

Differential effects of grazer species on periphyton of a temperate rocky shore

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ABSTRACT: Species-specific traits are relevant to understand variation in the effect of grazers on primary producers and are critical in determining their functional roles as controllers of productivity in marine ecosystems. In intertidal habitats, periphyton, composed of bacteria, cyanophytes, microalgae and early stages of macroalgae, constitutes the main food for many benthic grazers. Using field experiments, we examined the overall effect of grazers and the importance of direct and indirect effects of different grazer species on periphyton composition, richness, diversity and productivity. We manipulated the main grazer species present along Chilean rocky shores, enclosing adult individuals of the keyhole limpet *Fissurella crassa*, the chiton *Chiton granosus*, the scurrinid limpet *Scurria araucana* and the pulmonate limpet *Siphonaria lessoni*. These organisms overlap broadly in diet but exhibit differences in foraging behavior. Differences in the individual effects of grazer species on periphyton productivity, as well as on richness and diversity characterized the assemblage. *C. granosus* grazing led to lower periphyton productivity, richness and diversity compared with all other grazers, who had positive effects for all variables. Richness and diversity of the major periphyton groups were maximal at intermediate levels of total grazing pressure, highlighting the importance of ecological interactions within the periphyton assemblage. Our results demonstrate the complexity of functional roles and relationships of grazers involved in the control of composition and production of the periphyton assemblage. Given the importance of direct and indirect effects of grazers, understanding species interactions within the microbenthic community can shed light on the mechanisms through which herbivores modify community structure and productivity.

KEY WORDS: Grazer assemblage · Periphyton · Productivity · Diversity · Foraging behavior

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INTRODUCTION

The role of grazers in controlling primary production has been a major focus of experimental studies in coastal marine ecosystems around the world (see Lubchenco & Gaines 1981, Hawkins & Hartnoll 1983, Poore et al. 2012 for reviews). The mechanisms underlying observed variation in the effects on algal resources among different grazer species can be quite complex and variable from one place to another. Indeed, many individual- and population-

based traits of grazers and algae, as well as environmental conditions modify both the impact and the functional structure of the grazer assemblage (Schmitz 2008, Aguilera & Navarrete 2012a). A recent review of >600 experimental exclusions of grazers showed that morphological and taxonomical characteristics of producers can largely determine the magnitude of herbivores' effects in marine coastal habitats (Poore et al. 2012). Furthermore, experimental studies manipulating grazer species composition have generally shown that morphologi-

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cal and behavioral attributes of the grazers, including body size, in combination with general attributes of the macroalgal assemblage, modulate the role of different grazer species and the functional structure within the herbivore assemblage (Hawkins et al. 1992, Branch & Moreno 1994, Aguilera & Navarrete 2012a). Thus, the relative influence and functional roles of grazer species must be examined in the context of particular algal assemblages and successional stages (Aguilera & Navarrete 2012a).

Periphyton, or 'biofilm', is made up of benthic microalgae (diatoms, cyanophytes), sporelings and spores of macroalgae (Anderson 1995), and grazing frequently has negative effects on biomass and productivity (Liess & Hillebrand 2004). The role played by different grazing species and their specific attributes on periphyton composition and species richness is generally less clear (Liess & Hillebrand 2004).

Periphyton lies at the base of benthic aquatic food webs and appears to be critical for the establishment of benthic macroalgae (Park et al. 2011) and sessile invertebrate larvae (Harder et al. 2002, Dahms et al. 2004). In many cases, periphyton constitutes the main food source for a wide variety of benthic macrograzers, which usually are orders of magnitude larger than their algal prey (Liess & Hillebrand 2004). Several studies have evaluated the role of grazers and environmental factors (e.g. nutrient availability) on periphyton productivity (Underwood 1984, Dye & White 1991, Bustamante et al. 1995, Jenkins et al. 2001, Hillebrand 2008, Christofolletti et al. 2011), spatial distribution (Castenholz 1961, Nagarkar & Williams 1997, Williams et al. 2000, Hutchinson et al. 2006, Johnson et al. 2008, and see Hillebrand 2009 for review) and composition (Sommer 1999, Hillebrand et al. 2000, Kaehler & Froneman 2002, and see Liess & Hillebrand 2004 for review). In general, effects of grazers on periphyton and the microbenthic community have been observed to be highly variable, apparently due to differences in the identity of the grazers, grazing intensity, grazer mobility, periphyton composition and light and nutrient conditions (Sommer 1999, Hillebrand & Sommer 2000, Liess et al. 2009). Apparently, a few grazer species are able to graze selectively on different components of the periphyton (e.g. diatoms), and to evade less palatable or noxious taxa such as cyanophytes (Nicotri 1977, Hill & Hawkins 1991, Rosemond 1993). Although selectivity does not appear to be a general attribute of benthic grazers feeding on periphyton (Liess & Hillebrand 2004), it could explain the generally positive (indirect) effects of molluscan grazers on cyanophytes (e.g. *Nostocales*) on both tropical and tem-

perate coasts (Poore et al. 2012). This positive grazing effect can be understood as an indirect consequence of selective grazing on diatoms leading to competitive release by cyanophytes, much like keystone predation (Menge et al. 1994, Liess & Hillebrand 2004). Indeed, allelopathic interactions between blue-green algae and diatoms have been documented (Keating 1978, Fong et al. 1993). In this scenario and considering differential abilities of periphyton species to colonize and outcompete other species, a unimodal pattern of species richness and diversity can be expected as grazing pressure changes from weak (competitive species dominate the system as they escape grazing) to moderate (grazers keep abundance of dominant species under control) to the intense (grazers eliminate all but the fast colonizing or herbivore resistant species). This response has been observed in macroalgal (e.g. Lubchenco 1978) and plant assemblages (Olf & Ritchie 1998), but empirical evidence in some periphyton assemblages is equivocal (Liess & Hillebrand 2004). In addition to indirect effects propagated through the web of species interactions within the periphyton assemblage, grazers can have direct positive effects on some periphyton species. For instance, mucus trail and feces laid by different molluscan grazers can differentially trap diatom species and spores of macroalgae, enhancing growth and changing species dominance in the periphyton community (e.g. Santelices & Bobadilla 1996, Davies & Beckwith 1999). In summary, some grazer species can affect periphyton biomass, productivity and composition because of differential grazing on some species over others, or because they can stimulate settlement and growth of periphyton species, while other grazers have virtually no measurable effects (Connor 1986, and see Davies & Hawkins 1998 for review). Differences among grazer effects are attributable to species-specific traits, such as morphology of feeding apparatus, behavior, size and individual spatial distribution (Connor 1986). For instance, on Brazilian subtropical shores, fast-moving grazers (e.g. isopods) have been shown to drastically reduce periphyton biomass, while slow moving limpets and littorinid snails have marginal, if any, effects (Christofolletti et al. 2011). Similarly, differences in movement or foraging rates, closely associated to body size and feeding modes of individuals, can account for variation of per capita effects on periphyton richness. For example, Sommer (1999) found that grazers with foraging modes that enhance spatial heterogeneity of periphyton density can significantly increase periphyton richness.

Differences in foraging patterns, morphological attributes and body size lead to marked differences in the impact and functional roles of different grazer species on macroalgal assemblages (Aguilera & Navarrete 2011, 2012a, Poore et al. 2012). Recent experimental manipulations on the rocky shores of central Chile have shown that species-specific roles and the functional relationships within the grazer assemblage change depending on the stage of the macroalgal succession, shifting from redundancy in grazing effects during early succession, to a more compensatory and even a keystone functional structure in later successional stages (Aguilera & Navarrete 2012a). Despite the key roles played by the periphyton during early succession and the considerable information accumulated about grazer diets and effects on intertidal macroalgae (see Santelices 1990, Branch & Moreno 1994, Fernández et al. 2000, Aguilera 2011 for reviews), no studies in this system have evaluated grazer identity effects on microbenthic periphyton composition and primary productivity. Here, we take advantage of an experiment used to investigate the role of individual grazer species on macroalgae through succession (Aguilera & Navarrete 2012a) to examine the differential contribution of grazers on periphyton composition during the first few days after rock clearing and before macroalgal colonization.

Along Chilean rocky shores, periphyton constitute a 'transient' but frequent stage of the community, usually triggered by consumers (i.e. carnivores and herbivores) and to a lesser extent wave disturbance, which regularly remove both macroalgae and sessile invertebrates and generate bare rock patches (Navarrete & Castilla 2003). These bare rock patches are quickly (days) colonized by periphyton, but in a few weeks they are colonized by macroalgae and sessile invertebrates, which eventually overgrow the periphyton community (Santelices 1990, Fernández et al. 2000, Nielsen & Navarrete 2004). Thus, the periphyton community along the shores of central Chile might never reach late stages, as observed for instance along shores of subtropical Brazil (Christofoletti et al. 2011). Previous studies suggest that although periphyton may be 'transient' on these shores, it can be a key determinant of successional trajectories of the intertidal community, as well as in providing an important food source to a broad diversity of intertidal herbivores (Aguilera & Navarrete 2007, 2012a). In fact, all molluscan herbivores along the Chilean rocky shore could be considered 'periphyton-grazers' because they mostly consume diatoms, sporelings of macroalgae and small invertebrates (Santelices et al. 1986, Camus et al. 2008).

Since previous studies have shown that all grazer species considered here can have, in general, negative effects on early stages of macroalgal succession, which are dominated by ulvoids (Aguilera & Navarrete 2012a), we hypothesized that (1) all grazer species can negatively affect overall periphyton biomass. Moreover, since competitive interactions are expected among species and between major groups of periphyton, we hypothesize that (2) periphyton species richness and diversity will show a unimodal response to overall grazing pressure, as quantified through the abundance of bare rock or (inversely) the abundance of ulvoids in experimental plots. Conversely, differences in palatability or herbivore-resistance of the different periphyton groups could allow the expression of species-specific grazer traits and lead to differential effects among grazer species, as shown in later stages of macroalgal succession by Aguilera & Navarrete (2012a). Finally, we examine to what extent grazers' diet is associated with the net effect or impact of herbivores on periphyton composition. We hypothesize that (3) grazer effects result mostly from direct consumption of periphyton species, thus grazers' diet is associated with effects on periphyton abundance. Alternatively, if direct consumptive effects propagate in the periphyton through indirect interactions, no relationship between diet and impact should be found.

MATERIALS AND METHODS

Study site and focal assemblage

The study was conducted on the mid intertidal zone of a wave exposed rocky platform at Pelancura (33° 33' S, 71° 37' W) in central Chile. The benthic herbivore assemblage of the study site is characterized by ~8 species of molluscs (Aguilera & Navarrete 2011). The most common molluscan species in the mid shore include the fissurellid limpet *Fissurella crassa*, the chiton *Chiton granosus*, scurrinid limpets (mostly *Scurria araucana*, *S. ceciliana* and *S. zebrina* (Espoz et al. 2004) and a pulmonate limpet *Siphonaria lessoni*. Littorinid snails and an opisthobranch grazer (*Onchidiella* sp.) are common in the upper shore, while other fissurellid and scurrinid limpets (e.g. *F. limbata*, *F. costata*, *S. variabilis*, *S. plana*), chitons (e.g. *Acanthopleura echinata*), snails (*Tegula atra*), fish (*Scartichthys viridis*, *Syciases sanguineus*) and sea urchins (*Tetrapygus niger*, *Loxechinus albus*) can be found in the lower shore, but mid shore feeding is rarely observed. Of the mid shore molluscs,

only *F. crassa* is collected by humans and, therefore, their abundances are low in open-access areas compared to marine reserves (Oliva & Castilla 1986, Aguilera & Navarrete 2011). In our study, we evaluated the collective (total) effects of grazers as well as the individual effects of selected species, *C. granosus*, *S. araucana*, *F. crassa* and *Siphonaria lessoni*, on mid shore periphyton community composition and productivity.

Herbivore enclosure–exclusion experiments

To determine the collective and individual effects of grazers on periphyton composition (see below), we set up a replicated enclosure–exclusion experiments, selecting 28 plots 25 × 25 cm in size that included a crevice (3 cm wide and deep) where animals could find shelter. All plots were reset to the initial successional stage of bare rock by scraping the rock surface clean with drill-mounted brushes and manual chisels, thus removing all organisms including encrusting algal fragments. The following treatments were randomly assigned to experimental plots: enclosure of (1) 6 ind. of *Chiton granosus*, (2) 12 ind. of *Siphonaria lessoni*, (3) 1 ind. of *Fissurella crassa* and (4) 2 ind. of *Scurria araucana*. The number of individuals used in experimental enclosures closely matched the natural densities and patterns of aggregation or territoriality of the species in the field (Santelices et al. 1986, Aguilera & Navarrete 2011). We also set up a (5) total exclusion of benthic grazers (E_C) and (6) a control 'free access' plot to which all herbivores had access. In this design, we did not consider a 'procedural control', i.e. partial fencing, since a preliminary study showed partial fences are easily dislodged by waves, thus removing all organisms present inside plots (see Johnson 1992 for discussion). All treatments were replicated 4 times. Enclosures were made of stainless steel wire fences (8 cm high, 7 mm mesh opening) fastened to the rock with stainless steel screws. Fences have been shown to be effective in keeping animals enclosed and produce little disturbance to animal movement compared with natural conditions (Aguilera & Navarrete 2012a). The experiment started on March 27, 2008 and was terminated during August 2008 (austral fall to spring). Evaluation of grazer effects on periphyton community composition was restricted to the first weeks after the start of the experiment (sampling dates: April 7 and April 22, 2008) to avoid the confounding effects that settlement of macroalgae or sessile invertebrates may introduce on biofilm community

dynamics. Results for macroalgae are reported elsewhere (Aguilera & Navarrete 2012a).

Periphyton abundance and productivity

We quantified grazer effects on periphyton biomass and productivity (total biomass produced per day) using standardized units. We used 6.0 × 4.0 × 0.2 cm thick acrylic plates affixed to the rock with a flat-head screw flush to the plate surface and placed in all experimental plots. The top surface of plates were roughened with sand paper and, to facilitate herbivore access the sides of these plates, were filed to a 45° angle, leaving a vertical edge of 0.1 cm. Artificial substrates have been effectively used before to quantify epilithic biomass and area-specific productivity along central Chile's rocky shores (Aguilera & Navarrete 2007) and other rocky shore systems (e.g. Bustamante et al. 1995). Every 25 d between May and July 2008, we removed the plates, rinsed them with 1 ml filtered water, wrapped them in aluminum foil and deployed new ones. Plates were taken to the laboratory to be analyzed immediately, or frozen at –20°C for maximal 5 d before analysis. Chlorophyll *a* (chl *a*) extraction followed Thompson et al. (1999). Briefly, plates were placed in complete darkness with 50 ml of 100 % methanol for 10 to 15 h. A 1 ml sample was then taken and diluted in 5 ml of 100 % methanol before recording total fluorescence in a calibrated fluorometer (Turner Design AU-10) at 665 to 750 nm (Thompson et al. 1999). Data were transformed to μg of chl *a* cm^2 (HMSO 1986). Dividing this estimate of biomass per unit area by the number of days plates were exposed in the field yields a rate we use as a proxy of primary productivity specific to the periphyton, which considers both *in situ* production (actual algal growth) and recruitment of microalgae and spores onto the plates.

Herbivores' diet

To characterize the diet of each herbivore species, and to contrast the relative abundance of food items in the diet with local effects on periphyton observed within plots, we examined the stomach contents of individuals collected monthly in the field from March to August 2008. Rarefaction curves (Gotelli & Colwell 2001) indicated that a sample of 8 ind. of *Chiton granosus*, 7 of *Scurria araucana*, 6 *Fissurella crassa* and 12 ind. of *Siphonaria lessoni* was sufficient to characterize the diet of these species (Aguilera & Navarrete

2012a). Individuals were collected manually from the mid and high intertidal zone mainly during night and exclusively during daytime for *S. lessoni*, matching the foraging period of each grazer (Aguilera & Navarrete 2011). Specimens collected were immediately injected with a 10% formaldehyde solution to stop digestion, labeled and taken to the laboratory for analysis. The gut content of each individual was examined under a stereomicroscope. To estimate the relative abundance of each periphyton component, i.e. proportion of each taxa in the sample, we placed 1 ml of the sample on a glass slide reticulated with 50 points, recording the number of points intersected by each taxa in a phase contrast microscope. In this case, >1 taxa could intersect a given point; therefore, total abundance could be higher than 1.

Periphyton composition, richness and diversity

To characterize the composition of the periphyton, we sampled experimental plots during the first 2 wk of the experiment using the *in situ* sampling method proposed by MacLulich (1987). On each sampling date, 10 and 22 d after clearing the rock (April 6 and April 22), in 3 randomly chosen 5 × 5 cm areas inside each experimental plot, we collected all periphyton present on the rock substrate with a sterilized toothbrush that was then immersed in 5 ml filtered seawater inside a dark tube. The procedure was repeated 5 times for each area until the rock surface became 'whitish' (MacLulich 1987). No 5 × 5 cm area was sampled twice during the study. Immediately after collection, 1 ml of 37% formaldehyde was added to the tubes to preserve microalgal cellular structure, labeled and then frozen at -20°C before analysis. Samples were defrosted, centrifuged for 1 min and a 1 ml aliquot was placed on a microscope slide reticulated with 50 observational points (1 μm diameter). The number of intersected points by each taxon was then recorded under a light microscope with phase contrast illumination. With this procedure, we were able to identify to the level of genera all diatoms, cyanophytes and sporelings of macroalgae. Some microalgae were identified to species level, but we decided to use genera-level for analyses since small diatoms were difficult to assign to particular species.

Data analyses

We compared primary productivity (biomass cm⁻² d⁻¹) estimated from the acrylic plates installed inside

experimental treatments through separate one-way ANOVA for each sampling date, after log-transformation to meet variance homogeneity assumptions. In this case, we assumed that the new plates placed on each date were independent over time. In the case of significance of main effects, we used a *posteriori* Student-Newman-Keuls (SNK) test to determine which treatment differed from others. Moreover, for those dates in which treatment effects were significant, we calculated both per capita and per biomass (in g) effects of grazer species on periphyton productivity (see Supplement 1 available at www.int-res.com/articles/suppl/m484p063_supp.pdf). To this end, we calculated the effect size for each grazer species by subtracting periphyton abundance or productivity observed within enclosures from that observed in grazer exclusions and divided this effect size either by the number of individuals maintained in the plots (per capita effects or interaction strength sensu Navarrete & Menge 1996) or the total biomass of grazers in the plots (per biomass effects). In this manner, we could differentiate between the 'average' effects of grazers under the 'natural' densities and total biomass used in the experiments, from the expected effects of a single 'individual' of the species. Since individuals of the different grazer species differ widely in average body size (Aguilera & Navarrete 2011), expressing effects on a per gram basis allowed us to evaluate differences among grazer effects beyond those attributed to body size.

The abundance of different taxa of diatoms, cyanophytes, sporelings and spores of macroalgae recorded during the 2 sampling dates were used to calculate taxa richness (*S*, i.e. the number of taxa), Shannon's diversity (*H'*, Shannon & Wiener 1949) and evenness (*J'*) indices for each replicate sample. We used one-way ANOVA to test for differences in taxa richness, diversity and evenness among treatments. SNK *a posteriori* test was used to determine the pattern of treatment differences after significance in ANOVA. As before, we calculated per capita and per biomass effect of grazers on periphyton richness and diversity (see Supplement 1).

Differences in periphyton composition among grazer treatments were tested with a one-way permutational multivariate analysis of variance (PERMANOVA, Anderson 2001). We identified taxa with the largest contributions to differences among periphyton communities in different treatments through a similitude percentage analysis (SIMPER, Clarke 1993), using a Bray-Curtis dissimilarity matrix constructed with square-root transformed data on relative abundance of periphyton for each replicate plot.

For this analysis, we used per-plot averages across the 2 sampling dates. To determine the pattern of treatment differences, pair-wise *a posteriori* PERMANOVA comparisons between treatments were conducted after a significant main effect was observed ($\alpha = 0.05$), using Bonferroni correction of significance levels for multiple comparisons ($\alpha = 0.05/\text{number of comparisons}$). Additionally, non-metric multidimensional scaling (NMDS) was used to produce 2-dimensional ordinations (Clarke 1993) to compare periphyton assemblages among different treatments.

The relationship between average relative abundance of periphyton components (frequencies) inside experimental plots and that found in the gut contents of each herbivore species (pooled data) was examined with a Kolmogorov-Smirnov (K-S) likelihood 2-sample test.

Response to grazing pressure: indirect effects of grazing

To evaluate the effects of the overall intensity of grazing on periphyton taxa richness and diversity, i.e. whether the propagation of indirect effects on periphyton richness and diversity reached maximal levels at moderate, intermediate or maximal levels of grazing pressure exerted by each focal grazer, we correlated these periphyton variables against 2 semi-independent measures of grazing pressure. Previous studies show that the generation and maintenance of bare rock and the ability of grazers to reduce cover of ulvoid sporelings, readily consumed by all grazer species used in the study, are good indications of overall grazing pressure inside experimental plots (Aguilera & Navarrete 2012a). Thus, we fitted linear and quadratic polynomial regressions using ordinary least squares, considering overall microalgal taxa richness (S) and Shannon diversity (H') as the dependent variables, and bare rock and ulvoid sporelings cover as the predictors.

RESULTS

Periphyton abundance and productivity

One week after clearing the rock surface, periphyton (a yellow-brownish biofilm) represented the only sessile component of the intertidal community observed inside the experimental plots. No macroalgae were observed during this period. Periphyton biofilm cover peaked during the first week of April, 12 d after

rock clearance and then declined to near zero after 30 d in all treatments.

Periphyton productivity (chl *a* concentration per day in acrylic plates) showed a variable pattern for the different treatments and sampling dates (Fig. 1a). Significant treatment differences were observed only in May, around the time of peak periphyton cover in the plots. At this time productivity in control plots, in the presence of all grazers in the system, was significantly higher than in the grazer exclusion treatment (Table 1). This overall positive effect of grazers was in agreement with the patterns inside enclosures of *Scurria araucana*, *Fissurella crassa*, *Siphonaria lessoni* where periphyton productivity was not different from control plots, while they were significantly higher than grazer exclusions and enclosures of *Chiton granosus* (Table 1). Differences in the average effect of grazers observed when at 'natural' densities were reinforced with results expressed as per capita interaction strengths. All grazers had significantly positive per capita effects on periphyton production except *C. granosus* where we observed significant negative effects (Fig.1b). Per unit biomass effects were more evenly distributed among grazers and that the large per capita effect of *F. crassa* was much reduced when considering body size (Fig. 1c). In contrast, the small pulmonate limpet *S. lessoni* had the largest per gram, positive effect on periphyton (Fig.1c).

Grazer diets versus effects

Mean relative abundance of diatoms and cyanophytes recorded in the experimental plots and that were observed in the gut contents of grazers (pooled data) showed no significant differences (K-S 2 sample test: diatoms; $k = 0.500$, $p = 0.188$; cyanophytes; $k = 0.375$, $p = 0.519$). On the other hand, sporelings and spores of macroalgae measured inside plots and those found in the diet of grazers showed a weak but significant difference (K-S: $k = 0.6250$, $p = 0.0497$).

Periphyton composition, richness and diversity

Assemblage structure

At the time of peak periphyton cover, the periphyton assemblage within most grazer treatments was characterized primarily by diatoms and cyanophytes, and secondarily by spores and sporelings of macroalgae (Fig. 2). Across treatments, the dominant

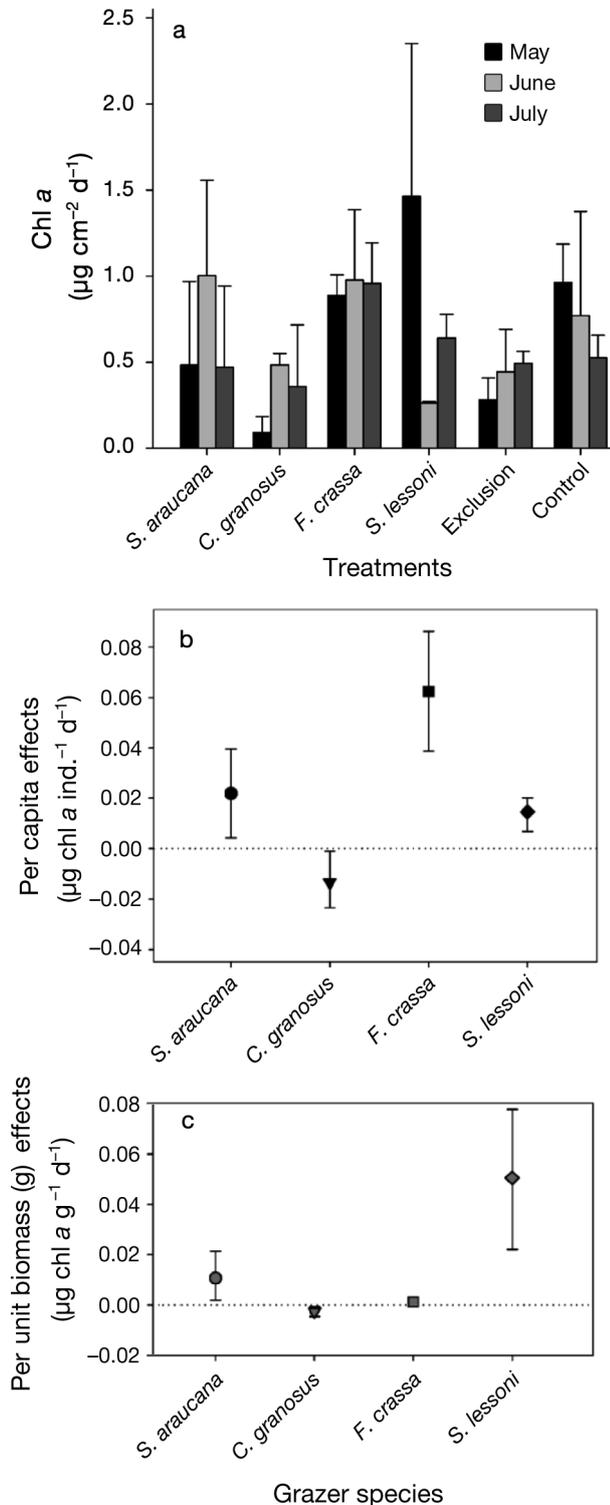


Fig. 1. Mean periphyton productivity recorded every 20 d between May and July 2008 on acrylic plates installed inside the experimental treatments; error bars = SE. (a) Periphyton productivity by month and treatment; (b) per capita effects of different grazers on periphyton production; (c) per unit biomass effects of different grazers on periphyton production. Grazer species: *Scurria araucana*, *Chiton granosus*, *Fissurella crassa*, *Siphonaria lessoni*

Table 1. One-way ANOVA on periphyton productivity ($\mu\text{g chl } a \text{ cm}^{-2} \text{ d}^{-1}$) recorded on acrylic plates installed and replaced every 20 d (May, June and July 2008) inside the experimental plots. Species names: see Fig. 1 legend. Significant values ($\alpha = 0.05$) in **bold**. SNK = Student-Newman-Keuls test, MSE = mean square error

Source	df	MS	F	p
May				
Treatment	5	0.2386	4.71	0.043
Error	6	0.0507		
SNK test: MSE = 0.0507				
Control = <i>S. lessoni</i> = <i>S. araucana</i> = <i>F. crassa</i> >				
<i>C. granosus</i> = Exclusion				
Jun				
Treatment	5	0.0111	0.62	0.690
Error	6	0.0177		
Jul				
Treatment	5	0.0071	0.53	0.748
Error	6	0.0125		

diatoms were *Gomphonema* (colonial cells), *Navicula* (both solitary and colonial cells), *Eunotia* and *Pinnularia*, while cyanophytes were represented mostly by *Lyngbya*, *Oscillatoria* and *Chroococcus* (Fig. 2a,b). Germlings of *Ulva* spp., *Bangia fuscopurpurea* and spores of macroalgae (unidentified species) were the most common identifiable taxa in this group (Fig. 2c). Notable differences between grazer enclosures, control and grazer exclusion areas were mostly due to different abundances of *Amphora*, *Gomphonema*, *Navicula*, *Lyngbya*, *Chroococals* and ulvoid sporelings (Fig. 2d), which together accounted for >75% of differences in composition among treatments.

PERMANOVA revealed significant differences in periphyton taxa composition among treatments (Table 2), which is in broad agreement with the patterns observed in the NMDS plot using all replicates from the different treatments (Fig. 3). SIMPER showed that the diatoms *Amphora* spp., *Gomphonema*, Naviculoids (solitary), and the cyanophyte *Lyngbya* spp. were the taxa that most contributed to dissimilarity among treatments (22.2, 14.1 and 12.24% on average, respectively; see also Fig. 2d). Specifically, NMDS analysis suggested that most treatments (controls and enclosures) had dissimilar compositional structure from enclosures of *Chiton granosus* (gray squares, Fig. 3) and from grazer exclusion plots (E_G , gray diamonds, Fig. 3). *A posteriori* pair-wise comparisons in PERMANOVA showed no significant differences between any grazer enclosures, controls or total exclusion (pair-wise comparisons, Table 2), probably due to low power.

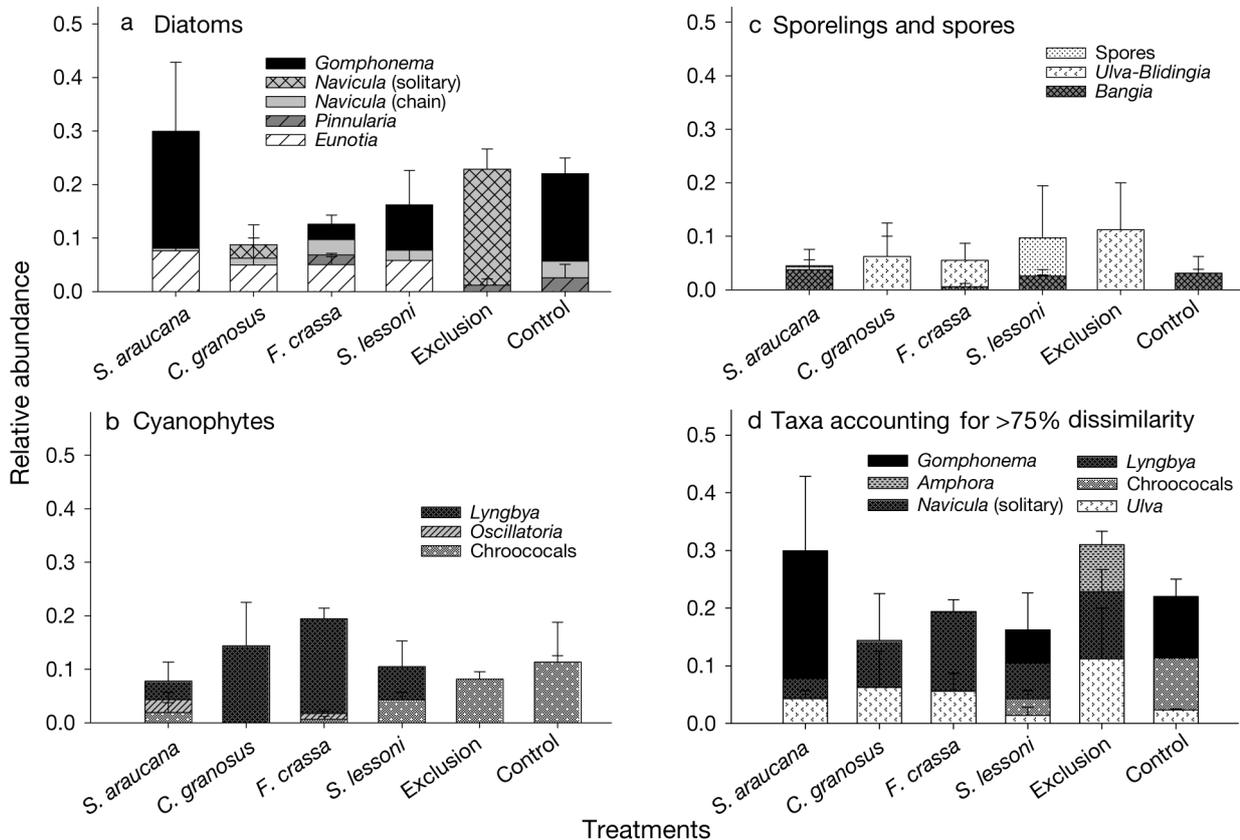


Fig. 2. Relative abundance (i.e. mean proportion of each taxa found in the total sample, +SE) of the main periphyton taxa observed inside the experimental areas: (a) diatoms, (b) cyanophytes, (c) sporelings and spores and (d) taxa accounting for >75 % dissimilarity. Species names: see Fig. 1 legend

Table 2. Permutational non-parametric MANOVA (PERMANOVA), using Bray-Curtis dissimilarity matrix on periphyton assemblage composition among grazer treatments during the first 2 wk of the field experiment. Comparisons: paired PERMANOVA treatment comparisons, with Bonferroni-adjusted p-values for multiple comparisons

Source	df	MS	F	p
Treatment	5	2.922	6.323	<0.001
Error	6	0.8039		
Comparisons			F	p
Control vs. Exclusion			9.980	0.104
<i>Scurria</i> vs. Exclusion			8.891	0.103
<i>Fissurella</i> vs. Exclusion			11.300	0.099
<i>Siphonaria</i> vs. Exclusion			9.435	0.096
<i>Chiton</i> vs. Exclusion			6.311	0.104

Periphyton taxa richness and Shannon diversity index (H') were relatively similar across all experimental treatments (Fig. 4a,b, Table 3), except enclosures of *Chiton granosus* and exclusions of all

grazers, where taxa richness and diversity were significantly lower than in all other treatments (Fig. 4a,b, Table 3). No treatment effects were observed in periphyton taxa evenness (J') (Fig. 4c, Table 3). In agreement with average effect of grazers, per capita and per gram effects showed mostly positive effects on periphyton taxa richness and diversity, with the exception of *C. granosus* (see Table S2 in the supplement).

Response to grazing pressure: indirect effects of grazing

At high grazing intensity (high bare rock cover, see insert Fig. 5a), diatom richness (S) and diversity (H') were low (white triangles and dashed line, Fig. 5a,c), while at intermediate to low levels of grazing pressure (low bare rock), diatom richness and diversity increased. Consequently, both quadratic and negative linear fits were significant (dashed and solid lines, Fig. 5a,c, Table 4). The cover of ulvoid sporelings inside the plots was also related to diatom diver-

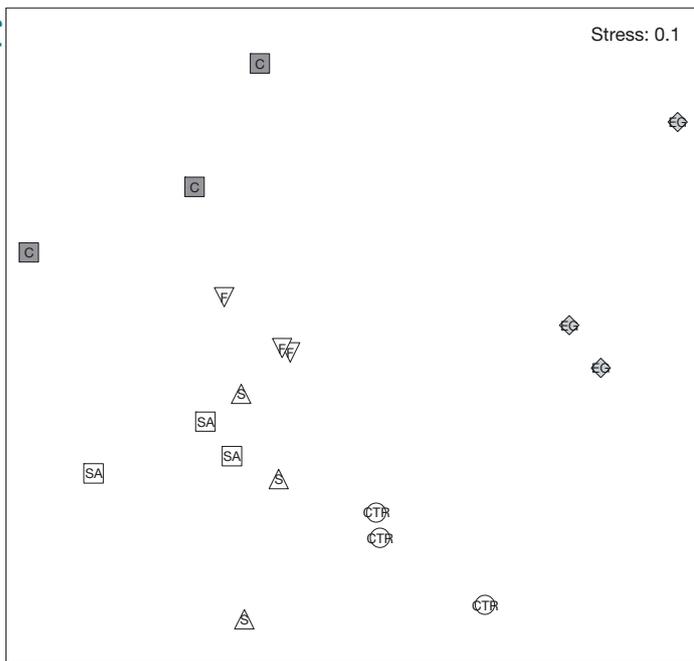


Fig. 3. Non-metric multidimensional scaling of average periphyton assemblage composition (pooling 2 sampling dates) recorded in the 6 experimental plots: \odot = Control ('all grazer access') areas; grazer enclosure treatments: \blacksquare = *Chiton granosus*, \square = *Scurria araucana*, \triangle = *Siphonaria lessoni*, ∇ = *Fissurella crassa*; \diamond = exclusion of grazers

sity and richness in a unimodal fashion, (see white triangles, dashed line, Fig. 5c,d), but only the latter was significant (Table 4). No significant relationship was observed between cyanophyte diversity or richness and the cover of bare rock (Fig. 5a,c, Table 4), but significant unimodal patterns of richness and diversity were observed with the cover of ulvoid sporelings (Fig. 5b,d, Table 4). If *Chiton granosus* (gray diamonds and circles in Fig. 5) was not considered in the analyses, the proportion of explained variance of bare rock and ulvoid cover on diatom richness and diversity declined (see Table S2 in the supplement), suggesting that the presence of this grazer species significantly alters interactions in the microalgae assemblage and the overall effect of the grazer guild.

DISCUSSION

Our experimental results showed overall positive effects of the grazer assemblage on periphyton biomass and productivity, as well as on richness and diversity, i.e. exclusion of the entire grazer assemblage led to significant reductions in all these variables. The results also demonstrated qualitative differences in

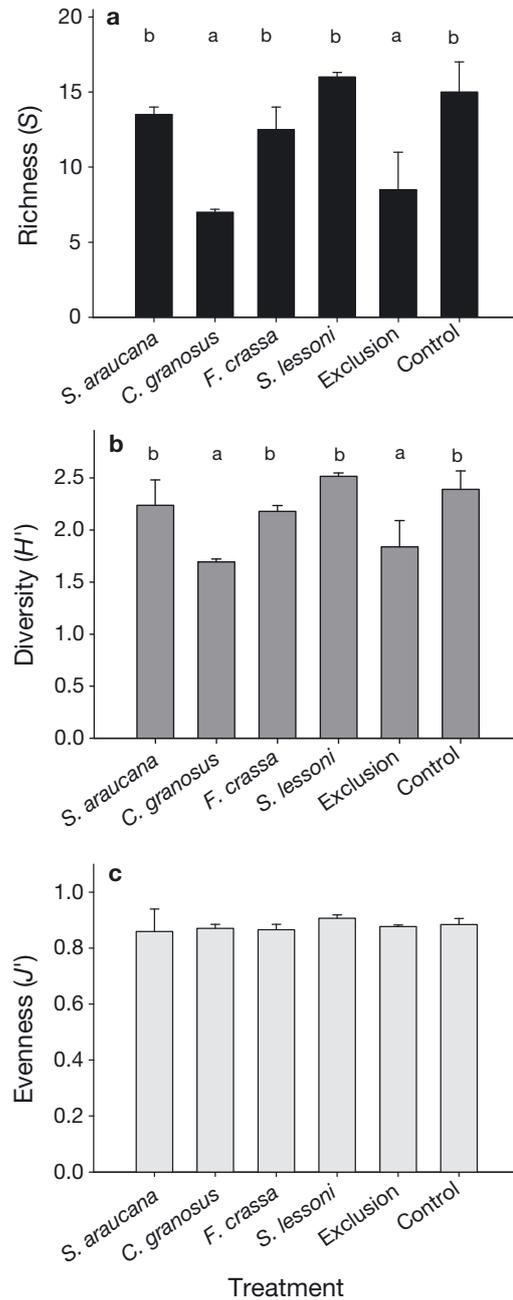


Fig. 4. (a) Periphyton taxa richness (S), (b), Shannon's diversity (H'), and (c) evenness (J') (mean + SE) in the 6 experimental treatments. Different letters above bars indicate significant differences ($p < 0.05$) among treatments (SNK test after one-way ANOVA). Species names: see Fig. 1 legend

the individual effects of different grazer species on periphyton. In particular, while *Chiton granosus* enclosures led to reduced periphyton productivity, taxa richness and diversity compared to the controls where all grazers had unrestricted access, while enclosures of all other grazer species led to significant positive effects. Qualitative differences among grazer species

Table 3. One-way ANOVA on periphyton taxa richness, Shannon's diversity (H') and Evenness (J') recorded inside the experimental treatments during first 2 wk of the experiment. Sampling of periphyton community was recorded in 3 haphazardly chosen replicated areas inside each plot. Analyses are based on H' and J' on log-transformed data per experimental plot. Significant values ($\alpha = 0.05$) in **bold**.

Species names: see Fig. 1 legend

Source	df	MS	F	p
Richness (S)				
Treatment	5	38.8250	18.27	<0.001
Error	12	2.1250		
SNK test: MSE = 2.1250				
Control = <i>S. lessoni</i> = <i>S. araucana</i> = <i>F. crassa</i> >				
<i>C. granosus</i> = Exclusion				
Diversity (H')				
Treatment	5	0.3026	11.37	<0.001
Error	12	0.0266		
SNK test: MSE = 0.0266				
Control = <i>S. lessoni</i> = <i>S. araucana</i> = <i>F. crassa</i> >				
<i>C. granosus</i> = Exclusion				
Evenness (J')				
Treatment	5	0.00085	0.66	0.661
Error	12	0.00129		

were even more striking when expressed on a per capita basis. Periphyton composition also changed in the presence of grazers when compared to grazer exclusions, and *C. granosus* was the species causing the most distinct changes in species composition of any grazer species. These grazer effects occurred when periphyton cover was at its peak and macroalgal species were not established in the plots. No significant differences were observed between diatoms and cyanophytes relative abundance in experimental plots or in the diet of the grazer species, but significant differences were detected for sporelings and spores of macroalgae suggesting direct consumption alone is not a good predictor of grazer effects. In general, intermediate levels of grazing pressure, when grazers appear to control dominant ulvoid algae yet they do not over-graze the surface, led to increased richness and diversity of diatoms and to a lesser extent of cyanophytes. Here, we discuss the potential direct and indirect mechanisms underlying experimental results and highlight the variability in the functional structure of the herbivore assemblage over different stages of community succession.

Periphyton biomass production and grazing

In our system, the periphyton community can be considered a transient state of community succession

Table 4. Polynomial quadratic and linear regression analyses on periphyton taxa diversity (H') and richness (S) (response variables [y]) using bare rock and ulvoid cover (%) as predictor variables (x). Dia = Diatoms; Cya = cyanophytes; $y(0)$ = origin; * $p < 0.05$, ** $p < 0.01$

	$y(0)$	a	b	R^2
Quadratic				
Bare rock (%)				
Dia H'	1.20	0.06	-1.316×10^{-4}	0.383*
Dia S	6.632	-0.032	-7.224×10^{-4}	0.240
Cya H'	0.462	-0.002	1.735×10^{-5}	0.009
Cya S	2.867	-0.017	1.938×10^{-4}	0.032
Ulvoids (%)				
Dia H'	0.988	0.013	-1.41×10^{-4}	0.097
Dia S	3.860	0.174	-0.002	0.366*
Cya H'	0.300	0.014	-1.559×10^{-4}	0.358*
Cya S	0.836	0.118	-0.001	0.489**
Linear				
Bare rock (%)				
Dia H'	1.28	-0.005		0.287*
Dia S	7.109	-0.029		0.173
Cya H'	0.450	-3.304×10^{-4}		0.003
Cya S	2.734	-3.018×10^{-5}		0.001
Ulvoids (%)				
Dia H'	1.124	1.792×10^{-4}		0.017
Dia S	6.416	-0.003		0.002
Cya H'	0.450	-2.574×10^{-4}		0.019
Cya S	2.484	0.004		0.008

toward dominance by macroalgae or sessile invertebrates. Previous field experiments (Nielsen & Navarrete 2004, Aguilera & Navarrete 2007), and our own observations, indicate that periphyton can dominate the rock surface from 2 to 4 wk following clearance of the rock surface. In some places, intense grazing on macroalgal sporelings and predation on invertebrates can prolong this early state (Navarrete & Castilla 2003, Aguilera & Navarrete 2007), but since all grazers consume the periphyton, the assemblage might never reach an 'established' or late successional stage as observed on other rocky shores, usually in the form of biofilm mats (e.g. Christofolletti et al. 2011). Nevertheless, periphyton can sustain a diverse grazer assemblage (Santelices et al. 1986, Camus et al. 2008) and seems to be relevant for colonization and settlement of macroscopic sessile organisms (Aguilera & Navarrete 2007, 2012a), potentially modulating successional trajectories of the entire intertidal community.

The rate of biomass production was significantly lower in grazer exclusions and in *Chiton granosus* enclosures than in controls or in any of the other grazer enclosures. In fact, periphyton biomass production measured in May was 4 times higher in controls and 10 times higher than in chiton enclosures. Although

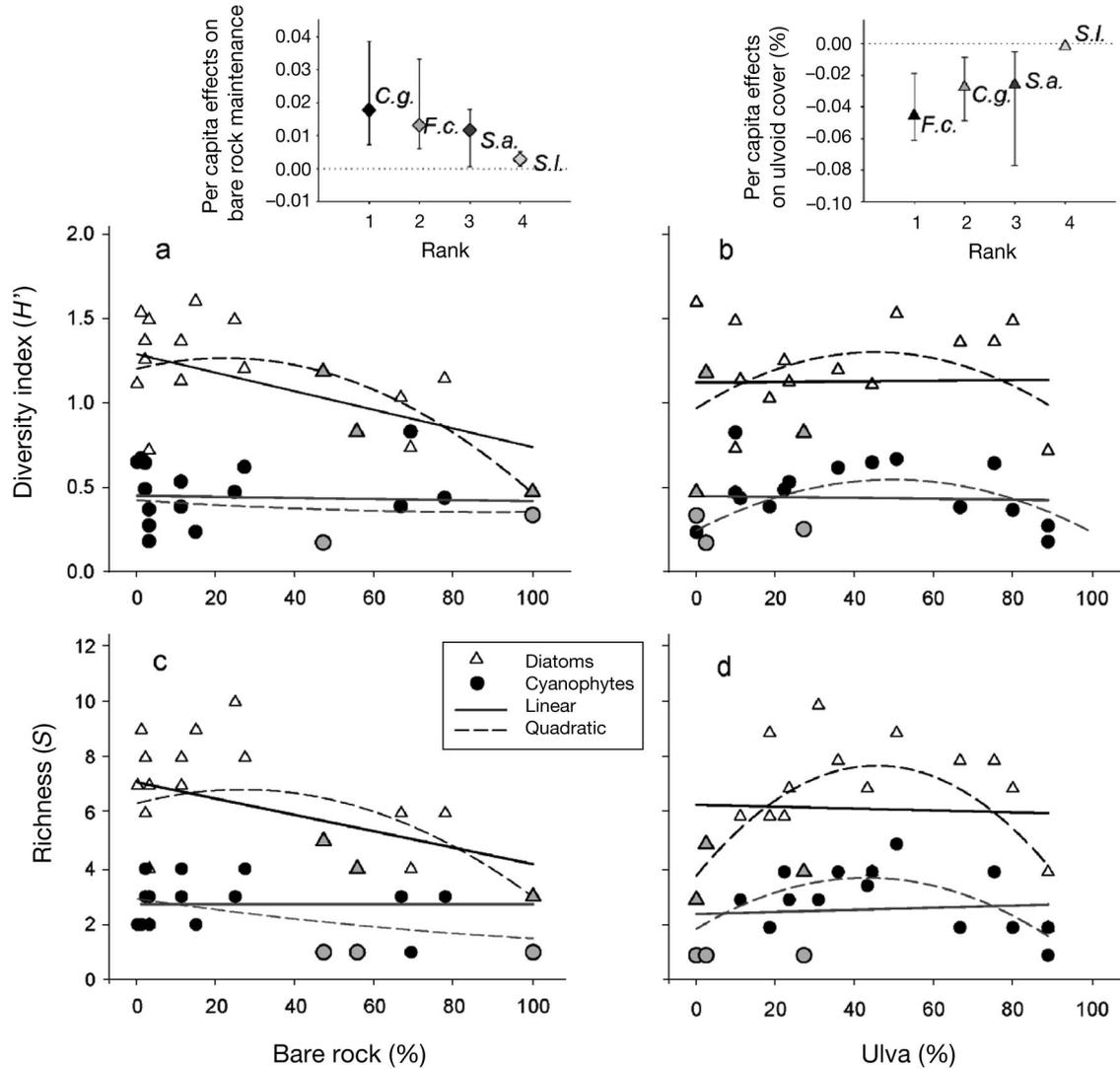


Fig. 5. Periphyton taxa diversity (Shannon's H') and richness (S) as a quadratic (dashed line) and linear (continuous line) function of percent cover (%) of bare rock (a, c) and ulvoid sporelings (b, d) inside experimental plots. H' and S were used as indicators of indirect effect of grazing during the first 3 wk of the experiment. Δ , \bullet : *Chiton granosus* enclosure plots for diatoms and cyanophytes, respectively. Insets correspond to per capita effects of the different study species, ranked according to their effects on bare rock maintenance and ulvoid cover (redrawn from Aguilera & Navarrete 2012a). *C.g.*: *Chiton granosus*; *F.c.*: *Fissurella crassa*; *S.a.*: *Scurria araucana*; *S.l.*: *Siphonaria lessoni*. Error bars: bootstrapped 95% CI

this result was significant only on 1 date (May), the general trend of positive effects of the grazer assemblage on periphyton productivity persisted in time. Studies on other shores have shown small or non-significant effects of grazers on periphyton biomass (Jenkins et al. 2001 and see, Liess & Hillebrand 2004 for review), but most studies have established that grazers have negative effects on microbenthic primary production (area specific productivity) on both tropical and temperate coasts (Castenholz 1961, Nicotri 1977, Mak & Williams 1999, Hillebrand et al.

2000, Christofolletti et al. 2011, Poore et al. 2012). Our results are, therefore, surprising for a consumer assemblage, which is expected to have overall negative or non-significant effects on resources when considering all species together (Paine 1992). It is unlikely that the positive grazing effects on periphyton productivity results from artifacts in the method used to measure productivity. (1) Field observations and laboratory assays showed that acrylic plates did not restrict movement or foraging of individuals of any of the species examined. The lowered profile of the

plate (0.1 cm at the edge) did not impede individuals of *Siphonaria lessoni*, the smallest grazer considered, or any other species from climbing upon the plates. (2) Potential cage (fence) artifacts should not affect these comparisons either as both, exclusions and enclosures, were under the same conditions. Thus, we interpret positive effects of the grazer assemblage as the propagation of indirect effects following changes in periphyton composition, i.e. grazers may favor the replacement of slow growing species for fast growing forms. For instance (see also effects on composition below), *Gomphonema* was the dominant diatom genus in the presence of grazers, while *Navicula* clearly dominated the diatom assemblage when all grazers were excluded. Similarly, sporelings of *Bangia* sp. were common in controls, while an association of *Ulva-Blidingia* sporelings dominated in the grazer exclusions (see Fig. 3). However, besides indirect positive effects, some direct positive effects of grazers may also play a role. Indeed, other experimental studies showed that mucus trail laid by molluscan grazers with varying behaviors can have differential effects in the stimulation of microalgae growth and spore attachment (Connor 1986, Santelices & Bobadilla 1996). Additionally, only some species of microalgae can be favored by grazers, thus changing dominance in the periphyton assemblage (Davies & Hawkins 1998, Davies & Beckwith 1999).

Observed differences among treatments imply that the strong effect of *Chiton granosus*, the only species with negative effects on periphyton productivity, does not compensate for the positive effects of all other grazers. As shown in other consumer systems (e.g. Duffy et al. 2001, Cardinale et al. 2006), species identity effects are important when considering periphyton productivity. The effects of grazer species were non-additive. Periphyton biomass production in controls ($0.783 \mu\text{g chl a cm}^{-2} \text{d}^{-1}$, averaged by date) was >3.5 times lower than the total production expected if grazers had additive effects on productivity (expected additive effect of the 3 grazer species equaled $2.790 \mu\text{g chl a cm}^{-2} \text{d}^{-1}$). This suggests that mid-shore grazer species not considered in the experiment (e.g. *Scurria zebrina*, *S. cecilians*) and particularly the interaction among grazers (Aguilera & Navarrete 2012b) are important factors affecting periphyton productivity in this community.

Periphyton composition, richness and diversity

One of the main traits that characterize our study system and other intertidal grazer assemblages is the

ability of individual species to scrape the substrate while foraging (e.g. Hawkins et al. 1992). A major overlap in diet is well documented for all focal species (Santelices et al. 1986, Camus et al. 2008, Aguilera & Navarrete 2012a), and corroborated by our results. While all species readily consume components of the periphyton assemblage and can be considered 'periphyton-grazers', we found contrasting periphyton relative abundances in the grazers' diet and available on the rock surface. No significant differences were observed for the abundances of either diatoms or cyanophytes, while weak but significant differences were observed for sporelings and spores of macroalgae in the grazers' diet and availability inside plots. Therefore, diet similarity does not correspond well with the diverse effects of herbivores on algal resources observed in our study and elsewhere (Aguilera & Navarrete 2012a). Indeed, since net grazer effects on algal resources are the result of both direct and indirect effects on different algal species (Liess & Hillebrand 2004), direct consumption could only partially explain their functional roles.

In our experiment, most grazers had negative effects on diatoms but positive effects on cyanophytes, in agreement with a recent compilation of experimental studies (Liess & Hillebrand 2004, Poore et al. 2012). This effect has been attributed to low palatability of cyanophytes to herbivores due to the presence of noxious compounds (e.g. Hill & Hawkins 1991, Rosemond 1993) or selectivity for nutritionally richer taxa (e.g. Lyngbya; Nagarkar et al. 2004). Our results suggest that palatability could be dissimilar within the same grazer assemblage. For example, the chiton *Chiton granosus* and the keyhole limpet *Fissurella crassa* had positive effects on the cyanophytes *Lyngbya* spp. while the other grazers had more variable but positive effects on other cyanophyte taxa (mainly *Oscillatoria* and *Chroococcus*). Thus, our results suggest that, within the same grazer assemblage, species can differentially affect periphyton components, triggering divergent effects on the overall microbenthic structure (Sommer 1999).

Effects of grazers on periphyton richness and diversity were similar to those observed on productivity; the presence of all grazers except for the chiton *Chiton granosus* had positive effects on periphyton richness and diversity. Both direct and indirect effects of grazers could enhance periphyton richness and diversity (Liess & Hillebrand 2004). In the absence of grazers, dominant periphyton taxa (e.g. ulvoids, *Amphora*) exclude other periphyton species, reducing overall richness.

Several experimental and theoretical models predict a unimodal relationship between grazing pressure and diversity (Lubchenco & Menge 1978, McNaughton 1986, Olf & Ritchie 1998), which is usually explained by the competitive-colonization tradeoff among the species in the assemblage (Paine & Vadas 1969, Connell 1978, Tilman 1994). In our experiment, diatom diversity increased at low to intermediate levels of bare rock cover, which was used as a proxy for overall grazing pressure. Diatom diversity was greater in the presence of *Siphonaria lessoni* and *Scurria araucana* and lower in presence of the chiton *Chiton granosus*, which has higher scraping capability and generates more bare space (Aguilera & Navarrete 2012a and see their insert in Fig.6a). At intermediate abundance of opportunistic ulvoid algae diatoms and cyanophytes taxa richness reached a maximum. We speculate that by reducing abundance of ulvoid sporelings to intermediate levels (i.e. 40% cover in plots), some grazer species could enhance spatial heterogeneity and favor a richer periphyton composition. The spatial heterogeneity created by intermediate grazing disturbance might, thus, balance competitive exclusion and colonization rates, generating niche opportunities for different microalgal taxa (Cardinale 2011). Previous studies have shown that allelopathic interactions between blue-green algae and diatoms are common (e.g. Keating 1978, Fong et al. 1993), and they could, indeed, play a role in the structure and response to grazing in the Chilean intertidal periphyton. Little is known about species interaction within the periphyton assemblage and further studies are required to examine our interpretations. Excluding *C. granosus* from the above analyses decreased the fraction of variance in diatoms diversity explained by bare rock cover or ulvoids, suggesting that the decline in periphyton diversity may be more closely related to types of grazer impacts rather than simply to measures of total grazing effect. More detailed experiments, probably within controlled mesocosms will be necessary to elucidate the mechanisms underlying changes in richness with grazer types and grazing pressure (e.g. Sommer 1999).

Periphyton and grazer functional structure

Recent evidence indicates that both functional roles and functional structure within herbivore assemblages can change through succession, depending on both herbivore and algal traits (Burkpile & Hay 2010, Aguilera & Navarrete 2012a). Our

results greatly improve basic knowledge about functional structure of consumer assemblages in general and intertidal molluscan grazers in particular. The differential role of grazers, i.e. 'identity effect', observed on periphyton richness, diversity and productivity is completely different to the patterns that have been observed for macroalgal assemblages. For example, the large keyhole limpet *Fissurella crassa* has a dominant, keystone effect on late macroalgal succession (Aguilera & Navarrete 2012a), but a relatively similar total or 'population effect' (sensu Navarrete & Menge 1996) to *Scurria araucana* and *Siphonaria lessoni* on periphyton productivity richness and diversity. Differences among grazer species on periphyton productivity were further stressed when calculating per capita interaction strengths (sensu Navarrete & Menge 1996). First, the significantly negative per capita effect of *Chiton granosus* was similar in magnitude to the significantly positive per capita effect of *S. araucana* and *S. lessoni*, but much smaller than the positive per capita effect of the large keyhole limpet *F. crassa*. Expressing effects in a per unit biomass basis changed this pattern and showed that the large effect of *F. crassa* appears to be largely due to its larger body size. Instead, per unit of body mass, the pulmonate limpet *S. lessoni* had the largest positive effect, 38 times higher than the large keyhole limpet, suggesting that behavioral or morphological traits (other than body size) of this species enhance periphyton productivity more than all other species.

The effects of *Chiton granosus* appear as the most distinct for the periphyton assemblage, but its role is similar to the other species on early macroalgal succession and absent in later successional stages (Aguilera & Navarrete 2012a). Even though foraging traits can be relevant for determining consumer effects (Schmitz 2008), no clear similarities or differences in traits alone could explain changes in functional roles of the focal grazers on the algal assemblages that co-occur during succession.

In summary, at the colonization phase, when the periphyton assemblages are the only primary producers on the rock surface, the herbivore assemblage seems to show limited redundancy in effects (sensu Hoey & Bellwood 2009). As succession progresses and small opportunistic macroalgae (e.g. ulvoids) colonize the surface, some level of redundancy is observed. As algae reach the adult-established phase, as well as in late succession, the functional relationships of the assemblage change to a keystone structure, dominated by the single-species effects (Aguilera & Navarrete 2012a). Thus, our results highlight

the complex functional roles and relationships of grazers involved in the control of structure and production of the periphyton assemblage. Periphyton appears to play a key role in community dynamics through its role as an early colonizer and facilitator of more productive species such as macroalgae and sessile invertebrates. Much needs to be learned about the basic ecology of periphyton species and their interactions in order to improve understanding of the mechanisms through which herbivores modify the structure and productivity.

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