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Macroalgae contribute to the diet of *Patella vulgata* from contrasting conditions of latitude
and wave exposure in the UK

Consumption of macroalgae by *Patella vulgata*

Gillian M. Notman*^{1,4,5}, Rona A. R. McGill², Stephen J. Hawkins^{3,5}
and Michael T. Burrows⁴

¹Department of Science, Natural Resources and Outdoor Studies, University of Cumbria,
Ambleside, UK, LA22 1BB (gill.notman@cumbria.ac.uk); ²NERC Life Sciences Mass
Spectrometry Facility, Scottish Universities Environmental Research Centre, East Kilbride,
UK, G75 0QF; ³Ocean and Earth Science, National Oceanography Centre Southampton,
University of Southampton, UK, SO14 3ZH; ⁴ Scottish Association for Marine Science,
Scottish Marine Institute, Oban, UK, PA37 1QA. ⁵ Marine Biological Association of the
UK, The Laboratory, Citadel Hill, Plymouth, UK, PL1 2PB.

16 **Abstract**

17 Analysis of gut contents and stable isotope composition of intertidal limpets (*Patella vulgata*)
18 showed a major contribution of macroalgae to their diet, along with microalgae and
19 invertebrates. Specimens were collected in areas with limited access to attached macroalgae,
20 suggesting a major dietary component of drift algae. Gut contents of 480 animals from two
21 moderately wave exposed and two sheltered rocky shores in each of two regions: western
22 Scotland (55-56°N) and southwest England (50°N), were analysed in two years (n = 30 per
23 site per year). The abundance of microalgae, macroalgae and invertebrates within the guts
24 was quantified using categorical abundance scales. Gut content composition was compared
25 among regions and wave exposure conditions; showing that the diet of *P. vulgata* changes
26 with both wave exposure and latitude. Microalgae were most abundant in limpet gut contents
27 in animals from southwest sites, whilst leathery/corticated macroalgae were more prevalent
28 and abundant in limpets from sheltered and northern sites. *P. vulgata* appears to have a more
29 flexible diet than previously appreciated and these keystone grazers consume not only
30 microalgae, but also large quantities of macroalgae and small invertebrates. To date, limpet
31 grazing studies have focussed on their role in controlling recruitment of macroalgae by
32 feeding on microscopic propagules and germlings. Consumption of adult algae suggests *P.*
33 *vulgata* may also directly control the biomass of attached macroalgae on the shore, whilst
34 consumption of drift algae indicates the species may play important roles in coupling subtidal
35 and intertidal production.

36

37 **Introduction**

38 The intertidal limpet, *Patella vulgata*, is the most abundant grazer on rocky shores in
39 the north east Atlantic and plays an important role in structuring intertidal communities
40 throughout its range (Hawkins & Hartnoll 1983, Hawkins et al. 1992). Classic limpet
41 removal experiments (Jones 1948, Lodge 1948, Burrows & Lodge 1950, Southward 1964),
42 observations following the mass limpet kills in the aftermath of the Torrey Canyon oil spill
43 clean-up (Southward & Southward 1978, Hawkins & Southward 1992) and limpet exclusion
44 experiments (Hawkins 1981, Hartnoll & Hawkins 1985, Jenkins et al. 2005, Coleman et al.
45 2006, Jonnson et al. 2006) have all shown that removal or exclusion of limpets produces lush
46 growth of canopy forming furoid macroalgae on wave exposed and moderately exposed
47 rocky shores. These indirect studies have led to the conclusion that *P. vulgata* is a keystone
48 species in the intertidal, preventing macroalgal growth through microphagous grazing of
49 epilithic biofilm and associated macroalgal propagules (spores and germlings) (Hill &
50 Hawkins 1991, Jenkins & Hartnoll 2001, Jenkins et al. 2005, Coleman et al. 2006, Jonnson et
51 al. 2006, Moore et al. 2007). This view is supported by observational studies of limpet
52 foraging behaviour (Hartnoll & Wright 1977, Little et al. 1988, Della Santina et al. 1994) and
53 several studies which directly examined limpet gut contents (Hawkins et al. 1989, Hill &
54 Hawkins 1991, Little et al. 1990).

55 More recent work suggests that *P. vulgata* may play an even more significant role in
56 structuring rocky shore ecosystems through direct grazing of mature macroalgae as well as
57 biofilm and propagules. On many more sheltered shores *P. vulgata* aggregate around
58 established stands of *Ascophyllum nodosum* and *Fucus vesiculosus* and ‘bite marks’ are
59 visually apparent on the algal fronds. The fronds are often much shorter than might be
60 expected, consistent with the ends being directly grazed away by the nearby limpets (Davies
61 et al. 2007, Davies et al. 2008, personal observation). Lorenzen (2007) also recorded *P.*

62 *vulgata* feeding on stranded macroalgae during periods of emersion, and the results of stable
63 isotope analyses from several sources provide strong evidence to suggest that macroalgae
64 may be a more significant source of organic carbon to *P. vulgata* than previously appreciated
65 (Campbell 2004, Riera et al. 2009, Schaal et al. 2010, Notman 2011).

66 The limited number of studies which have examined gut contents in *P. vulgata* to date
67 probably give an incomplete view of the dietary range of *P. vulgata*. Sample sizes for foregut
68 analysis were small and acid digestion methods may have provided biased information on
69 only a subset of the diet rather than representing whole gut contents (Hawkins et al. 1989,
70 Hill & Hawkins 1991). This has led to an overemphasis on grazing of the microalgal biofilm
71 by the species, and a potential underestimation of their capacity for feeding on adult or
72 detrital macroalgae. Quantification of whole diet composition without such bias is lacking,
73 and is crucial for a re-evaluation of the role of the species as a keystone grazer across its
74 range.

75 The overall aim of this study was to compare the diets of limpets between contrasting
76 latitudinal and wave exposure conditions. Specifically we quantified the relative importance
77 of microalgal biofilms and other food sources across these locations. We focussed on the diet
78 of limpets collected from bare rock microhabitats where access to other types of foods was
79 restricted. Gut contents of adult *P. vulgata* (30-50 mm in length) were examined from wave
80 exposed and wave sheltered sites in western Scotland and southwest England over two years.
81 Analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios from a subset of these
82 animals, together with their potential foods, was used to provide additional information on
83 limpet diets integrated over longer periods of time.

84

85 **Materials and Methods**

86 **Study sites & sample collection**

87 *Patella vulgata* were collected from mid-shore bare rock microhabitats at four sites in
88 southwest England, near Plymouth, Devon, and four sites in western Scotland, around Oban
89 and the Kintyre peninsula. Two moderately wave-exposed (300 – 500 km fetch) and two
90 sheltered (< 50 km fetch) sites were selected in each region, with the ranges of wave
91 exposure set using calculated values for wave fetch in accordance with Burrows *et al.* (2008).
92 Adult limpets were collected from areas of bare rock in spring 2005 and 2006. These habitats
93 seldom comprised entirely clean rock, but also often contained small areas of macroalgae and
94 barnacles. To investigate the potential influence of these alternate food sources on diet
95 composition, we visually estimated the percentage cover of bare rock, barnacle and attached
96 macroalgal cover in the mid-intertidal using twenty five 0.5 × 0.5 m quadrats at the eight sites
97 in each year. Limpets were immediately chilled upon collection to minimise digestion of gut
98 contents, and frozen (-20°C) on return to the laboratory. Thirty animals of similar size (mean
99 length 42 ± 4 mm) were selected from each site per year for gut contents analysis. A subset
100 of twelve limpets were randomly selected for stable isotope analysis from the different
101 locations and wave exposure conditions, and samples of potential foods (biofilm and
102 macroalgae) were collected at each site in each year. Macroalgal species included
103 *Ascophyllum nodosum*, *Fucus vesiculosus*, *F. serratus*, *F. spiralis*, *Himanthalia elongata*, and
104 detrital *L. digitata*.

105 **Dissection of gut contents**

106 The digestive organs of *P. vulgata* are long and complex, extending from the mouth
107 and buccal mass, through the crop and stomach to the long intestine embedded in the visceral
108 mass (Fretter & Graham, 1962). Limpets were dissected ventrally, whilst partially frozen,
109 with shells attached. The muscular foot and internal organs were completely removed to

110 reveal the crop and anterior portion of the stomach. Only the anterior sections of the
111 alimentary canal were excised as the posterior sections are difficult to separate from the
112 digestive gland and visceral mass.

113 **Examination of gut contents**

114 Dissected gut contents were initially examined at $\times 4$ magnification, and ingested algae
115 and invertebrates identified and quantified using categorical abundance scales (Table 1). It
116 was not possible to identify ingested macroalgae to species level. These were therefore
117 classified according to Steneck and Watling's (1982) functional group scheme (Table 1), with
118 categories 4 and 5 (corticated and leathery macroalgae) combined due to the difficulties in
119 objectively and consistently discriminating between these groups in gastropod guts (Raffaelli,
120 1985, personal observation). The abundance of microalgae (Group 1 including diatoms and
121 cyanobacteria) was assessed at $\times 20$ magnification using a compound microscope. A 1 cm²
122 subsample of material was covered with a glass coverslip and five fields of view were
123 randomly selected for closer examination.

124 **Stable isotope analysis**

125 Tissue from the muscular foot of *P. vulgata* was chosen for isotope analysis because
126 muscle tissues integrate consumer diets over long periods of time (Hobson & Clark 1992)
127 and, due to their low fat content, do not require lipid extraction (Pinnegar & Polunin 1999).
128 Dissected foot tissues from 48 of the limpets used in gut contents analysis were rinsed in
129 deionised water and freeze dried to constant weight for stable isotope analysis.

130 Biofilm was obtained from three 200 cm² rock samples (free from macroalgae and
131 encrusting organisms) from each site in each year. The rocks were soaked in 0.7 μm filtered
132 seawater for 15 minutes in the laboratory and the rehydrated biofilm removed by brushing
133 with an electric toothbrush. Biofilm was filtered onto glass fibre filter paper (Whatman
134 GF/F), frozen at -20°C and freeze dried to constant weight. Samples of corticated/leathery

135 macroalgae were rinsed in deionised water and epibiont-free fronds were frozen at -20°C
136 before freeze drying. The stable isotope values of barnacles were not measured due to the
137 difficulties inherent in analysing inorganic carbon (Soreide *et al* 2006).

138 Samples were ground into homogenous powders, weighed into tin capsules (0.7 mg
139 limpet tissue, 1.5 mg macroalgal tissue and two 9 mm discs of biofilm/filter paper),
140 randomised and loaded into an automatic carousel for simultaneous analysis of carbon and
141 nitrogen isotopes using continuous-flow isotope ratio mass spectrometry (CF-IRMS)
142 (Costech model ECS 4010 elemental analyser coupled with a ThermoFinnegan Delta Plus XP
143 mass spectrometer). Two laboratory standards were analysed every 10 samples allowing
144 instrument drift to be corrected if required.

145 **Data analysis**

146 Abundance of algae and invertebrates ingested by limpets was compared between the
147 two regions, between the two classes of wave exposure and among the sites (nested within
148 region and exposure), using ordinal logistic regression (OLR, Minitab version 15.1.20,
149 MINITAB Inc.) on ranked categorical abundance data to determine influences on the
150 probability of obtaining particular abundance categories. Best OLR models were selected by
151 comparisons of deviance likelihood ratios following step-wise exclusion of factors from a
152 saturated model (Notman 2011).

153 Multivariate analyses were used to examine patterns of gut content composition
154 between the sampling regions and wave exposure conditions. The Gower similarity
155 coefficient (S_{15}) was used to obtain a matrix of similarities for examination of ordinal
156 categories of limpet gut contents (Gower, 1971; Podani, 1999; Legendre & Legendre, 1998).
157 Normalisation was not necessary as similar abundance scales were used for each variable.
158 Non-metric multidimensional scaling (MDS, PRIMER 6, PRIMER-E Ltd.) was used to
159 produce a two-dimensional ordination of gut contents data from the similarity matrix (Clarke,

160 1993). Similarity indices were used to determine the effects of region and wave exposure on
161 composition of limpet gut contents (2-way ANOSIM), with the contribution of each taxon to
162 differences among levels of each factor assessed using similarity percentage analysis
163 (SIMPER, based on the Euclidian distance measure of association).

164 Consumption of small invertebrates was assessed using counts of ingested items in a
165 hierarchical cluster analysis. A matrix of similarities of ingested fauna was calculated for
166 each site using the Bray-Curtis similarity coefficient following square root transformation of
167 ingested faunal abundance data (Bray & Curtis, 1957) and analyses of similarity (ANOSIM)
168 were used to assess whether the observed patterns of ingested invertebrates differed
169 significantly between sampling regions and wave exposure conditions.

170 Stable carbon and nitrogen isotope ratios of biofilm, macroalgae and *P. vulgata* foot
171 tissue were compared among the two regions, the two classes of wave exposure and the sites
172 (nested within region and exposure) using analysis of variance. Site was treated as a random
173 factor, with all other factors fixed and orthogonal. Stable isotope ratios for macroalgae were
174 calculated for region and exposure type combinations as the mean and standard deviation
175 across all species of corticated/leathery macroalgae for each site.

176 Habitat composition was evaluated using Mann-Whitney U-tests to compare percentage
177 cover of bare rock at sampling sites among regions and Wilcoxon signed ranks tests to
178 evaluate bare rock cover among levels of wave exposure within each region (with Bonferroni
179 corrections for multiple comparisons) (SPSS version 22.0, IBM Corp).

180

181 **Results**

182 **Study Sites**

183 The percentage cover of bare rock was similar between moderately exposed sites in
184 both regions at 92 % (Mann-Whitney U-test, $p = 0.75$). Although bare microhabitats were
185 deliberately chosen at all sites, the percentage bare rock was lower on sheltered shores,
186 around 88 % in the north and 84 % in the south, and differed significantly between regions
187 (Mann-Whitney U-test, $p < 0.01$). Percentage macroalgal and barnacle cover was highest at
188 southern sheltered shores at around 3 and 13 % respectively ($p < 0.01$ for all comparisons).

189 **Main constituents of limpet gut contents**

190 Most of the 480 animals examined had quantifiable gut contents (82 %). It was not
191 possible to identify the species of algae ingested, but microalgae (Group 1) and
192 corticated/leathery algae (Groups 4/5) were present in almost all of these samples: 90 % and
193 95 % of gut contents respectively. Filamentous (Group 2) algae were found in around 36 %
194 of gut samples, and foliose (Group 3), articulated calcareous (Group 6) and crustose coralline
195 (Group 7) algae were only present in around 10 % of the material analysed (Fig. 1).
196 Invertebrates were present in 91 of the 392 limpet gut contents (23 % overall).

197 **Changes in diet with latitude**

198 There were significant differences in limpet diets between the two sampling regions.
199 Microalgae were almost five times more likely to be recorded in high abundance categories
200 (common, abundant and superabundant) in gut contents of southern limpets than northern
201 limpets (ordinal logistic regression region effect, log odds ratio 1.514, SE 0.367, $p < 0.001$,
202 Table 2, Fig. 2). In contrast, corticated/leathery macroalgae were around twice as likely to be
203 recorded in higher abundance categories in northern limpets (Fig. 3). The odds ratio of 0.55
204 given in Table 2 indicates that high abundance categories of corticated/leathery algae were
205 around half as likely in the south than in the north ($p < 0.01$). Abundance of other types of

206 algae (filamentous, foliose, articulated calcareous or crustose coralline) was not influenced by
207 latitude ($p > 0.05$ for all comparisons).

208 **Effect of wave exposure on diet**

209 Microalgae were more prevalent in guts of limpets from exposed shores than sheltered
210 shores in the south (Fig. 2) but not in the north, and no overall trend in the abundance of
211 microalgae with wave exposure was seen. In the south, higher abundance categories for
212 microalgae were more likely at the exposed shores at Andurn and Picklecombe (sites SE1 and
213 SE2) than at the sheltered sites Jennycliff or Cawsand (SS1 or SS2; $p < 0.01$, Fig. 2, Table 2).
214 There were no significant differences in microalgal abundance among the sites in the north,
215 but there was some evidence that the probability of higher microalgal abundances was greater
216 in samples from the moderately exposed Easdale (NE1) than for those from the nearby,
217 sheltered Ellenabeich (NS1; odds ratio = 1.95, $p = 0.079$, Table 2).

218 Corticated/leathery algae were almost twice as likely to be at least common (Table 1) in
219 the gut contents of limpets from sheltered sites than in those from exposed sites (odds ratio =
220 1.96, $p < 0.001$, Table 2 and Fig. 3). Abundance categories of filamentous, foliose,
221 articulated calcareous or crustose coralline algae were not significantly affected by wave
222 exposure ($p > 0.05$ for all comparisons).

223 **Ingestion of invertebrates**

224 Small invertebrates were present in 23 % of quantifiable limpet gut contents and were
225 found at every site (9 to 42 % of limpets examined). Barnacles were the most frequently
226 ingested taxon, found in 15 % of limpet guts. The small snail *Skeneopsis planorbis* was
227 ingested by 6 % of limpets, and acarid mites were found in 3% of guts. Other taxa included
228 bivalves (present in 4 individuals), foraminifera (3), ostracods (3), *Littorina mariae* (2),
229 copepods (1), hydroids (1), *Melaraphe neritoides* (1), and *Patella vulgata* itself (1). Few
230 limpets had ingested more than one type of invertebrate (only 4 %). Hierarchical cluster

231 analysis of ingested invertebrates and analyses of similarity indicated that there were no
232 significant effects of sampling region or wave exposure on the faunal composition of limpet
233 gut contents (ANOSIM region effect $r = -0.25$, $p = 0.78$; ANOSIM wave exposure effect $r = -$
234 0.25 , $p = 1.0$).

235 **Patterns of gut content composition in *P. vulgata***

236 Multidimensional ordination of the abundance of the functional groups of algae and
237 ingested fauna (Fig. 4) also showed that the composition of the diet of *P. vulgata* varied with
238 region and wave exposure (ANOSIM region effect $r = 0.017$, $p = 0.025$; ANOSIM wave
239 exposure effect $r = 0.03$, $p = 0.001$). Although no clear, discrete groupings of diet types are
240 immediately apparent from the ordination plot, there is a general pattern for individuals from
241 exposed sites in the south to be positioned towards the bottom right of the figure, and
242 individuals from sheltered sites in both regions to be positioned towards the top left. Samples
243 from northern exposed sites show less pronounced separation than other samples and overlap
244 considerably with other data. The MDS plot (Fig. 4) places individual limpets with high
245 abundance categories for microalgae (Fig. 4c) towards the right of the figure, and shows them
246 to be mainly from exposed and southern sites. Individuals with high abundance categories
247 for corticated/leathery algae (Fig. 4f) are positioned towards the top left of the MDS plot and
248 tend to be from sheltered sites in the north. Limpets which had ingested invertebrate fauna
249 are positioned towards the bottom of the ordination (Fig. 4b), and show no clear patterns
250 according to wave exposure conditions or sampling region. The few individuals with high
251 abundance categories for filamentous, foliose, articulated calcareous and encrusting coralline
252 algae (Groups 2, 3, 6 and 7; Figs. 4d, e, g and h respectively) appear as outliers in the MDS
253 plot and show no significant patterns according to the factors examined.

254 SIMPER analyses indicate that microalgae, corticated/leathery and filamentous algae
255 contribute most to differences in diet composition by region and wave exposure (Table 3).

256 Average abundance categories for microalgae and filamentous algae were higher for samples
257 from southern sites and average abundance categories for corticated/leathery algae were
258 higher for limpet gut contents from northern and sheltered sites. Higher abundance
259 categories of microalgae (Group 1 algae) were found in samples from wave exposed sites
260 than in those from sheltered areas.

261 **Stable isotopes**

262 Analysis of variance of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in *P. vulgata* foot tissues by region,
263 wave exposure and site (nested within region and exposure) showed a significant effect of
264 region on carbon isotope composition and significant effects of site on both carbon and
265 nitrogen isotope ratios (Table 4, Fig. 5). The average $\delta^{13}\text{C}$ values of animals from the south
266 were around 2 ‰ enriched in ^{13}C compared to animals from the north ($p < 0.01$). Variability
267 in $\delta^{15}\text{N}$ values of limpet tissues was largely due to differences between sites ($p < 0.01$) and,
268 although nitrogen isotope values in limpets from exposed sites in the north were slightly
269 higher than those of sheltered sites in this region, this trend was not significant ($p > 0.05$).
270 Average $\delta^{13}\text{C}$ values for corticated/leathery macroalgae differed significantly between the
271 sampling regions (-16.4 ‰ in the north and -17.2 to -18.5 ‰ in the south; $p < 0.01$; Fig. 5),
272 and average $\delta^{15}\text{N}$ values were lower in the north (5.3 to 6.2 ‰) compared to similar algae
273 from the south (7.4 to 7.6 ‰). No significant wave exposure effects were seen. The average
274 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of epilithic biofilm were -20.7 ‰ and 6.4 ‰ respectively and no
275 significant differences among region and wave exposure conditions were shown (Fig. 5).

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280 **Discussion**

281 Our work shows that the intertidal limpet *Patella vulgata* consumes not only
282 microalgae, but also large quantities of macroalgae and some small invertebrates. Intertidal
283 consumers are thought to exploit the most common food sources available in their immediate
284 surroundings (Raffaelli 1985; Steinarsdottir et al. 1995) and *P. vulgata* is a homing species
285 which tends to forage within 1 m of its 'home scar' (Hartnoll & Wright 1977, Della Santina
286 et al. 1995). Despite being collected from bare rock habitats, 95 % of the limpets examined
287 in this study had macroalgae in their guts, and the stable isotope evidence indicates that
288 assimilated carbon was from macroalgal sources. It is clear that these animals are exploiting
289 a macroalgal food resource that is not immediately apparent on the shore at low tide. *Patella*
290 *vulgata* has been observed feeding on drift algae stranded on the shore when exposed at low
291 water, particularly on dark, damp days and following storms (Lorenzen 2007, GMN, SJH
292 personal observations). South African limpets (*Patella argenvillei* and *P. granatina*) have
293 been documented capturing and feeding on drifting kelp directly from the water column
294 (Bustamante et al. 1995). Our findings suggest that *P. vulgata* may also be able to exploit
295 drift algae from the water column when the tide is in.

296 The relative abundance of ingested algal material in limpet guts is affected by both
297 latitude and wave exposure; microalgae are more common in the diet of limpets from
298 southern and moderately wave exposed sites, whereas corticated/leathery macroalgae are
299 more frequently encountered in animals from northern and sheltered coastlines. Stable
300 isotope analyses show that foot tissues from limpets from all sites are enriched in ^{13}C relative
301 to biofilm, also indicating that the organic carbon assimilated over time is largely derived
302 from macroalgal sources rather than microalgae.

303 *Patella vulgata* plays a major role in structuring intertidal communities along
304 gradients of wave exposure and latitude in the north-east Atlantic by ingestion of biofilm and

305 macroalgal propagules (Hawkins & Hartnoll 1983, Hawkins et al. 1992) and previous gut
306 contents analyses have supported this (Hawkins et al. 1989, Hill & Hawkins 1991). Ingestion
307 of invertebrates was reported in these studies at similar levels to those recorded here, but acid
308 digestion probably removed much of the macroalgal tissues from the analyses and the
309 presence of any residual macroalgal tissue (*Fucus* spp.) within the gut was interpreted as
310 consumption of juvenile algae, rather than sections of mature thalli (Hill & Hawkins 1991).
311 It is difficult dissect the limpet alimentary tract, and even harder to identify fragments of
312 algae in limpet gut contents (Raffaelli 1985, C. Maggs personal communication). Yet our
313 examination of gut contents, combined with stable carbon and nitrogen isotope analysis,
314 provides very strong evidence that *P. vulgata* routinely consumes mature macroalgae as well
315 as biofilm and macroalgal propagules. Use of live and detrital macroalgal foods may explain
316 the lack of clear relationships between biofilm standing stock, limpet density and grazing
317 activity which have been observed on a number of British shores (Jenkins et al. 2001,
318 Thompson et al. 2004).

319 Some of the ingested macroalgae in this study may be from *P. vulgata* directly
320 consuming adult furoid plants. In sheltered sea lochs and bays, where intertidal communities
321 are dominated by leathery, furoid macroalgae, *P. vulgata* may be directly feeding on the
322 fronds. On more exposed areas, headlands and breakwaters, where such algae are rare and
323 communities are characterised by barnacles and mussels (Lewis, 1964; Ballantine, 1961),
324 drift algae may enhance populations of these grazers and so maintain high grazing intensity
325 and prevent further escapes of furoids (Moore et al 2007). The greater availability of drift
326 algae in the north may explain the greater incidence of macroalgae in the diet of *P. vulgata*,
327 given than macroalgal cover increases with latitude. Furoids dominate most shores in
328 Norway whereas in northern Spain the algae are confined to only the most sheltered sites
329 (Ballantine 1961, Hawkins & Hartnoll 1983, Hawkins et al. 1992, Coleman et al. 2006).

330 By feeding on allochthonous drift algae, *P. vulgata* may be coupling subtidal and
331 intertidal production through feeding. Thus their ecological role may be much more
332 extensive than simply that of a microphagous grazer of rocky substrata. Such grazers may
333 therefore play crucial roles in facilitating nutrient flow in coastal environments and allowing
334 horizontal transport of resources between exposed and sheltered areas (Polis et al 1997).

335 Consumption of corticated/leathery algae is reported in many of the early accounts of
336 limpet biology (see Steneck and Watling (1982) for a comprehensive review), and is also
337 described in several more recent investigations (Davies et al., 2008; Davies et al., 2007;
338 Lorenzen, 2007). Lorenzen (2007) provides photographic evidence of rasping marks made
339 by *P. vulgata* on the thalli of *A. nodosum* from shores in Brittany and a video showing a low
340 shore individual feeding on attached *Laminaria digitata* was recorded by G. Notman in
341 Argyll in 2007 (<http://www.youtube.com/watch?v=79RvGRUdnwE>). Stable isotope
342 evidence from this and other studies also indicates that fucoid macroalgae are a significant
343 source of nutrition to *P. vulgata*; the limpets are not only ingesting macroalgal foods, but they
344 are also assimilating them into their tissues (Campbell, 2004; Notman et al. in preparation;
345 Riera et al., 2009; Schaal et al., 2010).

346 Despite limiting our investigation to relatively large limpets collected from open rock
347 habitats, and acknowledging that the diet of these molluscs may change seasonally and
348 ontogenetically, as well as in response to food availability, it is clear that the diet and the
349 ecological role of *P. vulgata* is not yet fully understood. *Patella vulgata* is considered to be a
350 keystone species on temperate rocky shores because its grazing activities prevent
351 establishment of mature algae by consumption of macroalgal propagules and germlings in the
352 biofilm. The assumption was that biofilm foods provided the main source of energy to these
353 animals. Our work suggests that the species may also play important roles in modifying
354 macroalgal cover, especially on more sheltered coastlines where fucoid algae commonly

355 occur. Moreover, previous work has interpreted the aggregation of *P. vulgata* under stands of
356 mature algae as being primarily a sheltering response, mitigating desiccation stress and
357 reducing predation (Hartnoll & Hawkins 1985, Coleman et al. 1999, Moore et al. 2007). This
358 work indicates that limpets associated with patches of attached macroalgae are likely to be
359 feeding on them too. The species may also be of great ecological importance in terms of
360 coupling sub- and intertidal production by ingesting allochthonous drift algae across a range
361 of wave exposure conditions and latitudes. This would help to explain the high biomass of
362 grazers which occurs on apparently bare shores across the north east Atlantic, which is
363 unlikely to be supported by epilithic microalgal production alone.

364

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Table 1. *Patella vulgata* gut contents. Taxa identified and abundance categories used for gut contents analysis. Algal functional groups follow the classification by Steneck and Watling (1982).

Group	Algal Type /Taxon	Representatives	Measure	S Super- abundant	A Abundant	C Common	F Frequent	O Occasional	R Rare	N None
1	Microalgae	Diatoms, cyanobacteria	No. cells per field of view	> 50	21 to 50	11 to 20	6 to 10	1 to 5	0.5 to 1	< 0.2
2	Filamentous Algae	<i>Cladophora</i> , <i>Ectocarpus</i>	No. of fragments	> 50	31 to 50	21 to 30	11 to 20	6 to 10	1 to 5	None
3	Foliose Algae	<i>Ulva</i> , <i>Porphyra</i>	No. of fragments	> 50	31 to 50	21 to 30	11 to 20	6 to 10	1 to 5	None
4	Corticated Macrophytes	<i>Bryothamnium</i> , <i>Chondria</i> /	No. of fragments	> 50	31 to 50	21 to 30	11 to 20	6 to 10	1 to 5	None
5	Leathery Macrophytes	<i>Laminaria</i> , <i>Fucus</i> , <i>Ascophyllum</i>	No. of fragments	> 50	31 to 50	21 to 30	11 to 20	6 to 10	1 to 5	None
6	Articulated Calcareous Algae	<i>Corallina</i> , <i>Halimeda</i>	No. of fragments	> 50	31 to 50	21 to 30	11 to 20	6 to 10	1 to 5	None
7	Crustose Coralline Algae	Lithothamnion, <i>Lithophyllum</i>	No. of fragments	> 50	31 to 50	21 to 30	11 to 20	6 to 10	1 to 5	None
Fauna	Ingested Invertebrates	Barnacle cyprids, <i>Skeneopsis planorbis</i> , acarinids	No. of individuals	> 50	31 to 50	21 to 30	11 to 20	6 to 10	1 to 5	None

489 Table 2. *Patella vulgata* gut contents. Comparison of abundance of microalgae and
 490 corticated/leathery macroalgae across regions, sites and wave exposure by ordinal logistic
 491 regression. Site type abbreviations: NE, northern exposed; NS, northern sheltered; SE,
 492 southern exposed; SS, southern sheltered. (***p < 0.001; **p < 0.01). Results are shown for
 493 best fit models (see text).
 494

Region or Site code (region)	Loge odds ratio	SE	z	P	Odds ratio	95 % confidence interval upper lower	
<i>Microalgae: Group 1</i>							
North vs South	1.514	0.367	4.13	< 0.001***	4.54	2.22	9.32
NE1 Easdale							
NE2 Putechan	0.016	0.400	0.04	0.968	1.02	0.46	2.23
NS1 Ellenabeich	0.666	0.379	1.75	0.079	1.95	0.93	4.09
NS2 Luing	0.514	0.369	1.39	0.164	1.67	0.81	3.45
SE1 Andurn							
SE2 Picklecombe	0.429	0.348	1.23	0.217	1.54	0.78	3.04
SS1 Jennycliff	1.150	0.345	3.34	0.001**	3.16	1.61	6.21
SS2 Cawsand	1.654	0.347	4.77	< 0.001***	5.23	2.65	10.32
<i>Corticated/leathery algae: Group 4/5</i>							
North vs South	-0.606	0.184	-3.29	0.001**	0.55	0.38	0.78
Exposed vs Sheltered	0.672	0.184	3.64	< 0.001***	1.96	1.36	2.81

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496 Table 3. *Patella vulgata* diet composition. Comparison of gut content composition between
 497 regions and wave exposures by SIMPER analysis using the Euclidian distance measure of
 498 association. Average abundance categories of algae are shown (categories N to S converted
 499 to numerical integers 0 to 7) with average squared Euclidian distance (D) and percentage
 500 contribution (C %) to differences between regions and wave exposures.
 501

Algal Group	Region				Wave exposure			
	North	South	D	C %	Exposed	Sheltered	D	C %
1 Microalgae	1.90	2.76	4.65	29	2.75	2.04	4.75	29
4/5 Corticated/Leathery	2.64	2.22	4.17	26	2.09	2.69	4.32	26
2 Filamentous	0.608	0.944	3.76	24	0.643	0.928	3.72	23
7 Encrusting coralline	0.08	0.315	1.11	7	0.346	0.087	1.34	8

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503 Table 4. ANOVA testing for differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *P. vulgata* foot tissue by
 504 region, wave exposure and site (nested within region and exposure). x denotes that the
 505 analysis could not perform an exact F test. (** p < 0.01) n = 12 per site.

Source	df	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
		MS	F	p	MS	F	p
Region	1	57.602	24.83	0.008** x	7.157	4.28	0.107 x
Exposure	1	1.172	0.51	0.517 x	3.934	2.36	0.200 x
Site (Reg Exp)	4	2.323	5.89	0.001**	1.674	4.47	0.004**
Region*Exposure	1	0.029	0.01	0.917 x	10.649	6.37	0.065 x
Error	44	0.394			0.374		

Levene's Test	1.22, p = 0.315	0.94, p = 0.491
Bartlett's Test	5.89, p = 0.553	10.52, p = 0.161
Variance Homogeneous	Yes	Yes

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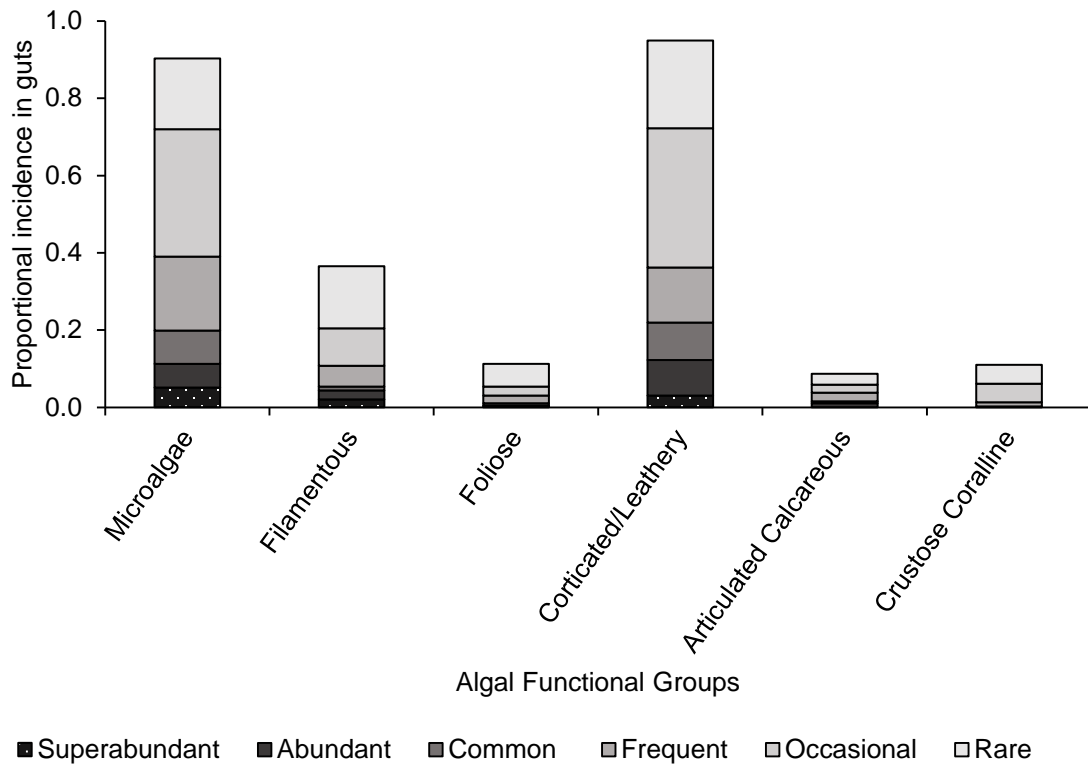
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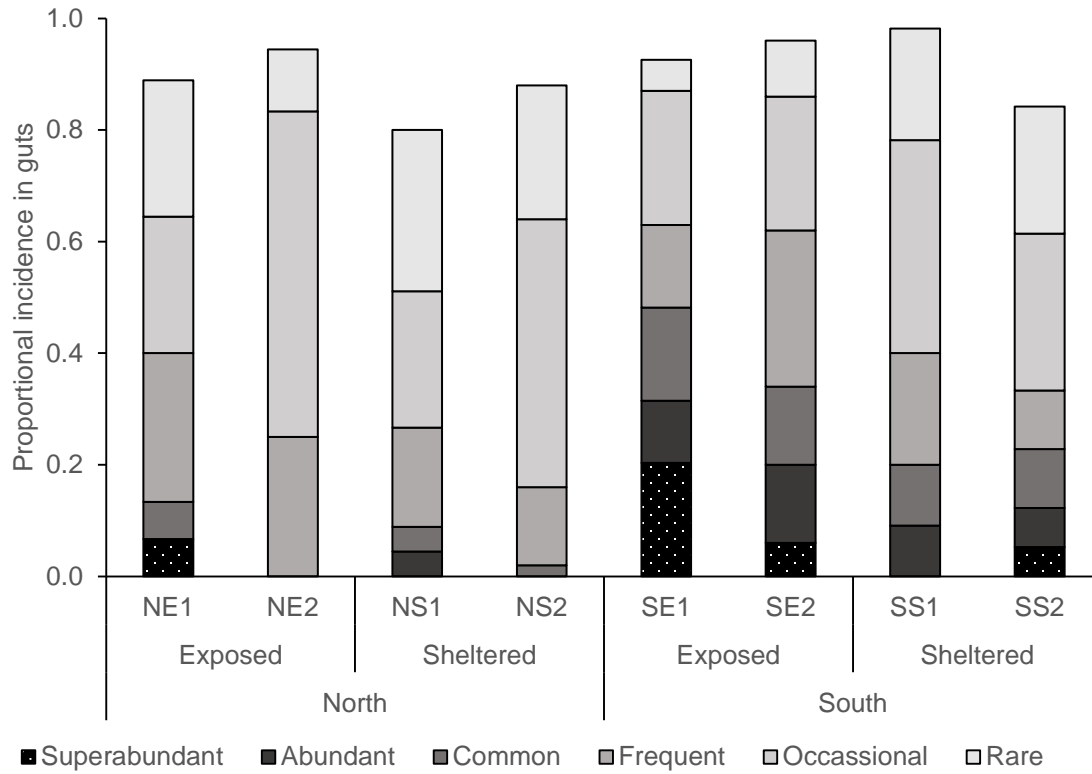
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515 Fig. 1. *Patella vulgata* gut contents (n = 392). Abundance of functional groups of algae in
 516 gut contents of *P. vulgata* shown by cumulative proportional incidence in abundance
 517 categories (defined in Table 1).

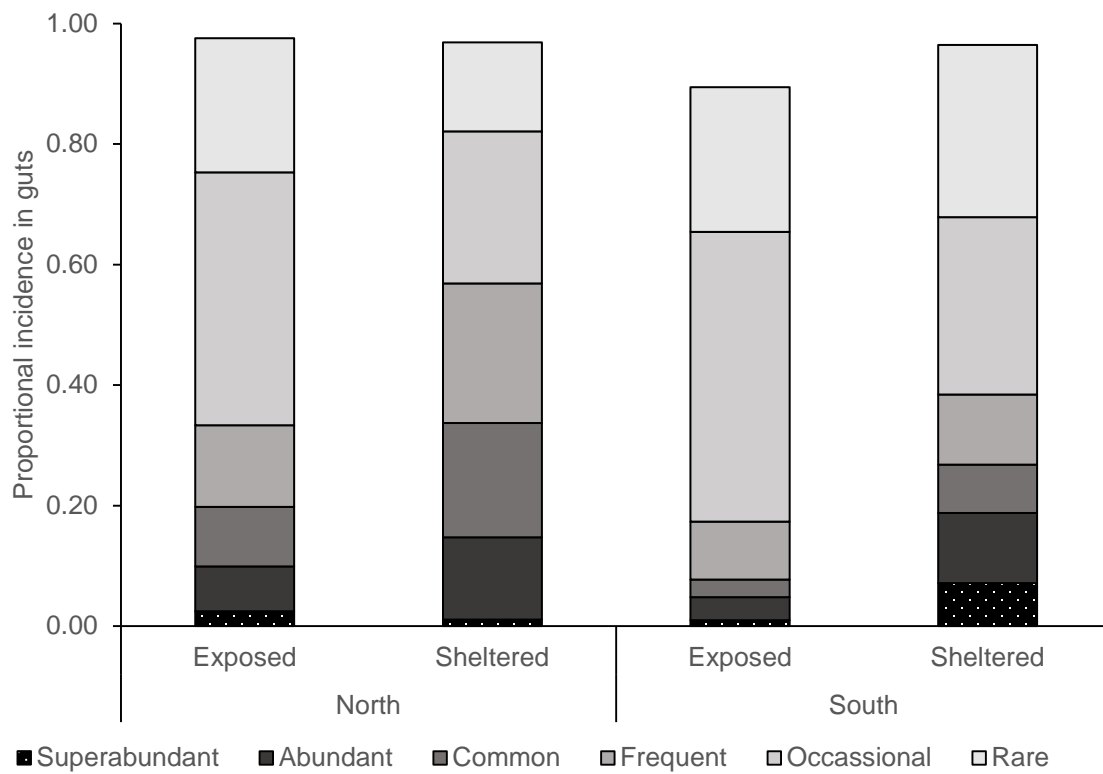


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519 Fig. 2. *Patella vulgata* gut contents (n = 392). Abundance of microalgae by proportional
 520 incidence in abundance categories (Table 1) by region, wave exposure and site. Site
 521 abbreviations are given in Table 2.

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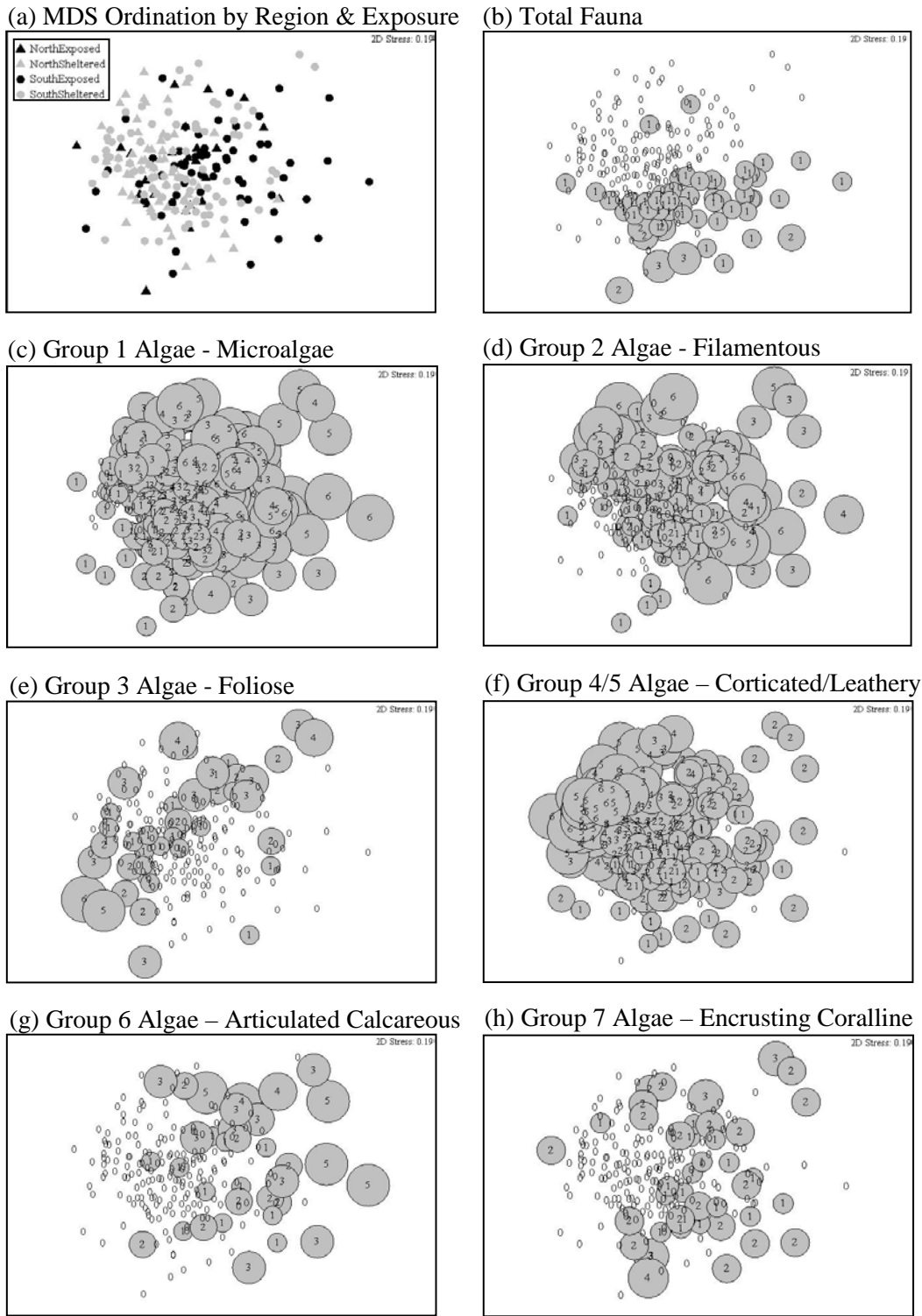


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525 Fig. 3. *Patella vulgata* gut contents (n = 392). Abundance of corticated/leathery macroalgae
526 as the proportional incidence in abundance categories (Table 1) by region and wave exposure.
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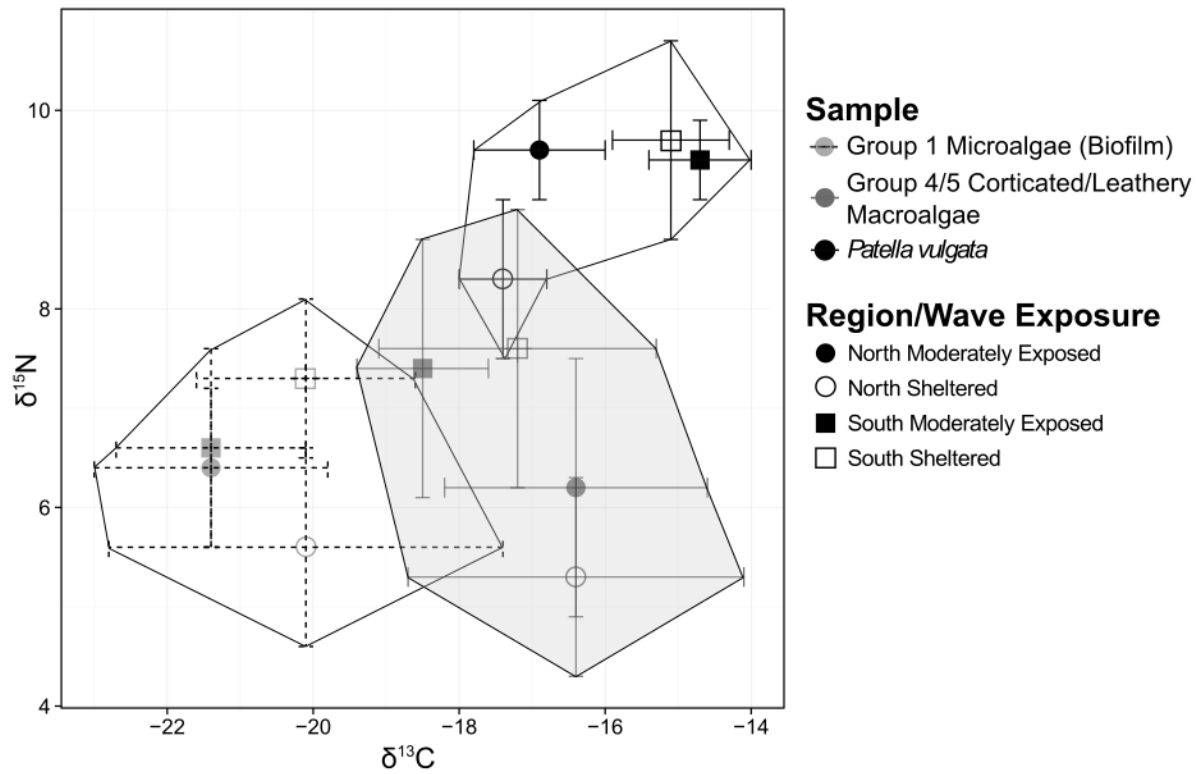
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571 ① Rare, ② Occasional, ③ Frequent, ④ Common, ⑤ Abundant and ⑥ Superabundant.
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573 Fig. 4. *P. vulgata* gut contents. MDS ordination of the composition of individual *P. vulgata*
 574 diets using abundance categories converted to numerical integers using Gower's S_{15}
 575 similarity matrix ($n = 392$). Subplots show (a) region and wave exposure of collection sites
 576 (b) the abundance category for total fauna as a varying sized symbol, (c) microalgae, (d)
 577 filamentous algae, (e) foliose algae, (f) corticated and leathery algae, (g) articulated
 578 calcareous algae and (h) encrusting coralline algae.



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Fig. 5. Stable isotope ratios of Microalgae, Corticated/Leathery Macroalgae and *P. vulgata* foot tissues. Mean $\delta^{13}\text{C}$ values are given against mean $\delta^{15}\text{N}$ values for tissues from moderately exposed and sheltered sites in the two study regions ± 1 SD.