Estuarine, Coastal and Shelf Science 212 (2018) 73–79

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Seagrass organic matter transfer in *Posidonia oceanica* macrophytodetritus accumulations

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ARTICLE INFO

Keywords:
Stable isotopes
Detrital pathway
Mixing models
Trophic level
Invertebrates
Mediterranean Sea
Food web

ABSTRACT

Seagrass ecosystems are net autotrophic systems which contribute to organic carbon burial in marine sediment. Dead seagrass leaves are often exported outside the seagrass beds and may form accumulations (exported macrophytodetritus accumulations, hereafter EMAs) from littoral zones to deepest canyons. Understanding how seagrass organic matter is channeled in its associated trophic web is necessary to assess the role of the seagrass ecosystem as blue carbon service providers. We used gut content and stable isotope analyses to delineate the *Posidonia oceanica* EMA food web structure and to determine the importance of detrital material in the diets of macrofauna. Evidence from gut contents and stable isotopes showed that this food web is fuelled mainly by two food sources found in the detritus accumulations: 1) *P. oceanica* detritus itself and 2) epiphytes and drift macroalgae. Dead leaves of *P. oceanica* enter the diet of dominant species, representing more than 60% of animal abundance. The food web is structured in five trophic levels with a numerical dominance of detritivore/herbivore species at the first consumer level. Animals act as a vector for seagrass organic matter transfer to upper trophic levels and this "dead seagrass signal" is followed through the entire food web. Seagrass primary production and seagrass organic matter processing by animals are spatially decoupled and this should be taken into account in assessments of seagrass ecosystems as key actors in C cycles in coastal areas.

1. Introduction

Accumulations of macrophytodetritus are ubiquitous features of marine ecosystems and are found from littoral zones to deepest canyons, and from high latitudes to tropical zones. These accumulations shelter specific and very abundant animal assemblages (e.g. Crawley and Hyndes, 2007; Gallmetzer et al., 2005; Vetter, 1995), acting as a faunal magnet (Duggins et al., 2016). They are commonly found associated to seagrass meadows. Seagrass meadows are net autotrophic ecosystems and key components of the carbon cycle in coastal areas (Champenois and Borges, 2012). They are now recognised for their importance in the burial of organic carbon in marine sediment and, consequently, in the mitigation of atmospheric CO_2 increase (i.e. blue carbon hypothesis) (Duarte and Krause-Jensen, 2017; Ewers Lewis et al., 2017; Lavery et al., 2013). Produced biomass is partly exported outside of the seagrass systems and forms accumulations of macrophytodetritus, mixed with other drift material (macroalgae, living leaves, uprooted rhizomes, dead organisms) ("exported macrophytodetritus accumulations", hereafter EMAs) (Pergent et al., 1997; Cebrian, 2002; Boudouresque et al., 2016). Therefore, seagrass ecosystems are often a net provider of dead organic material (macrophytodetritus) to unvegetated habitats (Duarte and Krause-Jensen, 2017) and act as trophic subsidies to various ecosystems (Heck et al., 2008).

As for many seagrasses worldwide, the detrital pathway is considered to be a very important route for the incorporation of the organic matter of the Neptune grass *Posidonia oceanica* (L. Delile, 1813) into coastal food webs, as a large proportion of the foliar primary production can end up in the detrital compartment (Boudouresque et al., 2016; Cebrian, 2002; Mateo and Romero, 1997; Pergent et al., 1997). The *P. oceanica* dead leaves are often exported out of the meadow to underwater unvegetated places (e.g., bare underwater sand patches).

These EMAs are colonised by meiofauna (38–1000 μm) (Mascart et al., 2015) and an abundant and diverse vagile macrofaunal (defined here as the fauna retained on 1 mm sieves and smaller than 5 cm) community (Como et al., 2008; Dimech et al., 2006; Gallmetzer et al., 2005; Remy, 2016). The EMAs' macrofauna consists of up to 115 species and is dominated by amphipod crustaceans, representing 80–97% of the total abundance (Gallmetzer et al., 2005; Remy, 2016). Because *P. oceanica* meadows are often impacted by human activities, these

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https://doi.org/10.1016/j.ecss.2018.07.001
Received 25 January 2018; Received in revised form 12 June 2018; Accepted 3 July 2018
Available online 04 July 2018
0272-7714/

particular communities are also potentially disturbed (Calizza et al., 2013).

The heterogeneous nature of the components of EMAs makes them a perfect candidate for a complex food web, with various food sources and distinct trophic preferences among the macrofauna species. Seagrass detritus could thus play a supportive role in these food webs, as was already suggested for certain invertebrates in the *P. oceanica* meadow (Lepoint et al., 2006; Michel et al., 2015; Vizzini, 2009).

Using a year-long sampling strategy, combining gut content analysis (GCA) and stable isotope analysis (hereafter SIA), we aimed 1. to describe the food web associated to *P. oceanica* macrophytodetritus accumulations and 2. to assess the role of animals living in these accumulations as vectors of seagrass-derived organic matter.

2. Materials and methods

To encompass the temporal and spatial heterogeneity of EMAs, samples were collected on 4 occasions between August 2011 and March 2012 at two shallow (i.e. 10 m depth) sampling sites near the STARESO oceanographic research station in Calvi Bay (42°35′N, 8°43′E, Corsica). The sites were approximately 700 m apart. Both sampled EMAs were on sandy substrate devoid of vegetation. A precise description of the sampled habitats may be found in Remy (2016).

The litter and associated macrofauna, defined here as the fauna retained on a 1 mm sieve and smaller than 5 cm (Table 2), were manually sampled while scuba diving, using large 30 L plastic bags. Samples were rinsed with seawater on 1 cm and 1 mm sieves to separate the animal fraction and the vegetal fraction. The vegetal fraction was retained on 1 cm mesh, corresponding to potential basal food sources (i.e. dead leaves, living leaves, drift macroalgae, epiphytes).

Suspended particulate organic matter (hereafter SPOM), sampled using Niskin bottles (2.5 L) underwater (1 metre above the EMA, i.e. 9 m depth), was collected on a GF/F glass fiber filter (pre-combusted at 400 °C). Potential food sources were frozen (−20 °C) until further analysis.

The animals in the 1 mm animal fraction (n = 566) were all identified and put individually in 4 mL glass vials and frozen (−20 °C) until further analysis. Isotopic and gut content analyses were performed for 19 species, allowing 90% of individual abundance at each season to be reached and representing all potential trophic levels found in the EMAs.

2.1. Gut content analysis

Gut content analyses were performed using the semi-quantitative technique described by Wilson and Bellwood (1997), adapted for the very small gut contents of vagile invertebrates. A 4 cm^2 grid composed of 100 squares of 4 mm^2 was used. Twenty-five squares were randomly chosen and marked 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 visually classified into five categories: (1) dead *Posidonia oceanica* leaves, (2) living *P. oceanica* leaves, (3) other vegetal material (macroalgae, epiphytes), (4) animal material, and (5) unknown material. Once the 25 squares were examined and the most dominant item noted for each, the relative abundance (%) of each category was calculated. Organisms presenting an empty gut or less than ten squares containing one of the determined items were excluded from further analysis.

2.2. Elemental and stable isotope analysis

After gut removal, each individual was dried (60 °C) for at least 96 h, and ground to form a homogenous powder. Epiphytes that are highly carbonated and crustaceans that may have carbonates in their cuticle were acidified under 37% HCl vapour for 15 h to limit the bias of carbonate content on tissue isotopic composition. After acidification, samples were dried again (60 °C) for 48 h, ground, and put in 6 mm^3 tin cups. For animals, individual measurements were performed (see Table 2 for sample numbers). The stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), and the elemental composition were determined using an isotopic ratio mass spectrometer (Isoprime 100™, Isoprime, UK) interfaced in continuous flow with an elemental analyser (vario MICRO cube™, Elementar). Isotope ratios for C and N 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 N_2:

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

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 for carbon and nitrogen respectively. Pure gases of CO_2 and N_2 were used and calibrated against certified reference materials, i.e., sucrose (IAEA-C6; $\delta^{13}C = -10.8 \pm 0.3‰$) and ammonium sulfate (IAEA-N2; $\delta^{15}N = 20.3 \pm 0.3‰$), obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria). The analytical precision was assessed by procedural blanks, internal replicates (i.e., glycine and in-house crustacean and seagrass reference material) and isotopic certified material (i.e., IAEA-C6 and IAEA-N2). Standard deviations of 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 $\delta^{15}N$. Isotopic data from harpacticoid copepods composing the "meiofauna" food source (hereafter, "COP") are from Mascart et al. (2018).

Table 1
Trophic enrichment factors (TEF) (i.e. net difference between the isotopic composition of this food source and the isotopic composition of consumer tissues) used to calculate the contribution of each aggregated food source to the macrofaunal diet.

Food Source	Acronym	TEF (mean ± SD)						Source
		$\delta^{13}C$			$\delta^{15}N$			
Dead *P. oceanica* leaves	DL	1.00	±	0.40	0.90	±	0.70	Remy et al., 2017
Living *P. oceanica* leaves	LL	1.00	±	0.40	0.90	±	0.70	Remy et al., 2017
Epiphytes/macroalgae	EPI	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
Harpacticoid copepods	COP	0.90	±	0.70	2.90	±	0.60	Remy et al., 2017
		0.50	±	0.10	2.30	±	0.20	McCutchan et al., 2003
Gammarella fucicola and *Melita hergensis*	GFMH	0.50	±	0.10	2.30	±	0.20	McCutchan et al., 2003
Gammarus aequicauda	GA	0.50	±	0.10	2.30	±	0.20	McCutchan et al., 2003
Omnivore invertebrates	POOL	0.50	±	0.10	2.30	±	0.20	McCutchan et al., 2003
Palaemon xiphias and *Processa edulis*	PX	0.50	±	0.10	2.30	±	0.20	McCutchan et al., 2003
Gobius spp.	GSPP	0.50	±	0.10	2.30	±	0.20	McCutchan et al., 2003

Table 2
$\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD), major feeding type and/or food item, and trophic positions of macrofauna inhabiting *Posidonia oceanica* dead leaf accumulations, using gut content analysis and stable isotope data.

Species (acronym)	n	$\delta^{13}C$	$\delta^{15}N$	Diet		Trophic position
		(‰)	(‰)	Gut contents	Stable isotopes	Stable isotopes
Gammarella fucicola (Gf)	82	−18.1 ± 1.5	1.9 ± 1.1	mixed vegetal (algae-dominated)	mixed vegetal	1.5
Gammarus aequicauda (Ga)	81	−15.4 ± 1.2	2.2 ± 0.7	dead leaves	dead leaves	1.8
Melita hergensis (Mh)	55	−19.0 ± 1.7	1.5 ± 1.0	mixed vegetal	mixed vegetal	1.2
Nototropis guttatus (Ngu)	30	−21.7 ± 1.0	2.3 ± 0.6	mixed vegetal	mixed vegetal	1.3
Idotea balthica (Ib)	27	−17.0 ± 1.1	3.1 ± 0.6	dead leaves	algae	1.7
Stenosoma lancifer (Sl)	7	−18.1 ± 0.3	4.6 ± 0.1	mixed vegetal (algae dominated)	omnivore	2.4
Apanthura corsica (Ac)	5	−19.0 ± 0.8	2.6 ± 0.7	/	mixed vegetal	1.5
Athanas nitescens (An)	61	−18.4 ± 0.7	4.4 ± 0.8	mixed vegetal	omnivore	2.3
Palaemon xiphias (Px)	52	−17.0 ± 1.4	6.1 ± 0.6	carnivore	carnivore 1	3.0
Processa edulis (Pe)	5	−17.7 ± 0.3	5.7 ± 0.7	/	carnivore 1	2.8
Hippolyte leptocerus (Hl)	9	−17.8 ± 0.4	4.1 ± 0.3	omnivore	omnivore	2.1
Macropodia linaresi (Ml)	5	−19.4 ± 0.5	4.2 ± 0.4	/	omnivore	2.2
Liocarcinus navigator (Ln)	19	−16.0 ± 2.0	5.1 ± 0.6	omnivore	carnivore 1	2.6
Liocarcinus holsatus (Lh)	22	−19.7 ± 2.6	4.0 ± 0.5	mixed vegetal (algae-dominated)	omnivore	2.1
Galathea intermedia (Gi)	13	−18.4 ± 0.5	2.5 ± 1.3	mixed vegetal (algae-dominated)	mixed vegetal	1.4
Nebalia strausi (Ns)	31	−17.5 ± 0.6	3.9 ± 0.7	mixed vegetal	omnivore	2.0
Polychaeta spp. (Pspp)	38	−18.2 ± 0.6	3.9 ± 0.8	/	omnivore	2.1
Bittium reticulatum (Br)	9	−13.8 ± 0.3	3.7 ± 0.6	/	dead leaves	2.0
Gobius spp. (Gspp)	9	−17.9 ± 1.6	8.1 ± 0.3	carnivore	carnivore 2	3.9

2.3. SIAR modelling

The Bayesian mixing model SIAR (Stable Isotope Analysis in R; Inger et al., 2010; Parnell et al., 2010) was used to give estimations of the contribution of every potential food source to the diet of the invertebrate consumers (Layman et al., 2012). The SIAR 4.2.2 package was fitted in R 3.3.2 (R Development Core Team, 2016), using the isotopic composition of each individual, the potential food sources (mean ± SD), and the trophic enrichment factors (hereafter, TEFs; expressed as mean ± SD). Here TEFs for both isotopic ratios were taken from literature reviews (McCutchan et al., 2003) and published laboratory feeding experiments (Remy et al., 2017; Michel et al., 2015) (food sources, acronyms, and TEFs are detailed in Table 1). The model was run with 10^6 iterations and "burn-in" size set as 10^5. Model outputs were presented as non-metric multidimensional scaling (nm-MDS) representations (+ANOSIM), or intervals of distribution of probability density functions (see statistics section).

2.4. tRophicPosition modelling

The Bayesian tRophicPosition model package (version 0.5.0.1000; Quezada-Romegialli et al., 2016) was used to estimate trophic position parameters of all sampled species in R 3.3.2. The model was run using $\delta^{13}C$ and $\delta^{15}N$ values of consumers, basal food sources, and TEFs from Remy et al. (2017) for the living and dead *P. oceanica* leaves, and from Michel et al. (2015) for epiphytes and SPOM (in red, Table 1). For each species, the model took two baseline items into account that were selected from SIAR model outputs. The two items displaying the highest mode (i.e. contributing the most to each species' diet) in the SIAR output were selected. For predators, the model took into account the baseline items consumed by their prey to remain consistent. The trophic position of these baselines were given the value of 1 ($\lambda = 1$). For each taxon, two parallel chains were sampled with 10000 adaptive iterations. Model solutions were presented using credibility intervals of probability density function distributions. When relevant, direct pairwise comparisons of model-estimated trophic positions were performed. These comparisons were considered meaningful when probability of occurrence exceeded 99%.

2.5. Statistical analyses

An nm-MDS ordination technique and ANOSIM analysis were performed on GCA data and SIAR outputs to distinguish potential temporal patterns and the trophic grouping of the samples. nm-MDS is based on an iterative procedure. In this study, we performed a 2D nm-MDS using the corresponding 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. Corresponding ANOSIM analysis was performed on relative proportion data using PRIMER v6.1.13 for Windows.

Preliminary ANOVA analyses to test the isotopic separation of sampling sites and potential food sources were performed using R 3.3.2 and all test results were considered significant when p was ≤ 0.01. Graphs were built with R 3.3.2 and Primer 6.

3. Results

3.1. Gut content analysis

Of the 566 organisms sampled, 24.39% had empty guts or did not present enough gut content material for useful observation. Guts from 428 individuals from 14 species were therefore examined, and the main ingested items identified.

From these 14 species, the nm-MDS and 1-way ANOSIM analysis (Fig. 1) highlighted five significant (ANOSIM, $p < 0.01$) grouping patterns (see Table 2) corresponding to five ingestion patterns: 1) "Litter consumers"; 2) "Algal consumers"; 3) "Mixed vegetal consumers"; 4) "Mixed omnivorous consumers"; 5) "Carnivores".

"Litter consumers", ingesting mostly dead leaves of *Posidonia oceanica*, consisted of the amphipod *Gammarus aequicauda* (Martynov, 1931) and the isopod *Idotea balthica* (Pallas, 1772). "Algal consumers", ingesting mostly algal material, consisted of the amphipod *Gammarella fucicola* (Leach, 1814), the decapods *Galathea intermedia* (Liljeborg, 1851) and *Liocarcinus holsatus* (Fabricius, 1798), and the isopod *Stenosoma lancifer* (Leach, 1814). "Mixed vegetal consumers", ingesting mostly a mix of dead leaves of *P. oceanica* and algal material, consisted of the amphipods *Nototropis guttatus* (Costa, 1853) and *Melita hergensis* (Reid, 1939), the decapod *Athanas nitescens* (Leach, 1813), and the leptostracean *Nebalia strausi* (Risso, 1826). "Mixed omnivorous consumers", ingesting vegetal but also non-negligible amounts of animal material, consisted of the decapods *Liocarcinus navigator* (Herbst, 1794) and *Hippolyte leptocerus* (Heller, 1863). It must be mentioned that the decapod *Palaemon xiphias* (Risso, 1816), ingesting almost exclusively

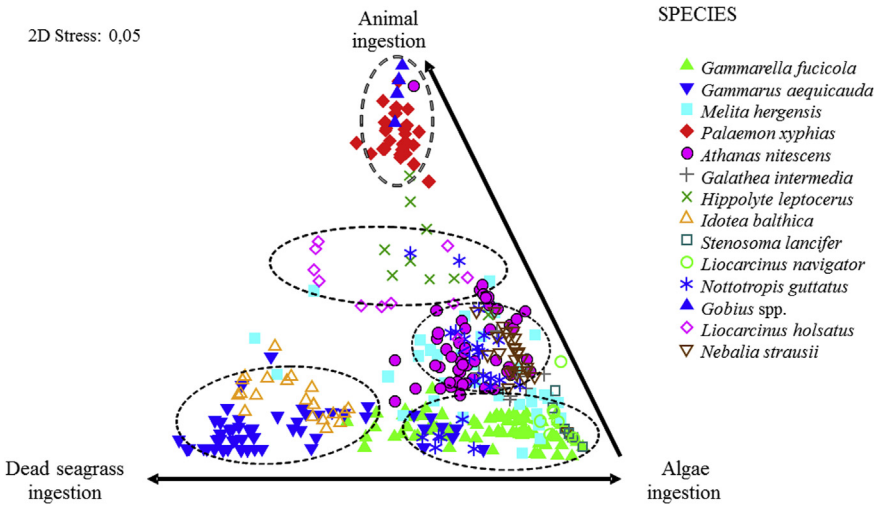

Fig. 1. 2D ordination of samples obtained via non-metric multidimensional scaling (nm-MDS), using Bray-Curtis similarities computed on relative proportion data from gut content examination of macrofauna inhabiting *Posidonia oceanica* dead leaf accumulations.

animal material, is the only representative of the "carnivores" group of this EMA macrofauna community. Due to very low sample size, *Gobius* spp. fishes were not included in the ANOSIM analysis but were grouped with *P. xiphias* in the nm-MDS ordination constituting a "group". No significant grouping according to sampling site was found.

3.2. Stable isotope analyses

The $\delta^{15}N$ and $\delta^{13}C$ values of the 19 studied macrofauna species (i.e. the 14 species used in GCA + the 5 species presenting empty guts, for a total of 566 individuals) ranged from −0.9 to 8.5‰ and from −23.3 to −13.0‰ respectively (Fig. 2). The $\delta^{15}N$ and $\delta^{13}C$ values of the five main basal food sources ranged from 1.0 to 2.2 and from −31.9 to −13.4‰ respectively (Fig. 2). Food sources displayed little or no significant differences in $\delta^{15}N$ (1-way ANOVA, $p > 0.01$) but displayed significant differences in $\delta^{13}C$ (1-way ANOVA, $p < 0.001$), except for the "Algae" and the "Epiphytes" sources (1-way ANOVA, $p = 0.322$). These two food sources, isotopically indistinguishable from each other, were thus pooled and treated as a single food source in all following analyses. Since no significant difference between the two sampling sites was identified (1-way ANOVA, $p > 0.01$), samples from both sites were also pooled for each species in all following analyses.

The SIAR model runs confirm the presence of different dietary preferences (Fig. 2, Table 3). The nm-MDS and ANOSIM (1-way ANOSIM, $p < 0.001$) analyses based on the SIAR outputs clearly showed the presence of 3 main significant groups: I, II and III (Fig. 2, Table 2). Group I corresponds to primary consumers and is composed of three sub-groups: dead leaf consumers (DL), mixed vegetal consumers (MIX), and *Idotea balthica* (TR) (Fig. 2). Group II is composed of two sub-groups: omnivore consumers (OMNI) and first order carnivorous predators (P1). Group III is composed of only one sub-group, second order carnivorous predators (P2). Overall, each sub-group corresponds to a given dietary preference (Fig. 2). In group I, the dead leaf consumers sub-group is composed of organisms assimilating mainly dead leaves of *P. oceanica*. The mixed vegetal consumers sub-group is composed of organisms ingesting mostly a mix of dead leaves of *P. oceanica* and epiphytes/algae. *I. balthica* is isolated which reflects the fact that it assimilates mostly vegetal items and small amounts of animal tissue (but less than omnivores). Interestingly, SIAR modelling does not retain detritus as an important food source, despite the fact that gut contents were often full of dead leaves. In group II, the omnivore sub-group is composed of organisms consuming a large proportion of animal prey

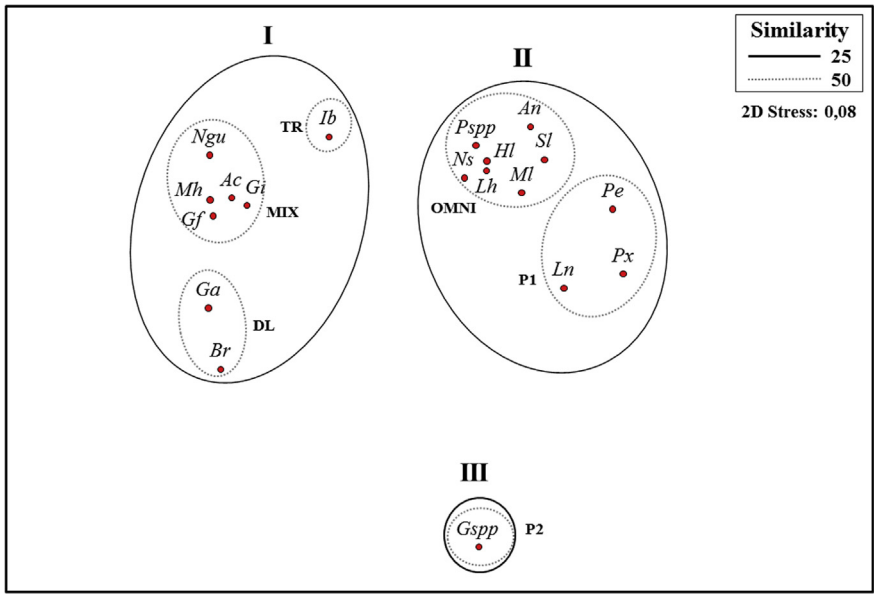

Fig. 2. 2D ordination of samples obtained via non-metric multidimensional scaling (nm-MDS) using Bray-Curtis similarities computed on SIAR (Stable Isotope Analysis in R) modelling output (Table 3). Trophic types were determined according to gut content analysis. DL: seagrass dead leaf consumer; MIX: consumer of both dead leaves and epiphytes; TR: diet transitional between first order consumers and omnivores; OMNI: omnivore; P1: first order carnivore; P2: second order carnivore. Species acronyms: *Apanthura corsica* (Ac), *Athanas nitescens* (An), *Bittium reticulatum* (Br), *Galathea intermedia* (Gi), *Gammarella fucicola* (Gf), *Gammarus aequicauda* (Ga), *Gobius* spp. (Gspp), *Hippolyte leptocerus* (Hl), *Idotea balthica* (Ib), *Liocarcinus holsatus* (Lh), *Liocarcinus navigator* (Ln), *Macropodia linaresi* (Ml), *Melita hergensis* (Mh), *Nebalia strausi* (Ns), *Nototropis guttatus* (Ngu) Polychaeta spp. (Pspp), *Processa edulis* (Pe), *Stenosoma lancifer* (Sl).

Table 3
Estimations of the contribution of potential food sources to the diet of the macrofauna species inhabiting *Posidonia oceanica* dead leaf accumulations calculated using the mixing model SIAR (Stable Isotope Analysis in R). Model output is presented as mode and inferior (CI_{95} inf) and superior (CI_{95} sup) limits of 95% credibility intervals of posterior probability density function distributions. Acronyms for food sources are the same as in Table 1.

Species names	Food sources	Food source contributions			Species names	Food sources	Food source contributions		
		CI_{95} inf	Mode	CI_{95} sup			CI_{95} inf	Mode	CI_{95} sup
Gamarella fucicola	Dead leaves	4.7	33.5	58.1	*Hippolyte leptocerus*	Dead leaves	0	0.9	10.3
	Epi	5.6	41.8	84.0		Epi	4.1	20.8	36.2
	SPOM	0.1	10.5	31.1		GFMH	7.0	29.1	48.8
	RMA	0	1.4	15.9		GA	0	8.3	23.8
	Cop	0	0.4	3.9		Cop	18.0	35.9	54.8
Gammarus aequicauda	Dead leaves	47.8	60.3	80.6	*Macropodia linaresi*	Dead leaves	0	1.5	21.3
	Epi	1.6	48.7	33.6		Epi	0	13.0	31.4
	SPOM	0	14.2	1.3		GFMH	0.8	27.9	48.2
	RMA	0	7.8	0.9		GA	0	23.3	38.7
	Cop	0	2.9	0.2		Cop	7.0	31.5	56.7
Melita hergensis	Dead leaves	5.1	34.1	55.03	*Liocarcinus navigator*	Living leaves	0	0.9	13.8
	Epi	1.6	34.3	72.3		Dead leaves	0	0.9	13.6
	SPOM	1.2	23.6	39.2		Epi	0	0.8	11.4
	RMA	0	1.8	20.9		GFMH	0	2.1	28.5
	Cop	0	0.5	5.3		GA	15.2	43.3	68.9
Nottotropis guttatus	Dead leaves	0.5	23.4	37.5		Cop	7.5	34.0	60.9
	Epi	1.0	29.0	52.7	*Liocarcinus holsatus*	Dead leaves	0	1.0	13.6
	SPOM	3.7	30.8	59.6		Epi	3.1	18.6	31.9
	RMA	0.1	20.0	34.6		GFMH	4.2	30.2	51.2
	Cop	0	0.7	5.9		GA	0	2.2	29.0
Idotea balthica	Dead leaves	0.2	9.1	20.3		Cop	15.1	35.6	58.1
	Epi	34.4	58.8	74.8	*Galathea intermedia*	Dead leaves	14.0	32.0	54.8
	GFMH	0	12.1	38.1		Epi	0.6	27.3	52.3
	GA	0	1.4	17.8		SPOM	0.1	19.6	30.5
	Cop	0	1.8	21.2		RMA	0	5.1	18.8
Stenosoma lancifer	Dead leaves	0	0.8	9.2		Cop	0	4.1	30.6
	Epi	0	3.1	20.9	*Nebalia strausi*	Dead leaves	0	8.5	0.9
	GFMH	3.0	32.8	52.1		Epi	15.4	42.2	29.2
	GA	0	9.0	29.4		GFMH	10.5	45.8	29.1
	Cop	22.6	45.0	70.0		GA	0.4	18.5	10.3
Apanthura corsica	Dead leaves	11.5	31.6	52.5		Cop	15.2	44.7	29.9
	Epi	0.4	27.9	52.6	Polychaetes (spp.)	Dead leaves	0	3.5	0.3
	SPOM	0.6	24.1	37.7		Epi	11.0	36.4	24.0
	RMA	0	4.6	22.2		GFMH	16.6	59.1	38.9
	Cop	0	2.5	26.6		GA	0	6.4	0.8
Athanas nitescens	Dead leaves	0	0.2	2.5		Cop	18.3	52.0	35.3
	Epi	0	4.2	14.3	*Bittium reticulatum*	Living leaves	0	52.4	12.8
	GFMH	25.6	44.3	59.5		Dead leaves	38.0	84.0	67.4
	GA	0	0.8	5.6		Epi	0	13.1	1.1
	Cop	33.3	48.0	62.2		SPOM	0	4.3	0.5
Palaemon xiphias	POOL	0	14.2	37.1		Cop	0	15.9	1.6
	GFMH	0	13.4	31.9	Gobidae (spp.)	POOL	0	23.5	2.1
	GA	17.0	28.8	39.5		GFMH	0	9.1	0.9
	GSPP	14.5	23.1	30.2		GA	0	8.5	0.7
	Cop	0	16.8	33.2		PX	65.5	92.6	81.6
Processa edulis	POOL	0.9	24.0	42.9		Cop	0	11.3	1.0
	GFMH	3.2	25.4	41.2					
	GA	1.1	18.1	30.3					
	GSPP	2.1	13.2	25.0					
	Cop	1.3	23.7	40.1					

but also a small amount of vegetal material, while first order predators represent pure carnivorous predators consuming only animal prey. Group III was only composed of sub-group P2, juvenile *Gobius* spp. fishes. This separation was potentially caused by their diet, composed mainly of animals from the first order carnivore sub-group.

The tRophicPosition model classified the 19 species into four significant "groups" (Fig. 3, Table 2). The first group displayed trophic positions with median values not significantly different from each other and between 1.2 and 1.8. It is composed of seven species (*G. fucicola*, *G. aequicauda*, *M. hergensis*, *N. guttatus*, *I. balthica*, *A. corsica*, and *G. intermedia*) which constitute the primary consumers. A second group composed of 8 species (*S. lancifer*, *A. nitescens*, *H. leptocerus*, *M. linaresi*, *L. holsatus*, *N. strausi*, Polychaetes, and *B. reticulatum*) showed trophic position median values between 2.0 and 2.4, representing the secondary consumers. A third group composed of three species (*Palaemon xiphias*, *Processa edulis*, and *Liocarcinus navigator*) had trophic position median values between 2.6 and 3.0 and thus represents the tertiary consumers. The fourth and last "group" is composed of only one species, the *Gobius* spp. juveniles, displayed a trophic position median value of 3.9, and represents the quaternary consumers.

4. Discussion

Our data highlighted both the important role of epiphytic/algal material but also of dead *P. oceanica* material to support the food web associated to *Posidonia* macrophytodetritus accumulations. In terms of numerical abundance (Remy, 2016), the trophic web is dominated by herbivores/detritivores. Herbivores/detritivores represented 50% of the EMA community (9.4 ± 23.6 ind. gDM^{-1}; Remy, 2016) with a diet consisting of up to 35% seagrass detritus. Moreover, the diet of the very

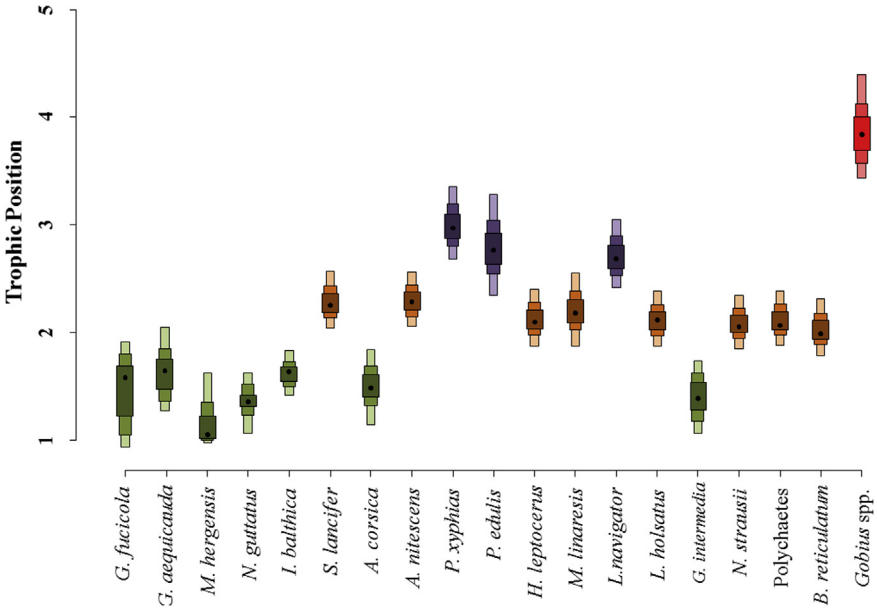

Fig. 3. Trophic position calculation using the tRophicPosition model of macrofauna species inhabiting *Posidonia oceanica* dead leaf accumulations. Dark, median, and light coloured boxes and black dots are respectively the 50%, 75%, and 95% credibility intervals and modes of model solutions' probability density function distributions. Species acronyms may be found in Table 2.

abundant *G. aequicauda* (8.05% of the EMA community, 2.7 ± 3.1 ind. gDM^{-1}; Remy, 2016) contained up to 80% seagrass detritus. Macrofauna consumption could therefore be a major vector of transmission of seagrass-derived organic matter in EMAs. This implies that this fauna participates not only in the fragmentation and degradation of macrophytodetritus, as revealed by gut contents, but also in the transfer of seagrass organic carbon to upper trophic levels, as revealed by stable isotopes. In terms of abundance (Remy, 2016), 60% of the community assimilates from 35 to 80% of consumed detrital seagrass material, which is far from negligible in terms of organic matter flux.

The role of detrital seagrasses as a potential food source for marine invertebrates has already been demonstrated in various temperate or tropical seagrass ecosystems (Kharlamenko et al., 2001; Vizzini et al., 2005; Vonk et al., 2008) or EMA systems (Kon et al., 2015; Hyndes and Lavery, 2005). Our study demonstrates that this assimilation, and therefore the seagrass organic matter transfer, is particularly important in *P. oceanica* dead leaf accumulations. This situation seems different from south-western Australia macrophytodetritus accumulations, where *Posidonia* spp. and *Amphibolis* spp. seagrass detritus are only weakly transferred in the trophic web (Hyndes and Lavery, 2005). In those EMAs, drift brown macroalgae are also abundant and this material is likely to make a greater contribution to the food web. This implies that EMA composition is likely to influence the associated trophic web.

In our study, the detritus is not the only food sources consumed in important amounts as epiphytes/macroalgae are also very important for community trophic support, like in the *P. oceanica* meadow itself (Michel et al., 2015). The presence of multiple food sources, available in variable amounts, is a key characteristic to maintain a diverse community with diverse diet preferences. The food web found in EMAs contrasts with the *P. oceanica* meadow itself, where the food web is dominated by small herbivorous species relying on the epiphytic community as food source (Lepoint et al., 2000; Vizzini, 2009). Detritivore amphipods are also present in the *P. oceanica* meadow but are generally not numerically dominant (Michel et al., 2015; Sturaro et al., 2015).

Therefore, detrital pathways occur mainly outside the meadow, in the exported macrophytodetritus accumulations of *P. oceanica* leaves that we have studied here. Seagrass primary production and seagrass organic matter processing by animals are therefore spatially decoupled, and this should be taken into account in assessments of seagrass ecosystems as key actors in C cycles in coastal areas.

According to the tRophicPosition model, this community encompassed 4 consumer levels, with primary consumers/detritivores, secondary omnivore species, first-order predators, and second-order predators. Few species display a more plant-based diet such as the isopod *Idotea balthica*, in agreement with a previous study focusing on idoteids of *P. oceanica* litter (Sturaro et al., 2010). Nevertheless, this is one species whose gut contents and stable isotopes are not in agreement. Indeed, *I. balthica* showed high levels of *Posidonia* detritus in their gut but stable isotope data showed that this detritus did not significantly contribute to the diet, meaning it is not assimilated. More likely, it is the epiphytes and microbes growing on leaves that are assimilated.

Species identified as primary consumers displayed different ingestion and assimilation preferences, but often with a non-negligible consumption of dead *P. oceanica*. For example, the amphipod *Gammarus aequicauda* showed massive (up to 80% of the diet) ingestion but also assimilation of dead *P. oceanica* fragments. The amphipod *Gammarella fucicola*, which is the most abundant species of this community (around 50% of individuals; Remy, 2016), and *Melita hergensis* assimilated large amounts of algae/epiphyte fragments but also assimilated dead *P. oceanica* leaves. This indicates that, in opposition to *G. aequicauda* which is specialised in seagrass litter consumption, these two amphipods rely equally on herbivory and on detritus feeding. This is also the case for two other crustaceans: the decapod *Galathea intermedia* and the isopod *Apanthura corsica*. They showed intermediate $\delta^{13}C$ values and present important overlaps with isotopic niches of *G. fucicola* and *M. hergensis*, indicating the equal consumption and assimilation of algae/epiphyte fragments and fragments of dead *P. oceanica* leaves. This highlights diet diversity among the detrivorous-herbivorous species, which do not share exactly the same trophic niches.

The omnivore group, composed of 8 species, was the more diverse but not the most abundant (Remy, 2016). The “typical” species of the group is the decapod *Athanas nitescens*. According to SIAR, this species assimilated equal amounts of first order consumers (mainly *Gamarella fucicola* as indicated by gut contents) and of harpacticoid copepods, and much less (5%) algae/epiphytes. Representing 8% of the total EMA community (Remy, 2016), these omnivores contribute to the transfer of seagrass organic matter via their consumption of detritivores and detritivore/herbivores. They play a crucial vector role in EMAs. This also shows the important role of meiofauna (i.e. animals with a body size between 38 μm and 1 mm) as an intermediary step in this trophic web (Mascart et al., 2015). In addition, meiofauna may also assimilate seagrass organic matter in these EMAs (Mascart et al., 2018) increasing

the potential amount of seagrass organic material transmitted to upper trophic levels.

The third trophic level was composed of 3 large carnivorous decapods: *Palaemon xiphias*, *Processa edulis*, and *Liocarcinus navigator*. These 3 species present quite well-defined niches, except for *P. edulis* that presents an intermediate niche overlapping with both *P. xiphias* and *L. navigator*. These 3 species shared a similar diet, assimilating a mix of herbivorous/detrivorous consumers, of *G. aequicauda*, of meiofauna, and, in the case of *P. xiphias*, a non-negligible amount of fish larvae. Even though these predators represent only 0.17% of the total EMA community (Remy, 2016), their isotopic composition evidences that, through their prey selection, they propagate organic matter-derived dead *P. oceanica* material from the bottom to the top of the food web.

The fourth and last consumer level was not composed of macroinvertebrates but of juvenile fishes of the *Gobius* genus. This niche corresponds to a diet composed mainly of predator crustaceans from the previous trophic position. Many other fishes are observed in the accumulations and, notably, include small Labridae and Mullidae that are known to feed on small crustaceans. Animals found in the EMAs act as a vector of seagrass organic material to the entire coastal food web, via fishes that feed both in the litter and in other compartments of the system (i.e. macroalgae and seagrass beds, sandy habitats, water column).

The food web described here appears to be based on multiple basal food sources (i.e. seagrass detritus and various pools of epiphytes or microbes). The abundance of detritivores and herbivore/detritivores that are actively consumed by omnivores and predators inside (but also outside) the EMAs make the transfer of seagrass organic material to other compartments of the coastal food web not only possible, but likely efficient. We therefore argue that macrofauna from EMAs can be seen to be major vectors of seagrass-derived organic matter.

Acknowledgements

The authors thank the STARESO field station staff for their assistance during dives and sampling setup. F. Remy acknowledges a F.R.I.A. grant (Belgian National Fund for Research training in Industry and in Agriculture). This study was conducted within the scope of FRS-FNRS research project FRFC 2.4511.09. G. Lepoint is a Research Associate of the FRS-FNRS. This is publication MARE number 379.

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