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

Gillian M. Notman[1,2,5,*], **Rona A. R. McGill**[3], **Stephen J. Hawkins**[2,4], **Michael T. Burrows**[1]

[1]**Scottish Association for Marine Science, Scottish Marine Institute, Oban PA37 1QA, UK**
[2]**Marine Biological Association of the UK, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK**
[3]**NERC Life Sciences Mass Spectrometry Facility, Scottish Universities Environmental Research Centre, East Kilbride G75 0QF, UK**
[4]**Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton SO14 3ZH, UK**

[5]*Present address:* **Department of Science, Natural Resources and Outdoor Studies, University of Cumbria, Ambleside LA22 1BB, UK**

ABSTRACT: Analysis of gut contents and stable isotope composition of intertidal limpets (*Patella vulgata*) showed a major contribution of macroalgae to their diet, along with microalgae and invertebrates. Specimens were collected in areas with limited access to attached macroalgae, suggesting a major dietary component of drift algae. Gut contents of 480 animals from 2 moderately wave-exposed and 2 sheltered rocky shores in each of 2 regions (western Scotland, 55–56° N; and southwest England, 50° N), were analysed in 2 years (n = 30 site^{-1} yr^{-1}). The abundance of microalgae, macroalgae and invertebrates within the guts was quantified using categorical abundance scales. Gut content composition was compared among regions and wave exposure conditions, showing that the diet of *P. vulgata* changes with both wave exposure and latitude. Microalgae were most abundant in limpet gut contents in animals from southwestern sites, whilst leathery/corticated macroalgae were more prevalent and abundant in limpets from sheltered and northern sites. *P. vulgata* appears to have a more flexible diet than previously appreciated, and these keystone grazers consume not only microalgae, but also large quantities of macroalgae and small invertebrates. To date, limpet grazing studies have focussed on their role in controlling recruitment of macroalgae by feeding on microscopic propagules and germlings. Consumption of adult algae suggests that *P. vulgata* may also directly control the biomass of attached macroalgae on the shore, whilst consumption of drift algae indicates that the species may play important roles in coupling subtidal and intertidal production.

KEY WORDS: Stable isotope · Gut contents analysis · Limpet · Trophic ecology · *Fucus* · *Ascophyllum* · Rocky shore

INTRODUCTION

The intertidal common European limpet *Patella vulgata* is the most abundant grazer on rocky shores in the northeast Atlantic and plays an important role in structuring intertidal communities throughout its range (Hawkins & Hartnoll 1983, Hawkins et al. 1992). Classic limpet removal experiments (Jones 1948, Lodge 1948, Burrows & Lodge 1950, Southward 1964), observations following the mass limpet kills in the aftermath of the 'Torrey Canyon' oil spill clean-up (Southward & Southward 1978, Hawkins &

*Corresponding author: gill.notman@cumbria.ac.uk

Southward 1992) and limpet exclusion experiments (Hawkins 1981, Hartnoll & Hawkins 1985, Jenkins et al. 2005, Coleman et al. 2006, Jonsson et al. 2006) have all shown that removal or exclusion of limpets produces lush growth of canopy-forming fucoid macroalgae on wave-exposed and moderately exposed rocky shores. These indirect studies have led to the conclusion that *P. vulgata* is a keystone species in the intertidal, preventing macroalgal growth through microphagous grazing of epilithic biofilm and associated macroalgal propagules (spores and germlings) (Hill & Hawkins 1991, Jenkins & Hartnoll 2001, Jenkins et al. 2005, Coleman et al. 2006, Jonsson et al. 2006, Moore et al. 2007). This view is supported by observational studies of limpet foraging behaviour (Hartnoll & Wright 1977, Little et al. 1988, Della Santina et al. 1994) and several studies which directly examined limpet gut contents (Hawkins et al. 1989, Little et al. 1990, Hill & Hawkins 1991).

More recent work suggests that *P. vulgata* may play an even more significant role in structuring rocky shore ecosystems through direct grazing of mature macroalgae as well as biofilm and propagules. On many more sheltered shores, *P. vulgata* aggregate around established stands of *Ascophyllum nodosum* and *Fucus vesiculosus*, and 'bite marks' are visually apparent on the algal fronds. The fronds are often much shorter than might be expected, consistent with the ends being directly grazed away by the nearby limpets (Davies et al. 2007, 2008, G. M. Notman & S. J. Hawkins pers. obs.). Lorenzen (2007) also recorded *P. vulgata* feeding on stranded macroalgae during periods of emersion, and the results of stable isotope analyses from several sources provide strong evidence to suggest that macroalgae may be a more significant source of organic carbon to *P. vulgata* than previously appreciated (Campbell 2004, Riera et al. 2009, Schaal et al. 2010, Notman 2011).

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

The overall aim of this study was to compare the diets of limpets between contrasting latitudinal and wave-exposure conditions. Specifically, we quantified the relative importance of microalgal biofilms and other food sources across these locations. We focussed on the diet of limpets collected from bare rock microhabitats where access to other types of foods was restricted. Gut contents of adult *P. vulgata* (30–50 mm in length) were examined from wave-exposed and wave-sheltered sites in western Scotland and southwest England over 2 years. Analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios from a subset of these animals, together with their potential foods, was used to provide additional information on limpet diets integrated over longer periods of time.

MATERIALS AND METHODS

Study sites and sample collection

Patella vulgata were collected from mid-shore bare rock microhabitats at 4 sites in southwest England, near Plymouth, Devon, and 4 sites in western Scotland, around Oban and the Kintyre peninsula. Two moderately wave-exposed (300–500 km fetch) and 2 sheltered (<50 km fetch) sites were selected in each region, with the ranges of wave exposure set using calculated values for wave fetch in accordance with Burrows et al. (2008). Adult limpets were collected from areas of bare rock in spring 2005 and 2006. These habitats seldom comprised entirely clean rock, but also often contained small areas of macroalgae and barnacles. To investigate the potential influence of these alternate food sources on diet composition, we visually estimated the percentage cover of bare rock, barnacle and attached macroalgal cover in the mid-intertidal using 25 quadrats (0.5 × 0.5 m) at the 8 sites in each year. Limpets were immediately chilled upon collection to minimise digestion of gut contents, and frozen (−20°C) upon return to the laboratory. Thirty animals of similar size (mean ±SE length 42 ± 4 mm) were selected from each site per year for gut content analysis. A subset of 12 limpets was randomly selected for stable isotope analysis from the different locations and wave exposure conditions, and samples of potential foods (biofilm and macroalgae) were collected at each site in each year. Macroalgal species included *Ascophyllum nodosum*, *Fucus vesiculosus*, *F. serratus*, *F. spiralis*, *Himanthalia elongata* and detrital *Laminaria digitata*.

Dissection and examination of gut contents

The digestive organs of *P. vulgata* are long and complex, extending from the mouth and buccal mass, through the crop and stomach to the long intestine embedded in the visceral mass (Fretter & Graham 1962). Limpets were dissected ventrally, whilst partially frozen, with shells attached. The muscular foot and internal organs were completely removed to reveal the crop and anterior portion of the stomach. Only the anterior sections of the alimentary canal were excised, as the posterior sections are difficult to separate from the digestive gland and visceral mass.

Dissected gut contents were initially examined at 4× magnification, and ingested algae and invertebrates were identified and quantified using categorical abundance scales (Table 1). It was not possible to identify ingested macroalgae to species level. These were therefore classified according to Steneck & Watling's (1982) functional group scheme (Table 1), with categories 4 and 5 (corticated and leathery macroalgae) combined due to the difficulties in objectively and consistently discriminating between these groups in gastropod guts (Raffaelli 1985, G. M. Notman pers. obs.). The abundance of microalgae (Group 1, including diatoms and cyanobacteria) was assessed at 20× magnification using a compound microscope. A 1 cm^2 subsample of material was covered with a glass coverslip, and 5 fields of view were randomly selected for closer examination.

Stable isotope analysis

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

Biofilm was obtained from 3 rock samples (each 200 cm^2 and free from macroalgae and encrusting organisms) from each site in each year. The rocks were soaked in 0.7 μm filtered seawater for 15 min in the laboratory, and the rehydrated biofilm was removed by brushing with an electric toothbrush. Biofilm was filtered onto glass fibre filter paper (Whatman GF/F), frozen at −20°C and freeze dried

Table 1. *Patella vulgata* gut contents. Taxa identified and abundance categories used for gut content analysis. Algal functional groups follow the classification by Steneck & Watling (1982). Abundance categories were converted into numbers for the multivariate analyses (SACFORN; S = 6, A = 5, C = 4, F = 3, O = 2, R = 1 and N = 0)

Group	Algal type/taxon	Representatives	Measure	Super-abundant (S)	Abundant (A)	Common (C)	Frequent (F)	Occasional (O)	Rare (R)	None (N)
1	Microalgae	Diatoms, cyanobacteria	No. of cells per field of view	>50	21–50	11–20	6–10	1–5	0.5–1	<0.2
2	Filamentous algae	*Cladophora, Ectocarpus*	No. of fragments	>50	31–50	21–30	11–20	6–10	1–5	None
3	Foliose algae	*Ulva, Porphyra*	No. of fragments	>50	31–50	21–30	11–20	6–10	1–5	None
4	Corticated macrophytes	*Bryothamnion, Chondria/*	No. of fragments	>50	31–50	21–30	11–20	6–10	1–5	None
5	Leathery macrophytes	*Laminaria, Fucus, Ascophyllum*	No. of fragments	>50	31–50	21–30	11–20	6–10	1–5	None
6	Articulated calcareous algae	*Corallina, Halimeda*	No. of fragments	>50	31–50	21–30	11–20	6–10	1–5	None
7	Crustose coralline algae	*Lithothamnion, Lithophyllum*	No. of fragments	>50	31–50	21–30	11–20	6–10	1–5	None
Fauna	Ingested invertebrates	Barnacle cyprids, *Skeneopsis planorbis*, acarinids	No. of individuals	>50	31–50	21–30	11–20	6–10	1–5	None

to constant weight. Samples of corticated/leathery macroalgae were rinsed in deionised water, and epibiont-free fronds were frozen at −20°C before freeze drying. The stable isotope values of barnacles were not measured due to the difficulties inherent in analysing inorganic carbon (Søreide et al. 2006).

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

Data analysis

Abundance of algae and invertebrates ingested by limpets was compared between the 2 regions, between the 2 classes of wave exposure and among the sites (nested within region and exposure), using ordinal logistic regression (OLR, Minitab version 15.1.20) on ranked categorical abundance data to determine influences on the probability of obtaining particular abundance categories. Best OLR models were selected by comparisons of deviance likelihood ratios following step-wise exclusion of factors from a saturated model (Notman 2011).

Multivariate analyses were used to examine patterns of gut content composition between the sampling regions and wave exposure conditions. The Gower similarity coefficient (S_{15}) was used to obtain a matrix of similarities for examination of ordinal categories of limpet gut contents (Gower 1971, Podani 1999, Legendre & Legendre 1998). Normalisation was not necessary as similar abundance scales were used for each variable. Non-metric multidimensional scaling (NMDS; PRIMER 6, PRIMER-E) was used to produce a 2-dimensional ordination of gut content data from the similarity matrix (Clarke 1993). Similarity indices were used to determine the effects of region and wave exposure on composition of limpet gut contents (2-way analysis of similarity, ANOSIM), with the contribution of each taxon to differences among levels of each factor assessed using similarity percentage analysis (SIMPER, based on the Euclidian distance measure of association).

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

Stable carbon and nitrogen isotope ratios of biofilm, macroalgae and *P. vulgata* foot tissue were compared among the 2 regions, the 2 classes of wave exposure and the sites (nested within region and exposure) using ANOVA. Site was treated as a random factor, with all other factors fixed and orthogonal. Stable isotope ratios for macroalgae were calculated for region and exposure type combinations as the mean and standard deviation across all species of corticated/leathery macroalgae for each site.

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

RESULTS

Study sites

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

Main constituents of limpet gut contents

Most of the 480 animals examined had quantifiable gut contents (82%). It was not possible to identify the species of algae ingested, but microalgae (Group 1) and corticated/leathery algae (Groups 4/5) were present in almost all of these samples: 90 and 95% of gut
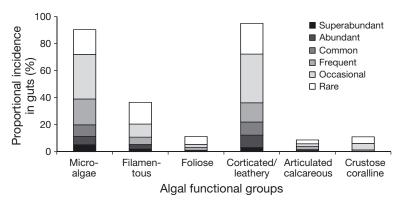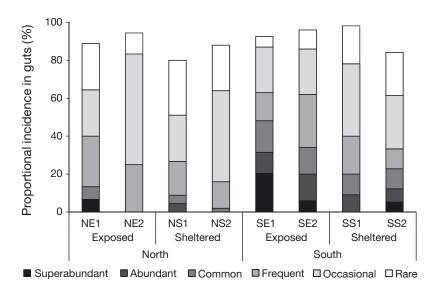
Fig. 1. *Patella vulgata* gut contents (n = 392). Abundance of functional groups of algae in gut contents of *P. vulgata* shown by cumulative proportional incidence in abundance categories (defined in Table 1)

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

Region or Site (region)	Log odds ratio	SE	z	p	Odds ratio	95% CI upper	lower
Microalgae: Group 1							
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
Corticated/leathery algae: Group 4/5							
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

Superabundant Abundant Common Frequent Occasional Rare

contents, respectively. Filamentous (Group 2) algae were found in around 36% of gut samples, and foliose (Group 3), articulated calcareous (Group 6) and crustose coralline (Group 7) algae were only present in around 10% of the material analysed (Fig. 1). Invertebrates were present in 91 of the 392 limpet gut contents (23% overall).

Changes in diet with latitude

There were significant differences in limpet diets between the 2 sampling regions. Microalgae were almost 5 times more likely to be recorded in high abundance categories (common, abundant and superabundant) in gut contents of southern limpets than northern limpets (ordinal logistic regression region effect, log odds ratio 1.514, SE: 0.367, p < 0.001, Table 2, Fig. 2). In contrast, corticated/leathery macroalgae were around twice as likely to be recorded in higher abundance categories in northern limpets (Fig. 3). The odds ratio of 0.55 given in Table 2 indicates that high abundance categories of corticated/leathery algae were around half as likely in the south as in the north (p < 0.01). Abundance of other types of algae (filamentous, foliose, articulated calcareous or crustose coralline) was not influenced by latitude (p > 0.05 for all comparisons).

Effect of wave exposure on diet

Microalgae were more prevalent in guts of limpets from exposed shores than sheltered shores in the south (Fig. 2) but not in the north, and no overall trend in the abundance of

Fig. 2. *Patella vulgata* gut contents (n = 392). Abundance of microalgae by proportional incidence in abundance categories (defined in Table 1) by region, wave exposure and site. Site abbreviations are given in Table 2
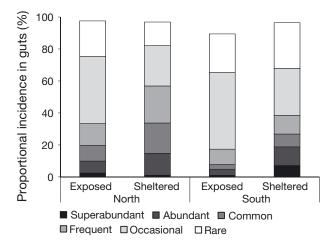
Fig. 3. *Patella vulgata* gut contents (n = 392). Abundance of corticated/leathery macroalgae as the proportional incidence in abundance categories (defined in Table 1) by region and wave exposure

microalgae with wave exposure was seen. In the south, higher abundance categories for microalgae were more likely at the exposed shores at Andurn and Picklecombe (sites SE1 and SE2) than at the sheltered sites Jennycliff or Cawsand (SS1 or SS2, p < 0.01; Fig. 2, Table 2). There were no significant differences in microalgal abundance among the sites in the north, but there was some evidence that the probability of higher microalgal abundances was greater in samples from the moderately exposed Easdale (NE1) than for those from the nearby, sheltered Ellenabeich (NS1; odds ratio = 1.95, p = 0.079, Table 2).

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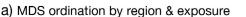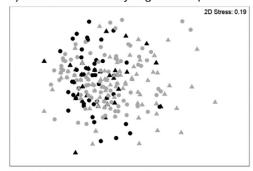
Ingestion of invertebrates

Small invertebrates were present in 23% of quantifiable limpet gut contents and were found at every site (9 to 42% of limpets examined). Barnacles were the most frequently ingested taxon, found in 15% of limpet guts. The small snail *Skeneopsis planorbis* was ingested by 6% of limpets, and acarinid mites were found in 3% of guts. Other taxa included bivalves (present in 4 individuals), foraminifera (3),

ostracods (3), *Littorina mariae* (2), copepods (1), hydroids (1), *Melaraphe neritoides* (1), and *Patella vulgata* itself (1). Few limpets had ingested more than 1 type of invertebrate (only 4%). Hierarchical cluster analysis of ingested invertebrates and ANOSIM indicated no significant effects of sampling region or wave exposure on the faunal composition of limpet gut contents (ANOSIM region effect r = −0.25, p = 0.78; ANOSIM wave exposure effect r = −0.25, p = 1.0).

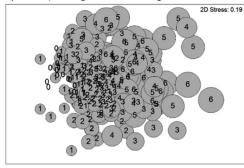## Patterns of gut content composition in *P. vulgata*

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

SIMPER analyses indicate that microalgae, corticated/leathery and filamentous algae contribute most to differences in diet composition by region and wave exposure (Table 3). Average abundance categories for microalgae and filamentous algae were higher for samples from southern sites and average abundance categories for corticated/leathery algae
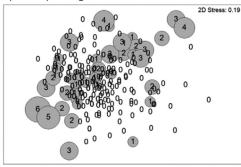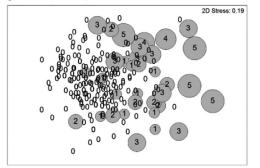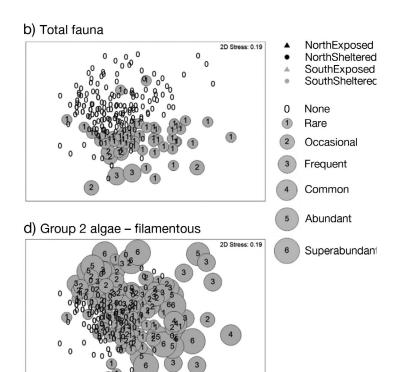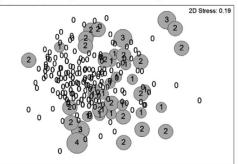
Fig. 4. *Patella vulgata* gut contents. NMDS ordination of the composition of individual *P. vulgata* diets using Gower's S15 simi-larity matrix (n = 392). Panels show (a) region and wave exposure of collection sites and the abundance categories for (b) total fauna (c) microalgae, (d) filamentous algae, (e) foliose algae, (f) corticated and leathery algae, (g) articulated calcareous algae and (h) encrusting coralline algae (varying sized symbols and numbers within represent abundance categories defined in Table 1)

Table 3. *Patella vulgata* diet composition. Comparison of gut content composition between regions and wave exposures by similarity percentage (SIMPER) analysis using the Euclidian distance measure of association (D_1). Data show average abundance categories of algae (converted to SACFORN numerical integers 0 to 6, as defined in Table 1), average squared Euclidian distance (D) and percentage contribution (C%) to differences

Algal group/taxon	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

Table 4. ANOVA testing for differences in $\delta^{13}C$ and $\delta^{15}N$ values of *Patella vulgata* foot tissue by region, wave exposure and site (nested within region and exposure)

Source	df	$\delta^{13}C$			$\delta^{15}N$		
		MS	F	p	MS	F	p
Region	1	57.602	24.83	0.008**,[a]	7.157	4.28	0.107[a]
Exposure	1	1.172	0.51	0.517[a]	3.934	2.36	0.200[a]
Site (Region Exposure)	4	2.323	5.89	0.001**	1.674	4.47	0.004**
Region × Exposure	1	0.029	0.01	0.917[a]	10.649	6.37	0.065[a]
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		

[a]Analysis could not perform an exact *F*-test; **p < 0.01, n = 12 per site

were higher for limpet gut contents from northern and sheltered sites. Higher abundance categories of microalgae (Group 1 algae) were found in samples from wave-exposed sites than in those from sheltered areas.

Stable isotopes

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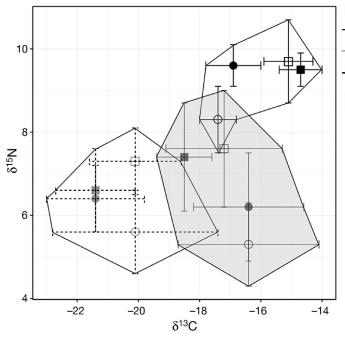
trend was not significant (p > 0.05). Average $\delta^{13}C$ values for corticated/ leathery macroalgae differed significantly between the sampling regions (−16.4‰ in the north and −17.2 to −18.5‰ in the south; p < 0.01; Fig. 5), and average $\delta^{15}N$ values were lower in the north (5.3 to 6.2‰)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

DISCUSSION

Our work shows that the intertidal limpet *Patella vulgata* consumes not only microalgae, but also large quantities of macroalgae and some small invertebrates. Intertidal consumers are thought to exploit the most common food sources available in their immediate surroundings (Raffaelli 1985, Steinarsdottir et al. 2009), and *P. vulgata* is a homing species which tends to forage within 1 m of its 'home scar' (Hartnoll & Wright 1977, Della Santina et al. 1995). Despite being collected from bare rock habitats, 95% of the limpets examined in this study had macroalgae in their guts, and the stable isotope evidence indicates that assimilated carbon was from macroalgal sources. It is clear that these animals are exploiting a macroalgal food resource that is not immediately apparent on the shore at low tide. *P. vulgata* has been observed feeding on drift algae stranded on the shore when exposed at low water, particularly on dark, damp days and following storms (Lorenzen 2007, G. M. Notman and S. J. Hawkins pers. obs.). The South African limpets *P. argenvillei* and *P. granatina* have been documented capturing and feeding on drifting kelp directly from the water column (Bustamante et al. 1995). Our findings suggest that *P. vulgata* may also be able to exploit drift algae from the water column when the tide is in.

The relative abundance of ingested algal material in limpet guts is affected by both latitude and wave exposure; microalgae are more common in the diet of limpets from southern and moderately wave-exposed

Fig. 5. Stable isotope ratios of microalgae, corticated/leathery macroalgae and *Patella vulgata* foot tissues. Mean $\delta^{13}C$ values are given against mean $\delta^{15}N$ values for tissues from moderately exposed and sheltered sites in the 2 study regions ± 1 SD

sites, whereas corticated/leathery macroalgae are more frequently encountered in animals from northern and sheltered coastlines. Stable isotope analyses show that foot tissues from limpets from all sites are enriched in ^{13}C relative to biofilm, also indicating that the organic carbon assimilated over time is largely derived from macroalgal sources rather than microalgae.

P. vulgata plays a major role in structuring intertidal communities along gradients of wave exposure and latitude in the northeast Atlantic by ingestion of biofilm and macroalgal propagules (Hawkins & Hartnoll 1983, Hawkins et al. 1992), and previous gut contents analyses have supported this (Hawkins et al. 1989, Hill & Hawkins 1991). Ingestion of invertebrates was reported in these studies at similar levels to those recorded here, but acid digestion probably removed much of the macroalgal tissue from the analyses, and the presence of any residual macroalgal tissue (*Fucus* spp.) within the gut was interpreted as consumption of juvenile algae, rather than sections of mature thalli (Hill & Hawkins 1991). It is difficult to dissect the limpet alimentary tract, and even harder to identify fragments of algae in limpet gut contents (Raffaelli 1985, C. Maggs pers. comm.). Yet our examination of gut contents, combined with stable carbon and nitrogen isotope analysis, provides very strong evidence that *P. vulgata* routinely consumes mature macroalgae as well as biofilm and macroalgal propagules. Use of live and detrital macroalgal foods may explain the lack of clear relation-

ships between biofilm standing stock, limpet density and grazing activity which have been observed on a number of British shores (Jenkins et al. 2001, Thompson et al. 2000).

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

By feeding on allochthonous drift algae, *P. vulgata* may be coupling subtidal and intertidal production through feeding. Thus, their ecological role may be much more extensive than simply that of a microphagous grazer of rocky substrata. Such grazers may therefore play crucial roles in facilitating nutrient flow in coastal environments and allowing horizontal

transport of resources between exposed and sheltered areas (Polis et al. 1997).

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

Despite limiting our investigation to relatively large limpets collected from open rock habitats, and acknowledging that the diet of these molluscs may change seasonally and ontogenetically, as well as in response to food availability, it is clear that the diet and the ecological role of *P. vulgata* are not yet fully understood. *P. vulgata* is considered to be a keystone species on temperate rocky shores because its grazing activities prevent establishment of mature algae by consumption of macroalgal propagules and germlings in the biofilm. The assumption was that biofilm foods provided the main source of energy to these animals. Our work suggests that the species may also play important roles in modifying macroalgal cover, especially on more sheltered coastlines where fucoid algae commonly occur. Moreover, previous work has interpreted the aggregation of *P. vulgata* under stands of mature algae as being primarily a sheltering response, mitigating desiccation stress and reducing predation (Hartnoll & Hawkins 1985, Coleman et al. 1999, Moore et al. 2007). Our work indicates that limpets associated with patches of attached macroalgae are likely to be feeding on them as well. The species may also be of great ecological importance in terms of coupling sub- and intertidal production by ingesting allochthonous drift algae across a range of wave-exposure conditions and latitudes. This would help to explain the high biomass of grazers which occurs on apparently bare shores across the northeast Atlantic, which is unlikely to be supported by epilithic microalgal production alone.

LITERATURE CITED

Ballantine WJ (1961) A biologically defined exposure scale for the comparative description of rocky shores. Field Stud 1:1–19

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr 27: 325–349

Burrows EM, Lodge SM (1950) Note on the interrelationships of *Patella, Balanus* and *Fucus* on a semi-exposed coast. Rep Mar Biol Stn Port Erin 62:30–34

Burrows MT, Harvey R, Robb L (2008) Wave exposure indices from digital coastlines and the prediction of rocky shore community structure. Mar Ecol Prog Ser 353:1–12

Bustamante RH, Branch GM, Eekhout S (1995) Maintenance of an exceptional intertidal grazer biomass in South Africa: subsidy by subtidal kelps. Ecology 76:2314–2329

Campbell EM (2004) Nitrogen assimilation by limpets. PhD dissertation, University of Glasgow

Clarke KR (1993) Nonparametric multivariate analyses of changes in community structure. Aust J Ecol 18:117–143

Coleman RA, Goss-Custard JD, dit Durell SELV, Hawkins SJ (1999) Limpet *Patella* spp. consumption by oystercatchers *Haematopus ostralegus*: a preference for solitary prey items. Mar Ecol Prog Ser 183:253–261

Coleman RA, Underwood AJ, Benedetti-Cecchi L, Aberg P and others (2006) A continental scale evaluation of the role of limpet grazing on rocky shores. Oecologia 147:556–564

Davies AJ, Johnson MP, Maggs CA (2007) Limpet grazing and loss of *Ascophyllum nodosum* canopies on decadal time scales. Mar Ecol Prog Ser 339:131–141

Davies AJ, Johnson MP, Maggs CA (2008) Subsidy by *Ascophyllum nodosum* increases growth rate and survivorship of *Patella vulgata.* Mar Ecol Prog Ser 366:43–48

Della Santina P, Naylor E, Chelazzi G (1994) Long-term field actography to assess the timing of foraging excursions in the limpet *Patella vulgata* L. J Exp Mar Biol Ecol 178: 193–203

Della Santina P, Santini G, Chelazzi G (1995) Factors affecting variability of foraging excursions in a population of the limpet *Patella vulgata* (Mollusca, Gastropoda). Mar Biol 122:265–270

Fretter V, Graham A (1962) British prosobranch molluscs, their functional anatomy and ecology. Ray Society, London

Gower JC (1971) A general coefficient of similarity and some of its properties. Biometrics 27:857–871

Hartnoll RG, Hawkins SJ (1985) Patchiness and fluctuations on moderately exposed rocky shores. Ophelia 24:53–63

Hartnoll RG, Wright JR (1977) Foraging movements and homing in the limpet *Patella vulgata* L. Anim Behav 25: 806–810

Hawkins SJ (1981) The influence of *Patella* grazing on the fucoid-barnacle mosaic on moderately exposed rocky shores. Kieler Meeresforsch 5:537–543

Hawkins SJ, Hartnoll RG (1983) Grazing of intertidal algae

by marine invertebrates. Oceanogr Mar Biol Annu Rev 21:195–282

Hawkins SJ, Southward A (1992) The Torrey Canyon oil spill: recovery of rocky shore communities. In: Thayer GW (ed) Restoring the nation's marine environment. Maryland Sea Grant, College Park, MD, p 583–631

Hawkins SJ, Watson DC, Hill AS, Harding SP, Kyriakides MA, Hutchinson S, Norton TA (1989) A comparison of feeding mechanisms in microphagous, herbivorous, intertidal, prosobranchs in relation to resource partitioning. J Molluscan Stud 55:151–165

Hawkins SJ, Hartnoll RG, Kain JM, Norton TA (1992) Plant–animal interactions on hard substrata in the north-west Atlantic. In: John DM, Hawkins SJ, Price JH (eds) Plant–animal interactions in the marine benthos. Clarendon Press, Oxford, p 1–32

Hill AS, Hawkins SJ (1991) Seasonal and spatial variation of epilithic microalgal distribution and abundance and its ingestion by *Patella vulgata* on a moderately exposed rocky shore. J Mar Biol Assoc UK 71:403–423

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes. 1. Turnover of ^{13}C in tissues. Condor 94: 181–188

Jenkins SR, Hartnoll RG (2001) Food supply, grazing activity and growth rate in the limpet *Patella vulgata* L.: a comparison between exposed and sheltered shores. J Exp Mar Biol Ecol 258:123–139

Jenkins SR, Arenas F, Arrontes J, Bussell J and others (2001) European-scale analysis of seasonal variability in limpet grazing activity and microalgal abundance. Mar Ecol Prog Ser 211:193–203

Jenkins SR, Coleman RA, Della Santina P, Hawkins SJ, Burrows MT, Hartnoll RG (2005) Regional scale differences in the determinism of grazing effects in the rocky intertidal. Mar Ecol Prog Ser 287:77–86

Jones NS (1948) Observations and experiments on the biology of *Patella vulgata* at Port St. Mary, Isle of Man. Proc Trans Liverpool Biol Soc 56:60–77

Jonsson PR, Granhag L, Moschella PS, Aberg P, Hawkins SJ, Thompson RC (2006) Interactions between wave action and grazing control the distribution of intertidal macroalgae. Ecology 87:1169–1178

Legendre P, Legendre L (eds) (1998) Numerical ecology. In: Developments in environmental modelling, Vol 20, 2nd English edn. Elsevier Science, Amsterdam, p 247–302

Lewis JR (1964) The ecology of rocky shores. English Universities Press, London

Little C, Williams GA, Morritt D, Perrins JM, Stirling P (1988) Foraging behaviour of *Patella vulgata* L. in an Irish sea-lough. J Exp Mar Biol Ecol 120:1–21

Little C, Morritt D, Paterson DM, Stirling P, Williams GA (1990) Preliminary observations on factors affecting foraging activity in the limpet *Patella vulgata.* J Mar Biol Assoc UK 70:181–195

Lodge SM (1948) Algal growth in the absence of *Patella* on an experimental strip of foreshore, Port St. Mary, Isle of Man. Proc Trans Liverpool Biol Soc 56:78–85

Lorenzen S (2007) The limpet *Patella vulgata* L. at night in air: effective feeding on *Ascophyllum nodosum* monocultures and stranded seaweeds. J Molluscan Stud 73: 267–274

Moore P, Thompson RC, Hawkins SJ (2007) Effects of grazer identity on the probability of escapes by a canopy-forming macroalga. J Exp Mar Biol Ecol 344: 170–180

Notman GM (2011) A comparison of the trophic ecology of grazing gastropods on the rocky shores of northern and southern Britain. PhD dissertation, University of Aberdeen

Pinnegar JK, Polunin NVC (1999) Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: implications for the study of trophic interactions. Funct Ecol 13:225–231

Podani J (1999) Extending Gower's general coefficient of similarity to ordinal characters. Taxon 48:331–340

Polis GA, Anderson WB, Holt RD (1997) Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annu Rev Ecol Syst 28: 289–316

Raffaelli D (1985) Functional feeding groups of some intertidal mollusks defined by gut contents analysis. J Molluscan Stud 51:233–239

Riera P, Escaravage C, Leroux C (2009) Trophic ecology of the rocky shore community associated with the *Ascophyllum nodosum* zone (Roscoff, France): a δ^{13}C vs δ^{15}N investigation. Estuar Coast Shelf Sci 81:143–148

Schaal G, Riera P, Leroux C (2010) Trophic ecology in a northern Brittany (Batz Island, France) kelp (*Laminaria digitata*) forest, as investigated through stable isotopes and chemical assays. J Sea Res 63:24–35

Søreide JE, Tamelander T, Hop H, Hobson KA, Johansen I (2006) Sample preparation effects on stable C and N isotope values: a comparison of methods in Arctic marine food web studies. Mar Ecol Prog Ser 328:17–28

Southward A (1964) Limpet grazing and the control of vegetation on rocky shores. In: Crisp DJ (ed) Grazing in terrestrial and marine environments. Blackwell Scientific Publications, Oxford, p 296–373

Southward AJ, Southward EC (1978) Recolonisation of rocky shores in Cornwall after use of toxic dispersants to clean up the Torrey Canyon spill. J Fish Res Board Can 35:682–706

Steinarsdóttir MB, Ingólfsson A, Ólafsson E (2009) Trophic relationships on a fucoid shore in south-western Iceland as revealed by stable isotope analyses, laboratory experiments, field observations and gut analyses. J Sea Res 61: 206–215

Steneck RS, Watling L (1982) Feeding capabilities and limitation of herbivorous mollusks — a functional-group approach. Mar Biol 68:299–319

Thompson RC, Roberts MF, Norton TA, Hawkins SJ (2000) Feast or famine for intertidal grazing molluscs: a mismatch between seasonal variations in grazing intensity and the abundance of microbial resources. Hydrobiologia 440:357–367