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4 . *Journal of the Marine Biological Association of the United Kingdom*, 99(8), 1779-
5 1786. doi:[10.1017/s0025315419000778](https://doi.org/10.1017/s0025315419000778)

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8 Running Head: limpet-barnacle interactions

9 **Barnacle cover modifies foraging behaviour of the intertidal limpet,**

10 ***Patella vulgata* L**

11

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31 *Limpets and barnacles are important components of intertidal assemblages*
32 *worldwide. This study examines the effects of barnacles on the foraging behaviour of*
33 *the limpet *Patella vulgata* , which is the main algal grazer in the North West Atlantic.*
34 *The behaviour of limpets on a vertical seawall on the Isle of Man (UK) was*
35 *investigated using autonomous radio-telemetry, comparing their activity patterns on*
36 *plots characterised by dense barnacle cover and plots from which the barnacles had*
37 *been removed. Additionally, the effects of the presence or absence of barnacles were*
38 *evaluated at two different levels on the shore. This experiment revealed a significant*
39 *effect of barnacle cover on the foraging activity of *P. vulgata*. Limpets on smooth*
40 *surfaces spent a greater proportion of total time active than did limpets on barnacles.*
41 *Foraging activity was also greater in areas that were lower down in the tidal range*
42 *In general, limpets were either predominantly active during diurnal high or low tides*
43 *and always avoided nocturnal high tides. Individuals on barnacles on the upper shore*
44 *concentrated their activity during nocturnal low water. All the other groups of limpets*
45 *(smooth surfaces on the upper level and all individuals on the lower shore) had more*
46 *excursions centred around daylight hours with an equal distribution of activity*
47 *between periods of low and high water. Inter-individual variability was, however,*
48 *pronounced.*

49

50 **Keywords: grazing, rocky shore, *Patella vulgate*, biotic interactions, behavioural**
51 **plasticity, timing of activity**

52

53 INTRODUCTION

54 Intertidal habitats are characterised by a cyclical change of abiotic stress factors
55 (temperature, desiccation, wave action) and biotic risks, primarily predation (Branch
56 1981; Hawkins & Hartnoll, 1983; Chapman & Underwood, 1992; Raffaelli and
57 Hawkins, 1996). Hence for mobile organisms, time is typically partitioned into
58 periods of foraging, when risks are greater, and periods in refuges that offer relative
59 shelter from predators and environmental stressors. Foraging, therefore, represents
60 trade-offs between the requirement for food resources, the quality of the food
61 available and the risks associated with acquiring these resources (Burrows *et al.*,
62 2000; Clark and Mangel, 2000; Santini *et al.*, 2014).

63 The direct effects of abiotic and biotic factors in influencing foraging
64 behaviour are well documented for a wide range of intertidal species (Hawkins and
65 Hartnoll, 1983; Chapman and Underwood, 1992; Raffaelli and Hawkins, 1996; Little
66 *et al.*, 2009 for reviews). These influences may be further modified as a result of the
67 indirect effects of other organisms. In some cases such effects may be relatively
68 predictable (e.g. a dense algal canopy can provide shelter from desiccation and refuge
69 from predators, Moore *et al.*, 2007), but sometimes relatively subtle differences in the
70 abundance of one species can have dramatic and often unpredictable indirect effects
71 on the behaviour and abundance of another species (Anderson, 1999; Trussell *et al.*,
72 2006; O'Connor *et al.*, 2013; Rashidul Alam and Noda, 2016). Moreover, the costs
73 and benefits of foraging are variable in both time and space, and to maximise benefits
74 and minimise risk at any given time it is important for an organism to be able to react
75 to locally changing conditions and modify its behaviour. The ability to do so varies
76 among individuals and among species according to the relative importance of their

77 endogenous rhythms and the ability to override them in response to exogenous cues
78 (Little, 1989; Santini *et al.*, 2004, 2005).

79 Limpets are an important component of intertidal assemblages worldwide and
80 their grazing is known to have a key role in the ecology of these habitats (Hawkins,
81 1981; Hawkins and Hartnoll, 1983; Jenkins *et al.*, 2005; Coleman *et al.*, 2006;
82 Burgos-Rubio *et al.*, 2015). Reducing the density of these grazers is consistently
83 followed by a proliferation of micro and then macroalgae and can lead to substantial
84 changes in assemblage composition and ecosystem functioning on rocky shores
85 (Hawkins *et al.*, 1992; Poore *et al.*, 2012). Barnacles are sessile filter feeders, being
86 major occupiers of space on exposed and moderately exposed shores and are common
87 worldwide (Lewis, 1964; Stephenson and Stephenson, 1972). Due to the importance
88 of both limpets and barnacles, investigating their interactions is essential for a better
89 understanding of the dynamics of rocky shore communities (Hartnoll and Hawkins,
90 1985; Johnson *et al.*, 1997,1998; Burrows and Hawkins, 1998; Hawkins *et al.*, 2008;
91 Jenkins *et al.*, 2008). Limpets are known to dislodge barnacle cyprids that have
92 recently settled from the plankton (e.g. Hawkins, 1983; Dungan, 1986; Jenkins *et al.*,
93 1999; Holmes *et al.*, 2005), but information on the effects of barnacles on limpets
94 living among barnacles are scarcer. Growth rates and maximum sizes of limpets can
95 be reduced where barnacles and other sessile organisms are abundant (Lewis and
96 Bowman, 1975; Choat, 1977; Thompson, 1980; Hawkins and Hartnoll, 1982;
97 Dunmore and Schiel, 2003) and there is anecdotal evidence that the rugosity of
98 barnacle dominated surfaces may impede the ability of limpets to deposit the mucous
99 trails along which they glide to facilitate locomotion, limiting their ability to adhere to
100 the substratum and hence to resist dislodgement by predators and wave action (Smith,
101 1991). Fraser *et al.* (2014) demonstrated that topographic complexity associated with

102 barnacles affects the resting orientation of limpets on vertical surfaces. Finally, there
103 is also evidence that barnacles may provide a greater resource of microalgae, on
104 which limpets feed, than that present on adjacent areas of open rock (Thompson *et al.*,
105 1996). Understanding the effect of the presence of barnacles on limpet behaviour
106 may help clarify the mechanisms underlying recruitment of algae to rocky shores. For
107 example, it is known that barnacles may promote furoid recruitment (e.g. Hawkins,
108 1981), but it is not yet clear whether this effect is entirely due to an increase of refugia
109 for algal propagules or if a modification of limpet behaviour may play a role.

110 Here we examine the effects of barnacles on the foraging behaviour of the
111 homing limpet *Patella vulgata* (Linnaeus, 1758). This species is a putative keystone
112 grazer on North-East Atlantic rocky shores (Jenkins *et al.*, 2005; Coleman *et al.*,
113 2006), often being used as a model organism to investigate the behaviour of intertidal
114 grazers and its influence on the dynamics of algal-grazer interactions (e.g., Thompson
115 *et al.*, 1997, 2004; Burrows and Hawkins, 1998; Johnson *et al.* 1998; Jenkins *et al.*,
116 2000; Jonsson *et al.*, 2006). Previous studies on the behaviour of *P. vulgata* have
117 revealed considerable variability in the timing of foraging activity. Populations from
118 different localities have been shown to be active during different phases of the tidal
119 and day/night cycles (e.g. Hawkins and Hartnoll, 1982, 1983; Little, 1989; Williams
120 *et al.*, 1999); but variability has also been described among limpets belonging to the
121 same population but resting in different micro-habitats (i.e., vertical versus horizontal
122 surfaces) (e.g. Della Santina *et al.*, 1994; Santini *et al.*, 2004). Several factors, such
123 as desiccation stress, dislodgement risks and/or predation have also been invoked to
124 explain the observed patterns at different sites, season of the year, micro-habitat or
125 level on the shore. Preliminary work by Hawkins and Hartnoll (1982) indicated that
126 barnacle cover can influence tide out foraging behaviour on a vertical barnacle

127 dominated surface, where limpets were observed foraging when the tide is out as well
128 as during both day and night provided conditions were damp, but not when raining. In
129 this paper, the behaviour of limpets was investigated using autonomous radio-
130 telemetry on the same vertical harbour wall described by Hawkins and Hartnoll
131 (1982). We compared their activity patterns on plots characterised by dense barnacle
132 cover and plots from which the barnacles were experimentally removed. Given that it
133 is known that the behaviour of limpet may change with height on the shore, we
134 explored the effect of barnacle cover at two different tidal heights.

135 We hypothesised that barnacle cover may affect limpet behaviour in two
136 different ways. The first detectable effect should be on the temporal budget of
137 activity. Since barnacles may offer greater resources of microalgae than smooth
138 surfaces, while at the same exposing limpets to greater risks of dislodgement, we
139 hypothesised that limpets on barnacles would be *time-minimizers* (Evans and
140 Williams, 1991; Santini and Chelazzi, 1996) when compared to limpets on smooth
141 concrete. A second hypothesised effect is that the presence of barnacles influences the
142 distribution of foraging effort among each of the four available activity windows (tide
143 out daytime, tide in daytime, tide out night-time, tide in night-time). Dense barnacle
144 cover is likely to affect the ability to adhere to the substratum when foraging, hence
145 increasing the risk of dislodgement and vulnerability to predation, forcing limpets to
146 be active during less risky time periods. In particular we expect that limpets living on
147 vertical barnacle covered surfaces will be more active during tide-out periods (lower
148 risks of dislodgement and predation), especially at night (reduced desiccation stress),
149 than limpets on smooth surfaces. As small limpets are easier to prey upon than larger
150 ones and lose water more readily, we expect that this pattern would be more
151 pronounced in smaller than larger limpets. Therefore, as a corollary to the second

152 hypothesis, we also checked whether body size has any subtle influence on the
153 behaviour of animals living on rough or smooth surfaces, despite the range of sizes
154 employed in this study being intentionally narrow. We thus expected small limpets on
155 barnacles to be more selective in the choice of the less risky foraging phase, than
156 larger limpets.

157

158 MATERIALS AND METHODS

159 The study was carried out at Port Erin, Isle of Man (UK). Data were collected during
160 three periods each of approximately two weeks over the spring-neap cycle during the
161 period 21st April to 11th June 1999. The study area consisted of a vertical harbour
162 wall, The Raglan Pier, which is faced in concrete, also used by Hawkins and Hartnoll
163 (1982). The base of this pier wall was covered by coarse sand just above mean low
164 water springs (MLWS) (0.75 m above lowest astronomical tide, LAT) and the wall
165 extends in height to well above mean high water springs (MHWS). The study was
166 conducted between approximately mid tide level (~ 2.8 m above LAT) and mean high
167 water neaps (MHWN) (4.6 m above LAT, Figure 1) in the region of the pier covered
168 with barnacles (> 95% cover). Tides are semidiurnal in this location with maximum
169 height of around 5.0 m above LAT during spring tides.

170 The concrete face of the pier wall had conveniently been constructed with 120
171 cm by 90 cm blocks. During early spring 1999 barnacles were scraped from the entire
172 surface of several blocks at each of two tidal levels. For each level four scraped, and
173 four interspersed unscraped blocks were used. Care was taken not to dislodge limpets
174 during the removal of barnacles. A period of one month was allowed for the limpets
175 and the biofilm on the scraped surface to recover from this disturbance before
176 behavioural recording started. The recordings ended 2.5 months from disturbance;

177 thus there should have been sufficient time for recovery of microalgae (Hill, 1990).
178 One month was sufficient for new limpet shell growth to occur to fit the concrete
179 around the home scar. However, we cannot exclude the possibility that microalgae
180 were still in the recovery phase when the recordings were carried out.

181 The response, in terms of limpet foraging behaviour, to the following factors was
182 considered:

- 183 1. Type of substratum ('substratum', SU): fixed factor with two levels
184 ("barnacle", "smooth").
- 185 2. Height on the shore ('height', HE): a fixed factor with two levels 'upper' and
186 'lower', each corresponding to one of the two rows of blocks used (Figure 1).

187 Limpet behaviour was monitored using the telemetric technique described by
188 Santini *et al.* (2001) that allows the long-term recording of activity rhythms for
189 homing species. Briefly, the system consisted of 30 reed switches each mounted on
190 metal support arms. Five limpets were selected in each of four blocks of each
191 treatment at each shore height. Individuals in the central portion of each block were
192 used wherever possible so as to minimise the influence of any edge effects. Reed
193 switches were positioned so as to overhang the home scars of these individuals, but
194 sufficiently far away so as not to interfere with access by the limpets to and from their
195 home scars. Small magnets (weight ~ 1.5 g) were attached with Milliput epoxy putty
196 to the shell of the limpet on each of these home scars and the length of each individual
197 recorded. Each of the 30 individual switches was connected via a cable to a
198 transmitter positioned well above MHWs. Sea level was monitored at 1 minute
199 intervals using Seamon TD water pressure loggers, positioned at the average tidal
200 height of the limpets in both upper and lower tidal levels (Figure 1). Every five
201 minutes the equipment assigned each limpet a home or away signal. This information

202 was transmitted to a Televilt RX-900 scanner/logger, located at the Port Erin Marine
203 Laboratory of the University of Liverpool (now closed). The data were then combined
204 with information on the state of the tide obtained from the Seamon loggers and the
205 time of dawn and dusk, obtained from tide tables, to give the activity status of each
206 individual in relation to the four temporal windows described. After two weeks the
207 reed switches were repositioned over different individuals and magnets attached to the
208 new individual as before. Thus each two week period, encompassing a spring-neap
209 tidal cycle, provided data on a different set of approximately 30 animals. In some
210 cases an animal did not yield sufficient useful information, for example because it
211 relocated home scar or went missing during the experiment. Data from these
212 individuals were discarded from subsequent analyses. The final fully balanced design
213 included fifteen individuals in each experimental condition (upper and lower shore,
214 plus and minus barnacles). Limpets used in the analyses were within the size range 25
215 – 36mm (mean 29.6) and the upper shore limpets were slightly larger (average values
216 \pm SE: upper = 30.2 ± 0.1 , upper = 28.8 ± 0.1 mm, t-test $P=0.036$), reflecting vertical
217 gradients in size in this species.

218 At the beginning of the sampling periods limpets that were to be monitored
219 with the telemetric apparatus, together with a further 20 individuals in each
220 experimental treatment, were uniquely labelled with micro-marker number tags. The
221 home scars of these individuals were also labelled. Records of the position of the
222 limpets as being home or away were made by visual observation twice during each
223 time window, either by accessing the site from the sand below the harbour wall at low
224 tide, or by SCUBA at high tide. For tagged individuals that were active at these times
225 the substratum on which the animal was foraging was noted. These data were used to
226 cross check the data provided by the Televilt equipment, but also to monitor if limpets

227 living on barnacles had moved to forage on smooth surfaces or the reverse. The
228 presence of any predators was also recorded during these visual observations.
229 Previous observations showed that the main tide-in predators were the crabs *Necora*
230 *puber* (Linnaeus, 1767), *Carcinus maenas* (Linnaeus, 1758) and *Cancer pagurus*
231 (Linnaeus, 1758) (Thompson *et al.*, 2000; confirmed by more recent work by Silva *et*
232 *al.*, 2008, 2010a,b). Predation by floating gulls on emersed limpets at the waterline
233 occurred very occasionally (personal observation).

234 Chippings from the surface of the pier were collected from each experimental
235 treatment (six replicates each) to obtain an estimate of microbial food resources by
236 chlorophyll extraction (Thompson *et al.*, 1999). In the case of the barnacle-covered
237 surfaces, the barnacle plates were scraped from the concrete and then the soft body
238 parts of each individual barnacle were removed with forceps so as to obtain an
239 estimate of the microbial biomass from the outside of the barnacle plates only. This
240 was only undertaken at the end of the final monitoring period because of its
241 destructive nature.

242 Two distinct aspects of behaviour were compared among treatments:

243 1. Long-term activity budget, for which the following variables were computed from
244 individual time series:

245 a. Proportion of time active (RT), computed as the ratio between the time spent
246 by each individual away from home and the total recording time;

247 b. Average duration of foraging bouts (DU, hours);

248 c. Number of foraging bouts/day (NB) performed by each limpet.

249 2. Selection for given tidal and diel phases, for which the following variables were
250 computed:

- 251 a. Proportion of activity performed during emersion periods (ET), computed as
252 the ratio between the time spent active during tide-out and total activity time;
253 b. Proportion of activity performed during night-time (NT), computed as the
254 ratio between the time spent active during the night and total activity time.

255 The effect of the two factors considered for the long-term budget of activity was
256 investigated through two-way ANOVA (Underwood, 1997) using the GMAV
257 software package. The relationship between activity, limpet length, height on the
258 shore and surface rugosity was analysed using ANCOVA and linear regression (Sokal
259 and Rohlf, 1995). The joint variation in the proportion of activity performed during
260 emersion periods and the proportion of activity during night-time (ET and NT,
261 respectively) was assessed through non-parametric multivariate analysis of variance
262 (PERMANOVA), according to Anderson (2001).

263

264 RESULTS

265 The analysis of chlorophyll content confirmed that barnacle-covered surfaces
266 (irrespective of tidal height) had considerably greater standing stock of microalgal
267 food than concrete surfaces (average chlorophyll content, 13.56 ± 0.67 vs $1.23 \pm$
268 $0.178 \mu\text{g}\cdot\text{cm}^{-2}$, $F_{1,20} = 291.06$, $P < 0.001$). There was no detectable effect of height on
269 the shore on chlorophyll content ($F_{1,20} = 0.01$, $P = 0.937$).

270 In terms of foraging activity across all possible tidal windows, the average
271 proportion of the total time recorded during which limpets were active (proportion
272 active, RT) was affected both by rugosity and height but not by their interaction
273 (Table 1; Figure 2). In particular, activity was greater on smooth surfaces than on
274 barnacles (Figure 2a) and on the lower shore than the upper shore (Figure 2b). The
275 comparison of the number of foraging excursions per day revealed a similar effect of

276 both substratum type (Table 1; Figure 2c) and tidal height (Figure 2d), but not of their
277 interaction. Finally the average duration of each foraging excursion (Table 1; Figure
278 2e-f) was affected by substratum but not by height on the shore or their interaction.

279 A summary of the analysis of the choice of foraging phases is shown in Figure
280 3 and Table 2. Timing of activity, measured as the joint change in the fraction of
281 activity performed during emersion (ET) and during night-time (NT), was very
282 variable but, in general, limpets were either predominantly active during diurnal high
283 tides or diurnal low tides. A few limpets were also active during diurnal low tides ,
284 especially on damp days. Whereas nocturnal high tides were always avoided (Figure
285 3a). Choice of the foraging phase was affected by a first order interaction between
286 presence/absence of barnacles and height on the shore, as revealed by PERMANOVA
287 (Table 2) and was evident from the phase-space portrait of average NT and ET values
288 from the different groups of limpets (barnacle upper-shore, smooth upper-shore,
289 barnacle lower-shore and smooth lower-shore) reported in Figure 3b. Individuals on
290 barnacles on the upper shore were generally more active during low water (ET ~70%)
291 at night (NT~60%); whilst individuals on smooth surfaces on the upper level and all
292 individuals on the lower shore tended to have more similar behaviour and favoured
293 excursions centred in daylight hours (NT~40%) with a reasonably equal distribution
294 of activity between periods of low and high water (ET ~50%).

295 Closer inspection of individual average activity reveals more subtle details on
296 the strategy followed by each individual limpet (Figure 3a). Limpets on barnacles on
297 the upper shore showed the greatest variability in the choice of activity phase and
298 despite the majority (n=9) being active during nocturnal low tides a few were clearly
299 more active during diurnal high tides (bottom left part of the plot), whereas others
300 seemed to adopt a mixed strategy (characterised by individual ET and NT values

301 close to 0.5). Individual, but less pronounced, variability was also present in other
302 groups.

303 The size range of the limpets used in these experiments was relatively narrow;
304 there were, however, significant relationships between the size of individuals and
305 their behaviour. ANCOVA showed that the fraction of activity performed during
306 emersion (ET) was influenced by the interaction between size of the limpets and
307 height on the shore, but not by the type of substratum (Table 3; Figure 4). In
308 particular, ET values decreased with increased size for limpets living on the high
309 shore; but not for those living on the lower part of the wall ($F_{1,28} = 0.38$, $P > 0.05$; $F_{1,28}$
310 $= 16.35$, $P < 0.001$, respectively).

311 Finally, the direct observation of limpet behaviour revealed that none of the
312 limpets on barnacles moved to forage on nearby smooth areas, whereas the reverse
313 was true. In fact, approximately 27% of foraging excursions of limpets living on
314 smooth surfaces were observed to occur on barnacle covered areas.

315

316 DISCUSSION

317 We revealed a significant effect of barnacle cover on the foraging behaviour of
318 *Patella vulgata*. Effects were detected for many of the components of its behaviour
319 (overall activity budget, choice of specific foraging time window) and varied between
320 limpets of different size.

321 Limpets living on barnacles spent a lower proportion (~19%) of their time
322 active than limpets on smooth surfaces (~26%), performed a lower number of
323 excursions each day and these were shorter than that of limpets on smooth surfaces,
324 thus confirming they were *time-minimizers*. The most likely determinant of this
325 pattern is the difference in microalgal standing stock, which was more than 10 times

326 higher on barnacles than on smooth surfaces: clearly the more rich the food supply
327 available the lower the time needed to obtain a specific energy level. A similar pattern
328 has been described previously when comparing the differences in standing stock and
329 grazing between sheltered and exposed shores (e.g. Jenkins and Hartnoll, 2001). That
330 barnacle-covered areas are more energy-rich and hence attractive to limpets seems to
331 have been confirmed by direct observations, which showed that 27% of tagged
332 limpets on smooth surfaces moved to forage on barnacle-covered blocks. In contrast,
333 movements in the opposite direction were never observed. Direct comparison of
334 standing crop between smooth and barnacle covered surfaces may, however, be
335 difficult as the exact surface area to be grazed is not known. On one hand, barnacles
336 increase the rugosity of the substrate and hence the surface available for algal growth.
337 On the other, however, it is likely that not all of the microbial material living in small
338 pits and cracks amongst the barnacle mosaic is available to the limpets (Hill and
339 Hawkins, 1991; Thompson *et al.*, 1996; Hutchinson *et al.*, 2006).

340 The second likely determinant of the difference in time budgets between
341 limpets on barnacle and non-barnacle covered surface is represented by increased
342 risks. Limpets are known to adhere to the substratum using a combination of suction
343 and gluing (Smith, 1991; Denny, 2000). Suction is suggested to be the main
344 mechanism whilst foraging (Smith, 1992), but for suction to work, the edge of the
345 foot must be sealed to the substratum. This is hard to achieve when moving on an
346 irregular surface. In addition, suction may provide good resistance to hydrodynamic
347 lift, but provides poor resistance to shear forces (Ellem *et al.*, 2002). On vertical
348 surfaces, where individuals are subject to a constant downward gravity force, the
349 reduction of adherence may easily become critical, making limpets more susceptible

350 to dislodgement by waves and predation (e.g. Coleman *et al.*, 1999; Thompson *et al.*,
351 2000; Silva *et al.*, 2008; Fraser *et al.*, 2014).

352 Choice of the diel/tidal window for foraging showed a preference for being
353 active either during daytime submersion or low-tide at night, a finding broadly in line
354 with previous work on the behaviour of this species (e.g. Hartnoll and Wright, 1977;
355 Hawkins and Hartnoll, 1982; Williams *et al.*, 1999; Santini *et al.*, 2004). This pattern
356 is dictated by a trade-off between different types of stresses and risks, for example,
357 desiccation during daytime tide-out periods and predation during nocturnal tide-in
358 periods. During the unused temporal window of high water at night (consistent with
359 Hawkins and Hartnoll, 1982), predatory crabs (*Necora puber*, *Carcinus maenas* and
360 *Cancer pagurus*) were commonly observed on the experimental blocks and on the
361 seabed beneath the pier wall during high water at night (see also work by Silva *et al.*,
362 2008; 2010a,b, 2014. on crab predation on limpets and foraging in the intertidal
363 zone). Marks from crab chelae (Thompson *et al.*, 2000b) were also observed on the
364 surface of the resin used to fix magnets on the limpets and on one occasion a crab was
365 seen eating a limpet..

366 Whilst confirming previous findings, our study provides new insights on the
367 choice of the temporal window for activity in *P. vulgata*. Limpets living on barnacles
368 on the upper shore clearly differed from all other groups, being the only group
369 spending most of their activity during nocturnal low tides: although considerable
370 within-group, inter-individual, differences were evident. Hawkins and Hartnoll (1982)
371 working at mid-shore showed that barnacle removal affected limpet behaviour,
372 reducing their foraging activity during emersion. Our results support this finding but
373 only for limpets on the upper shore and not for those on the lower shore, which
374 behaved similarly to limpets on smooth surfaces. Limpets from other treatment groups

375 appeared to be more day-active, although more unselective than previously reported
376 (e.g. Santini *et al.*, 2004). For these groups, in fact, the average proportion of activity
377 performed during emersion (ET) and night-time (NT) were in fact close to 0.5,
378 meaning that an “average” limpet had no specific preference for either high or low
379 tide or day and night. This average pattern was, however, dictated by a mix of inter-
380 individual differences (where each limpet had a preference for a specific temporal
381 windows but individual limpets adopting different behaviours coexisted within a
382 group) and intra-individual variation (when the same limpet is active during different
383 times). Interestingly, part of this variability is due to size-related effects although,
384 contrary to our expectations, no effect of substrate type was detected. However an
385 effect of height on the shore was evident, and a clear negative relationship between
386 the proportion of activity during low tide and size was detected in high shore limpets.
387 Size-related differences in the foraging of *P. vulgata* have been described in previous
388 studies (e.g. Little *et al.*, 1988; Della Santina *et al.*, 1995; Santini *et al.*, 1995) but
389 results of the present investigation add to this knowledge and reveal finer details not
390 described in previous studies. It is important to underline that the range of sizes used
391 in this study was relatively narrow (25-36 mm) if compared with that explored in
392 previous studies, (e.g. a range 25-56 mm was explored by Della Santina *et al.*, 1995)
393 thus suggesting greater influence than previously reported. Of course, large limpets
394 incur comparatively greater costs than smaller ones to be active during emersion,
395 given they are heavier and taller (and hence subject to a greater downward drag).
396 However, why this was evident only for those high but not low on the shore it is not
397 yet clear. Higher up they may be subject to less risk from tide in crab predation.

398 In conclusion, our study showed the importance of indirect effects of
399 biologically mediated substratum type - the presence of barnacle cover - on the

400 behaviour of *P. vulgata*. It also showed that individual differences in behaviour exist
401 and can be important. Such a variability may challenge the notion of Potential
402 Activity Phase (PAP, Evans and Williams, 1991), at least intended as a single time
403 window available to each limpet for foraging (see also Santini *et al.*, 2011). Despite
404 this the concept of PAP may be useful to understand the determinants of population
405 level behaviour within a specific time window, and may be a reasonable
406 simplification for solving optimization problems (Burrows *et al.*, 2000). Finally, our
407 findings may potentially be extended to the behaviour of other intertidal grazers.
408 Given the importance of both limpets and barnacles on rocky shores throughout the
409 Northeast Atlantic (see Hawkins *et al.*, 1992 and Jenkins *et al.*, 2008 for reviews),
410 information on their indirect and direct interactions is of considerable importance in
411 understanding the dynamics of these systems.

412

413 ACKNOWLEDGMENTS

414 Many thanks are due to Paolo Della Santina, Cosimo Tendi and Nicoletta Righini for
415 they help and encouragement during field work. Author contributions: RCT and SJH,
416 designed the experiment, RCT and SRJ collected the data; RCT and GS analysed the
417 data; GS, RCT, SJH wrote the manuscript with input from SRJ, RGH and GC; GC
418 developed the concepts and techniques and with RGH provided equipment and
419 logistical support. Many thanks are due to two anonymous referees, whose comments
420 greatly improved the manuscript.

421

422 FINANCIAL SUPPORT

423 Field work was funded by the European Union [Eurorock project (contract MAS3-
424 CT95-0012)]. R.C.T., S.J.H. and R.G.M. were also supported by a Leverhulme Grant
425 (F/180/AO) on limpet foraging behaviour.

426

427 REFERENCES

428 **Anderson M.J.** (1999) Distinguishing direct from indirect effects of grazers in
429 intertidal estuarine assemblages. *Journal of Experimental Marine Biology and*
430 *Ecology* 234:199–218.

431 **Anderson M.J.** (2001) A new method for non-parametric multivariate analysis of
432 variance. *Austral Ecology* 26:32–46.

433 **Branch G.M.** (1981) The biology of limpets: physical factors, energy flow and
434 ecological interactions. *Oceanography and Marine Biology, An Annual Review*
435 19:235–380.

436 **Burgos-Rubio V., De la Rosa J., Altamirano M. and Espinosa F.** (2015) The role of
437 patellid limpets as omnivorous grazers: a new insight into intertidal ecology.
438 *Marine Biology* 162: 2093–2106.

439 **Burrows M.T. and Hawkins S.J.** (1998) Modelling patch dynamics on rocky shores
440 using deterministic cellular automata. *Marine Ecology Progress Series* 167:1–13.

441 **Burrows MT, Santini G, Chelazzi G** (2000) A state-dependent model of activity
442 patterns in homing limpets: balancing energy returns and mortality risks under
443 constraints on digestion. *Journal of Animal Ecology* 69:290–300.

444 **Chapman M.G. and Underwood A.J.** (1992) Foraging behaviour of marine benthic
445 grazers. In: John DM, Hawkins SJ, Price JH (eds) *Plant-animal interactions in*
446 *the marine benthos*. Clarendon Press.

447 **Choat J.H.** (1977) The influence of sessile organisms on the population biology of
448 three species of acmaeid limpets. *Journal of Experimental Marine Biology and*
449 *Ecology* 26:1–26.

450 **Clark C.W. Mangel M.** (2000) *Dynamic state variable models in ecology: methods*
451 *and applications*. Oxford University Press, New York.

- 452 **Coleman R.A., Benedetti-Cecchi L., Åberg P., Arenas F., Arrontes J., Castro J.,**
453 **Hartnoll R.G., Jenkins S.R., Paula J., Della Santina P., Underwood A.J. and**
454 **Hawkins S.J.** (2006). A Continental scale evaluation of limpet grazing on rocky
455 shores. *Oecologia* 147:556–564.
- 456 **Coleman R.A., Goss-Custard J.D., Le V., Dit Durell S.E.A. and Hawkins S.J.**
457 (1999) Limpet *Patella* spp. consumption by oystercatchers *Haematopus*
458 *ostralegus*: a preference for solitary prey items. *Marine Ecology Progress Series*
459 183:253–261.
- 460 **Della Santina P., Santini G. and Chelazzi G.** (1995) Factors affecting variability of
461 foraging excursions in a population of *Patella vulgata* (Mollusca: Gastropoda).
462 *Marine Biology* 122: 65–270.
- 463 **Denny M.W.** (2000) Limits to optimization: Fluid dynamics, adhesive strength, and
464 the evolution of shape in limpet shells. *Journal of Experimental Biology*
465 203:2603–2622.
- 466 **Dungan M.L.** (1986) Three-way interactions: barnacles, limpets, and algae in a
467 Sonoran desert rocky intertidal zone. *The American Naturalist* 127:292–316.
- 468 **Dunmore R.A. and Schiel D.R.** (2003) Demography, competitive interactions and
469 grazing effects of intertidal limpets in southern New Zealand. *Journal of*
470 *Experimental Marine Biology and Ecology* 288:17–38.
- 471 **Ellem G.K., Furst J.E. and Zimmerman K.D.** (2002) Shell clamping behaviour in
472 the limpet *Cellana tramoserica*. *Journal of Experimental Biology* 205:539–547.
- 473 **Evans M.R. and Williams G.A.** (1991) Time partitioning of foraging in the limpet
474 *Patella vulgata*. *Journal of Animal Ecology* 60:563–575.
- 475 **Fraser C.M.L., Coleman R.A. and Seebacher F.** (2014) Trying to fit in: are patterns
476 of orientation of a keystone grazer set by behavioural responses to ecosystem
477 engineers or wave action? *Oecologia* 174:67–75.
- 478 **Hartnoll R.G. and Hawkins S.J.** (1985) Patchiness and fluctuations on moderately
479 exposed rocky shores. *Ophelia* 24:53–63.
- 480 **Hartnoll R.G. and Wright J.R.** (1977) Foraging movements and homing in the
481 limpet *Patella vulgata*. *Animal Behaviour* 25:806–810.

- 482 **Hawkins S.J.** (1981) The influence of season and barnacles on algal colonisation of
483 *Patella* exclusion zones. *Journal of the Marine Biological Association of the UK*
484 61:1–15.
- 485 **Hawkins S.J.** (1983) Interactions of *Patella* and macroalgae with settling
486 *Semibalanus balanoides* (L.). *Journal of Experimental Marine Biology and*
487 *Ecology* 71:55–72.
- 488 **Hawkins S.J. and Hartnoll R.G.** (1982) The influence of barnacle cover on the
489 numbers, growth and behaviour of *Patella vulgata* on a vertical pier. *Journal of the*
490 *Marine Biological Association of the UK* 62:855–867.
- 491 **Hawkins S.J. and Hartnoll R.G.** (1983) Grazing of intertidal algae by marine
492 invertebrates. *Oceanography and Marine Biology, An Annual Review* 21:195–282.
- 493 **Hawkins S.J., Hartnoll R.G., Kain J.M. and Norton T.A.** (1992) Plant-animal
494 interactions on hard substrata in the north-east Atlantic. In: John DM, Hawkins
495 SJ, Price JH (eds) *Plant-animal interactions in the marine benthos*. Clarendon
496 Press
- 497 **Hill A.S.** (1990) *The grazing of microbial films on moderately exposed shores on the*
498 *Isle of Man*. PhD Thesis, University of Liverpool, UK.
- 499 **Hill A.S. and Hawkins S.J.** (1991). Seasonal and spatial variation of epilithic
500 microalgal distribution and abundance and its ingestion by *Patella vulgata* on a
501 moderately exposed rocky shore. *Journal of the Marine Biological Association of*
502 *the UK* 71: 403-423.
- 503 **Hawkins S.J., Moore P.J., Burrows M.T., Poloczanska E., Mieszkowska N.,**
504 **Herbert R.J.H., Jenkins S.R., Thompson R.C., Genner M.J. and Southward**
505 **A.J.** (2008) Complex interactions in a rapidly changing world: responses of
506 rocky shore communities to recent climate change. *Climate Research* 37:123–
507 133.
- 508 **Holmes S.P., Walker G. and van der Meer J.** (2005) Barnacles, limpets and
509 periwinkles: the effects of direct and indirect interactions on cyprid settlement
510 and success. *Journal of Sea Research* 53:181–204.

- 511 **Hutchinson N., Nagarkar S., Aitchison J.C. and Williams G.A.** (2006)
512 Microspatial variation in marine biofilm abundance on intertidal rock surfaces.
513 *Aquatic Microbial Ecology* 42:187–197.
- 514 **Jenkins S.R., Coleman R.A., Hawkins S.J., Burrows M.T. and Hartnoll R.G.**
515 (2005) Regional scale differences in determinism of grazing effects in the rocky
516 intertidal. *Marine Ecology Progress Series* 287:77–86.
- 517 **Jenkins S.R. and Hartnoll R.C.** (2001) Food supply, grazing activity and growth in
518 the limpet *Patella vulgata*: a comparison between exposed and sheltered shores.
519 *Journal of Experimental Marine Biology and Ecology* 258:123–129.
- 520 **Jenkins S.R., Hawkins S.J. and Norton T.A.** (1999) Direct and indirect effects of a
521 macroalgal canopy and limpet grazing in structuring a sheltered inter-tidal
522 community. *Marine Ecology Progress Series* 188: 81–92.
- 523 **Jenkins S.R., Moore P., Burrows M.T., Garbary D.J., Hawkins S.J., Ingólfsson**
524 **A., Sebens K.P., Snelgrove P.V.R., Wethey D.S. and Woodin S.A.** (2008)
525 Comparative ecology of North Atlantic shores: do differences in players matter
526 for process? *Ecology* 89: S3–S23.
- 527 **Johnson M.P., Burrows M.T., Hartnoll R.G. and Hawkins S.J.** (1997) Spatial
528 structure on moderately exposed rocky shores: patch scales and the interactions
529 between limpets and algae *Marine Ecology Progress Series* 160:209–215.
- 530 **Johnson M.P., Burrows M.T. and Hawkins S.J.** (1998) Individual based simulations
531 of the direct and indirect effects of limpets on a rocky shore *Fucus* mosaic. *Marine*
532 *Ecology Progress Series* 169:179–188.
- 533 **Lewis J.R.** (1964) *The Ecology of Rocky Shores*. English Universities Press, London.
- 534 **Lewis J.R. and Bowman R.S.** (1975) Local habitat induced variations in the
535 population dynamics of *Patella vulgata* L. *Journal of Experimental Marine*
536 *Biology and Ecology* 17:165–203.
- 537 **Little C.** (1989) Factors governing patterns of foraging activity in littoral marine
538 herbivorous molluscs. *Journal of Molluscan Studies* 55:273–284.

- 539 **Little C., Williams G.A., Morritt D., Perrins J.M. and Stirling P.** (1988) Foraging
540 behaviour of *Patella vulgata* L. in an Irish sea-lough. *Journal of Experimental*
541 *Marine Biology and Ecology* 120:1–21.
- 542 **Little C., Williams G.A. and Trowbridge C.D.** (2009) *The biology of rocky shores.*
543 Second Edition, Biology of Habitats Series, Oxford University Press, Oxford.
- 544 **Moore P., Hawkins S.J. and Thompson R.C.** (2007) Role of biological habitat
545 amelioration in altering the relative responses of congeneric species to climate
546 change. *Marine Ecology Progress Series* 334:11–19.
- 547 **O'Connor N.E., Emmerson M.C., Crowe T.P. and Donohue I.** (2013),
548 Distinguishing between direct and indirect effects of predators in complex
549 ecosystems. *Journal of Animal Ecology* 82: 438–448.
- 550 **Poore A.G.B., Campbell A.H., Coleman R.A., Edgar G.J., Jormalainen V.,**
551 **Reynolds P.L., Sotka E.E., Stachowicz J.J., Taylor R.B., Vanderklift M.A.**
552 **and Duffy J.E.** (2012) Global patterns in the impact of marine herbivores on
553 benthic primary producers. *Ecology Letters* 15:912–922.
- 554 **Raffaelli D. and Hawkins S.J.** (1996) *Intertidal ecology.* Chapman and Hall, London.
- 555 **Rashidul Alam A.K.M. and Noda T.** (2016) An experimental evaluation of the direct
556 and indirect effects of endemic seaweeds, barnacles, and invertebrate predators on
557 the abundance of the introduced rocky intertidal barnacle *Balanus glandula.*
558 *Population Ecology* 58:507–514.
- 559 **Santini G. and Chelazzi G.** (1996) Energy Maximization Vs. Time Minimization in
560 the Foraging of the Limpet *Patella vulgata.* *Journal of Animal Ecology* 65:599–
561 605.
- 562 **Santini G., Chelazzi G. and Della Santina P.** (1995) Size-related functional and
563 energetic constraints in the foraging of the limpet *Patella vulgata* (Mollusca,
564 Gastropoda). *Functional Ecology* 9:551–558.
- 565 **Santini G., Ngan A., Burrows M.T., Chelazzi G., Williams G.A.** (2014) What drives
566 foraging behaviour of the intertidal limpet *Cellana grata*? A quantitative test of a
567 dynamic optimization model. *Functional Ecology* 28:963–972.

- 568 **Santini G., Ngan A. and Williams G.A.** (2011) Plasticity in the temporal organization
569 of behaviour in the limpet *Cellana grata*. *Marine Biology* 158:1377–1386.
- 570 **Santini G., Righini N. and Chelazzi G.** (2001) Automatic telemetry to monitor the
571 activity of limpets and sea level oscillations. *Journal of the Marine Biological*
572 *Association of the UK* 81:699–700.
- 573 **Santini G., Tendi C., Righini N., Thompson R.C. and Chelazzi G.** (2005)
574 Preliminary observation on intra-specific variability in the temporal organisation
575 of foraging of the limpet *Patella caerulea*. *Ethology Ecology & Evolution* 17:65–
576 75.
- 577 **Santini G., Thompson R.C., Tendi C., Hawkins S.J., Hartnoll R.G. and Chelazzi**
578 **G.** (2004) Intra-specific variability in the temporal organisation of foraging
579 activity in the limpet *Patella vulgata*. *Marine Biology* 144:1165–1172.
- 580 **Silva A.C.F., Boaventura D.M., Flores A., Ré P. and Hawkins S.J.** (2004) Rare
581 predation by the intertidal crab *Pachygrapsus marmoratus* on the limpet *Patella*
582 *depressa*. *Journal of the Marine Biological Association of the UK* 84:367–370.
- 583 **Silva A.C.F., Hawkins S.J., Boaventura D.M. and Thompson R.C.** (2008) Predation
584 by small mobile aquatic predators regulates populations of the intertidal limpet
585 *Patella vulgata* (L.). *Journal of Experimental Marine Biology and Ecology*
586 367:259–265.
- 587 **Silva A.C.F., Hawkins S.J., Boaventura D.M., Brewster E. and Thompson R.C.**
588 (2010a) Use of the intertidal zone by mobile predators: influence of wave
589 exposure, tidal phase and elevation on abundance and diet. *Marine Ecology*
590 *Progress Series* 406:197–210.
- 591 **Silva A.C.F., Hawkins S.J., Clarke K.R., Boaventura D.M., and Thompson R.C.**
592 (2010b) Preferential feeding by the crab *Necora puber* on differing sizes of the
593 intertidal limpet *Patella vulgata*. *Marine Ecology Progress Series* 416: 179–188.
- 594 **Smith A.M.** (1991) The role of suction in the adhesion of limpets. *Journal of*
595 *Experimental Biology* 161:151–169.
- 596 **Smith A.M.** (1992) Alternation between attachment mechanisms by limpets in the
597 field. *Journal of Experimental Marine Biology and Ecology* 160:205–220.

- 598 **Sokal, R.R. and Rohlf, F.J.** (1995) *Biometry: the principles and practice of statistics*
599 *in biological research*. 3rd Edition, W.H. Freeman and Co., New York.
- 600 **Stephenson T.A. and Stephenson A.** (1972) Life between tidemarks on rocky shores.
601 *Journal of Animal Ecology* 43:606–608.
- 602 **Thompson G.B.** (1980) Population dynamics of the limpet *Patella vulgata* L. in Bantry
603 Bay. *Journal of Experimental Marine Biology and Ecology* 45:173–217.
- 604 **Thompson R.C., Tobin M., Hawkins S.J. and Norton T.A.** (1999) Problems in
605 extraction and spectrophotometric determination of chlorophyll from epilithic
606 microbial biofilms: towards a standard method. *Journal of the Marine Biological*
607 *Association of the UK* 79:551–558.
- 608 **Thompson R.C., Wilson B.J., Tobin M.L., Hill A.S. and Hawkins S.J.** (1996)
609 Biologically generated habitat provision and diversity of rocky shore organisms at
610 a hierarchy of spatial scales. *Journal of Experimental Marine Biology and*
611 *Ecology* 202:73–84.
- 612 **Thompson R.C., Jenkins, S.R. and Bussell J.A.** (2000). A method for recording
613 predator-prey encounters between crabs and limpets using wax replicas. *Journal of*
614 *the Marine Biological Association of the UK* 80:633–638.
- 615 **Trussell G.C., Ewanchuk P.J. and Matassa C.M.** (2006) Habitat effects on the
616 relative importance of trait- and density-mediated indirect interactions. *Ecology*
617 *Letters* 9:1245–1252.
- 618 **Underwood A.J.** (1997) *Experiments in ecology*. Cambridge University Press,
619 Cambridge.
- 620 **Williams G.A., Little C., Morrill D., Stirling P., Teagle L., Miles A., Pilling G. and**
621 **Consalvey M.** (1999) Foraging in the limpet *Patella vulgata*: the influence of rock
622 slope on the timing of activity. *Journal of the Marine Biological Association of the*
623 *UK* 79:881–889.

624 Figure legends:

625

626 **Fig 1.** Schematic representation of the study site and sampling design. Grey blocks
627 are barnacle covered areas, whilst white blocks represent smooth surfaces.

628

629 **Fig 2.** Variation in the proportion of time active, mean number of excursion per day
630 and average duration of excursions, across surface type (a,c,e) and tidal level (b,d,f),
631 respectively. Average values and standard errors are shown. Asterisks show P values:
632 * P < 0.05; ** P < 0.01, otherwise non-significant

633

634 **Fig 3.** a) Mean individual proportion of activity performed during emersion (ET) and
635 night (NT) by limpets in the different groups (closed circles = barnacle top shore;
636 closed triangles = barnacles low shore; open circles = smooth surface top shore, open
637 triangles = smooth bottom shore). b) Mean (\pm SE) group ET and NT values.

638

639 **Fig 4.** Relationship between the proportion of activity performed during emersion
640 (ET) and limpet shell length, according to height on the shore and substratum type
641 (closed circles = barnacle top shore; closed triangles = barnacles low shore; open
642 circles = smooth surface top shore, open triangles = smooth bottom shore).
643 Continuous line = relationship observed for limpets high on the shore; dashed line =
644 relationship for low-shore limpets

Table 1. ANOVA on activity of *Patella vulgata* on surfaces with and without barnacles at each of two tidal on the Raglan Pier, Isle of Man, UK.

Source	df	Proportion active			Excursions per day			Duration of excursions		
		MS	F	P	MS	F	P	MS	F	P
Substratum (SU)	1	0.116	21.51	<0.001	0.214	9.12	0.0038	0.120	4.34	0.042
Height (HE)	1	0.066	12.26	<0.001	0.174	7.42	0.009	0.004	0.13	0.716
SUXHE	1	0.020	3.95	0.060	0.023	0.98	0.33	0.020	0.71	0.403
RES	56	0.005			0.023			0.028		
		Arcsine (proportion)			square root			log x+1		

Data were transformed as indicated at the bottom of the table. C, Cochran's test for homogeneity of variances; n.s. not significant.

Table 2. Results of PERMANOVA on the joint variation in the proportion of activity performed during emersion periods and the proportion of activity during night-time on surfaces with and without barnacles at each of two tidal levels.

Source	df	MS	F	<i>P</i>
SU	1	0.804	7.49	0.006
HE	1	1.369	12.76	<0.001
SUxHE	1	0.463	4.31	0.032
RES	56	0.107		

Euclidean distance dissimilarity was used and 4999 random permutations of residuals under the reduced model were performed. Variable names as in Table 1.

Table 3. Results of ANCOVA to evaluate the relationships between the size of limpets and their behaviour.

Source	df	MS	F	P
Size (SL)	1	0.022	0.913	0.344
SU	1	0.002	0.129	0.721
HE	1	0.137	5.700	0.021
SLxHE	1	0.102	4.213	0.045
SLxSU	1	0.002	0.083	0.775
SUxHE	1	0.010	0.399	0.530
LExSUxHE	1	0.006	0.259	0.613
RES	52	1.254		

Variable names as in Table 1.