

Mesograzers in *Posidonia oceanica* meadows: an update of data on gastropod-epiphyte-seagrass interactions

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Abstract

Information on dietary habits of mesograzers in *Posidonia oceanica* seagrass meadows is scarce and restricted to a few species. Here we provide data on the most likely food sources for eight gastropod species inferred from stable isotope data. We observed very similar isotopic signals for all species regardless of trophic guild category, indicating similar consumption behaviour with a main diet contribution from epiphytes. We also review the state of knowledge on gastropod-epiphyte-seagrass interactions, with particular emphasis on the scarcity of studies derived from Mediterranean systems. Laboratory experiments showed that under moderately nutrient-enriched conditions, two species of gastropods (*Bittium reticulatum* and *Jujubinus striatus*) were controlling epiphyte biomass at high grazer densities, with no consequences for seagrass performance. Finally, the results of a long-term *in situ* fertilisation experiment showed that the $\delta^{15}\text{N}$ signal for seagrass, epiphytes and gastropods reflects experimentally induced eutrophication, thus cascading up nutrient effects throughout the food web.

Keywords: epiphytes; gastropods; grazing; nutrients; *Posidonia oceanica*; stable isotopes.

Introduction

Seagrass meadows shelter an important diversity of flora and fauna (Hemminga and Duarte 2000). One important component of this diversity is the vagile fauna, a group associated with seagrass blades and comprising small animals (crustaceans, molluscs, polychaetes) with reduced mobility. In *Posidonia oceanica* (L.) Delile meadows, gastropod molluscs are among the most important elements of this vagile fauna in terms of both species richness (Mazzella and Russo 1989, Gambi et al. 1991) and numbers of individuals (Ledoyer 1968, Kikuchi and Peres 1977, Templado et al. 2004). Gastropods form

characteristic assemblages on leaves of *P. oceanica*, whereas leaf sheaths and rhizomes shelter species from the leaves and others from nearby infralittoral communities (macroalgal, coraligenous; Templado 1984a).

Seagrass leaf-associated gastropods generally feed on the epiphytic component, ingesting the biofilm composed of bacteria and diatoms, as well as various algal groups and animals (Van Montfrans et al. 1982, Kitting 1984, Mazzella et al. 1992). Gastropods have traditionally been assigned to different feeding guilds (Purchon 1968) coupled to the morphology of the feeding apparatus (radula) that constrains acquisition of food material and indirectly modulates the feeding resources for grazers (Steneck and Watling 1982) via selection of different food types (Peduzzi 1987, Mazzella and Russo 1989, Jernakoff and Nielsen 1997). These trophic abilities underlie our traditional understanding of feeding habits and dietary preferences of molluscs; for example, the diet of the herbivorous snail is based on the size, shape and toughness of a single algal species (Steneck and Watling 1982, Mazzella et al. 1992). Thus, trophic strategies of seagrass-associated gastropods are diverse and difficult to approach at the community level, but they can be relevant to the dynamics of the whole system (Phillippart 1995, Hughes et al. 2004).

Stable isotope analysis is an indirect method for identifying food pathways within complex ecosystems (Fry et al. 1987). In general, carbon (C) isotopes are used to identify the most likely carbon source of individual organisms (Fry and Sherr 1984), whereas nitrogen (N) isotopes are typically used to identify the trophic position of these organisms within the food web (Fry et al. 1987). The nitrogen isotopic signature at the consumer level can also be used to define foraging sites for organisms and the compositions of their diets relative to differences in N inputs (Shriver et al. 2002, Weiss et al. 2002, Carmichael et al. 2004, Vizzini and Mazzola 2004), and thus is a useful tool for tracking eutrophication phenomena (McClelland et al. 1997).

In *Posidonia oceanica* seagrass meadows, data on the relative contribution of different food sources have been investigated for macroherbivores (i.e., fish and sea urchins) using direct analyses of gut contents (Kemp 1962, Verlaque 1981), integrative stable isotope approaches (e.g., Havelange et al. 1997, Jennings et al. 1997, Vizzini and Mazzola 2004, 2006, Tomas et al. 2006, among others), or a combination of both (Pinnegar and Polunin 2000). Unfortunately, information on dietary habits of mesograzers is scarce, and for gastropods it is restricted to manipulative experiments designed to assess dietary preferences of *Gibbula* and *Jujubinus* spp. associated with *P. oceanica* (Peduzzi 1987, Mazzella and Russo 1989) and isolated data on stable isotopic compositions for a few species from the Bay of Calvi (Dauby 1989, Lepoint et al. 2000). Meadows of *Zostera marina* L.

have been studied more comprehensively, with direct microscopic observation of seagrass leaf scars after grazing (Van Montfrans et al. 1982, Neckles et al. 1993) and, most recently, a combination of stable isotopes and fatty acid biomarkers for several gastropod species (Kharlamenko et al. 2001, Alfaro et al. 2006).

Interactions between grazers and epiphytes result in reductions in epiphyte biomass, increases in production and a shift in species composition (see the review by Jernakoff et al. 1996). For gastropods, direct data on grazing activity are scarce but there is experimental work showing epiphyte control by gastropods on *Thalassia testudinum* Banks et Sol. ex Koenig from Florida (Frankovich et al. 2003, Peterson et al. 2007), *Zostera* species from North America and Hong Kong (Neckles et al. 1993, Nelson 1997, Fong et al. 2000) and *Posidonia sinuosa* Cambridge et Kuo from Western Australia (Jernakoff and Nielsen 1997). In the Mediterranean Sea, although seagrasses can accumulate large amounts of epiphytes (Cebrián et al. 1999, Tomas et al. 2005), particularly the long-lived *Posidonia oceanica*, we do not know of any study on potential control of seagrass epiphyte biomass communities by mesograzers. Regulation of epiphyte overgrowth by grazers is particularly relevant under high nutrient conditions and may result in enhancement of seagrass productivity and/or survival. Hughes et al. (2004) compiled data on the impact of nutrients and the effects of grazers in 35 meadows worldwide. They mention only one reference for the Mediterranean Sea, viz. epiphyte overgrowth on the seagrass *Cymodocea nodosa* (Ucria) Ascherson, but no information on interaction with grazers is provided. More recently, studies on *P. oceanica* have shown that nutrient inputs cause an increase in epiphyte biomass and a shift in species composition (Prado et al. 2008) and promote macrograzer pressure under experimental (Prado 2007) and anthropogenic nutrient input gradients (Kirkman and Young 1981, Ruiz et al. 2001). However, there is still no information on whether mesograzers play a significant role in this seagrass-epiphyte interaction in the Mediterranean Sea.

The goals of the present study were: 1) to assess trophic guilds of gastropod molluscs living as vagile fauna on seagrass *Posidonia oceanica* using stable isotopes; 2) to experimentally examine the role of gastropods in regulating epiphyte biomass and its potential interaction with seagrass *P. oceanica* under laboratory conditions; and 3) to assess whether mesograzers reflect experimentally induced eutrophication following a long-term *in situ* experiment.

Materials and methods

All specimens used in this study (gastropods, seagrass and epiphytes) were collected in a shallow (7 m depth) *Posidonia oceanica* meadow growing over sand located off Fenals Point (NW Mediterranean Sea; Figure 1). Gastropods were identified and classified into trophic guild categories following Purchon (1968) and Steneck and Watling (1982).

Trophic behaviour assessment using stable isotopes

We collected gastropods of different species (minimum length 15 mm) from the Fenals *Posidonia oceanica* meadow (Figure 1) and inferred food diet from the stable isotopic composition. Individuals were left for 24 h in seawater tanks to eliminate faeces and then stored frozen. To sort the snails, the shells were broken and the bodies extracted, making sure that there were no pieces of shell left. Gastropods were rinsed in distilled water, dried (24 h, 60°C) and ground to a fine powder. Samples were sealed in glass vials for further isotope analysis. All species were collected in summer 2004.

Isotope abundances were measured on a continuous-flow isotope radiomass spectrometer Delta C (Thermo Finnigan, Bremen, Germany) coupled to a Flash 1112 elemental analyser (Thermo Finnigan) through a ConFlo III interface. Carbon and nitrogen were analysed in dual isotope mode. Samples of reference material (internal standards) were used to calibrate the system and compensate for drift with time. Experimental precision, based on the standard deviation of replicates of the internal standard, was 0.5‰ for both isotopes. The isotope ratios are expressed relative to the Pee Dee Belemnite (PDB) standard for C and to N₂ in air for N. Isotope ratios were calculated as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}} - 1)] \times 1000$$

where R is the corresponding ¹³C/¹²C or ¹⁵N/¹⁴N ratio.

All analyses were performed at the Serveis Científic-Tècnics (University of Barcelona).

Interaction between meso-herbivores, epiphytes and *Posidonia oceanica* under laboratory conditions

To examine the interactions among gastropods, epiphytes and the seagrass *Posidonia oceanica*, we used 12 independent 10-l glass tanks filled with seawater. Ten *P. oceanica* shoots were placed in each tank, and four grazing treatments were allocated randomly: control (no grazers); 10 *Jujubinus striatus* L.; 20 *J. striatus*; and 20

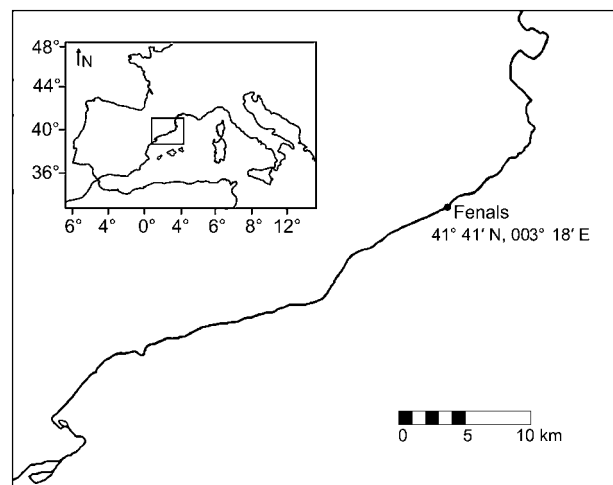


Figure 1 *Posidonia oceanica*: map of the study and sampling area at Fenals Point, northwestern Mediterranean Sea.

Bittium reticulatum da Costa individuals. These densities correspond to the maximum values recorded at Fenals meadow (10 individuals of this size in 20×20 cm²), plus two treatments with twice the maximum grazer densities recorded in the field. Tanks were kept under controlled light levels (60–160 μmol photons m⁻² s⁻¹) at a daylength of 14 h. The tank water was enriched with nutrients (100 μM NO₃⁻, 10 μM NH₄⁺ and 3.6 μM PO₄²⁻) to levels found at highly visited beaches nearby (Flo et al. 2007). The two gastropod species represent the most abundant herbivorous feeding guilds within seagrass meadows (grazing herbivores and herbivorous deposit-feeders) and, in contrast to natural conditions, they were applied separately in the different treatments. To estimate seagrass leaf growth (g dry wt [DW] shoot⁻¹), shoots were marked by the punch-hole method (Romero 1989) at the beginning of the experiment (August 13, 2005). The experiment ran for 25 days and the tank water was changed every 5 days. At the end of the experiment, leaf elongation and the length and width of the marked shoots were measured. Epiphytes from the shoots collected were removed by scraping with a razor blade (Alcoverro et al. 1997). Shoots and epiphytes were then dried (70°C to constant weight) and weighed separately. The leaf growth rate (g DW shoot⁻¹ day⁻¹) was determined by dividing the weight of new tissue (estimated from the relationship between leaf area and leaf DW) by the number of days elapsed since the marking event. Epiphyte load was determined as the epiphyte biomass per shoot (g DW shoot⁻¹). We used nested ANOVA [fixed factor, grazing pressure (GP); nested random factor, tank] to test for differences in epiphyte biomass and seagrass growth. There were no significant differences in the initial plant conditions (number of leaves per tank; ANOVA, F=0.92, n=12, p=0.52).

Mesograzers as markers of eutrophication

Following a long-term (1 year) *in situ* fertilisation experiment (Prado 2007), we investigated whether mesograzers would experimentally track induced eutrophication by collecting the most common gastropod (*Bittium reticulatum*) in the different treatment plots. The experimental design consisted of six seagrass patches (2–3 m²), three of them randomly assigned to a nutrient enrichment treatment, and the remaining three to controls. Nutrient enrichment was conducted monthly over 1 year using porous floating containers filled with a mixture of nutrient

salts or Osmocote (Panreac, Heerlen, The Netherlands) slow release fertiliser (see Prado et al. 2008 for a full methodological description). Nutrient loading was not measured during the experiment because strong effects were clearly detected in plant and epiphyte N contents (Prado et al. 2008). Here we provide information on seasonal variation in the composition of the epiphytic community for the two treatments based on a functional form approach (Littler et al. 1983) using multi-dimensional scaling ordination (MDS). For stable isotope analyses, we collected 4–5 seagrass shoots and 4–12 *B. reticulatum* individuals (depending on natural abundances) from each plot. We also analysed the δ¹⁵N signal of the fertiliser (see above for stable isotope analysis methodology). Owing to the limited amounts of gastropod material available, all individuals collected in a plot were pooled and thus differences in δ¹³C and δ¹⁵N between treatments (controls vs. fertilised) were assessed using a t-test. For seagrass blades and epiphytes, however, we conducted nested ANOVAs [factor treatment (T) fixed, factor plot (P) random and nested P(T)]. When necessary, if the factor plot was not statistically significant, we pooled P(T) with the residual to increase power with more degrees of freedom (Quinn and Keough 2002). Significant pairwise differences between means were tested using Tukey's test.

Results

Trophic groups in gastropods

We collected a total of 20 gastropods in eight taxa and three trophic guilds: grazing herbivore (GH), deposit-feeder herbivore (DFH) and benthic hunter (BH; Table 1). Representation of the different species in the biplot (Figure 2) shows that the carbon source for seagrass-associated gastropods (Table 2; mean±SD 18.52±0.51, n=20) is far from that for seagrass (13.69±0.70, n=10), and close to that for the epiphytic community (19.21±1.28, n=18). δ¹⁵N values were slightly enriched in the gastropods (Table 2; 5.59±0.61, n=20) compared to the primary producers (3.23±0.38, n=10 for *Posidonia oceanica* leaves and 4.15±0.44, n=18 for associated epiphytes). Within grazers, maximum variation in the δ¹⁵N signal was 1.86 units and differences within trophic guilds were not significant (Table 3; Figure 3).

Table 1 Feeding guilds and radula types to which species collected in a *Posidonia oceanica* bed off Fenals (northwestern Mediterranean Sea) were assigned.

Superorder	Family	Species	Feeding guild	Radula type
Archeogastropoda	Trochidae	<i>Jujubinus striatus</i>	GH	Rhipidoglossan
		<i>Gibbula</i> sp.	GH	Rhipidoglossan
Caenogastropoda	Calliostomatidae	<i>Calliostoma</i> sp.	GH	Rhipidoglossan
	Cerithiidae	<i>Cerithium vulgatum</i> Bruguière	DFH	Taenioglossan
		<i>Bittium reticulatum</i>	DFH	Taenioglossan
	Muricidae	<i>Muricopsis cristata</i> Brocchi	BH	Rachiglossan
	Nassaridae	<i>Nassarius incrassatus</i> Ström	BH	Rachiglossan
Conidae	<i>Mitromorpha</i> sp.	BH	Rachiglossan	

GH, grazing herbivore; DFH, deposit feeder herbivore; BH, benthic hunter.

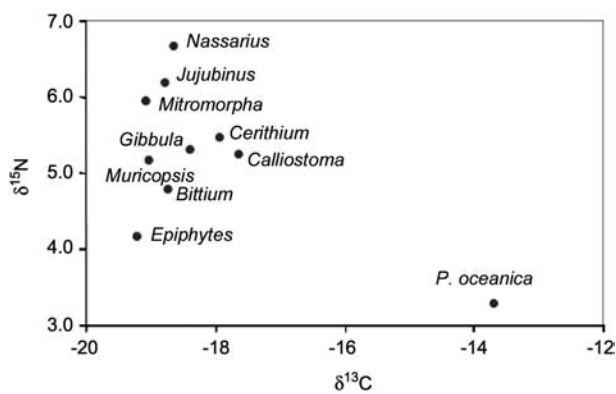


Figure 2 Stable isotope biplot for the different gastropod genera, epiphytes, and *Posidonia (P.) oceanica* from the Fenals meadow collected in summer 2004.

See Table 1 for more details on species identities.

Experimental manipulation of gastropods

After 25 days of experimental manipulation, we detected a significant effect of mesograzers in reducing epiphyte loading on *Posidonia oceanica* leaves (Table 4, Figure 4). The presence of gastropods reduced epiphyte biomass by 54–70% compared to controls; this effect was significant at high grazer densities (20 individuals) but not when 10 *Jujubinus striatus* individuals were present (Figure 4). When comparing the high grazer density treatments, epiphyte control by *J. striatus* was significantly higher than that by *Bittium reticulatum* (Figure 4). However, different gastropod densities did not result in differences in seagrass leaf production in terms of either biomass or surface area (Table 4).

Mesograzers as tracers of eutrophication

There were some differences in the composition of functional form groups of epiphytes from *Posidonia oceanica* undergoing *in situ* fertilisation compared to reference plots in spring and summer 2003 (Figure 5). Whereas bryozoans dominated in the control plots, filamentous algae proliferated in the fertilised plots in summer. Representation of the functional composition of the epiphytic community by treatments and season showed very similar epiphyte community composition across the experiment

Table 2 Means and standard deviations of the stable isotopic signals of mollusc species collected in a *Posidonia oceanica* meadow off Fenals.

Species	n	δ ¹³ C		δ ¹⁵ N	
		Mean	SD	Mean	SD
<i>Jujubinus striatus</i>	4	-18.76	0.13	6.17	0.19
<i>Gibbula</i> sp.	1	-18.39		5.30	
<i>Calliostoma</i> sp.	1	-17.64		5.25	
<i>Cerithium vulgatum</i>	1	-17.94		5.47	
<i>Bittium reticulatum</i>	10	-18.72	0.48	4.79	0.26
<i>Muricopsis cristata</i>	1	-19.03		5.16	
<i>Nassarius incrasatus</i>	1	-18.63		6.65	
<i>Mitromorpha</i> sp.	1	-19.06		5.94	
Epiphytes	18	-19.21	1.28	4.15	0.44
<i>Posidonia oceanica</i> leaves	10	-13.69	0.70	3.27	0.38

Data for epiphytes and *Posidonia oceanica* leaves are also included as a reference.

Table 3 Results of the paired t-tests comparing the C and N isotope signals among the different trophic guilds.

	δ ¹³ C			δ ¹⁵ N		
	GH	DFH	BH	GH	DFH	BH
GH	–			–		
DFH	0.91	–		0.42	–	
BH	0.17	0.39	–	0.54	0.24	–

Multiple pairwise comparisons without Bonferroni correction increase the possibility of a type I error. However, because there are no statistically significant differences at $p > 0.05$ among groups, this does not affect the result. Values correspond to the two-tailed p-value. GH, grazing herbivore; DFH, deposit feeder herbivore; BH, benthic hunter.

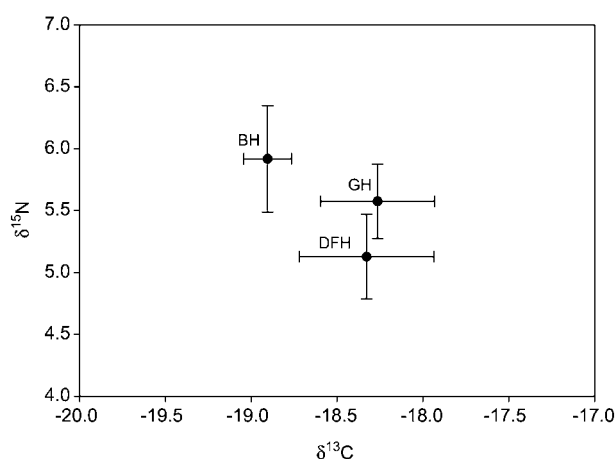


Figure 3 Mean ± SD values for the stable isotope biplots when grouping species by feeding guilds.

G, grazing herbivores (n=3 species); DFH, deposit feeder herbivores (n=2 species); BH, benthic hunter (n=3 species).

with the exception of samples from summer 2003 (Figure 6).

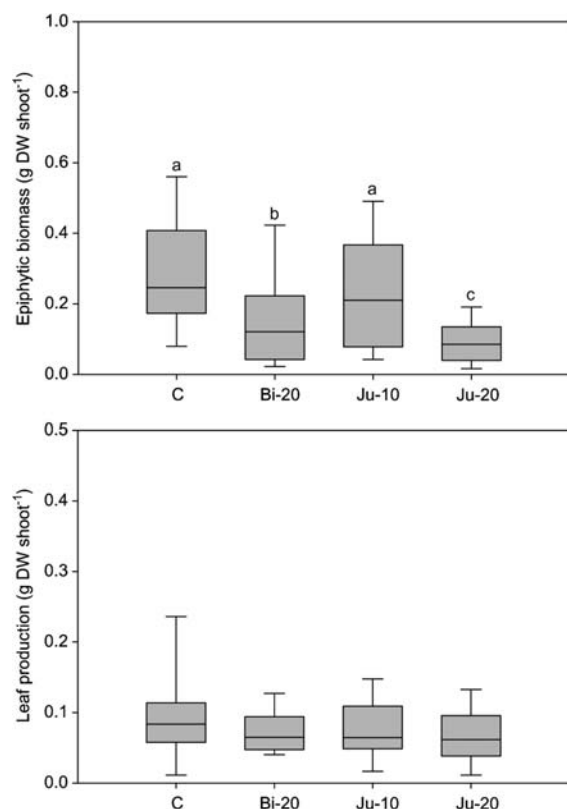
Fertilisation was detected in the δ¹⁵N signals of seagrass, epiphytes and gastropods, which were consistently and significantly lower in fertilised than in control plots and in accordance with fertiliser values (δ¹⁵N=0.4). However, we did not detect significant differences in δ¹³C among the different components of the food web (Table 5, Figure 7) in accordance with the lack of differences in the composition of epiphyte communities in control and

Table 4 Summary of the nested ANOVAs [factor treatment (T) fixed, factor plot (P) random and nested P(T)] to test for significant differences in epiphyte biomass, seagrass leaf elongation and leaf production during the laboratory experiment to test for gastropod-epiphyte-seagrass interactions.

ANOVA	df	MS	F	p
Epiphytic biomass				
Treatment=T	3	0.129	8.261	<0.00110
Tank=Tk (T)	8	0.023	1.47	0.187
Residual	60	0.0155		
Cochran's C		0.239		
Transformation		\sqrt{x}		
Leaf elongation				
Treatment=T	3	0.977	0.9	0.447
Tank=Tk (T)	8	5.48	5.059	<0.00110
Residual	60	1.0851		
Cochran's C		0.193		
Transformation		None		
Leaf production				
Treatment=T	3	0	0.321	0.81
Tank=Tk (T)	8	0.003	2.284	0.033
Residual	60	0.00143		
Cochran's C		0.130		
Transformation		None		

Grazing treatment (T), (no grazers, 10 *Jujubinus*, 20 *Jujubinus* and 20 *Bittium*); see Table 1 for species names. There were three tanks (P) per treatment containing 12 shoots each.

MS, mean square; df, degrees of freedom; p-values significant at 0.001. Cochran's test used to test for homogeneity of variances, with $p > 0.05$ observed for all values. Values in bold indicate significant differences for the factor considered.

**Figure 4** Epiphytic biomass and leaf production (in g DW shoot⁻¹) at the end of the gastropod-epiphyte-seagrass interactions laboratory experiment.

C, control; Bi-20, 20 *Bittium*; Ju-10, 10 *Jujubinus*; Ju-20, 20 *Jujubinus*. Boxes encompass 50% of the values, the horizontal line represents the median value, and the bars extend to the 95% confidence limits. Different letters indicate statistically significant pairwise differences (Scheffé rank test $p < 0.05$).

fertilised plots throughout the year, with the exception of summer 2003.

Discussion

Gastropods collected in Fenals meadow are common species of the molluscan taxocoenosis of *Posidonia oceanica* (Templado 1984b, Russo 1991) and belong to three representative feeding guilds of the seagrass-associated assemblage (grazing herbivorous, herbivorous-deposit feeders and benthic hunters). Stable isotope data suggest that the main carbon source for this mesofauna is close to that of epiphytes, which is a common pattern for other consumers in meadows of *P. oceanica* (Dauby 1989, Lepoint et al. 2000, Vizzini et al. 2002). Differences in the nitrogen isotopic signature between grazers and epiphytes were lower than expected, suggesting ingestion of an alternative substrate. Seagrass tissue is not likely to be an optional food source because the structural characteristics of *P. oceanica* represent a mechanical obstruction for the animals' feeding apparatus (radula; authors' unpublished data). The $\delta^{15}\text{N}$ signals for macroepiphytes are thought to be higher than those of the epiphytic biofilm, where the presence of cyanobacteria would lower the signal in relation to the overall community (Lepoint et al. 2000); gastropods (from eelgrass meadows) that consume large amounts of diatoms and bacteria have $\delta^{15}\text{N}$ signals close to those of epiphytes (Kharlamenko et al. 2001, Alfaro et al. 2006).

The similar C and N isotopic signatures among species belonging to different trophic guilds indicate that adult gastropods living in the Fenals seagrass meadow con-

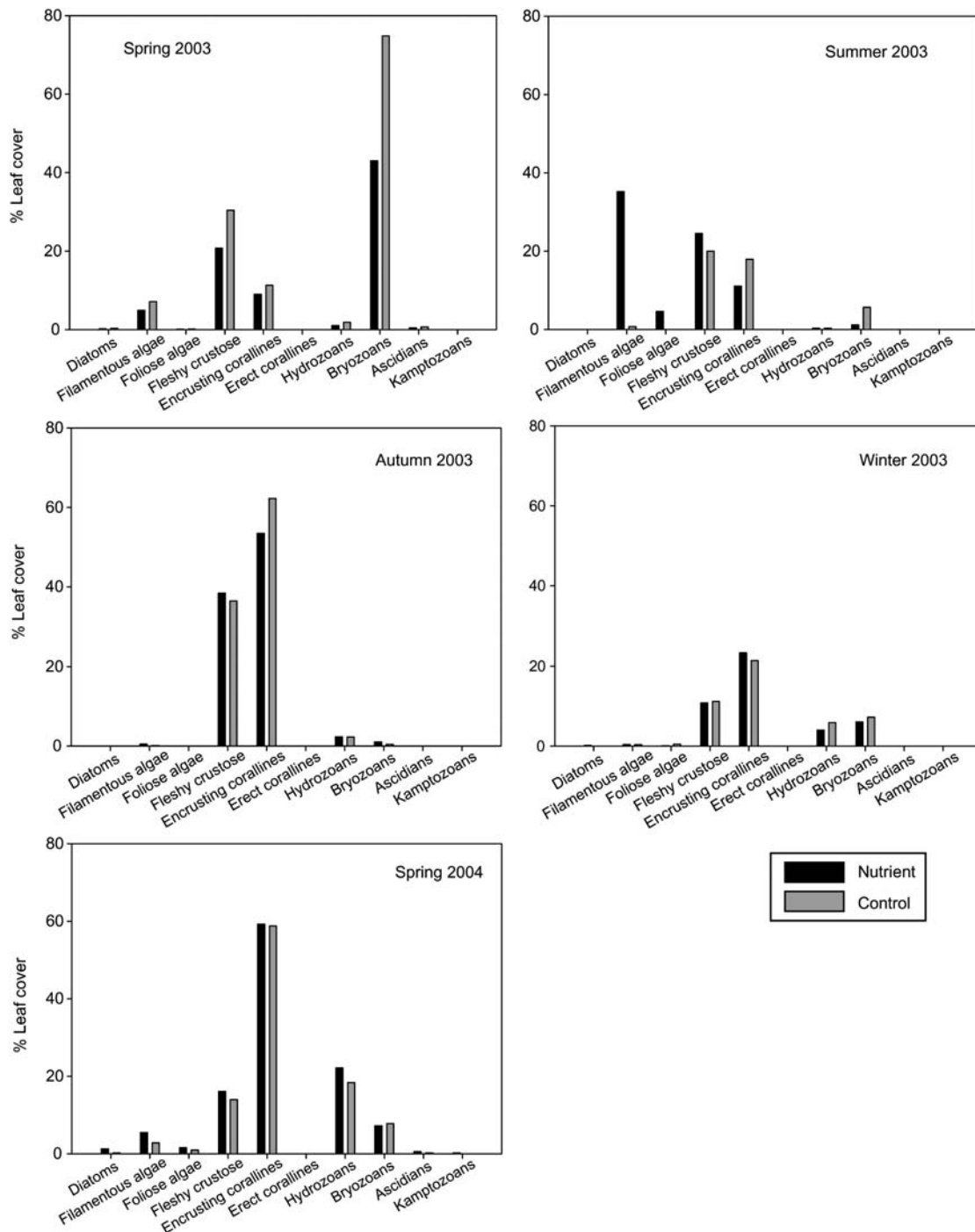


Figure 5 Impact of a year-long fertilisation experiment (see materials and methods section and Prado et al. 2008) on the functional group composition of the epiphytic community on *Posidonia oceanica*.

sume analogous substrates; thus, they do not conform to the feeding differentiation by trophic guild in traditional systems based on morphometric parameters (Steneck and Watling 1982). Similar results were found in a *Zostera capricorni* Asch. meadow, where $\delta^{15}\text{N}$ for predatory snails did not differ from that for grazing snails (Alfaro et al. 2006). Further studies should assess whether the feeding pattern encountered for gastropods associated with *Posidonia oceanica* is consistent throughout the year or varies seasonally with macro-epiphyte consumption (e.g., in eelgrass meadows, Neckles et al. 1993). Nevertheless, there is increasing evidence that the discrimination factors typically used to establish the relative

contribution of different food sources can vary substantially (Vanderklift and Ponsard 2003), and thus proper interpretation of field stable isotope data should consider controlled laboratory experiments that allow specific determination of enrichment factors between diets and consumers (Crowley et al. 2007). Furthermore, the manipulative experiment we conducted highlights the fact that gastropods can exert strong control on epiphyte biomass, at least under high densities in slightly eutrophic conditions. We did not record the composition of the epiphytic community, but differences in epiphyte biomass seem to support the notion that eutrophic conditions would favour food webs based on algae, whereas

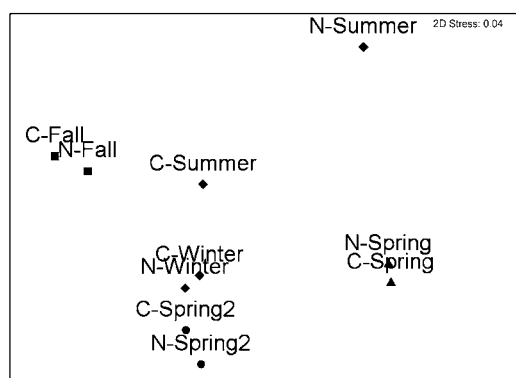


Figure 6 MDS ordination of the functional group composition in the *Posidonia oceanica* epiphytic community in the long-term fertilisation experiment.

N, nutrient enriched; C, control treatments.

oligotrophy would favour seagrass detritus and/or biofilm-based consumption (Dauby and Poulicek 1995). Similarly, Fry et al. (1987) pointed out that nutrient availability might govern the trophic importance of epiphytic algae in seagrass meadows.

The two trochids used in the laboratory experiment, *Bittium reticulatum* and *Jujubinus striatus*, were dominant mesograzers in the meadow studied and were commonly found during daylight hours on leaves of *Posidonia oceanica* at densities that would not have allowed significant control of the epiphyte biomass in our experiment. However, our experiment might have underestimated the potential effect of grazers on epiphyte control because numerous species with day-night migratory patterns from rhizomes to leaves (Templado et al. 2004) were not included. However, mesograzers-induced epiphyte reduction might not have had positive effects on the seagrass because epiphytes tend to accumulate on older leaves, which contribute least to the overall carbon balance of the plant (Alcoverro et al. 2004). A similar result with a lack of effect on the seagrass was found in manipulative experiments with the main invertebrate macrograzer in *P. oceanica* meadows [the sea urchin *Paracentrotus lividus* (Lamarck), Tomas et al. 2005]. Further studies examining the effect over a longer time period are necessary because *P. oceanica* growth is prone to inertia (Alcoverro et al. 2001), which might have hidden a shading effect by epiphytes. Alternatively, reduced light availability in the experimental conditions (levels below saturation; Alcoverro et al. 2001) might have constrained the growth potential of the plants in the grazed treatments.

Table 5 Summary of the nested ANOVAs to test for significant differences in carbon and nitrogen stable isotope signals in the long-term fertilisation experiment (see Prado et al. 2008 for more details).

Dependent variable	Treatment	$\delta^{13}\text{C}$			p	Treatment	$\delta^{15}\text{N}$			p	
		MS	df	F			MS	df	F		
Seagrass	Fertilisation	0.059	1	0.17	0.689*	Fertilisation	10.53	1	28.18	0.033	
	Error	0.353	18			Plot	0.38	2	3.45		0.057
						Error	0.11	16			
Epiphytes	Fertilisation	7.46	1	7.89	0.038**	Fertilisation	1.285	1	4.75	0.035*	
	Plot	0.88	4	0.33		0.856	Error	0.271	41		
	Error	2.67	37								

MS, mean square; df, degrees of freedom; significant differences between treatments at $p < 0.05$ are in bold.

* After pooling data; ** no homogeneity of variance achieved; $\alpha < 0.01$.

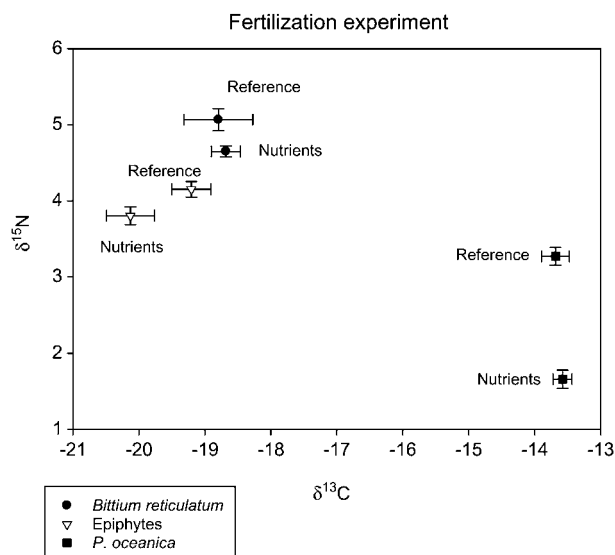


Figure 7 Isotopic signal biplot for seagrass, epiphytes and *Bittium reticulatum* collected in June 2004 in fertilised (nutrients) and reference plots after a 1-year fertilisation experiment.

Nutrients, three plots; reference, three plots; $n=4-12$ *B. reticulatum* individuals per plot. t -test for $\delta^{15}\text{N}$ $p < 0.04$ and for $\delta^{13}\text{C}$ $p=0.81$. Values are mean \pm SD; $n=10$ for *Posidonia* (*P.*) *oceanica* and $n=20$ for epiphytes.

Nitrogen isotopic signals were used to trace experimentally induced fertilisation in the system examined, although differences in $\delta^{15}\text{N}$ between control and fertilised treatments were not maintained throughout the food web. Whereas seagrass $\delta^{15}\text{N}$ was strongly influenced by the fertiliser signal and was very different from the signal for control plots, isotopic signals for epiphytes and mesograzers were closer between treatments (although the influence of the lower $\delta^{15}\text{N}$ from the fertiliser was still detectable in both cases). However, the lack of changes detected in $\delta^{13}\text{C}$ of epiphytes might reflect a similar community composition between control and nutrient-enriched experiments throughout the year (with the single exception of summer data).

Conclusion

The main adult gastropods associated with leaves of *Posidonia oceanica* seem to feed on the epiphytic component (based on their carbon isotopic signature) in a shallow meadow off the Catalan coast. Information on the nitrogen isotopic signature suggests that there is

selection for some of the epiphytes, with a major contribution from micro-epiphytes (diatoms and bacteria), which have lighter signatures. Carbon and nitrogen isotopic signatures of species belonging to different trophic guild categories did not show significant differences, indicating similar feeding substrates. Experimental testing of the potential effect of gastropods on modulating seagrass-epiphyte interaction following eutrophication revealed significant control of the epiphyte biomass by grazers at high gastropod densities, although this had no consequences for seagrass performance. This study highlights the necessity for further work to elucidate the diets of the overall gastropod assemblage associated with *P. oceanica* in a wide range of meadows and the use of additional tools (fatty acid biomarkers) to completely understand the feeding behaviour of gastropods in this seagrass ecosystem. Moreover, although preliminary data for high-density single-species treatments suggest that gastropods do not have any effect on *P. oceanica* growth, further studies over longer time periods and using more complete mesograzers assemblage should help to identify the role of grazers in seagrass-epiphyte interactions in the Mediterranean Sea. *Bittium reticulatum* did reflect experimentally induced eutrophication, cascading up nutrient effects throughout the food web over the long term in an *in situ* fertilisation experiment.

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