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Growth rates of Antarctic krill, *Euphausia superba*: Comparison of the instantaneous growth rate method with nitrogen and phosphorus stoichiometry

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Abstract

Zooplankton growth rates are hard to measure directly, and proxy measurements are desirable to encompass the variety of species and scales of interest. The *growth rate hypothesis* of stoichiometric theory states that a negative relationship exists between nitrogen : phosphorus (N : P) stoichiometry and growth rate, driven by cellular ribosomal ribonucleic acid (rRNA) content. Despite the wealth of literature on the growth rate hypothesis, there exist no practical demonstrations of its use in the marine literature. We thus investigated whether this hypothesis could be the basis of a technique to estimate growth rates of *Euphausia superba* by comparing, for the same individual krill, elemental stoichiometry and growth rates derived from the instantaneous growth rate (IGR) method. These growth rates were the first IGR measurements from South Georgia; from within a restricted area over the course of just 1 month, these rates were highly variable, from negative to near maximum rates recorded for the species. Although there were significant differences in N : P ratio and phosphorus content between individuals and schools, there was no relationship between N : P ratio and growth rate when data were grouped by school. Thus, our data do not support the predictions of the growth rate hypothesis at an intraspecific scale. However, when all data were pooled, the mean values of growth rate and N : P ratio did fit the interspecific relationship established previously for freshwater zooplankton. We suggest that krill maintain the biochemical machinery for high growth potential and maintain high growth in summer to take advantage of short-term fluctuations in food.

Antarctic krill, *Euphausia superba*, is an important species in the Southern Ocean ecosystem. In the waters around South Georgia, krill can contribute 50% of the zooplankton biomass (Atkinson et al. 2001), forming a direct link between primary production and the large top predators of the Antarctic. Thus, knowledge of the ecology and population dynamics of krill is fundamental to our understanding of the dynamics of the Southern Ocean ecosystem.

Central to any understanding of population dynamics is a knowledge of growth rate. Many data on krill growth have been gathered (e.g., Mackintosh 1972; Buchholz et al. 1989; Ross et al. 2000), yet the spatial and temporal variability in growth is poorly understood (Quetin et al. 1994). There are inherent difficulties in studying the growth of crustaceans, which retain no skeletal record of past growth. A variety of approaches have been used to investigate krill growth, including assessments of the length frequency of natural populations (Mackintosh 1972), those eaten by predators (Reid et al. 2002), and direct measurements with the instantaneous growth rate (IGR) method (Ross et al. 2000).

Each of these approaches has its strengths and weaknesses: field studies measuring growth directly with the IGR method yield invaluable results (Nicol et al. 1992; Ross et al. 2000) but are time consuming, and many krill need to be incubated individually. Changes in length frequency distributions in krill populations have been used to estimate growth rates, either through direct sampling (Rosenberg et al. 1986; Miller and Hampton 1989) or through the diet of predators (Reid et al. 2002). However, the results can be equivocal, relying on the assumptions that the same population is being sampled over time and that mortality is not size dependant. To improve our knowledge of growth rate responses in krill, we thus need alternative methods that are fast and efficient, in order to examine variability in growth over a wide range of scales.

Biochemical indicators of growth are one such possible alternative. For many organisms, including krill, the ribonucleic acid (RNA) content or its ratio to deoxyribonucleic acid (DNA) has been found to vary with growth rate (Sutcliffe 1970; Båmstedt and Skjoldal 1980; Ikeda 1989). More recently, studies of freshwater crustaceans have indicated a relationship between elemental composition (most notably phosphorus) and growth rate (Main et al. 1997; Elser et al. 2000*a*). Most phosphorus in zooplankton is bound in ribosomal RNA (rRNA) (Main et al. 1997), and organisms with high rRNA exhibit high maximum growth rates. This would indicate a consistent positive association between growth rate and RNA and, hence, phosphorus.

This relationship between nitrogen (N) and phosphorus (P) stoichiometry and growth rate has been termed the *growth rate hypothesis* (Elser et al. 2000*b*), but there have been few direct tests of this theory (Main et al. 1997; Elser et al. 2000*a*). The growth rate hypothesis states that ''differences in organismal C : N : P ratios are caused by differential allocations to RNA necessary to meet the protein syn-

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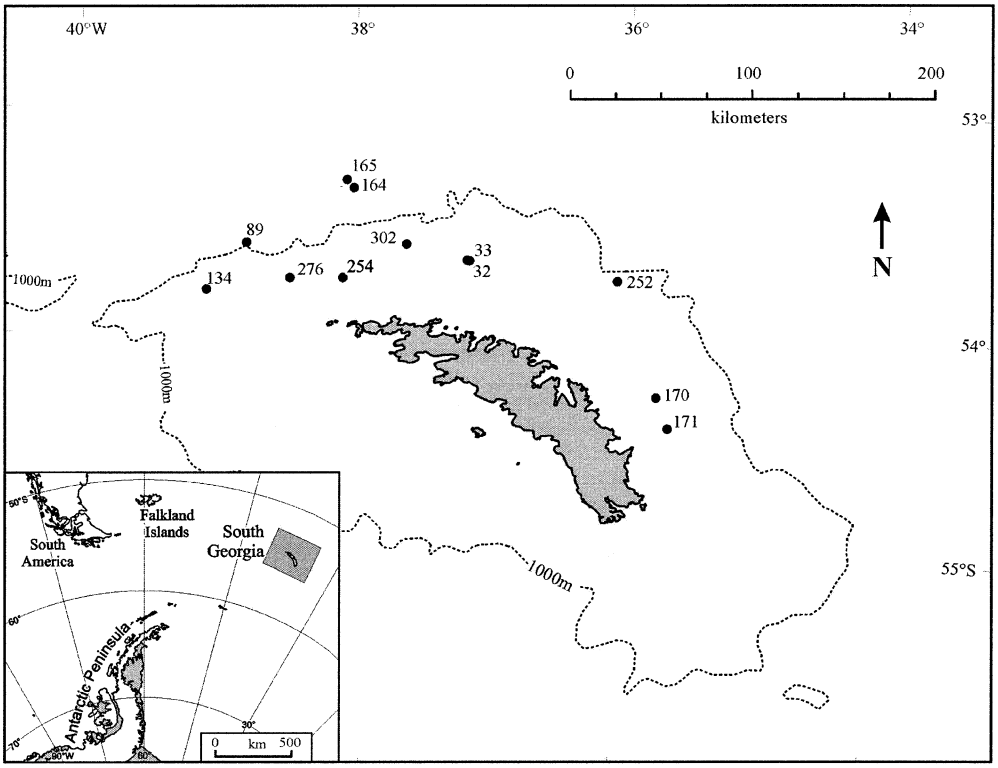

Fig. 1. Locations of sampling events for Antarctic krill, *Euphausia superba,* around South Georgia during January 2002. Filled circles represent discrete RMT8 hauls, with haul identifier shown. Dotted line indicates continental shelf boundary at 1,000 m.

thesis demands of rapid rates of biomass growth and development'' (Sterner and Elser 2002). The paradigm emerging from this hypothesis is that a negative relationship exists between molar N : P ratios and specific growth rates in organisms, driven by differences in rRNA, the machinery of protein synthesis required for growth. Most studies investigating this hypothesis have to date been focused at an interspecific level on freshwater zooplankton, and little work has examined whether such a relationship can be demonstrated on an intraspecific (between-individuals) basis. The only study to date that has considered intraspecific relationships (Main et al. 1997) showed a significant relationship between N : P and growth within individuals in only one of the three species of *Daphnia* studied.

In this study, we have undertaken the first test of the growth rate hypothesis in a marine zooplankton species, *Euphausia superba.* The hypothesis is tested on two scales: (1) Intraspecifically, where we test whether the N : P ratio of individual krill shows a relationship with their growth rate during the previous molt cycle (approximately 2 weeks), as measured with the instantaneous growth rate technique. That is, the N : P ratio will vary on the same spatial and temporal scales as observed growth rate; and (2) Interspecifically: here we examine whether the relationship determined in freshwater zooplankton species between N : P and growth rate encompasses mean data for *E. superba.*

Materials and methods

Experimental design—The instantaneous growth rate (IGR) method measures linear growth increments of krill maintained in laboratory incubations at the ambient temperatures encountered at the point of sample collection (Quetin and Ross 1991). The method assumes that growth increments (the percentage increase or decrease in krill length at the molt) measured during the first few days of incubation are representative of growth rates in the field (Nicol et al. 1992). Elemental analysis was conducted on individual krill whose growth was known: Each individual selected for analysis had molted within 5 d of capture and thus had a known growth increment derived from shipboard IGR experiments.

Field methods—Krill were sampled in the austral summer (January 2002) from Antarctic waters around South Georgia (54°S, 37°W) (Fig. 1). Live krill were caught using a Rectangular Midwater Trawl (RMT 8) with two nets, enabling the targeting of two separate schools in one haul. Each school is identified by a code comprising two numbers, the first corresponding to the haul number and the second to the net (e.g., 164.1 represents RMT haul 164, net 1). Hence, each code denotes a krill school, which increment sequentially with time. Fishing was carried out mainly at night on krill schools located and characterized with a Simrad EK 500 echosounder (38, 120, and 200 kHz). Sites both on and off the continental shelf and at both east and west ends of the island were sampled (Fig. 1), from which 15 krill schools were examined. We believe that this study thus presents a good field example with which to test the growth rate hypothesis, with samples collected within a limited area with limited seasonal effects.

To ensure experimental krill were in good physiological

Table 1. The correspondence between the sexual maturity stage codings of Makarov and Denys (1981) and Morris et al. (1988) and the characteristics that define these stages. Table adapted from Morris et al. (1988).

Morris et al. (1988)	Definition	Makarov and Denys (1981)	Characteristics
J	Juvenile	JI	No secondary sexual characteristics visible
MS1	Male subadult stage 1	MII A (1)	Petasma single, undivided lobe
MS2	Male subadult stage 2	MII A (2)	Two-lobed petasma
MS3	Male subadult stage 3	MII A (3)	Petasma also has wing present
MA1	Male adult stage 1	MIII A	Petasmae fully developed; ejaculatory ducts red
MA2	Male adult stage 2	MIII B	Fully formed spermatophores present in ejaculatory ducts
FS	Female subadult	FII B	Developing thelycum present, but color feeble or absent
FA1	Female adult stage 1	FIII A	Thelycum is fully developed, bright red, and visible through the gills
FA2	Female adult stage 2	FIII B	Thelycum dirty red, empty spermarophores present
FA3	Female adult stage 3	FIII C	Developing ovary fills body cavity, but body not swollen
FA4	Female adult stage 4	FIII D	Enlarged ovary clearly visible, thorax and first two abdominal segments swollen (gravid)
FA5	Female adult stage 5	FIII E	Thorax and first two abdominal segments swollen, but no enlarged ovary visible (postspawn)

condition, haul duration was no longer than 20 min, and a solid cod end was used. Freshly caught krill were transferred immediately to 150-liter circular bins containing seawater at ambient temperature. Healthy krill were then transferred individually to 300-ml perforated containers and held in tanks of free-flowing (1 L min^{-1}) filtered seawater maintained at ambient temperature. Krill were incubated in this way aboard ship for 5 d and were checked daily for molting. For all individuals that had molted, total length, length of uropod of the animal and the exuviae, and the sex and maturity stage were determined according to the method of Makarov and Denys (1981). To reduce measurement error, all measurements were carried out by one of us (R.S.) aboard ship under a binocular microscope (×12 magnification) fitted with an ocular micrometer (Shreeve et al. unpubl. data). These individuals were then frozen at −80°C for biochemical analysis in the United Kingdom.

When determining the sex and maturity stage, the classification codes of Makarov and Denys (1981) were recoded with a hierarchical alphanumeric code (after the method of Morris et al. 1988), in which the first letter represents sex, the second the developmental stage (subadult or adult), and the number the maturity stage; increasing maturity thus corresponds to an increasing numerical coding. The codes used are: J = juvenile, FS = female subadult, MS1–MS3 = male subadult, FA1–FA5 = female adult, and MA1–MA2 = male adult. Table 1 details the correspondence between the two codings (after Morris et al. 1988).

Growth rate—Individual growth rates were determined using the IGR method (Quetin and Ross 1991; Ross et al. 2000). The fraction of krill that molted during the incubations was recorded, and for each school, the intermoult period (IMP) was calculated from the total number of individuals molted in the experiments (N_{moult}), the total number incubated ($N_{incubated}$), and the duration of the experiments (t):

$$\text{IMP} = \frac{(t \times N_{incubated})}{N_{moult}}$$

Dead or dying krill and those that molted during the course of the incubations were removed from the tanks but were still included in the total number incubated ($N_{incubated}$).

The mean difference in the length of the uropods of molted exoskeletons and the postmolt animals was measured using an ocular micrometer to determine growth (or shrinkage) per intermoult period as a percentage of the initial length. Total length of the postmolt krill (LT_1) (mm) and the growth increment determined from the uropod measurements (*GI*) (%) were used to estimate the length of the krill at the beginning of the incubation (LT_0) (mm):

$$LT_0 = LT_1\left[\frac{(100 - GI)}{100}\right]$$

Lengths at T_0 were converted to dry mass (DMT_0) using the following equation:

$$DMT_0 = 0.0000106LT_0^{3.15}$$

This equation is given in appendix 3 of Morris et al. (1988), using their equation for all experimental krill, anterior-telson measurement. This equation was used because numbers of molted animals in this study were too few to derive an accurate regression from our data alone. Dry mass at T_1 (DMT_1) was as described in ''Methods, elemental analysis.'' It is worth noting here that krill 'shrinkage' or negative growth can be measured by this method and occurs when a negative growth increment (*GI*) is encountered (i.e., the uropod of the molted krill is smaller than that of its exuvium).

Table 2. Elemental and mineral ash composition (% dry mass) and growth rate (g d^{-1}) of *Euphausia superba*, for all individuals. Statistics are shown for one-way ANOVA by school. (SE, standard error; CV, coefficient of variation; n = total number of krill).

	Mean	SE	Range	CV%	n	df	F
Carbon	48.16	0.249	42.13–53.25	5.42	110	14,95	2.70**
Hydrogen	7.33	0.043	6.27–8.34	6.09	110	14,95	4.31*
Nitrogen	9.26	0.067	8.13–11.36	7.58	110	14,95	4.92*
Phosphorus	1.28	0.007	1.13–1.44	5.30	110	14,95	1.30†
Ash	11.23	0.226	5.05–19.84	23.79	101	14,86	1.38†
N : P	16.04	0.139	13.57–20.61	9.10	110	14,95	3.41*
Growth rate	0.01	0.001	−0.01–0.04	79.64	110	14,95	9.91*

* $p<0.001$; ** $p<0.005$.
† Not significant.

Specific growth rate (SGR) for each krill was then estimated as:

$$\mathrm{SGR} = \frac{[\ln(DMT_1) - \ln(DMT_0)]}{\mathrm{IMP}}$$

Elemental analysis—Krill frozen at sea were returned to the United Kingdom, dried to constant mass at 60°C, and ground individually to a homogeneous powder with a pestle and mortar. Triplicate subsamples of approximately 1 mg were taken from each animal and analyzed for carbon (C), N, and hydrogen (H) content in a CE Instruments EA 1108 CHN elemental analyzer. Acetanilide was used as the standard, with standard samples and blanks analyzed every 10 replicates. Mineral ash content of the remaining dried, homogenized krill was measured after ignition at 550°C for 12 h. C, H, and N were also measured in the resulting mineral ash and inorganic carbon determined. Triplicate subsamples of ~3 mg were also taken from each homogenized krill and analyzed for phosphorus content. Samples were digested in 70% perchloric acid at 180°C for 30 min and the resultant orthophosphate assayed colorimetrically with ammonium molybdate (Fiske and Subbarow 1925).

Statistics—All data were checked for normality using the Anderson–Darling normality test and were found not to deviate significantly from normal. Regression analysis and one-way analyses of variance (ANOVAs) were performed within the statistical software package MINTAB v.13 (Pennsylvania State University). The contributions of the *GI* and IMP to variance in SGR were estimated as follows:

$$\mathrm{Var[SGR]} \approx \mathrm{SGR}^2\left[\left(\frac{\mathrm{Var[IMP]}}{\mathrm{IMP}^2}\right) + \left(\frac{\mathrm{Var}[GI]}{GI^2}\right) - 2\left(\frac{\mathrm{Cov[IMP},\, GI]}{\mathrm{IMP} \times GI}\right)\right]$$

where SGR, IMP, and *GI* are the mean values for the variables, Var[SGR], Var[IMP], and Var[*GI*] are the variances and Cov[IMP, *GI*] is the covariance between *GI* and IMP (all values calculated from the pooled means from each school).

Results

IGR—Specific growth rate ranged from −0.010 to 0.041 g dry mass d^{-1}, with a coefficient of variation (CV) of 80%. Krill from separate schools displayed significantly different mean growth rates (ANOVA, $F_{14,95} = 9.91$, $p < 0.001$; Tables 2, 3). Growth rate was measured in krill sampled between 7 January and 31 January 2002, and the data indicate a general decrease in growth rate through the month (Fig. 2). The variation in SGR comprises both changes in the IMP and the variation in individual *GI*s. Plots of both IMP and *GI* as a function of sampling event indicate that variation in krill growth rate is driven mainly by changes in *GI* (Fig. 3), which contributes ~91% of the variation in SGR. As would be expected, a negative relationship was found between length and SGR ($F_{1,108} = 5.78$, $p = 0.018$, $r^2 = 5.1\%$). There was no significant relationship between growth rate and sexual maturity stage of the animals (ANOVA, $F_{4,105} = 1.74$, $p = 0.147$).

Chemical composition—Overall data: C content of all the krill studied varied from 42.13% to 53.25% of dry mass, N from 8.13% to 11.36%, P from 1.13% to 1.44%, and mineral ash from 5.05% to 19.84% (Table 2). Inorganic C was very low, ranging from 0% to 1.99%, with a mean of 0.26%. Less variation was observed in the phosphorus composition of krill than with nitrogen (CV P% = 5.3%, N% = 7.6%).

Variation with sexual maturity stage: Several of the sexual maturity stage classes contained few individuals, so animals were further pooled into coarser sexual maturity stage categories, disregarding the numerical maturity stage subdivisions (i.e., J, FS, MS, MA, and FA). Only N composition differed significantly between sex and maturity stage (ANOVA, $F_{4,105} = 2.57$, $p = 0.042$), with mature females displaying the highest %N and mature males the lowest. C and H content varied relatively little with maturity stage, but the data for mineral ash content showed some variation (not significant), with higher values for mature classifications (FA and MA) (Table 4).

Variation with size: Within the range of krill sampled (31 mm–58 mm), there were weak relationships between chemical composition and length (Fig. 4). Where relationships were statistically significant (C, N, ash), r^2 values were small (C%: $F_{1,108} = 15.68$, $p < 0.001$, $r^2 = 12.7\%$, N%: $F_{1,108} =$

Table 3. Summary of results by school. Growth rate is specific growth rate (g d^{-1}). Statistics are shown for one-way ANOVA by school. (SE, standard error; n = total number of krill).

	Length (mm)		Specific growth rate		Growth increment			
Event	Mean	Range	Mean	Range	Mean	Range	IMP	*n*
32.2	40.50	40–41	0.009	0.006–0.012	3.90	2.49–5.31	14	2
33.1	38.50	31–49	0.180	0.002–0.041	8.15	0.75–17.67	15	20
89.1	41.86	37–55	0.017	0.005–0.028	7.52	2.53–12.50	15	28
89.2	39.71	35–43	0.013	0.005–0.016	8.67	3.28–11.00	23	7
134.2	54.00	53–55	0.007	0.003–0.010	4.33	2.05–6.18	20	3
164.1	39.60	36–49	0.005	−0.001–0.010	3.54	−0.88–7.27	24	5
165.1	40.00	*	0.008	*	4.28	*	18	1
165.2	40.67	38–43	0.002	0.000–0.006	1.63	0.00–4.89	25	3
170.1	37.00	33–43	0.002	−0.003–0.006	1.17	−1.70–3.12	16	6
171.1	35.33	33–38	−0.003	−0.010–0.002	−1.41	−5.26–1.02	17	3
252.2	46.58	31–58	0.005	0.000–0.020	2.78	0.00–10.98	18	19
254.1	41.00	*	0.003	*	2.39	*	23	1
254.2	46.00	43–49	0.003	0.003–0.003	1.50	1.39–1.60	17	2
276.2	41.67	37–47	0.006	0.000–0.011	2.90	0.00–5.08	15	9
302.1	41.00	*	0.000	*	0.00	*	20	1

* No data (n<2) (results shown for n=1).

4.66, $p = 0.033$, $r^2 = 4.1\%$, and ash: $F_{1,99} = 5.60$, $p = 0.020$, $r^2 = 5.4\%$). Elemental composition was recalculated as a fraction of the ash-free dry mass (AFDM), with carbon expressed as organic C (C in mineral ash subtracted from total C); these organic data are summarized in Table 5. These data reveal a stronger correlation between organic carbon and total length ($F_{1,98} = 44.15$, $p < 0.001$, $r^2 = 31.1\%$), but the relationship between nitrogen and phosphorus as a percentage of AFDM was not significant.

Variation with school: When chemical data were analyzed between schools, significant differences were observed in %N (ANOVA, $F_{14,95} = 4.92$, $p < 0.001$), %C (ANOVA, $F_{14,95} = 2.70$, $p < 0.005$), and %H (ANOVA, $F_{14,95} = 4.31$, $p <$ 0.001) composition. The possibility existed to examine the differences between two schools sampled at the same location in three instances, but it was felt that sample sizes in these schools were insufficient to make any meaningful comparisons.

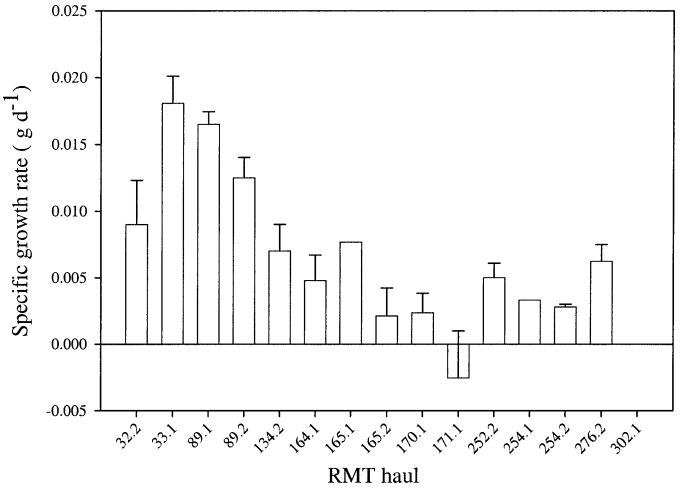

Fig. 2. Differences in specific daily growth rate (g d^{-1}) with sampling event. Data are means for separate schools. RMT hauls increment with time and cover schools sampled from 7–31 January 2002. Error bars display one standard error (no error bars shown for $n < 2$). The number of molters measured for each haul ranged from 1 to 28 (median, 5). Note that haul 302.1 has $n = 1$, SGR = 0.0.

Variation with growth rate: There were no significant relationships between percentage N, P, or molar N : P ratio and growth rate over all the individuals pooled by regression analysis ($n = 110$, $p > 0.05$ for all variables), even when the data was split by length (smaller krill having larger growth rates) (Fig. 5). However, the schools had different mean growth rates (Tables 2, 3), so the data were also analyzed after pooling by school. When this was done there was a significant variation in %N ($F_{14,95} = 4.92$, $p < 0.001$) and N : P ($F_{14,95} = 3.41$, $p < 0.001$) between schools (Fig. 6), but there was no significant relationship between N : P ratio or N% dry mass and SGR when data were pooled by school and analyzed by regression analysis. Data were also analyzed for relationships between growth rate and elemental composition within schools, but significant relationships were found in only two cases; event 164.1 (P%: $F_{1,3} = 44.82$, $p = 0.007$, $r^2 = 93.7\%$) and event 170.1 (N : P: $F_{1,4} = 10.01$, $p = 0.034$, $r^2 = 71.4\%$), although the number of individuals was small in both cases.

Discussion

This article has two main threads; krill growth rate and the relationship between growth rate and chemical composition (growth rate hypothesis). The discussion deals with the former first before discussing the latter.

Growth rate measured by the IGR method—This study is only the fifth study to measure krill growth directly with the IGR method, even though this is acknowledged as the best and most direct method available (Nicol 2000). The relative underutilization of the IGR method is probably related to its

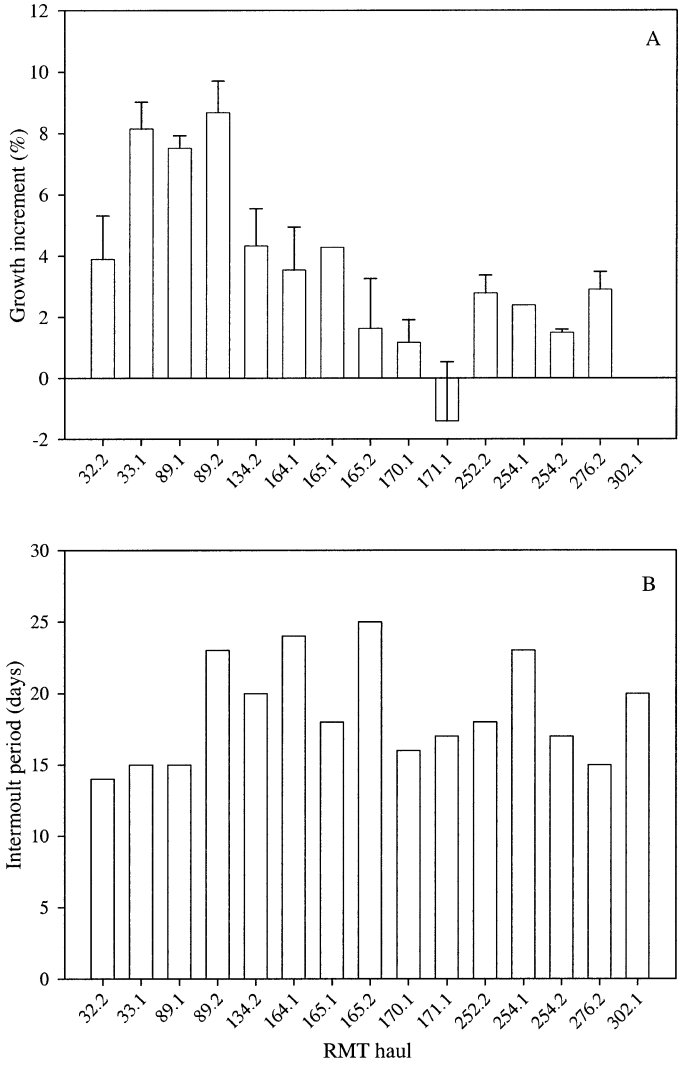

Fig. 3. Variation of (A) growth increment between schools and (B) intermoult period. Error bars display standard error (intermoult period is a mean value for each school).

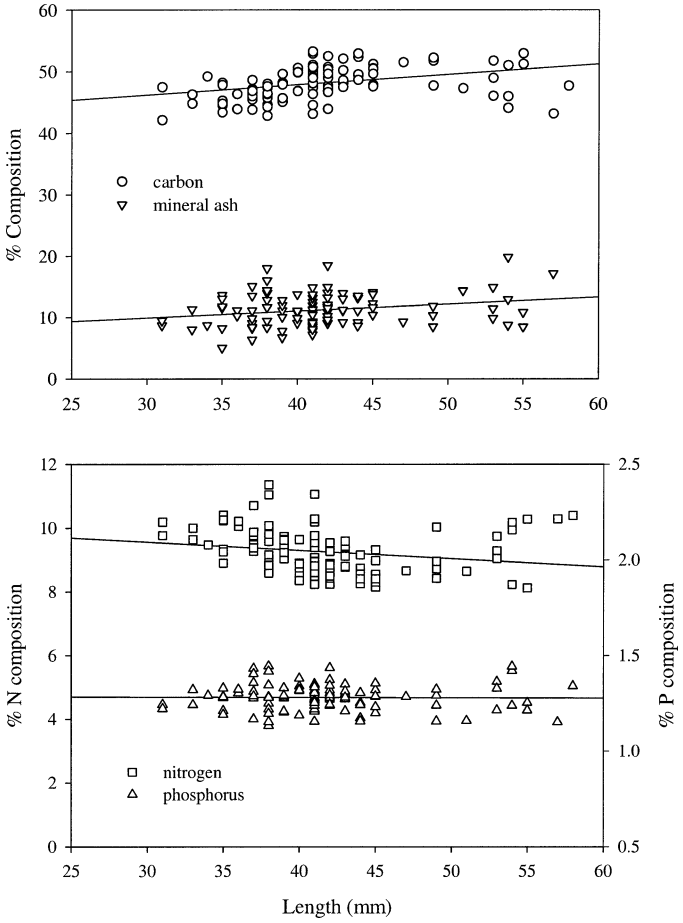

Fig. 4. *Euphausia superba:* relationship between elemental and mineral ash composition (% dry mass) and total length. Regressions significant for C% ($F_{1,108}$ = 15.68, p = <0.001, r^2 = 12.7%), N% ($F_{1,108}$ = 4.66, p = 0.033, r^2 = 4.1%), and ash ($F_{1,99}$ = 5.60, p = 0.020, r^2 = 5.4%), but not P% ($F_{1,108}$ = 0.02, p = 0.889, r^2 = 0%).

labor-intensive and time-consuming nature. Our results demonstrate how variable these observed growth rates are in krill, even within a single school. Individual krill showed great variability, from negative growth to rapid growth, within just a small scale of space and time. Of interest are the negative growth rates recorded in some individuals in high summer, even in a famously productive region (Atkinson et al. 2001). This could reflect either the different feeding histories of krill forming the school or individual variability in feeding ability within a school.

This study represents the first IGR study of krill around South Georgia. Krill growth varied markedly from individual to individual: In summer and in the same general area, krill

Table 4. Elemental and mineral ash composition (all data % dry mass) of Antarctic krill, *Euphausia superba*, shown by grouped sexual maturity stage (stages according to Makarov and Denys [1981] with MS including MS1–3, MA including MA1–2, and FA including FA1–5). (SE, standard error; n = total number of krill).

	% carbon		% nitrogen		% hydrogen		% phosphorus		% ash		
Stage	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	n
J	47.22	0.67	9.78	0.22	7.16	0.12	1.23	0.04	10.43	0.70	4
FS	48.36	0.41	9.23	0.12	7.34	0.07	1.29	0.01	11.03	0.35	39
MS	48.24	0.36	9.17	0.08	7.35	0.07	1.28	0.01	11.05	0.39	57
MA	47.28	*	8.65	*	7.50	*	1.16	*	14.40	*	1
FA	47.32	0.94	9.82	0.23	7.20	0.09	1.30	0.04	13.18	1.40	9

* No data (n<2) (results shown for n=1).

Table 5. Elemental composition of Antarctic krill, *Euphausia superba* (dry mass basis data, % dry mass; organic data, % ash-free dry mass). Statistics shown are for regression analysis with total length. (SE, standard error, n=total number of krill).

Component	Mean	SE	n	df	F
Dry mass basis					
Total carbon	48.16	0.25	110	1,108	15.68*
Nitrogen	9.26	0.07	110	1,108	4.66**
Phosphorus	1.28	0.01	110	1,108	0.02†
Ash	11.23	0.27	101	1,99	5.60**
Organic basis					
Organic carbon	53.87	0.27	100	1,98	44.15*
Nitrogen	10.42	0.08	101	1,99	2.56†
Phosphorus	1.44	0.01	101	1,99	0.38†

* $p<0.001$; ** $p<0.05$.
† Not significant.

growth varied from slight shrinkage to values near the maximum recorded for krill of this size (Nicol 2000). Our results (which are equivalent to 0.07 mm d^{-1}) are near the maximum *GI*s of 4.4% (Quetin and Ross 1991, fall), 15.7% (Nicol et al. 1992, summer), 6% (Nicol et al. 2000, summer), and 10% (Ross et al. 2000, summer) reported by the four previous IGR studies.

Three other studies using techniques other than IGR have previously reported krill growth from the South Georgia region, those of Clarke and Morris (1983) (0.33 mm d^{-1}), Rosenberg et al. (1986) (0.148 mm d^{-1}), and Reid (2001) (0.130 mm d^{-1}). These studies have each employed different techniques to measure growth rate, and the discrepancy between them shows that these methods could be prone to error. Clarke and Morris (1983) reported growth rates that were three times faster than any other published value (Quetin et al. 1994), and Reid (2001) suggested that krill in his study did not grow at all during January (compare with our observed January growth rates, Fig. 2).

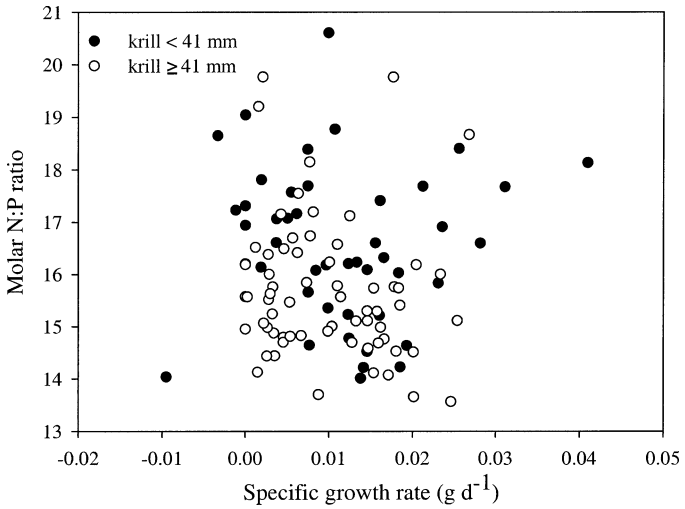

Fig. 5. Relationship between specific growth rate and N : P ratio in *Euphausia superba.* Closed circles are small krill (<41 mm); open circles are large krill (≥41 mm).

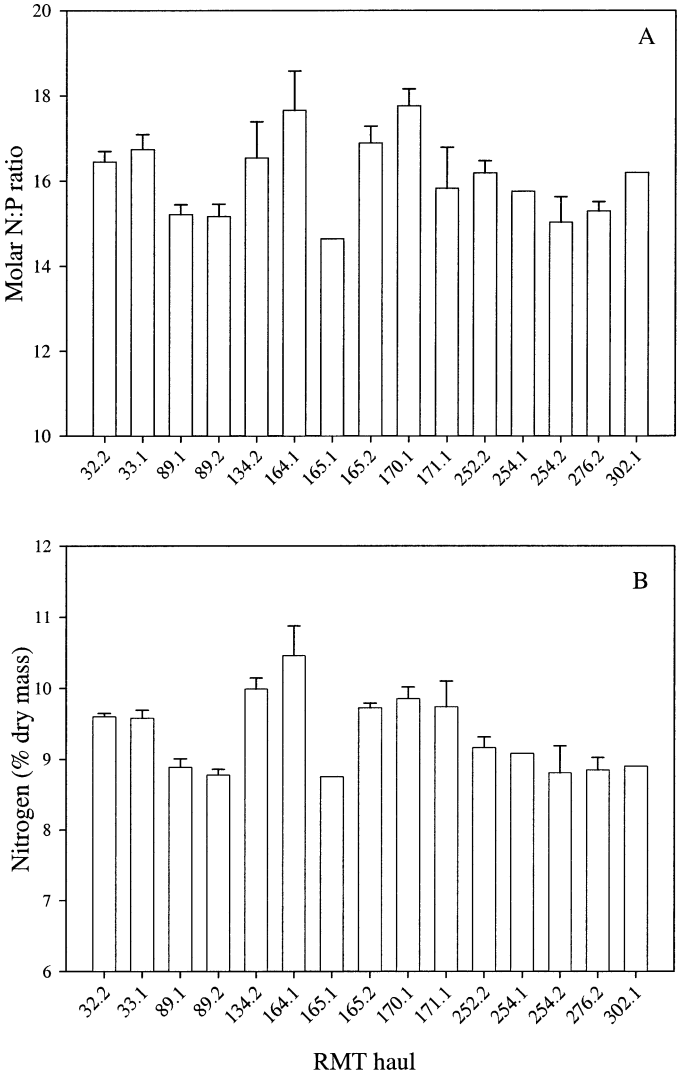

Fig. 6. *Euphausia superba:* differences in (A) molar N : P ratio and (B) N content (% dry mass) between schools. Error bars display standard error.

A significant correlation was observed between growth rate and sampling event (and, hence, date). The majority of this variation is attributable to growth increment at molt rather than molting frequency, although IMP did vary between schools. It is probable that the local environmental conditions at each sampling location, such as water temperature and food quality and availability, were triggering different rates of growth. Surface chlorophyll *a* values encountered during the cruise ranged from 0.19 to 18.67 mg m^{-3}. Provisional analysis with hourly chlorophyll samples and conductivity, temperature, depth (CTD) data from the cruise show a weak but statistically significant positive correlation with growth. However, it is difficult to relate observed growth to environmental variability, and it is beyond the scope of this study to do so. The apparent seasonal decline in growth rate evident in Fig. 2 cannot reliably be ascribed to temporal variation, as there are many other variables, such as chlorophyll *a* concentrations, spatial distribution, and water temperature, which may have also influenced the growth

rates observed. This variability in observed growth highlights the need for a better understanding of the causes of this variation and the development of a suitable proxy for growth to help us achieve this.

Chemical composition: Overall data—The chemical composition of Antarctic krill determined in this study is comparable to that reported previously (Ishii et al. 1987; Huntley et al. 1994; Torres et al. 1994). In contrast to previous stoichiometry studies on other zooplankton (Main et al. 1997; Elser et al. 2000*a*) there was less observed variation in the phosphorus composition of krill than was the case with nitrogen. It is possible that the higher variation in N composition could have resulted from the differing food conditions at which the krill were sampled. Studies of vascular plants (Shaver and Melillo 1984) and chlorophytes (Rhee 1978) have shown a significant relationship between their N composition and nutrient supply, although comparable studies on invertebrate zooplankton indicate that they tend to maintain relatively stable stoichiometric composition (Andersen and Hessen 1991; DeMott et al. 1998), even after different food manipulations in the laboratory (Hessen 1990).

Deposition of lipid commensurate with growth affects the C : N ratios of zooplankton (Sterner and Elser 2002), but this accumulation of C is unlikely to directly affect the N : P ratio, although it may dilute its component measurements (P and N, when measured as a function of dry mass). Although phosphorus is an important component of phospholipids, these typically contribute less than 5% of total body mass in most organisms (Sterner and Elser 2002) and therefore do not explain whole-animal patterns in a way that can be seen with RNA. In Antarctic krill, between 7% and 40% of total phosphorus is attributable to phospholipids. The highest proportions are confined to females with mature ovaries, as eggs contain large amounts of phospholipid in the egg yolk; in juveniles and males, the majority of P is contributed by RNA (British Antarctic Survey unpubl. data).

Variation with sexual maturity stage—Although the krill incubated represented a range of sexual maturity stages, most of those that molted were subadult females and males, which has resulted in the unbalanced nature of the sexual maturity stage categories in this study and the need to pool sex and maturity stages for statistical analysis. Only %N varied significantly with sexual maturity stage. The significant relationship between %N and sexual maturity stage is probably attributed to the low value for the mature male and high values for mature females contributing to most of the variation. This difference is driven by the large size of the ovary in gravid females (FA3 and FA4), which is rich in C and N (lipid and vitellogenin in yolk) but relatively low in P, despite the phospholipid component of yolk.

Variation with size—Weak relationships were shown between length and C, N, and ash composition. We would expect little variation in chemical composition with size of the animals when the results are expressed as percentage composition, as the animals are likely to have a broadly similar physiological make-up as they grow (Ikeda 1984). There are, however, life history reasons for some amount of variability, such as reproductive stage and season; thus, the observed change in ash content with length could be related to a size-dependant change in the proportion of the exoskeleton. However, we can see that differences in composition can be attributed to sexual maturity stage and that there is naturally an element of the size of the animal inherent in this distinction, as the two factors co-vary. The observed variations in elemental composition with size and maturity stage are, however, small.

Variation with growth rate—Between schools there was a significant difference in %N and N : P (Fig. 6), with the difference in N : P driven principally by variation in N and not in P (as discussed above). No relationship could be detected between SGR and either %N, %P, or N : P, whether these values were analyzed between events or for all data pooled together.

Intraspecific test of the growth rate hypothesis—Our results thus indicate that Antarctic krill at South Georgia in late summer do not support the growth rate hypothesis; there is no relationship between N : P ratio and growth rate when comparisons are made between individuals or when data are pooled by school. The most likely explanation for this is the lack of variation in phosphorus content. As all the krill were sampled during the summer in a period of maximum growth, it seems likely that they are all physiologically prepared to exploit the patches of good food quality and quantity with periods of rapid growth. As krill do not grow in length continually, but instead grow in bursts dictated by the molt cycle, a particular krill may have shown no growth during the duration of the experiment but may demonstrate positive growth on previous and, potentially, on its next molt. Furthermore, during this summer period it is unlikely that animals would alter the cellular concentration of ribosomes over short time-scales, as it would be costly to both reduce this and to then resynthesize ribosomes when conditions allow a period of high growth. Zooplankton typically live in a variable environment in which food is patchy, and they must be capable of exploiting food whenever they find it. To do so they maintain their capacity for growth (high ribosomal content) for the summer period, when they are likely to meet short-term concentrations of food. We speculate, therefore, that the N : P ratio may more accurately be described as reflecting potential growth rate, where a krill with a low N : P ratio has the potential to display high growth rates (apparatus for growth ready), although growth rate in the previous IMP has been low.

We predict, however, that a comparison between summer and winter krill from the same area would reveal variations in %P that could be attributed to long-term (seasonal) changes in cellular rRNA, which could in turn be correlated to a reduced growth rate observed during winter months. (Krill in the winter have a high N : P ratio, as they have a reduced potential for growth.) This prediction is supported by recent data from Antarctic krill sampled from Deception Island, where both RNA : DNA ratio and citrate synthase activity were lower in krill sampled in early spring (after overwintering) than in high summer (Cullen et al. 2003).

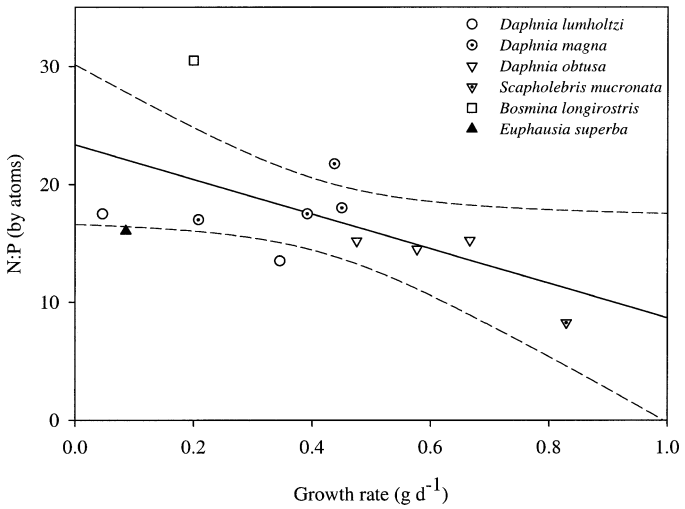

Fig. 7. Relationship between N : P ratio and growth rate from Main et al. (1997), with data from this study for *Euphausia superba* plotted. Solid line shows Main's regression, dashed lines = 95% confidence limits.

Interspecific test of the growth rate hypothesis—Although a relationship between growth rate and N : P could not be demonstrated for krill on an individual or school level, it is possible that the overall mean matches the growth rate hypothesis. To ensure comparability of data for this analysis, SGR data for krill have been recalculated to conform with the definition used by Main et al. (1997), namely

$$\mathrm{SGR} = 0.5\left[\ln\left(\frac{B_{final}}{B_{initial}}\right)\right]$$

where B_{final} is the final biomass and $B_{initial}$ the initial biomass.

When the mean data for *E. superba* from this study (all samples combined) are compared with the data for freshwater zooplankton (Main et al. 1997), the data from this study fit the general interspecific relationship between elemental stoichiometry and growth rate previously demonstrated (Fig. 7). The krill data from this study fall just within the 95% confidence levels for the overall regression, with a closer fit than data for *Bosmina longirostris.* This would indicate that at a population level, late-summer *E. superba* at South Georgia are in line with the overall data for freshwater zooplankton. However, it should be noted that the data in Fig. 7 mix several populations of some species, with an overall mean for Antarctic krill.

Despite the wealth of literature on theoretical support for the growth rate hypothesis (Sterner and Elser 2002, and references therein), there exist few practical demonstrations of its application outside of the field of freshwater zooplankton. In this light, the broad agreement observed on a larger scale indicates that further investigation of marine invertebrates will yield positive results. Comparisons of N and P content of summer and winter krill would therefore be of interest, because on a seasonal timescale we might expect the very large changes in growth rate to be reflected in the chemistry, although the analysis will be complicated by concomitant changes in body mass. In doing so, however, care needs to be taken to allow for the physiology of growth: short-term variations in growth rate may not be related to elemental composition. This means that elemental composition cannot provide a proxy for short-term variations in growth rate. It remains important, therefore, to pursue the discovery of a suitable biochemical proxy for growth rate in krill and to further test the suitability of this technique.

From this study we conclude that N and P stoichiometry do not provide a proxy for growth rate in *E. superba* on an intraspecific basis or over short time scales. However, if the data for summer krill are pooled to provide a long-term average for elemental composition and growth rate, the data for all individuals in this study are broadly comparable with the interspecific growth rate relationship demonstrated by Main et al. (1997) for five freshwater crustaceans (Fig. 7). This would indicate that when averaged over a period of a month, the population of krill at South Georgia do match the growth rate hypothesis and does lend some practical support to the idea that this hypothesis is a general relationship that can be applied over size, species, and habitat boundaries, as implied by stoichiometric theory.

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