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Diatom fatty acid biomarkers indicate recent growth rates in Antarctic krill

Abstract—We investigated the relationship between nutritional condition (levels of specific fatty acids) and growth increment (percentage growth per intermoult period, percentage IMP^{-1}) for Antarctic krill (*Euphausia superba*) collected from the vicinity of South Georgia in the austral summer 2002. There were correlations between percentage IMP^{-1} and the concentration (gram : gram dry weight) of the diatom biomarker fatty acids, 16 : 4(n-1) and 20 : 5(n-3) in tissues of individual krill, suggesting that the abundance of diatoms in the environment of the krill in the intermoult period prior to moulting was a key determinant of change in body length, a proxy for growth. This substantiates the view that diatoms are crucial for supporting high growth rates of krill, either as a direct food source or, indirectly, by enhancing production of microzooplankton and mesozooplankton based food webs.

Antarctic krill, *Euphausia superba,* are locally abundant in the Southern Ocean, where they are important for biogeochemical cycling and food web dynamics (Everson 2000; Atkinson et al. 2001). Understanding factors that control the growth and recruitment success of krill is an essential precursor to predictions of their distribution patterns and interannual variability. As in all crustaceans, growth rate in krill involves periodic moulting of the exoskeleton. Growth rate is thus a combination of moult frequency and the growth increment of moult. Quetin and Ross (1991) developed an instantaneous growth rate (IGR) technique to quantify krill growth rates that involves incubating animals individually for a few days after capture and, for those that moult, measuring the length increment at ecdysis. It is possible to calculate the growth increment, i.e., percentage growth per intermoult period (percentage IMP^{-1}), by measuring the difference in uropod length of both moult and animal. Application of the IGR technique during the Palmer long-term ecological research program (Ross et al. 2000; Quetin et al. 2003) indicated that highest growth rates of krill were associated with the latter stages of diatom blooms, whereas low growth was linked either to low phytoplankton biomass or blooms dominated by cryptophytes and prymnesiophytes.

Long-term, interannual, or high-resolution studies can be useful for characterizing the food environment of krill. However, such extensive coverage is rarely possible for open ocean biological oceanographers. Additionally, although it is relatively straightforward to characterize the potential food sources of krill, it is considerably more difficult to identify what krill are actually consuming. A marker within the krill that realistically reflects its food intake, both in terms of quantity and quality over at least an intermoult period (approximately 2–3 weeks in summer) and possibly longer is crucially required. Here we investigated the suitability of fatty acid biomarkers as a means of characterizing the nutritional condition of krill over intermediate timescales and how this influenced growth, as determined by the IGR technique. Fatty acids are increasingly used to identify specific

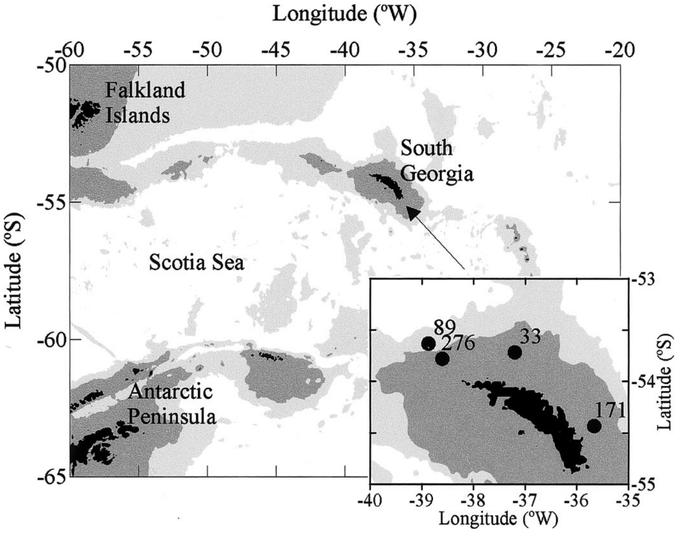

Fig. 1. Locations of the four stations off South Georgia where the krill growth experiments were conducted.

dietary sources in aquatic ecosystems, with some fatty acids being characteristic of specific microplankton taxa (Sargent et al. 1987; Pond et al. 1996; Müller-Navarra et al. 2000)

Methods—Krill were caught from four stations near South Georgia during a cruise of the James Clark Ross (JR70) in January and February 2002 (Fig. 1). Krill for IGR growth experiments were obtained using a rectangular midwater trawl (RMT8), and animals in good physiological condition were immediately transferred to 500-ml perforated plastic pots with screw cap lids. The pots were housed in 0.5-m^3 tanks in a controlled temperature room maintained at ambient seawater temperature (approximately 2°C). Filtered seawater was pumped through the tanks at 1 liter min^{-1} to provide aeration and remove excretory products.

Although the growth experiments were typically maintained for 6 d, only krill that had moulted within 24 or 48 h of collection were used for dry weight and lipid analysis, since growth increment decreased substantially after this time (Tarling et al. unpubl. data). Krill that had moulted were removed from the pots, and the uropods on both the moult and animal were measured using a binocular microscope. After determination of total length (front of eyes to end of uropods) and sex and maturity stage following Makarov and Denys (1981) and Morris et al. (1988), krill were stored at −80°C until analysis in the United Kingdom. Animals were initially freeze dried, then lipids extracted in chloroform : methanol (2 : 1 v : v) according to Folch et al. (1957). Fatty acid methyl esters (FAMEs) were prepared from aliquots of total lipid after the addition of an internal standard (21 : 0) to each aliquot. FAMEs were generated by transesterification of lipid samples in methanol containing 1.5% sulphuric acid at 50°C for 16 h (Christie 1982). FAMEs were then purified by thin layer chromatography using a hexane : diethyl ether : acetic acid solvent system (90 : 10 : 1, v : v : v) and analyzed on a Trace 2000 Carlo Erba gas chromatograph (GC). The GC was equipped with on-column injection, installed with a ZBWAX column (30 m × 0.32 mm), and hydrogen was used as the carrier gas.

Fatty acid and lipid data were standardized (g fatty acid/g krill dry weight) and subjected to pairwise regression analysis using the statistical package MINITAB v1.3 (Pennsylvania State University). Best subsets regression was then applied to a limited number of fatty acids with 16 : 1(n-7), 16 : 4(n-1), and 20 : 5(n-3) used as markers for diatoms, 22 : 1(n-11) for zooplankton (nauplii, copepodites, etc.), and 22 : 6(n-3) for flagellates (Sargent et al. 1987). 16 : 0, a fatty acid that is abundant in energy storage reserves of krill, was also included (Fricke et al. 1984). Best subsets regression analysis examines models containing 1, 2, 3, etc. explanatory variables and in each case selects the one with the largest R^2. Mallows' Cp statistic is also calculated in the analysis and gives an indication of the best fit model. If the model with p parameters is the true model, then Cp has an expected value close to p and is accordingly selected. The fatty acids detailed above were used as predictor variables with percentage IMP^{-1} as the response.

Results—A total of 31 krill were analyzed from four moulting experiments collected at four stations (Fig. 1). Krill from all sites were of similar size and predominantly subadult (Table 1). Between sites, there were clear differences in the mean percentage growth increment per intermoult period (IMP^{-1}), with the lowest values for krill from station 171 to the east of South Georgia (1.93% IMP^{-1}) compared with considerably higher values for krill at Stas. 33 and 89 to the west (mean values of 9.24 and 8.49% IMP^{-1} respectively, Table 1).

Fatty acid profiles of krill from the four stations were essentially similar and dominated by 16 : 0, comprising $\sim$24% of the total. Other abundant fatty acids were 14 : 0, 16 : 1(n-7), 18 : 1(n-9), 18 : 1(n-7), 20 : 5(n-3), and 22 : 6(n-3) (Table 2). 16 : 4(n-1), although only present in low amounts (0.7–0.9%), is notable since it is a reliable biomarker for diatoms (Sargent et al. 1987). No significant correlations were found between the percentage fatty acid compositions and growth increment of the krill. However, comparison of percentage IMP^{-1} with the actual concentration (g fatty acid/g krill dry weight) of specific fatty acids in the krill tissue indicated that the diatom biomarker fatty acids, 16 : 4(n-1) and 20 : 5(n-3), were positively correlated with growth (Fig. 2A,B). A significant correlation ($F = 10.69$, df = 30, R^2 0.29, $p = 0.03$) was also established between percentage IMP^{-1} and the concentration of 18 : 4(n-3), a fatty acid whose origin is more ambiguous, since it is synthesized in moderate amounts by both diatoms and flagellated microplankton (Sargent et al. 1995). The relationship between total (n-3) PUFA was also significant, although less so than for the individual diatom biomarker fatty acids ($F = 4.79$, df = 30, R^2 0.14, $p = 0.04$).

When the data were subjected to simple pairwise regression, no association was found between growth and the concentration of total lipid (g total lipid/g krill dry weight) in the tissue of the krill ($F = 2.94$, df = 30, $R^2 = 0.09$, $p = 0.1$). Similarly, 16 : 0, a saturated fatty acid that often forms the basis of energy storage reserves in marine zooplankton, was not correlated with growth ($F = 2.78$, df = 30, $R^2 =$

Table 1. Station information and basic biological parameters for samples of krill collected off South Georgia. (Sex and maturity stage according to Makarov and Denys 1981 and Morris et al. 1988). J, juvenille; fs, female subadult; fa1, female adult stage 1; ms1, male subadult stage 1; ms2, male subadult stage 2.

Station	Date	Sex and maturity stage	Length (mm)	Dry weight (g)	Growth increment (% IMP^{-1})	(SE)
33	7 Jan 02	fs	38	0.09667	5.88	
		j	39	0.13817	9.09	
		j	39	0.09085	7.41	
		ms1	41	0.11746	13.79	
		ms2	41	0.09388	3.51	
		ms1	41	0.09048	11.54	
		j	37	0.07526	13.46	
Mean			39	0.1004	9.24	(1.47)
89	11 Jan 02	ms1	38	0.1586	5.00	
		fs	43	0.1186	5.30	
		fs	42	0.0903	10.90	
		fs	41	0.1141	10.20	
		fs	37	0.1356	8.20	
		fs	41	0.142	8.30	
		fs	37	0.0915	10.90	
		fa1	42	0.129	9.10	
Mean			40	0.1225	8.49	(0.82)
171	19 Jan 02	fs	39	0.09272	0.85	
		ms1	42	0.13075	4.14	
		fs	39	0.09978	5.20	
		ms1	40	0.10268	3.48	
		fs	42	0.12947	2.41	
		ms1	38	0.08815	3.64	
		fs	38	0.08813	0	
		ms1	38	0.08628	1.79	
		ms1	39	0.08711	1.71	
		fs	34	0.08833	0	
		fs	34	0.06806	0	
		fs	38	0.0732	3.64	
		fs	39	0.09173	−1.72	
		ms1	42	0.1108	1.89	
Mean			39	0.0955	1.93	(0.52)
276	26 Jan 02	ms2	47	0.1093	6.10	
		fs	40	0.2444	4.10	
Mean			44	0.1769	5.10	
Overall mean			40	0.1088	5.48	(0.45)

0.1, $p = 0.1$). 22:6(n-3), a fatty acid whose origins in the food web are often ascribed to flagellated organisms, particularly prymnesiophytes, dinoflagellates, and ciliates, and which is known to be important for growth and reproduction in some marine zooplankton (Jónasdóttir et al. 1995; Pond et al. 1996), was also poorly correlated with growth ($F = 1.02$, df = 30, $R^2 = 0.3$, $p = 0.32$).

Best subsets regression analysis using key biomarker fatty acids also indicated that diatom biomarkers were important for explaining the variability in growth increment. The best fit model (Mallows' Cp statistic = 4.4) indicated that the diatom biomarkers 16:4(n-1) and 16:1(n-7) and the zooplankton marker 22:1(n-11) together accounted for 51.7% of the variability.

Discussion—Although growth of aquatic zooplankton can be influenced by a number of physical variables, notably temperature (Hirst et al. 2000), this work examines the effect of nutrition, both in terms of quantity and quality. The process of growth requires that a certain quantity of food is ingested, both to act as building blocks for new tissue and also to support the energy intensive metabolic demands associated with growth processes. The diet of krill also needs to provide a balance of elemental (C, N, P, etc.) and essential biochemical components (fatty acids, amino acids, vitamins, etc.). Essential dietary components in this context are defined as those that cannot be synthesized or are synthesized in inadequate amounts to sustain growth and survival and must therefore be obtained at least in part, but not necessarily wholly, from the diet (Spector 1999; Anderson and Pond 2000). Polyunsaturated fatty acids (PUFAs) are essential dietary components for many marine organisms and, in planktonic open ocean environments, are overwhelmingly derived from photosynthetic microplankton (Sargent et al. 1995). PUFAs are known to be essential dietary components for many marine crustacean, since they are required in high amounts during periods of growth to support tissue development. Müller-Navarra et al. (2000) found a significant and

Table 2. Mean percentage fatty acid composition of moulted krill from the four stations (standard errors in parentheses).

Fatty acid	Station 33 (n = 7)	 89 (n = 8)	 171 (n = 14)	 276 (n = 2)
14:0	9.7 (0.29)	10.6 (0.21)	9.8 (0.35)	11.2
15:0	0.3 (0.02)	0.3 (0.07)	0.6 (0.25)	0.3
16:0	24.9 (0.23)	24.8 (0.18)	24.4 (0.37)	24.7
16:1(n-9)	0.1 (0.02)	0.1 (0.02)	0.1 (0.02)	0.2
16:1(n-7)	10.0 (0.55)	10.0 (0.24)	10.5 (0.38)	10.2
16:2(n-3)	3.1 (0.08)	2.7 (0.07)	3.3 (0.10)	3.0
17:0	0.4 (0.02)	0.3 (0.02)	0.3 (0.02)	0.4
16:4(n-1)	0.9 (0.10)	0.7 (0.03)	0.7 (0.04)	0.7
18:0	1.3 (0.07)	1.5 (0.04)	1.1 (0.04)	1.4
18:1(n-9)	11.7 (0.41)	13.2 (0.33)	11.5 (0.27)	12.8
18:1(n-7)	7.0 (0.14)	6.3 (0.15)	7.2 (0.11)	7.0
18:2(n-6)	1.0 (0.08)	1.1 (0.15)	1.5 (0.04)	1.0
18:3(n-6)	0.1 (0.02)	0.2 (0.01)	0.2 (0.01)	0.1
18:4(n-3)	0.4 (0.02)	0.4 (0.01)	0.6 (0.05)	0.4
18:4(n-3)	1.8 (0.07)	2.3 (0.14)	1.7 (0.15)	1.8
20:0	0.1 (0.06)	0.1 (0.03)	0.1 (0.03)	0.0
20:1(n-9)	1.1 (0.06)	1.2 (0.04)	1.0 (0.04)	1.2
20:1(n-7)	0.3 (0.03)	0.2 (0.01)	0.3 (0.02)	0.4
20:4(n-6)	0.2 (0.04)	0.2 (0.01)	0.3 (0.01)	0.1
20:4(n-3)	0.3 (0.01)	0.3 (0.01)	0.3 (0.05)	0.3
20:5(n-3)	16.2 (0.77)	15.1 (0.47)	14.9 (0.50)	14.8
22:1(n-11)	0.9 (0.09)	0.8 (0.04)	0.6 (0.05)	1.1
21:5(n-3)	0.6 (0.04)	0.4 (0.06)	0.4 (0.03)	0.5
22:5(n-3)	0.1 (0.05)	0.0	0.1 (0.04)	0.0
22:6(n-3)	7.5 (0.39)	7.1 (0.31)	8.3 (0.47)	6.4

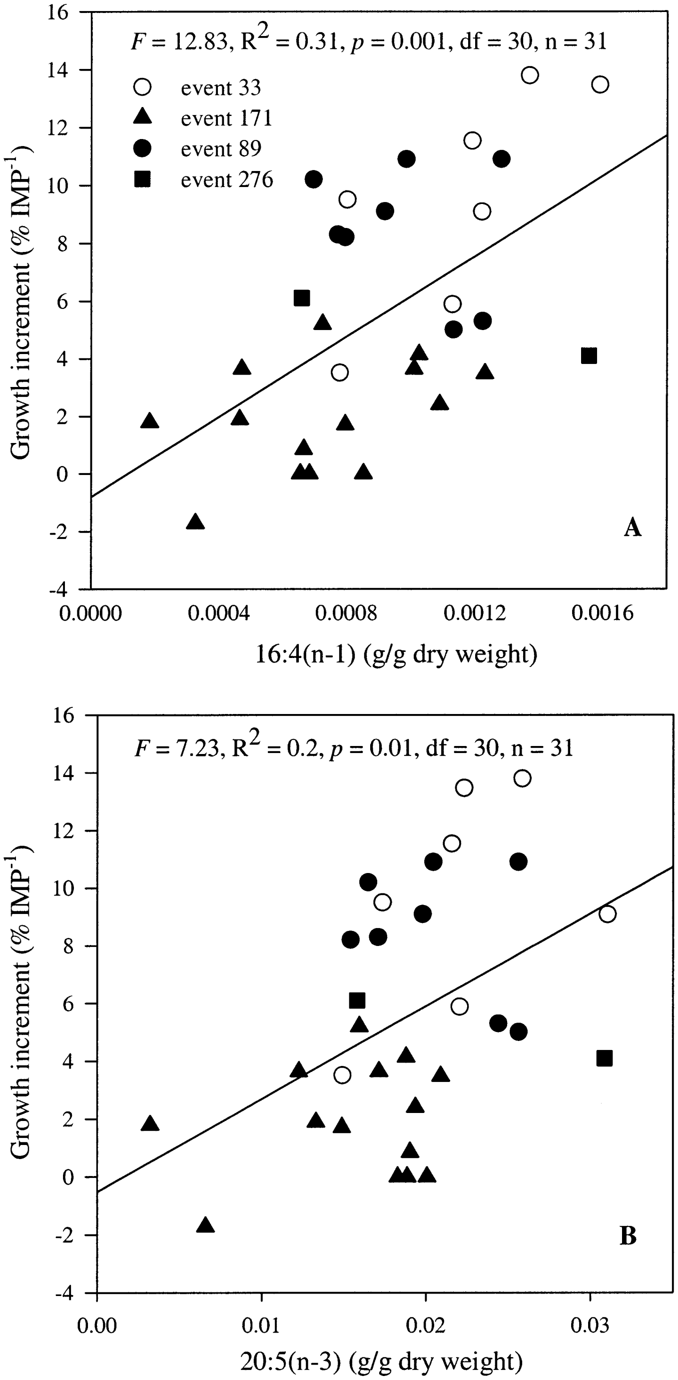

Fig. 2. Regression analysis of the concentration of diatom fatty acid biomarkers (g/g dry wt) in the tissues of the krill against growth increment for stations 33, 89, 171, and 276.

positive correlation between the concentration of a specific PUFA, the diatom biomarker 20:5(n-3) and growth of freshwater zooplankton. Other studies have implicated PUFA as being important for reproduction in marine calanoid copepods (Jónasdóttir et al. 1995; Pond et al. 1996; Anderson and Pond 2000).

Growth of krill does not appear to be simply related to nutritional condition in terms of bulk energy reserves since no correlation was apparent between the lipid content of the krill and growth increment (percentage IMP^{-1}). This finding suggests that krill do not continue to grow simply in response to accumulated storage reserves and points to shorter term factors being important (Buchholz 1991). Best subsets regression also indicated that the amount of 22:1(n-11) in the tissue of the krill off South Georgia, a fatty acid that is not present in photosynthetic microplankton but synthesized by zooplankton, was linked with growth. This could indicate that krill were ingesting nauplii and small copepods (Cripps and Atkinson 2000). However, since it is likely krill are able to synthesize this fatty acid themselves, since it is synthesized by many zooplankton, it is not possible to distinguish whether the levels of this fatty acid reflect endogenous biosynthesis or a zooplanktivorous component to the diet.

The correlation between growth increment and the amounts of total (n-3) polyunsaturated fatty acids in the krill is not surprising since these compounds are known to be important for growth and development in many aquatic organisms (Sargent et al. 1995). Perhaps more surprising is the clear importance of diatoms for supporting growth in krill, as evidenced by the association between percentage IMP^{-1} and the concentration of the diatom biomarker fatty acids 16:4(n-1) and 20:5(n-3). This finding highlights the specific importance of diatoms for supporting high growth rates in krill and supports the conclusion of Ross et al. (2000). From these data it is not possible to distinguish whether diatom fatty acid biomarkers in the krill reflected the direct ingestion of diatoms or the ingestion of diatom-derived fatty acids that had been transferred through the microzooplanktonic and mesozooplanktonic food web. However, what this finding does emphasize is the specific importance of diatoms for supporting high growth of krill, either by the provision of essential dietary nutrients or more generally by creating hot-spots of enhanced food web production.

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