

***Sarpa salpa* herbivory on shallow reaches of *Posidonia oceanica* beds**

L. Steele, K. M. Darnell, J. Cebrián & J. L. Sanchez–Lizaso

Steele, L., Darnell, K. M., Cebrián, J. & Sanchez–Lizaso, J. L., 2014. *Sarpa salpa* herbivory on shallow reaches of *Posidonia oceanica* beds. *Animal Biodiversity and Conservation*, 37.1: 49–57.

Abstract

Sarpa salpa herbivory on shallow reaches of *Posidonia oceanica* beds.— Here, we examined the temporal and small-scale spatial variability of grazing by the herbivorous fish *Sarpa salpa* on shallow beds of the temperate seagrass *Posidonia oceanica*. Herbivory intensity expressed as the percent of leaf area taken by fish bites was higher in September 2006 than in February 2007, and at 0.5 m than at 1.5 m during both sampling times. All *S. salpa* feeding at the shallow locations studied were juveniles, with bite sizes ranging from 0.03 to 0.62 cm². Juveniles feeding at 1.5 m were larger in February 2007 than in September 2006, as evidenced by significant differences in mean bite size per shoot. However, the larger juveniles feeding at 1.5 m in February 2007 did not appear to feed as frequently as the comparatively smaller juveniles feeding at the same depth in September 2006, as suggested by significant differences in number of bites per shoot. The number of bites per shoot was also lower at 1.5 m than at 0.5 m in February 2007, although mean bite size did not differ significantly between the two depths at that sampling time. In general *S. salpa* juveniles did not select a particular range of leaf ages when feeding in the study locations, although the juveniles feeding at 1.5 m in September 2006 appeared to select mid-aged leaves. Fish did not show a preference for more epiphytized leaves. These results show that grazing activity by *S. salpa* juveniles in shallow reaches of *P. oceanica* meadows may vary temporally and across small changes in depth, which in turn may affect the overall intensity of herbivory on the seagrass.

Key words: Grazing, Herbivory, Seagrass, *Sarpa salpa*, *Posidonia oceanica*.

Resumen

El herbivorismo de Sarpa salpa en los tramos someros de los lechos de Posidonia oceanica.— En el presente artículo analizamos la variabilidad temporal y espacial en pequeña escala de la actividad de alimentación del pez herbívoro *Sarpa salpa* en los lechos someros de la pradera submarina de clima templado *Posidonia oceanica*. La intensidad del herbivorismo expresada como el porcentaje de superficie foliar mordida por el pez fue superior en septiembre de 2006 que en febrero de 2007, y a una profundidad de 0,5 m que de 1,5 m durante los dos periodos de muestreo. Todos los individuos de *S. salpa* que se alimentaban en las zonas someras estudiadas eran juveniles y el tamaño de mordedura se situaba entre 0,03 y 0,62 cm². Los juveniles que se alimentaban a 1,5 m de profundidad fueron más grandes en febrero de 2007 que en septiembre de 2006, tal como ponen de relieve las diferencias significativas existentes en el tamaño medio de mordedura por haz. No obstante, los juveniles más grandes que se alimentaban a 1,5 m de profundidad en febrero de 2007 no parecían alimentarse con tanta frecuencia como los juveniles comparativamente más pequeños, que lo hacían a la misma profundidad en septiembre de 2006, tal como sugieren las diferencias significativas halladas en el número de mordeduras por haz. Asimismo, el número de mordeduras por haz fue inferior a 1,5 m de profundidad que a 0,5 m en febrero de 2007, si bien el tamaño medio de las mordeduras no difería en medida significativa entre las dos profundidades en aquel periodo de muestreo. En general, los juveniles de *S. salpa* no elegían un intervalo de edad concreto de las hojas a la hora de alimentarse en los lugares del estudio, a pesar de que los juveniles que se alimentaban a 1,5 m de profundidad en septiembre de 2006 parecían elegir hojas de edad mediana. Los peces no mostraron preferencia por las hojas con mayor cobertura de epífitos. Estos resultados muestran que la actividad de alimentación de los juveniles de *S. salpa* en los tramos someros de las praderas de *P. oceanica* puede variar con el tiempo y con pequeños cambios de profundidad, lo que a su vez puede afectar a la intensidad general del herbivorismo sobre las praderas submarinas.

Palabras clave: Alimentación, Herbivorismo, Praderas submarinas, *Sarpa salpa*, *Posidonia oceanica*.

Received: 11 III 13; Conditional acceptance: 18 VIII 13; Final acceptance: 17 IV 14

LaTina Steele & Just Cebrián, Dauphin Island Sea Lab, 101 Bienville Blvd., Dauphin Island, AL 36528, USA.– Kelly M. Darnell, Marine Science Inst., Univ. of Texas, 750 Channel View Dr., Port Aransas, TX 78373, USA.– Just Cebrián, Dept. of Marine Sciences, Univ. of South Alabama, 307 N. University Blvd., Mobile, AL 36688, USA.– Jose Luis Sanchez-Lizaso, Dept. of Marine Sciences and Applied Biology, Univ. of Alicante, Ap. 99, E-03080 Alicante, Spain.

Corresponding author: LaTina Steele, Sacred Heart Univ., Dept. of Biology, 5151 Park Ave., Fairfield, CT 06825, USA. E-mail: steelel@sacredheart.edu

Introduction

It is increasingly recognized that herbivory can influence the structure of seagrass populations (Mariani & Alcoverro, 1999; Alcoverro & Mariani, 2004; Valentine & Duffy, 2006; Heck & Valentine, 2006; Prado et al., 2007a), as well as that of seagrass-associated organisms (Prado et al., 2007a). Grazing of seagrasses has historically been perceived to be relatively unimportant in temperate species such as *Posidonia oceanica* (Vizzini et al., 2002; Cebrian et al., 1996a; Gobert et al., 2006; Mateo et al., 2006). However, studies have increasingly highlighted the important role of herbivory on beds of *P. oceanica* (Tomas et al., 2005; Verges et al., 2007a, 2007b; Prado et al., 2007b, Ruiz et al., 2009). In some cases, herbivory may even exacerbate damage to *P. oceanica* beds caused by other stressors, such as excessive input of organic matter, making herbivory particularly important to consider in this species (Ruiz et al., 2009).

The disagreement over the importance of herbivory may be attributed to considerable seasonal and spatial variation in grazing rates and amounts of tissue lost to herbivory in *P. oceanica* beds (Cebrián & Duarte, 1998; Boudouresque & Verlaque, 2001; Peirano et al., 2001). For example, Tomas et al. (2005) assessed patterns of grazing by the fish *Sarpa salpa* on *P. oceanica* and found higher grazing on seagrass leaves at 5 m depth than at 10 m, as well as a seasonal grazing peak in summer. Similarly, Peirano et al. (2001) and Prado et al. (2007b) documented higher levels of *S. salpa* herbivory in summer months.

Seagrass herbivory also varies at small spatial scales (among shoots, leaves, and other plant parts; Verges et al., 2007a). Some seagrass herbivores are known to feed selectively, based on leaf nutritional quality (McGlathery, 1995; Goecker et al., 2005) as well as structural and chemical deterrents (Verges et al., 2007a, 2007b; Prado & Heck, 2011; Steele & Valentine, unpublished data). Epiphyte cover can also be an important feeding cue for herbivorous fishes. For example, two fish species feeding on the Australian seagrass *Posidonia australis* displayed higher consumption of epiphytized leaves than of leaves with no epiphytes (Wressnig & Booth, 2007). Similarly, *S. salpa* and the sea urchin *Paracentrotus lividus* may selectively consume *P. oceanica* leaves with higher epiphyte loads (Cebrián et al., 1996a; Peirano et al., 2001).

Assessing the spatial and temporal variability of herbivory on *P. oceanica* beds, as well as patterns of feeding selectivity by seagrass grazers, is important to understand the role that grazers have on the structure and functioning of these Mediterranean ecosystems. Here we examined the variability in the magnitude and selectivity of herbivory by *S. salpa* across a 1–m depth range in shallow *P. oceanica* beds between summer and winter sampling dates. We focused on a finer spatial scale than previous studies, and simultaneously addressed spatial variability in both the intensity and leaf-age selectivity of grazing by juvenile *S. salpa* in two seasons.

Materials and methods

Posidonia oceanica shoots were collected from shallow beds in the southwestern Mediterranean near the town of Santa Pola (Spain), where the sea urchin *Paracentrotus lividus* only occurs in extremely low abundance (L. Steele, personal observation), in September 2006 and February 2007. These two collection dates captured most of the leaves produced throughout the year. Shoots collected in September 2006 bore leaves born since the previous spring (*i.e.* February–March 2006) through that summer, whereas the shoots collected in February 2007 bore leaves produced in between the previous fall and that winter (Ott, 1980; Cebrián et al., 1994).

On each sampling date (September 2006, February 2007), 25 shoots were collected from each of three sites at least 10 m apart at two depths, 0.5 m and 1.5 m, with a total of 75 shoots per depth per date. Collection sites were not marked so different sites within each depth were sampled on each date. Upon collection, the leaves on the shoots were separated and digitally photographed for later analysis. *S. salpa* leaves distinct, half-moon shaped bite marks on the leaf blades of *P. oceanica* that are clearly discernible (Boudouresque et al., 1984). Apparent herbivory intensity (defined as the percentage of total leaf area per shoot taken by bite marks visible at the moment of sampling), number of bites per shoot, and mean bite size per shoot were derived using Sigma Scan software. Epiphyte cover on the leaf (0–25%, 26–50%, 51–75%, or 76–100%) was also visually estimated from the photographs of shoots collected in September 2006.

Apparent herbivory intensity, number of bites per shoot, and mean bite size per shoot were compared between sampling times and depths using two-way ANOVA, with time and depth both as fixed factors. The focus of this study is to examine the overall variability between the two dates and depths sampled, and not within each depth, and thus sites were pooled for each depth and time. Post-hoc multiple comparisons were done with Tukey tests between times for each depth and between depths within each time (Quinn & Keough, 2002). For each depth in September 2006, a one-way ANOVA was used to determine if apparent herbivory intensity varied among the four groups of epiphyte cover, followed by post-hoc Tukey multiple comparison tests to determine which groups of epiphyte cover differed from each other.

We also inspected the relationship between apparent herbivory intensity on the leaf (expressed as percentage of leaf area taken by the bite marks visible at the time of sampling) and leaf age to determine whether *S. salpa* fed selectively on a certain leaf age range. Leaf age was calculated using the plastochrone interval (PI) method described by Cebrián et al. (1994). Briefly, this method uses the length of the sheath (Lsheath) of the oldest leaf and the length of the youngest (leaf 1) and second to youngest (leaf 2) leaves (Lleaf 1, Lleaf 2) to calculate the age of the youngest leaf on the shoot expressed as a fraction of one PI. Namely:

$$\text{Age leaf 1} = \frac{\ln(\text{Lleaf 1}) - \ln(\text{Lsheath})}{\ln(\text{Lleaf 2}) - \ln(\text{Lleaf 1})} \quad (1)$$

The age of the youngest leaf can then be converted to days by multiplying its fractional age in PI units by the average chronological equivalence of the PI (*i.e.* mean number of days per PI) at the time of sampling. Similarly, the age of any other leaf on the shoot can be estimated by summing the average chronological equivalences of the PIs for that given leaf and all younger leaves. In turn, the average chronological equivalence of each PI can be obtained from the well-established seasonal cycle of the PI chronological duration (Ott, 1980; Romero, 1985; Cebrián et al., 1994). This technique has been used to characterize leaf age-dependent processes in seagrasses (Sand-Jensen et al., 1994; Cebrián et al., 1996a, 1996b; Cebrián et al., 1999). For more details we direct the reader to Cebrián et al. (1994).

To reduce scatter in the relationship between apparent herbivory intensity and leaf age, and allow for better comparability among sampling depths and times, we first calculated the mean apparent herbivory intensity for leaves within 30-day age intervals (*i.e.* mean intensity for leaves with ages comprised between 0 and ≤ 30 days; mean intensity for ages comprised between 30 and ≤ 60 days, and so on). For each sampling depth and time we then identified the age interval at which consumption was first detected, and defined leaf age at the onset of herbivory as the lower limit of that interval. We subsequently plotted the mean apparent herbivory intensity for intervals with registered herbivory *versus* the upper limit of the interval after subtracting the age at the onset of herbivory (*i.e.* leaf age since the onset of herbivory, La). Herbivory intensity at the onset of herbivory was considered zero since all of the intervals beneath that age did not have any registered herbivory. This approach, which has been previously used in studies of seagrass herbivory (Cebrián et al., 1996a; Cebrián & Duarte, 1998), allows for the comparison of trends in herbivory intensity with increasing leaf age among seagrass populations regardless of the age at which herbivory first occurs.

We used regression analysis to assess whether the plotted relationships followed a linear, parabolic, sigmoidal, or exponential fit. The linear fit:

$$\text{Herbivory intensity} = a + b_1(\text{La}) \quad (2)$$

indicates no feeding selectivity by *S. salpa* on a certain leaf age range since herbivory intensity increases linearly with the time of exposure to herbivory (*i.e.* leaf age). The parabolic fit:

$$\text{Herbivory intensity} = a + b_1(\text{La}) - b_2(\text{La})^2 \quad (3)$$

and the sigmoidal fit:

$$\text{Herbivory intensity} = b_1 / (1 + e^{-b_2 \text{La}}) \quad (4)$$

indicate preference for mid-aged leaves, with herbivory intensity peaking equation (3) or leveling off

equation (4) at the mid-age range. The exponential fit:

$$\text{Herbivory intensity} = a + (\text{La})^{b_3} \quad (5)$$

indicates preference for old leaves, with non-proportionally higher consumption values at old ages in comparison with younger ages.

Results

Apparent herbivory intensity varied between the two sampling times and depths (fig. 1, table 1). Namely, it was higher in September 2006 than in February 2007 at each depth, and higher at 0.5 m than at 1.5 m within each sampling time (table 1). On average, shoots at 1.5 m had larger bites in February 2007 than in September 2006 (fig. 1, table 1). However, shoots at 1.5 m had a lower number of bites in February 2007 than in September 2006 (fig. 1, table 1). In addition, in February 2007 shoots at 0.5 m had more bites than those at 1.5 m, although mean bite size per shoot did not differ significantly between the two depths on that sampling time (table 1).

Regression analysis showed a linear relationship between apparent herbivory intensity and leaf age since the onset of herbivory at 0.5 m in September 2006 and at both 0.5 m and 1.5 m in February 2007 (fig. 2, table 2), indicating no feeding preference for a certain leaf age range. A parabolic curve was found to be the best fit at 1.5 m in September 2006 (fig. 2, table 2), indicating preference for mid-aged leaves.

ANOVA showed significant differences in apparent herbivory intensity across different levels of epiphyte cover (0–25%, 26–50%, 51–75%, or 76–100% of leaf surface covered by epiphytes) for both depths in September 2006 (0.5 m, $P < 0.01$; 1.5 m, $P < 0.01$). Multiple comparison tests revealed significantly lower herbivory intensity on leaves with 0–25% epiphyte cover than on leaves with > 25% cover, but no differences among leaves with 26–50%, 51–75%, and 76–100% cover. The innermost and youngest leaves on the shoot had the least epiphyte cover (0–25%) and lowest herbivory intensity.

Discussion

The majority of *Sarpa salpa* feeding in our study locations were juveniles, as indicated by the size of their bite marks (Tomas et al., 2005; Criscoli et al., 2006), since the locations were probably too shallow for the large shoals that adult *S. salpa* typically form at greater depths. Our study focuses on grazing by *S. salpa* juveniles within a narrow depth gradient (*i.e.* comparison between 0.5 m and 1.5 m). We showed that grazing activity and behavior by *S. salpa* juveniles in shallow *P. oceanica* beds may vary significantly over time and across small changes in depth. Namely, larger juveniles fed at 1.5 m in February 2007 than in September 2006 (as indicated by bite size), although not as intensively (as indicated by number of bites on the shoot). For the sampling date of February

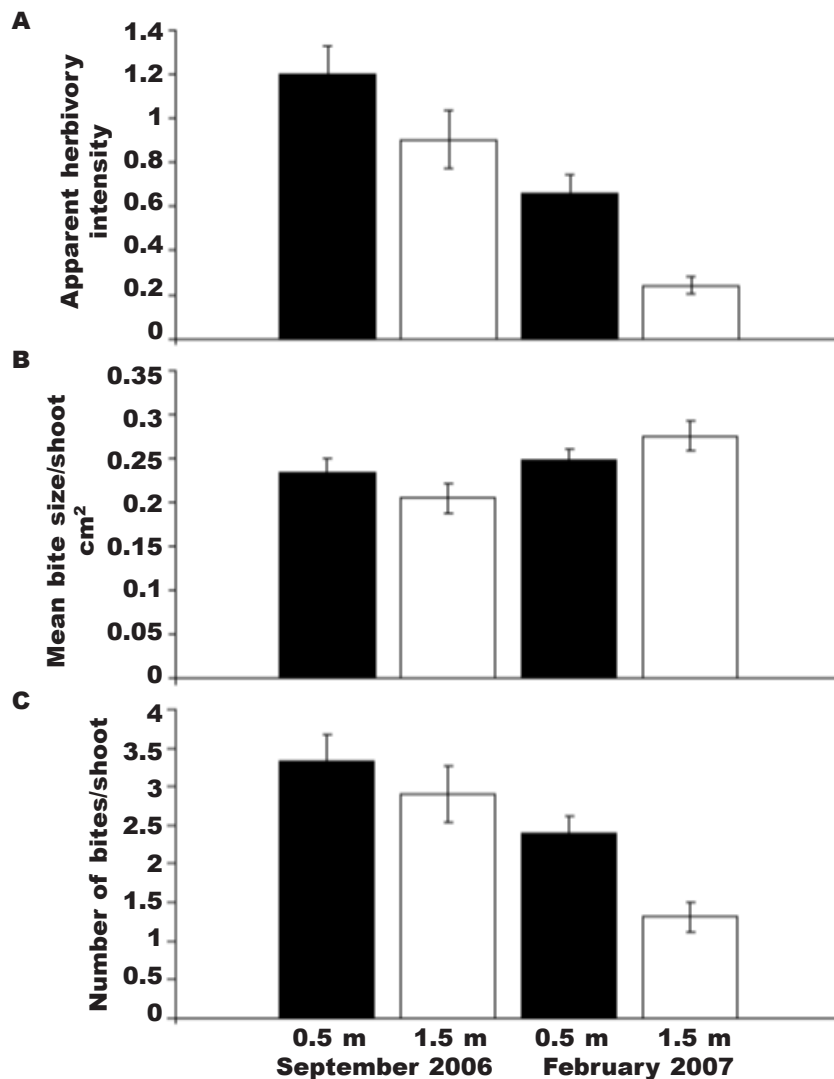


Fig. 1. Apparent herbivory intensity (A) (% leaf area per shoot taken by bite marks present at the sampling time), mean bite size per shoot (B), and number of bites per shoot (C) at 0.5 m and 1.5 m in September 2006 and February 2007. Bars denote mean values, and lines \pm SE, for the three sites at each combination of depth and time.

Fig. 1. Intensidad del herbivorismo visible (A) (% de superficie foliar por haz con marcas de mordeduras en el periodo de muestreo), tamaño medio de mordedura por haz (B) y número de mordeduras por haz (C) a 0,5 y 1,5 m de profundidad en septiembre de 2006 y febrero de 2007. Las barras denotan los valores medios y las líneas \pm ES, para los tres sitios en cada combinación de profundidad y tiempo.

2007, we also found differences between 0.5 m and 1.5 m in that juveniles fed more intensively at the shallower depth, although they seemed to be of similar size between the two depths (fig. 1). We recognize, however, that we would need direct observations of fish size at our study locations to fully demonstrate these inferences.

Other studies have focused on greater depth ranges and included both juvenile and adult *S. salpa*. For instance, Tomas et al. (2005) compared herbivory

by *S. salpa* on *P. oceanica* between 5 and 10 m in summer and winter months. They found that herbivory by juvenile *S. salpa* was higher at 5 m than at 10 m and, during the summer months when present at those depths (adult *S. salpa* move to deeper waters in winter to spawn, Peirano et al., 2001; Criscoli et al., 2006), grazing by adult *S. salpa* was also higher at 5 m. Here, we showed that changes in the grazing activity of juvenile *S. salpa* may occur at a much narrower depth gradient.

Table 1. Results of ANOVA and post-hoc Tukey tests. Tukey comparisons are between sampling times for each depth, and between depths for each sampling time. For instance, '(Sept '06 vs. Feb '07) 0.5 m' denotes the comparison between September 2006 and February 2007 for 0.5 m, and '(0.5 m vs. 1.5 m) Sept '06' the comparison between 0.5 and 1.5 m for September 2006.

Tabla 1. Resultados de la ANOVA y la prueba a posteriori de Tukey. Las comparaciones de Tukey se realizaron entre los periodos de muestreo para cada profundidad y entre las profundidades para cada periodo de muestreo. Por ejemplo, "(Sept '06 vs. Feb '07) 0.5 m" hace referencia a la comparación entre septiembre de 2006 y febrero de 2007 a 0,5 m de profundidad y "(0.5 m vs. 1.5 m) Sept '06", a la comparación entre 0,5 y 1,5 m para septiembre de 2006.

	Time	Depth	Interaction	Tukey tests
Herbivory intensity	< 0.01	< 0.01	0.359	(Sept '06 vs. Feb '07) 0.5 m: $P < \mathbf{0.01}$ (Sept '06 vs. Feb '07) 1.5 m: $P < \mathbf{0.01}$ (0.5 m vs. 1.5 m) Sept '06: $P = \mathbf{0.025}$ (0.5 m vs. 1.5 m) Feb '07: $P < \mathbf{0.01}$
Mean bite size	< 0.01	0.786	0.048	(Sept '06 vs. Feb '07) 0.5m: $P = 0.46$ (Sept '06 vs. Feb '07) 1.5 m: $P < \mathbf{0.01}$ (0.5 m vs. 1.5 m) Sept. '06: $P = 0.32$ (0.5 m vs. 1.5 m) Feb. '07: $P = 0.66$
Number of bites	< 0.01	< 0.01	0.264	(Sept '06 vs. Feb '07) 0.5 m: $P = 0.087$ (Sept '06 vs. Feb '07) 1.5 m: $P < \mathbf{0.01}$ (0.5 m vs. 1.5 m) Sept '06: $P = 0.17$ (0.5 m vs. 1.5 m) Feb '07: $P < \mathbf{0.01}$

Past reports have documented higher levels of herbivory by *S. salpa* on *P. oceanica* in summer than winter (Tomas et al., 2005; Peirano et al., 2001; Prado et al., 2007b). Here, we also found higher herbivory intensity in our summer sampling time (September 2006) in comparison with the winter sampling time (February 2007). However, this difference seemed more related to the presence of longer leaves (and thus smaller percent of leaf area accounted for by bite marks), rather than to lower grazing activity, in the winter sampling time. At any rate, our temporal analysis was limited because it was only based on two dates. It certainly did not allow for any inferences regarding seasonal variability in herbivory since we did not have replicated times per season. Therefore, our analysis shows that significant temporal variability in grazing activity by *S. salpa* juveniles does occur in the shallow reaches of *P. oceanica* beds, but it falls short at describing the nature and extent of that variability among seasons. Notwithstanding the temporal limitations of our study, our results suggest that small-scale changes in feeding activity by *S. salpa* juveniles may have important implications on the overall intensity of herbivory on *P. oceanica*; in February 2007 the more intense feeding rates at 0.5 m than at 1.5 m may have contributed to higher herbivory at the former depth.

Our results suggest preference for mid-aged leaves by *S. salpa* juveniles at 1.5 m on the shoots collected in September 2006, and no feeding preference in any

other case (0.5 m in September 2006, and 0.5 m and 1.5 m in February 2007; fig. 2). Cebrián et al. (1996b) examined a number of *P. oceanica* beds at depths ranging from 4 to 12 m along the Spanish Mediterranean coast during the summers of 1992 and 1993. They found that *S. salpa* showed preference for mid-aged leaves in some beds, but no preference for any particular leaf age range in others. However, the authors did not make any effort to determine the development stage of the fish based on the size of the bite marks. In concert, these results indicate that patterns of leaf age selectivity by *S. salpa* vary spatially and temporally. Differences in patterns of leaf age selectivity may have important implications for *P. oceanica* populations. Mid-age leaves are metabolically more active than old leaves (Alcoverro et al., 1998), and selective herbivory on mid-aged leaves may therefore have a greater impact on the productivity of *P. oceanica*.

In contrast to the results of Peirano et al. (2001), *S. salpa* at our study locations did not feed preferentially on leaves with higher epiphyte cover. We found no differences in apparent herbivory intensity among leaves with 26–50%, 51–75%, or 76–100% epiphyte cover. There was significantly more grazing on leaves with 26–100% epiphyte cover than on leaves with 0–25% epiphyte cover; however, the leaves with few epiphytes were young leaves located in the inner portion of the seagrass shoot, and they were less accessible to grazers than older leaves.

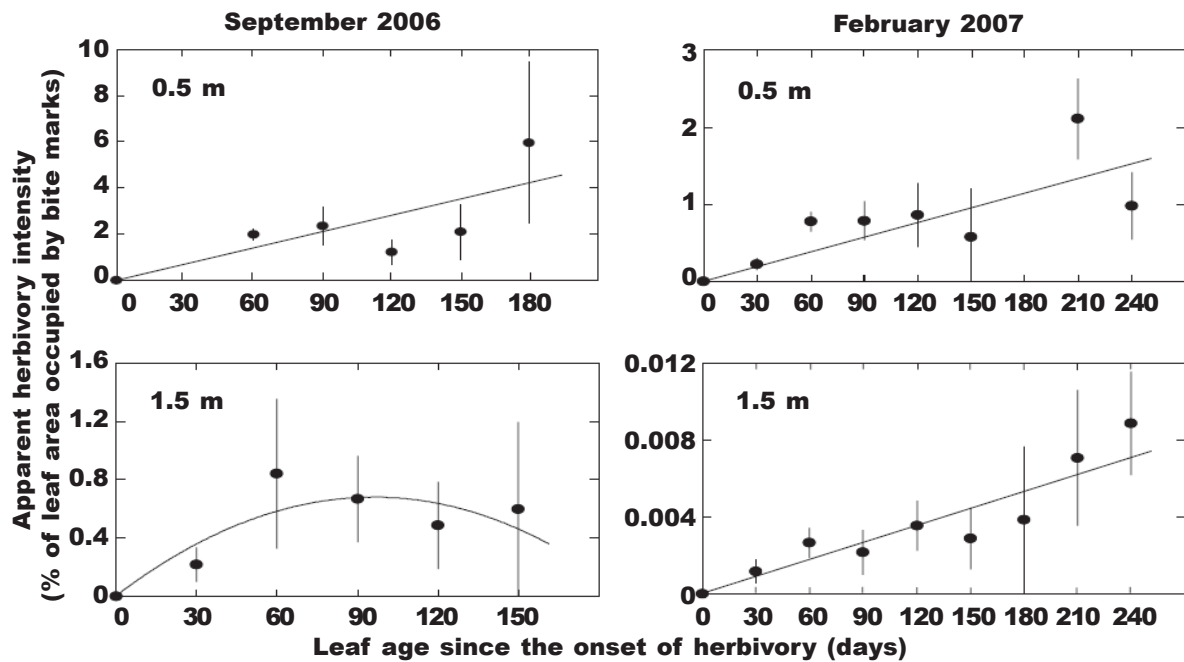


Fig. 2. Apparent herbivory intensity (% of leaf area per shoot taken by bite marks present at the sampling time) vs. leaf age since the onset of herbivory for *Posidonia oceanica* leaves at two depths in September 2006 and February 2007.

Fig. 2. Intensidad del herbivorismo visible (% de superficie foliar por haz con marcas de mordeduras en el período de muestreo) en función de la edad de las hojas desde el inicio del herbivorismo de *Posidonia oceanica* a las dos profundidades en septiembre de 2006 y febrero de 2007.

Our measurements of herbivory (*i.e.*, apparent herbivory intensity) most likely underestimated the true consumption by *S. salpa* because heavily grazed leaves tend to break off more easily and, thus, they may have been grossly under sampled. Indeed, prior studies (Kirsch et al., 2002; Tomas et al., 2005; Prado et al.,

2007b) have shown that the extent of underestimation generated by herbivory calculations based on extant bite marks on the leaves may be considerable. Yet our results should be robust, at least qualitatively. For the September 2006 samples, mean bite size and number of bites per shoot were similar between 0.5 and 1.5 m.

Table 2. Parameters and statistics of the equations describing the relationship between herbivory intensity and leaf age since the onset of herbivory (see text for details): A. Age at the onset of herbivory (in days).

Tabla 2. Parámetros y datos estadísticos de las ecuaciones que describen la relación entre la intensidad del herbivorismo y la edad de las hojas desde el inicio del herbivorismo (véase el texto para conocer más detalles): A. Edad en el inicio del herbivorismo (en días).

Time	Depth	R ²	A	b ₁ ± SE (% removed leaf ⁻¹ day ⁻¹)	b ₂ ± SE (% removed leaf ⁻¹ day ⁻²)
September 06	0.5 m	0.85	60	0.023 ± 0.004	–
	1.5 m	0.91	90	0.014 ± 0.003	0.000073 ± 0.000026
February 07	0.5 m	0.84	30	0.0066 ± 0.0011	–
	1.5 m	0.95	30	0.000030 ± 0.000003	–

Thus, herbivory should have been underestimated to a similar extent for the two depths on that sampling time since leaves should have had a similar likelihood of breakage based on the number and size of bites present. A similar underestimation for both depths should not negate the result that herbivory intensity was higher at 0.5 than at 1.5 m in September 2006. For the February 2007 samples, mean bite size was similar at the two depths, but there were more bites per shoot at 0.5 than at 1.5 m (fig. 1). On this basis, it could be expected that leaf breakage probability (and thus potential herbivory underestimation) would be higher at 0.5 m than at 1.5 m, but again this should not negate the result that herbivory intensity was higher at 0.5 m than at 1.5 m on this sampling date. Herbivory underestimation could perhaps affect the shape of leaf age–feeding selectivity relationships, as older leaves may sustain large cumulative herbivory and break off more easily. On these grounds, our three linear fits might have been misrepresentations of exponential curves, but this possibility seems rather unlikely judging by the levels of apparent grazing found on mid-aged leaves. Our parabolic fit, however, could be a misrepresentation of a linear process (*i.e.* no selectivity for any particular leaf age range).

In conclusion, we have shown that grazing activity and behavior by *S. salpa* juveniles may vary significantly over time and within small depth ranges in shallow *P. oceanica* beds. Such variations may have important implications on the overall levels of herbivory intensity on the seagrass. In these shallow reaches, *S. salpa* juveniles may feed preferentially on mid-age leaves, although they often showed no preference for a particular leaf age range. Our results add to previous findings of depth-related gradients in *S. salpa* herbivory. However, more accurate assessments of the temporal and spatial variability of feeding behavior by *S. salpa* juveniles in shallow *P. oceanica* beds are needed to better understand their role in these environments.

Acknowledgements

The authors would like to thank CIMAR in Santa Pola, Spain for their hospitality during our stay. In particular, we thank Alfonso Ramos for his guidance. Andres Izquierdo provided invaluable assistance with field collections in February 2007. Ashley McDonald helped with data treatment and figure preparation. The University of South Alabama Department of Marine Sciences and Dauphin Island Sea Lab provided travel funds and monetary support.

References

- Alcoverro, T., Manzanera, M. & Romero, J., 1998. Seasonal and age-dependent variability of *Posidonia oceanica* (L.) Delile photosynthetic parameters. *Journal of Experimental Marine Biology and Ecology*, 230: 1–13.
- Alcoverro, T. & Mariani, S., 2004. Patterns of fish and sea urchin grazing on tropical Indo-Pacific seagrass beds. *Ecography*, 27: 361–365.
- Boudouresque, C. F., Jeudy de Grissac, A. & Olivier, J. (Eds.), 1984. I. International workshop on *Posidonia oceanica* beds. GIS Posidonie, Marseille.
- Boudouresque, C. F. & Verlaque, M., 2001. Edible sea urchins: biology and ecology. In: *Developments in Aquaculture and Fisheries Science*: 177–216 (J. M. Lawrence, Ed.). Elsevier, Amsterdam.
- Cebrián, J. & Duarte, C. M., 1998. Patterns of leaf herbivory on seagrasses. *Aquatic Botany*, 60: 67–82.
- Cebrián, J., Duarte, C. M., Marba, N., Enriquez, S., Gallegos, M. & Olesen, B., 1996a. Herbivory on *Posidonia oceanica*: magnitude and variability in the Spanish Mediterranean. *Marine Ecology Progress Series*, 130: 147–155.
- Cebrián, J., Enriquez, S., Agawin, N., Duarte, C. M., Fortes, M. & Vermaat, J., 1999. Epiphyte accrual on *Posidonia oceanica* (L.) Delile leaves: implications on light absorption. *Botanica Marina*, 42: 123–128.
- Cebrián, J., Marba, N. & Duarte, C. M., 1994. Estimating leaf age of the seagrass *Posidonia oceanica* (L.) Delile using the plastochrone interval index. *Aquatic Botany*, 49: 59–65.
- 1996b. Herbivory on *Cymodocea nodosa* (Ucria) Ascherson in contrasting Spanish Mediterranean habitats. *Journal of Experimental Marine Biology and Ecology*, 204: 103–111.
- Criscoli, A., Colloca, F., Carpentieri, P., Belluscio, A. & Ardizzone, G., 2006. Observations on the reproductive cycle, age and growth of the salema, *Sarpa salpa* (Osteichthyes: Sparidae) along the western central coast of Italy. *Scientia Marina*, 70: 131–138.
- Gobert, S., Cambridge, M. L., Velimirov, B., Pergent, G., Lepoint, G., Bouquegneau, J. M., Dauby, P., Pergent-Martini, C. & Walker, D. I., 2006. Biology of *Posidonia*. In: *Seagrasses: Biology, Ecology, and Conservation* 387–408 (A. W. D. Larkum, R. J. Orth & C. M. Duarte, Eds.). Springer, Dordrecht.
- Goecker, M. E., Heck Jr., K. L. & Valentine, J. F., 2005. Effects of nitrogen concentrations in turtlegrass *Thalassia testudinum* on consumption by the bucktooth parrotfish *Sparisoma radians*. *Marine Ecology Progress Series*, 286: 239–248.
- Heck Jr., K. L. & Valentine, J. F., 2006. Plant–herbivore interactions in seagrass meadows. *Journal of Experimental Marine Biology and Ecology*, 330: 420–436.
- Kirsch, K. D., Valentine, J. F. & Heck, K. L., 2002. Parrotfish grazing on turtlegrass *Thalassia testudinum*: evidence for the importance of seagrass consumption in food web dynamics of the Florida Keys National Marine Sanctuary. *Mar. Ecol. Prog. Ser.*, 227: 71–85.
- Mariani, S. & Alcoverro, T., 1999. A multiple-choice feeding–preference experiment utilising seagrasses with a natural population of herbivorous fishes. *Marine Ecology Progress Series*, 189: 295–299.
- Mateo, M. A., Cebrián, J., Dunton, K. & Mutchier, T., 2006. Carbon flux in seagrass ecosystems. In: *Seagrasses: Biology, Ecology, and Conservation*: 159–192 (A. W. D. Larkum, R. J. Orth & C. M. Duarte, Eds.). Springer, Dordrecht.
- McGlathery, K. J., 1995. Nutrient and grazing influences on a subtropical seagrass community. *Marine Ecology Progress Series*, 122: 239–252.

- Ott, J. A., 1980. Growth and production in *Posidonia oceanica* (L.) Delile. *Marine Ecology*, 1: 47–64.
- Peirano, A., Niccolai, I., Mauro, R. & Bianci, C. N., 2001. Seasonal grazing and food preference of herbivores in a *Posidonia oceanica* meadow. *Scientia Marina*, 65: 367–374.
- Prado, P., Alcoverro, T., Martinez-Crego, B., Verges, A., Perez, M. & Romero, J., 2007b. Macrograzers strongly influence patterns of epiphytic assemblages in seagrass meadows. *Journal of Experimental Marine Biology and Ecology*, 350: 130–143.
- Prado, P. & Heck Jr., K. L., 2011. Seagrass selection by omnivorous and herbivorous consumers: determining factors. *Marine Ecology Progress Series*, 429: 45–55.
- Prado, P., Tomas, F., Alcoverro, T. & Romero, J., 2007a. Extensive direct measurements of *Posidonia oceanica* defoliation confirm the importance of herbivory in temperate seagrass meadows. *Marine Ecology Progress Series*, 340: 63–71.
- Quinn, G. P. & Keough, M. J., 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.
- Romero, J., 1985. Estudio ecológico de las fanerógamas marinas de la costa catalana: producción primaria de *Posidonia oceanica* (L.) Delile en las Islas Medes. Ph. D. Thesis, Universidad de Barcelona. (In Spanish.)
- Ruiz, J. M., Perez, M., Romero, J. & Tomas, F., 2009. The importance of herbivory in the decline of a seagrass (*Posidonia oceanica*) meadow near a fish farm: an experimental approach. *Botanica Marina*, 52: 449–458.
- Sand-Jensen, K., Jacobsen, D. & Duarte, C. M., 1994. Herbivory and resulting plant damage. *Oikos*, 69: 545–549.
- Tomas, F., Turon, X. & Romero, J., 2005. Seasonal and small-scale spatial variability of herbivory pressure on the temperate seagrass *Posidonia oceanica*. *Marine Ecology Progress Series*, 301: 95–107.
- Valentine, J. F. & Duffy, J. E., 2006. The central role of grazing in seagrass ecology. In: *Biology, Ecology, and Conservation Seagrasses*: 463–501 (A. W. D. Larkum, R. J. Orth & C. M. Duarte, Eds.). Springer, Dordrecht.
- Verges, A., Becerro, M. A., Alcoverro, T. & Romero, J., 2007a. Variation in multiple traits of vegetative and reproductive tissues influences plant-herbivore interactions. *Oecologia*, 151: 675–686.
- 2007b. Experimental evidence of chemical deterrence against multiple herbivores in the seagrass *Posidonia oceanica*. *Marine Ecology Progress Series*, 343: 107–114.
- Vizzini, S., Sara, G., Michener, R. H. & Mazzola, A., 2002. The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. *Acta OEcologica*, 23(4): 277–285.
- Wressnig, A. & Booth, D. J., 2007. Feeding preferences of two seagrass grazing monacanthid fishes. *Journal of Fish Biology*, 71: 272–278.
-