

Differential element assimilation by sea urchins *Paracentrotus lividus* in seagrass beds: implications for trophic interactions

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ABSTRACT: Despite increasing evidence that herbivory in seagrass systems is more important than previously considered, many factors regulating seagrass herbivory still need to be elucidated. In this study we evaluate the importance of epiphytes and seagrass blades in sea urchin nutrition using a multiple stable-isotope approach. Our aim is to contribute to the understanding of plant–herbivore interactions in seagrass beds. Stable-isotope ratios of carbon, nitrogen and sulphur of the sea urchin *Paracentrotus lividus* (Lamarck) were measured and compared to those of the potential food sources (i.e. the seagrass *Posidonia oceanica* [L.] Delile and its epiphytes) in a temperate seagrass meadow. Epiphytes and seagrass leaves had distinct $\delta^{13}\text{C}$ (–18.7 vs. –12.8‰, respectively) and $\delta^{15}\text{N}$ values (+6.4 vs. +3.6‰, respectively), while values for $\delta^{34}\text{S}$ were closer (+19.4 vs. +17.0‰, respectively). Data from $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ indicated that both food sources contributed in approximately equal proportions (50%) to the bulk organic matter assimilated by *Paracentrotus lividus*, whereas $\delta^{15}\text{N}$ measurements showed that approximately 90% of the nitrogen assimilated by the sea urchin is provided by epiphytes. Given the generally low nutritional quality of *P. oceanica* leaves, the data obtained suggest that epiphyte nitrogen is crucial in regulating the trophic relationships between the herbivore and the seagrass. Thus in *P. oceanica* meadows, epiphytes, particularly through nitrogen contribution, appear to be an essential component of the diet of important herbivores such as sea urchins.

KEY WORDS: Plant–herbivore interactions · Epiphytes · Stable isotopes · Nitrogen · Food quality · Mediterranean · *Posidonia oceanica*

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INTRODUCTION

Herbivory on seagrasses is generally not considered important in regulating plant abundance (Thayer et al. 1984, Valiela 1995) and the amount of plant ingested by grazers usually represents only a minor fraction of the annual net primary production (Thayer et al. 1984, Cebrián & Duarte 1998). The low consumption rates have been ascribed to the poor nutritional value of plant material expressed by high C:N ratios (Duarte 1990), the inability of most invertebrates to digest cellulose (Lawrence 1975), as well as to the presence of chemical deterrents (Zapata & McMillan 1979). Never-

theless, in connection with the large anthropogenically induced decline in large herbivorous vertebrates such as turtles, sirenians and large fishes, some works have pointed out the lack of historical perspective in examining the importance of herbivory in seagrass systems. These organisms were most probably keystone species in seagrass beds in the past (Thayer et al. 1984, Jackson et al. 2001).

In the Mediterranean, densities of the common sea urchin *Paracentrotus lividus* (Lamarck) in *Posidonia oceanica* (L.) Delile meadows are generally low (0 to 6 individuals m⁻²; see review by Boudouresque & Verlaque 2001), and seagrass abundance is controlled

mainly by primary resources (light, temperature and nutrients: Alcoverro et al. 1995). However, as in other seagrass species, some overgrazing events have been reported which, in most cases, have been associated with eutrophication phenomena and sea urchin outbreaks (e.g. Verlaque & Nédelec 1983, Ruiz et al. 2001).

As shown by feeding-preference experiments and gut-content analyses, in *Posidonia oceanica* meadows sea urchins feed preferentially on old epiphytised leaves rather than bare seagrass leaves (see review by Boudouresque & Verlaque 2001). Although sea urchins ingest seagrass leaves and epiphytes simultaneously, the epiphytic flora of *P. oceanica* leaves appear to be the preferred target of *Paracentrotus lividus* (see review by Boudouresque & Verlaque 2001), and experimental evidence exists indicating that, even at low densities, sea urchins can control epiphyte biomass (Tomas et al. 2005). This preference for epiphytes may be due to their higher palatability and richer nutritional quality (lower C:N ratios) in comparison to the seagrass material (Alcoverro et al. 1997 for epiphytes, Alcoverro et al. 2000 for seagrass), especially in old leaves, which have the highest C:N values (Alcoverro et al. 2000). The epiphytic community of *P. oceanica* leaves consists mainly of microscopic algae, such as crustose red algae (*Fosliella* spp.) and some typical brown algae of the genera *Myrionema*, *Giraudia* and *Cladosiphon*. Some small animals such as hydrozoans and bryozoans also appear, especially in deeper waters (Ballesteros et al. 1984, Romero 1989).

The alleged preference for epiphytes could indicate that epiphytes are the crucial food resource for sea urchins. However, this hypothesis has rarely been tested (but see Alcoverro et al. 1997 and Tomas et al. 2005) and has received little attention in most macroherbivore–seagrass studies (see reviews by Valentine & Heck 1999 or Williams & Heck 2001). A key point to elucidating such interactions is identification of the food sources, not only in terms of ingestion, but also (and mainly) in terms of assimilation. Stable isotopes can help in elucidating aspects of the origin and transformation of the organic matter ingested (e.g. Peterson et al. 1985, Kharlamenko et al. 2001) and can provide a time-integrated measure of trophic position (Vander Zanden & Rasmussen 2001).

This study evaluates the importance of epiphytes and seagrass blades in sea urchin nutrition using a multiple stable-isotope approach. Our aim is to provide new data for the understanding of plant–herbivore interactions in seagrass beds. Particularly, we compare, in a *Posidonia oceanica* meadow, the isotopic signatures of ^{13}C , ^{15}N and ^{34}S between sea urchins and seagrass leaf blades and epiphytes as a means to gaining understanding of diet preferences and the trophic

support of sea urchin populations in *P. oceanica* meadows. In all, our results contribute to elucidating the flow of organic matter from primary producers to herbivores in *P. oceanica* meadows.

MATERIALS AND METHODS

Study site. The study took place in a *Posidonia oceanica* meadow located at the Medes Islands (NW Mediterranean) between 5 and 6 m deep (see Alcoverro et al. 1995, 1997 for detailed description of site). Samples were collected by SCUBA divers at 3 different times during the seasonal cycle of seagrass growth (January and October 2001 and May 2002). May corresponds to the period of maximum leaf growth and production, October represents the period of senescence and loss of leaves and January corresponds to the period of formation of new leaves (Alcoverro et al. 1995). On each occasion, we harvested 6 *P. oceanica* shoots and 6 adult (5 to 7 cm test diameter [TD] without spines) sea urchins *Paracentrotus lividus*, representing the population structure present in this meadow (see Tomas et al. 2004 for more details). These were randomly collected over an area of ca. 500 m². To avoid any masking effect of development stage, we used exclusively adult sea urchins (>5 cm TD: when individuals reach 5 to 6 cm TD their growth rate strongly diminishes [Turon et al. 1995]). A sample size of 6 seagrass shoots per sampling was deemed adequate given the usually low levels of variation in isotopic signatures of Mediterranean seagrasses (e.g. Jennings et al. 1997, Lepoint et al. 2000, Pinnegar & Polunin 2000). Samples were frozen shortly after collection and further processed at the laboratory.

Laboratory processing and analyses. In the laboratory, sea urchins were dissected, and muscles from the Aristotle's lantern were carefully removed and used to perform stable-isotope analyses. Muscle was chosen instead of gonad (the other most abundant tissue) since lipid-rich tissues such as gonads are isotopically lighter owing to discrimination towards ^{12}C during lipogenesis (Van Dover et al. 1992), and this depletion in ^{13}C can potentially reduce the chances of correctly identifying types of source material (Polunin et al. 2001, Post 2002). In addition, lipid tissues have generally fast turnover rates and integrate short-term dietary histories (Polunin et al. 2001), whereas by analysing muscular tissue we can obtain a more integrative measure of assimilated food.

The epiphytes were scraped off the seagrass leaves with a razor blade (Alcoverro et al. 1997). Only the 2 oldest (outermost) leaves of every shoot were separated and used, as they are the most commonly eaten by *Paracentrotus lividus* under natural conditions

(Nédelec & Verlaque 1984). All samples were rinsed with distilled water (Ledent et al. 1995) and then dried at 70°C until constant weight (48 h approximately). After drying, samples were ground to a fine powder and then sealed in glass vials for later isotope analyses.

Due to the high content of carbonates in the epiphytes (Romero 1989), seagrass material and epiphytes were washed with diluted HCl (2N) to remove carbonates and analyse $^{13}\text{C}:^{12}\text{C}$ ratios of the organic material. As this chemical procedure has been reported to alter the $\delta^{15}\text{N}$ values (Bunn et al. 1995), for each sample of seagrass and epiphytes, half of the sample was submitted to an acid wash and half remained untreated. Samples washed with acid were used to analyse $\delta^{13}\text{C}$ and untreated samples were used to analyse $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$.

Measurements of stable-isotope abundances were performed using a continuous-flow isotope-ratio mass spectrometer Delta C (Thermo Finnigan) coupled to a flash 1112 elemental analyser (Thermo Finnigan) through a Conflo III interfase (Thermo Finnigan). Carbon and nitrogen were analysed in a dual isotope mode and sulphur was analysed separately. 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 $\delta^{13}\text{C}$, 0.5‰ for $\delta^{15}\text{N}$ and 0.2‰ for $\delta^{34}\text{S}$.

Isotope ratios were expressed relative to PeeDee Belemnite (PDB) standard for carbon, to N_2 in air for nitrogen, and to Canyon Diablo troilites standard for sulphur. Ratios were calculated as:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}} - 1)] \times 1000$$

where X is ^{13}C , ^{15}N or ^{34}S , and R is the corresponding ratio of $^{13}\text{C}:^{12}\text{C}$, $^{15}\text{N}:^{14}\text{N}$, $^{34}\text{S}:^{32}\text{S}$.

Analysis of data. For an optimal assessment of the importance of *Posidonia oceanica* and its epiphytes to *Paracentrotus lividus* nutrition, we used mixing models (Ben-David & Schell 2001, Phillips & Gregg 2001) to estimate the proportions of each element assimilated by *P. lividus* from each food source. Specifically, we pooled the values for all sampling times (see Table 1) to calculate the 2-source mixing model proposed by Phillips & Gregg (2001) and used the Excel spreadsheet provided by these authors (see www.epa.gov/wed/pages/models.htm):

$$\delta X_{\text{consumer}} = f_{s1} \delta X_{s1} + (1 - f_{s1}) \delta X_{s2}$$

where δX is the mean $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ or $\delta^{34}\text{S}$ value for the consumer or food sources, and f_{s1} and $1 - f_{s1}$ are the proportional inputs of the food sources.

To correct for isotope fractionation across trophic levels, we considered an enrichment of 0.4‰ for carbon (Michener & Schell 1994, Vander Zanden & Rasmussen 2001, Post 2002), of 3.4‰ for nitrogen

(Michener & Schell 1994, Post 2002) and no enrichment for sulphur (Fry 1988, Michener & Schell 1994, McCutchan et al. 2003).

To compare isotopic signatures between the 3 sampling times and the organisms, we used a 2-way ANOVA (fixed factors = time and organism). When overall significant differences were detected, a *posteriori* pairwise comparisons of means was performed using the Student-Newman-Keuls test (SNK: Zar 1984). Prior to statistical analyses, normality and homogeneity of variance were checked for all data (Kolmogorov-Smirnov test and Cochran's test, respectively). If we were unable to attain homoscedasticity even after trying several transformations of the variables studied (indicated in 'Results'), as samples were large, we considered ANOVA to be robust to departures from this assumption (Underwood 1997).

RESULTS

$\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ signatures were clearly and consistently different among the organisms (seagrass, epiphytes and sea urchins) (Fig. 1, Table 1), whereas a significant interaction between time and organism was found for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Table 1), indicating a different temporal trend of the C and N isotope signals amongst organisms. While seagrass and epiphytes exhibited a marked seasonal variability in $\delta^{13}\text{C}$, very low seasonality was detected for $\delta^{15}\text{N}$.

In the $\delta^{13}\text{C}$ versus $\delta^{34}\text{S}$ plot (Fig. 2a), generally considered the most informative in resolving food sources

Table 1. Summary of factorial 2-way ANOVA to assess significant differences in isotopic signatures between sampling times (October, January and May) and organisms (seagrass *Posidonia oceanica* leaves, epiphytes and sea urchins *Paracentrotus lividus*) and their interactions. ns = not significant

Variable	Effect	df	MS	F	p-level
$\delta^{13}\text{C}$	Time (T)	2	0.147	0.433	ns
	Organism (O)	2	148.168	435.241	<0.0001
	T × O	4	5.561	16.336	<0.0001
	Error	41	0.340		
$\delta^{15}\text{N}^a$	Time (T)	2	0.609	2.811	ns
	Organism (O)	2	147.963	682.394	<0.0001
	T × O	4	1.584	7.304	<0.0001
	Error	41	0.217		
$\delta^{34}\text{S}^a$	Time (T)	2	6.454	7.086	0.002
	Organism (O)	2	26.518	29.114	<0.0001
	T × O	4	2.168	2.380	ns
	Error	42	0.911		

^aNo homogeneity of variance achieved

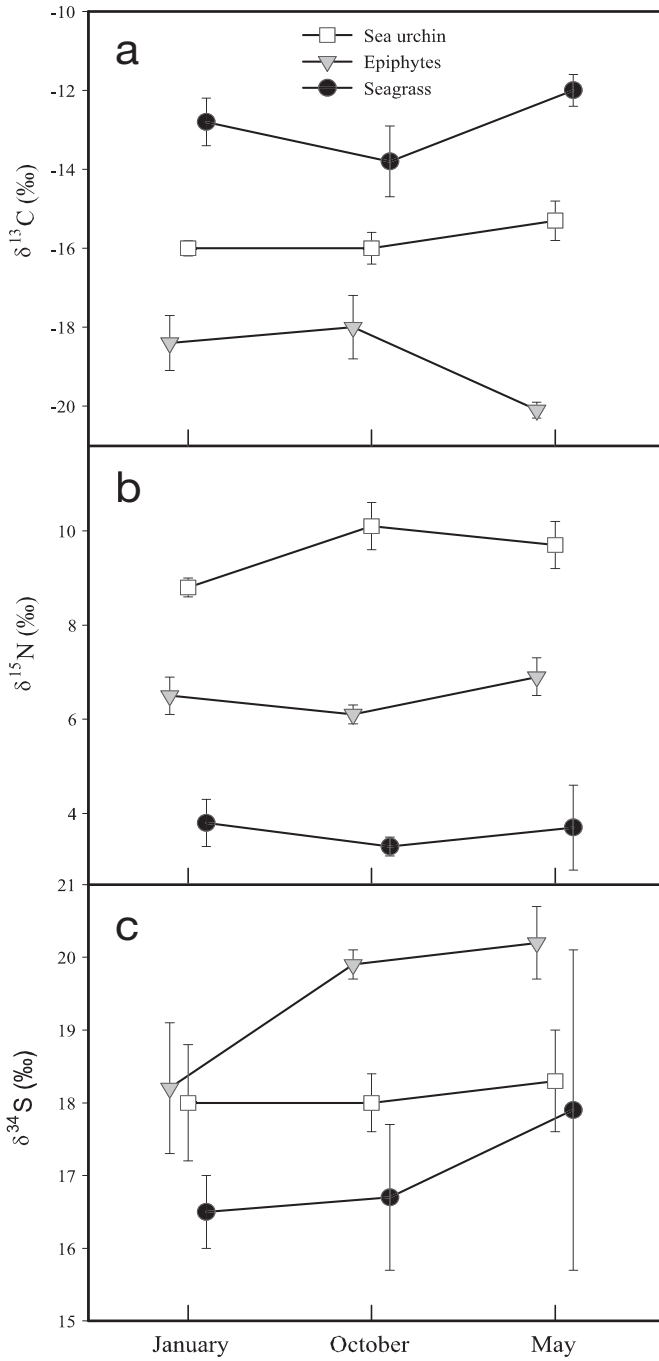


Fig. 1. *Paracentrotus lividus*. Time course of stable isotope ratios (mean ± SD) of (a) carbon, (b) nitrogen and (c) sulphur for sea urchin and its 2 main food sources, the seagrass *Posidonia oceanica* and its epiphytes

(Peterson et al. 1985, Kwak & Zedler 1997), *Paracentrotus lividus* composition appeared in an intermediate position relative to the potential food sources, indicating nearly equal proportions of both. In contrast, taking into account the trophic shift of 3.4‰, δ¹⁵N of the

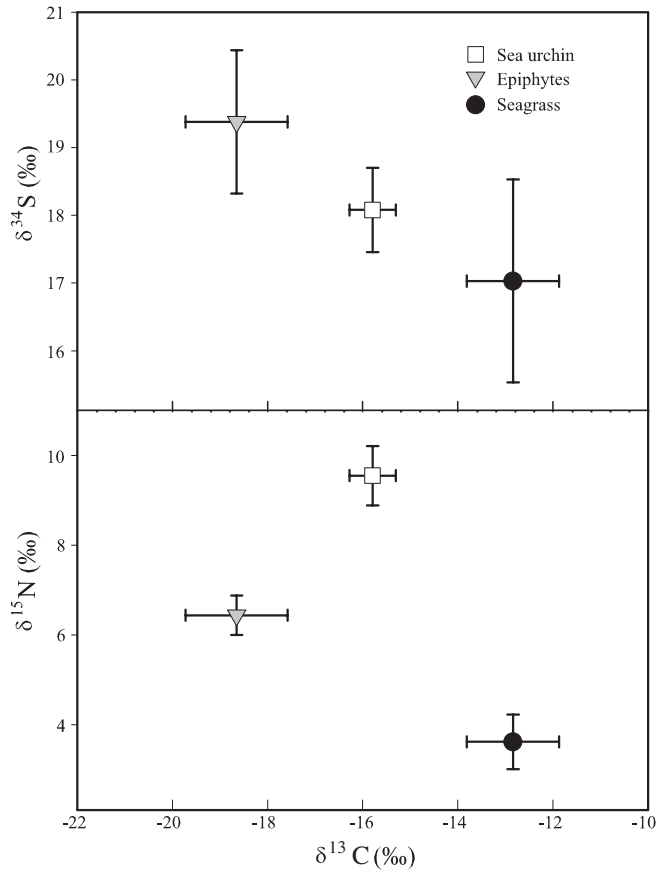


Fig. 2. *Paracentrotus lividus*. Stable-isotope plots of mean δ¹³C, δ¹⁵N and δ³⁴S (mean ± SD) for sea urchin and its 2 main food sources, the seagrass *Posidonia oceanica* and its epiphytes

sea urchin was much closer to epiphyte values than to seagrass values (Fig. 2b).

Using the linear model described by Phillips & Gregg (2001), we estimated that *Posidonia oceanica* provides 56 ± 4(SE)% of the carbon assimilated by *Paracentrotus lividus*, whereas the epiphytes provide the other 44 ± 4(SE)%. Similar results were obtained for S, i.e. the contribution of seagrass was 56 ± 11(SE)% and the contribution of epiphytes was 44 ± 11(SE)%. In contrast, the assimilation of nitrogen was noticeably skewed towards the epiphytes—90 ± 7(SE)% of the nitrogen being provided by the epiphytes and only 10 ± 7(SE)% by the seagrass itself.

DISCUSSION

The results of this study suggest that in *Posidonia oceanica* meadows the contribution of epiphytes to the diet of *Paracentrotus lividus* is very important, especially in regard to nitrogen. For carbon, the results

indicate that *P. lividus* obtains approximately half of its carbon from *P. oceanica* and the other half from the epiphytes. The same result was obtained by Kharlamenko et al. (2001) for grazing gastropods in a *Zostera marina* community. The results for sulphur confirm that both food sources contribute in approximately equal proportions to the bulk organic matter assimilated by sea urchins, while a markedly different pattern was observed for nitrogen, for which it seems that the contribution of the epiphytes greatly outweighs that of the seagrass.

In this study, we assumed that seagrass material and its epiphytes are the only 2 main food sources for *Paracentrotus lividus*. Nevertheless, other potential food sources that we have not taken into consideration (macroalgae attached to rhizomes or particulate organic matter) could have influenced the stable-isotope ratios observed. This influence, if any, should be only marginal. Macroalgae living on rhizomes are only rarely found in the guts of *P. lividus* (generally <1% dry weight: Nédelec & Verlaque 1984). Indeed, macroalgae on rhizomes are scarce in the meadow studied (Ballesteros et al. 1984) and can be dismissed as an important food source. We confirmed this by examining the gut contents of the sea urchins collected. The capacity to directly absorb particulate organic matter through the epidermis has been described for various echinoderm species (Péquignat 1972). However, this material is generally highly refractory and does not appear to be an attractive food source in terms of nutritional quality. In addition, if not highly processed, such organic matter should have a signature similar to its original material, mostly epiphytes or seagrass. *P. lividus* exhibits little migration (Dance 1987, Sala & Zabala 1996), especially in *Posidonia oceanica* meadows, where sea urchins travel significantly less than on rocky substrates (Boudouresque & Verlaque 2001). Therefore, it is unlikely that, in the meadow studied, *P. lividus* feeds on food sources other than seagrass and epiphytes, since the meadow is very homogeneous with no rocky outcrops to which urchins might migrate to feed.

Other factors which could also have modified our results are the enrichment values used in the mixing models. For C and S, the range of variation is relatively low (ca. 0.5 to 1‰ for C: Fry 1988, Michener & Schell 1994, McCutchan et al. 2003; ca. 0 to 1‰ for S: Peterson et al. 1985, Fry 1988, Van Dover et al. 1992, Michener & Schell 1994, McCutchan et al. 2003); and hence the conclusion of equivalent contributions of both food sources to the bulk of organic matter is valid. For nitrogen, the variation in the enrichment factor at each trophic level appears to be larger, generally ranging between ca. 2 and 4‰ (Fry 1988, Michener & Schell, 1994, McCutchan et al. 2003, Vanderklift & Ponsard

2003). Nevertheless, had we used a different enrichment factor within this range, the conclusion that epiphytes are the main suppliers of nitrogen assimilated by sea urchins would have remained, since the contribution of nitrogen would still have been markedly skewed towards epiphytes (ca. 70 to 100%).

Considering that nitrogen concentrations are similar in old *Posidonia oceanica* leaves and its epiphytes (1 to 2% dry weight: Alcoverro et al. 1997, 2000), the higher contribution of epiphytes to nitrogen in sea urchins suggests that, although *Paracentrotus lividus* is ingesting both seagrass leaves and its epiphytes, there is a differential assimilation of the elements. Furthermore, the C:N ratio of seagrass leaves is approximately double that of epiphytes (ca. 29 and 12, respectively: Alcoverro et al. 1997, 2000). Therefore, since the contribution by seagrass and epiphytes to sea urchin $\delta^{13}\text{C}$ is approximately 1:1, sea urchins appear to assimilate epiphyte nitrogen preferentially over seagrass nitrogen at a ratio (9:1) well above that which could be explained by the relatively higher availability of nitrogen per unit carbon for epiphytes compared with the seagrass (2.5:1). This supports our hypothesis of differential assimilation between epiphytes and seagrass nitrogen by sea urchins, as has been suggested for other animal species in diverse systems (Gannes et al. 1997, Koch & Phillips 2002, Post 2002).

Epiphytes appear to have a higher nutritional quality than seagrass. On the one hand (and as indicated by the C:N values), most of the total carbon content in seagrasses is in the form of cellulose and other structural components (Duarte 1990) that cannot be easily utilised by animals; on the other hand, the fraction of assimilable-nitrogen components is lower in old *Posidonia oceanica* leaves. On average, soluble nitrogen compounds in old leaves account for only 0.7% dry weight (Invers et al. 2002); hence a very important fraction of the nitrogen in old leaves available to sea urchins comprises structural refractory nitrogen. Therefore, although N concentrations are similar in both food sources, the availability of this N differs, which could explain the observed 'preferential' assimilation of epiphytic nitrogen.

It is generally believed that plant quality is a main factor limiting consumer feeding rates across communities and that communities in which plants have a higher nutritional value support higher losses to herbivory (Cebrián 1999). Some authors have also suggested that feeding intensity on seagrasses is determined by nitrogen availability, rather than by the carbon content of their leaves (Bjorndal 1980, McGlathery 1995, Ruiz 2000). Our results seem to support this, and point to nitrogen contained in epiphytes as a key factor in the trophic relationships in our system. Our results could explain the reported preference

of sea urchins for epiphytised leaves (see review by Boudouresque & Verlaque 2001). The importance of the nitrogen content of epiphytes has also been shown in a *Syringodium isoetifolium* seagrass bed by Yamamuro (1999), who observed a strong dependence of herbivores on epiphytic cyanobacteria and attributed this phenomenon to the relative differences in nitrogen content of the different organic materials.

In the system studied herein, the stable-isotope data obtained supports the hypothesis that grazing on seagrasses is driven by nitrogen availability, and specifically by the nitrogen content of epiphytes. Our study points to the importance of epiphytes in herbivore–seagrass interactions, a potential key factor that has not received full attention in the study of seagrass herbivory in other systems.

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