

Research

The role of flow velocity combined with habitat complexity as a top–down regulator in seagrass meadows

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Large-scale losses of seagrass areas have been associated with eutrophication events, which have led to an overproduction of photosynthetic organisms including epiphytes. Grazers that feed on epiphytes can exert a significant top–down control in the system, but the effects of physical factors on grazing activity and feeding behaviour have been rarely examined. We addressed the combination of hydrodynamic regime and seagrass shoot density can alter the feeding and foraging behaviours of mesograzers. A full factorial experiment, with flow velocity (high, medium and low) and shoot density (high versus low) as main factors, was conducted in a racetrack flume using artificial seagrass plots. The results showed that when high flow velocity conditions were combined with low shoot density, consumption of epiphytes by mesograzers was strongly reduced. In contrast, when flow velocity was low or shoot density was high, mesograzers exhibited high feeding rates and vigorous swimming behaviour. These results clearly indicate that hydrodynamic stress reduces the time that mesograzers can spend feeding, since it inhibits their swimming behaviour, and thus indirectly affecting to the density of epiphytes. Therefore, the triggering of trophic cascade effects in seagrass communities under these experimental conditions depended on the interrelationship and feedbacks among shoot density, abiotic (flow velocity) and biotic (epiphytes and mesograzers) compartments, with flow velocity exerting a top–down control on seagrass ecosystems.

Keywords: epiphytes, grazing, plant–herbivore interaction

Introduction

Over the last decades, a central focus in ecological research has been deciphering how environmental forcing and biological interactions are involved in the transfer of primary production to higher trophic levels (Duffy and Hay 2000, Duffy and Harvilicz 2001). Understanding the interplay between bottom–up and top–down processes is critical given the ongoing perturbations of environmental conditions and subsequent effects on food-web topology (Duffy 2003, Duffy et al. 2005, Byrnes et al. 2007, Poore et al. 2012). Seagrass ecosystems serve well as model



communities to study the importance of bottom–up and top–down processes in marine systems (Duffy et al. 2005, Heck and Valentine 2007, Burkholder et al. 2013) because they are highly threatened by both eutrophication (i.e. bottom–up) and overfishing (top–down) processes, besides other human activities (Waycott et al. 2009). In addition, seagrass ecosystems are hotspots for biological diversity since they are considered to be foundation species and, thus, can ameliorate biotic and/or abiotic stress, which facilitates the development of other species (Orth et al. 2006, van der Heide et al. 2012).

Earlier studies have revealed that a large number of seagrass-associated organisms feed heavily on marine epiphytic algae, which are important primary producers in seagrass ecosystems and make a significant contribution to food webs (Borowitzka et al. 2006, Myers and Heck 2013). Moreover, previous works have demonstrated that the presence of different species of epiphytes in seagrass leaves increases the consumption in these ecosystems by different consumers, such as sea urchins, fishes and mesograzers (Garcia et al. 1999, Jaschinski and Sommer 2008a, Marco-Méndez et al. 2015, Jiménez-Ramos et al. 2018a). Overproduction by epiphytic algae, however, has been associated with large-scale losses of seagrasses in eutrophic waters (Silberstein et al. 1986, Walker and McComb 1992). Grazers that feed on epiphytes can exert significant top–down control, preventing the negative consequences of epiphyte overgrowth (Jernakoff et al. 1996, Duffy et al. 2005, Borowitzka et al. 2006, Heck and Valentine 2006, Valentine and Duffy 2006, Poore et al. 2012). The regulation of the epiphytic community by grazers can initiate a trophic cascade, indirectly affecting seagrasses in eutrophicated areas (Williams and Ruckelshaus 1993, Philippart 1995, Schanz et al. 2002, Moksnes et al. 2008), since grazing improves the light environment and, thus, growth conditions while boosting the transfer of matter and energy to higher trophic levels (van Montfrans et al. 1984, Orth 1992, Jernakoff and Nielsen 1997, Valentine et al. 1997, Garcia et al. 1999, Lavery et al. 2007). The important role of grazers on seagrass ecosystems raises the question of which factors control mesograzers activity in such communities.

Mesograzers may have a large influence in the top–down processes in temperate seagrass communities, where they are often the dominant herbivores, consuming primarily epiphytic algae and detritus, and only rarely consuming seagrasses directly (Valentine and Duffy 2006, Whalen et al. 2013). However, their role in structuring seagrass communities and the influence of biotic and abiotic conditions on its feeding and foraging behaviour have remained unresolved for decades. This can be attributed to their small size, large mobility and complex taxonomy of the dominant mesograzers, which have made experimentation difficult (Jernakoff et al. 1996). Most studies on the regulation of epiphyte mesograzers have focused on how predation and habitat complexity influence their abundance and distribution patterns. For instance, the effects

of grazing by some mesograzers like amphipods, may vary because their populations and foraging behaviour fluctuate with environmental conditions, fish predation and food availability (Stoner 1980, Edgar 1990, Gambi et al. 1992, Amundrud et al. 2015, Hovel et al. 2016). Specially, the amphipod *Gammarus locusta* is a common mesograzer that naturally occurs in *Zostera* beds along Northeast Atlantic ocean (Costa and Costa 2000), but little is known about its general ecology (i.e. foraging and feeding preferences, population dynamics, etc). However, recent studies have shown that eutrophication and macroalgal blooms may have a positive effect on the feeding and foraging behaviour of this species (Kraufvelin et al. 2006, Moksnes et al. 2008). On the other hand, the effects of physical factors like water flow velocity on grazing activity of mesograzers have been rarely examined (but see Schanz et al. 2002, Lavery et al. 2007) even though hydrodynamics may be expected greatly influence both abiotic and biotic compartments. For instance, hydrodynamics are known to affect light conditions (Drake et al. 2003), nutrients and resource transfer within the canopy (Cornelisen and Thomas 2004, Morris et al. 2008, González-Ortiz et al. 2014b), which in turn can affect epiphytes (Borowitzka et al. 2006, Lavery et al. 2007), seagrasses (Koch 1994, De Los Santos et al. 2009, Egea et al. 2018) and fauna in positive or negative ways (Grizzle et al. 1992, Irlandi 1996, Schanz et al. 2002, González-Ortiz et al. 2014b). Therefore, hydrodynamics may be expected to affect bottom–up and top–down control processes in complex ways. The strength of the interactions involved may depend on the magnitude of both biotic and abiotic variables, such as the complexity of the seagrass canopy (i.e. shoot density) and physical forcing. In this study, we aim to untangle the interactive effects of seagrass canopy complexity and current velocity on the feeding and foraging behaviour of mesograzers under controlled hydrodynamic conditions (i.e. using a large race-track flume that generates an unidirectional flow). In doing so, we also aim to gain insight into the underlying mechanisms behind these processes.

Material and methods

Artificial canopy design

Seagrass mimics were designed to simulate the main physical properties of these macrophytes when underwater. We used two different designs for mimics depending on their function in the experiments:

- 1) *Bare plastic mimics* were used to create an artificial seagrass plot without epiphytes. These mimics were used to form matrices of contrasting complexity, in which a limited number of epiphytized mimics were subsequently placed. Shoots of the bare plastic mimics were simulated by using a group of leaf-like plastic straps, which were attached to a wooden stick simulating the rhizome–root system using a 4 × 0.4 cm plastic straw filled with adhesive silicon (imitating the leaf

sheath) (González-Ortiz et al. 2014a, 2016). Morphometric characteristics of the mimicked leaves (length, width and thickness; Fig. 1) resembled those of the main species thriving in European Atlantic coasts: *Zostera noltei*, *Z. marina* and *Cymodocea nodosa* (González-Ortiz et al. 2014a). Different treatments involved manipulating the complexity of the bed (e.g. shoot density), which was facilitated by the use of mimics (Bouma et al. 2005, Peralta et al. 2008, González-Ortiz et al. 2014a).

2) *Epiphytized mimics* were used to avoid any kind of potential biological interactions between the seagrass and animals, and between the seagrass and the epiphytes (e.g. grazing, herbivory or chemical interactions), thus allowing the study to be constrained by the interactions between epiphytes and mesograzers. These epiphytized mimics were constructed using a silicon tube sealed at both ends to resemble shoots, which was attached to a wooden stick and anchored to the sediment (Brun et al. 2012, González-Ortiz et al. 2014b; Fig. 1). The silicon tube floated underwater and bowed with the flow, since the central air chamber provided positive buoyancy. In order to induce epiphyte colonization of the mimics, they were kept in outdoor tanks filled with natural seawater renewed by tidal action for a month under natural light and temperature conditions. Care was taken to obtain similar epiphyte cover between mimics to enable homogenized experimental conditions. This procedure promotes the development of a dense epiphytic film cover of green filamentous algae over these mimics, as already observed in previous studies (González-Ortiz et al. 2014a).

Grazer selection

We used the amphipod *Gammarus locusta* as a model mesograzer in this study, since this species occurs naturally in seagrass beds on North Atlantic coasts (Baden and Pihl 1984, Fredriksen et al. 2005, Baden et al. 2010) and has a broad range of feeding preferences (i.e. marine macrophytes and its epiphytes; Costa and Costa 1999, 2000). Individuals were collected from the Oosterschelde Estuary (the Netherlands). Tidal currents for such estuarine marshes ranged from 5 to 40 cm·s⁻¹ (Bouma et al. 2005). Natural density of mesograzers was measured in the field using a 400 cm² quadrat at 10 different selected randomly areas into the *Zostera* beds. Mesograzers natural density was 15 ± 6.2 ind. m⁻². The experimental mesograzers were collected using ‘Ulva traps’. These traps comprising a series of mesh bags containing fresh *Ulva* sp., which is the major component of the natural *G. locusta* diet (Costa and Costa 2000), were placed at low tide and collected after a tidal cycle. A mesh size of 10 mm was used to collect only sexually mature *G. locusta* individuals (> 7 mm of length; Andersson et al. 2009). The length of the amphipods was measured as the distance between the anterior end of the rostrum and the posterior end of the last urosome, which is represented by the last three abdominal somites bearing modified appendages in amphipods (Moore and McCormick 1969), when the amphipod is in an extended position, i.e. pressed flat against a surface. *G. locusta* length was on average of 10.4 ± 1.2 mm (n=180). The amphipods were kept outdoors in 60 l flow-through tanks (100–200 individuals per tank) for at least five days before the experiment, with

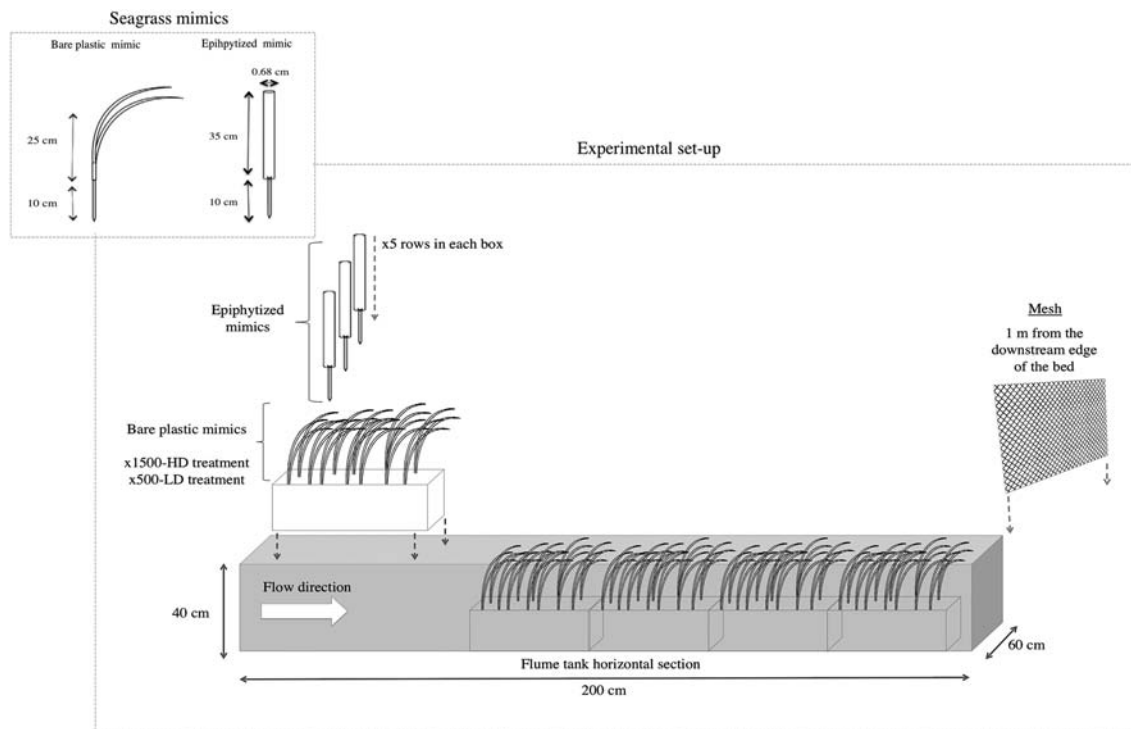


Figure 1. Drawing of the experimental set-up and the two different designs for seagrass mimics used in the feeding experiments.

Ulva sp. provided as both substratum and food source for each tank.

Pilot experiment

A series of pilot trials were conducted to ascertain that the feeding behaviour of *G. locusta* remained similar when offered mimics with epiphytes versus natural seagrass shoots with epiphytes. These treatments were designed to account for the effect of using mimics rather than natural seagrass shoots. *Epiphytized mimics* were selected from the pool of mimics produced in the outdoor tanks. Natural shoots of *Z. marina* were collected from *Zostera* meadows on the mudflats of Viane (51°39'N, 4°01'E), the Oosterschelde, in the south-western part of the Netherlands. After collection, they were cleaned of epiphytes and planted in the same outdoor reservoirs as explained above. Here, they were left to be colonized by epiphytes under the same conditions as described for the epiphytized mimics.

We tested the following treatments in the pilot experiment: 1) high density of epiphytized mimics (HD-EM, $n = 10$ mimics), 2) high density of naturally epiphytized *Z. marina* shoots (HD-EZ, $n = 10$ shoots), 3) low density of epiphytized mimics (LD-EM, $n = 5$ mimics) and 4) low density of naturally epiphytized *Z. marina* shoots (LD-EZ, $n = 5$ shoots). To estimate the initial epiphyte content, a total of 10 epiphytized mimics and 10 *Zostera marina* shoots were scraped and epiphyte biomass was quantified. Each treatment was carried out in triplicate and was conducted in tanks ($50 \times 35 \times 30$ cm) filled with natural seawater and sand. To minimize disturbance, no aquarium air pumps were used, and all aquaria were surrounded by opaque plastic. Before each trial, individuals of *G. locusta* were randomly selected from the reservoir tanks and kept without food for 24 h (maximum starvation time) to remove chlorophyll from the stomach content (i.e. starved grazers). At the beginning of each trial, five grazers were placed in each tank and after 6 h, they were removed from the water and immediately frozen at -20°C for further analysis (i.e. experimental grazers). In addition, another five *G. locusta* from the starved pool were also frozen before each run to estimate the initial chlorophyll content of the stomach (Zupo et al. 2001). Subsequently, at the beginning of the chlorophyll measurements, each sample was thawed in dark, transferred to a vessel, and gently blotted with filter paper to eliminate excess of water. Five ml of 90% acetone solution was added to each vessel and the mixture was shaken vigorously for 30–60 s with vortex mixers. Vessels were wrapped in aluminium foil, placed overnight in dark in a refrigerator at 5°C (12h). After this period, the solution was centrifuged (5 min, 4500 rpm), the supernatant transferred to a new tube, and remaining amphipod biomass was washed in distilled water, centrifuged again and after removing the water dried at 60°C for 24 h and weighed. The chlorophyll a (Chla) content in the supernatant acetone was analyzed by spectrophotometry and equations by Ritchie (2006) were used to calculate chlorophyll a concentration. Feeding rate was calculated as

the difference in Chla content between experimental grazers and starved grazers, taking into account the experimental period and finally expressed as $\mu\text{g Chla mg DW}^{-1} \text{ h}^{-1}$.

To estimate both the concentration of Chla in epiphytes and the epiphyte biomass in natural shoots and mimics, they were removed from shoots and mimics using a soft silicon stopper (Burkholder et al. 1990), excess of water removed with soft paper, fresh weighed, rinsed with filtered (0.2 mm) seawater, and collected by vacuum filtration onto a 2.5 cm Whatman GF/F glass fiber filter. Chla content in epiphytes was extracted from filters using acetone as solvent (90%). Filters were placed in disposable polypropylene plastic centrifuge tubes (10 ml), 2 ml of solvent was added and the mixture was shaken vigorously for 30–60 s by vortex mixers. Tubes were wrapped in aluminium foil, placed in dark in a freezer (at 5°C) and extracted overnight (ca 12 h). After extraction, samples were centrifuged (5 min, 4500 rpm). Chlorophyll a content in the supernatant was analyzed by spectrophotometry and equations provided by Ritchie (2006) were used to calculate its concentration. We calculated both the chlorophyll a content of the epiphytes ($\mu\text{g Chla g FW}^{-1}$) and the normalized epiphyte Chla concentration per surface area of shoot/mimic ($\mu\text{g Chla cm}^{-2}$; Jaschinski and Sommer 2008a).

Racetrack flume set-up

The experiment was run in a large unidirectional racetrack flume tank (Bouma et al. 2005) with a testing area of 200×60 cm and a total length of 1700 cm. The flume tank was filled with natural seawater (water column height of 0.4 m). Five wooden boxes ($40 \times 60 \times 10$ cm) were filled with natural sand and planted with plastic mimics (González-Ortiz et al. 2014b), creating artificial seagrass plots with two different densities (i.e. representing two different levels of habitat complexity): 1) high density (HD, 1500 mimics m^{-2}) and 2) low density (LD, 500 mimics m^{-2}). For each run, a total of 75 epiphytized mimics from the initial pool were placed in three rows every 25 cm within the artificial seagrass plots formed by five boxes, with a single density each one, along the flume tank, so that all experimental space had the same epiphyte load. Consequently, *G. locusta* were subjected to the same probability of finding food (i.e. epiphytized mimics) across the artificial canopy. At the beginning of each run, 10 epiphytized mimics from the initial pool of mimics were scraped to measure the initial epiphyte load for each trial. At the end of the channel (1 m far from the downstream edge of the bed) a net (mesh size of 5 mm) was placed to prevent the escape of grazers further downstream along the flume that could return to the leading edge of the reconstructed bed because of the circular shape of the flume (Fig. 1).

Racetrack flume experiment

A total of 18 tests were performed for two different canopy densities (HD and LD) under low (0.05 m s^{-1}), medium

(0.15 m s⁻¹) and high (0.30 m s⁻¹) unidirectional flow velocities (LV, MV and HV, respectively). The flume was run at the given speed for at least 15 min before starting the tests to stabilize the hydrodynamic regime. Within the flume tank, the X-, Y- and Z-axes are oriented respectively to the main flow direction (X), perpendicular to the lateral flume tank walls (Y), and vertically (Z) (Peralta et al. 2008, González-Ortiz et al. 2014b). After this stabilization period, 10 starved individuals of *G. locusta* were haphazardly introduced within the artificial seagrass plot. In addition, 10 starved *G. locusta* were frozen before each run to estimate the initial chlorophyll stomach content. The racetrack flume was left running for 6 h in each trial under dark conditions to minimize disturbance of the amphipods.

During the experimental period, the positions of *G. locusta* inside the artificial seagrass plot were checked four times along the transparent wall of the test section: observation 1 at 10 min, observation 2 at 1 h, observation 3 at 3 h, and observation 4 at 6 h, the end of the experiment. This set of data was collected manually by observing the organisms along the X, Y and Z axes of the flume tank, using a dim flashlight since the amphipods should remain in the dark as much as possible. We noted position and the number of individuals actively swimming during the observations. Once the experimental period ended, grazers were removed and immediately frozen at -20°C for Chla analysis (Ritchie 2006). Feeding rate was calculated as the difference in Chla content between grazers at the end of the experiment and initial starved grazers over the experimental period. Experimental epiphytized mimics were collected and scraped for epiphytes, which were then dried and weighed. Moreover, the same experimental set-up was run for all aforementioned treatments (n=18), but without including *G. locusta* (no-grazer controls), in order to determine the effects of hydrodynamic alone in epiphyte distribution.

Statistical analyses

Significant differences in Chla content in the pilot experiments were checked using a one-way ANOVA analyses. A two factorial ANOVA (two levels of shoot density and three hydrodynamic regimes) was used to test the effects of flow velocity and shoot density on feeding rate, epiphyte load and mesograzer positions in racetrack flume experiments. Tukey's test was used to compare significant effects. All data were checked for normality and homoscedasticity using Shapiro-Wilks and Barlett's tests respectively, and data were ln-transformed (ln (x + 1)) when necessary to assure the homogeneity of the variances (Quinn and Keough 2002). The level of significance was set at 5% ($\alpha=0.05$). Moreover, the 3D plane regression was added to graphical figure of mesograzer positions throughout the observational time. Data are presented as mean \pm SE and significance levels were set at $p=0.05$. Statistical analyses were computed with R statistical software ver. 3.0.2 (<www.r-project.org>).

Data deposition

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.8c86rf2>> (Jiménez-Ramos et al. 2018b).

Results

Pilot experiment

Epiphyte community was mainly dominated by green filamentous algae (i.e. *Ulva* sp. and *Cladophora* sp.) in both *Zostera* shoots and epiphytized mimics, with no visual differences between both. There were no significant differences in epiphyte load between natural *Zostera* shoots and epiphytized mimics from the initial pool (1.65 ± 0.27 versus 1.75 ± 0.13 g DW shoot⁻¹ or mimic⁻¹, respectively; p -value=0.06; $n=10$). The mean Chla content per gram of fresh weight of epiphyte was 0.6 ± 0.23 μ g Chla g FW⁻¹, while the epiphyte biomass per area was in natural shoots 0.39 ± 0.01 μ g Chla cm⁻² and 0.42 ± 0.023 μ g Chla cm⁻² for mimics. No significant differences were detected ($F=2.14$, $df=1.09$; $p=0.12$).

Our food preference experiment demonstrated that *Gammarus locusta* had the same feeding rate whether exposed to epiphytes on epiphytized mimics or natural *Zostera* shoots (p -value=0.255), independently of food availability (i.e. high or low food availability) (Fig. 2, Supplementary material Appendix 1 Table A1). Also, our experimental time (6 h) was sufficient to detect changes in Chla content between starved and experimental individuals of *G. locusta*.

Racetrack flume experiment: feeding behaviour of *G. locusta*

Flow velocity and shoot density (i.e. density of mimics) significantly influenced the feeding rate of *G. locusta* (p -value < 0.01 and p -value=0.018 respectively, Supplementary material Appendix 1 Table A2). In contrast, the interaction between flow velocity and density ($p=0.174$) was not significant. In all the HV treatments, independently of the shoot density, Chla content in *G. locusta* after 6 h was so low that it was not detectable with the method used, yielding a feeding rate of zero (Fig. 3, Supplementary material Appendix 1 Table A3). However, a remarkable increase of Chla in *G. locusta* was recorded under MV and in LV treatments, especially under HD conditions. Although shoot density had no influence during HV treatments, during MV and LV treatments, we found significantly higher feeding rates under high shoot density (both MV and LV, p -value < 0.01) compared with low shoot density (Fig. 3).

Change in epiphyte load along artificial seagrass plots

Epiphyte load showed a spatially explicit distribution depending on flow velocity and shoot density in the mesograzers

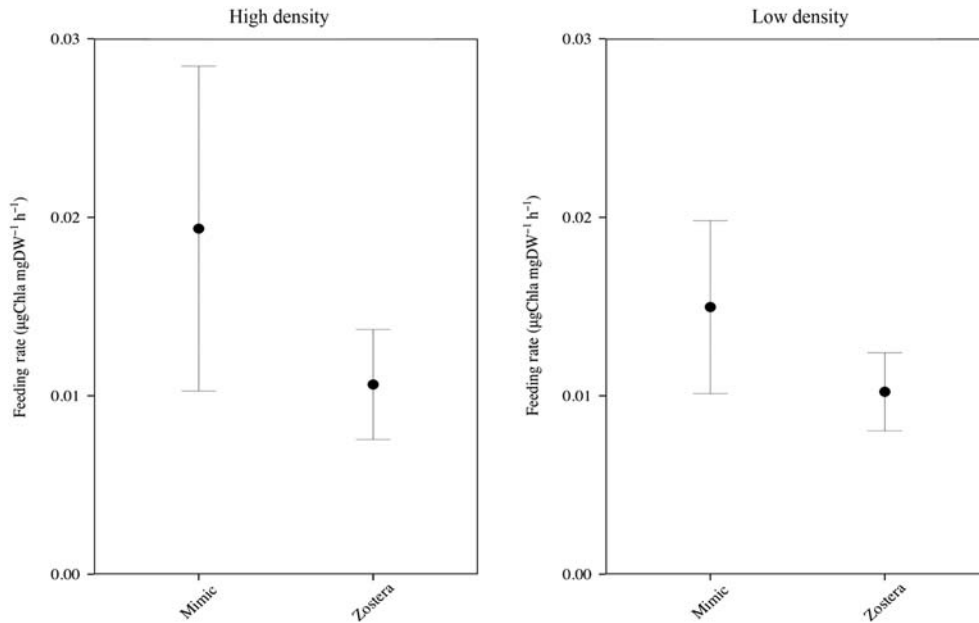


Figure 2. *Gammarus locusta* feeding rate ($n=3$) in the pilot trials at different levels of shoot density in *epiphytized mimic* and *Zostera* canopies ($n=12$). Values were normalized by epiphyte load in each tank ($g\ DW\ epiphytes$). Data are mean \pm SE.

trials (Supplementary material Appendix 1 Table A4). Overall, a significant decrease was found at the leading edge of the artificial seagrass plot (i.e. box 1, p -value < 0.01), especially under HV treatments (Fig. 4). Both flow velocity and flume position affected epiphyte load under LD treatments. However, hydrodynamic regime had no influence on epiphyte load under HD treatments (Fig. 4, Supplementary

material Appendix 1 Table A4; $p=0.267$). Similar results were found in the no-grazer controls, since an overall reduction (lower than $6 \pm 11.08\%$) in epiphyte load was found in the leading edge (box 1) of all the treatments, which was more noticeable under high velocity and low shoot density (Supplementary material Appendix 1 Table A4). In addition, hydrodynamics had not effects on epiphyte load under HD

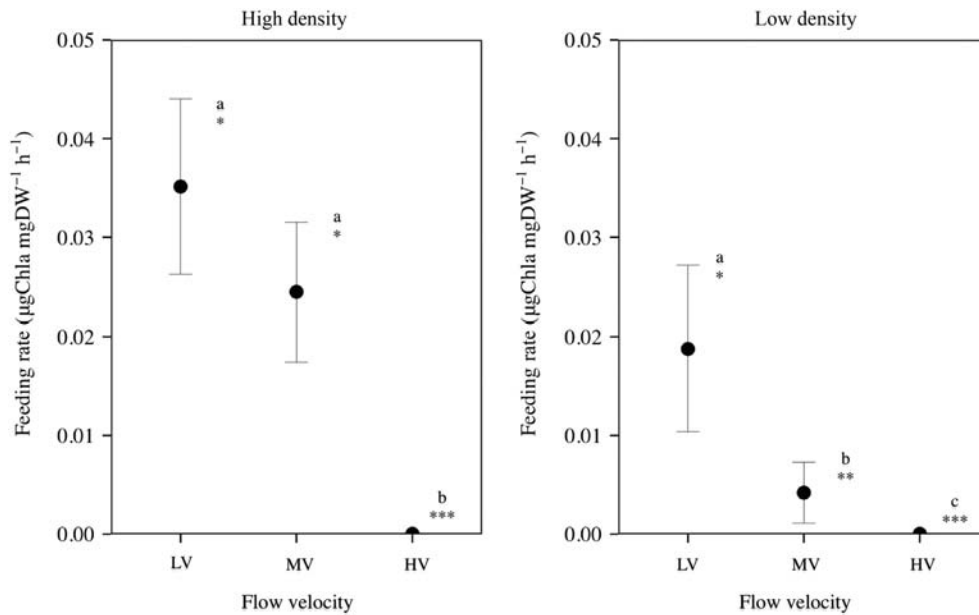


Figure 3. *Gammarus locusta* feeding rate ($n=3$) in the racetrack flume tank experiments for different unidirectional flow velocities (low (LV) = 0.015 , medium (MV) = 0.045 and high (HV) = $0.30\ m\ s^{-1}$) and shoot density (high and low density) ($n=18$). Letters indicate significant differences between flow velocities within each level of density. Asterisks indicate significant differences between density levels at the same velocity treatment (p -value < 0.05).

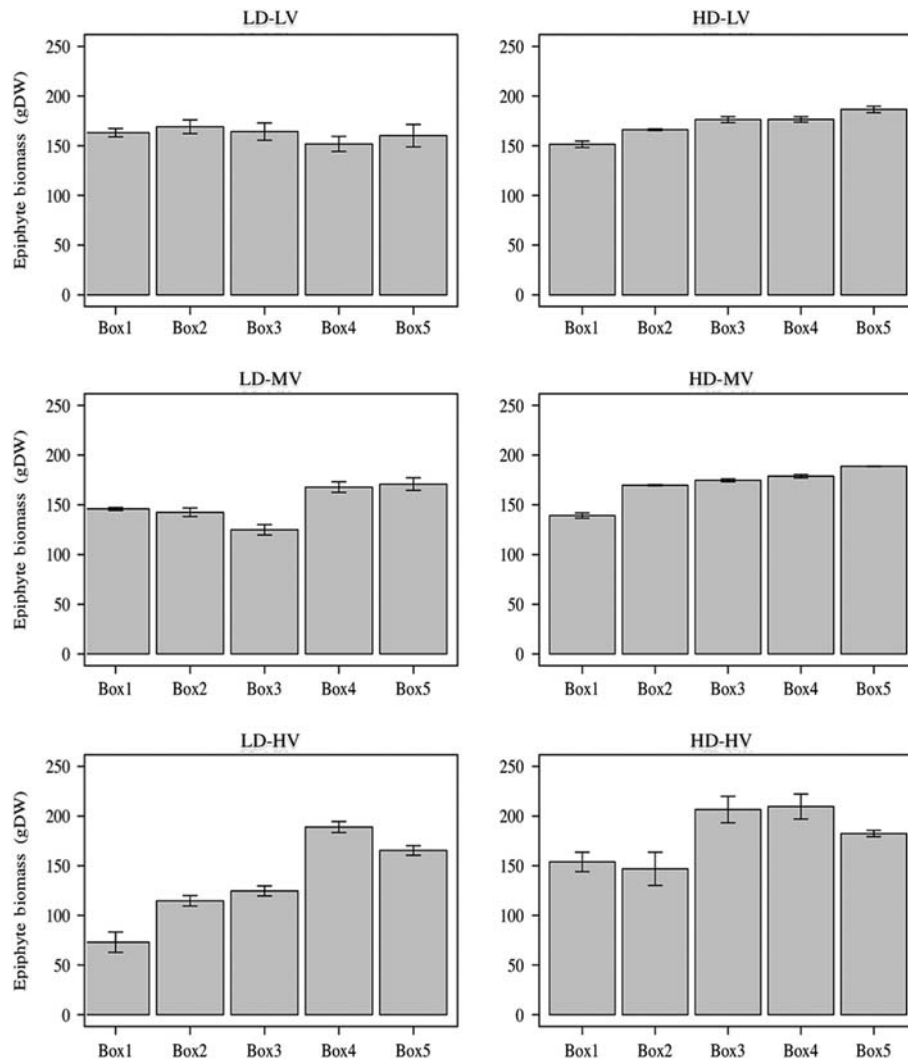


Figure 4. Mean \pm SE ($n = 3$) epiphyte load (g DW) in each box along the racetrack flume at the end of each run. Box 1 indicates the leading edge of the artificial seagrass plot.

treatments (p -value = 0.312) as already found when *G. locusta* was present (Supplementary material Appendix 1 Table A4).

Spatial position and swimming behaviour of *G. locusta*

Gammarus locusta location along the artificial seagrass plot was significantly affected by flow velocity, shoot density and time of observation (Fig. 5, Supplementary material Appendix 1 Table A5). In all the treatments, when individuals of *G. locusta* were released into the racetrack flume, they actively moved but the effectiveness of their swimming movements depended on experimental conditions (Supplementary material Appendix 1 Table A6). The swimming behaviour was most evident in treatments with low flow velocity (LV), since the amphipods tended to be distributed throughout the artificial seagrass plots (Fig. 5), especially under HD. In this scenario of LV, especially under low shoot density, higher percentages of *G. locusta* actively swimming were recorded (27.5 ± 4.18 % of individuals in the

whole experimental time) compared to percentages of individuals actively swimming under high shoot density (18.35 ± 13.70 %). In contrast, *G. locusta* usually showed limited mobility under HV treatments (Supplementary material Appendix 1 Table A6) and tended to aggregate downstream of the leading edge (Fig. 5).

Discussion

This experiment clearly indicates that water unidirectional flow conditions, shoot density and their interactions significantly influenced the feeding and foraging behaviour over epiphytes of mesograzers in (artificial) seagrass plots. This change in feeding and foraging behaviour also has major implications at the community level, affecting the density of epiphytes. The epiphyte assemblage on experimental shoots and mimics consisted mainly in filamentous green algae (mainly thin-tubular *Ulva* sp. and *Cladophora* sp.). This may

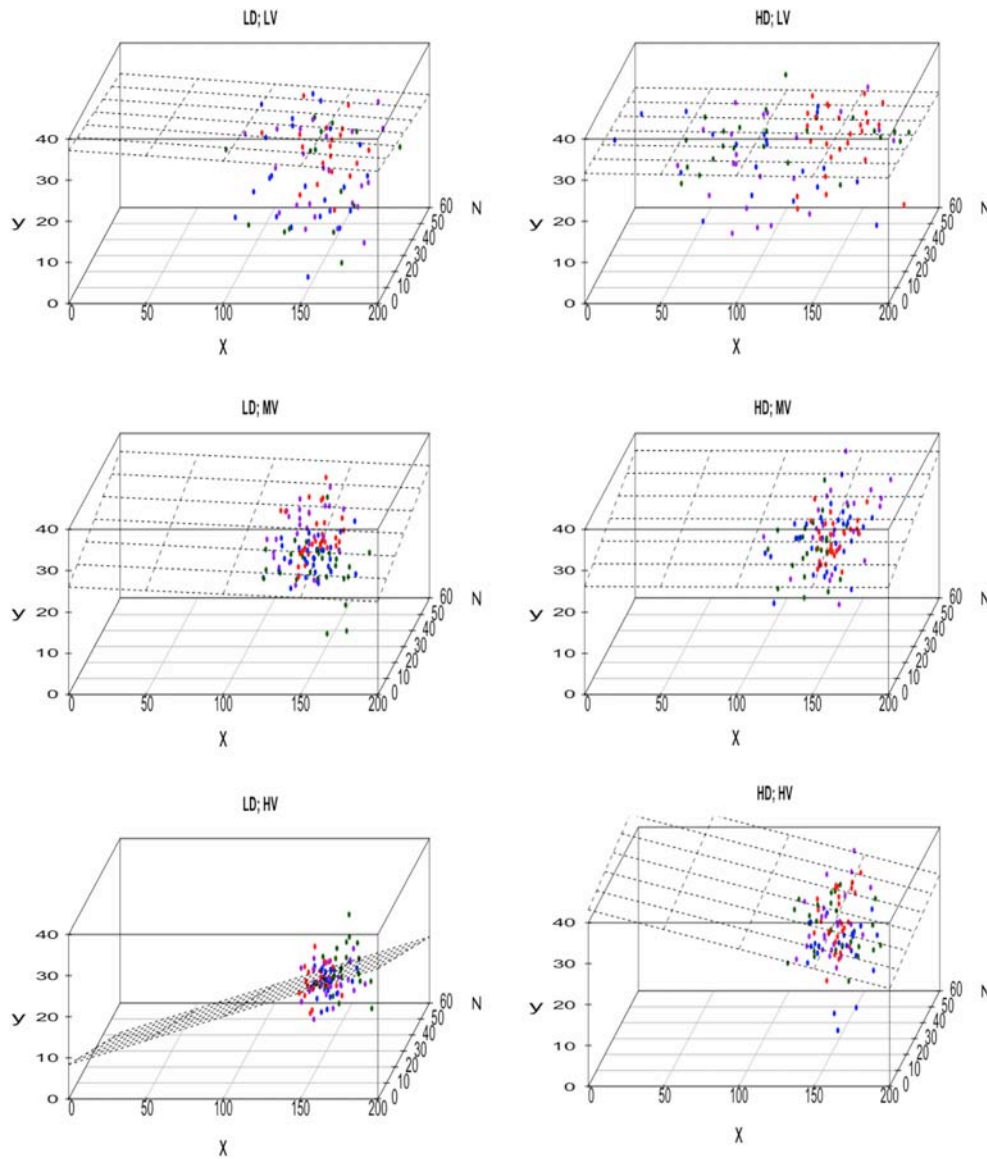


Figure 5. Three dimensional locations of *G. locusta* and the 3D regression plane in the racetrack flume at four observational times; 10 min (red circles), 1 h (purple circles), 3 h (blue circles) and 6 h (green circles). LD=low density, HD=high density, LV=low velocity, MV=medium velocity, HV=high velocity.

affect to the grazing capacity of the mesograzers, since filamentous green epiphytes use to be preferred by mesograzers as they bear lower resistant to grazing than others functional groups (Garcia et al. 1999, Andersson et al. 2009) due to the lower mechanical defences (i.e. carbon and fibers) and higher nutritional value (i.e. N content) (Klumpp et al. 1992). In fact, some studies showed that *Gammarus locusta* displayed higher growth rates and eggs production when feeding on nutrient-enriched algae (i.e. green filamentous algae) (Kraufvelin et al. 2006), while also bear higher individual density and biomass under such conditions (Moksnes et al. 2008). Notably, although some previous in situ works found differences between natural shoots versus mimics (Pinckney and Micheli 1998, Bologna and Heck Jr 1998), we did not

find differences in epiphytic species between natural shoots and mimics, perhaps because they were epiphytized in mesocosm tanks under the same conditions, where the bulk of epiphyte species diversity is lower. In addition, the lack of other types of epiphytic species in experimental shoots and mimics could be due to the experimental time needed for the growth of epiphytes. Therefore, using mimics with epiphytes seems to be a valid and valuable experimental approach to address the mechanisms underlying epiphyte-grazer interactions, since it allows the development of treatments controlled for differences resulting from plant-epiphyte interactions (e.g. differences in leaf surface, leaf span time, hydrodynamics, natural products; Bouma et al. 2005, Borowitzka et al. 2006, Teena et al. 2008, Gambi et al. 2011).

In our study, the filamentous green epiphytes from genus *Ulva* sp. and *Cladophora* growing in experimental shoots and mimics were a suitable food source for *G. locusta*, since their main diet consists in seaweeds (mainly *Ulva* sp.), seagrasses and epiphytes attached to their surface (Greze 1968, Costa and Costa 1999, 2000, Jephson et al. 2008, Andersson et al. 2009). Thus, the highly palatable epiphytes growing in the mimics made our methodology optimal for the objectives of the study. In addition, the Chla content in the stomach was an useful tool to assess feeding rates in our seagrass–epiphyte–grazer complex, since integrate the consumption of all species of epiphytic algae (all containing chlorophyll). However, it must be taken into account that in the case of a complex community of epiphytes (e.g. red, brown green, calcareous, etc) the estimation of the feeding rates using Chla concentrations does not discriminate between the type of epiphyte consumed. Therefore, if the goals of the study were to discriminate the individual consumption over each individual species, it would be advisable to use another type of methodology such as the use of stable isotopes (Lebreton et al. 2011, Michel et al. 2015) or the determination of specific pigments using HPLC analyses (Jaschinski and Sommer 2008a, Suffrian et al. 2008).

Can we explain the epiphyte effects by changes in swimming behaviour?

Virtually all species of Gammaridean amphipods can swim, with some travelling long distances at a continuous rate, while others escape predators or change microhabitats with short bursts of rapid swimming (Boudrias 1991). In this experiment, we observed individuals of *G. locusta* to move quickly from mimic to mimic. According to observations of classic works such as Vogel (1985), *G. locusta* possesses a rapid movement and together with other species of gammarids, such as *Hyale nilssonii* and *Corophium volutator*, possess a high swimming capacity. It is known that this swimming efficiency is due to a functional adaptation in the use of its tail and pleopods (Vogel 1985). However, in this study we have observed how the swimming capacity of *G. locusta* is limited by the flow velocity. That is, higher velocity resulted in shorter feeding time and lower swimming capacity, leading to lower rates of epiphyte removal. However, at low velocity, individuals of *G. locusta* tended to swim and disperse across the flume in scattered positions. This result indicates that the change in swimming behaviour and, thus, in the time spent in feeding, was responsible for the different rates of epiphyte removal. This result highlights the importance of deepening how mesograzers interact with the physical environment (bottom–up) and how the swimming capacity of can influence the top–down control into benthic habitats (Koehl and Cooper 2015).

Interacting feedback loops

Previous studies on the relationship between hydrodynamics and epiphyte–grazer systems found conflicting results on

the effect of flow on epiphyte load. On the one hand, epiphyte load was found to be lower under high flow conditions because of the washing out of epiphytes, (e.g. physical erosion; Lavery et al. 2007). On the other hand, the washing out of grazers (i.e. *Hydrobia ulvae*) was found to be actually responsible for the increase in epiphyte load (Schanz et al. 2002). Our results agree with both perspectives, since the relationship between shoot density and flow velocity has demonstrated that outcomes are contingent on the strength of such interactions (Fig. 6). For instance, high density may reduce physical stress for both epiphytes and mesograzers (Edgar and Roberston 1992, Moore and Hovel 2010, this work), reducing both the washing out of epiphytes and predation pressure on mesograzers while increasing the feeding time (Gilliam and Fraser 1987, Schanz et al. 2002, Borowitzka et al. 2006, Lavery et al. 2007, González-Ortiz et al. 2014b). Our results demonstrated that when hydrodynamic conditions were more gentle or shoot density (i.e. habitat complexity) was higher, the physical stress on the amphipods was lower. This allowed for investment in feeding and swimming (Pepper et al. 2015), leading to higher epiphyte removal from the canopy. In addition, at low velocity individuals of *G. locusta* tended to swim and disperse across the flume in scattered positions indicating that change in swimming behaviour and, thus, time spent in feeding, was responsible for the different rates of epiphyte removal.

Otherwise, high habitat complexity reduces light and nutrient availability for epiphytes (Lavery et al. 2007, Morris et al. 2008, 2013), which may decrease their occurrence frequency in the canopy. High flow velocity may increase nutrient transfer to epiphytes (Morris et al. 2008, 2013) while increasing self-shading because of the higher bowing of the canopy (Morris et al. 2008, González-Ortiz et al. 2014a), which in turn reduces light availability (Drake et al. 2003). Also, as demonstrated in this work when using an unidirectional flow, amphipods can be physically washed away from some locations (e.g. leading edge of

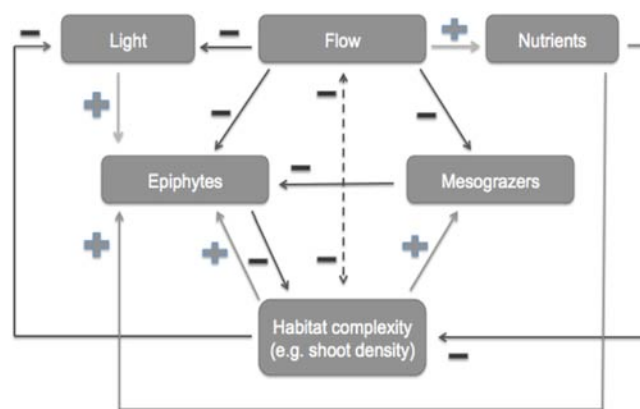


Figure 6. Conceptual diagram showing the positive or negative relationships among flow velocity, habitat complexity and biotic and abiotic factors. Black lines indicate negative relations. Grey lines indicate positive relations. Dashed line shows bidirectional relationship.

the patch) where higher flow velocity and turbulence values are expected (Morris et al. 2008, González-Ortiz et al. 2014a). Moreover, under such conditions *G. locusta* also tended to aggregate downstream of the leading edge of the reconstructed bed, where unidirectional flow velocity is highly reduced by the buffering capacity of the seagrass canopy (Bouma et al. 2005, Morris et al. 2008, González-Ortiz et al. 2014b); thus, the epiphyte consumption was restricted to these discrete areas where grazers tended to aggregate. Remarkably, most of these interrelationship paths are bidirectional and also affect habitat complexity (e.g. shoot density, leaf length, above/belowground ratio; Brun et al. 2006). In fact, modification of flow characteristics (e.g. velocity, turbulence, volumetric flow crossing the canopy, etc.) are known to depend on plants traits (e.g. density, morphometry, biomechanical properties; Bouma et al. 2005 and 2009, De los Santos et al. 2016, Morris et al. 2008, Peralta et al. 2008). In this regard, the existence of an allometric scaling among seagrass form, size and productivity in seagrasses (Duarte 1991, Duarte and Chiscano 1999), made our results of general applicability, but with some cautions. Higher densities are usually accomplished by fast growing species with fast aboveground biomass turnover rates, and the growth and turnover of leaves of seagrasses have a large influence in the epiphyte community (Borowitzka et al. 2006). For instance, Lavery and Vanderklift (2002) found that fast growing species bear short-sized epiphyte assemblages, while in slow-growing species such as *Posidonia oceanica* (Green and Short 2003) there was a temporal succession in the epiphyte community, starting with chlorophyta and phaeophyta and later rodhophyta algae, since the later needs longer times to appear (Borowitzka et al. 2006). On the other hand, differences in epiphytic composition also influence mesograzers community (Sommer 1999, Borowitzka et al. 2006), because epiphytes are not only a food source, but also a refuge against predators (Costa and Costa 2000, Boström and Mattila 1999). Therefore, in fast growing species bearing also high shoot density, where the epiphyte community is dominated by green filamentous species, the grazing activity may be mainly determined by flow velocity (as demonstrated in this work), while in slow-growing species with slow turnover rates, the higher complexity of the epiphyte cover, and then in the grazer community may make more complex the final outcome. For instance, some experiments have demonstrated that not all the grazer community bear the same feeding efficiency in seagrass epiphytes, and that an important species-specificity in the relationship epiphyte-grazer exists (Duffy and Harvilicz 2001, Duffy et al. 2003, Jaschinski and Sommer 2008b). Finally, all these paths may affect ecosystem properties, such as the transfer of matter and energy to higher trophic levels (Duffy et al. 2005, Heck et al. 2008, Poore et al. 2012) (Fig. 6), although caution should be taken when considering trophic cascade effects, since it may imply at least another level above the herbivores, which is not contemplated in this experimental approach.

Ecosystem implications

Eutrophication is a widespread disturbance that can severely impact coastal areas, including seagrass ecosystems, through several mechanisms, such as decreasing light availability by promoting macroalgae and epiphytic growth (Waycott et al. 2009). However, epiphyte removal by grazers has been found to enhance productivity and biomass of seagrasses and to buffer the negative consequences of eutrophication (Hootsmans and Vermaat 1985, Howard and Short 1986, Philippart 1995, Nelson and Waaland 1997, Duffy et al. 2005, Moksnes et al. 2008, Poore et al. 2012). Previous works have studied the role of herbivory as a primary determinant of the abundance and composition of marine primary producers, including seagrass epiphytes (Valentine and Heck 1999, Moncreiff and Sullivan 2001, Wressnig and Booth 2007), and epiphyte-grazers such as amphipods and isopods have the potential to relieve seagrasses from stress induced by increased eutrophication (Hughes et al. 2004, Heck and Valentine 2007). However, accurately measuring the effects of mesograzers herbivory has proven to be complicated, which has led to conflicting reports. For instance, some researchers have found that amphipods produce large impacts on primary producers (Neckles et al. 1993, Duffy and Hay 2000, Graham 2002, Hughes et al. 2004, Heck and Valentine 2006, Heck et al. 2006, Davenport and Anderson 2007, Moksnes et al. 2008, Baggett et al. 2010), while others have found that amphipods and other mesograzers have little or no effect on epiphyte or seagrass growth (Keuskamp 2004, Jaschinski and Sommer 2008b, Poore et al. 2009). This discrepancy may be partially explained by differences in the biology and life history of amphipod species, but it also likely results from differences in the experimental conditions of grazing studies (Hughes et al. 2004). On the other hand, our results highlights the overlooked effect of abiotic conditions such as hydrodynamics or habitat structure on feeding and foraging behaviour of mesograzers and the indirect consequences on epiphytic community.

This study clearly indicates that seagrasses act as foundation species, having a positive biological effect on mesograzers through facilitation (i.e. reducing hydrodynamic pressure). Not only does reducing hydrodynamic pressure have a direct impact on mesograzers, it also forms a feedback to the foundation species by reducing epiphyte load. By removing epiphyte biomass, grazers can substantially impact the persistence of seagrasses by promoting trophic cascade effects, thus stimulating the ecosystem services and functions of these foundation species (Klumpp et al. 1992, Philippart 1995, Duffy and Harvilicz 2001, Moksnes et al. 2008, Poore et al. 2012).

In conclusion, our work clearly demonstrates that the triggering of trophic cascades depends on the strength of the interrelationships and feedbacks among habitat complexity, abiotic and biotic compartments, with flow velocity acting as a top-down regulator (sensu Schanz et al. 2002) in seagrass ecosystems. Therefore, these complex relationships must be

considered in such habitats, since it may determine conditional outcomes at the level of communities and ecosystems. Furthermore, seasonal and spatial effects on these relationships should be addressed, since both are known to affect seagrass habitat complexity, hydrodynamic conditions and biotic compartments.

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Supplementary material (available online as Appendix oik-05452 at <www.oikosjournal.org/appendix/oik-05452>). Appendix 1.