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Growth in the slow lane: protein metabolism in the Antarctic limpet *Nacella concinna* (Strebel 1908)

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Summary

Growth rates in Antarctic ectotherms are generally considered to be low in comparison to temperate and tropical species. Food consumption plays a major role in determining animal growth rates, but once food is ingested soft tissue growth rates are largely determined by the protein synthesis retention efficiency (PSRE), a measure of the efficiency with which proteins are synthesised and retained as protein growth. The effect of water temperatures on the PSRE of polar organisms has not previously been investigated, and it is possible that reduced PSRE at polar water temperatures may at least partially explain low growth rates in Antarctic organisms. We also currently lack any information on the potential effects of predicted increases in seawater temperatures on protein metabolism in Antarctic ectotherms. We have measured seasonal protein synthesis, degradation and growth rates in free-ranging Antarctic limpets (*Nacella concinna*), together with protein synthesis rates at temperatures ranging between –1.5°C and 6.0°C. PSRE were not significantly different in summer (15.69±4.41%) or winter (20.59±4.45%), but values were considerably lower than those previously reported in temperate and tropical species. A meta-analysis of published ectotherm PSRE suggested there was a positive relationship with temperature (y=449.9–114.9x, r^2=28.8%, P<0.05). In turn, this suggests that temperature may be an important factor in determining ectotherm growth efficiency *via* an influence on PSRE. Maximal fractional and absolute protein synthesis rates occurred at ~1°C in *N. concinna*, the approximate summer water temperature at the study site, and protein synthesis rates decreased above this temperature. In the absence of adaptation, predicted increases in Antarctic water temperatures would result in reduced, rather than increased, rates of protein synthesis and, in turn, possibly growth.

Key words: polar, limpet, growth, protein synthesis.

Introduction

In most Antarctic ectotherms, annual growth rates are lower than temperate and tropical species (Clarke and North, 1991; Barnes, 1995; Clarke et al., 2004; Barnes et al., 2006; Bowden et al., 2006), although there are some reported exceptions (Dayton et al., 1974; Dayton, 1989; Rauschert, 1991; Barnes, 1995). Previous studies have suggested that low annual growth rates in Antarctic primary consumers are largely caused by highly seasonal primary productivity, which results in very low growth rates for the majority of the year (Clarke, 1985; Clarke, 1988; Clarke and Leakey, 1996; Fraser et al., 2004), although some studies do report strong temperature effects on growth (Heilmayer et al., 2004) and embryonic and larval development are markedly slowed due to temperature constraints (Hoegh-Guldberg and Pearse, 1995; Peck et al., 2006). Furthermore, during the austral summer, short-term growth rates in some polar species may approach those seen in temperate species (Nolan and Clarke, 1993; Peck and Bullough, 1993). Although reduced winter food availability is likely to be a major factor constraining annual growth in Antarctic ectotherms, it is possible that fundamental biochemical constraints may also limit growth at polar water temperatures.

Soft tissue somatic growth in an organism is primarily the result of protein growth, with the energetic cost of synthesising proteins dominating the overall costs of growth (Fraser and Rogers, 2007). The proportion of synthesised protein that is retained as protein growth is termed the protein synthesis retention efficiency (PSRE) (Houlihan et al., 1995) and is an important determinate of both growth rate and growth efficiency. PSRE can vary both within species, driven by factors such as food consumption and age, and between species (Houlihan et al., 1995; Carter and Houlihan, 2001). To date, no studies have examined protein synthesis and protein growth simultaneously in any adult Antarctic species. Although whole animal protein synthesis rates have been measured in several species (e.g. Whiteley et al., 1996; Marsh et al., 2001; Robertson et al., 2001a; Robertson et al., 2001b; Whiteley et al., 2001; Fraser et al., 2002a; Fraser et al., 2004), it is currently unclear whether temperature influences PSRE in polar ectotherms, and hence whether low growth rates in polar ectotherms could be partially determined by a reduced PSRE.

Global seawater temperatures are increasing in many regions, with Antarctic temperatures increasing at an above average rate, both in sub-surface water masses and in particular along the West Antarctic peninsula (Levitus et al., 2000; Gille, 2002; Meredith and King, 2005). In temperate ectotherms, an increase in temperature typically results in an increase in protein synthesis (Loughna and Goldspink, 1985; McCarthy et al., 1999). Current climate models predict a 2°C increase in global seawater temperatures over the next century, albeit with large confidence intervals, and sea temperatures in the Bellingshausen sea have increased by ~1°C in the last 50 years (Meridith and King, 2005). However, we have little idea what effect this will have on protein synthesis and, in turn, growth rates of Antarctic species. Previous studies have suggested that many Antarctic invertebrates have an extremely limited ability to acclimate to water temperatures even a few degrees above their normal summer maxima (Peck, 2002; Peck et al., 2004), although some fish species have been acclimated to temperatures of 4°C and above (Lowe and Davison, 2006).

The primary aim of this study was therefore to establish whether a thermally induced reduction in PSRE could be contributing to reported low annual growth rates in the Antarctic limpet *Nacella concinna* (Strebel 1908) (Clarke et al., 2004). To provide an environmental context for this work, we also examined whether an increase in seawater temperature could result in increased rates of protein synthesis and hence growth in this species.

Materials and methods

This study involved two sets of experiments examining protein metabolism in *Nacella concinna*. In the first set of experiments protein synthesis, protein growth and protein degradation were examined during summer and winter, with protein growth measured in free-ranging animals and protein synthesis measured in the laboratory. In the second set of experiments protein synthesis was measured at four water temperatures (–1.5, 1.0, 3.5 and 6.0°C) in laboratory maintained animals. All experimental work was carried out at Rothera Research Station (67°34′07″S, 68°07′30″W), Adelaide Island, Antarctica, in 1999 and 2000. *N. concinna* were collected from marked sites at water depths of 10–13 m in South Cove using SCUBA. Details of the general collection area are described elsewhere (Brown et al., 2004). Limpets were returned immediately to the laboratory and maintained in flow-through aquaria with ambient seawater. For temperature-controlled experiments, limpets were held in experimental aquaria with the seawater temperature maintained using jacketed tanks and thermocirculators (Grant Instruments, Cambridge, UK).

Seasonal protein turnover: experimental protocol

In December 1999 (the austral summer), 98 limpets were weighed (to ±1 mg), measured (to ±0.05 mm) using vernier callipers, and individually numbered with enamel paint (Humbrol, Kingston upon Hull, UK). Three days later the marked animals were returned to the field site from which they were collected. During the short period that the animals were held in the laboratory, they were maintained at similar water temperatures to those they experienced in the sea (mean water temperature during growth period, –0.47±0.12°C) and under a simulated natural photoperiod. After 64 days growing in the field, SCUBA divers collected as many of the marked animals as possible and returned them to the laboratory, where they were re-weighed and measured. A subset of 16 animals was selected to measure protein synthesis and protein growth. These animals were maintained in the aquarium overnight before protein synthesis was measured the next day (see below), and were not fed, although they were observed grazing biofilms on the sides of the tank.

In June 2000 (the austral winter), a second group of 67 unmarked limpets were collected from the same sampling site and returned to the laboratory. The limpets were weighed, measured and marked as described above before being returned to the collection site. After a 96-day field growth period, as many of the second group of marked limpets as possible were recovered, re-weighed and measured. A subset of 17 were selected to measure protein synthesis and protein growth (as above). The mean water temperature during the growth period was –1.62±0.16°C.

For both studies, further groups of approximately 100 animals were collected to allow the relationship between fresh mass (total mass less shell mass) and total body mass to be determined. The total protein content of these limpets was also measured (see below) to estimate the protein content of the marked animals at the start of the growth period.

Effect of temperature on protein synthesis: experimental protocol

In a separate study, freshly collected *N. concinna* were held in aerated water (temperature –1.3°C to –1.5°C) under a simulated natural photoperiod for 30 days prior to experimental work. The majority of the water in each tank was exchanged every 48 h with clean water of the same temperature. The animals were allowed to feed *ad libitum* on biofilms on the sides of the tanks, but were not provided with additional food sources. After the 30-day acclimatisation period, animals were transferred to experimental aquaria that contained water at the same temperature as the holding tanks. Experimental tank water temperatures were adjusted to the required temperatures (–1.5, 1.0, 3.5 and 6.0°C) at a rate of 0.5 K day^{-1}. After reaching the experimental water temperature, animals were allowed to acclimate for 30 days prior to the measurement of protein synthesis. Husbandry conditions, excluding water temperature, were as previously described. The day before protein synthesis was measured a unique identifying number was glued to each animal's shell with cyanacrolyte.

Measurement of protein synthesis

The flooding dose method has previously been validated for measurement of whole animal protein synthesis in *N. concinna*, as described in detail previously (Fraser et al., 2002a). In summary, each limpet was weighed (to ±1 mg) after surface drying with a tissue. The fresh mass was estimated from the previously determined relationship between total and fresh mass, and this value was used to calculate the required injection volume of radiolabelled amino acid (Fraser et al., 2002a). Each experimental animal was injected into the pedal sinus with a solution containing [^{3}H]-labelled and unlabelled phenylalanine

(Phe) (10 μl g^{-1} fresh mass of 135 mmol l^{-1} L-[2,6-^{3}H]Phe at 3.6 MBq ml^{-1}; Amersham, Little Chalfont, UK). After injection the animals were placed in a beaker containing 4 l of aerated seawater at the same water temperature at which the limpets were previously held. In each temperature experiment a time course (1, 2 and 4 h) of intracellular free-pool stability and protein-bound radiolabelling was determined, to validate the flooding dose methodology.

Sample analysis

At the end of the experiment limpets were removed from the beaker, shucked, and the fresh mass measured. The shucked body of the limpet was then homogenised (X120 Status homogeniser, PolyScience, Niles, IL, USA) in a known volume of ice-cold 0.2 mol l^{-1} perchloric acid (PCA) and the homogenate stored at 4°C prior to analysis. Sample analysis was carried out as described previously (Fraser et al., 2002a). The data were corrected for ^{3}H scintillation counting efficiency, which was 32±0.5% (Hionic Fluor scintillation fluid, LKB-Wallac Rack Beta scintillation counter). All radioactivities were expressed as disintegrations per minute (d.p.m.) per nmol Phe. Where free-pools were stable (all experiments with the exception of summer protein turnover animals), whole animal fractional protein synthesis rates were calculated using Eqn 1:

$$k_s = \frac{S_b}{S_a} \times \frac{100}{t} \times 1440\ , \qquad (1)$$

where k_s is the fractional protein synthesis rate (% day^{-1}), S_b is specific radioactivity of protein-incorporated radiolabel, S_a is specific radioactivity of intracellular free-pool (both as d.p.m. nmol^{-1} Phe), t is incorporation time (min) from injection to death, and 1440 is the number of min day^{-1}. Where the free-pools were decreasing linearly (summer protein turnover animals) then Eqn 2 was used (Garlick et al., 1983; Houlihan et al., 1995):

$$k_s = \left(\frac{S_{b(t_2)} - S_{b(t_1)}}{S_{a(t_2-t_1)}}\right) \times \left(\frac{1440}{t_2 - t_1}\right) \times 100\ , \qquad (2)$$

where $S_{b(t2)}$ is protein-bound specific radioactivity after t_2 min, $S_{b(t1)}$ is the protein-bound specific radioactivity at an earlier time point, t_1, $S_{a(t2-t1)}$ is the mean free-pool specific radioactivity over the same time period (again both as d.p.m. nmol^{-1} Phe) and other variables as for Eqn 1.

The absolute rate of protein synthesis (A_s) was calculated using the following equation:

$$A_s = \frac{k_s}{100} \times \text{protein mass}\ ,$$

where A_s is expressed as mg protein synthesised animal^{-1} day^{-1}, and protein mass is expressed as mg protein animal^{-1}.

The whole animal RNA concentration was expressed as the RNA:protein ratio (μg RNA mg^{-1} protein). The RNA activity, k_{RNA} (mg protein mg^{-1} RNA day^{-1}) was calculated using the following equation (Preedy et al., 1988):

$$k_{RNA} = \frac{10 \times k_s}{\text{RNA:protein ratio}}\ .$$

Whole animal protein growth rates (k_g, % day^{-1}) were calculated using the following equation (modified from Ricker, 1979; Houlihan et al., 1994):

$$k_g = \frac{\ln(w_2) - \ln(w_1)}{t} \times 100\ ,$$

where w_2 is the final protein mass, w_1 is the initial protein mass and t is time in days.

w_1 was calculated from the estimated initial protein concentration and fresh mass, and w_2 was directly measured. Protein synthesis retention efficiencies (PSRE, %) were calculated using the following equation, where k_s and k_g are expressed as % day^{-1}:

$$\text{PSRE} = \frac{k_g}{k_s} \times 100\ .$$

Statistical analysis

All data are expressed as mean ± s.e.m. The stability of intracellular free-pools was tested using ANOVA, and linear radiolabelling of bound protein was tested using least-squares regression analysis. Comparison of summer and winter physiological parameters was carried out using one- or two-sample tests where appropriate. In the temperature and protein synthesis experiments, comparisons between temperatures were carried out using ANOVA. The threshold for statistical significance was set at P<0.05.

Results

There were no significant differences in the initial total mass (including shell) or shell length in the groups of limpets used for the seasonal comparison of protein synthesis rates, or in the temperature study (all P<0.05: Table 1, Table 2).

Flooding dose validation

In summer limpets, intracellular free-pool specific radioactivities decreased linearly during the time course (Fig. 1A: F=5.59, P<0.05). Protein synthesis rates could therefore only be calculated using protein synthesis Eqn 2. In winter limpets, intracellular free-pool specific radioactivities did not significantly change during the time course measurement (Fig. 1B: P>0.05), and protein synthesis rates could be calculated with Eqn 1. In both summer (Fig. 1C) and winter (Fig. 1D) limpets, incorporation of radiolabelled Phe into proteins was both significant and linear (all P<0.001), and the intercepts of the regression lines were not significantly different from zero. To ensure that the flooding dose injection had successfully flooded the animal's intracellular free-pools, the post-injection increase in Phe was calculated. The pre-injection Phe concentration of *N. concinna* was

Table 1. *Biometrics of Nacella concinna used in the seasonal protein turnover experiments*

	Summer	Winter	T	P
Length (mm)	25.71±0.77	27.72±0.58	NS	
Total mass (g)	1.98±0.16	2.24±0.15	NS	
Protein (% tissue mass)	10.31±0.41	12.25±0.37	3.37	<0.01

Summer (N=16) and winter (N=17) values are means ± s.e.m.

Table 2. *Biometrics of Nacella concinna used in the effects of temperature on protein synthesis experiments*

	–1.5°C	1.0°C	3.5°C	6.0°C	*P*
Length (mm)	23.88±0.59	24.56±0.47	24.32±0.77	23.50±0.47	NS
Total mass (g)	1.50±0.12	1.59±0.12	1.55±0.16	1.21±0.09	NS
Protein (% tissue mass)	12.55±0.39	11.82±0.42	12.06±0.34	11.01±0.43	NS

Values are means ± s.e.m. (N=18 for all treatments).

0.34 nmol mg^{-1} fresh mass, the injection of radiolabelled and unlabelled Phe should therefore theoretically raise the free-pool concentrations by 1.35 nmol mg^{-1} fresh mass to a final concentration of 1.69 nmol mg^{-1} fresh mass (Fraser et al., 2002a). The mean Phe concentration after injection in both protein turnover experiments was 1.71±0.08 nmol mg^{-1} fresh mass: Phe concentrations had increased to 101% of the theoretical concentration, a 4.6-fold increase in tissue Phe levels, indicating that the free-pools had flooded successfully.

At all water temperatures, in the temperature and protein synthesis experiments, the time courses demonstrated that the underlying assumptions of the technique were met, as intracellular free-pool specific radioactivities were elevated and stable during the course of the protein synthesis measurements (all P>0.05). The increases in protein-bound radiolabelling with time were significant and linear, with the intercept of the regression lines not significantly different from zero (Table 3). The mean post-injection Phe concentration in limpets was 1.62 nmol mg^{-1} fresh mass, 96% of the theoretical post-injection Phe concentration (1.69 nmol mg^{-1} fresh mass); this represents a mean 4.75-fold increase in Phe concentrations after injection.

Seasonal protein turnover

The measured protein contents of the summer and winter limpets at the end of the experimental periods were significantly different (Table 1: T=–3.37, d.f.=30, P<0.01). Summer fractional and absolute protein growth rates were not significantly different to winter values (Table 4: P>0.05). Fractional protein synthesis rates in summer animals were 0.802% day^{-1} (error bars cannot be calculated due to the calculation method, see Eqn 2) and were significantly higher than winter protein synthesis rates of 0.55±0.06% day^{-1} (Table 4: T=–4.07, d.f.=16, P<0.001). While absolute protein synthesis rates and protein synthesis retention efficiencies were

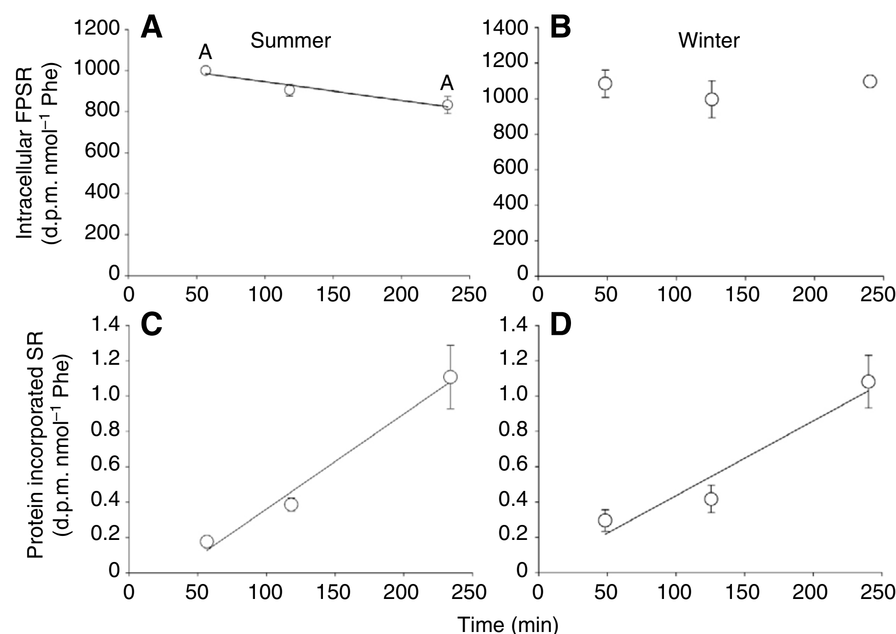

Fig. 1. Intracellular free-pool (FPSR) and protein-bound (SR) specific radioactivities in *Nacella concinna* at Rothera Research Station, Adelaide Island, Antarctica. (A) Summer intracellular free-pool (y=1037–0.9092x, r^2=44.4%, P<0.01). Letter A signifies data points that are significantly different to each other. (B) Winter intracellular free-pool. (C) Summer protein incorporated (y=–0.175+0.00538x; r^2=0.67). (D) Winter protein incorporated (y=–0.134+0.005x; r^2=0.67). All values are mean ± s.e.m. Each data point represents values from five (summer, 1 and 2 h; winter, 1 h time course) or six animals (summer, 4 h; winter, 2 and 4 h time course). Fitted regression lines were highly significant (P<0.001) with intercepts not significantly different to zero.

Table 3. *The relationship between radiolabelled bound protein and time in Nacella concinna, at a range of experimental temperatures*

Temperature (°C)	Regression equation	r^2 (%)	Intercept P	Slope P
–1.5	y=0.102+0.00287x	75.1	0.129	0.000
1.0	y=0.082+0.00489x	70.2	0.525	0.000
3.5	y=–0.0321+0.00410x	85.9	0.630	0.000
6.0	y =–0.0764+0.00489x	86.9	0.307	0.000

Table 4. *Seasonal variation in protein metabolism variables measured in Nacella concinna, Rothera 1999/2000*

	Summer	Winter	*T*	*P*
Directly calculated				
Absolute protein growth (mg day^{-1})	0.40±0.11	0.35±0.09	NS	
RNA:protein ratio	47.50±2.66	36.64±1.24	3.56	<0.01
Fractional protein synthesis (% day^{-1})	0.802	0.55±0.06	4.07	<0.001
Derived				
Fractional protein growth (% day^{-1})	0.13±0.04	0.09±0.02	NS	
Protein degradation (% day^{-1})	0.68±0.04	0.47±0.06	2.78	<0.01
Absolute protein synthesis (mg day^{-1})	1.33±0.10	1.03±0.10	NS	
PSRE (%)	15.69±4.41	20.59±4.45	NS	
k_{RNA}	0.18±0.01	0.16±0.02	NS	

Summer (*N*=16) and winter (*N*=17) values are means ± s.e.m.

not significantly different between seasons (Table 4: $P>0.05$), protein degradation, the difference between protein synthesis and protein growth, was significantly ($T=-2.78$, d.f.=24, $P<0.01$) higher in summer than winter.

RNA:protein ratios were significantly ($T=3.56$, d.f.=21, $P<0.01$) lower in winter than summer, while k_{RNA} was unchanged (Table 4: $T=0.95$, d.f.=22, $P>0.05$)

Effect of temperature on protein synthesis

Whole animal protein content was not affected by water temperature (Table 2; ANOVA, $F=2.50$, $P=0.067$). The mean limpet protein content for all water temperatures was 11.86±0.21%. Temperature had a significant effect on fractional protein synthesis rates (Fig. 2A) and RNA activity (Fig. 2D) with the rates at 1.0°C significantly higher than at the other experimental temperatures. The absolute protein synthesis rate at 1°C was significantly higher than at 6.0°C (Fig. 2B), while the RNA:protein ratio was significantly different at all measurement temperatures (Fig. 2C). RNA:protein ratios initially decreased with increasing water temperature, but after reaching a minimum value at 1.0°C, subsequently increased with temperature.

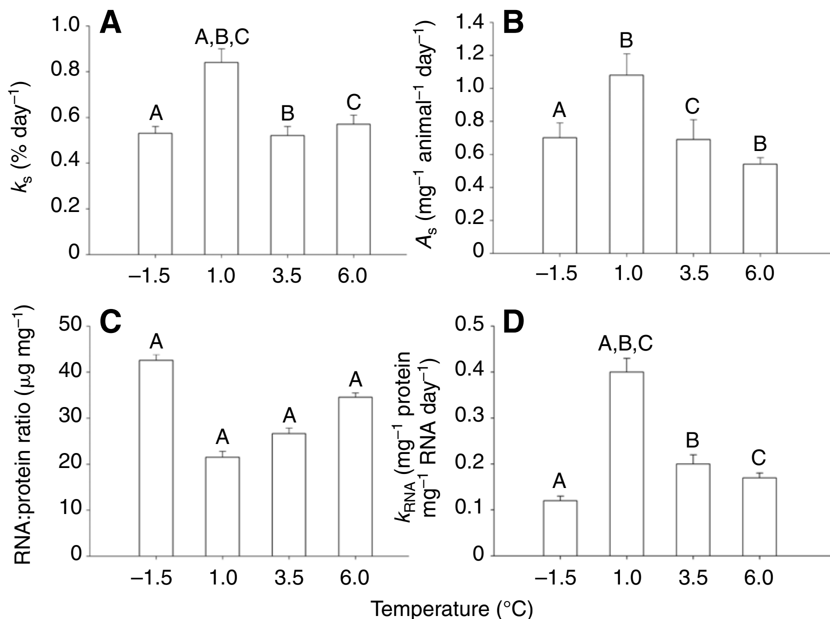

Fig. 2. The effect of temperature on protein and RNA metabolism in *Nacella concinna*, Rothera Research Station, Adelaide Island, Antarctica. (A) Fractional protein synthesis rate (k_s; ANOVA, $F=10.38$, $P<0.001$); (B) absolute protein synthesis rate (A_s; ANOVA, $F=4.80$, $P<0.01$); (C) RNA:protein ratio (ANOVA, $F=56.01$, $P<0.001$); (D) RNA activity (k_{RNA}; ANOVA, $F=36.64$, $P<0.001$). Bars with the same letter are significantly different to each other. For each bar, *N*=6, except for 6°C fractional and absolute protein synthesis rates, where *N*=5.

Discussion

This study presents the first adult protein metabolism data from any Antarctic ectotherm, and it also represents the first measurement of protein metabolism in a free-ranging ectotherm in its natural environment. Previous studies of temperate marine species have measured protein metabolism in laboratory maintained animals, in most cases fed artificial diets (e.g. Houlihan et al., 1990; Houlihan et al., 1994; Langar and Guillaume, 1994; Meyer-Burgdorff and Rosenow, 1995; Sveier et al., 2000).

Flooding dose validation

As there were no seasonal differences in the length or tissue mass of limpets in summer and winter, or between experimental groups held at different temperatures (Tables 1, 2) data were not corrected for mass prior to statistical comparisons. Stable (winter, Fig. 1B; –1.5 to 6.0°C temperature range) or linearly decreasing (summer, Fig. 1A) intracellular free-pools, significant and linear incorporation of radiolabelled Phe into protein in all experiments (Fig. 1C,D, Table 3), and the increase in intra-cellular free-pool Phe concentrations after injection, indicated that the criteria for the flooding dose technique (Houlihan et al., 1995; Fraser et al., 2002a; Fraser et al., 2004; Fraser and Rogers, 2007) had been fully met.

Seasonal protein turnover

Seasonal variations in the protein synthesis rate of *Nacella concinna* have previously been

reported, with reduced rates in winter, while faecal egestion rates in *N. concinna* were 11.9 and 7.35 mg dry mass animal^{-1} day^{-1} in February and October, respectively (Fraser et al., 2002a; Fraser et al., 2002b). Although faecal egestion rates are not a direct measure of food consumption they do provide an indication that food consumption decreases in winter. Protein synthesis rates in the current study were higher than those previously reported (Fraser et al., 2002a), probably as a result of inter-annual variability in food availability and consumption. Food consumption is known to have a significant effect on protein synthesis rates (Houlihan et al., 1989; Mente et al., 2001; Fraser et al., 2002a; Fraser et al., 2002b), and it is well established that both benthic and pelagic primary productivity show considerable inter-annual variability in the Antarctic (Clarke, 1988; Clarke et al., 1988; Gilbert, 1991; Fraser et al., 2004; Grange et al., 2004). Whole animal protein synthesis rates in *N. concinna* were very low in comparison to rates reported for temperate and tropical ectotherms, and a recent analysis (Fraser and Rogers, 2007) has suggested that protein synthesis rates decrease markedly below ~5°C (for reviews, see Houlihan, 1991; Houlihan et al., 1995; Carter and Houlihan, 2001).

Absolute protein synthesis rates did not vary seasonally (Table 4), and hence similar amounts of energy were allocated to synthesising protein in both summer and winter, presuming the thermodynamic cost of synthesising a unit of protein did not alter. Neither the PSRE nor k_g differed significantly between summer and winter, but the calculated PSRE values were very low in comparison to previously reported values, suggesting that only a small proportion of the proteins synthesised were retained as protein growth. Previously reported PSRE values in a wide selection of non-Antarctic ectotherms ranged between 25 and 95%, with a mean value of ~52%, and in most cases PSREs were considerably higher than the values of 16 and 21% reported in this study (Houlihan et al., 1995). The low protein synthesis rates reported here for *N. concinna*, coupled with the reduced PSRE, will result in both a low growth efficiency and a low overall growth rate.

Our observation of a very low PSRE in polar organisms poses the obvious question as to whether there is a general relationship between PSRE and body temperature, or whether *Nacella concinna* is simply an unusual organism. To examine whether ambient temperature has a general effect on PSRE, we compiled the available PSRE data from the literature. As both protein synthesis and protein growth scale with body mass, both variables were standardised to a body mass of 64.0 g (the mean value of all organisms in the compiled data set). The scaling coefficients used were calculated from the fitted relationship between fresh mass M and protein synthesis ($\ln k_s=1.869–0.2506\times\ln M$, $r^2=41.4\%$, $P<0.01$), and between fresh mass and protein growth ($\ln k_g=0.8389–0.1912\times\ln M$, $r^2=21.2\%$, $P<0.05$). Data were only utilised from studies in which the animals had been fed daily and not maintained under husbandry conditions likely to affect protein metabolism, such as exposure to pollution or temperatures outside of their normal thermal envelope. The data set is dominated by fish species with only two molluscs and a single crustacean species, and the analysis thus needs to be interpreted with caution, as the data set available is not phylogenetically diverse. However, there was a significant relationship between PSRE and temperature (Fig. 3). Animals living at polar temperatures (<0°C) retain as growth only about 30% of the protein they synthesise, whereas a typical tropical organism would retain about 70%, a difference of 2.3×. This finding has important implications, as it suggests that in ectotherms living at low temperatures, growth is far less efficient than at warmer temperatures, presumably as a result of fundamental biochemical constraints associated with the synthesis of proteins. This result is in contrast to earlier data reported (Heilmayer et al., 2004), which suggests that scallop growth efficiencies decrease with increasing temperature. Why a higher proportion of body proteins are degraded at low temperatures is currently unclear, although there is growing evidence of increased levels of cold-induced protein denaturation at polar water temperatures (Buckley et al., 2004; Place et al., 2004; Hofmann et al., 2005; Place and Hofmann, 2005).

Previous studies have reported high constitutive expression of the normally inducible heat shock protein 70 gene, together with elevated concentrations of ubiquitin-tagged proteins in Antarctic fish, both findings indicative of problems with protein folding and/or high levels of protein degradation (Buckley et al., 2004; Place et al., 2004; Hofmann et al., 2005; Place and Hofmann, 2005). In the wolfish, *Anarhichas lupus*, acclimatised to water temperatures ranging between 5 and 14°C, the relationship between PSRE and temperature was quadratic, with lower PSRE at temperatures near both the lower and upper extremes of its thermal range (McCarthy et al., 1999). It would therefore appear, that in *A. lupus*, protein degradation rates are either elevated at low temperatures or decrease at a slower rate than k_s. Further studies are required to obtain a better understanding of the effects of temperature on protein degradation. What is currently clear, however, is that the generally accepted view that Antarctic ectotherms grow slowly simply because of a highly seasonal food supply, is far from the complete story. In fact in the current study, protein growth rates, k_g, were not significantly different in summer or winter in spite of a previously reported tenfold difference in seasonal faecal egestion rates, a proxy measure of food consumption in this species (Fraser et al., 2002b). It therefore appears that temperature effects on fundamental biochemical factors may also limit the capacity of cold-water marine organisms to grow quickly and efficiently. It should be noted that other authors have suggested that growth efficiencies in scallops are elevated at low temperatures, and that stenothermal ectotherms maximise growth ability by minimising their standard metabolic rates, thereby maximising the energy available for growth (Heilmayer et al., 2004; Pörtner et al., 2005). However, protein growth efficiency, a major determinate of overall animal growth, appears to be lower, not higher, in Antarctic limpets. The reason for these conflicting results remains to be resolved.

The single data point (13) in Fig. 3 that does not appear to fit well within the data set is for the sole crustacean species available, *Homarus gammarus* (Mente et al., 2001). Growth in arthropods is achieved by periodic moulting and loss of the old exoskeleton; in turn this will result in the loss of some protein, thereby resulting in a reduction in the PSRE (O'Brien et al., 1991). Direct comparison of PSREs between arthropods and other animals is therefore problematic.

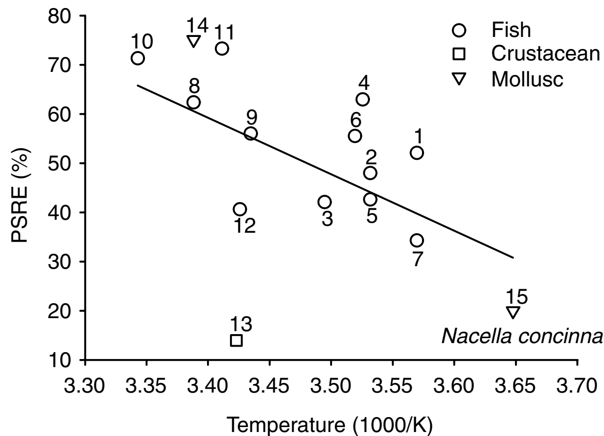

Fig. 3. The relationship between mass standardised protein synthesis retention efficiency (PSRE) and temperature in a range of ectotherm species. Temperatures are presented as an Arrhenius plot. The regression line was fitted using least-squares regression analysis (y=449.9–114.9x, r^2=28.8%, P<0.05). For data sources, see Table 5.

The RNA:protein ratios and k_{RNA} values measured in summer and winter animals in this study fall within the range of those previously reported in *N. concinna* (Fraser et al., 2002a).

Effect of temperature on protein synthesis

Previous studies have demonstrated the inability of Antarctic organisms to tolerate water temperatures even a few degrees above their normal summer maxima (Peck, 2002; Peck and Conway, 2000), with many species, including *Nacella concinna*, starting to lose some critical functions at water temperatures only slightly in excess of 0°C (Peck et al., 2004). The data presented in Fig. 2 suggest that the thermal optimum for k_s and A_s in *N. concinna* is around 1°C. Interestingly, although RNA:protein ratios initially decrease as water temperatures increase, as has been reported in many other studies, at water temperatures above 1°C, RNA:protein ratios start to increase again (Foster et al., 1992; Mathers et al., 1993; McCarthy and Houlihan, 1996; McCarthy et al., 1999; Fraser et al., 2002a). Conversely k_{RNA} increases with water temperature, reaching a maximum value at 1°C before then decreasing. In previous studies k_{RNA} was found typically to increase with temperature, while RNA:protein ratios decrease (McCarthy and Houlihan, 1996; McCarthy et al., 1999; Fraser et al., 2002a). It has been suggested that the typically observed linear increases in RNA:protein ratios with temperature occur to offset a thermally induced linear reduction in k_{RNA} (Fraser et al., 2002a), although it has been reported that in the Antarctic eelpout *Pachycara brachycephalum* and the Antarctic scallop *Adamussium colbecki*, low temperature compensation of protein synthesis is achieved primarily by cold-adaptation of the protein synthesis machinery rather than an increase in RNA concentration (Storch et al., 2003; Storch et al., 2005).

Why k_{RNA} decreases at water temperatures above 1°C is not clear. It is possible that evolutionary modifications have occurred to RNA in *N. concinna* to compensate protein synthesis rates at low water temperatures, with the trade-off for these modifications being a loss of function at even slightly elevated temperatures. Even though RNA:protein ratios increase at temperatures above 1°C, protein synthesis rates still decrease. Mean summer water temperatures, at the depths from which the limpets were sampled for this study, currently reach 1–2°C at Rothera Research station, around the optimum protein synthesis temperature for *N. concinna* measured in this study (Fraser et al., 2004). Therefore any future increase in seawater temperatures, even if only in the order of a few degrees, is at least in the short-term, likely to decrease, rather than increase summer protein synthesis rates. Extrapolating these data to absolute protein growth rates is complicated by our lack of knowledge of the effect of temperature on protein degradation rates in *N. concinna*. However, it is worth noting that the protein synthesis rates measured at –1.5 and 1.0°C in the temperature study are almost identical to those measured in the seasonal protein turnover study in winter and summer, respectively. In that study, although protein synthesis rates varied with season, protein growth rates did not, due to an increase in summer protein degradation rates. It is therefore not currently possible to speculate whether the measured decrease in protein synthesis rates at water temperatures above 1°C, will actually result in a decrease in protein growth and therefore overall growth rates. What is clear, however, is that protein and RNA metabolism in *N. concinna* are extremely sensitive to even small alterations in water temperature, with deleterious effects at water temperatures even a few degrees above the current summer maxima.

Table 5. *Sources of data used in Fig. 3, relating protein synthesis retention efficiency and temperature*

Data point label	Species	Author
1	*Limanda limanda*	(Houlihan et al., 1994)
2	*Gadus morhua*	(Houlihan et al., 1988; Houlihan et al., 1989)
3	*Salmo salar*	(Carter et al., 1993a)
4	*Hippoglossus hippoglossus*	(Fraser et al., 1998)
5	*Oncorhynchus mykiss*	(McCarthy et al., 1994)
6	*Anarhichas lupus*	(McCarthy et al., 1999
7	*Pleuronectes flesus*	(Carter et al., 1998)
8	*Ctenopharyngodon idella*	(Carter et al., 1993b)
9	*Dicentrarchus labrax*	(Langar et al., 1993)
10	*Oreochromis mossambicus*	(Houlihan et al., 1993)
11	*Chondrostoma nasus*	(Houlihan et al., 1992)
12	*Carassius auratus*	(Heba, 1992)
13	*Homarus gammarus*	(Mente et al., 2001)
14	*Octopus vulgaris*	(Houlihan et al., 1990)
15	*Nacella concinna*	This study

Conclusions

We have found that the PSRE in the polar limpet *N. concinna* is greatly reduced in comparison to temperate and tropical species. We have also for the first time demonstrated a significant relationship between PSRE and temperature, suggesting temperature has a fundamental influence on the efficiency of protein metabolism and thereby ectotherm growth. It therefore seems likely that although seasonal variability in food consumption undoubtedly reduces annual growth rates in Antarctic ectotherms, maximum growth rates (typically in

summer) are also constrained by inefficient protein metabolism. Over the range of temperatures examined it would appear that maximal protein synthesis rates in *N. concinna* occur at around 1°C, with rates decreasing at higher temperatures. This suggests, in turn, that any increase in water temperature along the Antarctic Peninsula, driven by the current regional warming (Clarke et al., 2007), is likely to result in reduced protein synthesis rates in *N. concinna*, which could result in reduced growth.

List of abbreviations

A_s	absolute protein synthesis rate
d.p.m.	disintegrations per minute
FPSR	intracellular free-pool specific radioactivity
k_d	fractional protein degradation rate
k_g	fractional protein growth rate
k_{RNA}	RNA activity
k_s	fractional protein synthesis rate
M	mass
PCA	perchloric acid
Phe	phenylalanine
PSRE	protein synthesis retention efficiency
SCUBA	self contained underwater breathing apparatus
SR	bound-protein specific radioactivity

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