GSPP= Gobius spp. Arrows indicate the dominance of animal material, algal material or dead P. oceanica material in the gut contents.

Among the 14 species, 5 (*Gammarella fucicola, Gammarus aequicauda, Melita hergensis, Athanas nitescens* and *Palaemon xiphias*) were present at every season. In order to assess the potential seasonal and spatial ordination of these 5 species according to their ingested food, bidimensional ordination (NM-MDS) and 2-way crossed ANOSIM analyses were performed for each of the 5 species (Figure 5.3). The stress values of all five ordinations were between 0.02 and 0.11 which can be considered satisfying.

For *Gammarella fucicola*, 2-way ANOSIM test showed that ingestion patterns from autumn 2011 and spring 2012 were significantly different ($p = 0.001$) from each other and from the other seasons. This difference corresponds to an increased ingestion of dead *P. oceanica* fragments in autumn 2011. Summer 2011 and winter 2012 formed a single group.

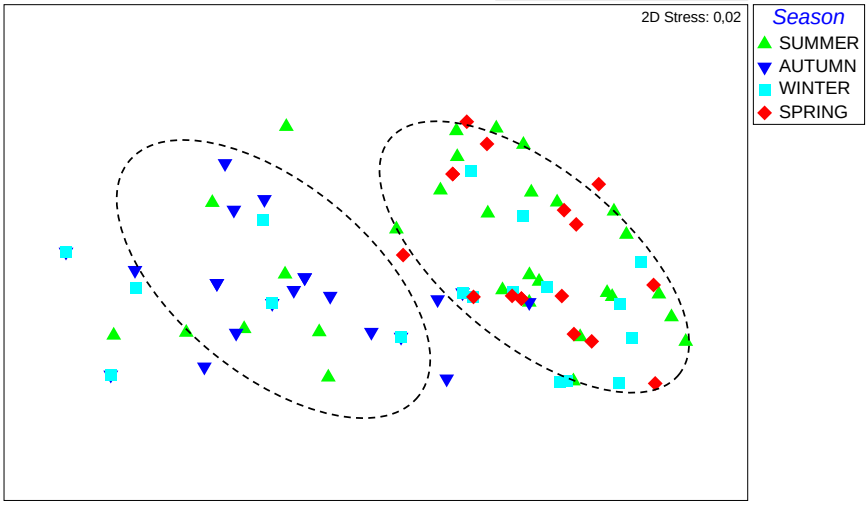
For *Gammarus aequicauda*, ingestion patterns from summer 2011 and winter 2012 were significantly different ($p < 0.01$) from each other and from the other seasons. This difference corresponds to an increased ingestion of dead *P. oceanica* fragments in winter 2012. Autumn 2011 and spring 2012 formed a single group.

For *Melita hergensis*, ingestion patterns from autumn 2011 and spring 2012 were significantly different ($p = 0.001$) from each other and from the other seasons. This difference corresponds to an increased ingestion of algal fragments in autumn 2012. Summer 2011 and winter 2012 formed a single group.

For *Athanas nitescens*, 2-way ANOSIM showed that ingestion patterns from summer 2011 and autumn 2011 were significantly different ($p < 0.01$) from each other and from the other seasons. This difference corresponds to an increased ingestion of dead *P. oceanica* fragments and a decrease of ingestion of animal material in autumn 2011. Winter 2012 and spring 2012 formed a single group.

2-way ANOSIM test on *Palaemon xiphias* did not show any significant spatiotemporal pattern.

Gammarella fucicola



Gammarus aequicauda

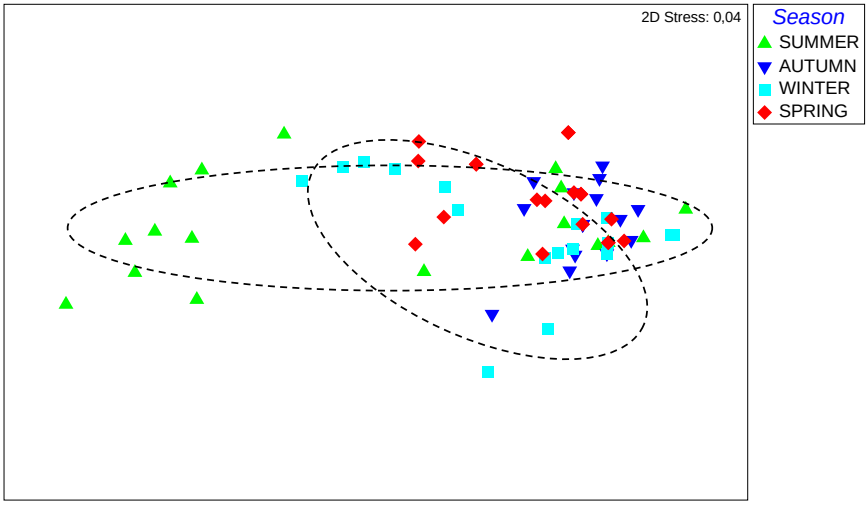
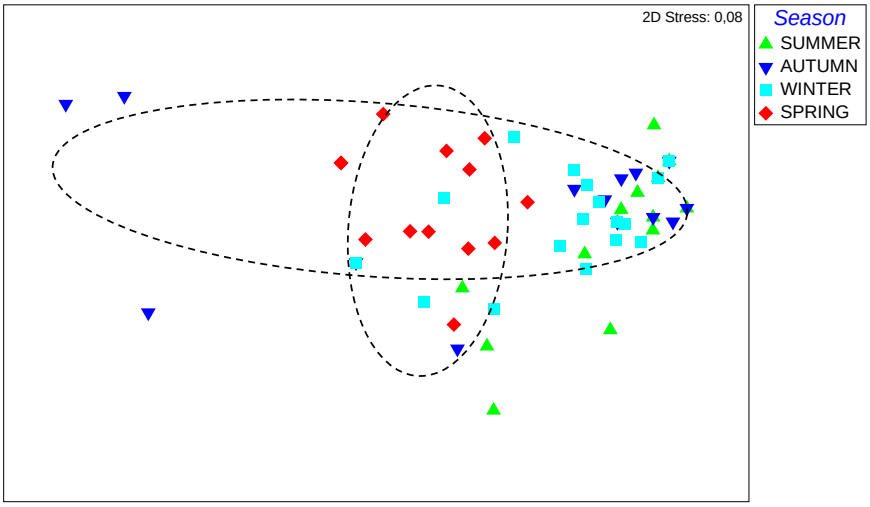


Figure 5.3A: 2D-ordination and superposed ANOSIM groups of ingestion pattern of Gammarella fucicola and Gammarus aequicauda sampled in 2011-2012. Black dotted ellipses represent the seasons significantly ($p \leq 0.01$) distinguishable from the other season according to the 2-way ANOSIM analysis.

Melita hergensis



Athanas nitescens

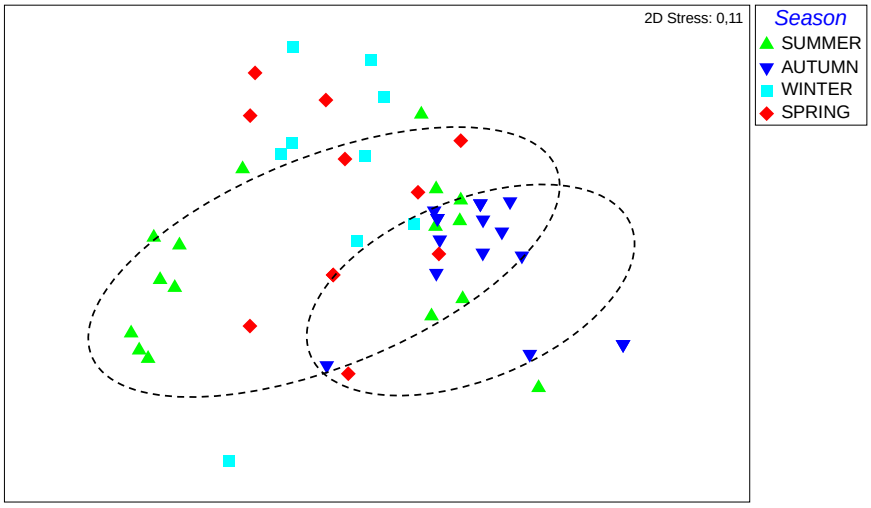


Figure 5.3B: 2D-ordination and superposed ANOSIM groups of ingestion pattern of Melita hergensis and Athanas nitescens sampled in 2011-2012. Black dotted ellipses represent the seasons significantly ($p \leq 0.01$) distinguishable from the other season according to the 2-way ANOSIM analysis.

Gammarella fucicola and *Gammarus aequicauda* presented differences (2-way ANOSIM, p= 0.001) of its ingesting patterns between HARBOR-site and OSCE-site. These spatial variations were observed in summer 2011, winter 2012 and spring 2012, but not in autumn 2011.

In turn, *Melita hergensis* showed spatial differences (2-way ANOSIM, p= 0.001) in summer 2011, autumn 2011 and winter 2012, but not in spring 2012.

Athanas nitescens presented spatial differences (2-way ANOSIM, p= 0.01) only in summer 2011 and spring 2012, but not in autumn 2011 and winter 2012.

Once again, 2-way ANOSIM test on *Palaemon xiphias* revealed no differences between the two sampling sites.

3.1.2. C and N stable isotopes

3.1.2.1. Global community considerations

As mentioned in the previous paragraph, 566 individuals were sampled for the first axis of the trophic ecology part of this PhD. These 566 individuals were composed of only 19 different species. Gut contents examination (when possible) and stable isotope analyses were performed individually on the same individual. The global isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of this whole community along with the potential basal food sources are given by Figure 5.4 (mean ± SD).

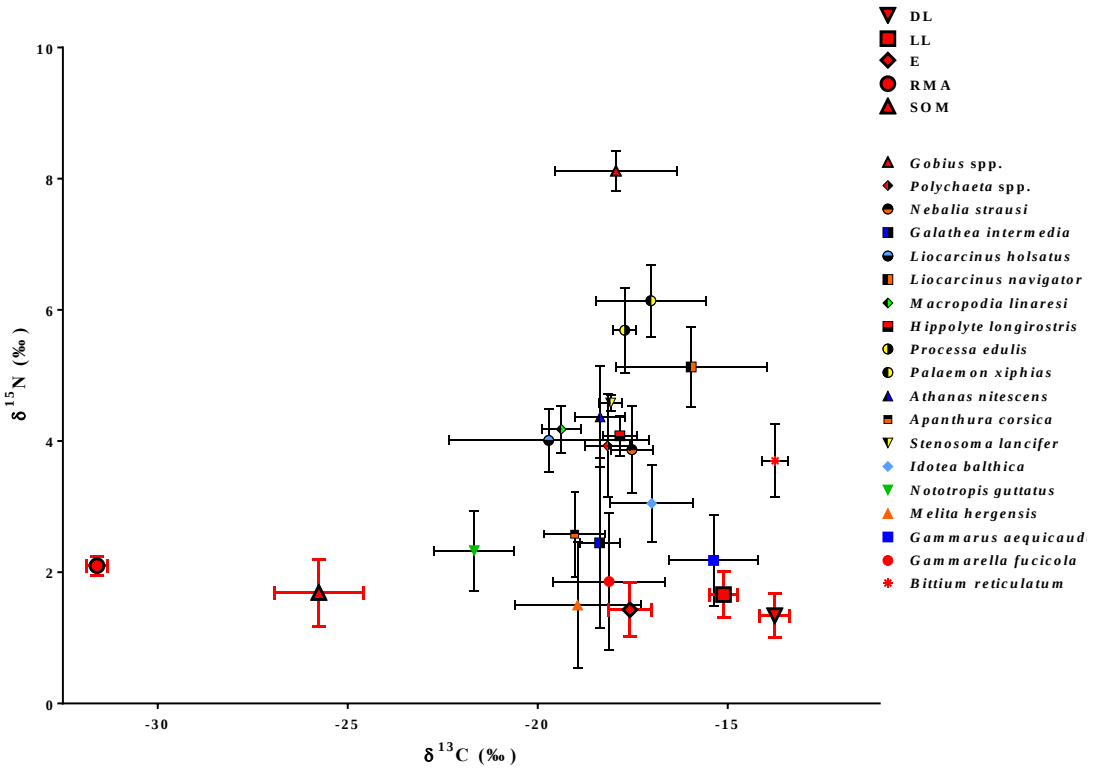


Figure 5.4: Global $\delta^{13}C$ vs. $\delta^{15}N$ biplot (‰) of macrofauna and potential basal food sources for the whole 2011-2012 period. All macrofauna data are individual measurements and basal food sources values are pooled measurements. Large red symbols with red error bars are the basal food sources. Food sources codes: E= epiphytes+various brown drift macroalgae; LL= Living P. oceanica leaves; DL= dead P. oceanica leaves; RMA= various red drift macroalgae; SPOM= suspended organic matter.

Five potential basal food sources were identified prior to the analyses: dead *P. oceanica* leaves (DL), living *P. oceanica* leaves (LL), epiphytes + brown photophilous macroalgae (E) (see § 2.3 of this chapter), red sciaphilous macroalgae (RMA) and suspended organic matter (SPOM). For $\delta^{13}C$, basal food sources were all negative and their isotopic compositions were very easily distinguishable from each other (no significant overlap between them). Dead leaves (DL) were the less negative food source, showing an average $\delta^{13}C$ value of -13.76 ± 0.40‰. Living leaves (LL) $\delta^{13}C$ showed an average value of -15.11 ± 0.17‰. Epiphytes (E) $\delta^{13}C$ showed an average value of -17.57 ± 0.56‰. The two most ^{13}C negative sources were SPOM and red macroalgae (RMA),

showing average values of -25.76 ± 1.18‰ and -31.60 ± 0.76‰ respectively (Figure 5.4).

For $\delta^{15}N$, the situation was different, showing positive values, less variability and a much more important overlap, with all the 5 sources ranging from 0.67 to 2.79‰.

The whole community presented most of the organisms with $\delta^{13}C$ values between -21.67 and -13.75‰ and with $\delta^{15}N$ values ranging from 1.50 to 7.94‰. The community presented much higher $\delta^{13}C$ variability at its base than its top. At the base of the community ($\delta^{15}N\approx$ 2.00‰), within amphipods, *Gammarus aequicauda* and *Nototropis guttatus* were the two extremes, well separated for $\delta^{13}C$ values, one on the less negative side of the isotopic space (-15.37 ± 1.17‰), the other on the more negative side (-21.67 ± 1.05‰), respectively. On the other hand, *Gammarella fucicola* and *Melita hergensis* presented an important overlap of their isotopic compositions. Above them ($\delta^{15}N\approx$ 4.00‰), most of the isopods, decapods and leptostraceans were found and all presented an important overlap of their $\delta^{13}C$ values. *Bittium reticulatum*, the only mollusk sampled for the trophic study, showed much less negative $\delta^{13}C$ values (-13.75 ± 0.34‰). Above ($\delta^{15}N\approx$ 6.00‰), there lay 3 species of decapods, *Palaemon xiphias* (-17.02 ± 1.44‰), *Processa edulis* (-17.71 ± 0.30‰) and *Liocarcinus navigator* (-15.96 ± 1.99‰). At the top, ($\delta^{15}N\approx$ 8.00‰), juvenile fishes, *Gobius spp*. were found (Figure 5.4). This trophic web potentially encompassed 3 to 4 trophic levels, from the primary consumers, to predator species of second order (all values detailed in Table 5.2).

Table 5.2: Summary table of global stable isotope ($\delta^{13}C$ and $\delta^{15}N$) compositions and C:N ratios of the 19 sampled consumer species and the 5 basal food sources. All values are expressed in mean ± standard deviation.

Consumers	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio	N
Gammarella fucicola (Gf)	-18.1 ± 1.5	1.9 ± 1.1	4.3 ± 0.5	82
Gammarus aequicauda (Ga)	-15.4 ± 1.2	2.2 ± 0.7	4.4 ± 0.5	81
Melita hergensis (Mh)	-19.0 ± 1.7	1.5 ± 1,0	4.2 ± 0.8	55
Nototropis guttatus (Ngu)	-21.7 ± 1,0	2.3 ± 0.6	4.7 ± 1.3	30
Idotea balthica (Ib)	-17.0 ± 1.1	3.1 ± 0.6	4.5 ± 1.3	27
Stenosoma lancifer (Sl)	-18.1 ± 0.3	4.6 ± 0.1	4.4 ± 1.8	7
Apanthura corsica (Ac)	-19.0 ± 0.8	2.6 ± 0.7	3.8 ± 0.4	5
Athanas nitescens (An)	-18.4 ± 0.7	4.4 ± 0.8	4.4 ± 1.2	61
Palaemon xiphias (Px)	-17.0 ± 1.4	6.1 ± 0.6	5.2 ± 0.7	52
Processa edulis (Pe)	-17.7 ± 0.3	5.7 ± 0.7	4.5 ± 0.1	5
Hypolite leptocerus (Hl)	-17.8 ± 0.4	4.1 ± 0.3	4.6 ± 0.3	9
Macropodia linaresi (Ml)	-19.4 ± 0.5	4.2 ± 0.4	5.3 ± 0.5	5
Liocarcinus navigator (Ln)	-16.0 ± 2.0	5.1 ± 0.6	4.7 ± 2.1	19
Liocarcinus holsatus (Lh)	-19.7 ± 2.6	4.0 ± 0.5	5.1 ± 0.3	22
Galathea intermedia (Gi)	-18.4 ± 0.5	2.5 ± 1.3	4.6 ± 0.6	13
Nebalia strausi (Ns)	-17.5 ± 0.6	3.9 ± 0.7	4.4 ± 1.7	31
Polychaeta spp. (Pspp)	-18.2 ± 0.6	3.9 ± 0.8	4.8 ± 0.5	38
Bittium reticulatum (Br)	-13.8 ± 0.3	3.7 ± 0.6	4.5 ± 0.5	9
Gobius spp. (Gspp)	-17.9 ± 1.6	8.1 ± 0.3	6.1 ± 0.5	9
Sources				
Dead *P.oceanica*	-13.8 ± 0.4	1.3 ± 0.3	51.3 ± 5.7	24
Living *P.oceanica*	-15.1 ± 0.2	1.7 ± 0.2	20.4 ± 3.6	24
Epiphytes + Brown algae	-17.6 ± 0.6	1.4 ± 0.4	11.3 ± 4.2	23
Red algae	-31.6 ± 0.3	2.1 ± 0.1	15.2 ± 2.1	24
SPOM	-25.8 ± 1.2	1.7 ± 0.5	5.2 ± 1.6	20

3.1.2.2. Spatio-temporal trend

Basal food sources showed important temporal variability $\delta^{13}C$, $\delta^{15}N$ and C:N ratio during the sampling period (presented in Table 5.3)

Dead *P. oceanica* leaves (DL) globally showed the highest C:N ratio and the highest $\delta^{13}C$ values during the whole sampling period. C:N ratio ranged from 34.25 ± 6.18 to 61.63 ± 1.24, $\delta^{13}C$ ranged from -12.65 ± 0.81‰ to -14.23 ± 0.3‰ and $\delta^{15}N$ ranged from 0.89 ± 0.47‰ to 1.96 ± 0.30 ‰.

Living *P. oceanica* leaves (LL) globally showed intermediate $\delta^{13}C$ values, between DL and E. C:N ratio ranged from 17.35 ± 2.42 to 22.37 ± 2.68, $\delta^{13}C$ ranged from -14.35 ± 0.47‰ to -16.04 ± 0.12‰ and $\delta^{15}N$ ranged from 1.07 ± 0.06‰ to 2.45 ± 0.23‰.

The pool of epiphytes and brown macroalgae (E) globally presented variable C:N ratios and quite constant isotopic compositions. C:N ratio ranged from 7.34 ± 2.31 to 14.84 ± 3.25, $\delta^{13}C$ ranged from -16.57 ± 0.34‰ to -18.85 ± 0.83‰ and $\delta^{15}N$ ranged from 0.81 ± 0.45‰ to 1.94 ± 0.91‰. It's important to note that epiphytes alone were much more variable in terms of $\delta^{15}N$, with values up to 3.00 ± 0.88‰.

Drift red macroalgae (RMA) globally showed the lowest $\delta^{13}C$ values during the whole sampling period. C:N ratio ranged from 13.32 ± 1.69 to 17.23 ± 3.42, $\delta^{13}C$ ranged from -29.38 ± 0.39‰ to -33.79 ± 0.51‰ and $\delta^{15}N$ ranged from 1.07 ± 0.09‰ to 2.79 ± 0.16‰.

Suspended organic matter (SOM) globally showed intermediate $\delta^{13}C$ values between E and RMA, and the lowest C:N ratio values during the whole sampling period. C:N ratio ranged from 3.48 ± 0.84 to 7.62 ± 3.02, $\delta^{13}C$ ranged from -24.76 ± 0.73‰ to -26.61 ± 1.31‰ and $\delta^{15}N$ ranged from 1.45 ± 0.58‰ to 2.14 ± 0.39‰.

A multivariate analysis (2-way MANOVA) on $\delta^{13}C$, $\delta^{15}N$ and C:N ratios showed a very significant ($p < 0.001$) influence of the seasonal factor for the 5 basal food sources for C:N ratios and both $\delta^{13}C$ and $\delta^{15}N$ values (Figure 5.5). The spatial factor showed much less influence, with a significant impact only for $\delta^{15}N$ of RMA and SPOM in spring 2012. This seasonality must be accounted in SIAR modelling as it constitutes an isotopic baseline shift, and therefore, each seasonal model was run with seasonal source data.

Table 5.3: Summary table of stable isotope ($\delta^{13}C$ and $\delta^{15}N$) compositions and C:N ratios of the 5 basal food sources (N=6) per season and site. All values are expressed in mean ± standard deviation. Food sources codes: E= epiphytes+various brown drift macroalgae; LL= Living P. oceanica leaves; DL= dead P. oceanica leaves; RMA= various red drift macroalgae; SPOM= suspended organic matter.

HARBOR-site

	SUMMER 2011			AUTUMN 2011		
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio
DL	-12.65 ± 0.81	1.33 ± 0.39	54.11 ± 2.33	-12.67 ± 0.37	1.77 ± 0.15	59.12 ± 2.53
LL	-15.35 ± 0.13	1.79 ± 0.32	17.35 ± 2.42	-15.46 ± 0.34	1.07 ± 0.06	18.31 ± 3.11
E	-18.85 ± 0.83	0.81 ± 0.45	12.34 ± 2.18	-17.64 ± 0.28	1.72 ± 0.59	14.12 ± 1.54
RMA	-31.37 ± 0.13	1.78 ± 0.09	15.38 ± 1.58	-29.38 ± 0.39	1.84 ± 0.20	17.23 ± 3.42
SPOM	-24.76 ± 0.73	1.75 ± 0.46	7.62 ± 3.02	-25.12 ± 1.32	1.74 ± 0.58	4.42 ± 1.13

	WINTER 2012			SPRING 2012		
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio
DL	-13.93 ± 0,40	1.40 ± 0.74	34.25 ± 6.18	-13.10 ± 0.26	1.07 ± 0.12	45.21 ± 3.47
LL	-16.04 ± 0.12	1.63 ± 0.08	19.84 ± 4.26	-14.63 ± 0.33	1.79 ± 0.03	22.37 ± 2.68
E	-16.91 ± 0.75	1.26 ± 0.31	8.31 ± 0.47	-17.61 ± 0.37	1.94 ± 0.91	7.87 ± 2.56
RMA	-33.79 ± 0.51	2.46 ± 0.24	13.32 ± 1.69	-30.10 ± 0.05	1.07 ± 0.09	14.58 ± 2.26
SPOM	-26.61 ± 1.31	1.87 ± 0.41	6.51 ± 2.91	-24.78 ± 0.74	2.14 ± 0.39	4.12 ± 0.57

OSCE-site

	SUMMER 2011			AUTUMN 2011		
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio
DL	-13.17 ± 0.60	1.96 ± 0.30	49.62 ± 6.13	-12.84 ± 0.13	1.20 ± 0.29	61.63 ± 1.24
LL	-15.50 ± 0.27	1.94 ± 0.23	18.03 ± 2.64	-15.22 ± 0.39	1.21 ± 0.22	18.79 ± 2.47
E	-17.51 ± 0.70	1.10 ± 0.44	13.03 ± 2.85	-17.38 ± 0.31	1.93 ± 0.60	14.84 ± 3.25
RMA	-32.36 ± 0.20	2.32 ± 0.84	16.31 ± 1.84	-30.17 ± 0.28	1.91 ± 0.19	16.84 ± 2.67
SPOM	-26.23 ± 1.02	1.47 ± 0.74	7.12 ± 2.21	-25.43 ± 1.18	1.71 ± 0.67	4.92 ± 0.63

	WINTER 2012			SPRING 2012		
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio
DL	-13.65 ± 0.42	1.32 ± 0.59	42.36 ± 4.3	-14.23 ± 0.30	0.89 ± 0.47	43.75 ± 3.62
LL	-15.71 ± 0.47	2.45 ± 0.23	18.57 ± 4.21	-14.35 ± 0.47	1.74 ± 0.12	21.98 ± 2.36
E	-18.26 ± 0.45	1.42 ± 0.16	8.94 ± 0.94	-16.57 ± 0.34	1.59 ± 0.16	7.34 ± 2.31
RMA	-33.43 ± 0.10	2.59 ± 0.11	14.02 ± 2.15	-31.17 ± 0.55	2.79 ± 0.16	15.21 ± 1.89
SPOM	-26.12 ± 0.88	1.68 ± 0.54	5.78 ± 1.65	-25.49 ± 1.28	1.45 ± 0.58	3.48 ± 0.84

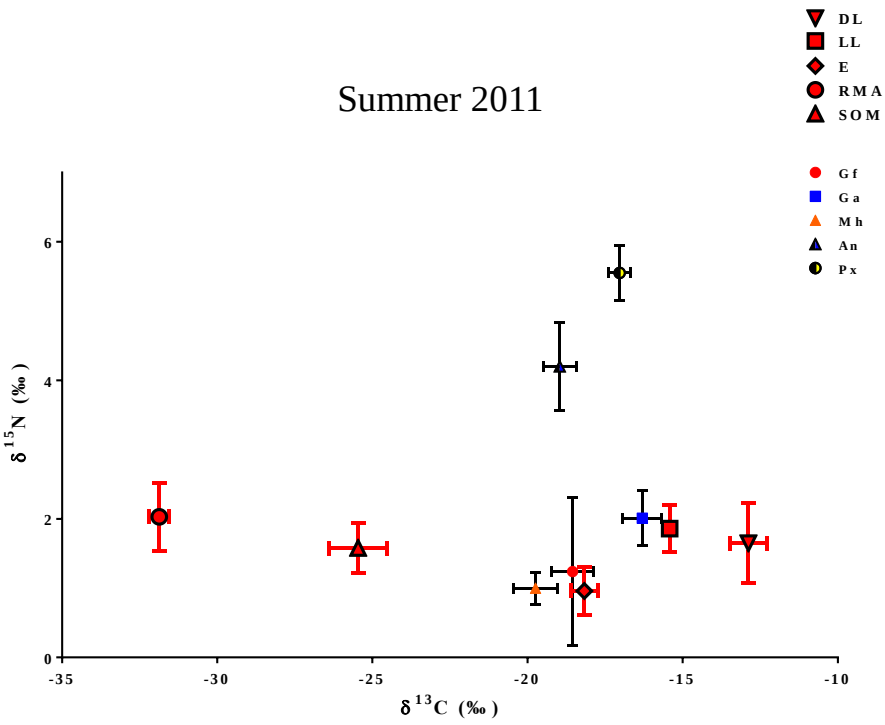
As for gut contents examination (see in this Chapter, §3.1.1), only 5 species out of the 19 were sampled all around the year at both sites and at every season: *Gammarella fucicola, Gammarus aequicauda, Melita hergensis, Athanas nitescens* and *Palaemon xiphias*.

Gammarella fucicola $\delta^{13}C$ values ranged from -16.18 ± 0.84‰ in autumn 2011 to -19.07 ± 0.64‰ in spring 2012, while $\delta^{15}N$ values ranged from 1.24 ± 1.07 ‰ in summer 2011 to 2.32 ± 0.88‰ in autumn 2011. *Gammarus aequicauda,* $\delta^{13}C$ values ranged from -13.90 ± 0.45‰ in autumn 2011 to -16.30 ± 0.63‰ in summer 2011, making it the least ^{13}C depleted amphipod of the community, while $\delta^{15}N$ values ranged from 1.76 ± 0.64 ‰ in autumn 2011 to 2.95 ± 0.45‰ in spring 2012 (Figure 5.5). *Melita hergensis* $\delta^{13}C$ values ranged from -16.23 ± 0.61‰ in autumn 2011 to -20.19 ± 0.77‰ in winter 2012, while $\delta^{15}N$ values ranged from 1.00 ± 0.23 ‰ in summer 2011 to 1.78 ± 1.35‰ in winter 2012 (Figure 5.5). *Athanas nitescens* $\delta^{13}C$ values ranged from -17.92 ± 0.40‰ in autumn 2011 to -18.96 ± 0.53‰ in summer 2011, while $\delta^{15}N$ values ranged from 3.72 ± 0.44 ‰ in summer 2011 to 5.37 ± 0.52‰ in winter 2012 (Figure 5.5). *Palaemon xiphias* $\delta^{13}C$ values ranged from -15.43 ± 0.93‰ in autumn 2011 to -19.18 ± 0.38‰ in spring 2012, while $\delta^{15}N$ values ranged from 5.55 ± 0.40 ‰ in summer 2011 to 6.44 ± 0.28‰ in spring 2012, making it the most ^{15}N enriched Arthropod of this community (Figure 5.5).

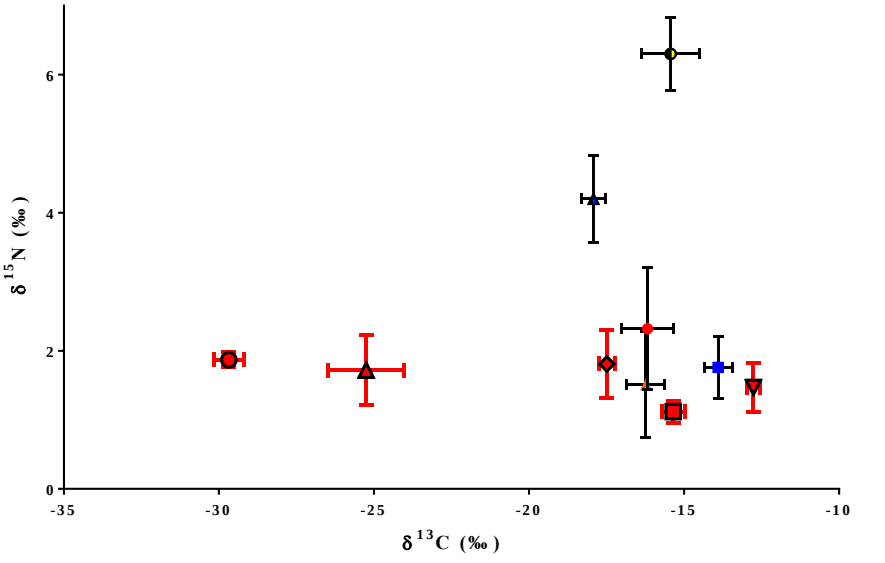
A multivariate analysis (2-way MANOVA) on $\delta^{13}C$, $\delta^{15}N$ and C:N ratios showed a very significant influence of the seasonal factor for the 5 species for both $\delta^{13}C$ and $\delta^{15}N$ values (Figure 5.5) but not for C:N ratios. The spatial factor showed much less influence, showing a significant influence only for *Melita hergensis* $\delta^{15}N$ values.

As the spatial factor only showed a limited impact only for both sources and species, only for $\delta^{15}N$, next analyses and graphical representations were performed only from a seasonal point of view.

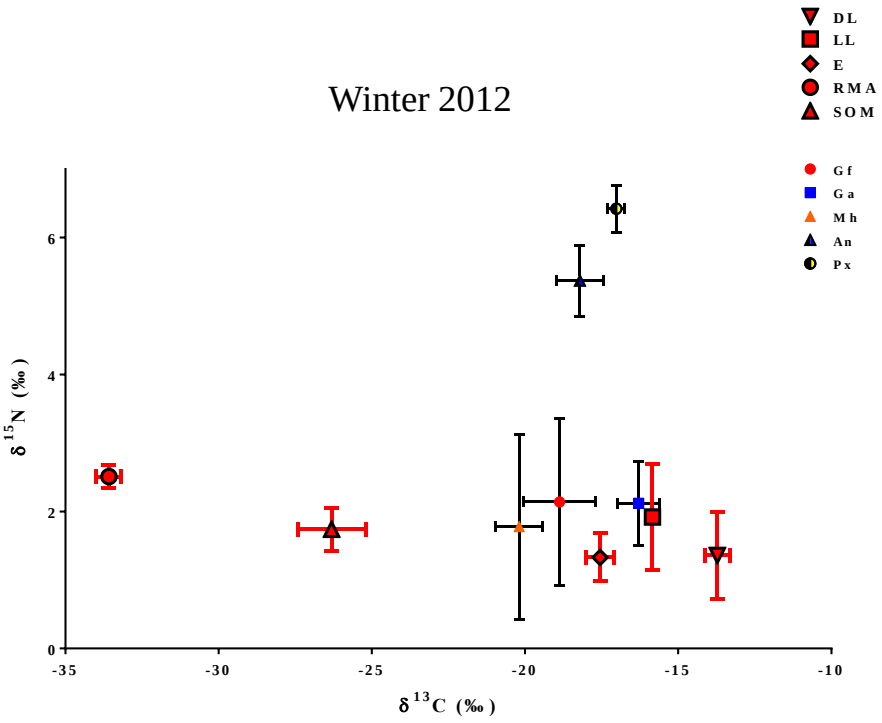
Summer 2011



Autumn 2011



Winter 2012



Spring 2012

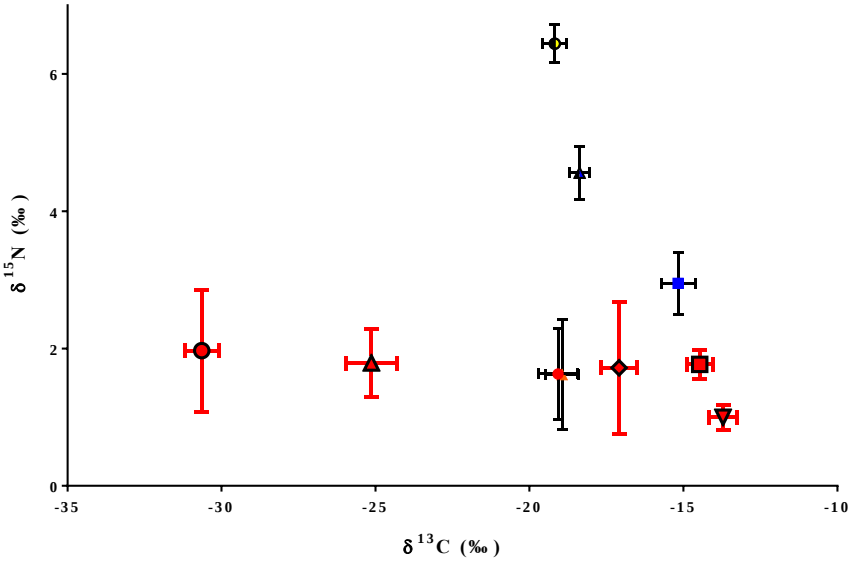


Figure 5.5: Seasonal $\delta^{13}C$ vs. $\delta^{15}N$ biplot (‰) of macrofauna and potential basal food sources for the whole 2011-2012 period. All macrofauna data are individual measurements and basal food sources values are pooled measurements. Large red symbols with red error bars are the basal food sources. Food sources codes: E= epiphytes+various brown drift macroalgae; LL= Living P. oceanica leaves; DL= dead P. oceanica leaves; RMA= various red drift macroalgae; SPOM= suspended organic matter. Species code: Gf= Gammarella fucicola; Ga= Gammarus aequicauda; Mh= Melita hergensis; Px= Palaemon xiphias; An= Athanas nitescens.

Figure 5.5 showed that food sources and the 5 species experienced non-negligible changes during the 2011-2012 sampling period. Between summer 2011 and autumn 2011, the reader's attention is drawn to the fact that all the food sources except DL shifted horizontally ($\delta^{13}C$) of about +2‰ to the less negative side of the isotopic space, and the 5 species also shifted horizontally of about +3‰. The sources, as well as the community, shifted back to their original position in the isotopic space between autumn 2011 and winter 2012. Between winter 2012 and spring 2012, RMA, SPOM, and LL shifted horizontally again of about +2 ‰, *Palaemon xiphias* shifted horizontally of about -2‰ to the more negative side of the isotopic space and *Gammarus aequicauda* shifted vertically ($\delta^{15}N$) of about +1‰ (Figure 5.5).

3.1.3. Mixing model and isotopic niche metrics

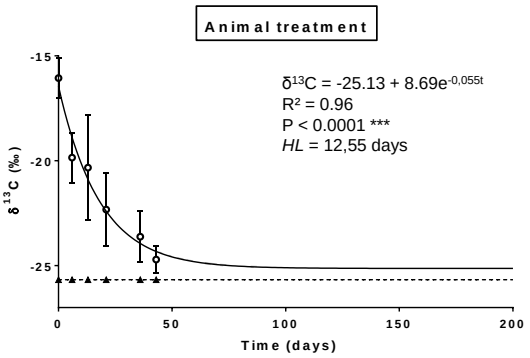
3.1.3.1. SIAR model: global community

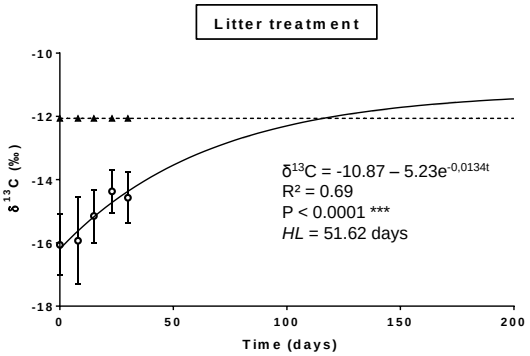
SIAR mixing model was used to determine the relative contributions of food sources to the average diet of the 19 species for the 2011-2012 period. We ran the model with individual $\delta^{13}C$, $\delta^{15}N$ and the TEFs presented in table 2.1 (see BOX 3).

BOX 3

Adapted from Remy F. *et al*., 2016 "Impact of food type on respiration, fractionation and turnover of carbon and nitrogen stable isotopes in the marine amphipod *Gammarus aequicauda* Martynov, 1931". Submitted to *J. Exp. Mar. Biol. Ecol*.

Trophic Enrichment Factors (TEFs) are parameters of major influence in trophic ecology. In addition to giving information about trophic levels of the different components of a given food web, this parameter is highly influencing the outputs of mixing models such as SIAR (Stable Isotope Analysis in R).



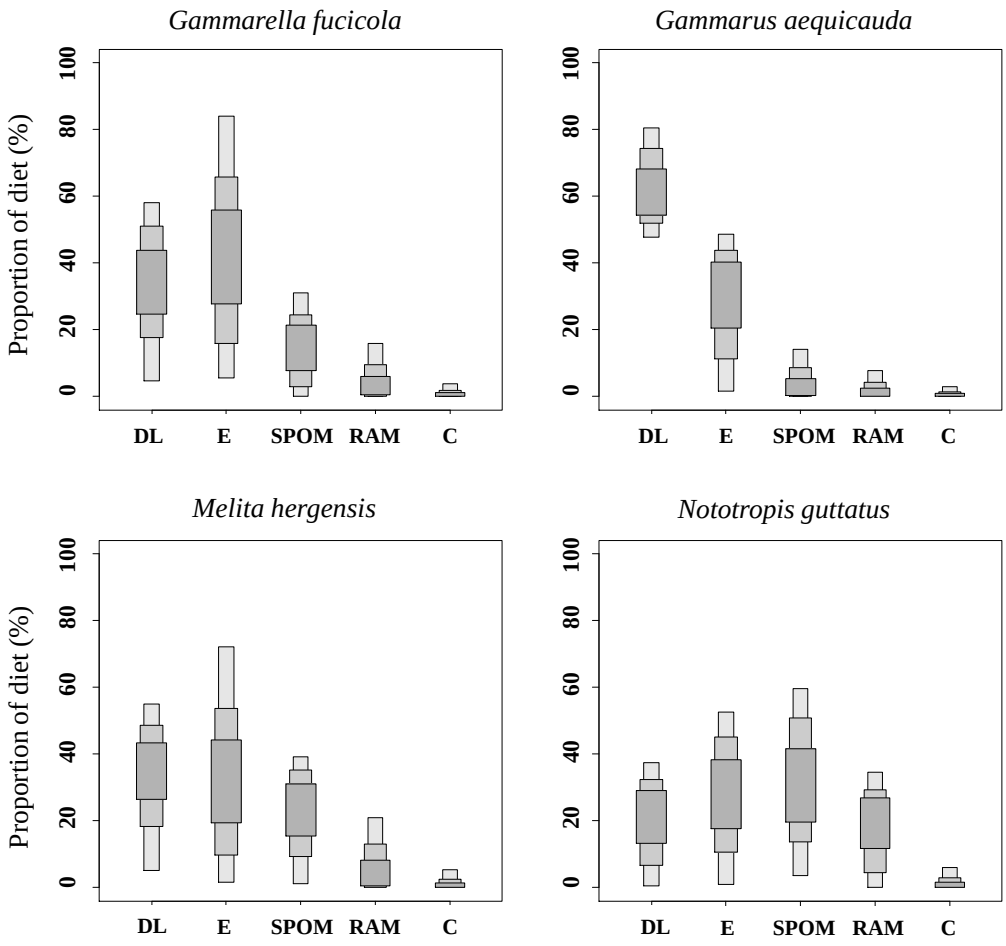


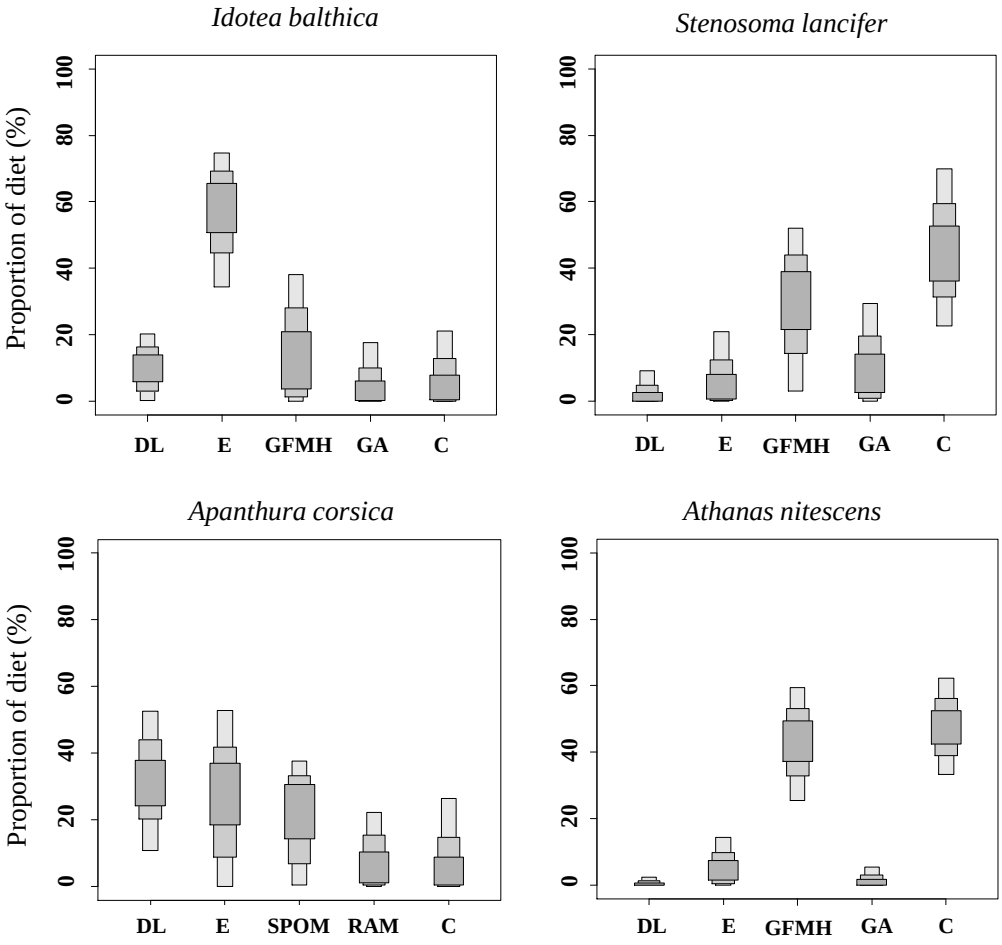
Gammarus aequicauda is the second most abundant amphipod of the exported *P. oceanica* detritus macrofauna community and is known to be a true detritivore, mainly ingesting and assimilating dead *P. oceanica* fragments. Knowing more about its TEFs and turnover speed for different potential food sources is thus of major importance for more robust interpretations of the *P. oceanica* detrital food web in its globality.

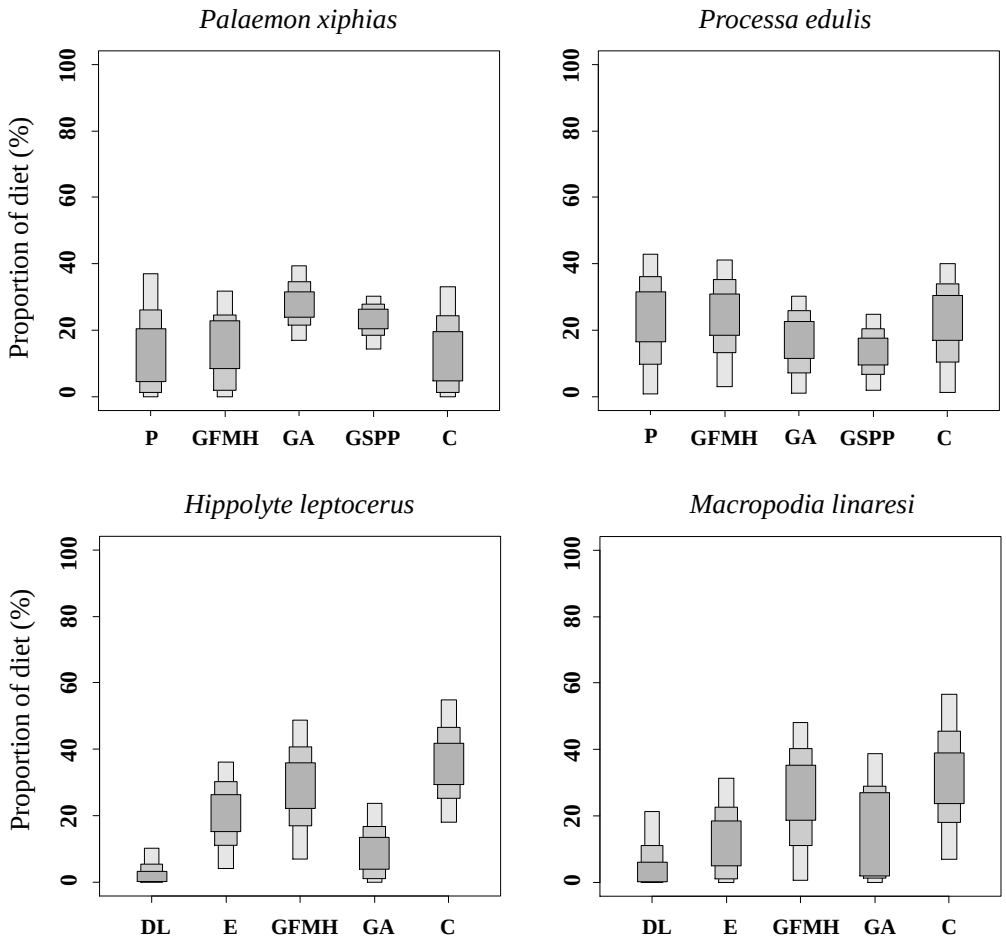
Three different food sources were tested during a controlled feeding experiment and turnover speed was found to be much faster for the food source of highest nutritional quality (animal treatment). A very interesting result was that Gammaru aequicauda displayed typical detritivore TEFs for the detrital food source (Litter treatment), but typical carnivore TEFs for the animal food source. Computing different TEF parameters for different food source types thus seemed compulsory for robust and reliable interpretations. Different mixing model runs performed with literature TEF values and our newly calculated TEFs demonstrated much logical outputs from the model and much more constrained results, indicating the importance of our results.

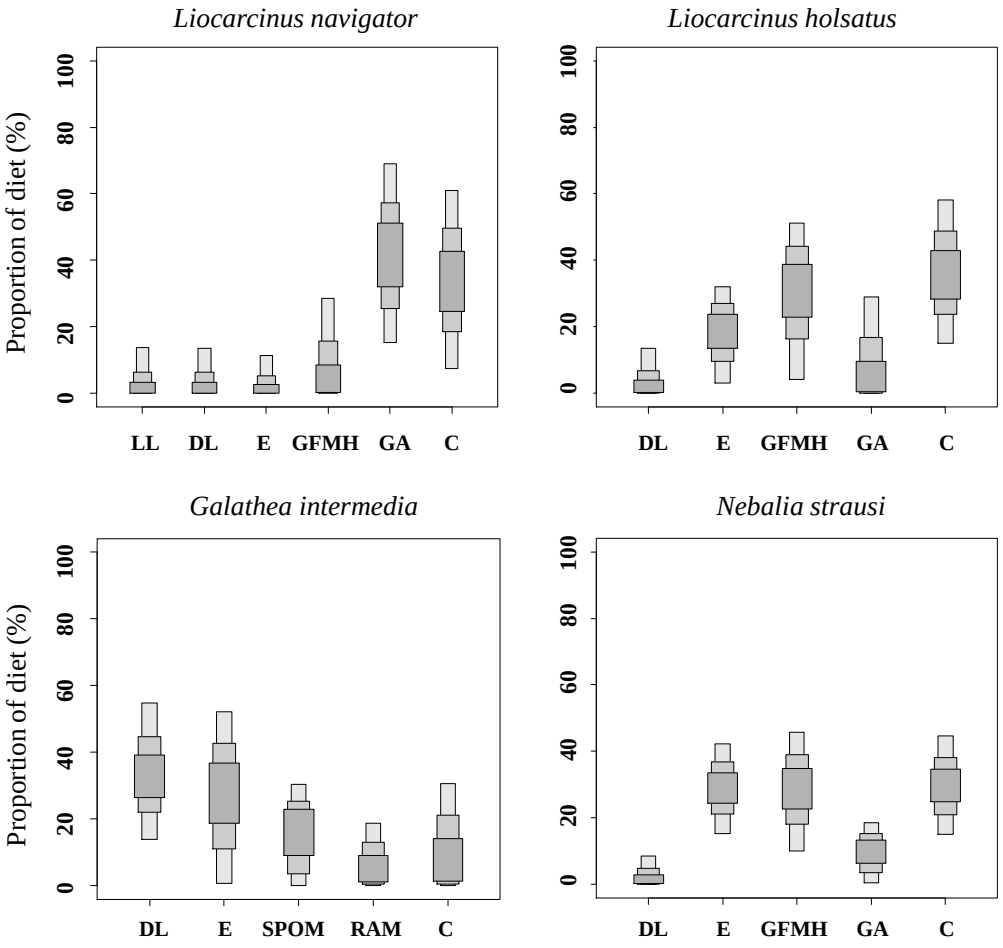
The basal potential food sources for the organisms situated at the bottom of the isotopic space ($\delta^{15}N \approx 2.00‰$) were the same as described § 3.1.2.1 of this chapter: dead *P. oceanica* leaves (DL), living *P. oceanica* leaves (LL), epiphytes + brown photophilous macroalgae (E) (see § 2.3 of this chapter), red sciaphilous macroalgae (RMA), suspended particulate organic matter (SPOM) and a pool of harpacticoid copepods (C) (from Mascart, 2015). For the organisms situated above them ($\delta^{15}N \approx 4.00‰$) DL and E were taken into account while RMA and SPOM were removed and replaced by *Gammarus aequicauda* (GA), a pool of *Gammarella fucicola* and *Melita hergensis* (GF and MH, respectively) and C, to take into account the higher proportion of animal material found in their digestive tracts (§3.1.1 of this chapter). For organisms above them ($\delta^{15}N \approx 6.00‰$) composed of *Palaemon xiphias* and *Processa edulis*, food sources were GA, GFMH, C, plus a pool of "intermediate organisms" (P) and the fishes (GSPP). For the top group ($\delta^{15}N \approx 8.00‰$) composed only of fishes, food sources were GA, GFMH, C, P and a pool of *Palaemon xiphias* and *Processa edulis* (PX) (Figure 5.6).

Nototropis guttatus, another primary consumer amphipod was not considered as a potential food source due to its very low abundance (~2% of global abundance) compared to *Gammarella fucicola* and *Gammarus aequicauda* abundances (more than 80%). This very low abundance should result in very low importance in terms of organic matter flux.


Gammarella fucicola
Gammarus aequicauda
Melita hergensis
Nototropis guttatus
Proportion of diet (%)
0
20
40
60
80
100
DL
E
SPOM
RAM
C


Idotea balthica
Stenosoma lancifer
Apanthura corsica
Athanas nitescens
Proportion of diet (%)
0
20
40
60
80
100
DL
E
GFMH
GA
C
SPOM
RAM


Palaemon xiphias
Processa edulis
Hippolyte leptocerus
Macropodia linaresi
Proportion of diet (%)
0
20
40
60
80
100
P
GFMH
GA
GSPP
C
DL
E


Liocarcinus navigator
Liocarcinus holsatus
Galathea intermedia
Nebalia strausi
Proportion of diet (%)
0
20
40
60
80
100
LL
DL
E
GFMH
GA
C
SPOM
RAM

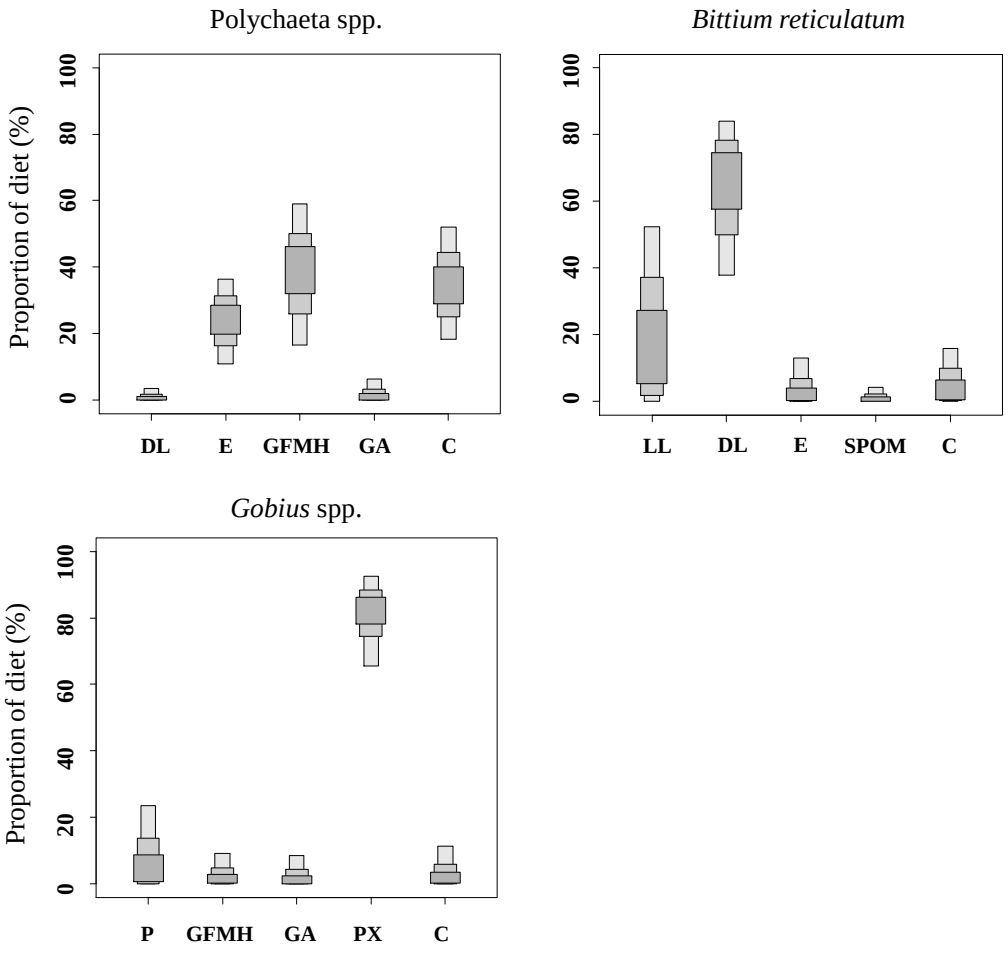


Figure 5.6: Boxplot of relative contributions of each food source to the diet of the 19 sampled species. Food sources codes: E= epiphytes+various brown drift macroalgae; LL= Living P. oceanica leaves; DL= dead P. oceanica leaves; RMA= various red drift macroalgae; SPOM= suspended organic matter; GA= Gammarus aequicauda; GFMH= pool of Gammarella fucicola and Melita hergensis; NGU= Nototropis guttatus; PX= pool of Palaemon xiphias and Processa edulis; GSPP= Gobius spp.; P= pool of Athanas nitescens, Nebalia strausi, Hippolyte leptocerus, Polychaeta spp., Liocarcinus holsatus, Macropodia linaresi and Stenosoma lancifer; C= pool of the 3 most abundant species of harpacticoid copepods found in P. oceanica exported litter (Mascart, 2015). Dark grey boxes are the 50% credibility intervals, medium grey boxes are the 75% credibility intervals, and light grey boxes are the 95% credibility intervals.

The diet of *Gammarella fucicola*, the most abundant macroinvertebrate present in the EMAs, showed a high contribution of both dead *P. oceanica* (CI_{95}: 4.65% - 58.14%; Mode: 33.48%) and epiphytes/macroalgae (CI_{95}: 5.55% - 84.02%; Mode: 41.82%), but also a non-negligible contribution of suspended organic matter (CI_{95}: 0.26% - 31.65%; Mode: 10.52%). RMA and C contributed much less (Mode: 1.39% and 0.36% respectively) to the diet. The reader's attention is drawn to the huge credibility interval (CI_{95}) for E.

Gammarus aequicauda showed a different diet, with dead *P. oceanica* contributing for most of the diet (CI_{95}: 47.76% - 80.56%; Mode: 60.32%), but also with epiphytes/macroalgae contributing for a non-negligible part of it (CI_{95}: 1.62% - 48.69%; Mode: 33.64%), while the contribution of SPOM, RMA and C were close to zero (Mode: 1.24%, 0.89% and 0.23% respectively).

Melita hergensis diet seemed to be constituted almost equally of dead *P. oceanica* (CI_{95}: 5.07% - 55.03%; Mode: 34.14%), epiphytes/macroalgae (CI_{95}: 1.58% - 72.26%; Mode: 34.33) and suspended organic matter (CI_{95}: 1.21% - 39.20%; Mode: 23.62%). RMA and C contribution were once again close to zero (Mode: 1.81% and 0.52% respectively).

Nototropis guttatus showed a much more diverse diet. Dead *P. oceanica* (CI_{95}: 0.51% - 37.49%; Mode: 23.40%), epiphytes/macroalgae (CI_{95}: 0.95% - 52.67%; Mode: 29.03%) and suspended organic matter (CI_{95}: 3.65% - 59.55%) and RMA (CI_{95}: 0.11% - 34.60%; Mode: 19.97%) all contributed significantly to its diet. C contribution was close to zero (Mode: 0.72%)

The diet of *Idotea balthica* showed important contribution of epiphytes/macroalgae (CI_{95}: 34.36% - 74.77%; Mode: 58.78%), GFMH (CI_{95}: 0.00% - 38.08%; Mode: 12.09%) and dead *P. oceanica* (CI_{95}: 0.15% - 20.30%; Mode: 9.06%). It also showed very small contributions of remaining animal potential food sources: GA (Mode: 1.41%) and C (Mode: 1.81%).

The diet of *Stenosoma lancifer* contrasted with the diet of the other Idoteidea. Dead *P. oceanica* and epiphytes/macroalgae showed contributions close to zero (Mode: 0.81% and 3.05% respectively) while animal food sources contributed much more. GA contributed modestly (CI_{95}: 0.00% - 29.37%; Mode: 9.04%) but GFMH (CI_{95}: 3.02% - 52.10%; Mode: 32.83%) and C (CI_{95}: 22.63% - 69.94%; Mode: 45.02%).

The diet of *Apanthura corsica* showed important but variable contributions of dead *P. oceanica* (CI_{95}: 11.53% - 52.52%; Mode: 31.60%), of epiphytes/macroalgae (CI_{95}: 0.4% - 52.56%; Mode: 27.92%) and of suspended organic matter (CI_{95}: 0.5% - 37.67%; Mode: 24.11%). RMA and C contributed much less to the diet (Mode: 4.56% and 2.45% respectively).

The diet of the *Athanas nitescens* shrimp showed only important contributions of animal food sources. GA contributed modestly (CI_{95}: 0.00% - 5.56%; Mode: 0.79%) while GFMH (CI_{95}: 25.59% - 59.44%; Mode: 44.29%) and C (CI_{95}: 33.30% - 62.20%; Mode: 47.94%) contributed more importantly to the diet.

Palaemon xiphias is the highest invertebrate in the isotopic space. Results showed relatively important contributions of GA (CI_{95}: 16.96% - 39.51%; Mode: 28.83%) and GSPP (CI_{95}: 14.49% - 30.18%; Mode: 23.13%) to the diet. P, GFMH and C presented variable and non-negligible contributions to the diet (Mode: 14.23%, 13.34% and 16.80% respectively).

Processa edulis showed a much less selective diet. Indeed, every animal food source contributed almost equally (15-25%) to its diet.

Hippolyte leptocerus also showed a very diverse diet. Dead *P. oceanica* and GA showed the lowest contributions (Mode: 0.93% and 8.33% respectively) while every other potential food source, E, GFMH and C showed important and quite similar contributions (Mode: 20.75%, 29.04% and 35.90% respectively).

Macropodia linaresi diet showed a diverse and mostly animal diet. Indeed major contributions of GFMH (CI_{95}: 0.79% - 48.17%; Mode: 27.94%), GA (CI_{95}: 0.00% - 38.68%; Mode: 23.32%) and C (CI_{95}: 6.99% - 56.66%; Mode: 31.46%) were observed. E and dead *P. oceanica* contributions were much more modest (Mode: 13.02% and 1.47%, respectively).

Liocarcinus navigator seemed to assimilate almost nothing from vegetal food sources, with LL, DL and E contributions close to zero (Mode: 0.94%, 0.93% and 0.79%, respectively). Two highest contributing food sources were GA (CI_{95}: 15.21% - 68.91%; Mode: 43.34%) and C (CI_{95}: 7.53% - 60.94%; Mode: 34.04%), while GFMH was a minor contributor to the diet (Mode: 2.06%).

Diet of *Liocarcinus holsatus* seemed to concentrate on 3 major contributors: C (CI_{95}: 15.07% - 58.07%; Mode: 35.62%), GFMH (CI_{95}: 4.18% - 51.20%; Mode: 30.22%) and epiphytes/macroalgae (CI_{95}: 3.11% - 31.19%; Mode: 18.54%). On the other hand, dead *P. oceanica* and GA contributions were close to zero (Mode: 1.04% and 2.17% respectively).

Galathea intermedia assimilation pattern showed important contributions of dead *P. oceanica* (CI_{95}: 13.99% - 54.83%; Mode: 32.00%), epiphytes/macroalgae (CI_{95}: 0.6% - 52.25%; Mode: 27.33%) and SPOM (CI_{95}: 0.05% - 30.56%; Mode: 19.64%), while RMA and C presented much more modest contributions (Mode: 5.05% and 4.10% respectively).

Nebalia strausi showed a very diverse diet, with almost similar contributions (~29%) of E, GFMH and C. GA contributed much less (CI_{95}: 0.41% - 18.48%; Mode: 18.54%) while dead *P. oceanica* contribution was close to zero.

The diet of Polychaeta spp. showed important contributions of epiphytes/macroalgae (CI_{95}: 10.98% - 36.36%; Mode: 23.94%), GFMH (CI_{95}: 16.63% - 59.05%; Mode: 38.91%) and C (CI_{95}: 18.30% - 52.01%; Mode: 35.34%). Dead *P. oceanica* and GA contributed much less to the diet (Mode: 2.98% and 8.05% respectively).

Bittium reticulatum showed a very high contribution of dead *P. oceanica* (CI_{95}: 37.97% - 84.00%; Mode: 67.41%) and variable and more modest contribution of LL (CI_{95}: 0.00% - 52.39%; Mode: 12.83%). E, SOM and C contributions were close to zero (Mode: 1.07%, 0.46% and 1.64% respectively).

Gobius spp. fishes showed a highly specialized diet, with massive contribution only of PX (CI_{95}: 65.51% - 92.57%; Mode: 81.58%). Other food sources, P, GFMH, GA and C presented much more anecdotic contributions to its diet (Mode: 2.10%, 0.86%, 0.73% and 1.01% respectively).

3.1.3.2. SIBER: global community

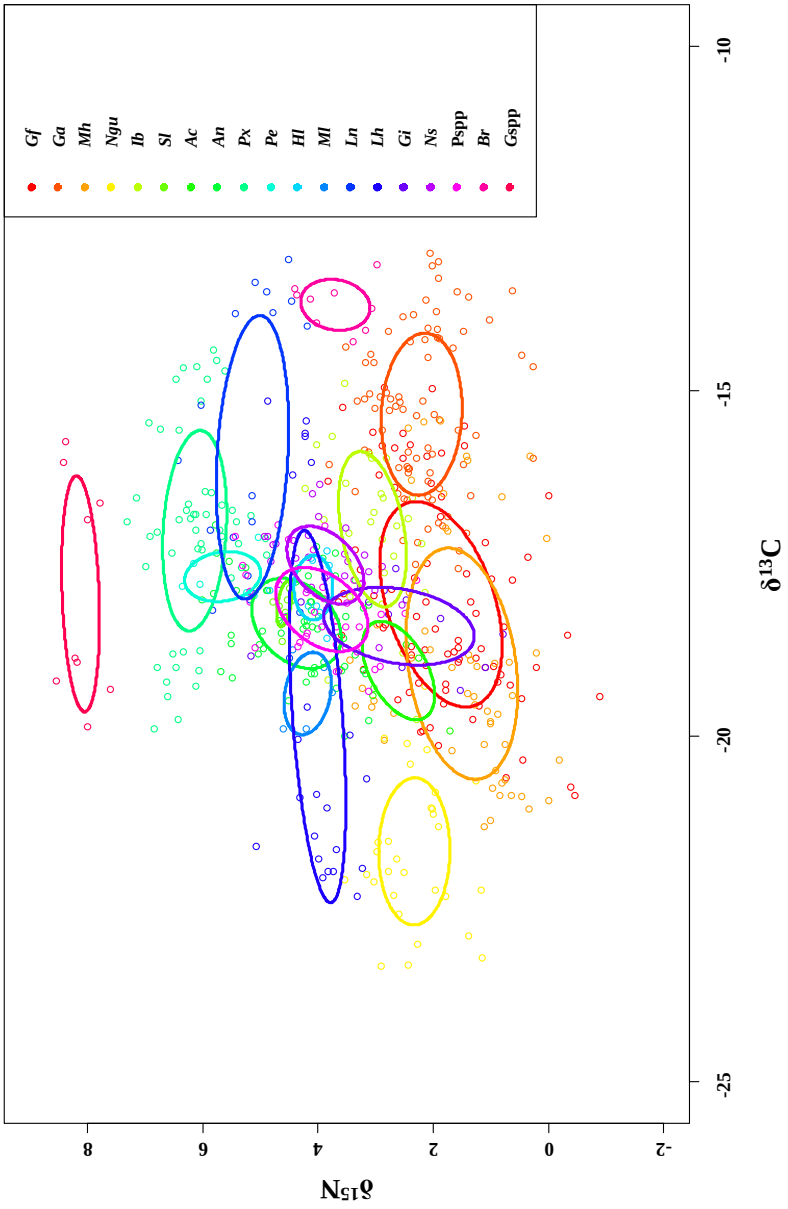


Figure 5.7: $\delta^{13}C$ vs. $\delta^{15}N$ biplot (‰) of the 19 macrofauna species. Thick colored lines: ~40% CI bivariate standard ellipses, corresponding to the global isotopic niches occupied by each of the 19 species. Species code: Gf= Gammarella fucicola; Ga= Gammarus aequicauda; Mh= Melita hergensis; Ngu= Nototropis guttatus; Px= Palaemon xiphias; Pe= Processa edulis; An= Athanas nitescens; Hl= Hippolyte leptocerus; Ml= Macropodia linaresi, Ln= Liocarcinus navigator; Lh= Liocarcinus holsatus; Gi= Galathea intermedia; Ac= Apanthura corsica; Ib= Idotea balthica; Sl= Stenosoma lancifer; Ns= Nebalia strausi; Br= Bittium reticulatum; Pspp= Polycheata spp.; Gspp= Gobius spp..

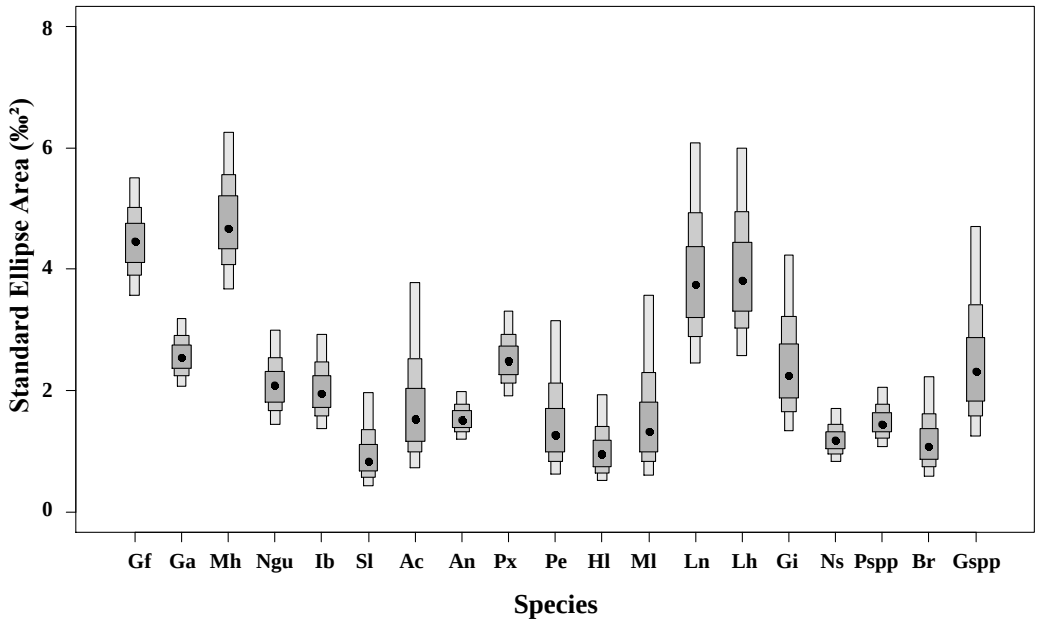


Figure 5.8: Boxplot of Standard Ellipse Areas of each of the 19 species. Species code: Gf= Gammarella fucicola; Ga= Gammarus aequicauda; Mh= Melita hergensis; Ngu= Nototropis guttatus; Px= Palaemon xiphias; Pe= Processa edulis; An= Athanas nitescens; Hl= Hippolyte leptocerus; Ml= Macropodia linareis, Ln= Liocarcinus navigator; Lh= Liocarcinus holsatus; Gi= Galathea intermedia; Ac= Apanthura corsica; Ib= Idotea balthica; Sl= Stenosoma lancifer; Ns= Nebalia strausi; Br= Bittium reticulatum; Pspp= Polycheata spp.; Gspp= Gobius spp.. Dark grey boxes are the 50 % credibility intervals, medium grey boxes are the 75 % credibility intervals, and light grey boxes are the 95 % credibility intervals. Black dots represent SEAc area.

SIBER isotopic niches representation showed a wide variety of niche dimensions and positions inside the isotopic space (Figures 5.7 and 5.8). *Gammarella fucicola* and *Melita hergensis* showed by far the widest niches among the community (SEAc: 4.52 ‰^{2} and 4.95 ‰^{2} respectively), while *Stenosoma lancifer* showed the smallest niche (SEAc: 0.11 ‰^{2}). While many niches were well individualized, 7 species situated above the primary consumers showed important overlap of their isotopic niches. *Nebalia strausi, Liocarcinus holsatus, Macropodia linaresi, Hippolyte leptocerus, Athanas nitescens, Stenosoma lancifer* and Polychaeta spp. presented important overlap of their niches and were all situated at the same place in the isotopic space.

3.1.3.3. SIAR model: seasonal trend

SIAR model was run with seasonal data from the 5 species present at every season: *Gammarella fucicola, Gammarus aequicauda, Melita hergensis, Athanas nitescens* and *Palaemon xiphias*. As the model took into account the food sources baseline variations, this part of the data analysis gave insights about true diet variations during the 2011-2012 sampling period.

Gammarella fucicola

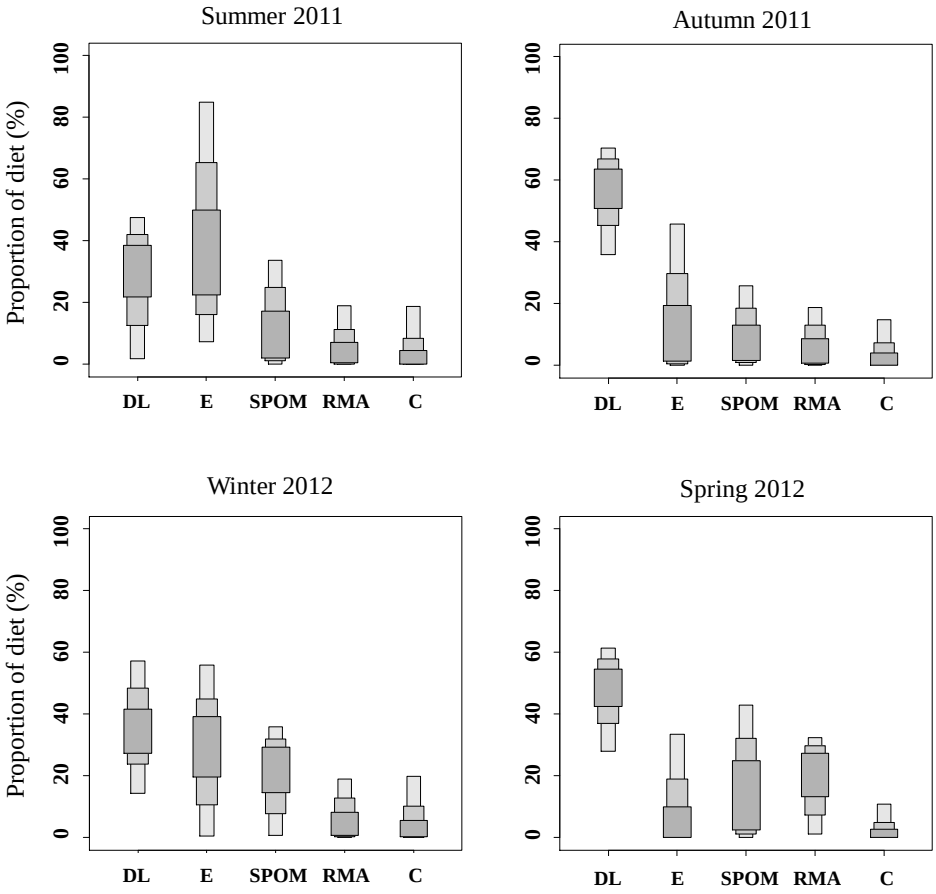


Figure 5.9: Seasonal boxplot of relative contributions of each food source to the diet of Gammarella fucicola. Food sources codes: E= epiphytes+various brown drift macroalgae; DL= dead P. oceanica leaves; RMA= various red drift macroalgae; SPOM= suspended organic matter; C= pool of the 3 most abundant species of harpacticoid copepods found in P. oceanica exported litter (Mascart, 2015). Dark grey boxes are the 50% credibility intervals, medium grey boxes are the 75% credibility intervals, and light grey boxes are the 95% credibility intervals.

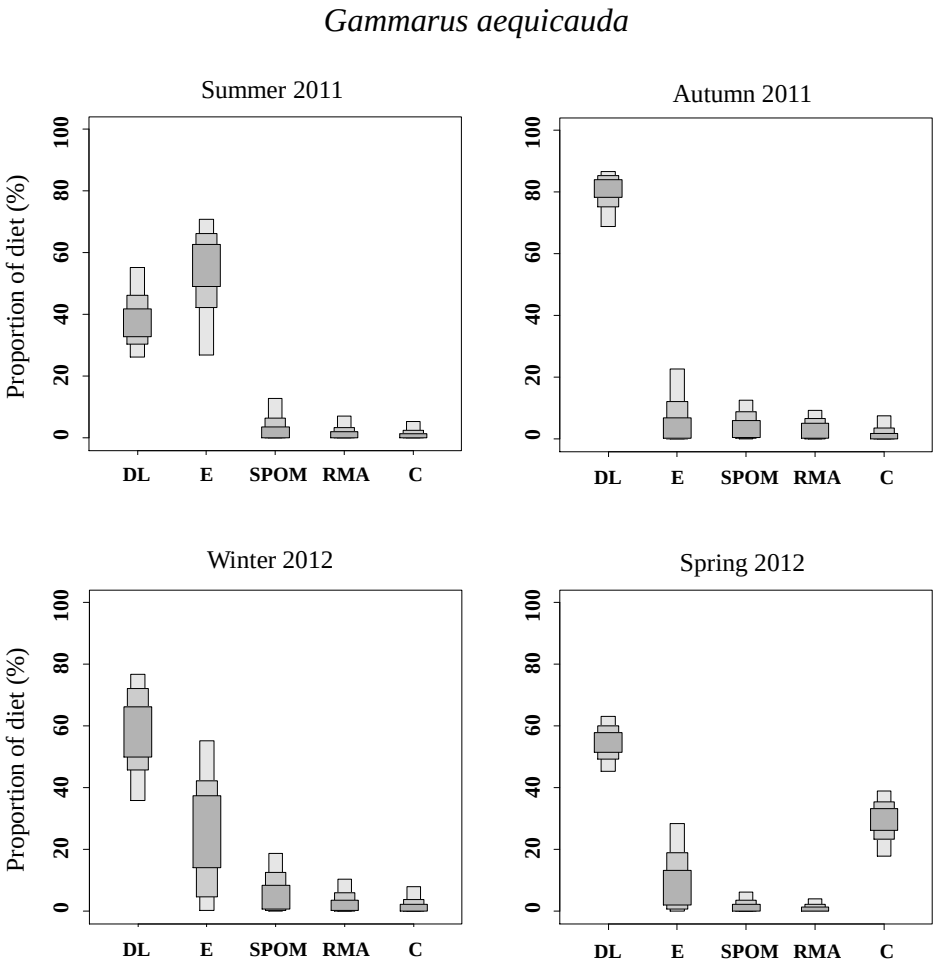


Figure 5.10: Seasonal boxplot of relative contributions of each food source to the diet of Gammarus aequicauda. Food sources codes: E= epiphytes+various brown drift macroalgae; DL= dead P. oceanica leaves; RMA= various red drift macroalgae; SPOM= suspended organic matter; C= pool of the 3 most abundant species of harpacticoid copepods found in P. oceanica exported litter (Mascart, 2015). Dark grey boxes are the 50% credibility intervals, medium grey boxes are the 75% credibility intervals, and light grey boxes are the 95% credibility intervals.

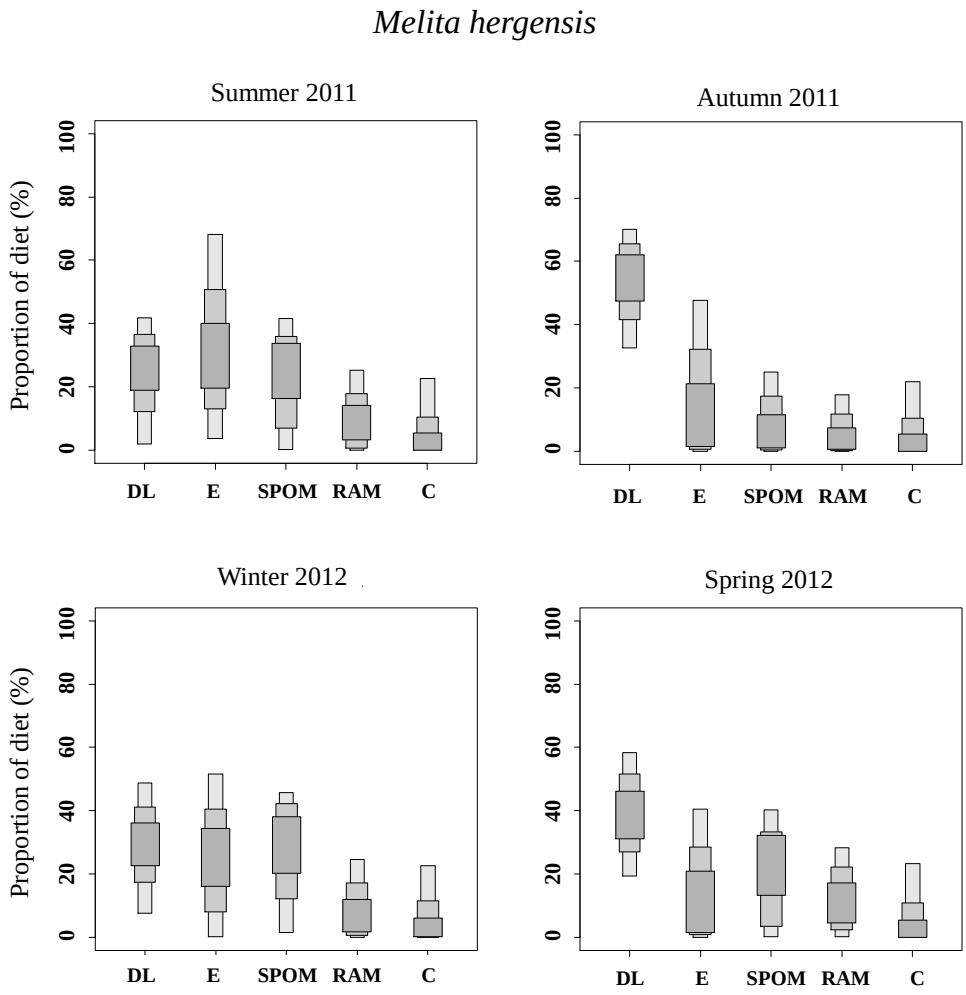


Figure 5.11: Seasonal boxplot of relative contributions of each food source to the diet of Melita hergensis. Food sources codes: E= epiphytes+various brown drift macroalgae; DL= dead P. oceanica leaves; RMA= various red drift macroalgae; SPOM= suspended organic matter; C= pool of the 3 most abundant species of harpacticoid copepods found in P. oceanica exported litter (Mascart, 2015). Dark grey boxes are the 50% credibility intervals, medium grey boxes are the 75% credibility intervals, and light grey boxes are the 95% credibility intervals.

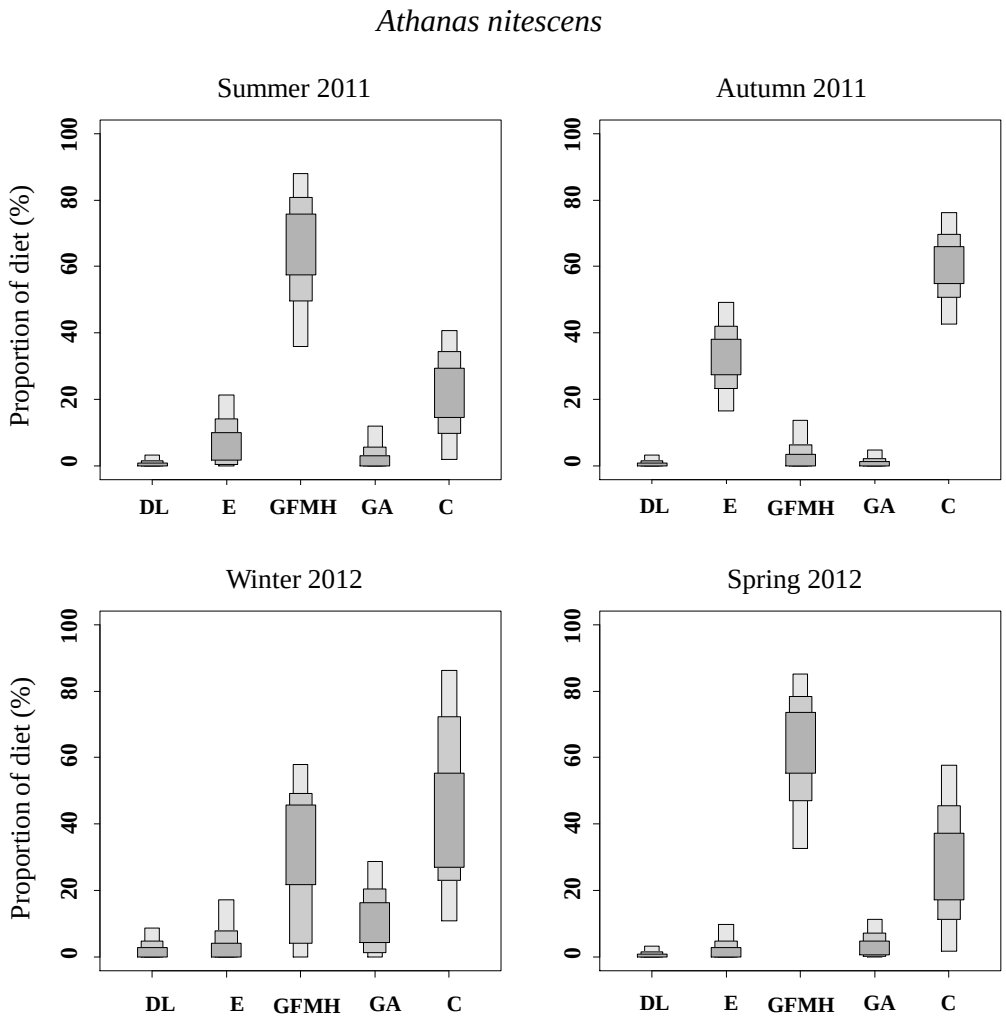


Figure 5.12: Seasonal boxplot of relative contributions of each food source to the diet of Athanas nitescens. Food sources codes: E= epiphytes+various brown drift macroalgae; DL= dead P. oceanica leaves; GA= Gammarus aequicauda; GFMH= pool of Gammarella fucicola and Melita hergensis; C= pool of the 3 most abundant species of harpacticoid copepods found in P. oceanica exported litter (Mascart, 2015). Dark grey boxes are the 50% credibility intervals, medium grey boxes are the 75% credibility intervals, and light grey boxes are the 95% credibility intervals.

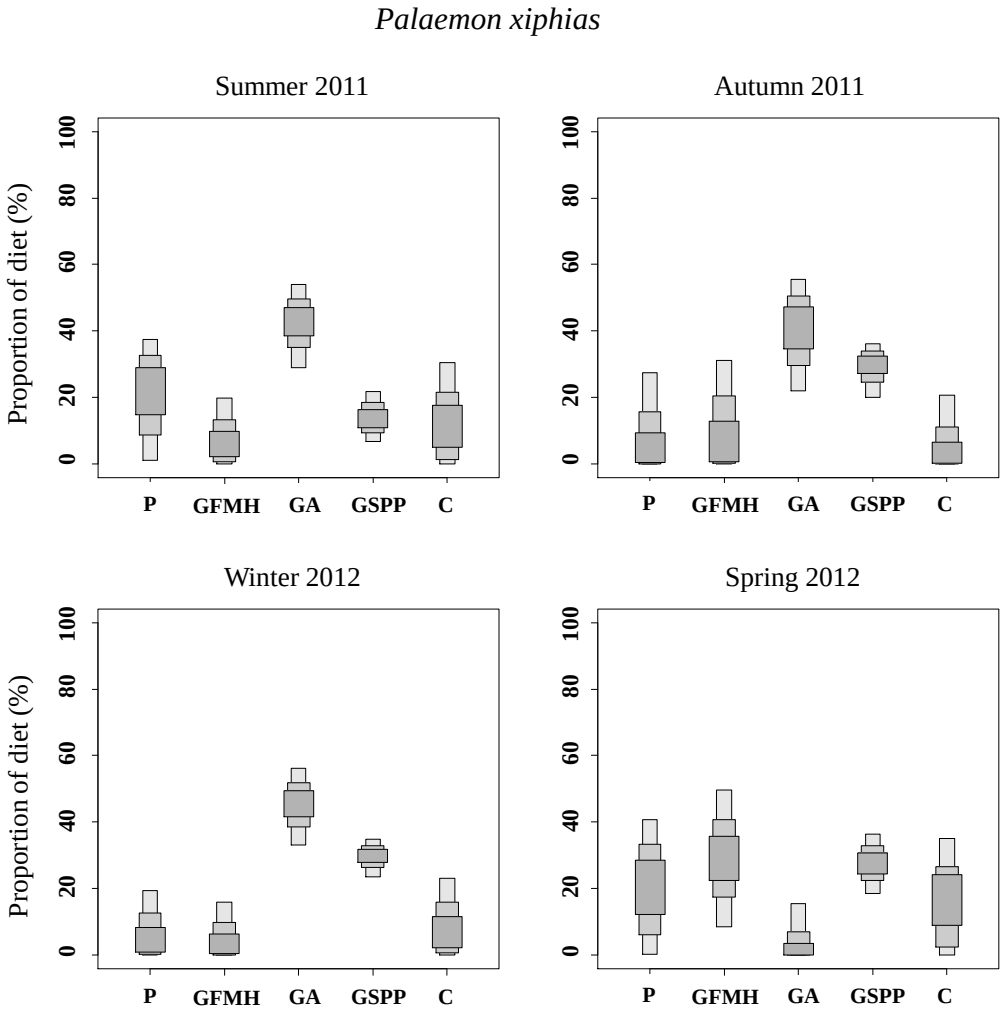


Figure 5.13: Seasonal boxplot of relative contributions of each food source to the diet of Palaemon xiphias. Food sources codes: P= pool of Athanas nitescens, Nebalia strausi, Hippolyte leptocerus, Polychaeta spp., Liocarcinus holsatus, Macropodia linaresi and Stenosoma lancifer; GA= Gammarus aequicauda; GFMH= pool of Gammarella fucicola and Melita hergensis; GSPP= Gobius spp.; C= pool of the 3 most abundant species of harpacticoid copepods found in P. oceanica exported litter (Mascart, 2015). Dark grey boxes are the 50% credibility intervals, medium grey boxes are the 75% credibility intervals, and light grey boxes are the 95% credibility intervals.

The diet of *Gammarella fucicola* (Figure 5.9), the most abundant amphipod of our community, always showed quite important and constant contributions of dead *P. oceanica*. Epiphytes/macroalgae were highly variable, showed important fluctuations between seasons and showed a maximum contribution in summer 2011 (CI_{95}: 7.44% - 84.89%; Mode: 25.52%) and a minimum contribution in spring 2012 (CI_{95}: 0.00% - 33.50%; Mode: 2.38%). Contributions of SPOM, RMA and C were low and quite constant at every season.

SIAR results about *Gammarus aequicauda* (Figure 5.10) showed very important but variable contributions of dead *P. oceanica*, with a maximum contribution in autumn 2011 (CI_{95}: 68.95% - 86.71%; Mode: 82.21%) and a minimum in summer 2011 (CI_{95}: 26.15% - 55.23%; Mode: 37.47%). Epiphytes/macroalgae showed a globally much more modest, but highly variable contribution to the diet, even exceeding the DL contribution in summer 2011 (CI_{95}: 26.83% - 70.91%; Mode: 56.56%). Contributions of SPOM and RMA were always close to zero. The contributions of C were close to zero in summer 2011, autumn 2011 and winter 2012, but presented a non-negligible increase between winter 2012 (Mode: 0.76%) and spring 2012 (CI_{95}: 17.84% - 38.95%; Mode: 29.77%).

The diet of *Melita hergensis* (Figure 5.11) was globally quite constant, except for epiphytes/macroalgae, showing variable contributions. E contribution increased between summer 2011 (CI_{95}: 3.72% - 68.18%; Mode: 29.38%) and autumn 2011 (CI_{95}: 0.00% - 47.77%; Mode: 3.79%) and between winter 2012 (CI_{95}: 0.01% - 51.50%; Mode: 26.49%) and spring 2012 (CI_{95}: 0.00% - 40.58%; Mode: 3.81%). Contributions of dead *P. oceanica* and SPOM were always non-negligible and much less variable, while contributions of RMA and C were always close to zero, no matter the season.

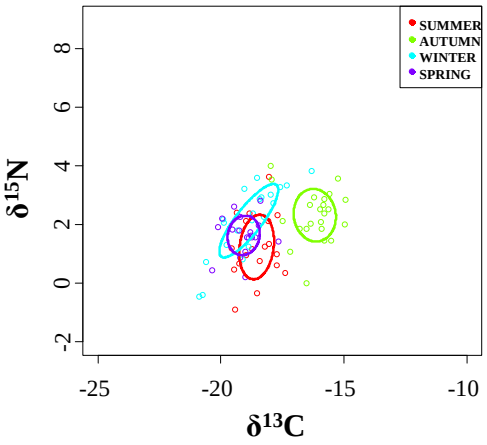
Athanas nitescens (Figure 5.12) never seemed to assimilate dead *P. oceanica*, showing contributions close to zero, no matter the season. Epiphytes/macroalgae showed variable contributions depending on the season, with maximum contributions in autumn 2011 (CI_{95}: 16.53% - 49.13%; Mode: 33.43%) but contributions close to zero in summer 2011, winter 2012 and spring 2012. GFMH was assimilated importantly in summer 2011, winter 2012 and spring 2012 (25 - 68%), but not at all in autumn 2011 (CI_{95}: 0.00% - 13.73%; Mode: 1.01%). C food source was assimilated non-negligibly no matter the season, with a marked maximum in autumn 2011 (CI_{95}: 42.71% -

76.27%; Mode: 60.07%). GA food source showed very modest contributions, no matter the season (0.4 - 10%).

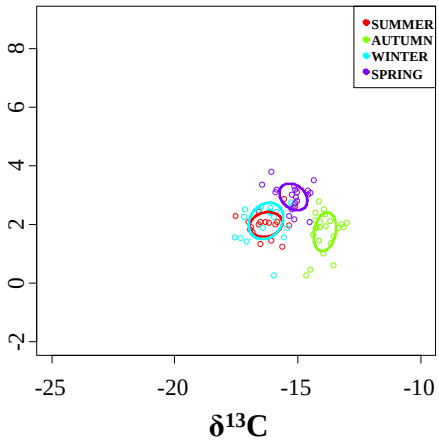
SIAR results about *Palaemon xiphias* diet (Figure 5.13) showed that GFMH food source always presented low but non-negligible contributions to the diet, except in spring 2012 (CI_{95}: 8.57% - 49.57%; Mode: 28.24%). C food source followed almost exactly the same pattern. GA food source contributions were variable, showing important values in summer 2011, autumn 2011 and winter 2012 (41 - 46%), but a much lower value in spring 2012 (CI_{95}: 0.00% - 15.59%; Mode: 1.01%). GSPP always showed quite important and constant contributions to the diet at every season. P showed very low contributions to the diet in autumn 2011 (CI_{95}: 0.00% - 27.54%; Mode: 2.15%) and winter 2012 (CI_{95}: 0.00% - 19.47%; Mode: 2.87%), and higher but variable contributions in summer 2011 (CI_{95}: 1.08% - 37.45%; Mode: 21.76%) and spring 2012 (CI_{95}: 0.20% - 40.69%; Mode: 23.53%).

3.1.3.4. SIBER: seasonal trend

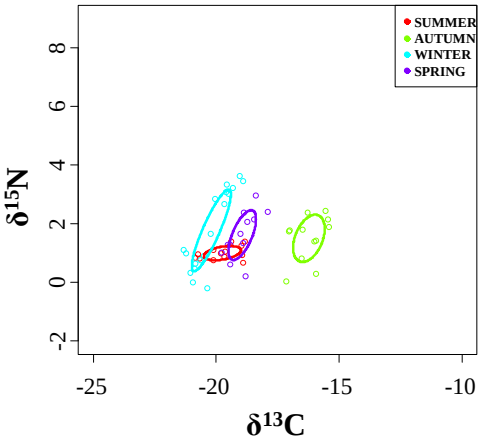
Gammarella fucicola



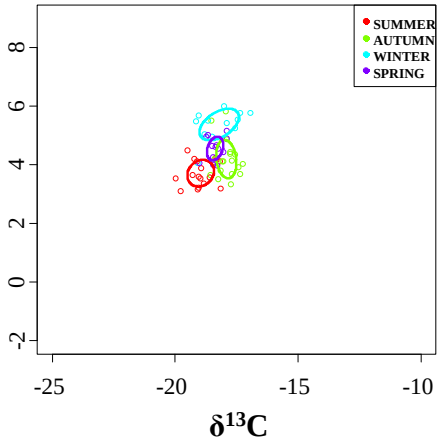
Gammarus aequicauda



Melita hergensis



Athanas nitescens



Palaemon xiphias

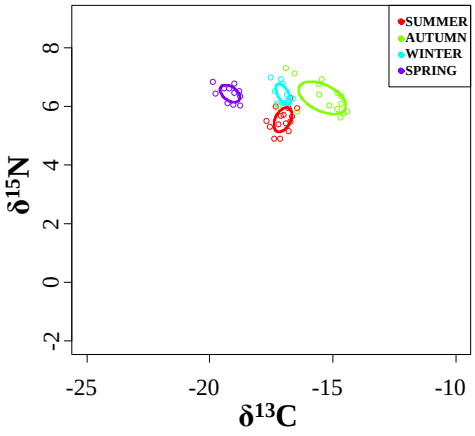


Figure 5.14: Seasonal $\delta^{13}C$ vs. $\delta^{15}N$ biplots (‰) of Gammarella fucicola, Gammarus aequicauda, Melita hergensis, Athanas nitescens and Palaemon xiphias. Thick colored lines: ~40% CI bivariate standard ellipses, corresponding to the global isotopic niches occupied by each species at each season.

SIBER seasonal niches representation (Figure 5.14) gave interesting insights about seasonal changes of niche areas and niche positions inside the isotopic space.

For *Gammarella fucicola*, the only niche that was completely individualized was in autumn 2011 which was situated on the less negative side of the graph. Spring 2012 niches presented important overlap with both summer 2011 and winter 2012 niches (0.73 and 1.04 ‰²). Winter 2012 and summer 2011 presented less overlap (< 0.5 ‰²), indicating much more separated isotopic niches. No significant difference between niche areas (SEAc ranging from 1.39 to 2.55 ‰²) was observed among seasons ($p > 0.05$).

For *Gammarus aequicauda*, summer 2011 and winter 2012 niches presented very important overlap (0.87 ‰²). Spring 2012 isotopic niche was situated higher in the isotopic spece. Autumn 2011 niche which was situated on the less negative side of the graph just as for *Gammarella fucicola*. No significant difference between niche areas (SEAc ranging from 0.78 to 1.35 ‰²) was observed among seasons ($p > 0.05$).

Melita hergensis presented niches of very different sizes and positions depending on the season. Winter 2012 niche area (SEAc: 0.52‰²) was significantly ($p = 0.025$) larger than summer 2011 niche area (SEAc: 1.64‰²), while the other niche areas presented no significant differences. Summer 2011 niche and spring 2012 niche presented important overlap (0.15 ‰²) while autumn 2011 niche and winter 2012 niche were well individualized. Autumn 2011 niche was on the less negative side of the graph just as for *Gammarella fucicola* or *Gammarus aequicauda* and winter 2012 niche was on the more negative side of the graph.

Athanas nitescens occupied a globally less important place in the isotopic space than any other of the 5 species. Autumn 2011 and spring 2012 niches presented non-negligible overlap (0.11 ‰²) while summer 2011 and winter 2012 niches were well individualized. Winter 2012 isotopic niche was situated higher in the isotopic space, while summer 2011 isotopic niche was situated lower in the isotopic space. No significant difference between niche areas (SEAc ranging from 0.41 to 1.20 ‰²) was observed among seasons ($p > 0.05$).

Palaemon xiphias niches were all well individualized, showing no overlap, indicating marked isotopic variations all around the 2011-2012 period. Moreover, autumn 2011 niche area (SEAc: 1.45‰²) was significantly larger than areas from summer 2011 (SEAc: 0.43‰²; p = 0.019), winter 2012 (SEAc: 0.27‰²; p = 0.032) and spring 2012 (SEAc: 0.33‰²; p = 0.041). This indicated higher inter-individual isotopic composition variations in autumn 2011. Summer 2011 isotopic niche was situated lower than any other season in the isotopic space. Autumn 2011 niche was on the less negative side of the graph just as for *Gammarella fucicola*, *Gammarus aequicauda* or *Melita hergensis*, while spring 2012 was on the more negative side of the graph.

3.2. Weekly determination of feeding preferences of two very abundant amphipod species

3.2.1. Gut contents examination

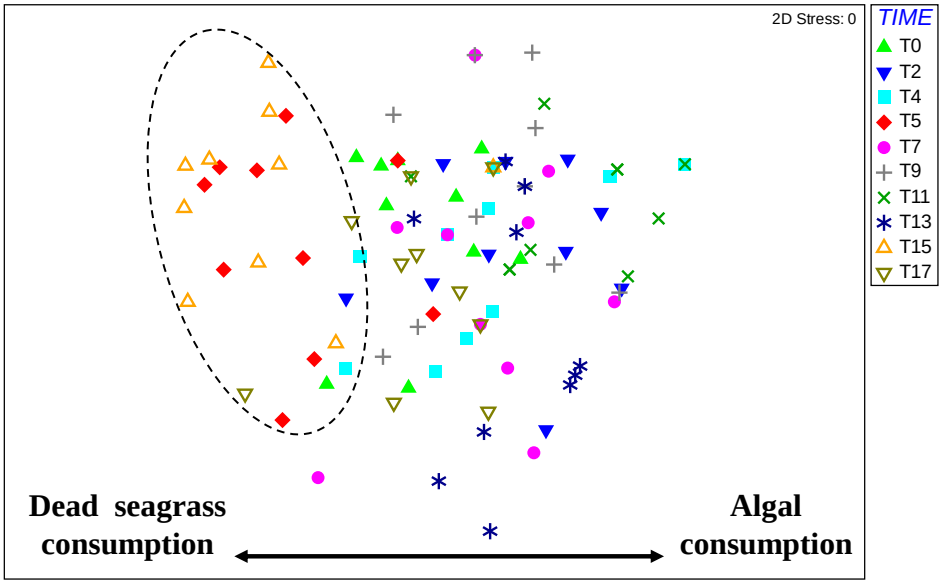
For this weekly study, we chose to focus only on the two most abundant and dominant macrofauna species: *Gammarella fucicola* and *Gammarus aequicauda.* Sampled every two weeks between September 2012 and May 2013, they showed quite constant ingestion patterns, very similar to what was found during the first seasonal trophic axis of this PhD (§ 3.1.1 of this chapter). In terms of organic matter flux, these two species were clearly dominant.

Gammarella fucicola globally ingested mostly algal material (67.35 ± 10.00 %) along with a smaller amount of dead *P. oceanica* fragments (14.90 ± 10.12%) and a non-negligible part of unidentifiable material (17.74 ± 9.20%). *Gammarus aequicauda* globally ingested mostly dead *P. oceanica* fragments (69.24 ± 12.27%) along with a more modest amount of algal material (19.65 ± 9.32%) and rarely animal material (1.74 ± 3.55%).

In order to assess the temporal ordination of the gut content examination data of *Gammarella fucicola* and *Gammarus aequicauda*, NM-MDS and 1-way ANOSIM analysis were performed. The stress value of the ordination was very satisfying (0 - 0.06).

Results showed very constant and very variable ingestion patterns for both Amphipods, with no clear temporal positioning of the data. Only T5 showed an ingestion pattern significantly different (1-way ANOSIM, $p = 0.001$) from all the other sampling dates for *Gammarus aequicauda*. For *Gammarella fucicola*, T5 and T15 presented significantly different (1-way ANOSIM, $p = 0.002$) ingestion patterns from all the other sampling dates. T5 and T15 were not different from each other. The reader's attention is drawn to the fact that these two sampling dates correspond to the two important perturbation events identified on November 11th, 2012 and May 13th, 2013 in Chapter 3. Between T4 and T5, as well as between T13 and T15, one striking result is the increase of dead *P. oceanica* fragments ingestion of about 20% for both species. The algal material ingestion decreased of the same proportion. It must be noted that these two periods showed an important import of litter on this accumulation (Chapter 3, §3.2.1).

Gammarella fucicola



Gammarus aequicauda

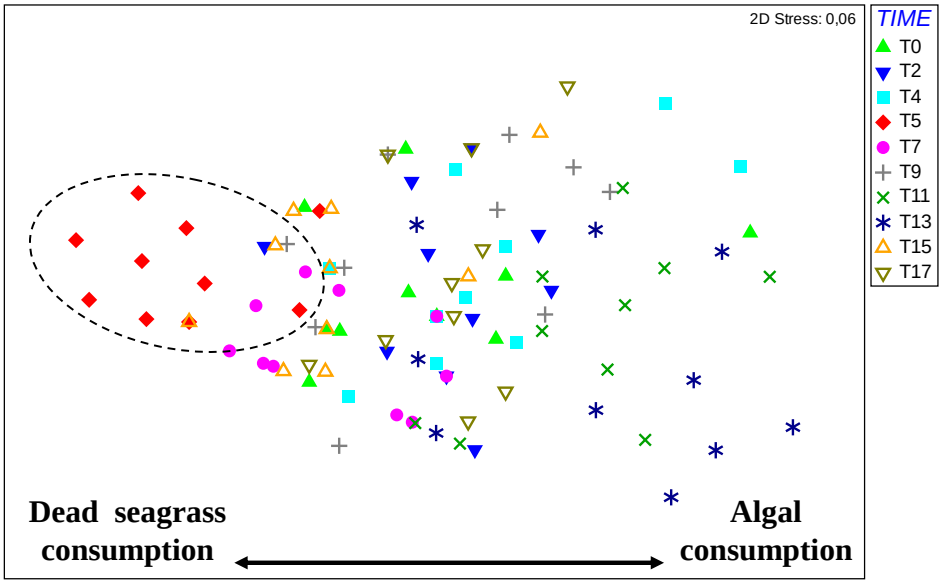


Figure 5.15: 2D-ordination and superposed main ANOSIM groups of the ingestion patterns of Gammarella fucicola and Gammarus aequicauda, sampled in 2012-2013. Black dotted ellipses represent the samples significantly ($p \leq 0.01$) distinguishable from all the other samples according to the 1-way ANOSIM analysis.

3.2.2. C and N stable isotopes:

Potential food sources for *Gammarella fucicola* and *Gammarus aequicauda* presented constant $\delta^{13}C$ and $\delta^{15}N$ values all around the sampling period and were perfectly in the range found in § 3.1.2.2 of this chapter. Global $\delta^{13}C$ and $\delta^{15}N$ values for DL are -13.55 ± 0.84‰ and 1.13 ± 1.06‰ respectively; $\delta^{13}C$ and $\delta^{15}N$ values for E are -18.31 ± 1.54‰ and 2.23 ± 1.12‰ respectively; $\delta^{13}C$ and $\delta^{15}N$ values for SPOM are -25.17 ± 2.15‰ and 2.31 ± 0.74‰ respectively, $\delta^{13}C$ and $\delta^{15}N$ values for RMA are -31.67 ± 1.56‰ and 1.91 ± 0.68‰ respectively; $\delta^{13}C$ and $\delta^{15}N$ values for C are -25.17 ± 2.31‰ and 2.15 ± 0.74 ‰ respectively (average values from Mascart, 2015).

Concerning *Gammarella fucicola* and *Gammarus aequicauda*, their elemental and isotopic values were perfectly in the range found in § 3.1.2.2 of this chapter. C:N ratios and $\delta^{15}N$ values showed almost no variation during the sampling period.

$\delta^{13}C$ values showed a slightly different pattern (Figure 5.16), with a decrease between January 2013 and April 2013. An important result is that $\delta^{13}C$ values of both species jumped of about 1.5‰ after the two events of November 11th, 2012 and May 13th, 2013.

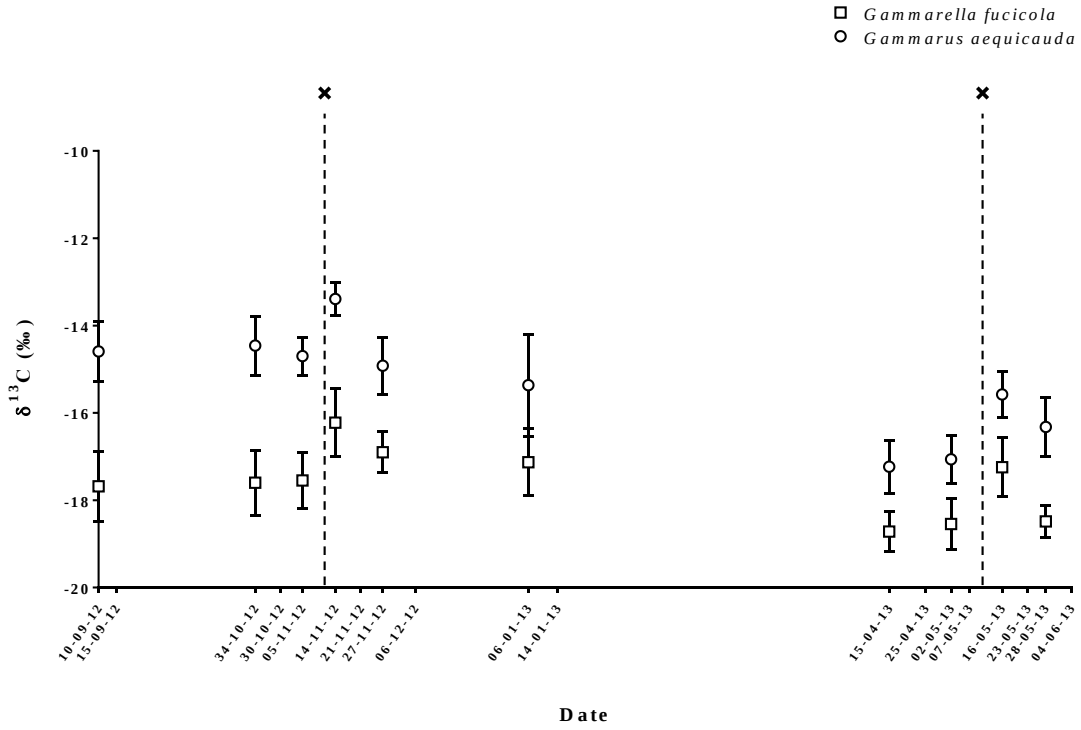


Figure 5.16: plot of $\delta^{13}C$ values of Gammarella fucicola and Gammarus aequicauda during the sampling period. Dotted black lines and crosses represent the events of November 11th, 2012 and May 13th, 2013.

3.2.3. SIAR model

Due to the constant pattern observed except at the two events of November 11th, 2012 and May 13th, 2013, and not to dilute the most interesting information, we chose to concentrate on these events.

Since the SIAR results were noticeably identical for both events and since detailing both of them was not considered essential, only the November 2012 event will be presented in this paragraph. Note that both events will of course be discussed further in this manuscript.

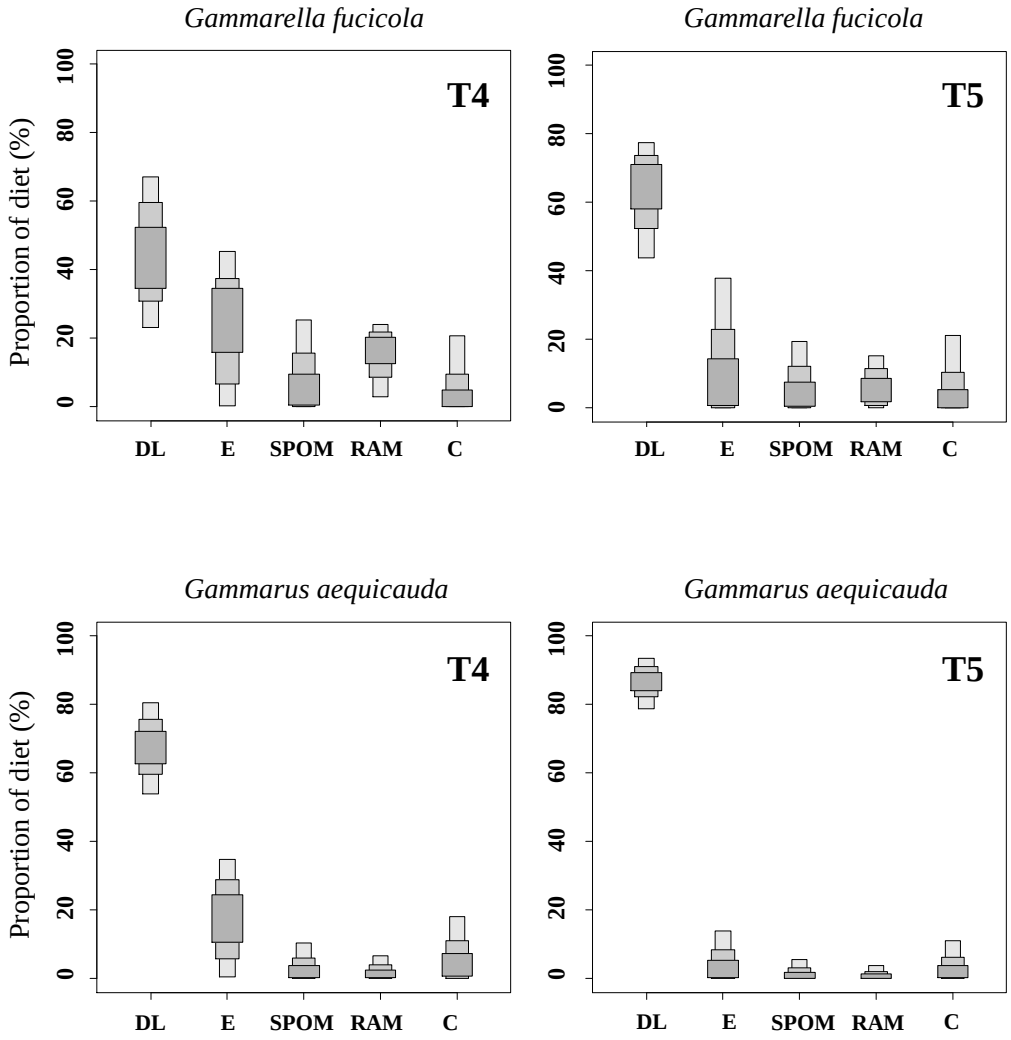


Figure 5.17: Boxplot of relative contributions of each food source to the diet of Gammarella fucicola and Gammarus aequicauda before (T4) and after (T5) the Novermber 11th, 2012 event. Food sources codes: E= epiphytes+various brown drift macroalgae; DL= dead P. oceanica leaves; RMA= various red drift macroalgae; SPOM= suspended organic matter; C= pool of the 3 most abundant species of harpacticoid copepods found in P. oceanica exported litter (Mascart, 2015). Dark grey boxes are the 50 % credibility intervals, medium grey boxes are the 75 % credibility intervals, and light grey boxes are the 95 % credibility intervals.

SIAR results showed that a non-negligible diet change occurred (Figure 5.17) after this event. Indeed, both species showed higher contributions of dead *P. oceanica* and lower contributions of epiphytes/macroalgae to their diet, indicating that both species relied a little more on dead *P. oceanica* leaves after this event.

The contribution of dead *P. oceanica* for *Gammarella fucicola* increased from 41.84% (CI_{95}: 23.10% - 67.15%) to 65.67% (CI_{95}: 43.76% - 77.53%) between T4 and T5. The opposite pattern was observed for epiphytes/macroalgae, decreasing from 28.21% (CI_{95}: 0.19% - 45.25%) to 3.41% (CI_{95}: 0.00% - 37.91%) between T4 and T5.

For *Gammarus aequicauda*, the contribution of dead *P. oceanica* to its diet increased from 67.77% (CI_{95}: 53.95% - 80.51%) to 86.82% (CI_{95}: 78.72% - 93.43%) between T4 and T5. The opposite pattern was observed for epiphytes/macroalgae, decreasing from 14.63% (CI_{95}: 0.44% - 34.78%) to 1.22% (CI_{95}: 0.00% - 13.83%) between T4 and T5. Results were analog for the second event.

3.2.4. SIBER niches

Due to the roughly constant pattern observed except at the two events of November 11th, 2012 and May 13th, 2013, and not to dilute the most interesting information, we chose to concentrate on these events.

Since the SIBER results were noticeably identical for both events and since detailing both of them was not considered essential, only the November 2012 event will be presented in this paragraph. Note that both events will of course be discussed further in this manuscript.

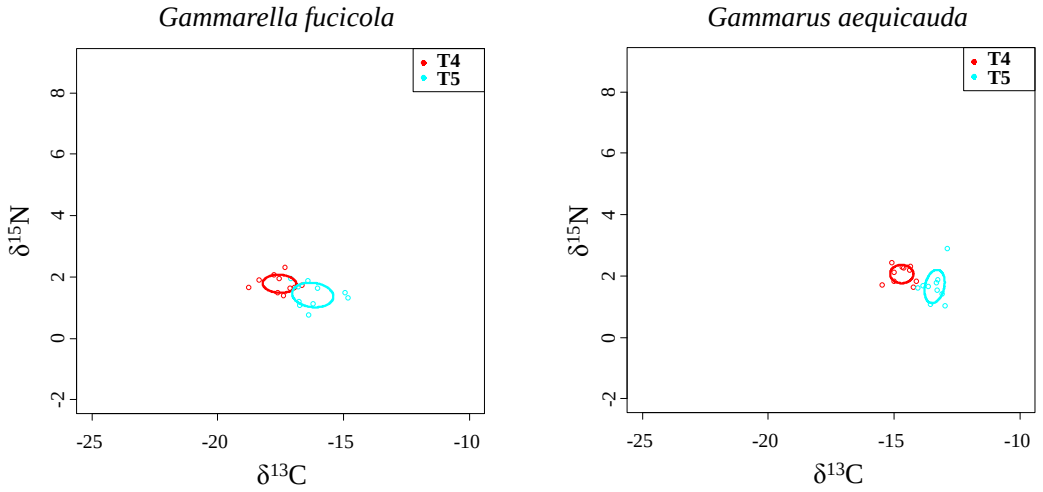


Figure 5.18: Seasonal $\delta^{13}C$ vs. $\delta^{15}N$ biplots (‰) of Gammarella fucicola and Gammarus aequicauda before (T4) and after (T5) the Novermber 11th, 2012 event. Thick colored lines: ~40% CI bivariate standard ellipses, corresponding to the global isotopic niches occupied by each species at T4 and T5.

SIBER niche representations (Figure 5.18) confirmed the isotopic changes and diet modifications that *Gammarella fucicola* and *Gammarus aequicauda* dealt with during the November event.

SIBER results for both species showed that niche areas did not vary significantly from T4 to T5 (SEAc for *Gammarella fucicola*: from 0.62 ‰² to 1.02‰²; SEAc for *Gammarus aequicauda*: from 0.43 ‰² to 0.67‰²). An interesting result is that niches in T5 for both species presented no overlap with their niches in T4, indicating that both species did not occupy the same position in the isotopic space in T4 and T5. Between T4 and T5, *Gammarella fucicola* and *Gammarus aequicauda* isotopic niches moved to the less negative side of the isotopic space (Figure 5.18). Results are analog for the second event which occurred in May 2013.

4. Discussion

4.1. Seasonal characterization of the food web

First of all, 19 species could seem a strangely limited number of species since 115 species were sampled in the seasonal community samples which were taken at the same time (see Chapter 3). However, the trophic sampling was not performed using the same technique, or aiming at the same goals as the seasonal community study. First, rare species were not taken into account since quite an important number of individuals was required for robust mixing model use. Moreover, the processing routine was not meant to be as exhaustive as the seasonal diversity identification routine. Very small species that were only separated from detrital material using magnifying glass were thus potentially under-sampled. Nonetheless, since many of these 19 species were also found in the 19 most abundant species representing more than 90% of the community (see Chapter 3), it was assumed that we presented here a satisfying global view of the food web composed by the most abundant macrofauna species present in the exported *P. oceanica* litter accumulations, also representing most of the organic matter fluxes.

4.1.1. Global view of the *P. oceanica* litter trophic web: insights from gut content examinations and stable isotope analysis

This study first demonstrated that we observed much less unidentifiable material than other studies on macraofauna communities. Indeed, Michel *et al*. (2015) observed that up to 80% of the gut contents of invertebrates from the *P. oceanica* meadow could be composed of such undetermined material. This contrasted a lot with our findings for closely related or similar organisms, and this might potentially be explained partly by the different methods used. First, Michel *et al*. (2015) used a more exhaustive technique, observing the entire gut content of every sampled organism. This method consisted in the discoloration of the body wall of the organisms (after a method from Guerra-Garcia and Tierna De Figueroa, 2009) and the examination of the entire gut content through the discolored organism, giving ingestion patterns in terms of surface proportion (%) occupied by the different items. Compared to our fast semi-quantitative technique (described in details in this chapter, § 2.2), this *in toto*

protocol could have potentially overestimated undetermined material, primarily because of the impossibility to isolate and manipulate the items during identification. This technique allows only the observation of a single lateral profile of the gut contents, making the identification of tightly packed items potentially hazardous. The sometimes very small size of organisms analyzed by Michel *et al*. (2015) could also partly explain the complexity of items identification. Our protocol was not perfect either. Since it consisted in the examination of randomly chosen areas of every spread gut contents, areas not examined might contain any item, including unidentifiable material so that our method potentially underestimated the proportion of amorphous material.

Despite these obvious methodology-based differences, we demonstrated in this seasonal study that the different species analyzed presented very different ingestion patterns, some ingested important amounts of detrital *P. oceanica* material and displaying detritivore patterns. This is in accordance with literature (Lepoint *et al*. 2006; Sturaro *et al*., 2010; Michel *et al*., 2015), mentioning the importance of detritus in the diet of species such as *Gammarella fucicola*, *Gammarus aequicauda* or various idoteids. Since *Gammarus aequicauda* and *Idotea balthica* are the only species presenting high (60-75%) proportions of detritus in their guts, they could be hypothesized to be the only true detritivore species sampled for this study.

Most of the sampled species presented important amounts of algal material in their guts, thus potentially displaying herbivore feeding preferences. These algal pieces, from unknown origin, might come from degraded drift erected macroalgae (Lepoint *et al*., 2006; Michel *et al.*, 2015) or from the epiphytes growing on the dead *P. oceanica* leaves. However, even if the real origin of this algal material remains unknown, this result indicated that a large part of the sampled species presented a certain tendency to herbivory (see next paragraph for nuance).

In the "algal material eaters", many species also ingested a certain amount of dead *Posidonia* leaf or animal material, more precisely, crustacean fragments and remains. Inside gut contents from these "omnivore" species, such as *Athanas nitescens*, *Hippolyte leptocerus* or *Nebalia strausi* remains of various crustaceans were identified, probably from amphipods or copepods. This mixed diet is in accordance with Cruz-Rivera and Hay (2000) who found that mixed diets improved the fitness of some amphipod species. Since

cannibalism and exuviae ingestion is not rare for marine invertebrates (Bellan-Santini, 1999; Michel, 2011), it could be possible that some crustacean material from the gut contents come from these "practices".

Two species were considered to be purely carnivore species: *Palaemon xiphias*, and *Gobius* spp. While not part of macrofauna, juvenile *Gobius* spp. were included to this study to see the feeding habits of a potential "top predator" spending a part of its life in exported litter accumulations. The carnivore ingestion pattern of *Palaemon xiphias* was in accordance with what was found by Guerao (1995) for *Palaemon xiphias* from *Cymodocea nodosa* meadows.

One particularly striking result from these gut contents was *Liocarcinus navigator*, which was the only species to ingest significant amounts of living *P. oceanica* fragments. This ingestion was highly variable but 3 individuals out of the 19 sampled (16% of the sampled individuals) presented living leaves constituting up to 45% of their gut contents, the remaining 55% corresponded to animal material, algal material and dead *P. oceanica* fragments. To our knowledge, this is the first time that ingestion of living *P. oceanica* is reported for this species. This was a surprising result since other species of the same genus are reported to be predators of small invertebrates of mussels (Lee and Seed, 1992; Freire, 1996).

Stable isotopes revealed a community supported mainly by 3 different food sources: dead *P. oceanica* leaves, epiphytes/macroalgae and suspended particulate organic matter, SPOM. Living leaves and drift red macroalgae were almost not assimilated by organisms of our community. Isotopic values for carbon and nitrogen obtained for these potential basal food sources were all in accordance with literature on that subject (Cooper and DeNiro, 1989; Dauby, 1989; Lepoint *et al.*, 2000; Vizzini *et al*., 2002; Lepoint *et al*., 2006; Sturaro *et al*., 2010; Mascart, 2015; Michel *et al*., 2015). As stated earlier in this chapter, the community ranged from -21.67 to -13.75‰ for $\delta^{13}C$ and from 1.50 to 7.94‰ for $\delta^{15}N$. This result signified that our food web encompassed different trophic levels: primary consumers, intermediate consumers/omnivores, first order predators and second order predators. The primary consumers were represented by the amphipods *Gammarus aequicauda*, *Gammarella fucicola*, *Melita hergensis* and *Nototropis guttatus*, but also of the decapod *Galathea*

intermedia and the isopod *Apanthura corsica*. Omnivore/intermediate organisms were composed of the isopods *Idothea balthica* and *Stenosoma lancifer*, the decapods *Athanas nitescens*, *Liocarcinus holstaus*, *Macropodia linaresi* and *Hippolyte leptocerus*, the leptostracean *Nebalia strausi* and the pool of polychaetes. The first order predators mainly comprised the three decapods *Liocarcinus navigator*, *Palaemon xiphias* and *Processa edulis*. The second order predators consisted of the juvenile fishes from the *Gobius* genus (see Figure 5.19 and 5.21). SIBER and Clustering Dendrogram Based on SIAR contributions (Figure 5.19) confirmed this general pattern, with primary consumers occupying two distinct isotopic niches; a narrow one for detritus consumers and the other, much larger, for the consumers of both epiphytes/macroalgae and detritus. Another important group of organisms occupying overlapping niches constituted the omnivore species, consuming vegetal and animal food sources and presenting trophic redundancy in the middle of the isotopic space. SIBER highlighted two other distinct groups: a group of first order decapod predators and a last group of "top predators" composed of fishes from the *Gobius* genus, also confirmed by the Clustering Dendrogram.

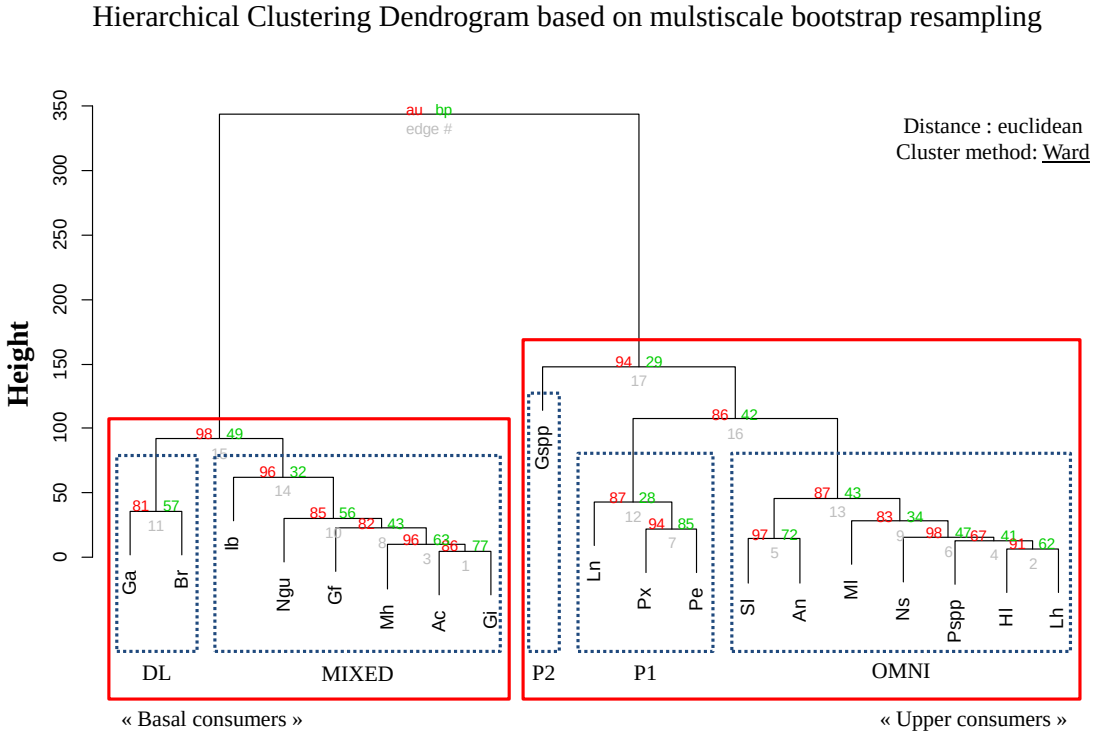


Figure 5.19: Hierarchical clustering dendrogram using Euclidean distances and Ward grouping method. Based on mode values of SIAR mixing model contribution outputs for each species and each food source. Species code: Gf= Gammarella fucicola; Ga= Gammarus aequicauda; Mh= Melita hergensis; Ngu= Nototropis guttatus; Px= Palaemon xiphias; Pe= Processa edulis; An= Athanas nitescens; Hl= Hippolyte leptocerus; Ln= Liocarcinus navigator; Lh= Liocarcinus holsatus; Ml= Macropodia linaresi; Gi= Galathea intermedia; Ib= Idotea balthica; Ac= Apanthura corsica; Sl= Stenosoma lancifer; Ns= Nebalia strausi; Pspp= Polychaeta spp.; Br= Bittium reticulatum; Gspp= Gobius spp.. Cluster code: DL= P. oceanica detritus consumers; MIXED= consumers of a mixed diet composed of P. oceanica detritus, SPOM and epiphytes/macroalgae; OMNI= consumers of both animal and vegetal food sources; P1= first order predators consuming only animal food sources; P2= second order predators consuming mainly P1 food sources. The Y axis represents the Euclidean distance between the samples. Red numbers can be interpreted as the probability a cluster has been formed during the 10000 iteration of the bootstrap resampling process (values above 75 are considered as "high"). Green numbers are the bootstrap value.

Apart from *Gammarus aequicauda* and *Nototropis guttatus*, the other primary consumers laid right in the middle of the isotopic space occupied by our community, right above the epiphyte/macroalgae basal food source, potentially indicating a diet composed mainly of epiphytes/macroalgae. Gut contents and mixing model analysis confirmed this, showing epiphytes/macroalgae was the most important contributor to their diet (30-45%). However, an interesting result is that dead *P. oceanica* leaves also constitute a non-negligible contributor (up to 25-30%) to their diet, indicating that these basal primary consumers all presented mixed diets (Figure 5.19), composed of both algal and seagrass food sources. This evidence of mixed diet is coherent with the mixed diet of *Gammarella fucicola* assessed by Lepoint *et al*. (2006) and Michel *et al*. (2015). To our knowledge, data concerning *Galathea intermedia* or *Apanthura corsica* are very scarce. The only published trophic study for these two species is about *Galathea intermedia* in Norway fjords assessing the detritus-feeder status of the species (Samuelsen, 1970). Our study suggested a much mixed diet, with contributions from both detritus and epiphytes/macroalgae.

Gammarus aequicauda was the less negative primary consumer of our community for carbon and laid close to the "*Posidonia*" food sources. Gut contents and mixing model analysis demonstrated that dead *P. oceanica* fragments constituted the most important contributor of the diet of *Gammarus aequicauda* (60%) which is in accordance with literature (Lepoint *et al*., 2006; Michel *et al*., 2015). This result is of major importance in the perspective of organic matter flux from the *P. oceanica* meadow to the coastal food webs, since *Gammarus aequicauda* is the second most abundant vagile macroinvertebrate encountered in the exported litter accumulations (Chapter 3).

In summary, seagrass eaters, ranging from detritus specialist to occasional detritus assimilators and/or ingestors, constitute the majority of fauna inhabiting EMAs. Therefore, these species could potentially constitute an important link between the *P. oceanica* meadow and the coastal food webs, through the "detrital pathway", demonstrating their potentially important role in coastal organic matter fluxes.

Nototropis guttatus was the most negative primary consumer sampled for carbon, and this species laid right between suspended particulate organic matter and epiphytes/macroalgae food sources, suggesting a consumption of both of them which is not in accordance with studies from Gambi *et al.* (1992)

or Scipione (1998), who classified this species as a consumer of *P. oceanica* detritus. The mixing model confirmed this consumption of both suspended particulate organic matter and epiphyte/macroalgae (30% each), but also of dead *P. oceanica* leaves (20%).

Results for these primary consumers are particularly interesting, reflecting various diet preferences, but more importantly, the non-negligible contribution of *P. oceanica* detritus (the food source presenting the highest C:N ratio and thus the lowest nutritional quality) to these diets. All these organisms are thus hypothesized to be an important entrance path for the *P. oceanica* organic matter in the coastal ecosystems.

Even if *Idotea balthica* was in the "MIXED cluster" (Figure 5.19), we considered this species as a member of the "omnivore group" anyway, due to the existing information about it (Sturaro *et al.*, 2010). In the omnivore group, *Idotea balthica* was a particular case, showing high contributions of epiphytes/macroalgae (58%) and low contributions of dead *P. oceanica* leaves as well as low contribution of the GFMH pool or meiofauna copepods. However, gut contents revealed an important ingestion of dead *P. oceanica* leaves. This result, associated to the important contribution of epiphytes/macroalgae revealed by mixing model analysis, could potentially indicate that dead *P. oceanica* fragments were ingested only for the epiphytes present on them. *P. oceanica* detrital organic matter was not assimilated, but the epiphytes were assimilated by *Idotea blathica*. This was in accordance with Sturaro *et al*. (2010), demonstrating similar preferences for exported litter specimens of *Idotea blathica*. Another idoteid, *Stenosoma lancifer*, displayed a very different and contrasted diet. While gut contents suggested important ingestion of epiphytes/macroalgae, mixing model analysis revealed that stable isotope results indicated a much more carnivore/omnivore diet, with low contributions of epiphytes/macroalgae and *P. oceanica* detritus (1-3%), but high contributions of the GFMH pool and of harpacticoid copepods (30-45%). This highlighted the need to confront the two techniques not to misinterpret gut contents or isotopic results. Several hypotheses could explain these contradictory results. First, the gut content technique used in this study could under-estimate the presence of non-dominant items, such as small copepods. Secondly, the epiphytes/macroalgae food source is a pool of both epiphytes and drift brown macroalgae, not isotopically distinguishable at each season). Epiphytes themselves are a very heterogeneous group which showed highly variable isotopic values, mainly because of the variations of the presence of

epiflora and epifauna (Borowitzka and Lethbridge, 1989; Michel *et al*., 2015). These studies revealed that epifauna was situated higher in the isotopic space than epiflora, showing values close to those found in this study for the GFMH pool. It could thus be hypothesized that *Stenosoma lancifer* assimilated mostly animal epiphytes, and probably not so much other crustacean species.

Athanas nitescens was the typical omnivore species of our community, showing important ingestion of animal and algal material. Mixing model analysis revealed that this species was in fact much more focused on animal food sources, assimilating mainly GFMH pool and harpacticoid copepods (45% each) and a modest proportion of epiphytes (5%). Such diet preferences indicate that species like *Athanas nitescens* could potentially constitute a trophic link between the macrofauna and the important meiofauna community (Mascart *et al*. 2015a). Apart from *Macropodia linaresi* and *Nebalia strausi*, other species from this "omnivore group" present diets close to *Athanas nitescens*, assimilating mainly GFMH pool and harpacticoid copepods, but also a more variable part of epiphytes/macroalgae. *Macropodia linaresi* and *Nebalia strausi* were two special cases, since they presented important contributions of *Gammarus aequicauda* (GA) food source (15-35%). These two species could thus constitute another link in the transmission of the *P. oceanica* detrital organic matter from the primary consumers to higher trophic levels in our community.

Higher in the trophic web, were observed the biggest shrimp and crab species: *Palaemon xiphias, Processa edulis* and *Liocarcinus navigator*. *Palaemon* presented gut contents composed almost exclusively of animal material, mainly crustacean fragments which corresponds to what Guerao (1995) found, with guts of *Palaemon xiphias* containing mostly amphipod and isopod fragments. We could not examine guts from *Processa*, but Guerao (1996) found mostly fragments of crustaceans and annelids. Mixing model analyses corroborated these gut results, indicating a diet composed of a mixture of most animal food sources. *Palaemon xiphias* was a little bit different from *Processa edulis*, assimilating a non-negligible contribution from fishes (*Gobbius* spp. food source). This could potentially indicate a more opportunistic behavior of *Palaemon*, sometimes predating on juvenile fish (ambush predator) or scavenging on fish carcasses.

Liocarcinus navigator was a little bit apart from these two predators. Indeed, as already mentioned earlier in this discussion, it was the only species presenting variable but non-negligible amounts of living green *P. oceanica*

fragments in its guts. However, mixing model analysis indicated that living leaves contributed for only 0.9% to its diet, which was considered unlikely, since contribution of epiphytes/macroalgae was also close to zero. Indeed, why would *Liocarcinus navigator*, quite a big swimming crab, ingest fragments of living *P. oceanica* leaves, if not for the epiphytes growing on them? One hypothesis came from ghost shrimps feeding habits. Indeed, these Thalassinidae decapods are known to "store" living and detrital seagrass leaves in their burrows and feed on them. Kneer *et al*. (2008) hypothesized that these shrimps should display $\Delta^{15}N$ values between 3 and 4 ‰ to reflect their important ingestion of living and detrital seagrass material in their isotopic values. We thus hypothesized that for *Liocarcinus navigator*, $\Delta^{15}N$ values used in SIAR might potentially have been far too low. We thus used $\Delta^{15}N$ values of 3.9 ± 0.5 ‰ from Yokoyama *et al*. (2005) and ran SIAR again (Figure 5.20, scenario 2). In this new scenario, with new TEF values, living *P. oceanica* leaves displayed a contribution of more than 45% to the diet of *Liocarcinus navigator*, followed by the contribution of harpactocoid copepods of about 25%. This result would much more reflect the important contribution of living leaves of some individuals, but since only 3 presented a high living leaves ingestion pattern, we hypothesized that the actual contribution of living *P. oceanica* is a compromise between the two mixing model scenarios, reflecting a modest but non-negligible of about 10% of living *P. oceanica* leaves, but also an important contribution of about 30-35% of GA and copepod food sources for *Liocarcinus navigator*. This "first order predator group" is thus very interesting, reflecting the assimilation of various crustaceans from lower trophic levels, but also of fish material, potentially from scavenging. Another result is the important assimilation of organisms which were themselves demonstrated as important consumers of dead *P. oceanica* fragments. This highlighted the transmission of the dead *P. oceanica* basal food source through different trophic levels inside our community, which is one more indication of the potentially important trophic role of *P. oceanica* detritus, supporting partly the whole macrofauna community.

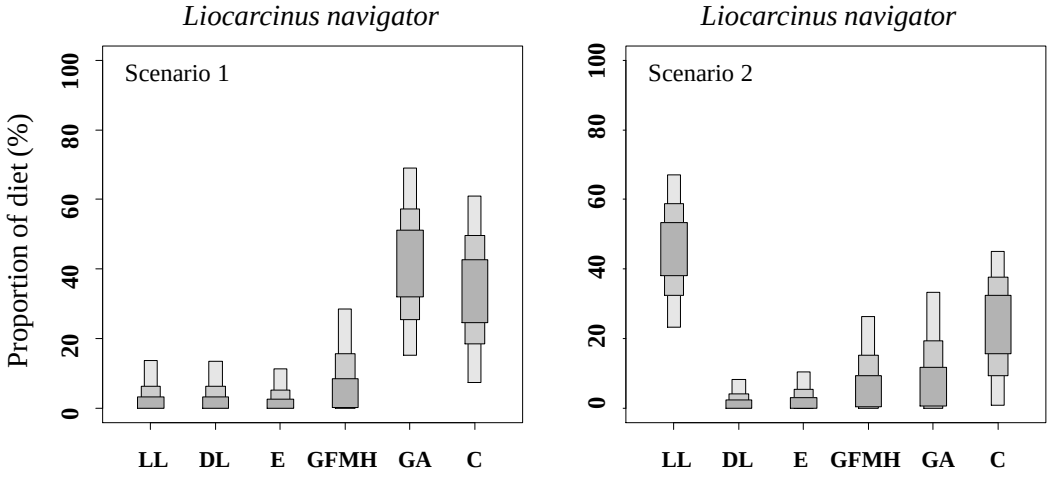


Figure 5.20: Boxplot of relative contributions of each food source to the diet of Liocarcinus navigator in the two different TEF scenarios hypothesized. Food sources codes: E= epiphytes+various brown drift macroalgae; DL= dead P. oceanica leaves; LL= living P. oceanica leaves; GA= Gammarus aequicauda; GFMH= pool of Gammarella fucicola and Melita hergensis; C= pool of the 3 most abundant species of harpacticoid copepods found in P. oceanica exported litter (Mascart, 2015). Dark grey boxes are the 50% credibility intervals, medium grey boxes are the 75% credibility intervals, and light grey boxes are the 95% credibility intervals.

The highest trophic level encountered did not include vagile macrofauna species, but fishes from the *Gobbius* genus. Nonetheless, we decided to include them to the analyses to assess the diet of these juvenile fishes, playing a role of potential "top predators" in the litter trophic web. Moreover, juvenile fishes were very abundant in *P. oceanica* litter (personal observations). Some species were also observed as adult, foraging in the litter (*e.g. Symphodus* spp., *Chelon labrosus*, *Mullus surmuletus*, *Coris julis*, *Lepadogaster* spp.,…) These juvenile *Gobius* spp., representing juvenile fishes, were above the macroinvertebrates in the isotopic space, indicating a potential predatory behavior on these organisms. This was confirmed by their gut contents, mainly containing crustacean fragments. Mixing models confirmed the second order predator status of juvenile fishes sampled during this study, reporting a massive contribution of decapod predators to their diet (81%). Even if these juvenile *Gobius* spp. could not be identified to the specific level, our results are coherent with what Hajji *et al*. (2013) found for *Gobbius niger* in Tunisia,

demonstrating that smaller individuals (< 10cm) showed a clear preference for crustacean and mollusk preys, occupying a trophic level (TL) between 3.5 and 4. This enhanced the fact that EMA invertebrate fauna constitutes an important food source for juvenile, and potentially some adult, fish species.

In conclusion, we thus described a macrofauna community based mainly on 4 food sources, epiphytes/macroalgae, *P. oceanica* detritus, meiofauna and SPOM. The species composing this community occupied defined isotopic niches except for an important omnivore group, presenting overlapping niches, indicating a certain trophic redundancy. A major result was the importance of detrital material for many species of primary consumers. The other major result is that this detrital "signal" was potentially transferred to the top of the food web through more than 2 trophic levels, which is a proof of the importance of the "detrital pathway" for the transfer of organic matter produced by the *P. oceanica* meadow itself to the coastal Mediterranean food webs through the vagile macroinvertebrates associated to the exported *P. oceanica* detritus accumulations. Potential consumption of harpacticoid copepods was also assessed, highlighting the non-negligible trophic link between the macrofauna and the meiofauna (Mascart *et al.*, 2015) in these detritus accumulations.

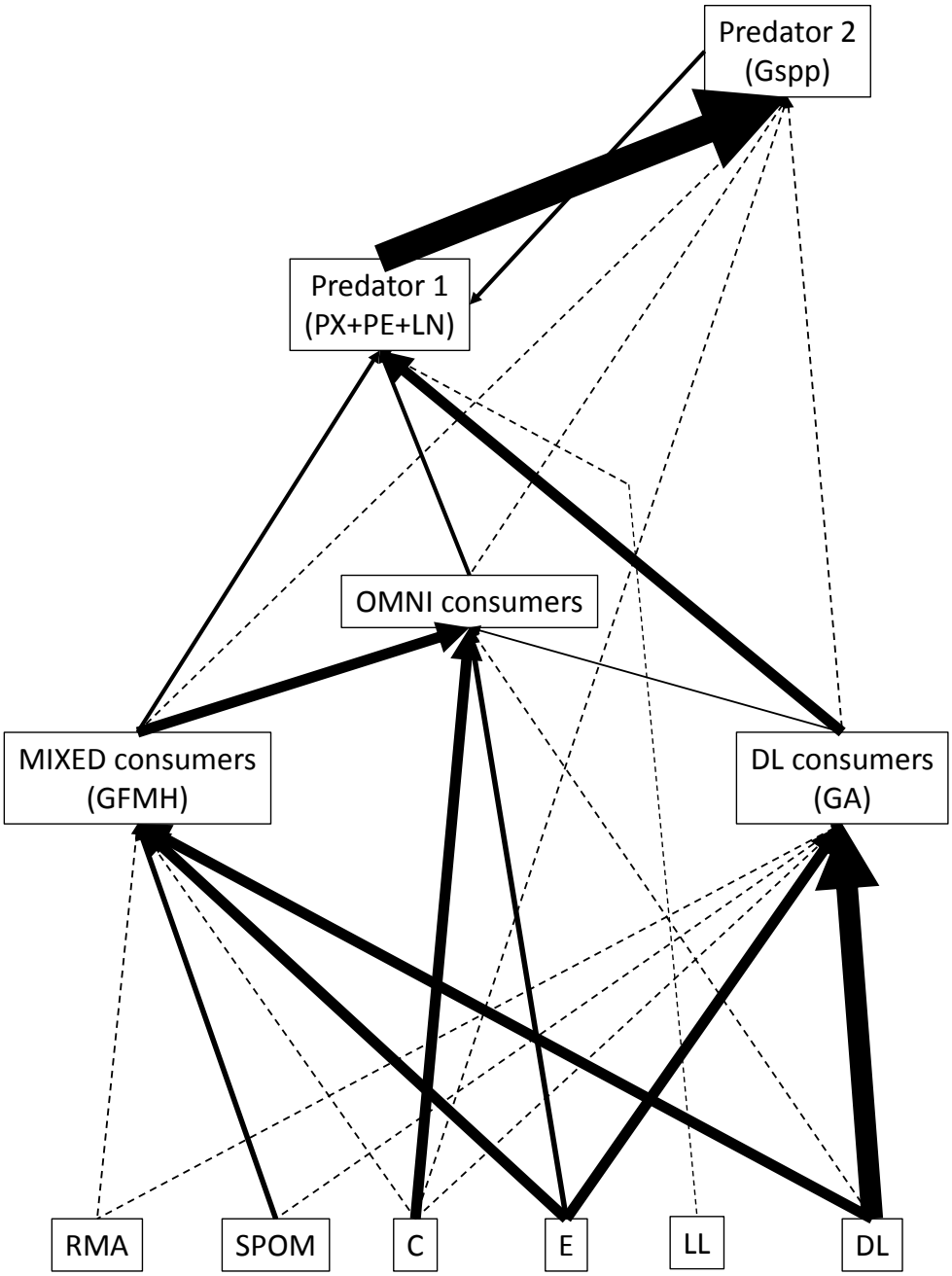

Predator 2
(Gspp)
Predator 1
(PX+PE+LN)
OMNI consumers
MIXED consumers
(GFMH)
DL consumers
(GA)
RMA
SPOM
C
E
LL
DL

Figure 5.21: conceptual model of the exported detritus food web comprising basal food sources, the two primary consumer groups, intermediate omnivore species as well as first and second order predators. Sources code: E= epiphytes+various brown drift macroalgae; LL= Living P. oceanica leaves; DL= dead P. oceanica leaves; RMA= various red drift macroalgae; SPOM= suspended organic matter; C= pool of the 3 most abundant species of harpacticoid copepods found in P. oceanica exported litter (Mascart, 2015). DL consumers= P. oceanica detritus consumers; MIXED consumers= consumers of a mixed diet composed of P. oceanica detritus, SPOM and epiphytes/macroalgae; OMNI consumers= consumers of both animal and vegetal food sources; Predator 1= first order predators consuming only animal food sources; Predator2= second order predators consuming mainly P1 food sources. Thickness of arrows is proportional to the source's contribution.

4.1.2. Seasonal variations: true diet changes of baseline shifts?

This seasonal study showed that both food sources and 5 dominant species experienced significant changes of isotopic compositions, mainly depending on the season of sampling. These seasonal changes are reported to be caused by various changes in the inorganic or organic C and N pools (Hemminga and Mateo, 1996, Costanzo *et al*., 2001). Changes observed for epiphytes might also be caused by important variations in the specific composition of the epiphytes (Mazella *et al*., 1989; Lepoint *et al.*, 2000; Prado *et al*., 2008). SIBER also showed that isotopic niches vary according to the season. Such variations of isotopic compositions of sources and invertebrates from the *P. oceanica* meadow or food sources were already reported by Hemminga and Mateo (1996) and Vizzini *et al*. (2003). This variability constitutes what we called isotopic baseline shift. These marked differences were also observed in these 5 abundant species in terms of position and space occupied in the isotopic space. However, two very different hypotheses, implying very different mechanisms could explain these observed variations. First, it could be due to actual feeding preferences modifications, *i.e.* diet changes. Second, it could be related to baseline shifts of the basal food sources. SIBER and simple stable isotope bi-plots examination could not precise one hypothesis or another. However, SIAR model was a good way to obtain that answer, as seasonal food sources isotopic compositions are taken into account in the model. Baseline shifts could thus be identified, corresponding to variations of SIBER ellipses positions and areas, but not to food sources contributions in SIAR. Actual diet changes corresponded to variations observed in both SIBER ellipses and SIAR food sources contributions.

Gammarella fucicola showed an important niche horizontal translation to the least negative side of the isotopic space between summer and autumn. This difference appeared to be caused by a diet shift, from a diet composed of equal contributions of epiphytes/macroalgae and dead *P. oceanica* fragments, to a diet composed mostly of dead *P. oceanica* fragments. After the autumn, the diet came back to what it originally was, composed of a mixture of epiphytes/macroalgae and dead *P. oceanica* fragments. Summer and winter presented niche position variations, but this was not reflected in the SIAR outputs, indicating that variations between summer and winter are potentially due to isotopic baseline shift of the food sources, and not to an actual diet change of *Gammarella fucicola*. Winter and summer niches presented no significant area variations, whereas spring niche was situated lower in the isotopic space. This potential lower position of *Gammarella fucicola* could result in the lower contribution of epiphytes to its diet in spring.

Gammarus aequicauda, the most significant *P. oceanica* detritus consumer of our community, also showed important isotopic composition and niches seasonal variations. Just like *Gammarella fucicola*, *Gammarus aequicauda* showed an important niche position horizontal translation between summer and autumn, and this niche translation to the less negative side of the isotopic space seemed due to increased consumption of dead *P. oceanica* leaves as well. One striking result is the increase in model uncertainty concerning food sources contributions between autumn and winter, potentially caused by an increased inter-individual variability, food sources less important isotopic separation and model struggling. The spring niche was different from the others and situated higher, and SIAR seemed to confirm that this was the consequence of a true diet change of *Gammarus aequicauda*, consuming much more harpacticoid copepods during that season than during all the others.

Melita hergensis showed quite important niche variations at each season, but this was not reflected in drastic diet modifications. Autumn is the only season that showed a significant diet modification. *Melita hergensis* seemed to assimilate much more *P. oceanica* detritus at that season. In all the other seasons, *Melita hergensis* showed quite constant diets. This is an important result, confirming the importance and complementarity of both approaches in order to identify isotopic compositions changes caused by diet modifications from those caused by the isotopic baseline shift of the food sources.

The decapod *Athanas nitescens*, an abundant omnivore species, showed quite narrow isotopic niches, but also showed clear seasonal variations of niche

position. The autumn niche was much more on the less negative side than the summer one, and this niche horizontal translation might actually be caused by an apparently less important contribution of GFMH pool to the diet and a much greater contribution of epiphytes/macroalgae and harpacticoid copepods. Winter was characterized by an important uncertainty of the SIAR outputs, leading to particularly important credibility intervals for the contribution of the GFMH pool and harpacticoid copepods food sources. However there seemed to be a true diet change between autumn and winter in terms of epiphytes/macroalgae consumption. An important result is the fact that, despite the season niche variations observed for *Athanas nitescens*, only autumn seemed to reveal a real diet change, while all the other seasons seemed to reflect a much more important influence of the baseline shift.

Palaemon xiphias isotopic niches experienced important variations throughout the year. However, this species seemed to have a particularly specialized and invariable diet, consuming important amounts of *Gammarus aequicauda* during almost every season, associated with a more modest but significant consumption of the other animal food sources. The exception was spring. During this season, *Palaemon xiphias* seemed to switch from *Gammarus aequicauda* preys to GFMH preys. This reflects the fact that *Palaemon xiphias*'s diet was quite constant. Its favorite preys seemed to be *Gammarus aequicauda*, which was itself subject to important diet modifications. The diet modifications of *Gammarus aequicauda* might explain the niche variations observed for *Palaemon xhipias*, situated above *Gammarus aequicauda* in the isotopic space and in the food web.

These seasonal results from both SIBER ellipses and SIAR model were particularly interesting and confirmed that 5 of the most frequent macrofauna species encountered in the exported *P. oceanica* litter accumulations were subject to diet modifications throughout the year. However, we also demonstrated that variations observed in terms of niche position and size might not always reflect real diet changes, but sometimes only baseline shifts of the food sources. In such cases, ellipses modifications reflect only that consumers' isotopic composition followed the isotopic composition of their food sources. These results indicated that careful food sources sampling simultaneously to community sampling is compulsory to identify diet changes and distinguish them from simple baseline shifts.

4.2. A weekly sampling of two very abundant exported litter amphipods: the impact of two dramatic resource pulse events

4.2.1. General patterns

This weekly study demonstrated a relative stability of the gut contents and stable isotope patterns during the 10 sampling dates. The only general pattern was a 1 ‰ decrease of both species and food sources between the January period and the April period. Isotopic values in the September-January period were in the range of values found for autumn during the seasonal part of the thesis, while the values from the April-June period were in accordance with the range of values found for the spring period in the seasonal part. The general feeding patterns of both species were identical to what was found during the seasonal part and to literature (Lepoint *et al*., 2006; Michel *et al*., 2015). *Gammarella fucicola* ingested mostly algal material while *Gammarus aequicauda* ingested mostly dead *P. oceanica* fragments. SIAR outputs were in accordance with previous results concerning both species, with *Gammarella fucicola* displaying a mixed diet with important contributions of both epiphytes/algae and dead *P. oceanica* fragments, and *Gammarus aequicauda* assimilating mostly dead *P. oceanica* fragments.

4.2.2. Pulsed events: what consequences on amphipod diets?

As mentioned earlier in this chapter (and in Chapter 3), 2 strong, brief and random events occurred during this weekly sampling, one in November 2012, the other one in May 2013. Due to these characteristics, these events were considered as true resource pulses (*sensu* Ostfeld and Keesing, 2000). These events corresponded to two major stormy events characterized in detail in Chapter 3.

These 2 events were the only moments when dietary parameters displayed important variations. The NM-MDS and ANOSIM analysis showed that ingestion patterns changed significantly just after these two resource pulses, corresponding to increased ingestion of dead leaves. SIBER ellipses corroborated that important changes occurred, showing niche position horizontal changes to the less negative side of the isotopic space. SIAR outputs demonstrated that these niche variations corresponded to true diet modification

after the resource pulses. Both species assimilated more dead *P. oceanica* leaves after these resource pulses, which seemed coherent with the results of the seasonal study, and literature. Indeed, *Gammarus aequicauda* was described as quite a specialized detritivore species, mainly assimilating its organic matter from dead *P. oceanica* litter. Since Yang *et al*. (2008) and Yee and Juliano (2012) stated that mobile specialists could take much more advantage of resource pulses than other organisms and that detritus feeders could benefit more than any other feeding group from such important pulses, it was hypothesized that *Gammarus aequicauda* responded to the increased availability of its preferred food source by focusing on it for its diet. The diet switch of *Gammarella fucicola*, described as a more generalist species assimilating both epiphytes/macroalgae and dead *P. oceanica* leaves, could be a possible response of this species to the resource pulses. Indeed, this would be congruent with Chapter 3 and what Ostfeld and Keesing (2008) and Nowlin *et al*. (2008) suggested for terrestrial generalist species which could be the most subject to resource pulse utilization through diet switching. These dietary responses could potentially be another demonstration of the common characteristics shared by exported *P. oceanica* litter accumulations and terrestrial detrital-driven ecosystems.

In conclusion, this weekly study demonstrated that dietary responses to strong resource pulses could potentially be observed for the two most abundant amphipods encountered in the exported *P. oceanica* detritus accumulations which are very important in terms of organic matter flux. The observed responses could highlight the potential effect of resource pulses on macrofauna, but also on *P. oceanica* litter degradation itself. Indeed, frequent but stochastic resource pulses could enhance the long-term degradation, fragmentation and assimilation of dead *P. oceanica* leaves through ingestion and assimilation patterns modification of macrofauna species such as *Gammarella fucicola* and *Gammarus aequicauda*. It could thus be hypothesized that, in addition to the inherent hydrodynamics-driven mechanical fragmentation occurring during such stormy events, dietary responses of the litter macrofauna might also potentially play an important role in global litter degradation and organic matter transfer from the *P. oceanica* meadow to the coastal ecosystems, through the "detrital pathway".

Chapter 6

General discussion, novel findings and future developments

The general aim of this PhD thesis was to characterize the vagile macrofauna community present inside exported *Posidonia oceanica* litter accumulations in the angle of resource pulses and dynamic conditions potentially occurring inside their habitat, and determine the trophic ecology of the most abundant species encountered in this coastal Mediterranean macrophytodetritus habitat.

This last chapter will be divided into 3 main sections. In the first one, we will summarize and integrate the main advances and findings developed in the 3 preceding chapters. In the second one, we will detach ourselves a little bit from the *P. oceanica* point of view and put this thesis and its main outcomes in the general resource pulse perspective. In the third one we will finally discuss the importance of exported *P. oceanica* litter in the coastal organic matter flux and as a habitat and food source for the macrofauna community. A last part detailing general conclusions, novel findings, and future perspectives will put an end to this manuscript.

1. Macrophytodetritus: a variable habitat for a variable invertebrate community

1.1. *Posidonia oceanica* and the detrital pathway

The Neptune grass *Posidonia oceanica* is a highly productive coastal plant which covers most of the Mediterranean costal (0-40 m) zones (Ott, 1980; Pergent and Pergent-Martini, 1991; Pergent *et al*., 1994; Pergent-Martini *et al*., 1994; Pasqualini *et al*., 1998; Gobert *et al*., 2006). Often ranked among the most productive ecosystems on the planet (Duarte and Chiscano, 1999), Neptune grass meadows present a sort of paradox: direct herbivory consumption of green leaves is rarely important (Thomas *et al*., 2005). This massive organic matter source of the Mediterranean coastal areas is so indigestible and contains so much deterrent and phenolic compounds (Duarte, 1990; Vergès *et al*., 2007; Vizzini, 2009) that only a few fishes and sea urchins are able to ingest and assimilate it with various efficiencies. The massive autumnal leaves shedding is the beginning of a new life for these unconsumed leaves, entering the "detrital pathway". In this new detrital life, *P. oceanica* decaying leaves will be transported to various adjacent marine and terrestrial places (e.g. beach "banquettes" in Boudouresque and Meinesz, 1982). In the

marine environment, dead *P. oceanica* leaves will often deposit on unvegetated areas such as bare sand patches to form exported *P. oceanica* detritus accumulations. There, mixed with drift algae, uprooted living *P. oceanica* leaves and shoots, fine sediment and dead organisms, the *P. oceanica* dead leaves form what is called exported litter accumulations (Anesio *et al*., 2003; Boudouresque *et al*., 2006). General studies about detritus and their impact on unvegetated areas such as underwater sandy patches demonstrated the structuring role of the detritus accumulations on these areas in terms of habitat creation, physico-chemical conditions modification and food provider (Hyndes *et al*., 2014).

1.2. Temporal variation time scale: a key concept to comprehend exported litter accumulations

We demonstrated that even if exported litter was almost always present on the studied sand patches, important temporal fluctuation of biomass, composition, fragmentations and cover occurred all year long. We also demonstrated that these important variations were observable at the annual and seasonal time scale, but also at a much shorter time scale, from one week to another. One of the most important fluctuations was the massive litter input occurring every year in autumn, corresponding to this already well known annual leaves shedding event (Cebrian *et al*., 1997). After that autumnal maximum of biomass, we demonstrated that litter biomass was gradually decreasing to reach a minimum in spring. This continuous biomass decrease occurred simultaneously to important litter degradation and fragmentation during winter, spring and summer. This general pattern was in accordance with the negative exponential decomposition pattern observed by Mateo *et al*. (2006) and comprising leaching of dissolved compounds from decaying material, decomposition and fragmentation of refractory phases. Litter composition and complexity was also highly variable, showing a maximum in summer, with a litter composed of a mixture of degraded *P. oceanica* leaves, various drift macroalgae, abundant epiphytes and living *P. oceanica* leaves. In parallel with these drastic modifications of the purely physical parameters of the exported litter habitat, we demonstrated its role in terms of physico-chemical driver for the O_2 and nutrients concentrations (NO_X, NH_4 and PO_4). While water column and water just above the litter accumulations showed quite constant physico-chemical parameters all year long, water inside the litter was

proved to be highly variable, indicating a potential "buffer" role of litter between the water column and the underlying sediment (Fig). We demonstrated that O_2 and nutrients concentrations varied a lot inside the exported litter accumulations, but that even if significantly linked to weather conditions, no clear seasonal pattern could be emphasized. Nutrients rich and hypoxic conditions were more frequent in spring and summer, but were nonetheless also randomly encountered in winter or autumn. These parameters were thus hypothesized to depend on more than just the litter itself.

However, we also demonstrated that another weekly time scale variation of litter parameters and physico-chemical parameters was observable. Weekly measurements of these parameters reflected a clear pattern with maximum litter biomass and minimum fragmentation in autumn, followed by a continuous and progressive biomass decrease and fragmentation increase between autumn and spring. At this weekly time scale, litter O_2 concentration was strongly linked to litter biomass, indicating the potential role of litter itself as a temporary driver of the physico-chemical conditions inside a litter accumulation.

The difference observed between the two different time scales leads to one of the main hypotheses of this PhD, which is that conditions inside an exported litter accumulation could potentially be regulated by different processes, playing important roles alternately at different moments of the year. Autumn, winter and early spring would be seasons when litter abundance is the main driver of the physico-chemical parameters such as litter O_2 concentration. That period of the year, low temperatures and microbial activity (Sarmento *et al*., 2010; Champenois and Borges, 2012) associated to perturbed weather, is not favorable to induce oxygen level variations inside the litter accumulation. However, an abundant and thick litter could act as a barrier against the disrupted and well-oxygenated water from the water column and favor clam and steady conditions inside the litter accumulation. If maintained long enough, these calm conditions might influence the oxygen level inside the litter accumulation and ultimately provoke hypoxia. However, litter biomass as driver of physico-chemical conditions might prevail on other parameters only during the "cold season". In late spring and summer, the temperature rises, stimulating the microbial activity and the organic matter decomposition (Rice and Tenore, 1981) and the weather is much calmer. These conditions are very favorable for hypoxia to develop. During the "hot season", an abundant and thick litter could not be "required" anymore to influence the physico-chemical parameters. At that period of the year, hypoxia could thus occur no matter the

litter abundance if the other parameters are favorable. This hypothesis was particularly interesting since being aware of the existence of different processes occurring at different periods of the year could be very important in terms of interpretation.

1.3. Vagile macrofauna: an inconstantly globally constant community

This PhD demonstrated the continuous presence of an abundant macrofauna community inside exported *P. oceanica* litter accumulations. We described a community largely dominated by arthropods (77%), annelids (12%) and mollusks (7%), which on one hand corresponded to what Gallmetzer *et al*. (2005) described in his preliminary study, but was very different on the other hand, since we described a community composed of 115 species, while Gallmetzer *et al*. (2005) mentioned only 80 species. Despite the presence of 115 species, only 19 represented 90% of the community, meaning that only a few species are tremendously dominant in this community, which was particularly the case for the amphipod *Gammarella fucicola*, representing 40-55% of the whole community.

This community was globally highly variable and very constant at the same time. It was variable because most of the 19 dominant species showed important seasonal variations and because the global biodiversity was also highly variable, showing a maximum in summer and a minimum in winter. But despite these important species-specific variations, the global community actually experienced few changes at a high taxa level in terms of global abundance, relative abundance or dominance, and was present throughout the year at both sampling sites.

1.4. Environmental parameters, macrofauna and stratification: from hypotheses to experimental proof

Despite this "constant-inconstant" characteristic, several environmental measured parameters appeared to be very important drivers for some species. Litter O_2 concentration and NH_4 concentration seemed to be one important influencing factor for several very abundant species. Even if NH_4 is known to be negatively linked to oxygen concentration through the organic matter decomposition, anaerobic bacterial nitrogen fixation and nitrate reduction to ammonium by NR-SOB (linked to the oxidation of H_2S to SO_4^{2-}) (Gruber, 2004; Bonaglia *et al.*, 2014), no significant collinearity was found in statistical analysis for the seasonal and weekly sampling. Oxygen thus seemed to impact exported litter macrofauna species in 3 different ways. First, most species, including the two most abundant amphipods *Gammarella fucicola* and *Gammarus aequicauda*, did not seem impacted at all by these parameters. Secondly, some species were positively linked to litter O_2 concentration. And finally, two or three species were negatively influenced by litter O_2 concentration. The hypothesis concerning the impact of O_2 on these species was that hypoxia tolerant species only colonized the litter accumulations during hypoxic periods, while hypoxia intolerant ones simply did the opposite and colonized *P. oceanica* detritus only during well-oxygenated periods.

However, this hypothesis could be considered incomplete since many organisms, presumably not really hypoxia tolerant (Diaz and Rosenberg, 1995; Gambi *et al*., 2009; Levin *et al*., 2009; Hernàndez-Miranda *et al*., 2012; Veas *et al*., 2012) were found all year round in the exported litter accumulations. Moreover, even if some species could be simply well adapted to detrital habitats, and complete their life cycle independently from litter O_2 concentration, one question remained: how did these presumed hypoxia intolerant species cope with low oxygen conditions occurring inside their habitat? A part of the answer was given by the in situ experimentation performed in October 2014 (see Chapter 4). Indeed, we demonstrated for the first time that physico-chemical conditions inside a *P. oceanica* litter accumulation are not similar in every layer of the accumulation. Top layers present conditions approaching those found in the water column in terms of O_2 and nutrients concentrations, while bottom layers, deeper in the accumulation, presented very different conditions, with O_2 levels reaching hypoxia threshold and dramatically increasing nutrients. This stratification occurred very quickly,

even in autumn, in less than 48 hours, indicating that even in seasons unfavorable in terms of hypoxia, short periods of calm weather are sufficient to induce stratification and ultimately hypoxia in deep layers of litter. This might fill partly the gap of our hypothesis. Indeed, even during moments we considered hypoxic, oxygenated layers might remain available for hypoxia intolerant organisms, explaining the presence of these intolerant species even when conditions are hypoxic in deeper layers. This spatial segregation of species would also explain partly why summer is the season presenting the highest biodiversity. Indeed, literature concerning encountered several species life cycles (or closely related species) indicates that their maximum abundance occurs in summer, when conditions (mainly temperature) are favorable for growth, reproduction and feeding (Karakiri and Nicolaidou, 1987; Gueraro and Ribera, 2000; Prato and Biandolino, 2003; Hyne *et al*., 2005). Since litter is not completely hypoxic during low oxygen periods, these species, although not especially tolerant to hypoxia, could survive in the oxic top layers even during summer.

More than litter biomass, litter "complexity" could be one important driver of global biodiversity in the exported *P. oceanica* litter macrofauna community. Indeed, fragmented dead leaves associated to the presence of living leaves and rhizomes and drift macroalgae enhance the complexity of the litter accumulation itself, and since complexity is a major parameter for invertebrates diversity and abundance in various environments (Bell *et al*., 2001; Fahrig, 2003; Hovel, 2003; Atilla *et al*., 2005; Matias, 2013) the high complexity present in summer associated to the simultaneous stratification of environmental conditions could partly explain the high diversity found at that moment of the year.

Another potential effect is the effect of *P. oceanica* in terms of physical habitat availability. Indeed, since *Gammarella fucicola* presented a weak but significant positive link with litter biomass, it could be possible that for some other species also dependent on habitat availability, litter quantity present on the accumulation constitutes an important factor influencing their presence and abundance.

We thus highlighted 3 main different strategies. First, species like *Gammarella fucicola,* potentially representing an important part of the encountered species, don't really respond to the parameters we measured, indicating a sort of indifference for the O_2 parameter and that other regulators influence their life cycles. Habitat availability could be one of them, as well as

temperature, prey-predator relationships or natural life cycle. Secondly, hypoxia tolerant species such as the leptostracean *Nebalia strausi* or the decapod *Athanas nitescens* present important hypoxia tolerance and colonize only oxygen-depleted layers of the exported *P. oceanica* litter. These species avoid the oxic zones, avoiding at the same time competition and predation occurring in these more "crowded" layers. Thirdly, hypoxia intolerant species like *Melita hergensis* or *Microdeutopus chelifer* colonize only oxic zones of litter accumulations. These species, potentially more vulnerable to competition and predation during stratified hypoxic moments, see their abundances decrease dramatically during these periods.

These results were congruent with the recent study of Mascart *et al*. (2015) who found nauplius larvae to be very abundant in winter-spring, juvenile amphipods to be more abundant in spring and adult copepods to be highly abundant in spring-summer. These results are in accordance with the general increased diversity and abundance observed in late spring and summer of the macrofauna, indicating larval recruitment at the end of winter and spring, and adults being highly abundant in the summer season. Moreover, Mascart *et al*. (2015) also stated that meiofauna community was encountered throughout the year inside exported *P. oceanica* litter accumulations, just like the macrofauna. This result indicated that seagrass macrophytodetritus are a habitat for quite diverse and linked meiofauna and macrofauna communities, which are subject to temporal variations caused by different environmental parameters.

1.5. Feeding habits are another variation source

In addition to the temporal physico-chemical parameters and biological parameters variations, the litter macrofauna food web was demonstrated to be also quite inconstant.

The study of the global community allowed the characterization of a multi trophic level food web, based on *P. oceanica* detritus, algae, epiphytes, suspended organic matter and meiofauna. One general finding was that dead *P. oceanica* leaves play a double role on unvegetated areas, first as a habitat provider for various invertebrates species, and secondly as a food source provider for a majority of primary and secondary consumers.

Despite this general statement, non-negligible temporal variations of isotopic values and isotopic niche parameters were observed in the 5 most

sampled species, *Gammarella fucicola, Gammarus aequicauda, Melita hergensis, Athanas nitescens* and *Palaemon xiphias*. SIAR mixing model allowed a clear separation between baseline-driven modification and true diet-driven modification. Since litter composition and abundance also experienced important temporal variations, we hypothesized that these diet modifications might be linked to the different food sources abundance variations.

The species classified earlier in this PhD as "mixed-diet" feeders such as *Gammarella fucicola* and *Melita hergensis*, were able to ingest and assimilate both "epiphytes/macroaglae" and dead *P. oceanica* leaves. During autumn and spring, these species assimilate a larger part of dead *P. oceanica* leaves and less epiphytes/macroalgae. In summer and winter a much more mixed diet was observed, with high contributions of epiphytes/macroalgae. Since dead *P. oceanica* experienced a major abundance increase in autumn due to the autumnal leaves shedding (Bay, 1984) it seemed coherent that these two very abundant generalist species assimilate more of this overabundant food source at that moment of the year. In spring, dead leaves are much less abundant, but much more degraded and potentially of higher nutritional quality and palatability for these species (lower C:N ratio). The high assimilation of this food source despite its lower abundance might be a sign that different processes occurred. In autumn, dead *P. oceanica* leaves are of lower nutritional quality and palatability but so abundant that "mixed diet" feeders could potentially take advantage of this highly abundant food source by increasing their ingestion and digestion time. They could ingest dead *P. oceanica* fragments and digest them longer to cope with the low nutritional value of this food source and thus still present high assimilation contributions. Literature mentioned this phenomenon for many different organisms fed with food sources of different qualities (Taghon, 1981; Prop and Vulink, 1992). This longer digestion time could induce an over-representation of the less palatable item in their digestive tracts. We observed similar results, with higher presence of dead *P. oceanica* fragments in gut contents observed in autumn compared to gut contents from spring. On the other hand, epiphytes/macroalgae food source was less assimilated in autumn and spring. Epiphytes were nearly absent in spring, potentially explaining the very low contribution observed for that food source at that season. However, epiphytes were much more abundant in autumn, indicating that the two species preferred low nutritional quality food source at that moment of the year, independently of the epiphytes/macroalgae abundance, yet of better nutritional quality (low C:N ratio).

Epiphytes/macroalgae food source is in reality a very heterogeneous food source due to the presence of epifauna, epiflora and macroalgae in various proportions throughout the year (Lepoint *et al.*, 2000; Michel, 2011; Piazzi *et al*., 2016). Since our results showed that drift macroalgae were absent of the litter accumulation in autumn 2011, almost only epiphytes and probably epiflora constituted the epiphyte/macroalgae food source at that season, which was confirmed by the high C:N ratio of epiphytes in autumn 2011. It could thus be hypothesized that epiphyte composition present in autumn 2011 was simply not adapted to *Gammarella fucicola* and *Melita hergensis* diet preferences. This could also explain why they fed preferentially on the highly abundant but low nutritional quality food source at that moment of the year.

Gammarus aequicauda, which is already known to ingest and assimilate a large proportion of dead *P. oceanica* detritus (Lepoint *et al*., 2006; Michel *et al*., 2015), was found to ingest and assimilate globally about 60% of dead *P. oceanica* detritus. However, this assimilation was variable depending on the moment of the year. Summer 2011 and spring 2012 showed interesting patterns. In summer 2011, *Gammarus aequicauda* presented a mixed diet, with important contributions of both dead *P. oceanica* and epiphytes/macroalgae. Since this species was demonstrated to be quite specialized in detritus assimilation, summer could be a season of limited food availability. Indeed, summer was the season when litter biomass was at its minimum value (Chapter 3). Since summer was also a season favorable for hypoxic and stratified conditions, and since *Gammarus aequicauda* was not encountered during the "stratification" *in situ* experiment, questions remain about its ability to colonize every layer during hypoxic periods. *Gammarus aequicauda* could potentially be confined to oxygenated layers and thus be submitted to increased competition, especially competition for food at that moment of high macrofauna diversity and abundance but lack of food. To cope with these limiting conditions, *Gammarus aequicauda* could potentially simply migrate to other adjacent habitats like the *P. oceanica* meadow itself, which? has been found in literature (Gambi *et al*., 1992; Michel *et al*., 2015). This active migration out of the litter could partly explain the very low abundance of *Gammarus aequicauda* in summer (Chapter 3). This very low abundance could also be caused by the important competition and predation impacting *Gammarus aequicauda*. However, a few *Gammarus aequicauda* remained in the exported *P. oceanica* accumulations in summer and these remaining individuals might have needed to shift to a less preferred food source to cope

with unfavorable living and feeding conditions. These strategies of diet modification and migration due to habitat modifications have been observed for other specialist organisms (Lofaldli *et al*., 1992; Watt *et al*., 2013) and could thus explain what was observed in summer for *Gammarus aequicauda*. Spring 2012 was also interesting, since *Gammarus aequicauda* diet also contained a non-negligible amount (30%) of harpacticoid copepods, indicating the increased tendency of *Gammarus* to predation. Since *Gammarus aequicauda* abundance displayed maximum values in spring 2012, and since sampled individuals were bigger in spring than in the other seasons (personal visual observation), it could be hypothesized that the larger individuals present more omnivore diet preferences in spring. This hypothesis would be in accordance with the species-specific correlation found between body size and trophic position of various marine organisms (Layman *et al*., 2013; Romero-Romero *et al.*, 2016) and this could explain the higher contribution of harpacticoid copepods in *Gammarus aequicauda* diet during the season when sampled individuals are the biggest.

Athanas nitescens presented only one important diet change in autumn 2011. During that season, this omnivore species appeared to neglect one of its preferred food sources, the GFMH pool, to feed more on epiphytes/macroalgae. This diet variation could potentially be linked to the massive input of litter and epiphytes in autumn on the sampling sites, indicating a shift of *Athanas nitescens* towards a very abundant food source. The important and quite constant contribution of harpactocoid copepods to the diet of *Athanas nitescens* also demonstrated the trophic link existing between the meiofauna community and the macrofauna.

Palaemon xiphias was the most abundant true carnivore invertebrate species sampled in the exported *P. oceanica* litter during this PhD. This big predatory shrimp presented a mixed diet composed of all possible animal food sources. This species, already known to feed on various amphipods, isopods, copepods and decapods (Guerao, 1994), also presented a tendency to assimilate a non-negligible part of fishes, indicating a potential scavenger behavior in this habitat mainly composed of decaying vegetal and animal organic matter. The only important diet change is the lower contribution of the amphipod *Gammarus aequicauda* and the higher contribution of the GFMH pool to its diet in spring 2012. As mentioned above, *Gammarus aequicauda* individuals were bigger in spring 2012. This could potentially make them harder to catch for *Palaemon xiphias* since bigger *Gammarus aequicauda* would potentially

swim faster. With the important contributions of *Gammarus aequicauda* and the lower but non-negligible contribution of harpactocoid copepods to its diet, *Palaemon xiphias* demonstrated the trophic link existing between meiofauna and macrofauna, but also between the *P. oceanica* meadow and the exported detritus community, showing the propagation of the "litter signal", from the dead leaves to the upper trophic levels of the food web.

2. Resource pulses are important structuring processes

2.1. Community potential impact

In addition to the other temporal variations observed inside the exported *P. oceanica* litter accumulations during this PhD, another type of variation was detected and observed. These "variations" were caused by stormy weather conditions and were very short and random episodes of overabundance of dead leaves. These characteristics enter fully in the standards proposed by Ostfeld and Keesing (2000) to define "resource pulses". This type of event has been demonstrated to play an important role in the structuration and functioning of various ecosystems (Yang *et al*., 2008; Yang *et al.*, 2010). Even if exported *P. oceanica* detritus accumulations are known to be transient and inconstant habitats, this PhD was, to our current knowledge, the first attempt to evaluate the potential impact of resource pulses on this compartment and on the encountered vagile macrofauna community.

During the weekly sampling part of this PhD, we identified 2 major stormy events potentially constituting resource pulses, one in November and the other in May. Even if storms occur throughout the year in the studied area, storms are more frequent between autumn and spring, while summer is generally quite calm. These two events occurred during strong north-eastern wind storms, confirming the strong link existing between exported litter accumulations and weather and local hydrodynamics (see Chapter 3).

These stormy events caused mainly 2 different phenomena: massive litter departure from the accumulation, or massive litter input on the accumulation. These 2 types of events happened in 12-14 hours, confirming their fast and brief character. The two types of events impacted the litter habitat and the associated macrofauna very differently.

The first type of event occurred only on November 1st. It corresponded to massive litter departure from the accumulation, inducing a massive concentration of the macrofauna inside the remaining litter patches, more precisely, of the very abundant amphipod species *Gammarella fucicola*. Most other dominant species showed no response at all to this litter departure However, species richness decreased by about 33% after that event, indicating the departure (migration or death) of several less abundant species. This highlighted the potential extreme adaptation and dependence of *Gammarella fucicola* to the litter habitat. Indeed, habitat loss is known to generally negatively impact various ecosystems (Eggleston *et al.*, 1999; Hovel, 2003; Farhig, 2003; Devore, 2014) but sort of "refuge effect" has already been observed for very specialized species (Eggleston *et al*., 1999). While other species departed from the remaining fragmented litter patches to other adjacent habitats (*e.g*. *P. oceanica* meadow, vegetated rocky areas…), *Gammarella fucicola* concentrated in these "refuge patches", potentially enduring increased habitat and food source competition due to the higher density encountered (Buchmann *et al*., 2013). This "a-captain-always-goes-down-with-his-ship" type of response of *Gammarella fucicola* preferring to stay in the litter instead of migrating to less "crowded" areas, was a potential indication of its dependence to the litter habitat, preferring a temporary increased competition to migration. One last result to remember is that even if *Gammarella fucicola* concentrated in the remaining litter and if several modestly abundant species migrated out of the remaining litter, most abundant species displayed no significant response to this litter departure. The fact that many abundant species displayed neither increase nor decrease of abundance could potentially indicate that some species could simply be passively carried away along with the departing dead leaves to other accumulation places during storms. This would explain the quite constant density patterns observed for these species.

The second type of event, corresponding to massive "fresh" litter input on the accumulations corresponded really to what Ostfeld and Keesing (2000) defined as true "resource pulses". Responses of the community were completely different for that type of event. Most species, including *Gammarella fucicola* showed no clear density response to this important litter input, while only two other very abundant species, the amphipod *Gammarus aequicauda* and the leptostracean *Nebalia strausi* showed marked density increase just after the two events that occurred on November 11th and May 13th. Since this type of event was corresponding perfectly to the definition of true

resource pulses, we tried to experimentally demonstrate its potential effects on the *P. oceanica* litter macrofauna community in *in situ* mesocosms. We tested two different treatments: defaunated litter input (litter only without macrofauna), and "natural" litter (litter with associated macrofauna). First, the experimental design in itself (mesocosm effect) was negligible since macrofauna samples taken in the litter accumulation outside the mesocosms at the end of the 14-day experiment were not significantly different from the macrofauna encountered inside the control mesocosms at the end of the experimentation. Secondly, the two treatments were significantly different from the controls, but not from each other, indicating that the principal effect was the addition of "fresh" litter to the mesocosms. This absence of effect of the input of highly nutritive quality animal resources was surprising. Not to be redundant, refer to the §4.2 in Chapter 4, to examine the hypothesis potentially explaining this apparently negligible effect. The effects of the addition of "fresh" litter to the mesocosms were quite important for the abundant and dominant species. Global diversity increased in both treatments, while densities of *Gammarus aequicauda*, *Nebalia strausi*, *Palaemon xiphias* and *Athanas nitescens* increased importantly. On the other hand, the most abundant amphipod, *Gammarella fucicola*, displayed a massive density decrease inside both treatments. The 7-fold increase of density of the most important true detritivore amphipod, *Gammarus aequicauda*, during this experiment was also observed during the weekly sampling after November and May events.

This result was really what changed our vision of the potential impact of resource pulses because we realized that this result had already been observed in apparently very different ecosystems: terrestrial macrophyte-driven ecosystems (Nowlin *et al*., 2008). Indeed, it appeared that exported *P.oceanica* litter accumulations present important similarities with terrestrial macrophyte-driven ecosystems from the resource pulses angle. Like most terrestrial plants, *Posidonia oceanica* is a flower plant which sheds its leaves in autumn. Like most terrestrial macrophyte-driven ecosystems, these leaves form an important detrital pool with associated well-developed "detrital" food webs. Observing our results from this new angle was very important for our comprehension of what could happen during litter pulses. After natural and experimental resource pulses, we thus observed a drastic increase of *Gammarus aequicauda*, which was in accordance with Nowlin *et al*. (2008), Yang *et al*. (2008) and Yee and Juliano (2012) who demonstrated that detritus feeders and mobile specialists take much more advantage of resource pulses than any other organism in terms

of food source availability. We also observed an important increase of *Nebalia strausi* density after both natural and experimental resource pulses. Since *Nebalia strausi* was absent from oxygenated samples/layers and strictly bound to hypoxic conditions, we suspected that the litter input of a resource pulse favored the establishment of a hypoxic layer inside the two treatments, inducing the active colonization of these layers by *Nebalia strausi*. We also observed the increase of density of two shrimps, *Palaemon xiphias* and *Athanas nitescens* during the experimental litter pulse. *Palaemon xiphias* is known to feed on various crustacean species, and the density increase could be linked to the drastic increase of abundance of *Gammarus aequicauda,* one of its preferred food sources. This would be in accordance with literature (Ostfeld and Keesing, 2000; Chesson *et al*., 2008; Yang *et al*., 2010), which demonstrated the lagged top-down response of predators after a resource pulse. This lagged response can occur quite fast when the community is composed of organisms with short generation times (Nowlin *et al*., 2008), which is what we observed here in less than 14 days, and that shorter lag is typically a characteristic of aquatic ecosystems subject to resource pulses. *Athanas nitescens* could also respond to the increase of abundance of *Gammarus aequicauda* but also potentially to the increased abundance of epiphytes, another potential food source of this omnivore species. *Athanas nitescens* was also demonstrated to be linked to hypoxic periods/layers, and the potential presence of hypoxic conditions in the two treatments, caused by the litter input, could also influence the colonization of the mesocosms by this species. Finally, we observed another important result: the massive drop of *Gammarus aequicauda* density after the experimental pulse. This drop was massive, and *Gammarella fucicola,* which was apparently always tremendously dominant in our seasonal and weekly samples, represented only 30-32% of the community in the two treatments, while it represented 65-70% of the same community in the control. This drop was hypothesized to be potentially caused by different things. First, as it was mentioned earlier when discussing *Gammarus aequicauda,* Nowlin *et al*. (2008), Yang *et al*. (2008) and Yee and Juliano (2012) demonstrated that generalist herbivore species don't respond as positively as detritivore specialists to resource pulses. The observed density increase of *Gammarus aequicauda* could impact *Gammarella fucicola* in terms of increased competition. Moreover, the density increase of *Palaemon xiphias* and *Athanas nitescens* might also have played a role in terms of increased predation. Associated to the potential increase of competitiveness of

Gammarus aequicauda for space and food, this could explain the density decrease observed for *Gammarella fucicola*, corresponding to active migration out of the mesocosms, or decreased survival inside.

2.2. Trophic ecology potential impact

In addition to the community impact, results concerning the weekly sampling also highlighted dietary effects, potentially linked to the resource pulses events. These trophic changes were observed for *Gammarella fucicola* and *Gammarus aequicauda*, the two most abundant species of the litter macrofauna community. First, despite the important litter departure of November 1st, the two species did not seem to experience ingestion of assimilation pattern modifications; their abundance patterns were modified, but apparently not their dietary preferences. The result was different in the case of the massive litter inputs of both November 11th and May 13th events, resulting in measurable trophic changes.

Indeed, after both events, *Gammarella fucicola* and *Gammarus aequicauda* presented a non-negligible horizontal isotopic niche shift to the least negative side of the isotopic space. The fact that niches before and after the pulses events did not present overlap confirmed the importance of these modifications. Moreover, SIAR outputs confirmed that these niches modifications were due to real diet modifications and not only to baseline shifts of the food sources. After the two massive litter input events, both species appeared to assimilate a more important part of their organic matter from dead *P. oceanica* leaves. For *Gammarus aequicauda*, this result confirmed what was observed for the community-level changes in natural and experimental conditions. *Gammarus aequicauda* potentially focused on its preferred food source after the resource pulses, resulting in a potentially increased competitiveness and an increase of its density inside the two treatments. *Gammarella fucicola* was demonstrated to be a "mixed diet" feeder in Chapter 5. This diet modification was congruent with Ostfeld and Keesing (2000) and Nowlin *et al*. (2008), who stated that many generalist species respond to resource pulses through diet switching. However, this diet switch might also be a potential explanation of what was observed in terms of density decrease. Dead *P.oceanica* leaves might not be an optimal food source for *Gammarella fucicola* when consumed alone, and this would be in accordance with literature (Evans et al., 1999; Cruz-Rivera and Hay 2000; Moreau et al., 2003). These

authors stated that generalist species display improved fitness when consuming mixed diet, sometimes including alternative and non-optimal food sources. This reduced fitness after resource pulses could impact *Gammarella fucicola* directly with increased mortality or decreased competitiveness, or indirectly, with increased top-down effect of predation due to the decreased fitness.

Resource pulses could thus potentially impact the exported *P. oceanica* litter accumulations in terms of habitat and food availability, in terms of macrofauna assemblage but also in terms of diet modifications. *P. oceanica* exported litter accumulations presented similarities with both terrestrial and aquatic environments. These "intermediate" characteristics induced different potential responses. Some were often observed in terrestrial ecosystems, and several others were often observed in aquatic ecosystems, making *P. oceanica* exported litter accumulations a peculiar compartment.

3. Litter degradation: a history of "macro-micro-benthic loop", temporal variation and natural perturbations.

As demonstrated in every chapter of this PhD, the dead *P. oceanica* detritus, their inconstant availability and the macrofauna are strongly mutually linked, in terms of community structure, assemblage, and trophic ecology. Indeed despite the very different ingestion and assimilation patterns observed among the species of the macrofauna community of the litter, up to 85% of them ingested significant amounts of dead *P. oceanica* fragments. This result was of major importance for the comprehension of the functioning of the exported litter compartment. Even if all these organisms did not really assimilate large amounts of organic matter from dead *P. oceanica* fragments, most of them play a non-negligible role in the purely mechanical fragmentation of the *P. oceanica* dead leaves. In terrestrial and stream ecosystems, detritivore organisms ("shredders") are known to feed preferentially on litter colonized by bacteria and fungi, probably because of the presence of biofilm and hyphae of higher nutritional quality than the leaves themselves. In addition to the assimilation of plant material, these shredders also enhance the physical fragmentation of the leaves (Graça, 2001; Graça *et al*., 2001; Ferreira *et al*., 2006; Yang *et al*., 2012). This mechanical fragmentation, in turn, enhances the bacterial and fungal colonization and chemical degradation of the plant, constituting sort of a degradation cycle. It was thus hypothesized that most of the species composing the litter macrofauna community might play a

significant role in the dead *P. oceanica* leaves degradation in terms of mechanical fragmentation, potentially enhancing the microbial and fungal activity (see Cuomo *et al*., 1985). This possible enhanced microbial and fungal activity could, in turn, enhance the chemical degradation of the dead leaves, making them more attractive for the detritivore macrofauna. This could constitute, apart from the "physico-chemical-dependent" degradation (see next paragraph) and the true assimilation of dead leaves organic matter, an important mechanical invertebrate-driven degradation pathway of the dead *P. oceanica* leaves. More importantly, we "traced" the trophic signal of dead leaves through multiple trophic levels up to juvenile fishes, indicating that detrital *P. oceanica* material partly supports an important coastal food web, contributing to the transmission of *P. oceanica* organic matter into the upper trophic levels.

In addition to the influence of this "macro-micro-benthic loop" on litter degradation, we also highlighted the impact of the constant inconstancy of the litter and of the physico-chemical conditions encountered inside the litter accumulations. We demonstrated that calm and hot periods such as most of late spring and summer were moments of high nutrients abundance, low oxygen availability, "layering" and potentially important microbial activity inside the exported litter accumulations. Nutrients were potentially influenced by the presence of the macrofauna community (excretion), litter leaching, sediment, organic matter decomposition and remineralization. Nutrients are known (Greenwood *et al*., 2007; Apostolaki *et al*., 2009) to enhance the detritus degradation through the microbial activity. This microbial (*e.g.* bacteria, fungi, microalgae, protozoans) activity in turn enhanced the litter degradation (Anesio *et al*., 2001; Vähätalo *et al*., 2003; Romani *et al*., 2006; Mancinelli *et al*., 2009, Vohník *et al*., 2015), but also the oxygen concentration inside the deepest layers of the litter, also impacting the community. This litter microbial activity is also known to be highly influenced by temperature, indicating that summer could be the season when the impact of microbial degradation is maximum (Mateo and Romero, 1996). The natural life cycle of *P. oceanica* itself also impacted the litter strongly, with the massive autumnal litter input and also the more modest but continuous litter input occurring throughout the year (Lepoint *et al*., 2006; Champenois and Borges, 2012).

Another level of perturbation potentially impacted strongly the litter degradation: hydrodynamics. Indeed, hydrodynamics constitutes a first and quickly occurring phase of direct mechanical degradation just after leaf

shedding, during exportation (see Romero *et al*., 1992). Moreover, we also demonstrated that important storms could induce resource pulses on exported *P. oceanica* litter accumulations. In addition to the mechanical degradation occurring during the storm itself, resource pulses could enhance the litter degradation through the macrofauna community as well as microbial activity. Indeed, very abundant amphipods appeared to feed preferentially on dead *P. oceanica* leaves just after each resource pulse. It could thus be hypothesized that every time a resource pulse would occur, litter ingestion and assimilation would increase for some species, resulting in a more important degradation just after each resource pulse. Since resource pulses occur preferentially in autumn and winter, such violent stormy events could be an important driver of litter degradation when microbial activity is lower.

The links between the different compartments are summarized on Figure 6.1.

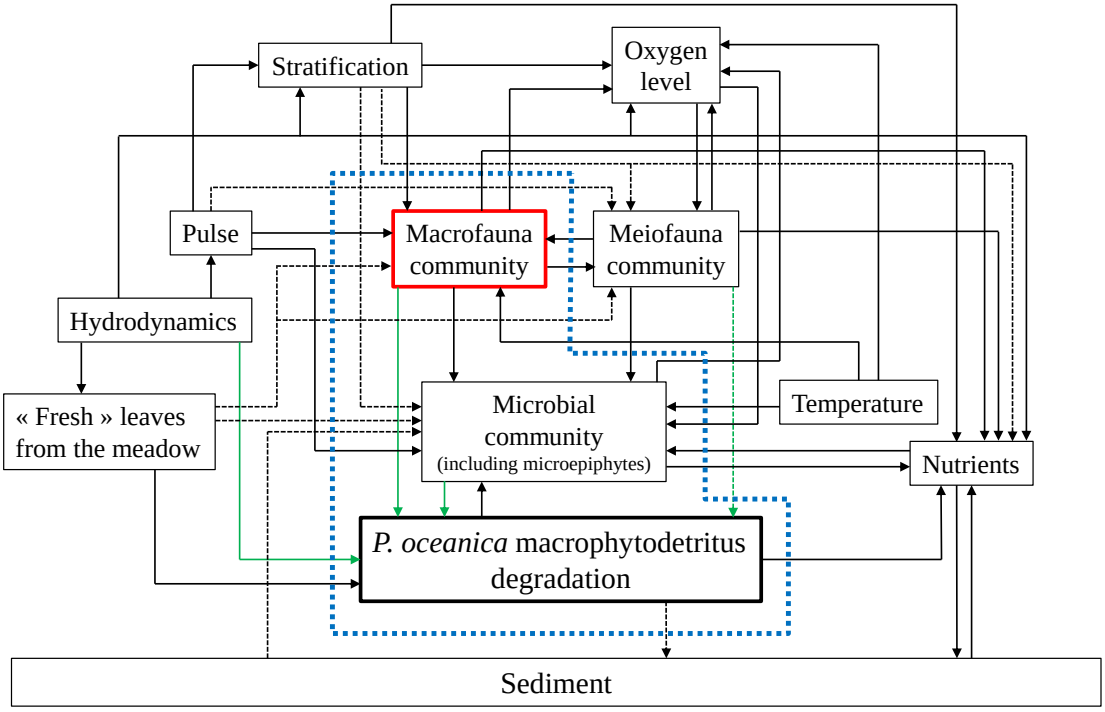


Figure 6.1: Simplified representation of links between all the compartments and Posidonia oceanica exported litter degradation. Full green arrows: direct litter degradation action. Full black arrows: direct impact of one compartment on the other, not implying direct litter degradation. Dotted green arrow: potential direct litter degradation action. Dotted black arrows: potential impact of one compartment on the other, not implying direct litter degradation. Blue dotted polygon: "macro-micro-benthic loop".

4. General conclusion

The general objective of this PhD was to characterize the exported *P. oceanica* litter macrofauna community and to assess its dynamics and trophic ecology in Calvi Bay, Corsica. To achieve this goal, we tried to fulfill the following specific objectives:

i. Characterize for the first time the macrofauna community, using a multi-year, multi-season and multi-site sampling (Chapter 3).

We demonstrated that the community was composed of 115 species, tremendously dominated by arthropods (mostly amphipods, decapods and isopods, followed by annelids (mostly polychaetes) and mollusks (mostly gastropods). This community was quite different from the community found in *P. oceanica* meadow or drift macroalgae detritus. However, even some species are also found living in other adjacent coastal habitats, *P. oceanica* litter community displayed its own assemblage and abundance dominance pattern. One species to remember: the amphipod *Gammarella fucicola*, the most typical litter amphipod of the community, representing 40-50% of the global abundance.

ii. Using this global baseline, evaluate the spatiotemporal changes occurring at two different time scales in the detritus themselves and in the macrofauna community (Chapter 3).

iii. Trying to evaluate the relationships between environmental parameters and the variations we observe at the community and the specific level (Chapter 3).

We demonstrated non-negligible temporal variations of both litter environmental parameters and community. However, even if clear global abundance or diversity patterns were highlighted at the year and seasonal time scale but also at the week time scale, a species-specific look at the most abundant species demonstrated that many species were only modestly influenced by the parameters we measured. Only oxygen concentration inside the litter (strongly influenced by the weather just before sampling), and nutrients (mostly NH_4) seemed to impact quite a limited number of species. We highlighted species presenting hypoxia tolerance, avoiding oxic conditions, and

others presenting very limited hypoxia tolerance, avoiding hypoxic conditions. Very abundant species presented seasonal variations that were linked to none of the measured parameters indicating the influence of other factors in their natural life cycles. Photographic and faunistic sampling at the week time scale allowed us to identify another level of perturbation occurring in exported litter accumulations: resource pulses. These random, strong and brief events corresponding to dramatic decrease or increase of litter biomass on litter accumulations and environmental parameters modifications impacted strongly the community, in particular the most abundant species. The litter biomass drastic decrease induced the extreme concentration of *Gammarella fucicola* inside the remaining litter, while the litter biomass increase induced the important hypoxia inside the litter (attracting hypoxia tolerant species), but also the density increase of the most abundant detritivore species, taking advantage of this food overabundance.

iv. Experimentally demonstrate the stratification occurring in a stable P. oceanica litter accumulation, the impact of this stratification on environmental conditions and on the macrofauna (Chapter 4).

We demonstrated experimentally a fast and important stratification of environmental parameters inside the litter, occurring in less than 48 hours. This stratification of living conditions induced a stratification of the species encountered. Oxygen played once again a role for several species, some of them being sampled only in oxic zones, others only in hypoxic zones, and most of the others sampled indistinctly in every layer, reflecting the different strategies and behavioral responses of the encountered species to these layered living conditions. This layering could explain the quite high diversity in the sample? defined as “hypoxic” previously. This presence of both hypoxic and oxic conditions inside the same litter accumulation could also explain the high abundance of presumed intolerant species during “hypoxic” moments of the year. These different layers potentially created different micro-habitats inside the litter accumulations, potentially allowing the presence of a more important diversity of organisms presenting various environmental preferences.

v. Experimentally demonstrate the impact of resource pulses on the exported P. oceanica litter macrofauna community (Chapters 4 and 5).

During the weekly sampling we demonstrated the occurrence of resource pulses in the exported *P. oceanica* litter accumulations. After that, we demonstrated experimentally the impact of "fresh" litter input on the macrofauna, simulating a moderate resource pulse in mesocosms. The results were striking: the density of the most dominant amphipod, *Gammarella fucicola*, dropped drastically while the density of the detritivore amphipod *Gammarus aequicauda* increased, as well as other predator species and hypoxia tolerant species. These responses were partly characteristic of typical terrestrial ecosystems resource pulses, and partly characteristic of typical aquatic ecosystems resource pulses. It appeared that the "*P. oceanica* meadow - *P. oceanica* litter "complex"? shares common characteristics with both types of environment (nature of the dead leaves, importance of the "detrital pathway", short-lived organisms-based community), potentially inducing intermediate responses. We predicted? the following potential general responses of this ecosystem to resource pulses: 1) very fast increase of mobile detritivore species density; 2) fast but moderate responses of predators; 3) persistent effect of the resource pulse on the community; 4) increased dominance of detritivore species and decrease of herbivore competitiveness; 5) diet modification of generalist invertebrates; 6) increased chances of hypoxia and creation of micro-habitats due to the resulting O_2/nutrients stratification; 7) longer-term increase of the total biodiversity, 8) increased degradation of dead *P. oceanica* leaves due to successive pulse events.

vi. Unravel for the first time the global P. oceanica litter macrofauna food web, using a multi-season and multi-site sampling (Chapter 5).

We described a food web based on 4 basal food sources and composed of 3-4 trophic levels, from the primary detritivore/herbivore organisms to the second order predators. We demonstrated the importance of the *P. oceanica* detrital material for this food web, and also the importance of this community in the *P. oceanica* dead leaves degradation and fragmentation since around 85% of the sampled organisms ingested or assimilated *P. oceanica* detritus. We also highlighted the trophic link between meiofauna and macrofauna since omnivore and carnivore crustaceans seemed to consume a non-negligible part of harpacticoid copepods found abundantly in the *P. oceanica* litter accumulations (Mascart *et al.*, 2015a).

vii. Evaluate the spatiotemporal changes of diet preferences of 5 very abundant species and determine if the observed changes are really synonym of true diet changes (Chapter 5).

We demonstrated that temporal trophic niche modifications occurred for the 5 most abundant species found at every season. We also demonstrated that these isotopic niche modifications were sometimes caused only by food sources baseline shifts, but more often caused by real diet modifications. These diet modifications were potentially mainly caused by food source abundance, potential prey abundance, but also the ability of the 5 species to cope with inconstant resource availability and sometimes the need to feed on potentially non-optimal food sources. Trophic responses of the two most dominant amphipods *Gammarella fucicola* and *Gammarus aequicauda* to resource pulses were non-negligible. Both species appeared to ingest and assimilate a larger amount of *dead P. oceanica* after resource pulses indicating potentially a certain trophic plasticity and the ability to change their diet to take advantage of a resource overabundance.

5. Novel findings

During this PhD, several major novel findings were put forward:

- The vagile macrofauna community associated to exported *P. oceanica* detritus accumulation in Corsica was described in detail for the first time on a spatio-temporal scale. So far, only one study carried out on one sampling site at a single season tried to describe this community (Gallmetzer *et al*., 2005).

- We described for the first time on a spatio-temporal scale the global food web of this vagile macrofauna community and highlighted the incorporation of *P. oceanica* detritus in this food web through multiple trophic levels, demonstrating its importance as a coastal food source. So far, the only published trophic studies on exported *P. oceanica* detritus focused on limited numbers of species (Lepoint *et al*., 2006; Sturaro *et al*., 2010).

- We demonstrated experimentally for the first time the important stratification occurring in the exported *P. oceanica* detritus accumulation and the impact of this stratification on ambient living conditions, especially oxygen concentration, impacting consequently the associated macrofauna community. So far, no other demonstration of such stratification has been published.

- We demonstrated for the first time the occurrence and potential impact of resource pulses on exported *P. oceanica* detritus accumulations and associated vagile macrofauna community. A preliminary experimental study also highlighted the similarities between exported *P. oceanica* detritus accumulations and terrestrial forest ecosystems in terms of resource pulses characteristics and potential responses. To our knowledge, this was the first time that exported *P. oceanica* detritus accumulations were observed in the angle of the "resource pulse theory".

6. Future Perspectives

Based on the new outcomes presented in the previous paragraph, recommendations and new questions arose for further researches:

- Trophic ecology and more particularly mixing models use is highly dependent on sampled food sources, but also on the possibility to distinguish them in terms of isotopic compositions. The case of epiphytes was quite impossible to solve during this PhD, since separating physically bacteria, fungi, or diatoms is not possible. Other trophic markers such as Fatty Acids, and/or compound specific stable isotopes on marker fatty acids could potentially bring new important information to delineate more precisely the exported *P. oceanica* litter food web.

- Few studies about exported *P. oceanica* detritus are done in Europe, and since the associated vagile macrofauna was proven to be a potentially very important pathway of the organic matter flux from the *P. oceanica* meadow itself to the coastal marine food webs, more should be done at large spatial scale, to precise the importance of this macrofauna community at the scale of the whole Mediterranean basin.

- The preliminary study conducted during this PhD about resource pulses occurring in exported *P. oceanica* detritus revealed potentially important effect on the physico-chemical conditions measured inside the litter accumulation, on the community, but also on the global use of dead *P. oceanica* as a food source by some macrofauna species. This preliminary study should encourage other researches to precise the impact and potential structuring role of pulsed perturbations on the *P. oceanica* detrital compartments, on the associated macrofauna, but also on other organisms present in non-negligible amounts such as meiofauna.

- One general finding of this PhD was that we probably missed several important regulating environmental parameters. More researches should include parameters such as temperature in general, but also on a more precise scale at different depth inside litter accumulations. Precise measures of local currents and sunlight received by these different layers could also be an interesting parameter.

- Preliminary study of resource pulses occurring in exported *P. oceanica* detritus revealed that active migration could occur quite rapidly in the case of new habitat and dietary resource input. This active colonization of "fresh" dead *P. oceanica* could be interesting to understand better who recolonization after a major disturbance occur and which organisms are the most efficient colonists.

- The exported *P. oceanica* detritus vagile macrofauna was demonstrated to be importantly impacted by habitat availability, food source availability and variations, but also by random and strong resource pulses events. Global change could potentially impact and modify these parameters (*e.g.* more frequent storms, ocean acidification impact on carbonate depositing organisms). The assessment of these potential affect should thus be carefully investigated experimentally to describe the magnitude of these modifications and the potential responses of the macrofauna. This would give important information about the potential future impact of global change on this important detrital transfer of organic matter flux from the *P. oceanica* meadow itself to the global Mediterranean coastal food webs.

Addendum 1:

Summary table of all 115 encountered species during the seasonal sampling. All values expressed are average ± standard deviations. Scientific names presented here were all marked as "accepted" by WoRMS (World Register of Marine Species) on the date of the last check (28/03/2016). Possible minor changes might have occurred since that last check.

Order	Family	Species	2010			2011				2012	
			SPRING	SUMMER	AUTUMN	WINTER	SPRING	SUMMER	AUTUMN	WINTER	SPRING
			Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Amphipoda	Nuuanuidae	*Gammarella fucicola*	2.627 ± 1.553	0.718 ± 0.934	0.154 ± 0.176	0.294 ± 0.496	1.6 ± 1.337	2.008 ± 1.586	0.437 ± 0.395	0.961 ± 0.68	1.143 ± 1.383
	Melitidae	*Melita hergensis*	0.013 ± 0.014	0.01 ± 0.014	0.008 ± 0.015	0.139 ± 0.167	0.01 ± 0.023	0.08 ± 0.087	0.027 ± 0.031	0.127 ± 0.116	0.17 ± 0.098
	Gammaridae	*Gammarus insensibilis*								0.004 ± 0.013	0.076 ± 0.078
		Gammarus aequicauda	0.263 ± 0.277	0.012 ± 0.029	0.023 ± 0.034	0.028 ± 0.025	0.604 ± 0.887	0.006 ± 0.012	0.031 ± 0.079	0.048 ± 0.112	0.87 ± 1.627
	Phliantidae	*Pereionotus testudo*						0.002 ± 0.006			
	Dexaminidae	*Dexamine spiniventris*								0.001 ± 0.005	
		Dexamine spinosa						0.01 ± 0.025			0.115 ± 0.127
	Atylidae	*Atylus guttatus*	0.197 ± 0.163	0.021 ± 0.033	0.071 ± 0.095	0.057 ± 0.075		0.007 ± 0.014		0.144 ± 0.145	0.194 ± 0.227
	Leucothoidae	*Leucothoe incisa*								0.011 ± 0.033	
	Calliopiidae	*Apherusa chiereghinii*	0.224 ± 0.545				0.002 ± 0.001				0.002 ± 0.008
	Uristidae	*Tmetonyx nardonis*						0.003 ± 0.011			
	Hyalidae	*Hyale camptonyx*	0.059 ± 0.055					0.031 ± 0.084		0.001 ± 0.005	0.044 ± 0.079
	Amphilochidae	*Apolochus neapolitanus*									0.008 ± 0.026
	Stenothoidae	*Stenothoe monoculoides*								0.004 ± 0.01	0.001 ± 0.004
	Iphimediidae	*Iphimedia obesa*			0.006 ± 0.015	0.002 ± 0.006		0.002 ± 0.008			
	Lysianassidae	*Socarnes filicornis*						0.002 ± 0.008			
		Lysianassa costae	0.056 ± 0.039	0.009 ± 0.021	0.006 ± 0.009	0.019 ± 0.03	0.058 ± 0.069	0.017 ± 0.031	0.005 ± 0.007	0.023 ± 0.035	0.069 ± 0.105
	Maeridae	*Maera grossimana*				0.002 ± 0.006		0.014 ± 0.028			
	Corophiidae	*Leptocheirus pectinatus*						0.002 ± 0.001			
		Leptocheirus guttatus						0.003 ± 0.008			
	Ischyroceridae	*Ericthonius punctatus*						0.003 ± 0.012			
	Aoridae	*Microdeutopus chelifer*	0.035 ± 0.062	0.022 ± 0.035	0.076 ± 0.082			0.038 ± 0.052		0.131 ± 0.113	0.094 ± 0.127
	Caprellidae	*Caprella acanthifera*	0.002 ± 0.006					0.013 ± 0.02			0.028 ± 0.049
		Phtisica marina	0.006 ± 0.015					0.002 ± 0.005			0.013 ± 0.036
Leptostraca	Nebaliidae	*Nebalia strausi*	0.087 ± 0.064	0.232 ± 0.296		0.032 ± 0.052	0.239 ± 0.178	0.119 ± 0.12	0.089 ± 0.143	0.001 ± 0.005	0.112 ± 0.138
Tanaidacea	Paratanaoidea incertae sedis	*Pseudoparatanais batei*									0.009 ±
Isopoda	Anthuridae	*Apanthura corsica*	0.016 ± 0.03	0.025 ± 0.031	0.002 ± 0.004	0.028 ± 0.068	0.068 ± 0.07	0.023 ± 0.024	0.001 ± 0.004	0.01 ± 0.021	0.021
	Sphaeromatidae	*Cymodoce truncata*						0.015 ± 0.021	0.005 ± 0.009		0.007 ±
	Holognathidae	*Cleantis prismatica*						0.004 ± 0.012		0.001 ± 0.005	0.078
	Janiridae	*Jaera (Jaera) nordmanni*	0.011 ± 0.023	0.095 ± 0.105			0.04 ± 0.048	0.468 ± 0.433	0.02 ± 0.035	0.113 ± 0.098	0.055 ±
	Limnoriidae	*Limnoria quadripunctata*						0.004 ± 0.01		0.003 ± 0.01	
	Gnathiidae	*Gnathia vorax*			0.002 ± 0.004			0.002 ± 0.005		0.006 ± 0.012	0.035
	Idoteidae	*Idotea balthica*	0.012 ± 0.023	0.043 ± 0.053	0.002 ± 0.006		0.029 ± 0.047		0.001 ± 0.004		0.022 ± 0.069
		Stenosoma capito									0.029 ± 0.014
		Stenosoma appendiculatum	0.016 ± 0.03	0.026 ± 0.047	0.002 ± 0.006			0.026 ± 0.033	0.003 ± 0.01		0.005 ±
Decapoda	Galatheidae	*Galathea intermedia*		0.062 ± 0.045	0.002 ± 0.006		0.074 ± 0.075	0.094 ± 0.125	0.038 ± 0.051		
	Epialtidae	*Pisa tetraodon*	0.002 ± 0.006	0.009 ± 0.016				0.002 ± 0.005			0.018
	Inachidae	*Macropodia deflexa*									0.005 ± 0.004
		Macropodia linaresi									0.001 ± 0.004
	Alpheidae	*Athanas nitescens*	0.062 ± 0.063	0.101 ± 0.098		0.011 ± 0.027	0.068 ± 0.064	0.107 ± 0.134	0.061 ± 0.1		0.001 ±
	Leucosiidae	*Ebalia cranchii*	0.01 ± 0.023	0.012 ± 0.029	0.002 ± 0.006		0.051 ± 0.073	0.003 ± 0.011			0.01
	Hippolytidae	*Hippolyte leptocerus*						0.004 ± 0.01			0.003 ±
	Processidae	*Processa edulis edulis*	0.002 ± 0.006		0.002 ± 0.006			0.002 ± 0.008			
	Palaemonidae	*Palaemon xiphias*	0.04 ± 0.067	0.002 ± 0.005		0.002 ± 0.006	0.057 ± 0.051	0.004 ± 0.01		0.001 ± 0.005	
	Paguridae	*Anapagurus chiroacanthus*						0.082 ± 0.198			0.004
	Polybiidae	*Liocarcinus navigator*	0.021 ± 0.024	0.024 ± 0.048	0.004 ± 0.006		0.051 ± 0.073	0.006 ± 0.015			0.001 ±
	-	*Decapoda spp.*						0.017 ± 0.04			
Trombidiformes	Halacaridae	*Thalassarachna affinis*						0.001 ± 0.003			0.018
Myodocopida	Cypridinidae	*Skogsbergia mediterranea*								0.011 ± 0.038	0.005 ± 0.17

Phyllodocida	Nereididae	*Platynereis dumerilii*	0.423 ± 0.447	0.051 ± 0.043	0.005 ± 0.012	0.05 ± 0.078	0.188 ± 0.251	0.222 ± 0.248	0.013 ± 0.018	0.008 ± 0.018	0.158 ±
		Ceratonereis (Composetia) costae						0.001 ± 0.001			0.046
		Nereis caudata						0.032 ± 0.092			0.03 ± 0.004
		Nereis rava						0.002 ± 0.008			0.001 ±
		Websterinereis glauca								0.011 ± 0.022	
		Perinereis sp.						0.002 ± 0.001			0.027
	Chrysopetalidae	*Chrysopetalum debile*	0.069 ± 0.063	0.045 ± 0.092	0.007 ± 0.008	0.003 ± 0.006	0.042 ± 0.067	0.02 ± 0.024	0.008 ± 0.021	0.017 ± 0.022	0.015 ±
	Polynoidae	*Harmothoe spinifera*	0.009 ± 0.016	0.009 ± 0.021	0.003 ± 0.005			0.032 ± 0.047			
		Polynoinae sp.						0.002 ± 0.008			0.009
	Hesionidae	*Hesione pantherina*						0.007 ± 0.013			0.004 ± 0.069
		Hesiospina aurantiaca	0.033 ± 0.05		0.049 ± 0.087	0.024 ± 0.028		0.049 ± 0.028	0.02 ± 0.027	0.097 ± 0.098	0.109 ± 0.006
	Phyllodocidae	*Sige macroceros*						0.008 ± 0.018	0.002 ± 0.007	0.006 ± 0.013	0.002 ± 0.008
		Eulalia viridis			0.002 ± 0.006		0.001 ± 0.001	0.012 ± 0.016			0.002 ±
	Glyceridae	*Glycera rouxii*	0.016 ± 0.025						0.003 ± 0.006		
		Glycera tesselata								0.002 ± 0.006	
	Syllidae	*Myrianida prolifera*					0.004 ± 0.009			0.002 ± 0.007	0.013
		Syllis prolifera	0.025 ± 0.045					0.064 ± 0.179	0.003 ± 0.006	0.007 ± 0.016	0.009 ± 0.008
		Eurysyllis tuberculata									0.002 ± 0.009
		Branchiosyllis exilis									0.004 ±
		Salvatoria limbata								0.001 ± 0.005	0.012
		Odontosyllis gibba								0.004 ± 0.013	0.004 ±
		Trypanosyllis zebra								0.003 ± 0.01	
		Trypanosyllis (Trypanosyllis) coeliaca						0.003 ± 0.011		0.005 ± 0.013	0.011
		Syllis gracilis	0.018 ± 0.024	0.009 ± 0.021			0.009 ± 0.023	0.01 ± 0.02		0.005 ± 0.009	0.003 ±
	Sphaerodoridae	*Sphaerodoridium claparedii*						0.002 ± 0.009			
	Lumbrineridae	*Lumbrineris coccinea*								0.007 ± 0.013	0.004
Eunicida	Eunicidae	*Eunice vittata*						0.004 ± 0.011			0.001 ± 0.004
		Lysidice hebes						0.002 ± 0.001			0.001 ±
		Eunice oerstedii						0.002 ± 0.009			
	Dorvilleidae	*Protodorvillea kefersteini*	0.039 ± 0.032	0.016 ± 0.026	0.003 ± 0.008		0.051 ± 0.061	0.008 ± 0.026			
		Schistomeringos rudolphi						0.002 ± 0.005			
	Oenonidae	*Arabella iricolor*								0.002 ± 0.007	
	Onuphidae	*Nothria conchylega*						0.005 ± 0.013			0.523
-	Opheliidae	*Polyophthalmus pictus*	0.17 ± 0.184	0.03 ± 0.043	0.003 ± 0.005	0.039 ± 0.068	0.076 ± 0.097	0.289 ± 0.527		0.033 ± 0.047	0.232 ±
-	-	Annelida spp.						0.01 ± 0.028			
-	-	Platyhelminthes spp.	0.022 ± 0.034	0.008 ± 0.019	0.002 ± 0.004		0.001 ± 0.001	0.011 ± 0.017		0.004 ± 0.011	0.068
-	-	Nemertea spp.	0.076 ± 0.053	0.03 ± 0.035	0.02 ± 0.029		0.013 ± 0.023	0.043 ± 0.048		0.013 ± 0.025	0.036 ±
Chitonida	Acanthochitonidae	*Acanthochitona crinita*	0.009 ± 0.016				0.011 ± 0.027	0.002 ± 0.006			0.004
	Chitonidae	*Chiton olivaceus*						0.002 ± 0.005			0.001 ±
-	Fissurellidae	*Emarginula octaviana*						0.002 ± 0.001			
Neogastropoda	Conidae	*Conus sp.*						0.004 ± 0.012			
	Raphitomidae	*Raphitoma linearis*						0.002 ± 0.008			
	Muricidae	Muricidae sp.						0.003 ± 0.009			
Neogastropoda	Mangeliidae	*Bela zonata*						0.003 ± 0.009			
-	Trochidae	*Gibbula varia*						0.001 ± 0.003	0.024 ± 0.065		0.008
-		*Jujubinus gravinae*						0.003 ± 0.011			0.002 ±
-		*Jujubinus exasperatus*							0.003 ± 0.006		
Littorinimorpha	Rissoidae	*Rissoa violacea*								0.003 ± 0.011	
		Rissoa ventricosa						0.009 ± 0.019	0.006 ± 0.01	0.001 ± 0.005	
		Rissoa guerinii							0.003 ± 0.01		0.027
		Alvania lineata						0.031 ± 0.063	0.009 ± 0.012	0.007 ± 0.021	0.01 ±
		Rissoa auriscalpium						0.006 ± 0.017			
	Rissoinidae	*Rissoina bruguieri*						0.003 ± 0.009			
	Eulimidae	Eulimidae sp.						0.002 ± 0.009	0.001 ± 0.002		

Arcida	Arcidae	*Arca noae*							0.003 ± 0.01			0.197
Caenogastropoda	Cerithiidae	*Bittium reticulatum*	0.202 ± 0.11		0.003 ± 0.008	0.035 ± 0.067	0.17 ± 0.202	0.293 ± 0.26	0.132 ± 0.182	0.119 ± 0.092	0.139 ±	
-	Phasianellidae	*Tricolia pullus*							0.005 ± 0.011	0.001 ± 0.005		
-	Phasianellidae	*Tricolia speciosa*							0.001 ± 0.004			0.049
-	Phasianellidae	*Tricolia tenuis*	0.026 ± 0.03	0.002 ± 0.005	0.002 ± 0.004		0.091 ± 0.098	0.017 ± 0.035	0.009 ± 0.021		0.036 ±	
Nudibranchia	Flabellinidae	Flabellinidae sp.						0.002 ± 0.001				
-	Pyramidellidae	*Odostomia sp.*						0.001 ± 0.004				
-	-	Gastropoda sp.						0.008 ± 0.016				0.016
Ophiurida	Amphiuridae	*Amphipholis squamata*	0.002 ± 0.006	0.016 ± 0.021			0.025 ± 0.053	0.047 ± 0.081				0.01 ± 0.004
	Ophiomyxidae	*Ophiomyxa pentagona*	0.012 ± 0.018				0.01 ± 0.023	0.002 ± 0.005				0.001 ± 0.007
Perciformes	Gobiidae	*Gobiidae* spp.	0.011 ± 0.018	0.012 ± 0.029			0.015 ± 0.025	0.009 ± 0.025	0.002 ± 0.004	0.001 ± 0.005	0.002 ± 0.026	
	Labridae	*Labridae* spp.	0.004 ± 0.007				0.002 ± 0.001	0.001 ± 0.003	0.001 ± 0.002	0.002 ± 0.005	0.008 ± 0.002	

Addendum 2:

Summary table of all the encountered species during the oxygen impact experiment. All values expressed are average ± standard deviations.

	Layer 5	Layer 10	Layer 15	Layer 20
	Avg. ± SD	Avg. ± SD	Avg. ± SD	Avg. ± SD
Gammarella fucicola	2.63 ± 0.57	2.16 ± 0.74	2.00 ± 0.96	1.64 ± 0.57
Melita hergensis			0.03 ± 0.05	0.01 ± 0.04
Apherusa chiereghinii	0.01 ± 0.01	0.01 ± 0.01		
Microdeutopus chelifer		0.06 ± 0.1	0.17 ± 0.13	0.37 ± 0.08
Lysianassa costae	0.01 ± 0.01		0.01 ± 0.01	0.02 ± 0.03
Stenothoe monoculoides		0.01 ± 0.02		
Nototropis guttatus	0.01 ± 0.01	0.01 ± 0.03	0.09 ± 0.10	0.14 ± 0.11
Phtisica marina			0.01 ± 0.02	0.06 ± 0.11
Maera grossimana		0.01 ± 0.01		0.01 ± 0.02
Dexamine spinosa				0.01 ± 0.01
Athanas nitescens	0.29 ± 0.14	0.19 ± 0.13	0.06 ± 0.06	0.04 ± 0.05
Hippolyte leptocerus		0.01 ± 0.01	0.16 ± 0.17	0.26 ± 0.24
Palaemon xiphias			0.01 ± 0.01	0.08 ± 0.05
Alpheus glaber		0.01 ± 0.03	0.01 ± 0.02	0.01 ± 0.01
Galathea intermedia			0.07 ± 0.14	0.17 ± 0.13
Anapagurus chiroacanthus			0.01 ± 0.04	0.11 ± 0.07
Macropodia deflexa				0.01 ± 0.03
Pisa tetraodon			0.01 ± 0.02	
Liocarcinus navigator		0.01 ± 0.01	0.01 ± 0.02	0.01 ± 0.03
Jaera nordmanni			0.05 ± 0.07	0.03 ± 0.07
Stenosoma lancifer			0.01 ± 0.03	0.09 ± 0.08
Cymodoce truncata			0.06 ± 0.11	0.18 ± 0.22
Sphaeroma serratum				0.01 ± 0.01
Apanthura corsica			0.03 ± 0.04	0.02 ± 0.04
Nebalia strausi	0.43 ± 0.16	0.13 ± 0.12	0.02 ± 0.04	
Achelia echinata				0.01 ± 0.01
Platynereis dumerilii	0.05 ± 0.03	0.1 ± 0.08	0.32 ± 0.09	0.42 ± 0.23
Polyophthalmus pictus		0.07 ± 0.07	0.18 ± 0.10	0.24 ± 0.11
Hesiospina autantiaca				0.06 ± 0.14
Hesione panthernia			0.01 ± 0.01	
Bittium reticulatum	0.01 ± 0.01	0.02 ± 0.02	0.33 ± 0.27	0.50 ± 0.23
Tricolia tenuis			0.01 ± 0.02	0.21 ± 0.20
Rissoa violacea				0.03 ± 0.06
Cerithium vulgatum			0.01 ± 0.02	
Chiton olivaceus				0.01 ± 0.01
Octopus vulgaris (juvenile)				0.01 ± 0.01
Ampipholis squamata			0.03 ± 0.03	0.06 ± 0.04
Astropecten spinulosus				
Echinoidea sp.			0.01 ± 0.02	0.01 ± 0.02
Gobius spp.			0.02 ± 0.02	0.04 ± 0.04

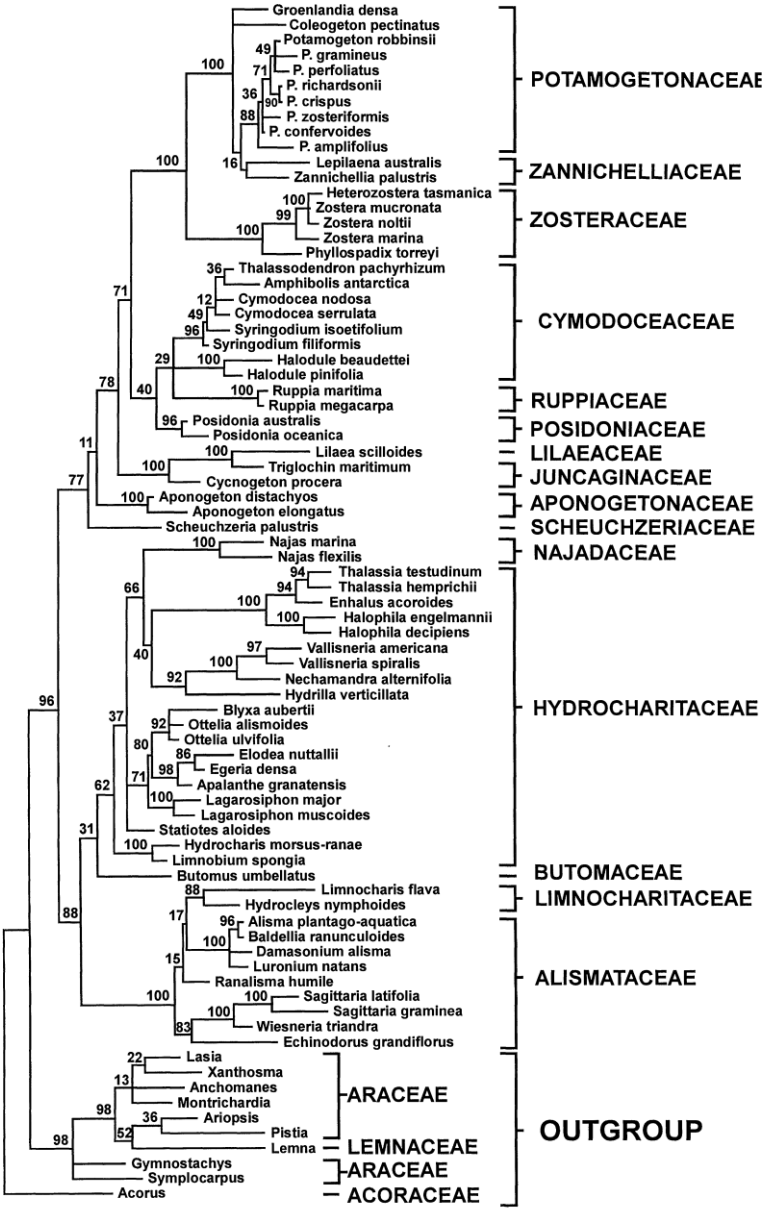
Addendum 3:

Summary table of all the encountered species during the pulse impact experiment. All values expressed are average ± standard deviations.

	T final		Control		T-defaun		T-fauna	
	Avg.	± SD	Avg.	± SD	Avg.	± SD	Avg.	± SD
Gammarella fucicola	3.28	± 0.51	3.5	± 1	1.14	± 0.19	1.08	± 0.19
Gammarus aequicauda	0.13	± 0.04	0.11	± 0.06	0.7	± 0.18	0.72	± 0.12
Melita hergensis	0.17	± 0.12	0.19	± 0.16	0.13	± 0.08	0.11	± 0.04
Apherusa chiereghinii	0.03	± 0.04	0.01	± 0.02	0.01	± 0.01	0.03	± 0.02
Leptocheirus guttatus	0.01	± 0.01	0.01	± 0.01	0.01	± 0.02	0.02	± 0.03
Microdeutopus chelifer	0.21	± 0.11	0.25	± 0.2	0.11	± 0.06	0.04	± 0.03
Stenothoe monoculoides	0.01	± 0.02	0.02	± 0.03	0.01	± 0.01	0.01	± 0.01
Lysianassa costae					0.02	± 0.03	0.02	± 0.03
Nototropis guttatus	0.05	± 0.03	0.06	± 0.04	0.03	± 0.02	0.09	± 0.04
Maera grossimana	0.01	± 0.01	0.01	± 0.01	0.01	± 0.02	0.01	± 0.01
Dexamine spinosa	0.03	± 0.03	0.02	± 0.02	0.01	± 0.02	0.02	± 0.03
Athanas nitescens	0.02	± 0.03	0.03	± 0.04	0.13	± 0.02	0.15	± 0.06
Palaemon xiphias	0.04	± 0.02	0.05	± 0.04	0.08	± 0.01	0.18	± 0.07
Alpheus glaber	0.01	± 0.01	0	± 0.01	0.02	± 0.05	0.03	± 0.02
Galathea intermedia	0.02	± 0.03	0.01	± 0.02	0.01	± 0.02	0.07	± 0.03
Anapagurus chiroacanthus					0.07	± 0.06	0.07	± 0.03
Pisa tetraodon	0.01	± 0.01	0.01	± 0.01			0.01	± 0.01
Liocarcinus navigator	0.02	± 0.03	0.02	± 0.04	0.01	± 0.01	0.01	± 0.02
Jaera nordmanni	0.13	± 0.09	0.15	± 0.12	0.2	± 0.05	0.24	± 0.04
Idotea balthica					0.02	± 0.02	0.02	± 0.03
Stenosoma lancifer	0.02	± 0.03	0.02	± 0.04	0.03	± 0.04	0.01	± 0.02
Cymodoce truncata					0.02	± 0.01	0.02	± 0.02
Nebalia strausi					0.19	± 0.08	0.24	± 0.06
Achelia echinata			0.01	± 0.01	0.01	± 0.02	0.01	± 0.01
Platynereis dumerilii	0.24	± 0.13	0.3	± 0.21	0.08	± 0.04	0.15	± 0.03
Polyophthalmus pictus	0.16	± 0.04	0.15	± 0.04	0.08	± 0.05	0.08	± 0.05
Hesiospina autantiaca					0.03	± 0.02	0.04	± 0.03
Chrysopetalum debile					0.03	± 0.02	0.05	± 0.04
Protodorvillea kefersteini					0.02	± 0.02	0.02	± 0.02
Hesione panthernia					0.01	± 0.01	0.01	± 0.01
Bittium reticulatum	0.15	± 0.1	0.17	± 0.14	0.11	± 0.05	0.12	± 0.06
Tricolia tenuis	0.02	± 0.03	0.03	± 0.04	0.03	± 0.03	0.03	± 0.04
Rissoa violacea	0.01	± 0.02	0.01	± 0.02	0.01	± 0.02	0.01	± 0.01
Chiton olivaceus					0.01	± 0.01	0.01	± 0.01
Ampipholis squamata	0.03	± 0.03	0.02	± 0.02	0.08	± 0.05	0.09	± 0.03
Holoturie	0.01	± 0.02	0.01	± 0.02	0.01	± 0.02	0.01	± 0.01

Addendum 4

Maximum parsimony *rbcL* cladogram representing the phylogeny of the seagrasses. Cladogram taken from Les *et al.*, 1997.



Publication list

Peer reviewed Articles

First author

Remy, F.* , Collard, F.* , Gilbert, B., Compère, P., Eppe, G., & Lepoint, G. (2015). When microplastic is not plastic: the ingestion of artificial cellulose fibers by macrofauna living in seagrass macro-phytodetritus. *Environmental Science & Technology*, *49*, 11158 − 11166.
* Ces auteurs ont contribué de façon équivalente à la publication.

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Co-author

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.

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Remy, F., Michel, L., Sturaro, N., Mascart, T., & Lepoint, G. (2016). *The secret life of a Mediterranean seagrass litter macrofauna community : a history of oxygen*. Oral presentation presented at Vliz 16th Marine Scientists Day, Bruges, Belgique.

Remy, F., Mascart, T., Dauby, P., Gobert, S., & Lepoint, G. (2015). *Changes of macrofauna stable isotope compositions in a very inconstant seagrass detritic habitat: actual diet modification or baseline shift?* Oral comunication presented at 4th Mediterranean Seagrass Workshop, Oristano, Italy.
Best Young Scientist Oral Presentation Award 2015.

Remy, F., Darchambeau, F., Dauby, P., Melchior, A., Gobert, S., & Lepoint, G. (2015). *Application of stable isotopes in trophic ecology: importance of TEF and seasonal baseline for robust interpretations*. Oral comunication presented at BASIS 2015 Meeting, Utrecht, Pays-Bas.
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Remy, F., Melchior, A., Darchambeau, F., & Lepoint, G. (2014). *Turnover rates of carbon and nitrogen stable isotopes in the amphipod Gammarus aequicauda: insights for trophic studies of Mediterranean macrophytodetritus accumulation*. Oral presentation presented at 9th IsoEcol, International Conference on the Applications of Stable Isotope Techniques to Ecological Studies, Perth, Australie.

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

Remy, F., Borges, A., Darchambeau, F., Dauby, P., Gobert, S., & Lepoint, G. (2010). *Trophic structure and diversity of macro organisms associated with Posidonia oceanica litter in the bay of Calvi*. Oral presentation presented at 17th Benelux Congress of Zoology (GENT 22-23 October 2010), Gent, Belgium.

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