Estuarine, Coastal and Shelf Science 92 (2011) 339–346

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Isotopic shift for defining habitat exploitation by the Antarctic limpet *Nacella concinna* from rocky coastal habitats (Marian Cove, King George Island)

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ARTICLE INFO

Article history:
Received 17 August 2010
Accepted 14 January 2011
Available online 1 February 2011

Keywords:
food availability
Antarctic limpet *Nacella concinna*
phytobenthos
tidal pools
rocky shores
King George Island

ABSTRACT

$\delta^{13}C$ and $\delta^{15}N$ of the Antarctic limpet *Nacella concinna* tissues and their potential food sources were used to determine their dietary origins and their movements between diverse habitats of intertidal and subtidal rocky shores and tide pools of Marian Cove, King George Island, Antarctica in the austral summer. $\delta^{13}C$ and $\delta^{15}N$ of the organic matter sources of epilithic microalgae, macroalgae and suspended particulate organic matter (SPOM) were readily distinguishable to discern their relative contribution to the limpet diets, with the most depleted values being found in SPOM and the most enriched in macroalgae. The limpets exhibited a spatial trend in distribution due to their seasonal migration, with smaller individuals in the subtidal zone compared with larger ones on the intertidal sites. The limpet isotopes had relatively broad ranges of $\delta^{13}C$ and $\delta^{15}N$ (−26.6 to −12.8‰ and 2.6–7.1‰, respectively), suggesting a dietary shift between habitats as well as size classes. The stable isotope ratios for each habitat seem likely to reflect the differing availabilities of the three potential food sources. Isotope mixing model results indicate a spatial shift in dietary mixture between habitats as well as limpet size classes. Epilithic microalgae and phytoplankton made great contributions to the diet of the subtidal limpets. Together with epilithic microalgae, macroalgae were significant contributors to the intertidal limpets where macroalgae were abundant. A higher contribution of macroalgae to the limpet diets was found in the tide pools. In contrast, while phytoplankton was an important food source for the limpet spat, a great dietary dependence on epilithic microalgae was found in the small-size limpets from the lower intertidal zone. Our results suggest that limpet grazing can determine microalgal and/or macroalgal abundance and coverage on the Antarctic rocky-shore ecosystem, and trophic structure of benthic food web can change along environmental gradients even at spatial scales of tens or hundreds of metres in the Antarctic.

1. Introduction

The patellid limpet *Nacella concinna* (Strebel, 1908) is one of the most common macrobenthic invertebrates colonizing the Antarctic and sub-Antarctic rocky shores (Picken, 1980; Davenport, 1988). The Antarctic limpet, which is a benthic grazer, displays the most important biomass of intertidal invertebrates in King George Island (Fraser, 1989). This limpet resource represents an important dietary component to the energy requirements of Antarctic kelp gulls (Fraser, 1989; Favero et al., 1997). Limpets may, therefore, play a crucial role as a trophic mediator between primary producers and predators in the Antarctic rocky-shore food web.

As previously shown by Walker (1972) for the limpet *Patinigera polaris* (identified as the same species with *N. concinna* by Powell (1973)) found around Signy Island (Antarctic), *N. concinna* consists of two subpopulations in the rocky coast of King George Island. Whereas the one migrates seasonally from the subtidal to the intertidal zone in spring and back to the subtidal zone in autumn, the other remains in the subtidal all the year. They have distinct characteristics regarding size and morphology. The subtidal nonmigrant population consists of individuals with a relatively small shell size, whereas the intertidal migrant population exhibits a larger shell size of >20 mm length, with larger shell height-to-length ratios (Walker, 1972; Brêthes et al., 1994; Kim, 2001). Several factors, including competition, environmental changes, predation and food availability, may be considered to be responsible for such a limpet migration pattern (Fletcher, 1987; De Aranzamendi et al., 2008). Firstly, the food availability in the intertidal zone has long been recognized as an important factor

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0272-7714/$ – see front matter
doi:10.1016/j.ecss.2011.01.009

determining the migration of the limpets towards it (Walker, 1972; Brêthes et al., 1994; Kim, 2001).

When considering the availability of food for the growth of the Antarctic limpet, most studies have considered microphytobenthos as an important food source (Iken, 1995; Brêthes et al., 1994; Corbisier et al., 2004). As suggested by Brêthes et al. (1994), investigations on the trophic role of alternative food sources may help to better identify the factors of migration. In this respect, macroalgae may be one of the most likely candidates (Jernakoff, 1985; Iken, 1995). Indeed, the dominant macroalgae *Adenocystis utricularis* and *Iridaea cordata* are observed in the middle intertidal and at edges of the tide pool in summer and in the lower intertidal throughout the year on the coast of King George Island (Kim, 2001). This author also shows that the density of propagules of macroalgae may be regulated by the grazing of the limpets. Moreover, settled phytoplankton and ice algae may also be available to benthic grazers (Brêthes et al., 1994).

Although an investigation of the standing stock of microphytobenthos may assess part of the limpet food availability, such a study would give little information on the food assimilated. The analysis of the stable isotopes of carbon and nitrogen can provide indications on the origin of organic matter sources and further flows through the food web (Fry and Sherr, 1984; Michener and Schell, 1994). Slight enrichment of heavier isotopes in the tissues of organisms during the course of metabolism occurs, and these metabolic fractionations are predictable (0.8‰ for carbon, Fry and Sherr, 1984; 2–5‰ for nitrogen, Vander Zanden and Rasmussen, 2001; McCutchan et al., 2003). Stable isotopes have therefore proven to be a powerful tool in the interpretation of food assimilated by consumers over time.

The present study investigated the stable isotope ratios of carbon and nitrogen in the dominant organic matter sources of epilithic microalgae, macroalgae and phytoplankton, together with the limpet tissues in Marian Cove, King George Island, Antarctica. Stable isotope ratios can discriminate between different sources of diet for limpets (Dunton, 2001). In order to understand more accurately the role of limpets in the food web of the sub-Antarctic rocky-shore ecosystem and their population responses to food availability, it is necessary to have information on their trophodynamics. The aims of the present work were to determine the food sources of the limpet populations and to assess their dietary shift according to site conditions among different habitats.

2. Materials and methods

2.1. Study area

King George Island is in western Antarctic and is the largest of the South Shetland Islands. Maxwell Bay is one of the deep, U-profile fjords along the southern margin of the island. Several subsidiary embayments are developed within the fjord, including Marian Cove and Potter Cove in the northeast and Collins Harbour in the north (Fig. 1). Marian Cove, 3.5 km long and 1.2 km wide, where King Sejong Research Station (62°13′S, 58°47′W) is located, is one of the fjords within Maxwell Bay that receives a substantial amount of glacier-melt water during the austral summer.

The main study area is located on the coast of the Barton Peninsula at the side of Marian Cove and is characterized by cobbles lying on sand and gravel in the littoral fringe between the intertidal and subtidal zones. This stony beach extends from the supralittoral down to the uppermost subtidal level to a depth of ca 0.5–1 m below the low-water line. Beneath the cobble layer, there is patchy gravel and pebbles, the surface of which, at the time of sampling, was covered by relatively abundant epilithic algae. The upper intertidal is characterized by disturbance of ice foot and ice scouring on the rocky shore of Maxwell Bay on the Barton Peninsula (Kim, 2001). The red alga *Porphyra endiviifolium* and filamentous algae, such as *Urosopora penicilliformis*, *Ulothrix* sp., and *Bangia atropurea,* occupy predominantly the upper intertidal zone on the coastal rocks. However, the limpet *Nacella concinna* are absent in the upper intertidal. Filamentous algae occur in the middle intertidal in spring, but disappear during summer. Thalli of *Adenocystis utricularis* and *Iridaea cordata* grow in rock crevices and tide pools in the middle intertidal. The limpet *N. concinna* migrates in this zone during summer. The lower intertidal is characterized by the absence of ice foot in winter. *N. concinna* occurs throughout the year, and *A. utricularis* and *I. cordata* are also observed all year round in this zone. For comparison, an additional study area was chosen in the intertidal zone of Weaver Peninsula on the opposite side of Marian Cove.

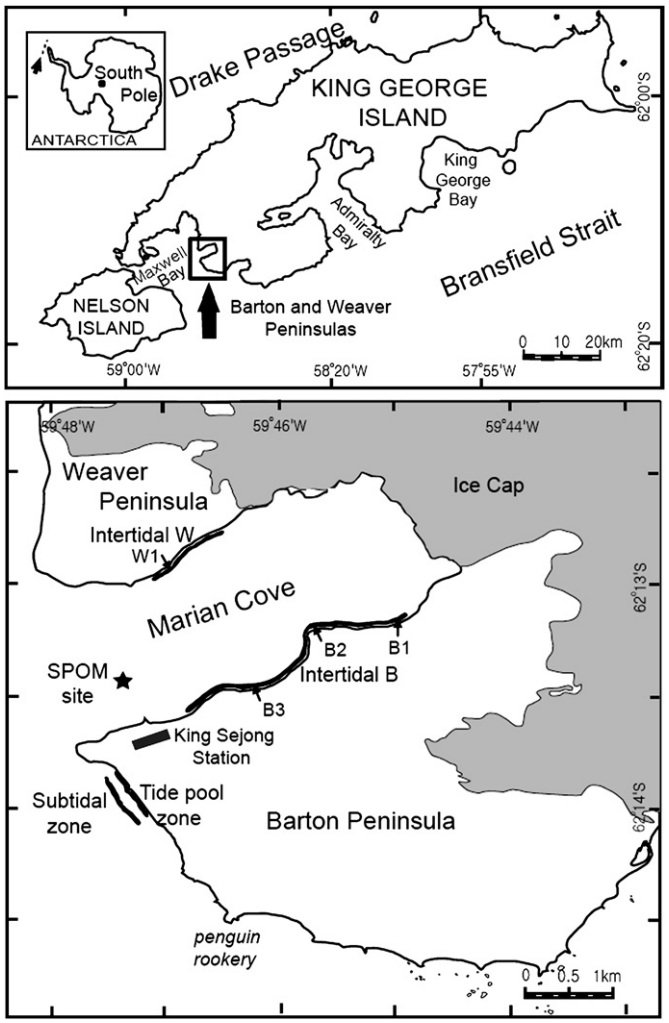

Fig. 1. Map of the study area in Maxwell Bay of King George Island. B and W, intertidal stations of Barton Peninsula and Weaver Peninsula, respectively.

2.2. Sample collection

The field work was carried out from January 29 to February 13, 2009, and the limpets were collected mainly during the short period of February 10–13, 2009. To characterize suspended particulate organic matter (SPOM) entering the bay from offshore, 20-L water samples were collected at high tide at the entrance of the bay from a depth of about 50 cm below the water surface (Fig. 1). At low tide, the dominant macroalgae *Adenocystis utricularis* and *Iridaea cordata*

were randomly collected by hand at three sites on the bare flat (hereafter Intertidal B) and tide pools of Tide pool zone in Barton Peninsula. Samples of epilithic microalgae were also randomly obtained by scraping the rock surface with a metal brush at two sites on Intertidal B, one site on the bare intertidal flat in Weaver Peninsula (hereafter Intertidal W), tide pools of the intertidal zone, and the subtidal zone in Barton Peninsula. The limpets, *Nacella concinna*, were also randomly collected by hand from four habitats along the shoreline of the cove during ebb tide: the bare intertidal flats of Barton and Weaver Peninsulas (Intertidal B and W); tide pools on the intertidal zone of Barton Peninsula; and the subtidal zone of Barton Peninsula.

2.3. Sample preparation

SPOM was obtained by filtering seawater on precombusted (6 h, 550 °C) Whatman GF/F glass-fiber filters. The seawater was pre-filtered with a 200-μm mesh net to remove any zooplankton and large particles, and collected in acid-washed plastic bottles. Water from these samples was then passed through a 20-μm sieve to obtain coarse suspended particulate organic matter comprised of mainly micro-sized phytoplankton. The remaining water was filtered once again through precombusted Whatman GF/F glass-fiber filters (nominal pore size = 0.7 μm) to obtain fine suspended particulate organic matter representing pico- and nano-plankton. The sieved and filtered particulates for organic carbon isotope analysis were quickly acidified with 2–3 drops of 1 N HCl to remove inorganic carbonates and then rinsed with Milli-Q water. As acid washing can affect the nitrogen isotope ratios of organic material (Bunn et al., 1995), samples for nitrogen isotope analysis were not acidified. The collected macroalgal leaves were scraped with a razor blade to remove epibionts, rinsed with fresh water to eliminate salts and epibionts, and then rinsed again with Milli-Q water. The limpets collected were kept alive for 24 h in the laboratory to allow the evacuation of gut content. After being depurated, they were frozen for ease of dissection, and the shell was removed from each half-frozen limpet. Shell dimensions were determined to the nearest 0.01 mm with vernier calipers. While the adults of the limpet were individually analyzed, the spat were pooled for 20 individuals to ensure sufficient biomass for stable isotope analysis. All samples for stable isotope analysis were freeze-dried for about 48 h. Dried specimen of macroalgae and limpets were ground to a fine powder and then all the samples were kept frozen (−30 °C) until analysis.

2.4. Stable isotope analyses

The samples for isotope analysis were analyzed using a system that coupled an elemental analyzer (EuroVector 3000 Series) with a continuous-flow isotope ratio mass spectrometer (CF-IRMS; Isoprime, GV Instruments, U.K.). The samples wrapped in tin capsules were combusted at 1030 °C in the elemental analyzer, and the percentage C and N compositions were directly determined, the instrument being calibrated with an acetanilide standard (C, H, $N = 71.09, 6.71, 10.36\%$). Then the resultant CO_2 and N_2 gases were introduced into the CF-IRMS.

Stable isotope abundance is expressed in delta (δ) notation as the deviation from the conventional standard Pee Dee Belemnite (PDB) for carbon and air N_2 for nitrogen in parts per thousand (‰), according to the equation: $\delta X = ([R_{sample}/R_{standard}] - 1) \times 10^3$, where X is ^{13}C or ^{15}N and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio, respectively (Fry and Sherr, 1984). Sucrose (ANU $C_{12}H_{22}O_{11}$; NIST, Gaithersburg, MD) and ammonium sulfate ($[NH_4]_2SO_4$; NIST) were used for the internal ^{13}C and ^{15}N calibration, respectively, and were analyzed twice after every six samples. The analytical reproducibility, based on the standard deviations of at least triplicate analyses for each sample, was approximately ±0.1‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$.

2.5. Statistical analysis

Because of the small sizes of replications, spatial trends in $\delta^{13}C$ and $\delta^{15}N$ values for epilithic microalgae and macroalgae were tested using the Kruskal–Wallis test. To test the interaction between algal sources and sampling sites, two-way analysis of variance (two-way ANOVA) was performed for macroalgae (brown alga *Adenocystis utricularis* and red alga *Iridea cordata*) and epilithic microalgae collected at the same sites. Differences in $\delta^{13}C$ and $\delta^{15}N$ among the organic matter sources (i.e. SPOM, epilithic microalgae and macroalgae) were then analyzed using one-way ANOVA, and appropriate means were compared using Tukey *post hoc* tests. Normality was tested using the Shapiro–Wilk test, and the heterogeneity of variance was determined using Levene's test prior to ANOVA. We also investigated whether shell-length distribution of the limpets differed among sampling sites. Mean $\delta^{13}C$ and $\delta^{15}N$ of the limpet tissues among four habitats were compared using the same procedures. For this test, the limpets from the intertidal B and the subtidal were separated into two size classes. The SPSS 12.0 statistical software package was used to perform all statistical tests.

The relative contribution of three dominant primary producers (phytoplankton, epilithic microalgae and macroalgae) to the limpet food source and the SPOM composition was estimated using the concentration-weighted mixing model (Phillips and Koch, 2002). In this calculation, the $\delta^{13}C$ and $\delta^{15}N$ values of the limpet were assumed to increase 0.8‰ and 2.2‰ from the diet, respectively (Dunton, 2001). These trophic fractionation values were very close to mean shifts (−0.4 and +1.3 for $\delta^{13}C$; +2.5 and +2.2 for $\delta^{15}N$, respectively) suggested for herbivores by Vander Zanden and Rasmussen, (2001) and McCutchan et al. (2003). Habitat- and size-specific mean $\delta^{13}C$ and $\delta^{15}N$ values for the limpets were incorporated into the mixing model. Mean isotopic values and C and N concentrations determined for epilithic microalgae and macroalgae in this study were used as end-member values. The mean $\delta^{13}C$ and $\delta^{15}N$ values (−27.7‰ and 0.4‰, respectively) in the previous studies were used as end-member values for phytoplankton (Wada et al., 1987; Dunton, 2001). It was assumed that C and N concentrations for phytoplankton are the same as those for SPOM.

3. Results

3.1. Isotope ratios of organic matter sources

Although $\delta^{15}N$ values of suspended particulate organic matter (SPOM), epilithic microalgae and macroalgae fell within a very narrow range (mean ± 1 SD, 1.3 ± 0.6 to 3.9 ± 1.0‰), $\delta^{13}C$ values separated these groups fairly well (range: −24.1 ± 0.2 to −13.5 ± 3.6‰; Table 1). There were no significant spatial differences in the $\delta^{13}C$ and $\delta^{15}N$ values of epilithic microalgae (Kruskal–Wallis ANOVA, $\chi^2 = 3.420$ and 7.596, $p = 0.331$ and 0.055, $n = 14$, for C and N, respectively). No significant spatial trends were found even in the macroalgal $\delta^{13}C$ and $\delta^{15}N$ values (Kruskal–Wallis ANOVA, $\chi^2 = 6.038$ and 5.581, $p = 0.110$ and 0.134, $n = 14$, for C and N of *A. utricularis*; $\chi^2 = 2.733$ and 4.133, $p = 0.435$ and 0.247, $n = 9$, for C and N of *I. cordata*, respectively). The 2-way ANOVA tests on the $\delta^{13}C$ and $\delta^{15}N$ values of two macroalgal species (*Adenocystis utricularis* and *Iridea cordata*) and epilithic microalgae showed that there was a significant difference in the $\delta^{13}C$ among species ($p = 0.001$) but no significant difference among sampling sites ($p = 0.522$; Table 2). There was no significant interaction in the $\delta^{13}C$ between the algal source and the sampling site ($p = 0.607$), indicating consistent difference in the $\delta^{13}C$ between algal sources at all sampling sites.

Table 1
Mean stable isotope ratios (‰) of potential food sources and *Nacella concinna*.

	Station	$\delta^{13}C$	SD	*n*	$\delta^{15}N$	SD	*n*	Shell length (mm)
SPOM		−24.1	0.2	6	1.3	0.6	4	
Epilithic microalgae		−19.5	2.1	14	4.0	0.7	14	
(mean)	Intertidal B1	−20.2	2.1	2	3.7	0.0	2	
	Intertidal B3	−20.5	2.6	4	3.7	0.8	4	
	Intertidal W	−18.2	1.8	5	4.6	0.4	5	
	Tide pool	−19.8	2.1	3	3.7	0.8	3	
Macroalgae (mean)		−13.5	3.6	23	3.9	1.0	23	
Adenocystis utricularis	Intertidal B1	−13.0	3.3	4	3.3	0.9	4	
	Intertidal B2	−13.8	0.2	2	3.9	0.6	2	
	Intertidal B3	−16.3	4.6	6	3.8	0.6	6	
	Tide pool	−11.0	5.1	2	4.7	0.8	2	
Iridea cordata	Intertidal B1	−12.7	2.5	3	3.3	1.0	3	
	Intertidal B2	−12.1	0.1	2	2.9	2.3	2	
	Intertidal B3	−13.5	0.6	2	4.5	0.7	2	
	Tide pool	−14.4	0.1	2	4.9	0.6	2	
Nacella concinna	Intertidal B	-17.8^{a}	1.2	29	6.0^{g}	0.7	29	23.2–13.6
	Intertidal B	-22.1^{c}	0.6	12	5.2^{fg}	0.5	12	10.7–19.0
	Tide pool	-16.5^{a}	1.5	27	5.0^{f}	0.6	27	26.3–35.7
	Intertidal W	-19.6^{b}	1.4	11	4.9^{f}	0.6	11	30.6–10.7
	Subtidal	-20.6^{bc}	1.1	24	5.0^{f}	0.5	24	14.4–30.2
	Subtidal	-26.3^{d}	0.3	3	2.7^{e}	0.1	3	<4

The same superscript letters (a–g) indicate no significant difference (Tukey test, $p > 0.05$).

Moreover, Tukey *post hoc* test showed that while two macroalgal species displayed no significant difference in their $\delta^{13}C$ values ($p = 0.845$), epilithic microalgae had significantly lower $\delta^{13}C$ than that of macroalgae ($p < 0.05$). The $\delta^{13}C$ values of epilithic microalgae ranged from −22.9 to −16.7‰ with a mean (±1 SD) of −19.5 (±2.1) ‰ (Table 1). Two macroalgal species had the most positive values of the organic matter sources, ranging from −19.7 to −7.4‰ with a pooled mean of −13.5 (±3.6)‰ (one-way ANOVA, $F_{3, 39} = 25.906$, $p < 0.001$; Tukey *post hoc* test, $p < 0.05$). The epilithic microalgae and macroalgae had similar $\delta^{15}N$ with mean values of 4.0 (±0.7)‰ and 3.9 (±1.0)‰, respectively (Tukey *post hoc* test, $p = 0.974$). As fine and coarse particulate organic matter from the water column had very similar $\delta^{13}C$ and $\delta^{15}N$ values, the data were pooled. SPOM exhibited the most negative $\delta^{13}C$ value with a mean of −24.1 (±0.2)‰ and the lowest $\delta^{15}N$ value of 1.3 (±0.6)‰ (Tukey *post hoc* test, $p < 0.05$).

3.2. Isotope ratios of the limpets

Despite some overlap in shell length between the intertidal and subtidal sites, the shell length–frequency diagram exhibits a clear trend in the distribution of the limpet *N. concinna*, the former group having larger individuals than the latter group (one-way ANOVA, $F_{3,416} = 198.6$, $p < 0.001$; Fig. 2). Stable isotope values of the limpets were quite variable, ranging from −26.6 to −12.8‰ and 2.6–7.1‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively (Table 1, Fig. 3). The individuals from the intertidal B and tide pool were uniformly more ^{13}C-enriched than those from the subtidal and the intertidal W.

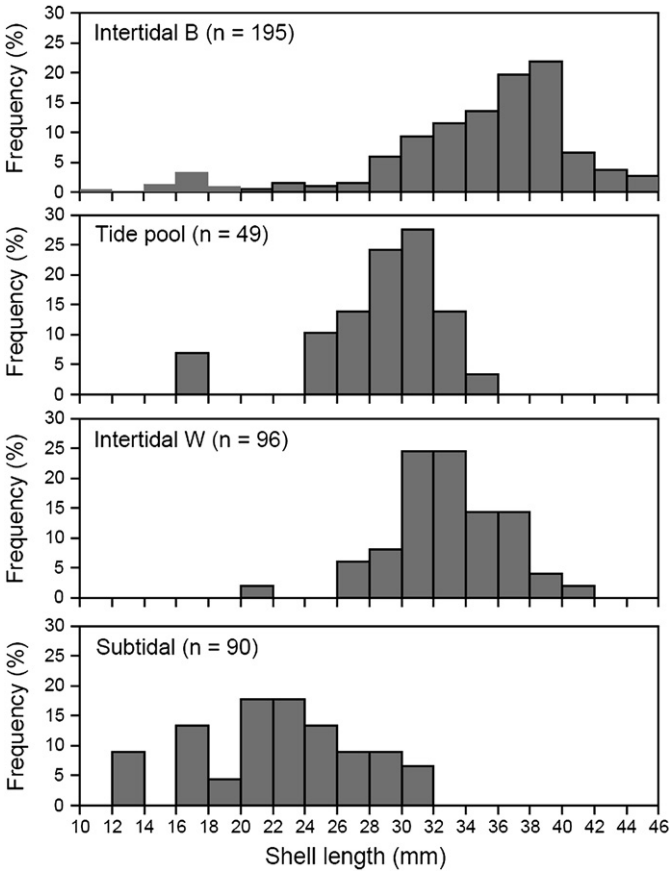

Fig. 2. Size frequency of the Antarctic limpet *Nacella concinna* collected in different habitats of King George Island on February 10–13, 2009.

Results of the one-way ANOVA revealed evidence for a significant site effect on the observed differences in the limpet $\delta^{13}C$ values ($F_{5, 100} = 72.912$, $p < 0.001$; Table 1). The limpets collected from the intertidal B sites (a mean of −20.6 ± 1.1‰) displayed more ^{13}C-enriched values than those collected from the subtidal zone (mean −17.8 ± 1.2‰; Tukey *post hoc* test, $p < 0.05$) and from the intertidal W (mean −19.6 ± 1.4‰; $p < 0.05$), but were similar to those from the tide pool (mean −16.5 ± 1.5‰; $p = 0.228$). Between-site differences in the $\delta^{15}N$ values of the limpets were significant ($F_{5,100} = 21.905$, $p < 0.001$). The difference was relatively small (about 1‰) between individuals from the intertidal B and from the other sites (Tukey *post hoc* test, $p < 0.05$).

The ANOVA results also showed a significant size effect on the $\delta^{13}C$ differences in the limpet tissues (Table 1; Fig. 3). By chance during the sampling, small-sized limpets of 10.7–19.0 mm were collected in an intertidal rock crevice in the lower part of the intertidal B. These smaller individuals had a mean $\delta^{13}C$ of −22.11 (±0.56)‰, which was more ^{13}C-depleted than larger specimens more commonly collected from the B intertidal zone (Tukey *post hoc* test, $p < 0.05$) but similar to those collected from the B subtidal region ($p = 0.106$). We also collected many subtidal limpet spat of 3–5 mm shell length. Both the $\delta^{13}C$ and $\delta^{15}N$ values of the spat (mean of −26.3 ± 0.3‰ and 2.7 ± 0.1‰, respectively) were the most depleted among the limpets analyzed (Tukey *post hoc* test, $p < 0.05$ for $\delta^{13}C$ and $\delta^{15}N$).

A dual isotope plot of $\delta^{13}C$ versus $\delta^{15}N$ for the potential food sources and the consumers enables us to interpret the food sources

Table 2
2-way ANOVA of effects of source and sampling site on the $\delta^{13}C$ and $\delta^{15}N$ values of algal sources (*Adenocystis utricularis, Iridea cordata* and epilithic microalgae) collected concurrently

Source of variation	df	$\delta^{13}C$			$\delta^{15}C$		
		MS	*F*	P	MS	*F*	P
Within + Residual	19	11.06			0.54		
Source	2	123.90	11.20	0.001	0.57	1.05	0.369
Sampling site	2	7.45	0.67	0.522	1.85	3.40	0.055
Source × Sampling site	4	7.64	0.69	0.607	0.62	1.13	0.372
Model	8	37.10	3.35	0.014	0.87	1.61	0.188
Total	27						

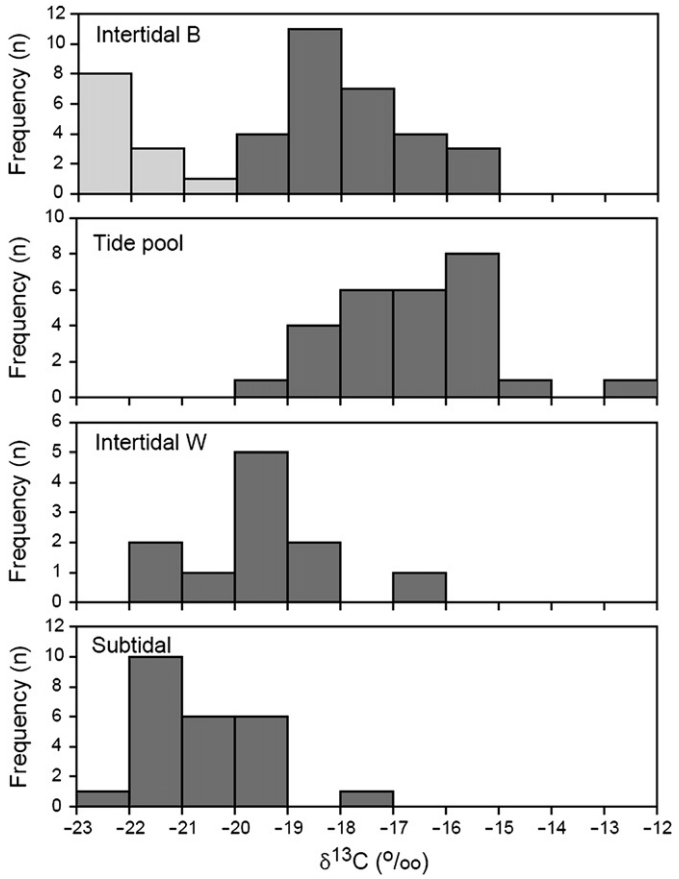

Fig. 3. Frequency distribution of $\delta^{13}C$ values of the Antarctic limpet *Nacella concinna* collected in different habitats of King George Island on February 10–13, 2009. Gray bar represents small-size limpets of 10.7–18.1 mm in shell length collected in the intertidal B station.

assimilated (Fig. 4). The $\delta^{13}C$ values of the limpet spat were very close to those of SPOM. The limpet $\delta^{13}C$ values from the subtidal B zone, the intertidal W, and the smaller-size animals from the intertidal B zone were aligned with those of epilithic microalgae. However, the values of the limpets commonly collected in the intertidal B and tide pools were close to those of macroalgae and positioned between those of epilithic microalgae and macroalgae.

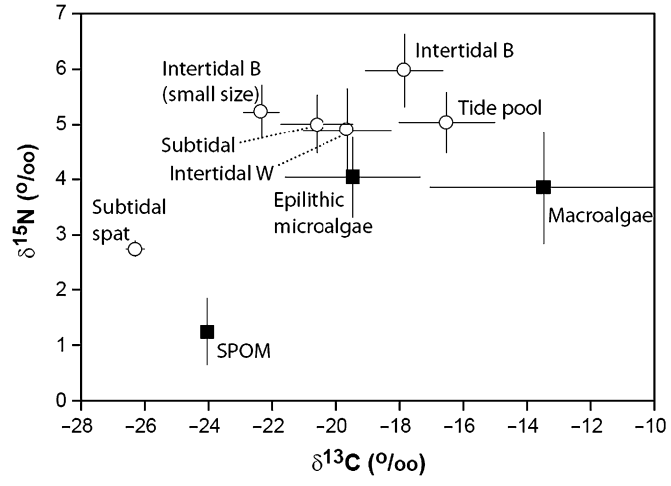

Fig. 4. Dual plot of $\delta^{13}C$ and $\delta^{15}N$ values of organic matter sources (square) and the limpets (circle) collected in different habitats of King George Island in February 2009.

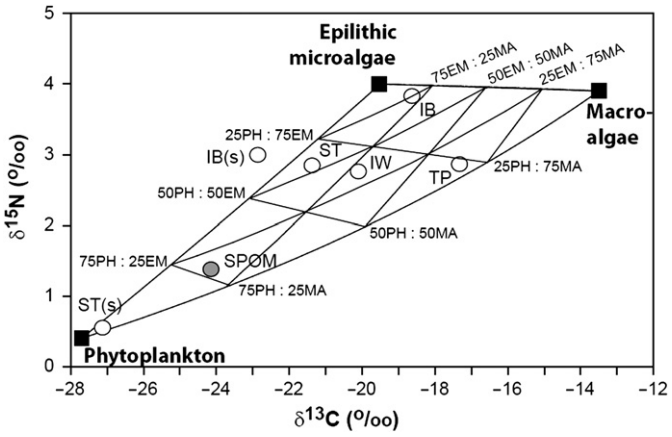

Fig. 5. Mixing triangle for the concentration-weighted model. Variations in percentage contribution of phytoplankton (PH), epilithic microalgae (EM), and macroalgae (MA) are shown along the edges of the mixing triangles. IB, IB(s), ST, ST(s), IW, and TP indicate the presumed diets for the limpets of intertidal B, intertidal B (small-size), subtidal, subtidal (spat), intertidal W, and tide pool, respectively.

Based on trophic fractionation of +0.8‰ and +2.2‰ for limpet-tissue $\delta^{13}C$ and $\delta^{15}N$ from the diet, $\delta^{13}C$ and $\delta^{15}N$ plots for the presumed diet of the limpets showed considerable shifts among habitats (Fig. 5). Therefore, a three-source mixing model suggested spatial variation in relative contribution of primary organic matter sources to the limpet diet (Table 3). The result indicated that epilithic microalgae and phytoplankton made great contributions (54% and 36%, respectively) to the diet of the subtidal limpets, with a minor contribution of 10% by macroalgae. Together with the high contribution (72%) of epilithic microalgae, macroalgae may be a significant contributor (22%) to the large-sized limpet diet of the intertidal B zone. A higher contribution (67%) of macroalgae to the limpet diets was found in the tide pool site, with a considerable contribution (28%) of epilithic microalgae. Epilithic microalgae and phytoplankton made nearly equal contribution (about 37%) to the diet of the limpets was detected on the intertidal W, with an important contribution (27%) of macroalgae. On the other hand, while epilithic microalgae occupied the major part (83%) of the diet of the small-sized limpets with a shell length of 10.7–19.0 mm collected in the lower part of the intertidal B, the contribution of macroalgae was estimated as negative. In contrast, phytoplankton were the only sources contributing to the diet of the limpet spat with a shell length of 3–5 mm collected at the subtidal zone. Model estimation also suggested that phytoplankton primarily occupied SPOM. However, epilithic microalgae and macroalgal debris also constituted considerable amounts (10 and 16%, respectively) of SPOM.

Table 3
Relative contribution of 3 food sources (phytoplankton. epilithic microalgae and macroalgae) to the limpet diet, as based on 2 isotopes of $\delta^{13}C$ and $\delta^{15}N$ (after correcting trophic fractionation of +0.8‰ for $\delta^{13}C$ and +2.2‰ for $\delta^{15}N$)

Subpopulation	Phytoplankton	Epilithic microalgae	Macroalgae	Shell length (mm)
Inter-tidal B	0.06	0.72	0.22	23.2–43.6
Intertidal B (small)	0.34	0.83	−0.17	10.7–19.0
Tide pool	0.28	0.05	0.67	26.3–35.7
Intertidal W	0.36	0.37	0.27	30.6–40.7
Subtidal	0.36	0.54	0.10	14.4–30.2
Subtidal (spat)	0.96	0	0.04	<4
SPOM	0.74	0.10	0.16	

4. Discussion

4.1. Isotope ratios of potential food sources

The mean $\delta^{13}C$ ($-24.1 \pm 0.2‰$) and $\delta^{15}N$ ($1.3 \pm 0.6‰$) of suspended particulate organic matter (SPOM) found in this study were within ranges previously measured in Antarctica (Mincks et al., 2008). A ^{13}C depletion in Antarctic SPOM when compared with temperate SPOM ($-22.3‰$, see the reviews of Rau et al., 1982; Fischer, 1991; France, 1995) is generally accepted. Low temperature (below 2 °C), low light intensity and high water CO_2 (aq) values lead to very low ^{13}C content in the phytoplankton (Rau et al., 1989, 1991a). Thomson and Calvert (1994) also suggest a substantial role of irradiance rather than of CO_2 (aq) in the physiology of ^{13}C incorporation in marine diatoms. As a result, the low $\delta^{13}C$ and $\delta^{15}N$ values of SPOM may be explained by slow grow rates of phytoplankton (Cifuentes et al., 1988; Fry, 1996; Goering et al., 1990), together with the typical environmental characteristics of Antarctica. In contrast, the remarkably wide $\delta^{13}C$ range (-19.7 to $-7.4‰$, mean: $-13.5 \pm 3.6‰$ in this study) of macroalgae is commonly determined along the rocky shores of the Antarctic Peninsula (Fischer and Wiencke, 1992; Dunton, 2001). Large inter- and intraspecific variations as well as different isotopic composition in different parts of the same thallus is due to variation in specific growth rate depending on varying environmental condition (Fischer and Wiencke, 1992). The $\delta^{13}C$ and $\delta^{15}N$ of the two dominant macroalgal species, *Adenocystis utricularis* and *Iridea cordata*, were very close to those previously found in the Antarctic Peninsula (Fischer and Wiencke, 1992; Dunton, 2001). On the other hand, variation in $\delta^{13}C$ of the epilithic microalgae was also slightly high in an intermediate range (-16.7 to $-22.9‰$, mean: $-19.5 \pm 2.1‰$) between macroalgae and phytoplankton. The mean value was slightly (about $2‰$) lower than that of epiphytic diatoms (largely *Navicula schefterae*), probably because of different species composition (Dunton, 2001). Their $\delta^{15}N$ (mean: $4.0 \pm 0.7‰$) was very similar to that of macroalgae. Although the $\delta^{13}C$ values of macroalgae and epilithic microalgae varied somewhat widely within each source, it was possible to discriminate among the three food sources to discern their relative contribution of organic matter.

There is a higher standing stock of benthic invertebrates in the Antarctic intertidal zone, where the ice cover varies throughout the year, than on continental shelves (Grebmeier and Barry, 1991). This suggests a close relationship between pelagic and benthic productivity in some nearshore parts of the Antarctic Peninsula. Primary productivity within the nearshore water column in Antarctica is extremely seasonal and limited to the short period of summer (Clarke et al., 1988; Clarke and Leakey, 1996). The highest rates ($0.04–0.56$ g C m^{-2} d^{-1}) of primary production and SPOM concentration found in the austral summer are generally low compared with the temperate and tropical coastal regions of other continents (Kim et al., 1998). During summer, dense populations of the Antarctic limpet *Nacella concinna* occur both subtidally and intertidally. Ahn (1993) suggested that the summer phytoplankton production and SPOM levels of the water column are not sufficient to support all the benthic organisms, and that supplementary food sources are necessary.

In addition to the pelagic source of organic matter, other primary sources of food, such as benthic microalgae, macroalgae and ice-associated microalgae, may also contribute to secondary production in the polar nearshore environment (Dayton et al., 1986; Kaehler et al., 2000; Dunton, 2001; Corbisier et al., 2004). Indeed, the biomass of benthic microalgae reaches a maximum during Spring–Summer (October–February) in the intertidal zone of the Barton Peninsula of King George Island (Kim, 2001). Previous studies on the phytoplankton community in the Marian Cove waters have revealed that half of the annual chlorophyll *a* was present in the two months of the austral summer (November and December), and epiphytic or epilithic diatoms such as *Fragilaria striatula*, *Achnanthes brevipes*, and *Licmophora* spp. then account for a considerable part of the water-column microalgal populations because of resuspension of benthic microalgae (Ahn et al., 1997, 2004; Kang et al., 2002). Although macroalgae and filamentous algae are relatively abundant on the upper intertidal zone of the rocky shore of Maxwell Bay on the Barton Peninsula (Kim, 2001), the limpets are absent from the upper intertidal zone. In contrast, the brown alga *Adenocystis utricularis* and the red alga *Iridea cordata* are the dominant macroalgal species in the middle and lower intertidal zones, where the limpets are abundant. These macroalgal species display great abundance and coverage in the middle part of the intertidal zone during summer, whereas the lower part is nearly bare of algae in the course of summer. On the other hand, visible mats of epilithic microalgae were found in the intertidal zone of the Weaver Peninsula, with macroalgal mats in patches during the sampling period.

Ice-associated microalgae may be one of the important food resources to the Antarctic food web and thus the limpet diet (Wada et al., 1987; Rau et al., 1991b; Corbisier et al., 2004; Norkko et al., 2007). *N. concinna* immigrates to the intertidal zone during the austral summer when ice cover is free. Furthermore, the absence of ice foot on the lower intertidal even in winter may indicate low availability of the ice algae to the intertidal limpet. Nevertheless, this source may have been assimilated just after the ice-break (early summer). In addition, although the samples in this study were collected a long time after the ice-break in the spring, the ice algae may still influence the isotope signal of low turnover tissue (Peck and Veal, 2001). However, when ice algae release from the sea ice, they can mix with epilithic microalgae. Indeed, as ice algae have ^{13}C-enriched values similar to epilithic microalgae (Norkko et al., 2007), their contribution to the limpet diet can be reflected in the mixing model calculation through the end-member value of epilithic microalgae in this study.

4.2. Isotopic shift between limpet habitats

The most interesting finding in the stable isotope analysis of the limpets is the large range in the tissue $\delta^{13}C$ and $\delta^{15}N$ (-26.6 to $-12.8‰$ and $2.6–7.1‰$, respectively), suggesting a dietary shift between sites as well as size classes. As earlier expected from the availabilities of primary producers at each limpet habitat, our stable isotope analysis indicates that epilithic microalgae and macroalgae might be important dietary sources for the limpets, but their importance varied between sites (Figs. 3 and 4). The $0.8‰$ trophic enrichment by limpets is generally accepted in food web studies (Dunton, 2001). The trophic enrichment for *N. concinna* was presumed to be $2.2‰$ (Dunton, 2001), which corresponds to the lower limit of $2–5‰$ generally mentioned for marine consumers (Vander Zanden and Rasmussen, 2001; McCutchan et al., 2003). The trophic enrichment of ^{15}N can be quite variable, with differences occurring because of the major form of nitrogenous waste, the C:N ratio or protein content of the diet, and the taxonomic class (Vanderkliff and Ponsard, 2003; McCutchan et al., 2003). McCutchan et al. (2003) show that the trophic shift in $\delta^{15}N$ for consumers ranges from $-0.8‰$ to $+5.9‰$. While N excretion increases postprandially in most marine ectotherms, NH_3 elimination declines in *N. concinna*, strongly reflecting a tendency to metabolize more ingested lipids and/or carbohydrates (Peck and Veal, 2001). These authors also find slow growth and development rates, low levels of activity and reduced metabolic rates as compared with temperate species. Considering such metabolizing characteristics in the Antarctic limpet, $\delta^{15}N$ enrichment as low as about $+2‰$ seems probable compared to traditional enrichment factor of $+3.4‰$ (Minagawa and

Wada, 1984). Trophic enrichment of 1–2‰ in $\delta^{15}N$ may be broadly accepted for herbivorous consumers (Brito et al., 2006).

Therefore, considering the $\delta^{13}C$ and $\delta^{15}N$ trophic enrichment values of +0.8‰ and +2.2‰, respectively, $\delta^{13}C$ and $\delta^{15}N$ of the presumed diet of the limpets changed considerably from site to site, indicating that the limpets utilize different mixes of dietary sources (Fig. 5). The result of the concentration-weighted mixing model indicates that the subtidal limpets utilize epilithic microalgae and phytoplankton as their major dietary sources. High grazing on microphytobenthos by the limpet *N. concinna* has been well established in the subtidal of the Antarctic Peninsula (Dunton, 2001; Corbisier et al., 2004). Macroalgae-derived organic matter also constitutes part (10%) of their diet, confirming the contribution of macroalgal fragments, together with sedimentary organic matter, to subtidal food web of the King George Basin (Dunton, 2001; Fischer and Wiencke, 1992; Corbisier et al., 2004). Although most of the diet of the large-sized limpets on the intertidal B site comes from epilithic microalgae, macroalgae also constitute their important dietary component (22%). A slightly more important contribution (27%) of macroalgae to the limpet diet was also detected on the intertidal W site. This result may be confirmed by the existence of the boundary between the mat-forming area and the area grazed by *N. concinna* (see photographs in Kang et al., 2002), and also by grazing activity regulating the density of propagules of macroalgae (Kim, 2001). Epilithic microalgae are still highly important as dietary component of the limpets on the intertidal W site. However, it is difficult to explain trophic contribution of phytoplankton comparable to that in the subtidal in this study. The timing of immigration of the limpets from the subtidal to the intertidal and thereby reflecting prior feeding condition may be a possible explanation regarding their isotopic difference between the intertidal B and W zones. In contrast, macroalgae are the most important contributors (67%) to the limpet diet in the tide pools. In this habitat, while a substantial contribution (28%) of phytoplankton supplements the limpet diet, the contribution of epilithic microalgae is minimal (5%).

4.3. Isotopic shift depending on limpet ontogeny

In addition, there was a significant change in the diets of the limpets depending on their size. SPOM seemed to be an important food source for the limpet spat with a shell length of 3–5 mm collected at the subtidal site. The stable isotope values of the spat were very close to those of the suspension feeders, such as bivalves, ascidians and some cnidarians in the Antarctic nearshore zone (Dunton, 2001; Corbisier et al., 2004; Norkko et al., 2007). Actually, the spat isotope values were very close to those (mean -25.3 ± 0.3‰ and 2.4 ± 0.8‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, unpubl. data) of the suspension-feeding bivalve *Laternula elliptica*, which utilizes exclusively phytoplankton in the subtidal zone of the study area (Ahn, 1993, 2004). The $\delta^{13}C$ range of marine particulate organic matter, which is derived from phytoplankton, is known to be −28.0 to −30.4‰ (Dunton, 2001), so our SPOM data may represent a mixture of true phytoplankton and more ^{13}C-enriched sources. The existence of resuspended epiphytic or epilithic diatoms may account for such a ^{13}C-enrichment of SPOM in Marian Cove (Ahn et al., 1997; Kang et al., 2002). As a result, the slightly more depleted $\delta^{13}C$ in the spat tissues than that in the SPOM may reflect the spat feeding on true marine phytoplankton prior to their settlement on the bottom, considering their slow growth, low metabolic rates and thereby probably their slow turnover of tissues (Peck and Veal, 2001).

Conversely, despite a considerable proportion of macroalgae in the diet of the larger limpets in the middle part of the intertidal zone, the small-sized limpets collected in the lower part of the intertidal B have a great dietary dependence (83%) on epilithic microalgae without any contribution of macroalgae. The considerable contribution (6–36%) of SPOM to nutrition of the limpets suggests that they probably browse on the rocky surfaces when ungrazed phytoplankton falls to the seabed during the intense spring phytoplankton bloom (Table 3). The availabilities of the potential food sources seem to lead to a size-dependent variation in the diet of the limpets. The subtidal and the lower intertidal sites where the small-size limpets were collected were free from macroalgae. Hence, a minimal contribution of macroalgae to the diet of the small-size limpets may be attributable to the low macroalgal availability in their habitats. In contrast, the increasing contribution of macroalgae to the larger limpet diet in the middle intertidal area may reflect high macroalgal availability. It may also be the case that the structure of the radula may be more specialized in larger limpet individuals so that they feed on large food items (Ruppert et al., 2004).

5. Conclusions

The limpet *N. concinna* in Marian Cove, King George Island exhibited a spatial trend in distribution due to seasonal migration, with smaller individuals in the subtidal zone as compared with larger ones at the intertidal sites. The availabilities of the three potential food sources of epilithic microalgae, macroalgae and phytoplankton varied within each habitat. Our stable isotope analysis and mixing-model estimation indicate a change in dietary mixture between habitats as well as limpet size classes due to the difference in the food availability among habitats. Our results also suggest that grazing by limpets can determine, if available, microalgal and/or macroalgal abundance and coverage in the Antarctic rocky-shore ecosystems. Finally, this study highlights that trophic structure of benthic food web can change along environmental gradients even at spatial scales of dozens or hundreds of metres, as shown at large spatial scales of dozens or hundreds of kilometres in the Antarctic (Norkko et al., 2007).

Acknowledgements

This work was carried out as a part of the project “Status and Changes of Polar Indicator Species and Coastal/Terrestrial Ecosystems (PE10040) and “Studies on biodiversity and changing ecosystems in King George Island, Antarctica (PE11030)” funded by the Korea Polar Research Institute (KOPRI). The authors are grateful to Mrs. Jung Hyun Kwak and Hyun Je Park for stable isotopic analysis at the Isotope Ecology Laboratory, Pohang University of Science and Technology.

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