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Effects of grazing and fertilization on epiphyte growth dynamics under moderately eutrophic conditions: implications for grazing rate estimates

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ABSTRACT: The effects of grazing and nutrients on epiphyte biomass in seagrass beds have received much attention, yet less is known about effects on other metrics of epiphyte growth dynamics, such as epiphyte productivity and biomass turnover rates. To help address this gap, we present here a number of mesocosm experiments in which we manipulate grazer presence and nutrient concentrations under initially moderate eutrophic conditions. We also examine the potential bias contained in estimates of epiphyte grazing that disregard the effects of grazing on epiphyte productivity. The effects of grazing and nutrient enrichment on the epiphyte growth metrics examined were disparate. Namely, the effects of grazing on epiphyte biomass predominated over those of nutrient enrichment, but grazing and nutrients had similar importance as controls of epiphyte productivity and biomass turnover rates. The results illustrate that the effects of environmental and biological factors on one given metric of epiphyte growth dynamics can be quite different from those on other metrics. Thus, we suggest that the biomass-centered view mostly used to date in studies of epiphyte dynamics should perhaps shift toward a broader approach including other metrics of epiphyte growth, such as productivity and turnover rates, to better understand epiphyte dynamics in coastal systems and cascading functional consequences in the system. Another risk of biomass-centered studies lies in the calculation of grazing rates since, as also shown here, estimates of grazing rates derived as the difference in biomass accrual rates between non-grazed and grazed conditions can be highly biased.

KEY WORDS: Seagrass · Epiphyte · *Halodule wrightii* · Eutrophication · Grazing

INTRODUCTION

Seagrass fauna represent a unique and diverse assemblage of organisms that directly or indirectly rely on the meadow as a source of energy and/or shelter (Virnstein et al. 1983, Heck et al. 2003). The seagrass itself, sediment microalgae, epiphytic algae, loose macroalgal mats and phytoplankton constitute the trophic base of food webs in seagrass beds (Cebrian 2004, Hauxwell & Valiela 2004). Understanding the complex set of trophic interactions between seagrass fauna and their prey has presented a challenge (Hemminga & Duarte 2000, Duffy et al. 2003, Mateo et al. 2006, Douglass et al. 2010). Anthropogenic eutrophication often stimulates epiphytic and macroalgal growth, further complicating these interactions (Sand-Jensen & Borum 1991, Duarte 1995, Borum & Sand-Jensen 1996). In some instances, the algae accumulate in quantities large enough to cause substantial shading and hypoxic/anoxic conditions and subsequent seagrass mortality (Sfriso & Marcomini 1997, Hauxwell et al. 2001, 2003,

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Orth et al. 2006), but in other instances, grazers can control nutrient-induced algal growth and dampen the deleterious effects on the seagrass (Heck et al. 2000, 2006, Hughes et al. 2004).

Epiphytic algae can be highly productive and account for up to 60% of the total primary productivity in seagrass beds (Morgan & Kitting 1984, Silberstein et al. 1986, Moncreiff et al. 1992). Epiphyte grazers include species of crustaceans, gastropods, polychaetes, sea urchins and small fish (Luczkovich et al. 1995, Jernakoff et al. 1996, Hillebrand et al. 2000, Tomas et al. 2005). Epiphyte grazers are the preferred prey for many consumers, thus representing a trophic cornerstone for food webs in seagrass beds (Orth & Van Montfrans 1984, Van Montfrans et al. 1984, Jernakoff & Nielsen 1997, Moore & Wetzel 2000). They also constitute important intermediaries in the cycling of carbon and nutrients in seagrass beds (Klumpp et al. 1992, 1993, Jernakoff et al. 1996).

In the present study, we focus on the interactions between grazers and epiphytic algae and the resulting effects on the seagrass host. Here, we add to a series of studies that have focused on the effects on epiphyte biomass accrual (e.g. Williams & Ruckelshaus 1993, Neckles et al. 1993, 1994, Heck et al. 2006, Baggett et al. 2010) by examining how varying grazing and nutrient enrichment affects the productivity of epiphytes, both on an epiphyte weight-specific basis and on a leaf weight-specific basis, as well as the net accumulation of epiphyte biomass on seagrass leaf blades and the resulting effects on seagrass growth. This adds to our understanding of how different conditions of nutrient availability and grazing intensity may affect primary productivity and biomass, trophic support of secondary and higher-level consumers and the cycling of carbon and nutrients in seagrass beds.

We pursued a second goal with the present study: the critical evaluation of conventional estimates of epiphyte grazing based on differences in epiphyte biomass accrual between grazed and non-grazed treatments. Epiphyte grazing has been addressed experimentally through a variety of techniques, such as analyses of gut contents, fecal pellets, radionuclide labeling and stable isotope signatures, but the most prevalent method is the use of enclosure/exclosure experimental designs (Klumpp et al. 1992, Neckles et al. 1993, Jernakoff & Nielsen 1997, Bostrom & Mattila 1999, Duffy & Hay 2000, Heck et al. 2000). The difference in epiphyte biomass accrual between non-grazed and grazed treatments observed in enclosure/exclosure experiments may be regarded as an estimate of epiphyte grazing (for a review, see Cebrian 2004). The rationale for this is as follows. Under grazed conditions, the rate of epiphyte biomass accrual corresponds to:

$$(\text{Epiphyte biomass accrual})_{\text{GRAZED}} = (\text{Epiphyte productivity})_{\text{GRAZED}} - (\text{Grazing})_{\text{GRAZED}} - (\text{Other losses})_{\text{GRAZED}} \quad (1)$$

where $(\text{Epiphyte productivity})_{\text{GRAZED}}$ denotes the rate of epiphyte productivity, $(\text{Grazing})_{\text{GRAZED}}$ is the rate of biomass loss to consumption by grazers, and $(\text{Other losses})_{\text{GRAZED}}$ is the rate of other (i.e. non-consumptive) biomass losses, such as sloughing, under the presence of grazers. Following the same logic, the rate of epiphyte biomass accrual in the absence of grazers (non-grazed conditions) corresponds to:

$$(\text{Epiphyte biomass accrual})_{\text{NON-GRAZED}} = (\text{Epiphyte productivity})_{\text{NON-GRAZED}} - (\text{Other losses})_{\text{NON-GRAZED}} \quad (2)$$

Rearranging Eqs. (1) & (2), we can derive grazing rates as follows:

$$(\text{Grazing})_{\text{GRAZED}} = (\text{Epiphyte biomass accrual})_{\text{NON-GRAZED}} - (\text{Epiphyte biomass accrual})_{\text{GRAZED}} + (\text{Epiphyte productivity})_{\text{GRAZED}} - (\text{Epiphyte productivity})_{\text{NON-GRAZED}} + (\text{Other losses})_{\text{NON-GRAZED}} - (\text{Other losses})_{\text{GRAZED}} \quad (3)$$

Thus, estimates of epiphyte grazing rates simply derived as the difference in epiphyte biomass accrual rates between non-grazed and grazed conditions may be substantially biased depending on how much the rates of epiphyte productivity and non-consumptive losses of epiphyte biomass differ between non-grazed and grazed conditions. Namely, it is clear that if grazers have a strong influence on rates of epiphyte productivity and/or non-consumptive losses (i.e. either one or both of them simultaneously) through direct or indirect mechanisms, then estimates of grazing rates that are simply calculated as the difference in epiphyte biomass accrual rates between non-grazed and grazed conditions may be largely equivocal. Here, we run a series of experiments to set quantitative limits to the potential extent of such bias.

MATERIALS AND METHODS

Experimental setup

We collected shoalgrass *Halodule wrightii*, epiphytes and grazers from a bed located at Pointe Aux Pins, ~26 km northwest from the Dauphin Island Sea

Lab (Alabama, USA). Epiphyte and grazer abundance vary seasonally at this site, offering several grazer/epiphyte combinations for comparison (Stutes 2000, Anton et al. 2009). To investigate grazer and nutrient effects on epiphyte biomass and productivity, a radio-labeling (^{14}C) technique (Wetzel 1964, Morgan & Kitting 1984, Moncreiff et al. 1992) was employed in conjunction with a mesocosm study. Three experiments were performed seasonally during 2004 (Table 1), representing a range of environmental conditions and interactions among nutrient availability, grazers, epiphytes and the host seagrass. The experiments were performed in twelve 19 l glass aquaria placed in an outdoor mesocosm facility at the Dauphin Island Sea Lab. The experiments examined grazing effects (no grazers or *in situ* grazer density), nutrient effects (ambient or increased nutrients) and their interaction, with a total of 4 treatments and 3 replicates per treatment in each experiment.

The sediment used in the aquaria was collected from the study site and defaunated (i.e. the infauna was killed by storing the sediment outdoors for 1 wk prior to starting the experiment) to prevent side effects of infaunal patchiness (i.e. bioturbation) and help control for the grazer abundances seeded in the aquaria. Upon defaunation, sediment was placed and leveled in each of the aquaria to a height ~5 cm from the bottom.

The aquaria were subsequently filled to ~5.0 cm beneath the top edge with 20 µm-filtered bay seawater that had been collected near (<100 m) the mesocosm facility. The water used to fill up the aquaria was moderately eutrophic, similar to the water at the seagrass bed from which the samples were obtained. Work carried out in 2005 (Table 2; for more details, see Cebrian et al. 2008, Anton et al. 2009, 2011, Plutchak et al. 2010) showed substantial overlap in seawater concentrations of dissolved inorganic nitrogen (DIN) and phosphate (DIP) between the seagrass bed and the site where the mesocosm water was collected, although mean values were slightly higher at the mesocosm water collection site.

The aquaria were placed outdoors in a bath with running ambient, unfiltered bay seawater that flowed around the aquaria (surrounding the aquaria completely and approximately extending up to two-thirds of the aquarium height) to achieve ambient water temperature and diel light cycles (Duffy & Hay 2000, Pilon et al. 2003). The water within the aquaria was aerated with air stones throughout the experiment to maintain adequate oxygen levels and water circulation. Typical levels of light attenuation as observed at the study site were simulated with shade cloth placed over the aquaria (Table 1). The sediment was allowed to settle for 1 d after the aquaria had been filled up with water.

Shoots of *Halodule wrightii* were collected by hand from different locations across the bed and pooled in a large container. Care was taken to preserve epiphytes and rhizomes in the samples collected. Grazers (in this case, a composite of amphipod species from the genera *Gammarus, Ampithoe, Ampelisca*

Table 1. Specific dates, light irradiance (µmol m^{-2} s^{-1} just above the water surface, mean ± SE), transmittance (% of irradiance reaching the top of the seagrass canopy, mean ± SE), mean densities of seagrass shoots, amphipods and gastropods (in number per aquarium or number per square meter in parentheses), and the ratio of grazer (sum of amphipods and gastropods) to shoot density in the 3 experiments done in 2004

	Specific dates (mm/dd)	Irradiance	Transmittance	Seagrass shoot density	Amphipod density	Gastropod density	Ratio of grazers to shoot density
Spring	5/7–5/14	2642.7 ± 30.2	42.7 ± 1.2	290 (2900)	330 (3300)	21 (210)	1.2
Summer	8/13–8/20	2378.0 ± 46.1	57.3 ± 1.9	110 (1100)	20 (200)	9 (90)	0.26
Fall	11/12–11/19	2078.3 ± 25.8	49.3 ± 0.9	53 (530)	6 (60)	2 (20)	0.15

Table 2. Environmental conditions at the seagrass bed and the bay site where the water used for the mesocosms was collected. Values were obtained from a series of unrelated experiments conducted at the same locations in 2005. See 'Experimental setup' for details. DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphate

	Temperature (°C)		Salinity (ppt)		DIN (µM)		DIP (µM)	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Seagrass bed	12.3–31.3	25.9 ± 0.9	21.3–30.3	27.3 ± 0.5	0.10–4.92	1.1 ± 0.2	0.06–0.52	0.14 ± 0.01
Bay seawater collection site	14.2–33.7	24.9 ± 0.8	7.4–29.4	16.5 ± 0.7	0.03–16.46	4.5 ± 0.2	0.05–0.92	0.26 ± 0.01

and *Corophium* and 1 gastropod species, *Neritina usnea*) were collected using air conditioner filters. The filters offer a highly complex habitat that attracts amphipods, gastropods and other small crustaceans. This method has been proven successful for collecting mesograzers in seagrass beds with relatively little effort and practically no disturbance to the seagrass bed, in contrast to coring, which would involve substantial removal of seagrass biomass in order to collect similar mesograzer quantities as collected with the air filters (Heck et al. 2000, Spitzer et al. 2003). Upon collection, the filters were rinsed and sieved through a 500 µm mesh, and the amphipods and gastropods retained on the sieve were then separated.

Shoots were collected on the day after the sediment in the aquaria settled and were planted the same day. Three samples consisting of 5 shoots per sample were randomly taken from the pooled seagrass shoots to determine the epiphyte load at the beginning of the experiment. Air conditioner filters were deployed in the field for 4 to 6 d prior to starting the experiment. Grazers were collected 1 d after planting the shoots and stocked in the aquaria that same day (<3 h elapsed between collection and stocking). Shoots and grazers were both stocked at *in situ* densities, which were determined a few days before starting the experiment by haphazardly taking five 15.5 cm inner-diameter PVC cores in the bed (Table 1).

We measured the concentration of DIN and DIP in the filtered water used to fill up the aquaria. These concentrations were measured with a Skalar SAN$^+$ autoanalyzer following standard procedures (Strickland & Parsons 1972, Pennock & Cowan 2001). Based on those measurements, we then calculated the quantity of nutrient solution to be added to each fertilized aquarium in order to increase the ambient concentrations of DIN and DIP ~3-fold. This corresponds to a moderate level of increase in relation to the levels applied in other fertilization experiments (Downing et al. 1999, Worm et al. 2000). Nutrients were added shortly after the grazers. Measurements taken 1 h after adding the nutrients revealed that immediate increases in nutrient concentration were variable (Table 3), ranging on average from 2- to 9-fold among experiments for DIN and from 2- to 5-fold for DIP. Discrepancies between targeted and realized increases in nutrient concentration often occur in fertilization experiments due to factors that are not well known and/or cannot be controlled with accuracy (Worm et al. 2000, Stutes et al. 2006, Cebrian et al. 2009). Here, it is possible that nutrient efflux from the settling sediment into the overlying water within the aquaria may have contributed to the higher level of enrichment found in some of the experiments (Sundbäck et al. 1991, Rizzo et al. 1992, Hardison et al. 2010). In spite of this, the level of increase in nutrient concentration attained with our initial additions still remains moderate in relation to other fertilization experiments (Downing et al. 1999, Worm et al. 2000).

Table 3. Nutrient concentrations (µM) measured in non-enriched and enriched aquaria 1 h after adding nutrients to the enriched aquaria (see 'Materials and methods'). Values represent mean ± SE for pooled non-grazed and grazed aquaria (n = 6). DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphate

	DIN (non-enriched)	DIN (enriched)	DIP (non-enriched)	DIP (enriched)
Spring	1.7 ± 0.5	15.7 ± 3.3	0.13 ± 0.01	0.22 ± 0.06
Summer	2.5 ± 0.7	7.2 ± 0.8	0.11 ± 0.02	0.38 ± 0.05
Fall	14.8 ± 0.8	28.4 ± 1.4	0.14 ± 0.01	0.66 ± 0.06

Prior similar experiments (Stutes 2000) showed that seagrass and attached epiphytes can absorb substantial amounts of water-column nutrients in closed aquaria (i.e. no flow through) over the course of 1 wk (which corresponds to the duration of our experiments). Thus, to prevent nutrient limitation in the control aquaria and maintain elevated nutrient concentrations in the fertilized in relation to control aquaria throughout the duration of the present study, we spiked additional nutrients midway during the experiment (Day 3) in all aquaria (both control and fertilized). These additional quantities were calculated from the temporal dynamics of nutrient concentration in non-fertilized and fertilized closed aquaria containing seagrass and epiphytes that were observed in the prior experiments mentioned above (Stutes 2000). In the present study, we did not measure nutrient concentrations shortly after spiking the additional quantities or at the end of the experiments. However, based on our previous work (Stutes 2000), our initial and mid-way nutrient addition should prevent nutrient limitation in the control aquaria and maintained elevated nutrient levels in the fertilized in relation to non-fertilized aquaria throughout the duration of the experiments, with these differences corresponding to moderate levels of enrichment in comparison with other studies. Also, these extra quantities added midway through the experiment should not enrich the control aquaria in relation

to ambient conditions but rather prevented nutrient limitation. Salinity in the aquaria was monitored daily and maintained within 0.5 ppt of initial conditions using deionized water to make up for evaporative losses.

The aquaria were incubated for 7 d. At the end of this period, 16 shoots and attached epiphytes were randomly sampled from each aquarium. Ten of the 16 shoots were frozen for determination of epiphyte biomass, and the other 6 shoots were used for the radio-labeling (^{14}C) assay. Although we did not separate and count grazers in the aquaria at the end of the experiments, careful visual inspection at that time revealed that non-grazed aquaria contained virtually no grazers and that most of the grazers initially placed in grazed aquaria were alive and active.

Response variables

We used the ^{14}C incubation method to measure the productivity of the epiphytes and the host seagrass. The method was first introduced by Steemann Nielsen (1951) for phytoplankton and has since been adapted for aquatic macrophytes and attached epiphytes (Wetzel 1964, Capone et al. 1979, Morgan & Kitting 1984, Moncreiff et al. 1992). The method relies on measuring carbon uptake by the primary producers using the radioactive marker ^{14}C. When corrected for inactive (e.g. adsorption of inorganic carbon to particles) and chemotrophic carbon uptake (e.g. marine nitrifiers), it provides a direct measurement of the inorganic carbon actively taken up by primary producers for their growth (i.e. a direct measurement of primary productivity). Such correction is best achieved using the compound dichlorophenyl dimethyl urea (DCMU), which specifically inhibits active uptake by primary producers (Legendre et al. 1983, Markager 1998). Thus, the difference in carbon uptake between DCMU-free and DCMU-spiked clear incubations corresponds to the amount of carbon actively taken by primary producers. DMCU is regarded as a better corrective choice than dark incubations because some primary producer metabolical processes that lead to active carbon uptake, such as the Wood-Werkman pathway, only occur in dark conditions (Legendre et al. 1983). Controversy regarding the use of the ^{14}C method arises when different versions of the method (e.g. using dark bottles to correct for inactive and chemotrophic carbon uptake), or when this and other methods to measure primary productivity (e.g. oxygen evolution), are combined in the same experiment, but if the same ^{14}C procedure based on DCMU blanks is used throughout the experiment, the method provides robust, well-validated measurements of primary productivity (Arthur & Rigler 1967, Peterson 1980, Kemp et al. 1986).

For the ^{14}C experiments, 3 shoots and attached epiphytes were placed in each of twelve 50 ml centrifuge tubes (1 tube per aquarium) with 45.0 ml of filtered aquarium seawater labeled with 4.0 µCi of $NaH^{14}CO_3$. Immediately after transfer, the tubes were inverted and placed back in their respective aquaria. A second set of twelve 50 ml tubes containing 3 shoots each (also 1 tube for each aquarium) were spiked with DCMU to a final concentration of 10^{-5} M. Before placement, we made sure none of these chemicals leaked out of the tubes. The tubes were incubated for 3.0 h beginning at 09:00 h on Day 8 of the experiment. All incubations in all experiments started approximately at that time. In tropical and subtropical latitudes, such as our experimental sites, incubations for primary productivity during early to mid morning may help avoid elevated photorespiration at midday under intense sunlight (Ralph et al. 1998, Ralph 1999). The incubations were terminated by adding 4% formalin solution.

After incubation, the seagrass leaves with attached epiphytes were removed and washed with ~25 ml of 2.0% HCL onto a pre-weighed 0.7 µm pore size glass-fiber filter to remove unincorporated label and catch any loosely attached epiphytes. Seagrass and epiphytes were separated using a dulled razor blade on a glass plate. Separation of epiphytes from seagrass blades through scraping works well in cases where, as here, most epiphytes are soft-bodied (i.e. mostly consisting of filamentous algae and/or microalgae, such as diatoms and cyanobacteria, embedded in a mucilaginous periphyton matrix and with little to no occurrence of calcareous encrusting algae; Dauby & Poulicek 1995, Antón et al. 2011). The epiphytes were added to the filter, and the seagrass leaves were placed in a pre-weighed drying tin. Both were dried at 80°C, re-weighed and then placed into separate 15 ml Pyrex™ digestion vials. The samples were then digested with 10.0 ml of concentrated nitric acid for a minimum of 24 h. After digestion, the vials were centrifuged, and 0.5 ml of supernatant was added to 4.5 ml of Tris buffer solution. Then, 0.5 ml of that mixture was added to 4.5 ml of scintillation cocktail (EcoLume). A Packard Tri-Carb 2500-TR scintillation counter was used to record the radioactivity of each sample as decays per minute (dpm), which was then standardized per unit of biomass dry weight (DW) for both regular samples and DCMU blanks. The DCMU

blank was then subtracted out, and the reading was converted into units of weight-specific productivity (SP) or mg C g^{-1} DW h^{-1} for each producer type (i.e. epiphyte or seagrass) using the following equation:

$$SP = (D_c \times V \times Alk \times CT \times DF) / (t \times D_a) \quad (4)$$

where D_c is the DCMU-corrected sample activity in dpm (g^{-1} DW), V is the volume incubated in the tube (0.045 l), Alk is the alkalinity of the incubated water in mg C l^{-1}, CT is the correction term to account for differential isotopic uptake (1.064), DF is the dilution factor (200), t is the incubation time (3 h), and D_a is the absolute activity in the incubation tube (8.88×10^6 dpm). Alkalinity was measured in each aquarium at the time of incubation using a Shimadzu TOC-500 fitted with a non-dispersive IR detector. To calculate epiphyte absolute productivity (mg C g^{-1} seagrass DW h^{-1}), both for regular samples and DCMU blanks, we divided the epiphyte dpm by the weight of the corresponding leaves from which the epiphytes had been collected, subtracted out the DCMU blank from the regular sample and applied Eq. (4).

To determine epiphyte biomass, the epiphyte material was removed from the leaf surface using a dulled razor blade, and epiphytes and seagrass leaves were placed into separate pre-weighed drying tins. Samples were dried at 80°C, re-weighed, and the epiphyte biomass was standardized per unit of seagrass biomass (i.e. g DW epiphyte biomass per g DW seagrass biomass). The 10 shoots from each aquarium were pooled (i.e. sum of total epiphyte biomass for the 10 shoots divided by the total weight of the 10 shoots) into 1 true replicate for statistical analysis.

Statistical analysis

For each of the response variables (final epiphyte biomass, weight-specific epiphyte and seagrass productivity and absolute epiphyte productivity), separate 2-way ANOVAs were run for each experiment (spring, summer and fall) with nutrients (control or increased concentrations) and grazing (no grazing or *in situ* grazing) as fixed factors. Data sets were tested for normality and heterocedasticity and natural log-transformed when necessary in order to meet the assumptions of ANOVA. Post-hoc comparisons were done with the Tukey's pairwise multiple comparison test. Differences were considered significant at $\alpha = 0.05$. The statistical analyses were done with Minitab 13.0 (SPSS).

RESULTS

Epiphyte biomass

We detected a significant main effect of grazing but not of nutrient enrichment in the spring and summer trials (Table 4). There was not a significant inter-

Table 4. Two-way ANOVA (G: grazing; N: nutrients) and Tukey tests (NG: non-grazed; G: grazed; NF: non-fertilized; F: fertilized)

Response variable / Experiment	2-way ANOVA		Tukey tests
Epiphyte biomass			
Spring	G	$p < 0.05$	NG > G
	N	$p = 0.59$	
	G × N	$p = 0.09$	
Summer	G	$p < 0.05$	NG > G
	N	$p = 0.44$	
	G × N	$p = 0.08$	
Fall	G	$p < 0.05$	NGNF > GNF
	N	$p < 0.05$	NGF = GF
	G × N	$p < 0.05$	NGNF = NGF
			GNF < GF
Epiphyte absolute productivity			
Spring	G	$p < 0.05$	NG > G
	N	$p = 0.91$	
	G × N	$p = 0.92$	
Summer	G	$p = 0.06$	NF < F
	N	$p < 0.05$	
	G × N	$p = 0.07$	
Fall	G	$p < 0.05$	NGNF > GNF
	N	$p < 0.05$	NGF > GF
	G × N	$p < 0.05$	NGNF < NGF
			GNF = GF
Epiphyte weight-specific productivity			
Spring	G	$p < 0.05$	NGNF < GNF
	N	$p = 0.31$	NGF = GF
	G × N	$p < 0.05$	NGNF = NGF
			GNF = GF
Summer	G	$p = 0.68$	NF < F
	N	$p < 0.05$	
	G × N	$p = 0.07$	
Fall	G	$p < 0.05$	NGNF < GNF
	N	$p = 0.5$	NGF = GF
	G × N	$p < 0.05$	NGNF = NGF
			GNF = GF
Seagrass weight-specific productivity			
Spring	G	$p = 0.91$	NGNF = GNF
	N	$p < 0.05$	NGF = GF
	G × N	$p < 0.05$	NGNF = NGF
			GNF > GF
Summer	G	$p < 0.05$	NG > G
	N	$p = 0.86$	
	G × N	$p = 0.44$	
Fall	G	$p = 0.70$	
	N	$p = 0.87$	
	G × N	$p = 0.70$	

action between grazing and nutrients in these trials. Indeed, grazer presence reduced biomass in comparison with non-grazed conditions to a similar extent, regardless of whether the aquaria had been enriched with nutrients or not (Fig. 1). However, in the fall trial, we detected a significant interaction between grazing and nutrients. Post-hoc analyses revealed that the presence of grazers reduced epiphyte biomass in relation to non-grazed conditions only under ambient nutrient conditions, but not under enriched nutrient conditions. Nutrient enrichment increased epiphyte biomass if grazers were present, but not when they were absent, in relation to ambient nutrient conditions (Fig. 1, Table 4).

Epiphyte absolute productivity

Grazer presence significantly decreased epiphyte absolute productivity in the spring trial. There was neither a significant main effect of nutrients nor a significant interaction between grazing and nutrients in this trial (Fig. 2, Table 4). In the summer trial, nutrient enrichment significantly increased epiphyte absolute productivity in relation to ambient nutrient conditions, and there was no significant main effect of grazing or interaction between the 2 factors. We found a significant interaction between grazing and nutrients in the fall trial. Post-hoc analyses showed that grazer presence reduced epiphyte absolute productivity in comparison with non-grazed conditions both under ambient and enriched nutrient conditions, but nutrient enrichment increased epiphyte absolute productivity in relation to ambient nutrient conditions only when grazers were absent, not when they were present (Fig. 2, Table 4).

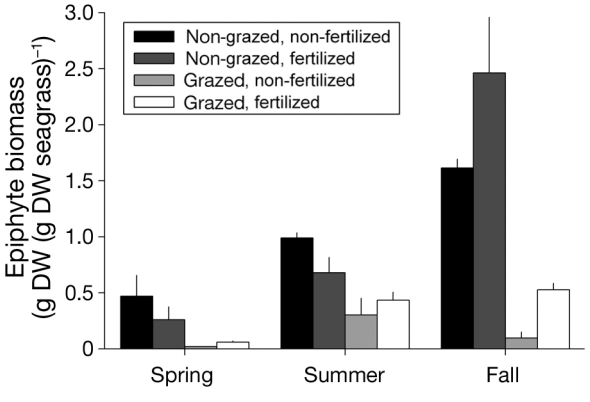

Fig. 1. Epiphyte biomass (g DW g^{-1} DW seagrass) in the spring, summer and fall experiments. Bars are means ± SE

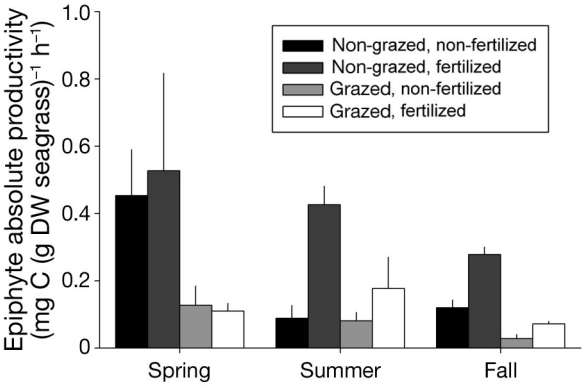

Fig. 2. Epiphyte absolute productivity (mg C g^{-1} DW seagrass h^{-1}) in the spring, summer and fall experiments. Bars are means ± SE

Epiphyte weight-specific productivity

We found a significant interaction of grazing and nutrients on epiphyte weight-specific productivity in the spring trial. Post-hoc tests indicated that grazer presence increased epiphyte weight-specific productivity in comparison with non-grazed conditions under ambient nutrient conditions but not under enriched nutrient conditions. Nutrient enrichment did not increase or decrease epiphyte weight-specific productivity in relation to ambient nutrient conditions, regardless of whether grazers were present or absent (Fig. 3, Table 4). In the summer trial, nutrient enrichment significantly increased epiphyte weight-specific productivity in relation to ambient nutrient conditions, but there was no significant main effect of grazing or interaction between the 2 factors. The results for the fall trial were analogous to the results for the spring trial (Fig. 3, Table 4).

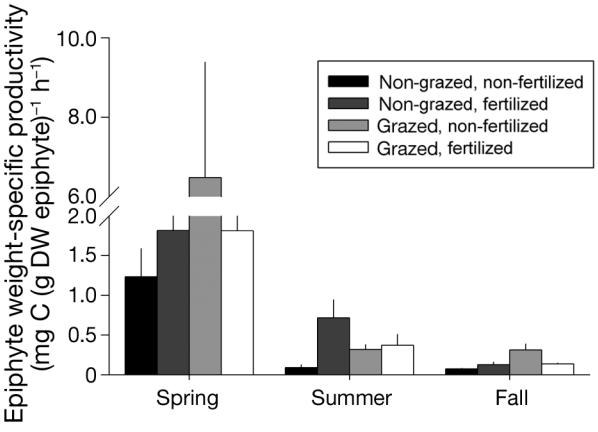

Fig. 3. Epiphyte weight-specific productivity (mg C g^{-1} DW seagrass h^{-1}) in the spring, summer and fall experiments. Bars are means ± SE

Seagrass weight-specific productivity

In the spring trial, we found a significant interaction between grazing and nutrients on seagrass weight-specific productivity. Nutrient enrichment decreased seagrass-specific productivity in relation to ambient nutrient conditions when grazers were present, but not when they were absent. Grazing did not increase or decrease seagrass weight-specific productivity in relation to non-grazed conditions, regardless of whether the aquaria had been enriched with nutrients or not (Fig. 4, Table 4). In the summer trial, grazing significantly decreased seagrass weight-specific productivity in relation to non-grazed conditions. There was no significant main effect of nutrient enrichment or significant interaction between the 2 factors in this trial. We found no significant main grazing and nutrient effects or interactions between the 2 factors in the fall trial (Fig. 4, Table 4).

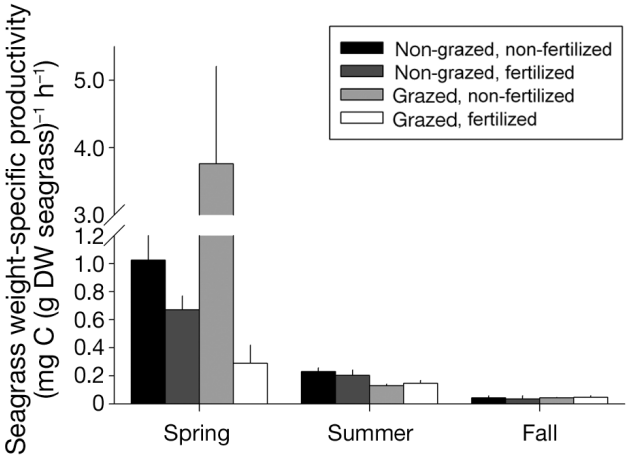

Fig. 4. Seagrass weight-specific productivity (mg C g^{-1} DW seagrass h^{-1}) in the spring, summer and fall experiments. Bars are means ± SE

Derivation of epiphyte grazing rates

Eq. (3) illustrates how epiphyte grazing rates can be derived from grazer-exclosure experiments, such as the ones performed here. However, rates of absolute epiphyte productivity and non-consumptive losses of epiphyte biomass (e.g. sloughing) are rarely measured along with changes in epiphyte biomass in grazer-exclosure experiments. Thus, epiphyte grazing rates in most grazer-exclosure experiments can only be estimated from the difference in the rates of epiphyte biomass accrual between non-grazed and grazed conditions. Here, we have not made any attempt to measure rates of non-consumptive losses of epiphyte biomass, but our measurements allow us to set qualitative bounds to the extent of the potential bias in estimates of epiphyte grazing rates derived solely as the difference in the rates of epiphyte biomass accrual between non-grazed and grazed conditions. The results are summarized in Table 5. Namely, estimates of epiphyte grazing rates that include the effect of grazers on the rates of epiphyte absolute productivity found in our experiments could be ~2 to 30% lower than estimates derived solely as the difference in epiphyte biomass accrual rates between non-grazed and grazed conditions, assuming that the rates of non-consumptive losses under non-grazed and grazed conditions cancel each other out. These differences would be larger if rates of non-consumptive losses are higher in grazed than in non-grazed treatments.

Table 5. Estimates of epiphyte grazing rates derived without and with consideration of effects on productivity and non-consumptive losses. All estimates are expressed as g DW epiphytes g^{-1} DW seagrass d^{-1}. Estimates without consideration of effects on productivity and non-consumptive losses were simply derived as the difference in epiphyte biomass accrual between non-grazed and grazed treatments divided by the duration of the experiment (7 d), i.e. [(final epiphyte biomass – initial epiphyte biomass)non-grazed – (final epiphyte biomass – initial epiphyte biomass)grazed] / 7. Estimates which take those effects into consideration were derived using Eq. (3). Rates of absolute epiphyte productivity for non-grazed and grazed conditions (Epiphyte productivity$_{NON\text{-}GRAZED}$ and Epiphyte productivity$_{GRAZED}$) were derived from the measurements done on the last day of the experiments and expressed as g DW epiphytes g^{-1} DW seagrass d^{-1} using C to DW conversion factors obtained from the literature (Cebrian 2004) and the length of daylight (number of light hours per day) on the incubation day. For sim-plification, values for non-fertilized and fertilized aquaria were pooled

	Without consideration of effects on productivity and non-consumptive losses	With consideration of effects on productivity and non-consumptive losses
Spring	0.043	0.043 – 0.013 + (Other losses)$_{NON\text{-}GRAZED}$ – (Other losses)$_{GRAZED}$
Summer	0.086	0.086 – 0.007 + (Other losses)$_{NON\text{-}GRAZED}$ – (Other losses)$_{GRAZED}$
Fall	0.243	0.243 – 0.004 + (Other losses)$_{NON\text{-}GRAZED}$ – (Other losses)$_{GRAZED}$

DISCUSSION

Our results suggest that the effects of grazing and nutrient enrichment on diverse metrics of epiphyte growth (i.e. biomass, absolute productivity or productivity per leaf weight unit of the seagrass host, and weight-specific productivity or productivity per epiphyte weight unit) can be disparate. Grazing had a preponderant effect on biomass in comparison with the effect of nutrient enrichment. In 2 of the 3 trials, grazer presence caused large reductions in epiphyte biomass, and nutrient enrichment did not have any significant effects. In the third trial, we found an interaction between the 2 factors, but the reduction in epiphyte biomass due to grazing under ambient nutrient conditions was larger in magnitude (as indicated by the respective *F*-values) than the increase in epiphyte biomass that occurred following nutrient enrichment when grazers were present. Interestingly, we did not find a clear predominance of grazing effects over nutrient effects on the other 2 metrics of epiphyte growth examined, i.e. absolute epiphyte productivity and weight-specific epiphyte productivity. Regarding absolute epiphyte productivity, one trial showed significant decreases due to grazing but no effect of nutrients, another trial showed significant increases due to nutrients but no effect of grazing, and the third trial showed a complex interaction with significant reductions due to grazing, regardless of nutrient condition, and a significant increase due to nutrients only when grazers were absent. For weight-specific productivity, 2 of the trials showed significant increases due to grazing under ambient but not under enriched nutrient conditions, and no increases or decreases due to nutrient enrichment with grazers present or absent, whereas the other trial showed significant increases due to nutrients but no effect of grazing.

In concert, these results illustrate that the effects of grazing and nutrients on a given measure of epiphyte growth do not necessarily cascade equivalently onto other growth measures. For instance, nutrient enrichment had little impact overall on epiphyte biomass. Out of the 3 trials, we only found 1 instance of significant effects of nutrient enrichment on epiphyte biomass, whereby nutrient enrichment increased biomass when grazers were present in the fall trial. Interestingly, nutrient enrichment never increased epiphyte biomass when grazers were absent, which contrasts with results from previous experiments (Neckles et al. 1993, 1994, Williams & Ruckelshaus 1993, Peterson et al. 2007). The lack of a major consistent impact of nutrient enrichment on epiphyte biomass in our trials could be due to the background eutrophic conditions in our mesocosms and in the seagrass beds where the samples were collected (Cebrian et al. 2008, Anton et al. 2009, Plutchak et al. 2010). Consistent with these naturally eutrophic levels, the levels of epiphyte biomass in the samples incubated in the mesocosms were high in comparison with values reported in the literature (Borum 1987, Neckles et al. 1993, Cebrian et al. 1999, Hauxwell et al. 2001, 2003, Stutes et al. 2007, Jaschinski & Sommer 2010), even when those levels were reduced under the presence of grazers. High levels of epiphyte biomass may imply thick and dense canopies, which through intense self-shading and limited nutrient and gas diffusion may dampen a positive response of epiphyte biomass to nutrient enrichment (Sand-Jensen & Borum 1984, 1991, Van Montfrans et al. 1984, Duarte 1995). The only instance of positive biomass response to nutrient enrichment found under the presence of grazers suggests that grazers may somewhat alleviate the restraining effects of thick epiphyte canopies and allow for significant increases of epiphyte biomass. These results are qualitatively similar to previous experiments that have shown little effect of nutrient enrichment on periphyton biomass under eutrophic conditions (Hillebrand & Kahlert 2002, Cebrian et al. 2009, 2012).

However, in clear opposition to the lack of major, consistent effects of nutrient enrichment on epiphyte biomass, nutrient enrichment did significantly increase epiphyte productivity per gram of seagrass leaf (epiphyte absolute productivity) in the summer trial and in the fall trial when grazers were not present, but this did not translate into parallel changes in biomass. This indicates that the increased carbon uptake that occurred under nutrient enrichment during those trials did not result in significant increases in the production of new epiphytic biomass (i.e. larger biomass accrual). Perhaps the increased amount of carbon taken up by photosynthesis under nutrient-enriched conditions during those trials, rather than resulting in the formation of new biomass, was mostly used for the metabolical support of bottom parts of thick epiphyte canopies subject to intense self-shading and limited diffusion (Agustí et al. 1994, Duarte 1995, Enríquez et al. 1996, Enríquez 2005). The effects of grazing and nutrients on epiphyte productivity per gram of epiphyte (epiphyte weight-specific productivity) appeared to be a combination of propagated effects on biomass and absolute productivity. In the spring and fall trials, the lowest values of biomass due to grazing under non-enriched

conditions appeared to drive the significantly higher values of epiphyte weight-specific productivity found in those trials, and in the summer trial, the increase in absolute productivity observed with nutrient enrichment seemed to drive a parallel increase in epiphyte weight-specific productivity.

Therefore, caution should be exerted when inferring implications on epiphyte growth dynamics and cascade effects on the system from studies that only consider one of the growth metrics. For instance, here we find a preponderant effect of grazing on epiphyte biomass, which is in agreement with the large control of epiphyte biomass by grazers found in other studies (Neckles et al. 1993, Heck et al. 2000, 2006, Hughes et al. 2004, Gruner et al. 2008). However, these results do not necessarily provide much information about the effects of grazing and other environmental controls (e.g. nutrients) on other metrics of epiphyte growth (e.g. epiphyte productivity on a per leaf or per epiphyte weight basis), and as such, the implications and inferences of biomass-based results on the dynamics of epiphyte populations, and feedbacks on elemental cycling and trophic transfer in the system, are limited if other metrics of epiphyte growth are not considered. Epiphyte biomass is indicative of the quantity of carbon stored by epiphytes. Epiphyte absolute productivity (productivity on a per leaf weight basis) is indicative of the amount of carbon taken up by epiphytes through photosynthesis per unit of host biomass, and epiphyte weight-specific productivity (productivity on a per epiphyte weight basis) reflects carbon turnover through the epiphyte pool. On this basis, we show that in our experiments, grazing is a predominant factor over nutrient enrichment in the control of carbon storage as epiphyte biomass, but grazing and nutrient enrichment are more similar in terms of their overall importance in the control of photosynthetic carbon uptake by epiphytes and rates of carbon cycling through the epiphyte pool.

In the present study, we did not find cascading positive effects on seagrass weight-specific productivity from the large reductions in epiphyte biomass caused by grazing, unlike the results of other studies (Orth & Van Montfrans 1984, Van Montfrans et al. 1984, Jernakoff et al. 1996). Perhaps the reduction of epiphyte biomass under grazed conditions observed here was in general not large enough to elicit significant increases in seagrass weight-specific productivity. This possibility is supported by the observation that the lowest mean value of epiphyte biomass, recorded for the grazed, ambient nutrient treatment in the spring trial, coincided with the highest mean value of seagrass weight-specific productivity, which was several-fold higher than any other mean value of seagrass weight-specific productivity in our study. However, other treatments with low values of epiphyte biomass, albeit not as low as the grazed, ambient nutrient treatment in the spring trial, did not result in comparatively enhanced values of seagrass weight-specific productivity.

Our results also set qualitative bounds to the potential bias committed when epiphyte grazing rates are estimated simply as the difference in the rates of epiphyte biomass accrual between non-grazed and grazed treatments. We did not measure non-consumptive losses, but our calculations suggest that when the reduction in epiphyte absolute productivity by grazers found in our experiments is accounted for, estimates of epiphyte grazing rates may be 2 to 30% lower than estimates solely derived as the difference in the rates of epiphyte biomass accrual between non-grazed and grazed treatments. The extent of these differences would be larger if rates of non-consumptive losses are enhanced in the presence of grazers. However, our calculations are only approximations since they rely on a number of untested assumptions, i.e. (1) that rates of non-consumptive losses under non-grazed and grazed conditions cancel each other out, which seems to be a rather unrealistic assumption, (2) that daily rates of epiphyte biomass accrual are constant throughout the experiment, and (3) that the rates of epiphyte absolute productivity measured during the incubation period (from 09:00 to 12:00 h) are applicable to all daylight hours on the incubation day. At any rate, and despite the uncertainty of these untested assumptions, our calculations suggest that caution should be exerted when using epiphyte grazing estimates solely based on the difference in the rates of epiphyte biomass accrual between non-grazed and grazed conditions since such estimates may be considerably biased.

In conclusion, we show here that the effects of grazing and nutrient enrichment on diverse metrics of epiphyte growth can be disparate. Under the ambient eutrophic conditions of our experiments, further nutrient enrichment affected epiphyte biomass little in comparison with the effects of grazing. We found a significant increase of epiphyte biomass following nutrient enrichment only in 1 trial and under the presence of grazers, but decreases of epiphyte biomass due to grazing were more common throughout our experiments. In contrast, grazing and nutrients appeared to have an overall similar importance as controls of epiphyte productivity both expressed on a leaf or epiphyte weight unit basis. Thus, these

results illustrate that studies aimed at examining the effects of environmental and biological controls on epiphyte growth dynamics and functional implications for the system should consider several epiphyte growth metrics since the effects on one given metric may be quite different from those on other metrics. Perhaps studies of grazing and nutrient effects on epiphyte dynamics should shift from the biomass-centric view mostly applied to date to a broader approach in which other metrics of epiphyte growth, such as productivity, are also considered. Indeed, such biomass-centric views, also have other risks since we show here that grazing rate estimates solely derived as the difference in biomass accrual rates between non-grazed and grazed rates can be considerably biased.

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