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Quantifying the top-down effects of grazers on a rocky shore: selective grazing and the potential for competition

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1 **Quantifying the top-down effects of grazers on a rocky shore: selective grazing and**  
2 **the potential for competition**

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15  
16 **Abstract**

17 Grazers affect the structure of primary producer assemblages, and the details of these  
18 interactions have been well described for terrestrial habitats. By contrast, the effect of grazers on the  
19 diversity, distribution, and composition of their principal food source has rarely been described for the  
20 high intertidal zone of rocky shores, a model system for studying the potential effects of climate  
21 change. Along rocky, wave-swept shores in central California, the microphytobenthos (MPB) supports  
22 diverse assemblages of limpets and littorine snails, which, at current benign temperatures, could  
23 potentially partition food resources in a complementary fashion, thereby enhancing secondary

24 productivity. Two limpet species in particular, *Lottia scabra* and *L. austrodigitalis*, may partition  
25 components of the MPB, and are likely to affect the composition of the MPB on which they graze. In  
26 this study, we describe the composition, nutritional value (C:N ratio), and fluorescence (an index of  
27 chlorophyll density) of ungrazed, *L. scabra*-grazed and *L. austrodigitalis*-grazed MPB assemblages,  
28 each as function of temperature. Fluorescence decreased with increased average daily maximum  
29 temperature for ungrazed MPB, but temperature had no discernible effects on either fluorescence or the  
30 composition of the MPB of grazed assemblages. *L. austrodigitalis* and *L. scabra* did not partition the  
31 MPB, and did not exhibit complementarity. Both species exhibited an ordered grazing scheme, in  
32 which limpets grazed down certain components of the MPB before others, and grazing increased the  
33 C:N ratio of the MPB, decreasing its nutritional value. Taken together, these results suggest that *L.*  
34 *austrodigitalis* and *L. scabra* may experience increased competition as warming temperatures reduce  
35 the available MPB.

36

37 **Running page head:** Selective grazing and competition on rocky shores.

38 **Keywords:** Selective grazing; intertidal zone; biofilm; microalgae; microphytobenthos; *Lottia*; limpets

39

## 40 **Introduction**

41 The distribution and diversity of primary producers play a major role in structuring  
42 communities. In terrestrial environments, plant assemblages and the biological and physical factors  
43 that determine their structure have been extensively studied (e.g. Harper 1977, Tilman 1988). The  
44 diversity, distribution and abundance of plant species affect a number of ecosystem processes that  
45 include productivity (Naeem et al. 1994, Tilman et al. 1996, Hector et al. 1999, Cardinale et al. 2007),  
46 functional stability (McNaughton 1977), the distribution and abundance of consumers (MacArthur &

47 Pianka 1966, Tahvanainen & Root 1972) and resistance to invasive species (Fargione & Tilman 2005,  
48 but see Levine & D'Antonio 1999).

49 Numerous studies have examined the effects of intertidal grazers on the structure of macroalgal  
50 assemblages in the wave-swept, rocky intertidal zone, a model system for experimental ecology (see  
51 Underwood 1979, Coleman et al. 2006 and Poore et al. 2012 for relevant syntheses; Dayton 1975,  
52 Littler & Littler 1984, Gee & Warwick 1994, Williams et al. 2013, Best et al. 2014 for specific  
53 examples). However, relatively few have investigated the role of grazing on the epilithic  
54 microphytobenthos (MPB) that dominates the upper intertidal environment (but see Castenholz 1961,  
55 Nicotri 1977, Underwood 1984b and Underwood & Murphy 2008), and the composition and  
56 distribution of the MPB in the upper intertidal environment has yet to be described at scales relevant to  
57 intertidal grazers.

58 The intertidal MPB is composed of a diverse assemblage of organisms, including  
59 cyanobacteria, diatoms, macroalgal spores, germlings and juveniles and invertebrate larvae, all  
60 embedded in an extracellular matrix (Wahl 1989, Underwood 1984, Davey & OToole 2000). The  
61 MPB coats all hard surfaces submerged in water (Sekar et al. 2004), and extends to the top of the  
62 splash zone in the intertidal environment (Ricketts et al. 1968). In the upper intertidal zone, where the  
63 MPB is the only source of benthic primary productivity, it supports a diverse assemblage of limpets and  
64 littorine snails (Ricketts et al. 1968). However, the composition and distribution of components within  
65 the MPB, the potential for partitioning of these components by grazers, and the feedbacks between top-  
66 down consumption by grazers and bottom-up availability of nutrients in the MPB remain largely  
67 unknown.

68 In diverse terrestrial plant assemblages, niche partitioning can reduce interspecific competition,  
69 resulting in enhanced primary productivity relative to the performance of the most productive single-  
70 species assemblage (Tilman 2000, Naeem 2002, Hooper et al. 2005, Cardinale et al. 2011). This idea,

71 called complementarity, is generalizable across trophic levels: increased predator diversity is also  
72 associated with higher rates of prey capture due to resource partitioning (Ives et al. 2005, Byrnes et al.  
73 2006; Griffin et al. 2008). Although early studies linking biodiversity to ecosystem functioning  
74 highlighted the role of complementarity in temperate grassland assemblages, it also underpins  
75 relationships between diversity and productivity in intertidal systems (Griffin et al. 2008, Byrnes &  
76 Stachowicz 2009, Cardinale et al. 2011, Bracken & Williams 2013). For example, Griffin et al. (2008)  
77 used a factorial mesocosm experiment to explore how the species richness of prey assemblages affects  
78 the rate of prey consumption by three species of intertidal crabs. They found that, due to  
79 complementarity, all species of crabs ingested more food per individual when their prey was in  
80 polyculture than in any single monoculture. Through complementarity, diverse assemblages may also  
81 show increased resistance to the effects of climate change (Loreau et al. 2001): if resources become  
82 more scarce (as a result of increasing temperature, for instance), organisms that partition resources are  
83 more likely to survive and thrive. If upper intertidal grazers are complementary in their consumption  
84 of components of the MPB, it may have implications for how those species coexist now, and how they  
85 will be influenced by future climate shifts.

86 Nicotri (1977) measured the effects of foraging by the intertidal limpets *Lottia pelta* Rathke,  
87 *Lottia strigatella* Carpenter and *Lottia scutum* Rathke and the littorine snail *Littorina scutulata* Gould  
88 on MPB and observed that niche partitioning did not occur. She used transmitted light and scanning  
89 electron microscopy to determine that these four species grazed statistically indistinguishable quantities  
90 of the same species of cyanobacteria and diatoms from the MPB. However, her study organisms  
91 typically exist in different microhabitats, and thus may not always have been in direct competition for  
92 food resources. In addition, all four gastropod species supplement their diets with macroalgae, and so  
93 may not have experienced evolutionary pressure to specialize on the MPB. Similarly, Hawkins et al.  
94 (1989) examined three European gastropods, *Gibbula umbilicalis* da Costa, *Littorina littorea* Linnaeus

95 and *Patella vulgata* Linné, and found that their diets were indistinguishable despite differences in  
96 radular structure and feeding movements. But again, these species overlap only in a subset of locations  
97 across wide geographic ranges (Hawkins et al. 1989), and *G. umbilicalis* and *L. littorea* supplement  
98 their diets with detritus and macroalgae, respectively. Organisms that specialize entirely on the MPB  
99 and share the same microhabitat may be more likely to exhibit niche partitioning, and thus  
100 complementarity.

101         Recently, strong evidence has emerged that three intertidal gastropod species native to Italy may  
102 exhibit complementarity through resource partitioning (Sanz-Lázaro et al. 2015). Sanz-Lázaro et al.  
103 (2015) manipulated the density and richness of two limpet species (*Patella ulyssiponensis* and *P.*  
104 *rustica*) and one topshell (*Porchus turbinatus*) and observed the effects on the photosynthetic efficiency  
105 and photosynthetic biomass of the MPB. These gastropods share the same microhabitat in the  
106 intertidal environment, and they specialize on microalgae as their primary food resource. When two of  
107 these species grazed in concert, the photosynthetic efficiency of the MPB significantly increased while  
108 photosynthetic biomass did not, suggesting complementarity via resource partitioning. However, these  
109 effects were not additive: all three species grazing together reduced photosynthetic efficiency back to  
110 levels typical of individual grazers.

111         Along the central coast of California, two limpet species, *Lottia scabra* Gould and *Lottia*  
112 *austrodigitalis* Murphy, share the upper intertidal habitat, living above the *Mytilus californianus* mussel  
113 zone and often beyond the upper extent of the macroalga *Endocladia muricata* (Wolcott 1973). They  
114 share this habitat with small barnacles (*Chthamalus* spp.) and a variety of littorine snails (*Littorina*  
115 spp.) (Ricketts et al. 1968). Both *L. scabra* and *L. austrodigitalis* live on vertical rock surfaces,  
116 although *L. scabra* can also be found on horizontal surfaces (Collins 1976, Hahn & Denny 1989). Both  
117 species forage for microalgae twice per day during high tides, and exhibit a general dietary preference  
118 for diatoms (Nicotri 1977).

119 Previous research suggests that *L. austrodigitalis* and *L. scabra* are in direct competition for at  
120 least some food resources, and this competition could be driven by several factors. Haven (1973)  
121 observed that when *L. austrodigitalis* were excluded from a plot containing *L. scabra* in the intertidal  
122 zone at Hopkins Marine Station (HMS) in Pacific Grove, California, the density of the MPB increased,  
123 as did *L. scabra* growth rates. This result provides evidence that at least some portion of the MPB is a  
124 shared resource between the two species. However, it does not exclude the possibility that there are  
125 other components of the MPB that may be partitioned between *L. austrodigitalis* and *L. scabra*.

126 Food quality might also drive competition between *L. austrodigitalis* and *L. scabra*. Gastropod  
127 grazers, including limpets, are frequently selective in their preferences for intertidal algae (Della  
128 Santina et al. 1993, Bracken & Low 2012). Often, the preferred species are those with higher internal  
129 nitrogen concentrations or lower carbon-to-nitrogen (C:N) ratios (Pedersen & Borum 1996, Bracken et  
130 al. 2011, Bracken et al. 2014). This preference for higher nitrogen food sources is consistent with  
131 limitation of gastropod abundance and growth by nitrogen availability (Nielsen 2001, Sommer 2001).  
132 Differences between the C:N ratios of MPB grazed by *L. austrodigitalis* and *L. scabra* would suggest  
133 that they are targeting different food resources.

134 Lastly, temperature may influence the intensity of competition between these two *Lottia* species  
135 by reducing the availability, composition, or quality of an already limited food resource. Dark-adapted  
136 fluorescence of intertidal MPB can be used as a proxy for chlorophyll *a* density and is known to  
137 decrease as temperature increases (Miller et al. 2015). This suggests that less food is available to  
138 epilithic grazers like limpets when rock surface temperatures are high. Additionally, temperature may  
139 affect the composition and quality of the MPB. For example, Jackson et al. (2010) found that intertidal  
140 MPB on an Australian subtropical beach contained fewer cyanobacteria than that on a temperate beach.  
141 Diatoms may also be less plentiful at higher temperatures; they tend to be more abundant lower on  
142 shore (Castenholz 1961) and decrease with increasing insolation (Thompson et al. 2004). Because

143 diatoms are a nitrogen-rich food source for intertidal grazers, an increase in temperature may lead to a  
144 reduction in food quality, as well as food availability.

145 In this study, we investigated the effects of limpet grazing on the composition and quality of the  
146 upper intertidal MPB. Specifically, we asked:

147

- 148 1) How does temperature influence the density and quality of the MPB?
- 149 2) How do *L. austrodigitalis* and *L. scabra* affect the density and composition of the MPB?
- 150 3) Do *L. scabra* and *L. austrodigitalis* exhibit complementarity by partitioning components of the  
151 MPB during grazing?
- 152 4) How does grazing by *L. austrodigitalis* and *L. scabra* affect the nutritional quality of the MPB?

153

154 To answer these questions, we examined the dark-adapted fluorescence (a proxy for chlorophyll  
155 density) and C:N ratio (an index of food quality) of MPB as a function of average maximum  
156 temperature of the substratum. We also compared the dark-adapted fluorescence and composition of  
157 MPB that had not been grazed with MPB that had been grazed by *L. scabra*, MPB that had been grazed  
158 by *L. austrodigitalis*, and MPB that had been grazed by the natural suite of upper intertidal gastropods.  
159 Lastly, we quantified the percent cover of morphotypes in the MPB from each grazing treatment, and  
160 compared the results between treatments. We expected that increased temperatures and limpet grazing  
161 would decrease dark-adapted fluorescence values, change the composition of the MPB, and increase  
162 the C:N ratio. Additionally, if resource partitioning was occurring at the microscopic level, we  
163 expected that the composition of the *L. scabra*-grazed MPB would differ from that of the *L.*  
164 *austrodigitalis*-grazed MPB.

165



166

## 167 **Methods**

### 168 *Field experiment*

169         We selected six vertically-oriented field sites in the rocky, wave-swept intertidal zone adjacent  
170 to HMS (36.6217° N, 121.9043° W). Sites were between 3 m and 35 m apart. At each site, we  
171 deployed four circular aluminum plates (10 cm in diameter, 12 mm thick) in horizontal transects at 1.7  
172 m above mean lower low water (MLLW). The average diurnal tidal range at this site is 1.6 m. We  
173 covered each plate with a thin layer of light gray rubber grip tape (Safety Walk Tape, 3M) to encourage  
174 microalgal growth. Each plate was attached to the granite substratum with a central bolt, and a cement  
175 pad was constructed beneath each plate to ensure good thermal contact with the underlying rock. At  
176 each site, one plate held four *L. scabra* and one held four *L. austrodigitalis*. This density (500 limpets  
177 m<sup>-2</sup>) corresponded to that observed in limpet-rich areas of the intertidal zone at HMS (Morelissen and  
178 Harley 2007). Individuals of about 1 cm in shell length were selected for the experiment, and were  
179 corralled using 20 mm tall, stainless steel mesh fences (5.5 mm square mesh size). These fences  
180 deterred most limpets from leaving the plates, but an average of  $0.11 \pm 0.15$  *L. scabra* and  $1.31 \pm 0.94$   
181 *L. austrodigitalis* were still lost per month. A third plate at each site had an identical fence and served  
182 as a grazer exclusion control, while the fourth plate lacked a fence altogether and allowed access by  
183 grazers living on the neighboring natural rock surface. We deployed the plates in June of 2013, and  
184 they remained in the field through December of that year. Fences were cleared of any macroalgal  
185 growth in the late fall.

186         It is important to note that in Monterey Bay, *L. austrodigitalis* overlaps with a cryptic congener,  
187 *L. digitalis* Rathke. The two species can be reliably identified only through genetic methods.  
188 However, prior research at our field site suggests that *L. austrodigitalis* are most commonly found on

189 vertical rocky surfaces, while *L. digitalis* is associated with the micro-environment provided by the  
190 gooseneck barnacle, *Pollicipes polymerus* Sowerby (Crummett & Eernisse 2007). More recent work  
191 has also shown that *L. austrodigitalis* makes up 88-89% of the cryptic species pair in high shore  
192 habitats at HMS (Dong et al. 2008, Dong & Somero 2009), where our limpets were collected. We refer  
193 to *L. austrodigitalis* throughout the remainder of this study, acknowledging that a small percentage of  
194 our organisms may in fact be *L. digitalis*.

195

#### 196 *Temperature*

197 Temperature measurements were obtained for each plate using iButton temperature dataloggers  
198 (0.5°C resolution, Maxim Integrated, San Jose, CA, USA). We machined a circular pocket in the back  
199 of each plate to hold the iButton. The iButtons recorded temperature every 12 minutes, and we  
200 downloaded the data every 2 weeks.

201 At the conclusion of the experiment, the average maximum daily temperature for December  
202 2013 was calculated for each plate. We checked that these temperatures were consistent across sites  
203 and grazing treatments using 2-factor ANOVA and Tukey's HSD test for multiple comparisons. Visual  
204 checks were performed to ensure that model assumptions of normality and equal variances were met.  
205 Analyses were carried out in MATLAB 2014a.

206

#### 207 *Dark-adapted fluorescence*

208 We surveyed plates on July 10, August 6, September 6, October 6, November 6 and December  
209 1, 2013 during favorable tides and wave conditions. During each survey, we replaced any limpets lost  
210 during the previous month and measured the density of the MPB. The density of the MPB was  
211 approximated using a PAM fluorometer (Diving-PAM, Walz, Effeltrich, Germany). We took six dark-  
212 adapted fluorescence measurements haphazardly on each plate during night time low tides. The dark-

213 adapted fluorescence value ( $F_0$ ) from the fluorometer serves as a proxy for the chlorophyll  $a$  density of  
214 the MPB (Barranguet & Kromkamp 2000, Honeywill et al. 2002, Serodio et al. 2008). The tip of the  
215 fiber-optic measuring head of the fluorometer was fitted with a 10 mm spacer to maintain a fixed  
216 distance from the experimental plate, and the opening covered an area of 53 mm<sup>2</sup>. The tip was held in  
217 place at each measurement site until the  $F_0$  value stabilized (typically 3-5 seconds) before recording a  
218 value, as recommended by the manufacturer. Because changes in surface moisture can affect  $F_0$  values  
219 (Maggi et al. 2013), we restricted sampling to periods when the plates were moist but not actively  
220 splashed or submerged by the tide.

221 2-factor ANOVA and Tukey's HSD test for multiple comparisons were used to compare dark-  
222 adapted fluorescence for December 2013 across sites and grazing treatments. Temperature and dark-  
223 adapted fluorescence are known to be negatively correlated (Miller et al. 2015); analysis of covariance  
224 was used to determine whether the relationship between temperature and dark-adapted fluorescence  
225 was influenced by grazing treatment. Visual checks were performed to ensure that model assumptions  
226 of normality and equal variances were met. Analyses were carried out in MATLAB 2014a.

227

### 228 *Scanning Electron Microscopy*

229 After plates were removed from the field, we haphazardly selected and cut three 1 cm<sup>2</sup> samples  
230 of rubber tape from each plate. The samples were soaked in a 2.5% gluteraldehyde solution for 15-45  
231 minutes to preserve cell shape, washed in phosphate-buffered solution (pH 7.9) for 15 minutes, rinsed  
232 in de-ionized water and air dried. Samples were then gold-coated using a SPI-MODULE sputter coater  
233 (Structure Probe Inc., West Chester, PA, USA), and were viewed under a scanning electron microscope  
234 (SEM; FEI Quanta 200,  $h\nu = 25\text{kV}$ , spot size = 4 nm) at a magnification of 300x. This magnification  
235 resulted in a 0.159 mm<sup>2</sup> field of view. Nine fields were chosen haphazardly from each sample and  
236 photographed, for a total of 648 images (162 images per treatment).

237

238 *Identification and Quantification of Morphotypes*

239 We processed each image using the photoQuad image analysis program

240 (<http://www.mar.aegean.gr/sonarlab/photoquad/index.php>). A spatially stratified, random field of 50  
241 points was overlaid on the entire image, from which percent cover of the components of the MPB was  
242 calculated. Points that fell in areas that were not in focus were excluded from the analysis. We chose  
243 the magnification of 300x to visualize diatoms and cyanobacteria, which are the components of the  
244 MPB thought to be the preferred food sources for *Lottia* spp. (Castenholz 1961, Haven 1973, Nicotri  
245 1977, Connor 1984). This resolution did not allow us to identify each organism at the species level.  
246 Instead, components of the MPB were classified by morphotypes, which took into account general  
247 shape, size and other features observable at 300x (Figure 1). These morphotypes were based on the  
248 organisms described, identified and pictured by Nagarkar and Williams (1997) and Hawkins et al.  
249 (1989). Morphotypes 1-5 were long, narrow organisms and were classified as filamentous  
250 cyanobacteria. Morphotypes 6-12 were smaller, boxy or rod-like organisms and were classified as  
251 diatoms. Organisms described as morphotype 13 formed encrusting mats with individual components  
252 that were impossible to distinguish at 300x. Morphotype 14 were also small encrusting organisms, but  
253 they grew in a characteristic diamond pattern. This pattern did not correspond in scale to the  
254 topographical features of the rubber grip tape or the radulae of *L. scabra* or *L. austrodigitalis*.  
255 Morphotype 15 were small, round organisms, and were classified as coccoidal cyanobacteria. Lastly,  
256 morphotype 16 described any other organism observed, while morphotype 17 indicated empty rubber  
257 grip tape.

258 From the random field of 50 points, percent cover of each morphotype was calculated as the  
259 number of points covering a particular morphotype divided by the total number of points (usually 50,  
260 minus any points not scored due to focus issues). Percent cover of organism type (filamentous

261 cyanobacteria, diatoms, encrusting organisms or coccoidal cyanobacteria) was also calculated. We  
262 averaged percent cover of morphotypes and organism types within each plate (n = 27) and we  
263 calculated average percent cover of morphotypes and organism types for each grazing treatment across  
264 sites (n = 6). To determine a) how grazing by *L. scabra* and *L. austrodigitalis* affected MPB  
265 composition, and b) whether the two species were partitioning the MPB, we used PRIMER v6 software  
266 (<http://www.primers-e.com/>) to generate MDS plots to visualize organism- and morphotype-level  
267 variation in assemblage structure among grazing treatments. Analysis of similarity (ANOSIM) was  
268 used to assess the relative size of these differences (Clarke & Warwick 2001). Because untransformed  
269 data place heavy emphasis on similarities in abundant organisms between assemblages (Clarke &  
270 Warwick 2001), square-root and fourth-root transformations of the raw data were also performed.  
271 These transformations put increasing emphasis on similarities in rare organisms between assemblages.  
272 Finally, the top contributors to differences in MPB composition were identified using similarity  
273 percentage tests (SIMPER; Clarke & Warwick 2001).

274

#### 275 *Carbon and Nitrogen Elemental Analysis*

276 A second, larger field experiment was deployed from June 2014 through January of 2015. It  
277 was assembled as described above, except six replicate plates of seven grazing treatments were placed  
278 at each field site: an ungrazed treatment, three *L. scabra*-grazed treatments with four, eight or twelve  
279 limpets, and three *L. austrodigitalis*-grazed treatments with four, eight or twelve limpets. A subset of  
280 these plates with visually apparent MPB were used to evaluate the effects of limpet grazing on the  
281 nutritional content of the MPB.

282 Scrapings of the MPB were obtained from 34 ungrazed, 10 *L. scabra*-grazed and 9 *L.*  
283 *austrodigitalis*-grazed plates with sufficient MPB to permit sampling. To determine internal carbon  
284 and nitrogen concentrations, scrapings were dried to constant mass (60 °C for 72 hours), ground to a

285 fine powder in stainless steel microvials (Retsch Mixer Mill MM 400, Verder Scientific, Newtown,  
286 Pennsylvania, USA) and analyzed on a Flash 2000 Elemental Analyzer configured for analyses of C  
287 and N in organic samples (Thermo Fisher, Cambridge, UK). Carbon-to-nitrogen (C:N) ratios were then  
288 calculated for each plate.

289 We tested a set of linear models using likelihood ratio tests to determine whether  $F_0$ , grazing  
290 treatment, average daily maximum temperature, or an interaction between these factors helped  
291 determine C:N ratios. Due to the relatively small number of limpet-grazed plates with visually  
292 apparent MPB growth for the C:N analysis, the number of limpets per plate for limpet-grazed  
293 treatments could not be included in our model. ANOVA and Tukey's HSD tests were used to  
294 determine differences in log-transformed C:N ratios among grazing treatments. Visual checks were  
295 performed to ensure that model assumptions of normality and equal variances were met. Analyses  
296 were carried out in R 3.1.1.

297

### 298 *Effects of Limpet Density*

299 Although each limpet-grazed plate was initially seeded with four limpets in the 2013  
300 experiment, some animals either escaped from or fell off the plates each month. This prompted us to  
301 explore the effects of limpet loss on the composition of the MPB. We examined the relationships  
302 between the number of limpets remaining on a plate the month prior to plate recovery from the field  
303 (December 2013) and the percent cover of morphotypes at recovery using ANOVA and Tukey's HSD  
304 multiple comparisons test. Visual checks were performed to ensure that model assumptions of  
305 normality and equal variances were met. Analyses were carried out in MATLAB 2014a.

306

307

308

## 309 **Results**

### 310 *Temperature*

311           Grazing treatments in the 2013 experiment experienced average daily maximum temperatures  
312 that were statistically indistinguishable from each other (mean  $\pm$  SE =  $14.68 \pm 0.42^\circ\text{C}$ ;  $F_{3,15} = 0.71$ ,  $p =$   
313  $0.561$ ). However, the average daily maximum temperature at site six ( $18.73 \pm 0.51^\circ\text{C}$ ) was  
314 significantly higher than that at the five other sites ( $13.87 \pm 0.03^\circ\text{C}$ ;  $F_{5,15} = 20.62$ ,  $p < 0.001$ ).

315           To determine whether temperature might be influencing the composition of the MPB, we  
316 divided our ungrazed plates into cool samples (average maximum daily temperature of less than  $14^\circ\text{C}$ )  
317 and warm samples (average maximum daily temperature of  $14^\circ\text{C}$  or greater). An analysis of similarity  
318 was performed. We found no evidence of a difference between the composition of the MPB on warmer  
319 and cooler ungrazed plates ( $R = -0.179$ ,  $p = 0.667$ ), although our sample size for this test was small ( $n$   
320  $= 4$  cool plates,  $n = 2$  warm plates). Additionally, the average difference in daily maximum  
321 temperature between warm and cool plates was only  $3^\circ\text{C}$ .

322

### 323 *Dark-adapted fluorescence*

324            $F_0$ , our proxy for microalgal density, was significantly higher for the ungrazed treatment than  
325 for the natural-grazed, *L. scabra*-grazed and *L. austrodigitalis*-grazed treatments ( $F_{3,15} = 5.94$ ,  $p =$   
326  $0.005$ ) (Figure 2). Site was not a significant factor in this analysis ( $F_{5,15} = 2.62$ ,  $p = 0.068$ ).  $F_0$  was  
327 affected by an interaction between grazing treatment and average maximum daily temperature ( $F_{1,16} =$   
328  $9.47$ ,  $p < 0.001$ );  $F_0$  was inversely proportional to temperature for the ungrazed treatment (slope of  
329 linear model =  $-27.23 \text{ }^\circ\text{C}^{-1}$ ), but the two variables were uncorrelated for the three grazed treatments  
330 (Figure 3).

331

332 *Organism-Level Assemblage Data*

333 Analysis of similarity on untransformed data indicated that the MPB of the ungrazed treatment  
334 was significantly different from that of either the *L. scabra*-grazed treatment ( $R = 0.284$ ,  $p = 0.035$ ) or  
335 the *L. austrodigitalis*-grazed treatment ( $R = 0.247$ ,  $p = 0.048$ ) (Figure 4). Additionally, the MPB of the  
336 natural-grazed treatment was different from that of the *L. scabra*-grazed treatment ( $R = 0.185$ ,  $p =$   
337  $0.041$ ), but not from the other treatments. No significant difference was observed between the *L.*  
338 *scabra* and *L. austrodigitalis*-grazed treatments ( $R = 0.117$ ,  $p = 0.123$ ). As increasing emphasis was  
339 placed on the presence or absence of rare organisms by square-root and fourth-root transforming the  
340 data, the ungrazed MPB differed more from the three grazed MPBs (Table 1).

341 Similarity percentage tests indicated that differences between the ungrazed MPB and the *L.*  
342 *scabra*-grazed MPB were driven by percent cover of filamentous cyanobacteria, diatoms and  
343 encrusting organisms. On average, the ungrazed MPB exhibited a higher percent cover of filamentous  
344 cyanobacteria and diatoms, and a lower percent cover of encrusting organisms (Table 2). Similar  
345 differences were identified between the ungrazed MPB and the *L. austrodigitalis*-grazed MPB, except  
346 these differences were also driven by percent cover of coccoidal cyanobacteria, which was more  
347 abundant in the *L. austrodigitalis*-grazed MPB. Differences between the natural-grazed MPB and the *L.*  
348 *scabra*-grazed MPB were driven by filamentous and coccoidal cyanobacteria. The former assemblage  
349 exhibited higher percent cover of both organisms relative to the latter.

350

351 *Morphotype-Level Assemblage Data*

352 Similar trends were exhibited at the morphotype level as those at the organism-level. The  
353 ungrazed treatment was significantly different from both the *L. scabra*-grazed treatment ( $R = 0.559$ ,  $p$   
354  $= 0.002$ ) and the *L. austrodigitalis*-grazed treatment ( $R = 0.274$ ,  $p = 0.006$ ) when analyses were done  
355 on untransformed data (Figure 5). Once again, the natural-grazed treatment and the *L. scabra*-grazed



356 treatment also differed ( $R = 0.293$ ,  $p = 0.039$ ), although this difference became non-significant after  
357 transforming the data (Table 1). The specific morphotypes driving these differences in composition  
358 were identified. Limpet-grazed treatments exhibited lower percent cover in morphotypes 2 and 5  
359 (cyanobacteria), 8, 9 and 11 (diatoms) and 13 (encrusting organisms), but higher percent cover in  
360 morphotype 14 (encrusting organisms) (Table 2).

361

### 362 *Evidence for Resource Partitioning*

363 Both the organism-level and morphotype-level data indicate no significant differences in  
364 composition between the MPB grazed by *L. scabra* and that grazed by *L. austrodigitalis* (Table 1;  $R =$   
365  $0.117$ ,  $p = 0.123$  and  $R = -0.069$ ,  $p = 0.652$  respectively). Emphasizing rare organisms by transforming  
366 the MPB composition data makes these communities appear more similar (Table 2). An MDS plot was  
367 generated using only *L. scabra*-grazed and *L. austrodigitalis*-grazed treatments that had the full quota  
368 of four limpets during the month prior to sample collection ( $n = 8$  out of 12 limpet-grazed plates); after  
369 controlling for limpet density, there was no significant difference between *L. scabra* and *L.*  
370 *austrodigitalis*-grazed plates ( $R = -0.031$ ,  $p = 0.393$ ).

371

### 372 *Nutrient Concentrations and Ratios*

373 The best linear model describing trends in the log-transformed C:N ratio data included no  
374 significant interactions between  $F_0$ , average maximum daily temperature and the grazing treatment.  
375 However, C:N ratios were related independently to both  $F_0$  and the grazing treatment ( $F_{1,49} = 4.236$ ,  $p$   
376  $= 0.045$  and  $F_{2,49} = 12.977$ ,  $p < 0.001$  respectively). C:N ratios were found to be negatively related to  
377 increased  $F_0$ , while *L. scabra*-grazed plates were found to be significantly depleted in nitrogen (C:N =  
378  $2.832 \pm 0.201$ ) relative to ungrazed plates (C:N =  $2.180 \pm 0.029$ ), resulting in higher C:N ratios ( $p <$   
379  $0.001$ ) (Figure 6). *L. austrodigitalis*-grazed plates (C:N =  $2.501 \pm 0.138$ ), however, were not

380 significantly different from either the *L. scabra*-grazed plates or the ungrazed plates ( $p = 0.0634$ ,  $p =$   
381  $0.127$  respectively).

382

### 383 *Effects of Limpet Density*

384 Although each limpet-grazed plate was initially seeded with four limpets in the 2013  
385 experiment, some animals either escaped from or fell off the plates each month. These animals were  
386 replaced during monthly surveys. The month prior to SEM sample collection (December 2013), no *L.*  
387 *scabra* were lost, but the *L. austrodigitalis* plates lost between 0 and 2 individuals, resulting in an  
388 average retention of  $3.167 \pm 0.753$  limpets per plate (approximately 400 limpets  $m^{-2}$ ). Significant  
389 correlations were found between the number of limpets on the plates at the end of December 2013 and  
390 the percent cover of coccoidal cyanobacteria and encrusting organisms (Figure 7). Percent cover of  
391 coccoidal cyanobacteria was highest at the intermediate densities of 2 or 3 limpets per plate. Percent  
392 cover of encrusting organisms was positively related to limpet density ( $F_{10,12} = 5.200$ ,  $p = 0.046$ );  
393 however, at the morphotype level, morphotype 13 decreased with limpet density while morphotype 14  
394 increased. Finally, during both the 2013 and 2014 experiments, significant negative correlations were  
395 found between  $F_0$  and the number of limpets remaining on the plates during the final month of each  
396 experiment ( $F_{1,10} = 5.57$ ,  $p = 0.04$  and  $F_{1,17} = 6.9$ ,  $p = 0.012$  respectively). In 2013, increasing grazer  
397 density from approximately 250 limpets  $m^{-2}$  (2 limpets per plate) to 400 limpets  $m^{-2}$  (4 limpets per  
398 plate) resulted in a decrease in  $F_0$  from 77.167 ( $n = 1$ ) to  $48.083 \pm 1.977$  ( $n = 8$ ). In 2014, increasing  
399 grazer density from approximately 125 limpets  $m^{-2}$  (1 limpet per plate) to 1000 limpets  $m^{-2}$  (8 limpets  
400 per plate) resulted in a decrease in  $F_0$  from 82.667 ( $n = 1$ ) to 17.833 ( $n = 1$ ).

401

402

## 403 **Discussion**

404           On the ungrazed plates, high average maximum daily temperatures were correlated with low  $F_0$   
405 values. MPB is reduced in biomass and therefore less fluorescent when exposed to warm conditions  
406 (Jackson et al. 2010, Miller et al. 2015). However, limpet grazing reduced  $F_0$  values to those typical of  
407 warm, ungrazed plates, regardless of temperature, suggesting that in intertidal regions where grazers  
408 are very sparse,  $F_0$  values will be low if the region is warm, and high if the region is cool. The  
409 presence of grazers masks this signal, corroborating prior observations that limpet grazing suppresses  
410 natural variation in the density of the MPB along intertidal shores (Miller et al. 2015). Our results also  
411 indicate that a low  $F_0$  value on a warm, ungrazed plate does not necessarily signify a change in  
412 assemblage structure at the microscopic level. However, a more extensive study designed to observe  
413 the composition of the MPB across a larger range of temperatures and with higher replication would  
414 better address this question.

415

### 416 *Dark-adapted fluorescence*

417           Dark-adapted fluorescence ( $F_0$ ), our proxy for chlorophyll *a* density within the MPB, was  
418 significantly higher on ungrazed plates than on grazed plates, indicating that intertidal grazers reduce  
419 the abundance of photosynthesizing organisms in the MPB. However, the average grazed  $F_0$  (mean  $\pm$   
420 SE =  $55.8 \pm 18.22$ ) was much greater than the zero value obtained from new clean safety walk grip  
421 tape, suggesting that photosynthesizing organisms are still plentiful even under constant grazing  
422 pressure. Small organisms that limpets are unable to graze may be the primary contributors to this  $F_0$   
423 value, and it may represent a baseline signifying the limit to which grazers can deplete food resources.  
424 Herbivorous intertidal sea stars, in addition to a variety of gastropod grazers, have been found to have  
425 limited ability to deplete epilithic MPB, possibly because certain components are inaccessible, toxic or

426 indigestible (Nicotri 1977, Underwood 1984, Jackson et al. 2009). However, higher densities of *L.*  
427 *scabra* and *L. austrodigitalis* than our experimental density of about 500 limpets m<sup>-2</sup> might graze down  
428 photosynthesizing organisms even further. Our data from 2014 indicated that densities of 1000 limpets  
429 m<sup>-2</sup> should deplete  $F_0$  to about half the level observed at 500 limpets m<sup>-2</sup>. However, this observation  
430 was based on data from a single plate. Excluding this sample, the negative correlation between limpet  
431 density and  $F_0$  was not statistically significant; limpet densities between 125 and 500 m<sup>-2</sup> grazed the  
432 MPB down to an average  $F_0$  value of  $44.5 \pm 0.9$ . It is worth noting that all of these limpet densities  
433 have been observed at HMS. Although Morelissen and Harley (2007) observed 515 *L. scabra* m<sup>-2</sup> in  
434 limpet-rich regions of the intertidal zone at HMS, both *L. scabra* and *L. austrodigitalis* have clumpy  
435 distributions that can sometimes result in even higher local densities (D. LaScala-Gruenewald, personal  
436 observation).

437

#### 438 *Effects of Grazers and Grazer Density*

439         The composition of grazed MPB was significantly different from that of ungrazed MPB. The  
440 limpet-grazed MPB contained fewer diatoms and filamentous cyanobacteria in particular, which may  
441 be the preferred food sources of *Lottia* spp. (Nicotri 1977, Connor 1984, Haven 1973, Hahn & Denny  
442 1989). On plates where fewer than four limpets grazed, there were higher percentages of coccoidal  
443 cyanobacteria, and lower percentages of encrusting organisms. This suggests an ordered grazing  
444 scheme, where filamentous cyanobacteria and diatoms are consumed first, followed by coccoidal  
445 cyanobacteria. This pattern may be due to animal preference (Schmitt 1996), but it is more likely due  
446 to topographical constraints. Lateral teeth in *L. scabra* and *L. austrodigitalis* are typically 100  $\mu\text{m}$  long  
447 and 50  $\mu\text{m}$  wide at the widest point, while tooth tips are typically separated by distances of 100  $\mu\text{m}$  in  
448 the cross-radula direction and 200  $\mu\text{m}$  in the along-radula direction (D. LaScala-Gruenewald,  
449 unpublished data). In contrast, coccoidal cyanobacteria and some other organisms common on grazed

450 plates (morphotypes 5 and 9 for example) are under 30  $\mu\text{m}$  in maximum dimension, and are possibly  
451 difficult to consume, especially if the substratum is rugose at the  $\mu\text{m}$ -scale.

452         Plates open to the natural suite of upper intertidal grazers had compositions that were  
453 intermediate between ungrazed and limpet-grazed plates. Although the number and species of grazers  
454 on the natural-grazed plates were not monitored throughout the experiment, both *Littorina* spp. and  
455 *Lottia* spp. were capable of ascending the 12 mm plate edge (D. LaScala-Gruenewald, personal  
456 observation), and the former were occasionally observed foraging on the plates during monthly  
457 surveys. Because no limpets were confined on the natural-grazed plates, they probably experienced  
458 reduced grazing pressure, resulting in higher percent cover of filamentous cyanobacteria and diatoms  
459 relative to the limpet-grazed plates. These results indicate that, at HMS, we should expect to see  
460 assemblages of primarily encrusting organisms where limpets are very dense, and assemblages of  
461 primarily filamentous cyanobacteria and diatoms in regions where limpets and other grazers are very  
462 sparse.

463

#### 464 *Evidence for Resource Partitioning*

465         No significant differences were observed between the composition of the MPB grazed by *L.*  
466 *scabra* and that grazed by *L. austrodigitalis*. This result is consistent with previous findings (Haven  
467 1973) indicating that these two species are in direct competition for food resources, and fails to support  
468 our hypothesis of complementarity. However, microscopic-level analyses of the MPB may be  
469 important in determining resource partitioning in other scenarios. *L. scabra* and *L. austrodigitalis* have  
470 very similar morphology; in fact, their radulae show only very subtle differences in lateral tooth shape,  
471 and in the length of their ribbon segments (Lindberg 1981, D. LaScala-Gruenewald personal  
472 observation). Complementarity might still be observed if a wider array of grazers were considered. In  
473 particular, the abundant upper intertidal periwinkle, *Littorina keenae* Phillippi, which was not included

474 in this experiment, has distinctly different radula morphology, radula hardness, and feeding behavior,  
475 and may be able to access and consume components of the MPB that are unavailable to limpets  
476 (Goodwin & Fish 1977, Hawkins et al. 1989).

477

#### 478 *Effects of Grazing on Nutrient Ratios*

479 Limpet grazing also affected the C:N ratio of the MPB. Cyanobacteria have been hypothesized  
480 to be nutritionally inferior to diatoms (Hargrave 1970, Nicotri 1977), and we found that the ratio of the  
481 percent cover of diatoms to the percent cover of cyanobacteria was significantly reduced in MPB  
482 grazed by *L. scabra* and *L. austrodigitalis* (ANOVA;  $F_{3,20} = 4.150$ ,  $p = 0.019$ ). *L. scabra*-grazed  
483 samples also had significantly increased C:N ratios relative to ungrazed samples, indicating preferential  
484 consumption of high-nitrogen portions of the MPB. However, *L. austrodigitalis*-grazed samples had  
485 C:N ratios that were intermediate between *L. scabra*-grazed and ungrazed samples. Due to the  
486 relatively low number of limpet-grazed plates that had sufficient coverage of MPB to be used for the  
487 C:N analysis, we grouped plates foraged by four, eight and twelve limpets, which raises the possibility  
488 that differences in C:N ratios on limpet-grazed plates were driven by limpet density. The *L.*  
489 *austrodigitalis*-grazed plates suitable for C:N analysis were initially seeded with an average of 8.4  
490 limpets while the *L. scabra*-grazed plates were seeded only with 4.4 limpets, but by the end of our  
491 study, the number of limpets on *L. austrodigitalis* and *L. scabra*-grazed plates were statistically  
492 indistinguishable ( $3.53 \pm 0.35$ ). This was likely due to the fact that *L. austrodigitalis* have a higher  
493 propensity for escaping our field enclosures than *L. scabra* (Miller et al. 2015). Additionally, no  
494 relationship was found between the number of limpets remaining on the plates at the end of the study  
495 and C:N ratio.

496 Prior research does not indicate that cyanobacteria have increased C:N ratios relative to  
497 diatoms; both groups of organisms tend to have C:N ratios between 3 and 30 (Brzezinski 1985, Fukuda

498 et al. 1998, Geider & La Roche 2002, Fu et al. 2007). However, C:N ratios are species- and location-  
499 specific, and vary with nutrient availability and physical parameters such as temperature (Geider & La  
500 Roche 2002). More specific data on C:N ratios of diatoms and cyanobacteria native to the upper  
501 intertidal zone at HMS would be necessary to better evaluate our hypothesis of depleted nutritional  
502 quality.

503

#### 504 *Relevance and Implications*

505 Our results corroborate previous work investigating potential partitioning of the MPB by  
506 intertidal gastropods. Nicotri (1977) showed that the limpets *L. pelta*, *L. strigatella* and *L. scutum*, in  
507 addition to the littorine snail *L. scutulata*, did not partition food resources at the microscopic scale.  
508 Similarly, Hawkins et al. (1989) examined three European gastropods, *G. umbilicalis*, *L. littorea* and *P.*  
509 *vulgata*, and found that their diets were indistinguishable despite differences in radula structure and  
510 feeding movements. The species in Nicotri's (1977) study occupy different microenvironments, and  
511 both studies considered some species that supplement the MPB in their diets with macroalgae.  
512 However, it appears that their results are generalizable to species that compete in the field for the  
513 entirety of their food resources. This is somewhat surprising: if food is limiting in the upper intertidal  
514 environment (Haven 1973, Branch & Branch 1981, Underwood 1979, Underwood 1984a, b, c), there  
515 should be strong selective pressure favoring individuals who can partition the MPB. Nicotri suggests  
516 that the MPB may be too difficult to partition successfully, and the relative sizes of lateral teeth, ventral  
517 plates and maximum dimension of the components of the MPB considered here are consistent with that  
518 conclusion. However, *Lottia* spp. may still be partitioning resources with the littorine snails *Littorina*  
519 *keenae*, *L. scutulata* and *L. plena*, which graze on the MPB in the upper intertidal environment at HMS  
520 but have different radula structure. It would be interesting to observe whether the overlaps in diet  
521 reported by Nicotri (1977) and Hawkins (1989) are also true for *L. austrodigitalis*, *L. scabra* and

522 *Littorina* spp.

523           In general, we conclude that *L. scabra* and *L. austrodigitalis* are in direct competition for  
524 resources, and that their density affects the composition of the MPB. However, this conclusion has a  
525 number of caveats. First, although a large number of SEM images were taken for each treatment, the  
526 percent cover of each plate that was imaged was very small (< 1%). It is possible that the lack of  
527 significant differences between grazing treatments reported in our results was affected by low sampling  
528 area, although we doubt that our general conclusions would change given wider sampling.  
529 Additionally, we were unable to identify organisms to the species level. Again, doing so would be  
530 unlikely to change our general results, but more information might be provided by performing genomic  
531 analyses to determine the species involved. Finally, all of our experiments took place on rubber grip  
532 tape rather than the limpets natural rock habitat. While this difference in substratum likely influenced  
533 the composition of both the ungrazed and grazed MPB, it allowed for consistency across sites and  
534 treatments. The native substratum at HMS is granite, an igneous rock containing feldspar, quartz and  
535 an array of other rock types that vary based on where and when the rock was formed. Each of these  
536 rock types has unique chemical and physical properties, which may affect patterns of growth in the  
537 MPB (Cattaneo-Vietti et al. 2002, Faimali et al. 2004, Perotti 2008). The granite at HMS is also  
538 topographically variable on scales ranging from meters to millimeters, and topography has been shown  
539 to influence the settlement and growth of MPB (Johnson 1994, Schmitt 1996, Keough et al. 1997,  
540 Hutchinson et al. 2006). Using rubber grip tape as the substratum for this experiment eliminated  
541 potential variability due to rock type and topography, which might otherwise have masked differences  
542 in the composition of the MPB due to grazing.

543           Rubber grip tape as an experimental substratum has some drawbacks. Although it is highly  
544 rugose across small scales, it does not mimic the larger (5-10 mm) topographic features typical of the  
545 native granite substratum at HMS. Additionally, granite is much harder than rubber tape. Prior



546 research has found that it takes less force to remove crustose algae from a harder surface (Padilla  
547 1985), suggesting that limpets might feed more efficiently on granite. However, granite cannot be  
548 deformed by a radula, and limpets on granite may not be able to completely remove the MPB from  
549 small-scale topographical features. It is likely that both the rugosity and flexibility of rubber tape  
550 influenced the MPB composition observed on both grazed and ungrazed plates.

551         The deposition of pedal mucus by limpet grazers may also have had an effect on the  
552 compositions of the observed MPB. Limpet locomotion leaves behind a thin mucus layer that, in some  
553 species, has been shown to persist in the field and attract components of the MPB relative to adjacent  
554 mucus-free substratum (Davies & Hawkins 1998). Connor and Quinn (1984) and Connor (1986)  
555 examined the capacity for mucus from *L. scabra* and *L. austrodigitalis* to act as a persistent adhesive  
556 trap for components of the MPB. They found that mucus from both species do recruit components of  
557 the MPB at +1.7 m above MLLW in the intertidal zone adjacent to Bodega Bay Marine Laboratory in  
558 Sonoma County, California, but mucus from *L. scabra* was more effective in doing so. Additionally,  
559 they found that the median persistence time for trails from both species in the field was 7 days.  
560 Research on another limpet species, *Patella vulgata*, indicated that its mucus was especially effective  
561 in trapping diatoms relative to other components of the MPB, and especially effective during the first  
562 24 hours after deposition (Davies et al. 1992). It is highly likely that our limpet-grazed plates were  
563 coated in pedal mucus throughout the duration of our field study, and that this mucus recruited  
564 additional MPB to grazed areas. However, the effects of this mucus were not sufficient to counter the  
565 effects of grazing. *L. scabra* and *L. austrodigitalis*-grazed plates showed statistically indistinguishable  
566 percentages of diatoms and cyanobacteria, and dark-adapted fluorescence. Additionally, *L. scabra*  
567 exhibited a significantly lower growth rate than *L. austrodigitalis* throughout the study, which fails to  
568 reflect a relative increase in food consumed (D. LaScala-Gruenewald, unpublished data). Differences  
569 in mucus properties between our study species were likely masked by grazing intensity. Both limpets

570 cover an average area of about 50 cm<sup>2</sup> during a foraging bout (D. LaScala-Gruenewald, unpublished  
571 data); the plate area (78.54 cm<sup>2</sup>) was too small to accommodate four limpets without regrazing.

572         The fact that *L. scabra* and *L. austrodigitalis* appear not to partition resources may have  
573 implications in the face of future climate change. Prior research indicates that moderate increases in  
574 temperature may create a temporary benefit for ectothermic organisms such as limpets (Miller et al.  
575 2015). Increases in limpet growth rate have been observed at higher temperatures, and it has been  
576 hypothesized that either temperature effects on physiological processes or a shift towards consuming  
577 higher value food items may be mechanistically responsible (Miller et al. 2015). Our data indicate that  
578 the former is more likely, since the composition of MPB does not appear to vary across a small  
579 temperature range (13-16°C). Provided that sufficient food resources are available, moderate increases  
580 in temperature during aerial exposure should result in increases in limpet metabolism and growth  
581 (Sanford 2002, Miller et al. 2015). However, our study also suggests that it may be possible for  
582 moderate densities of limpets (200 – 500 m<sup>-2</sup>) to reduce the MPB to a level where little or no food is  
583 available. Furthermore, the MPB that remains is of lower quality (i.e., higher C:N). Limpets under  
584 future climate change conditions may not be able to obtain sufficient resources at current spatial  
585 densities, and we may observe declines in densities of upper-intertidal limpets. We might also expect  
586 to see more success from organisms that do partition resources; complementarity has been documented  
587 between a number of intertidal organisms (e.g. Byrnes & Stachowicz 2009, Griffin et al. 2008, Sanz-  
588 Lázaro et al. 2015) and is a leading explanation for how assemblages may cope with a changing  
589 climate (Loreau et al. 2001). Additionally, because increased temperatures correspond to decreases in  
590  $F_0$ , less food may be available as the climate warms, particularly in the summer. The density and  
591 composition of the MPB vary seasonally (Underwood 1984, Jackson et al. 2010), and our samples for  
592 this study were collected in December, when the MPB biomass was relatively high. As the MPB  
593 decreases over the summer months, there will be a corresponding decrease in food availability

594 (Thompson 2000). Individuals with limited access to resources are at greater risk of temperature stress,  
595 as they are less able to mitigate potential costs through physiological mechanisms such as the heat  
596 shock response (Fitzgerald-DeHoog et al. 2012).

597         Although *L. scabra* and *L. austrodigitalis* do not appear to partition resources at the  
598 microscopic level, the density of grazers does have an effect on the composition of the MPB. Both  
599 species consume large filamentous cyanobacteria and diatoms before eating the smaller filamentous  
600 and coccoidal cyanobacteria. Therefore, we should expect to see MPB relatively rich in filamentous  
601 cyanobacteria and diatoms in regions where limpets are sparse. It is also likely that small filamentous  
602 and coccoidal cyanobacteria form some baseline density of the MPB that is unavailable to upper  
603 intertidal grazers. Prior research suggests that differences in feeding behavior and radula morphology  
604 between limpet species may result in differing abilities to graze on certain species of macroalgae  
605 (Padilla 1985). Similarly, *L. scabra* and *L. austrodigitalis* may graze on certain morphotypes in the  
606 MPB first because of preference, or because of radula morphology and function. The microtopography  
607 of the substratum may also play a role. Hutchinson et al. (2006) examined the effects of substratum  
608 roughness on the composition of the MPB and molluscan grazing patterns using SEM. Diatoms were  
609 more abundant on rougher substrata, and both diatoms and filamentous cyanobacteria were more  
610 abundant around surface features. Finally, different species of molluscan grazers varied in their  
611 abilities to remove certain diatoms and cyanobacteria. It is likely that an interaction between grazing  
612 behavior, radula morphology and physical properties of the substratum (such as roughness and  
613 hardness) are responsible for our observed grazing patterns.

614         In summary, although the MPB contributes strongly to the primary productivity that fuels the  
615 upper intertidal ecosystem (Nicotri 1977, Underwood 1984, Thompson et al. 2000), relatively few  
616 studies have considered the composition of intertidal MPB at the microscopic level (Nagarkar &  
617 Williams 1997, Hill & Hawkins 1990, Hutchinson et al. 2006), and even fewer have examined the

618 impacts of environmental factors on the composition of the MPB (but see Nicotri 1977, Underwood  
619 1984, Thompson et al. 2004). Here we found that two limpet species, *L. scabra* and *L. austrodigitalis*,  
620 appear to compete directly for food resources even at the microscopic scale, and that grazer density  
621 influences the composition of the MPB. C:N ratios on grazed and ungrazed plates suggest that limpet  
622 grazing may reduce the nutritional content of the MPB via selective consumption of high-N organisms.  
623 Additionally, we found that moderate increases in temperature decreased the density of the MPB, but  
624 did not change its composition. As the climate continues to warm, we expect to see decreases in the  
625 density of the MPB and consequent decreases in grazer density, especially among species that do not  
626 partition resources at the microscopic level.

627

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633

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841

842

843 **Tables**

844

845 **Table 1. Transforming MPB composition data results in larger differences between grazed and**  
846 **ungrazed assemblages.** P-values are displayed from analysis of similarity tests for the treatments  
847 being compared when the data underwent no transformation, a square-root transformation, and a  
848 fourth-root transformation. These transformations place increasing emphasis on the presence of rare  
849 species in assemblages. Data from both the organism-level analysis and morphotype-level analysis are  
850 shown. Significant p-values are in bold type; associated R-values are in parentheses.

851

Treatments being compared	Organism-level			Morphotype-level		
	No transformation	Square-root	Fourth-root	No transformation	Square-root	Fourth-root
Ungrazed and Natural	0.093 (0.127)	0.087 (0.159)	0.093 (0.144)	0.301 (0.026)	<b>0.035</b> (0.281)	<b>0.011</b> (0.393)
Ungrazed and <i>L. scabra</i>	<b>0.035</b> (0.284)	<b>0.024</b> (0.306)	<b>0.015</b> (0.294)	<b>0.002</b> (0.559)	<b>0.002</b> (0.711)	<b>0.002</b> (0.661)
Ungrazed and <i>L. austrodigitalis</i>	<b>0.048</b> (0.247)	<b>0.028</b> (0.285)	<b>0.022</b> (0.233)	<b>0.006</b> (0.274)	<b>0.004</b> (0.515)	<b>0.002</b> (0.631)
Natural and <i>L. scabra</i>	<b>0.041</b> (0.185)	0.069 (0.191)	0.067 (0.250)	<b>0.039</b> (0.293)	<b>0.026</b> (0.307)	0.169 (0.122)
Natural and <i>L. austrodigitalis</i>	0.766 (-0.068)	0.814 (-0.076)	0.461 (0.004)	0.229 (0.078)	0.331 (0.026)	0.297 (0.044)
<i>L. scabra</i> and <i>L. austrodigitalis</i>	0.123 (0.117)	0.249 (0.069)	0.372 (0.002)	0.652 (-0.069)	0.578 (-0.044)	0.645 (-0.054)

852

853

854 **Table 2. Comparison of percent cover of organisms and morphotypes across grazing treatments.**

855 In general, grazed treatments showed fewer filamentous cyanobacteria (morphotypes 2 and 5) and  
 856 diatoms (morphotypes 8, 9 and 11) than the ungrazed treatment. Percent cover of encrusting organisms  
 857 were also higher on average, due to the larger percentage of morphotype 14. Coccoidal cyanobacteria  
 858 (morphotype 15) were higher in the natural-grazed and *L. austrodigitalis*-grazed treatments.  
 859 Organisms that were relatively abundant in grazed treatments are in bold type, while those that were  
 860 relatively scarce in grazed treatments are italicized.

861

	Ungrazed	Natural	<i>L. scabra</i>	<i>L. austrodigitalis</i>
<b>Organism-level</b>				
Filamentous cyanobacteria	<i>20.83</i>	<i>16.00</i>	<i>9.83</i>	<i>12.83</i>
Encrusting organisms	<b>63.83</b>	<b>71.83</b>	<b>84.17</b>	<b>76.50</b>
Coccoidal cyanobacteria	<i>3.50</i>	<i>9.17</i>	<i>4.67</i>	<i>9.50</i>
Diatoms	<i>10.00</i>	<i>3.00</i>	--	<i>1.00</i>
<b>Morphotype-level</b>				
2	<i>1.60</i>	<i>0.02</i>	<i>0.05</i>	<i>0.02</i>
5	<i>17.11</i>	<i>15.15</i>	<i>8.92</i>	<i>11.56</i>
8	<i>1.68</i>	<i>0.83</i>	<i>0.14</i>	<i>0.12</i>
9	<i>6.18</i>	<i>1.11</i>	<i>0.36</i>	<i>0.52</i>
11	<i>2.10</i>	<i>0.65</i>	<i>0.74</i>	<i>0.19</i>
13	<i>60.53</i>	<i>58.76</i>	<i>44.61</i>	<i>45.01</i>
14	<b>3.09</b>	<b>12.83</b>	<b>39.05</b>	<b>32.21</b>
15	<i>3.58</i>	<i>9.35</i>	<i>4.88</i>	<i>9.12</i>

862

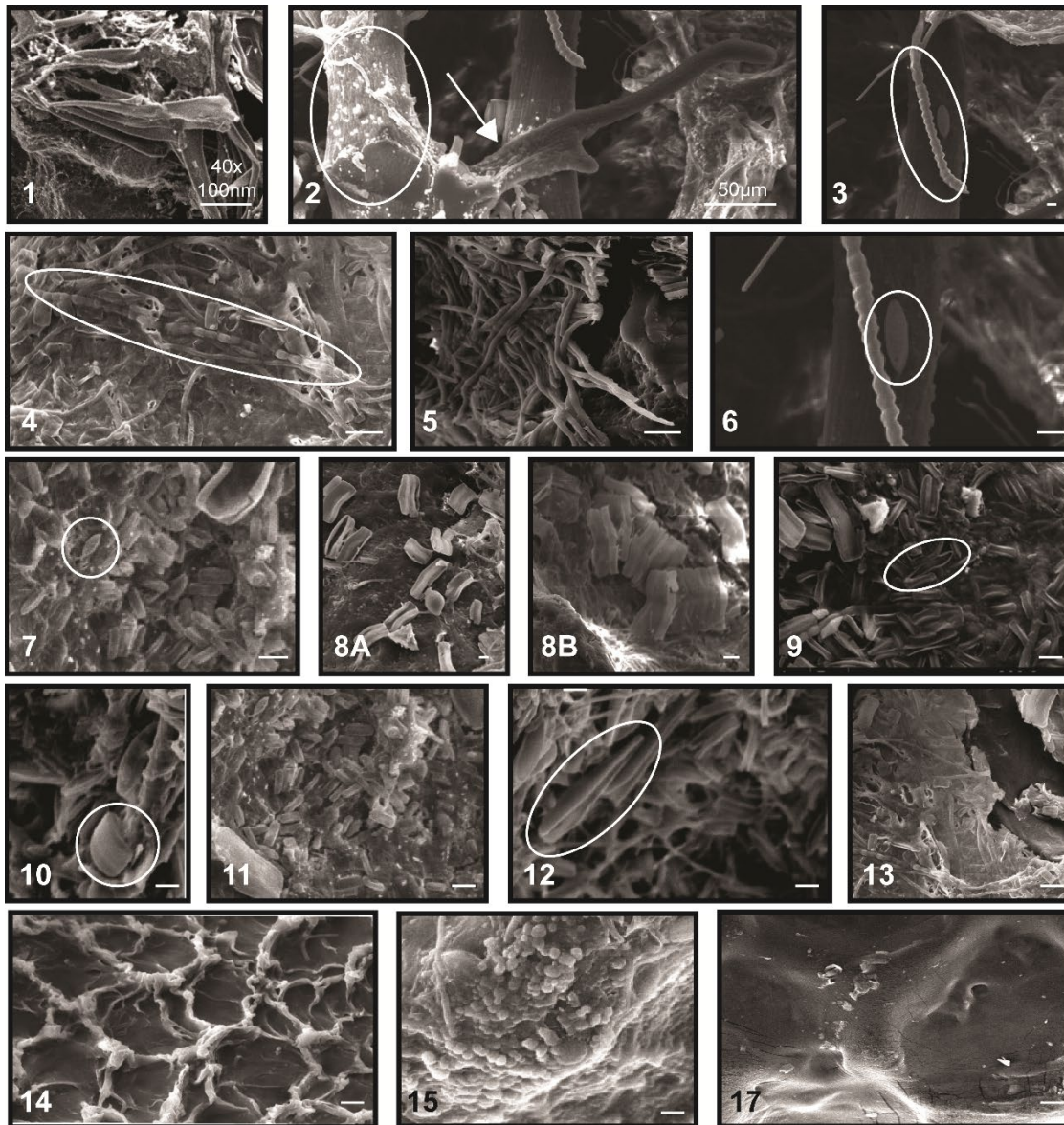
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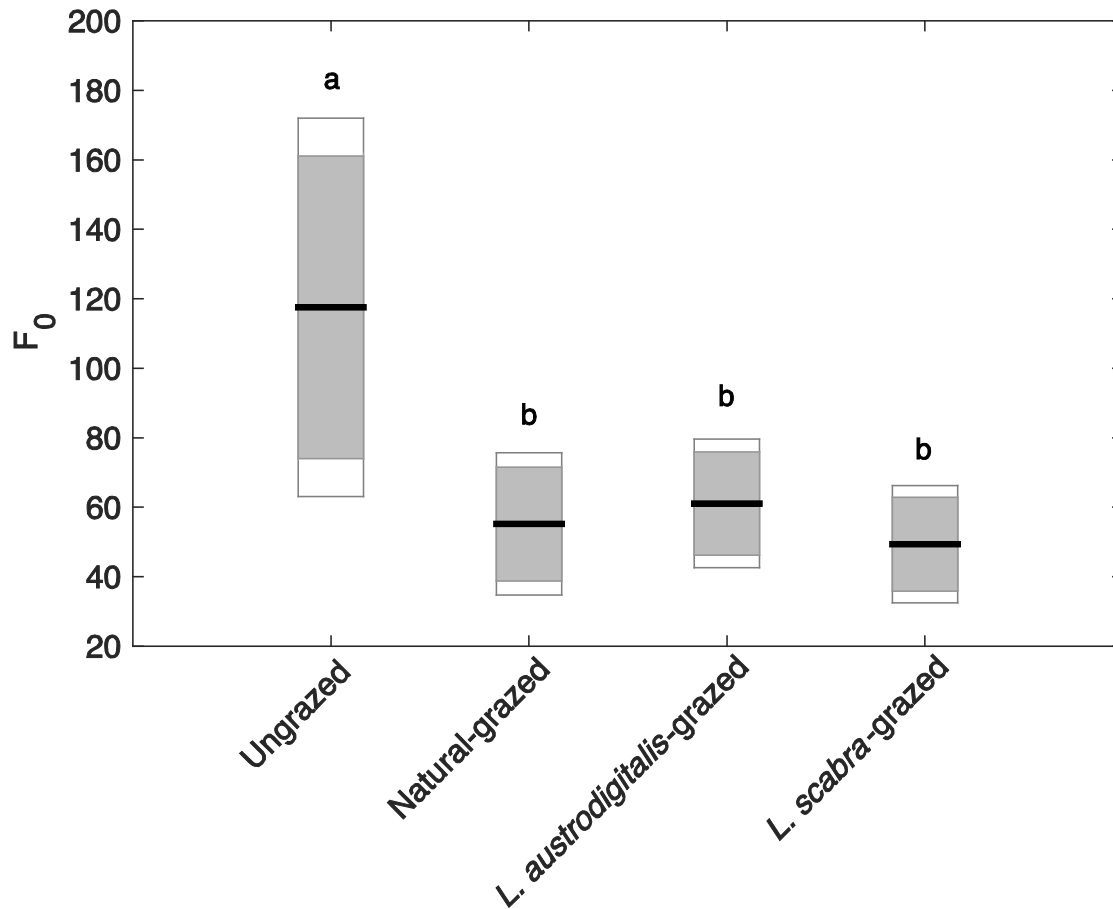
<p><b>Filamentous cyanobacteria:</b>  <b>1</b> - 800nm x 150nm  <b>2</b> - 200µm x 50µm, includes branches (see arrow)  <b>3</b> - bead diameter 10µm  <b>4</b> - bead diameter &lt; 10µm  <b>5</b> - width &lt; 7µm</p>	<p><b>7</b> - Pointed, 10µm x 5µm  <b>8A</b> - Boxy, 40µm x 20µm  <b>8B</b> - Can form chains, as shown  <b>9</b> - Can form chains, 25µm x 7µm  <b>10</b> - Can form chains, 13µm x 8µm  <b>11</b> - Less than 10µm x 5µm  <b>12</b> - Tapered, length 70µm</p>	<p><b>14</b> - Diamond pattern, length ~ 30-60µm  <b>Coccoidal Cyanobacteria:</b>  <b>15</b> - Diameter &lt; 5µm  <b>Non-Biological:</b>  <b>16</b> - Other, not shown  <b>17</b> - Rubber grip tape (biofilm substrate)</p>
<p><b>Diatoms:</b>  <b>6</b> - Pointed, 30µm x 10µm</p>	<p><b>Encrusting Organisms:</b>  <b>13</b> - Encrusting, undefined shape</p>	

869

870 **Figure 1. Descriptions and images of morphotypes observed in the MPB.** Each morphotype is  
 871 identified by a number (1-17), and pictured above at 300x unless otherwise indicated. Scale bars are



872 10µm unless otherwise indicated, circles highlight the specific organism being described and all  
873 measurements provided are approximate. Arrows point to features referred to in the right-hand panel.  
874 The bottom panel includes each morphotype number, in addition to the organisms broader  
875 classification (filamentous cyanobacteria, diatom, encrusting organism or coccoidal cyanobacteria) and  
876 a summary of the shape and size details used to identify the organism.  
877



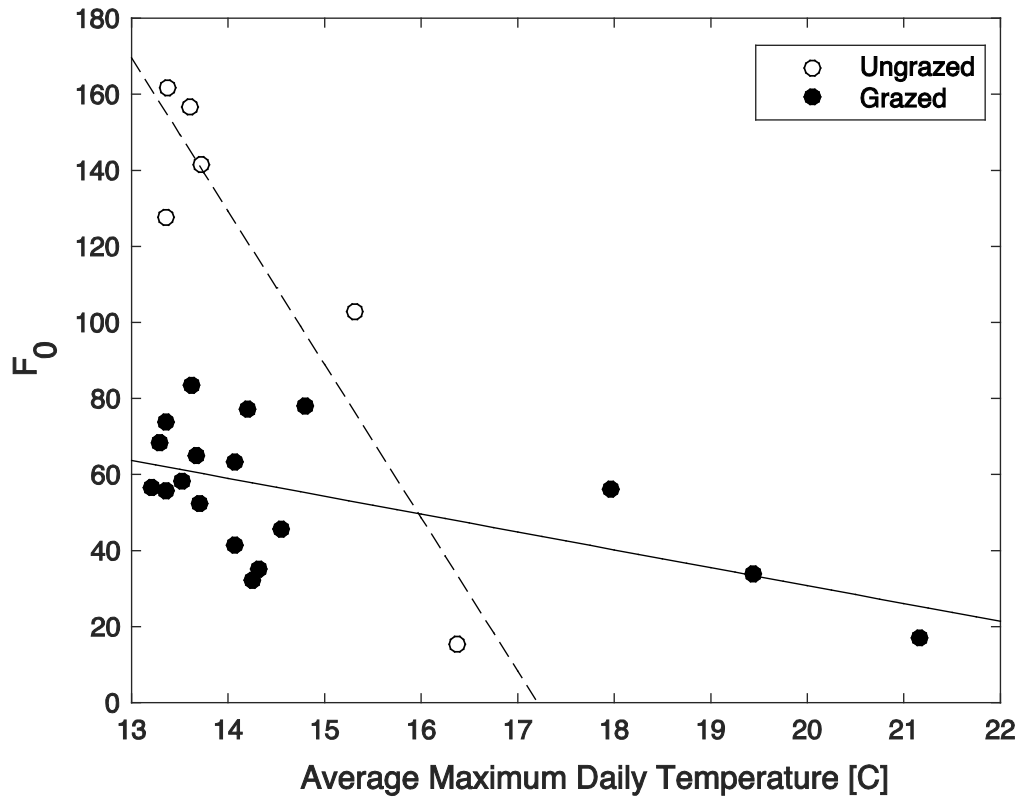
878

879 **Figure 2. The effect of grazing treatment on  $F_0$ .** The *L. scabra*-grazed, *L. austrodigitalis*-grazed and  
 880 natural-grazed treatments exhibited decreased chlorophyll density ( $F_0 = 55.2 \pm 5.2$ ) relative to the  
 881 ungrazed treatment ( $F_0 = 117.5 \pm 22.2$ ) during the month prior to SEM sample collection. Black lines  
 882 indicate the mean values for each treatment, grey rectangles represent one standard deviation and white  
 883 rectangles represent 95% confidence intervals (n = 6 for all treatments). Letters indicate significantly  
 884 different  $F_0$  values, as determined by Tukey's HSD test for multiple comparisons.

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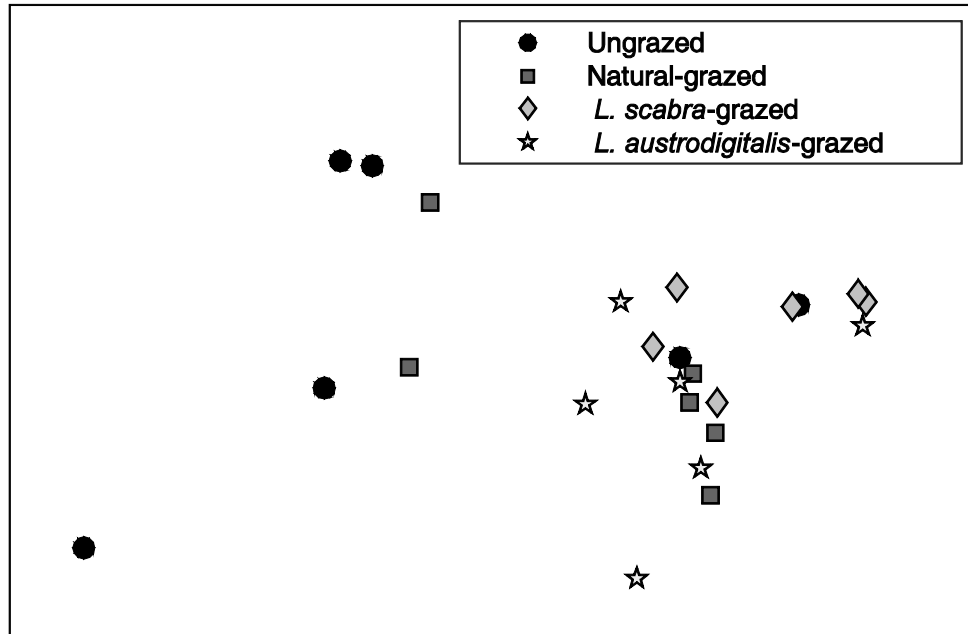


888

889 **Figure 3. Correlations between average maximum daily temperature and  $F_0$  differed between**  
 890 **treatments.** Average maximum daily temperature and  $F_0$  were significantly more negatively correlated  
 891 on ungrazed plates ( $n = 6$ ) than on grazed plates ( $n = 18$ ,  $F_{3,16} = 9.47$ ,  $p < 0.001$ ). Temperatures above  
 892  $16^\circ\text{C}$  reduced  $F_0$  values on ungrazed plates to those typical of grazed plates. Grazing masked this  
 893 effect of temperature, keeping  $F_0$  values at about half of those on ungrazed plates even when  
 894 temperatures were less than  $16^\circ\text{C}$ .

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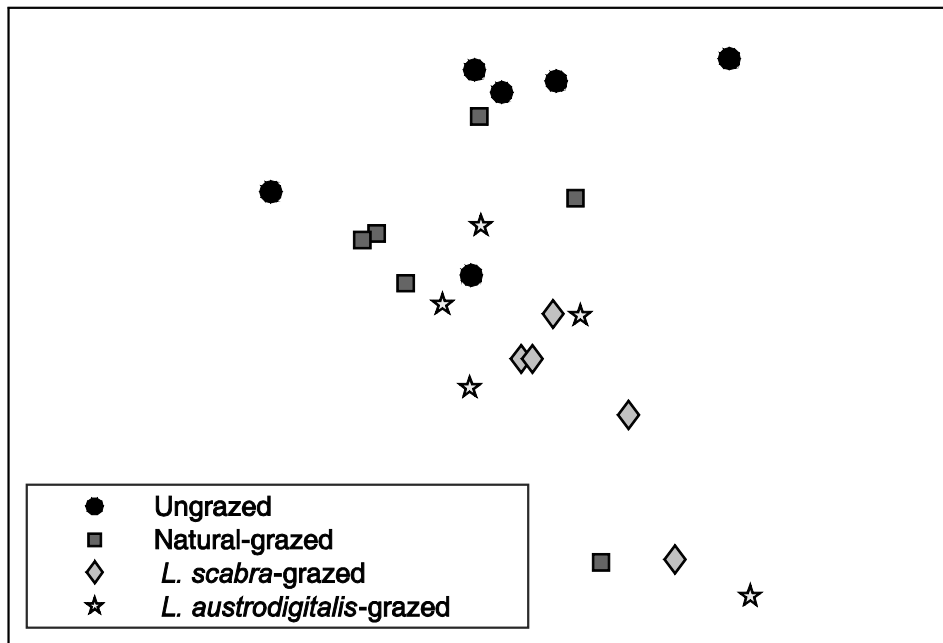


897

898 **Figure 4. Ungrazed MPB was significantly different from grazed MPB at the organism level.** The  
 899 assemblage structure of the ungrazed treatment was significantly different from that of the *L. scabra*-  
 900 grazed and *L. austrodigitalis*-grazed treatments when considering untransformed data ( $R = 0.284$ ,  $p =$   
 901  $0.035$  and  $R = 0.247$ ,  $p = 0.048$  respectively); the natural-grazed treatment was significantly different  
 902 from the *L. scabra*-grazed but not the *L. austrodigitalis*-grazed treatment ( $R = 0.185$ ,  $p = 0.041$  and  $R =$   
 903  $-0.068$ ,  $p = 0.766$  respectively); and no significant difference was observed between *L. scabra* and *L.*  
 904 *austrodigitalis*-grazed assemblages ( $R = 0.117$ ,  $p = 0.123$ ).

905

906



907

908 **Figure 5. Ungrazed MPB was significantly different from grazed MPB at the morphotype level.**

909 The assemblage structure of the ungrazed treatment was significantly different from that of the *L.*

910 *scabra*-grazed and *L. austrodigitalis*-grazed treatments when considering untransformed data ( $R =$

911  $0.559$ ,  $p = 0.002$  and  $R = 0.274$ ,  $p = 0.006$  respectively); the natural-grazed treatment was significantly

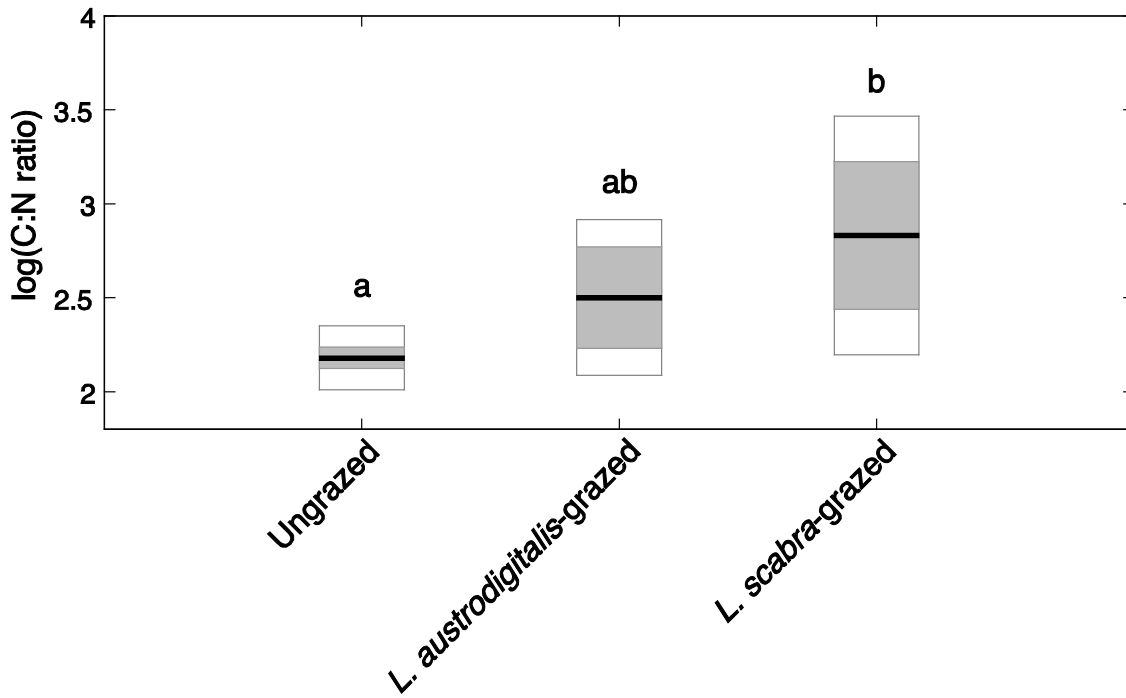
912 different from the *L. scabra*-grazed but not the *L. austrodigitalis*-grazed treatments ( $R = 0.293$ ,  $p =$

913  $0.039$  and  $R = 0.078$ ,  $p = 0.229$  respectively); and no significant difference was observed between *L.*

914 *scabra* and *L. austrodigitalis*-grazed assemblages ( $R = -0.069$ ,  $p = 0.652$ ).

915

916



917

918 **Figure 6. Effects of grazing treatment on C:N ratio.** Grazing by *L. scabra* significantly increased  
 919 the log(C:N) ratio of the MPB ( $F_{2,45} = 12.98$ ,  $p < 0.001$ ). However, C:N ratios of *L. austrodigitalis*-  
 920 grazed samples were intermediate between ungrazed and *L. scabra*-grazed samples, and were not  
 921 significantly different from either sample. Site was not a significant factor in this analysis ( $F_{5,45} = 1.38$ ,  
 922  $p = 0.248$ ), and Tukey's HSD multiple comparisons test was used to determine differences between  
 923 grazing treatments. Black lines indicate the mean values for each treatment, grey rectangles represent  
 924 one standard deviation and white rectangles represent 95% confidence intervals ( $n_{\text{ungrazed}} = 34$ ,  $n_{L. scabra-}$   
 925  $\text{grazed}} = 10$ ,  $n_{L. austrodigitalis-}$   
 926  $\text{grazed}} = 9$ ). Letters indicate significantly different C:N ratios.

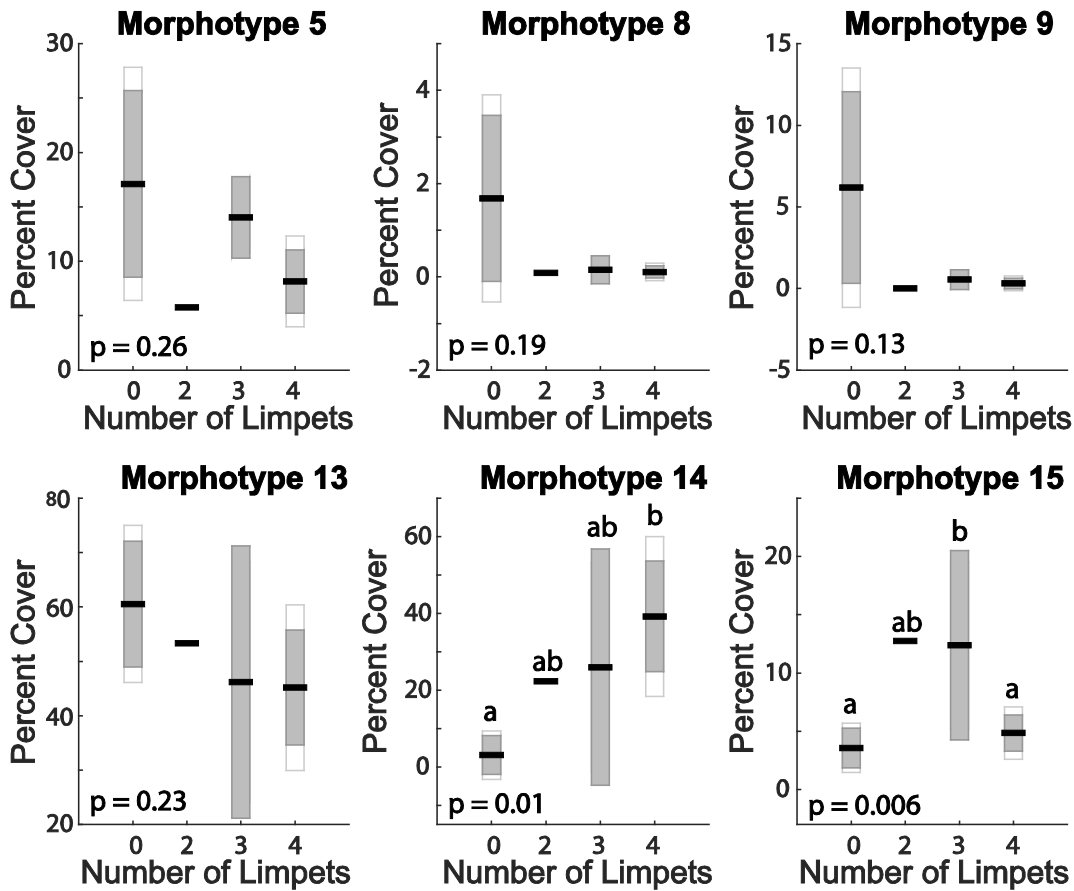
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933 **Figure 7. Effects of number of grazers on percent cover of selected morphotypes.** On average,  
 934 more limpet grazers resulted in lower percent cover of encrusting organisms. This was primarily due to  
 935 increases in morphotype 14; morphotype 13 decreased as the number of grazers increased. Diatoms  
 936 (morphotypes 8 and 9) were present in very low percentages if any grazers were present. The percent  
 937 cover of filamentous cyanobacteria (morphotype 5) was, on average, reduced on grazed plates, but  
 938 exhibited no correlation with number of grazers. Finally, percent cover of coccoidal cyanobacteria  
 939 (morphotype 15) was highest at intermediate numbers of grazers. Tukey's HSC multiple comparisons  
 940 test was used to determine differences between limpet densities. Black lines indicate the mean values  
 941 for each treatment, grey rectangles represent one standard deviation and white rectangles represent

942 95% confidence intervals ( $n_0 = 6$ ,  $n_2 = 1$ ,  $n_3 = 3$ ,  $n_4 = 8$ ). Letters indicate significantly different percent  
943 covers of morphotypes.



944 **Supplementary Materials**

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946 **Table S1. ANOVA table for Figure 4.**

Source	SS	df	MS	F	Prob>F
Grazing Treatment	17879.2	3	5959.74	5.94	0.0045
Site	9341.9	5	1868.38	2.62	0.068
Error	10709.5	15	713.97		
Total	37930.7	23			

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948 **Table S2. ANOVA table for Figure 5.**

Source	SS	df	MS	F	Prob>F
Grazing Treatment	15375.4	3	5125.13	15.55	0.0001
Temperature	5412.3	1	5412.3	16.42	0.0009
Interaction	9364.9	3	3121.63	9.47	0.0008
Error	5274.2	16	329.64		
Total	35426.8	23			

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950 **Table S3. ANOVA table for Figure 7.**

Source	SS	df	MS	F	Prob>F
Grazing Treatment	3.3804	2	1.6902	14.73	0
Site	0.7939	5	0.15878	1.38	0.2484
Error	5.16375	45	0.11475		
Total	9.46316	52			

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