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Low-temperature protein metabolism: seasonal changes in protein synthesis and RNA dynamics in the Antarctic limpet *Nacella concinna* Strebel 1908

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Summary

Protein synthesis is a fundamental and energetically expensive physiological process in all living organisms. Very few studies have examined the specific challenges of manufacturing proteins at low ambient temperatures. At high southern latitudes, water temperatures are continually below or near freezing and are highly stable, while food availability is very seasonal. To examine the effects of low temperature and a highly seasonal food supply on protein metabolism, we have measured whole-body protein synthesis, RNA concentrations, RNA:protein ratios and RNA translational efficiencies in the Antarctic limpet *Nacella concinna* at four times of the year. From summer to winter, protein synthesis rates decreased by 52%, RNA concentrations decreased by 55% and RNA:protein ratios decreased by 68%, while RNA translational efficiencies were low and very variable. Protein synthesis rates in *N. concinna* approached those measured in temperate mussels, while RNA:protein ratios were considerably higher than in temperate species. Inter-specific comparisons show that species living at low temperatures have elevated RNA:protein ratios, which are probably needed to counteract a thermally induced reduction in RNA translational efficiency. Calculations using theoretical energetic costs of protein synthesis suggest that Antarctic species may allocate a larger proportion of their metabolic budget to protein synthesis than do temperate or tropical species.

Key words: protein synthesis, RNA:protein ratio, Antarctic limpet, *Nacella concinna*, temperature.

Introduction

Proteins are essential in all living organisms, performing roles ranging from structural to catalytic. The synthesis and degradation of proteins is therefore a fundamental physiological process, and an animal's protein pool is in a continual state of flux, with new proteins entering the pool via protein synthesis and being removed via protein degradation. Protein synthesis is energetically expensive, accounting for 11–42% of basal metabolism in a range of ecto- and endotherms, and is therefore a major component of overall animal energetics (Houlihan et al., 1995a). The continual synthesis and degradation of proteins is not only vital for tissue maintenance and animal growth but is also important in allowing animals to adapt to changing environmental conditions, to replace denatured or damaged proteins, to mobilize amino acids and to allow metabolic regulation (Hawkins, 1991).

Recent analyses of resting respiration rates in fish and bivalves have demonstrated a positive non-linear relationship with temperature (Clarke and Johnston, 1999; Peck and Conway, 2000). Therefore, polar animals have low resting metabolic rates but must still generate sufficient ATP for the major cellular energy sinks (protein synthesis, RNA/DNA synthesis, proton leak, Na^+/K^+-ATPase and Ca^{2+}-ATPase) while balancing their metabolic budgets (Buttgereit and Brand, 1995). Some cellular parameters, such as the cycling of microtubules and red muscle mitochondrial densities, are cold adapted in polar species (Detrich et al., 1989; Johnston et al., 1994). Interestingly, the temperate fish *Gadus morhua* and the isopod *Saduria entomon* increase tissue RNA concentrations when acclimated to low temperatures in the laboratory (Foster et al., 1992; Robertson et al., 2001a). If a major cellular process is cold adapted in a polar species (i.e. the rate is maintained) then it seems likely that the process will require a larger proportion of the metabolic budget and there will therefore be a shift in the balance of ATP consumption between cellular processes.

In vivo protein synthesis rates have only been measured in a few Antarctic species e.g. the giant isopod *Glyptonotus antarcticus* and the sea urchin *Sterechinus neumayeri* (Whiteley et al., 1996; Marsh et al., 2001; Robertson et al., 2001b). Earlier studies have examined protein synthesis rates in a range of fish species (Smith and Haschemeyer, 1980; Haschemeyer, 1983). Evidence so far suggests that protein synthesis rates in polar species are similar to those in temperate species. All of these studies have concentrated on answering specific physiological questions relating to protein synthesis,

but none has examined seasonal changes in protein synthesis related to the highly variable Antarctic environment (Clarke, 1983).

Several studies have examined various aspects of the seasonal physiology and ecology of Antarctic marine invertebrates, including feeding (Barnes and Clarke, 1994; Brêthes et al., 1994), oxygen consumption and nitrogen excretion (Brockington and Clarke, 2001; Brockington and Peck, 2001), reproduction (Picken, 1980; Kim, 2001) and growth (Barnes, 1995; Peck et al., 1997). In all cases, a strongly seasonal pattern was evident. The primary aims of the current study were to rigorously validate the flooding dose methodology for measurements of protein synthesis at sub-zero water temperatures and to answer the following questions using the Antarctic limpet *Nacella concinna* as a model species: (1) does protein synthesis vary seasonally; (2) do tissue RNA concentrations and RNA:protein ratios change with season and do they show temperature adaptation; and (3) does *N. concinna* allocate a similar proportion of its metabolic budget to protein synthesis as do temperate and tropical species?

Materials and methods

Sampling

Experimental work was carried out at Rothera Research Station (67°34′07″S, 68°07′30″W), Adelaide Island, Antarctic Peninsula between February and December 1999. *Nacella concinna* Strebel 1908 were collected in February, July, October and December by SCUBA divers from South Cove, Rothera Point at depths between 6 m and 15 m (for details of the site, see Brockington and Peck, 2001). Immediately after collection, limpets were returned to the laboratory and placed in a through-flow aquarium under a simulated local light:dark cycle. Predicted sunrise and sunset time (POLTIPS 3, Proudman Oceanographic Laboratory, Birkenhead, UK) were used in conjunction with a mechanical timer to control the lighting regime. Aquarium mean water temperatures varied daily between 0.48°C and 0.68°C in February, –1.31°C and –1.40°C in July, –1.03°C and –1.07°C in October, and –0.28°C and –0.43°C in December. Animals were maintained in the aquarium for a maximum of 24 h prior to use in experiments. Collected limpets were not fed directly but were observed grazing biofilms on the sides of the tank.

Measurement of fractional protein synthesis rates, tissue RNA concentrations, RNA:protein ratios and RNA translational efficiencies

Limpet fractional protein synthesis rates were measured using a modification of the flooding dose technique (Garlick et al., 1980; Houlihan et al., 1986). The length of each animal was measured to the nearest 0.05 mm using vernier calipers. After surface drying with paper tissues, the animal (including shell) was weighed to the nearest mg. Each limpet was then individually labelled with a plastic number glued to the shell using cyanoacrylate adhesive. A preliminary sample of limpets was dissected to establish the relationship between flesh mass, y (mass excluding shell) and total mass, x (y=0.84x–0.14, r^2=99.2%, P<0.001, N=88). This scaling relationship was used to estimate the flesh mass of the experimental animals used in protein synthesis measurements and, hence, to adjust the isotope dose. A preliminary group of limpets was injected in the pedal sinus with a solution of Alcian blue (Sigma, Poole, UK) and dissected to examine whether the injectate was successfully delivered to the pedal sinus. In all cases, the injections were successful in delivering the injectate to the pedal sinus. Each experimental animal was injected in the pedal sinus with a solution containing unlabelled and ^{3}H-labelled phenylalanine [10 μl g^{-1} flesh mass of 135 mmol l^{-1} L-[2,6-^{3}H] phenylalanine at 3.6 MBq ml^{-1} (100 μCi ml^{-1}); Amersham, Little Chalfont, UK]. After injection, limpets were placed in a beaker containing 4 l of seawater, maintained at a temperature of –1.1±0.6°C. After 1 h, 2 h and 4 h, six or seven limpets were removed from the beaker, had their shells removed, and the flesh mass weighed and homogenised (X120 Status homogeniser) in a known volume of ice-cold 0.2 mol l^{-1} perchloric acid (PCA, 2 ml per 100 mg flesh mass). Homogenised limpets were stored at 4°C prior to analysis. A group of ten non-injected limpets were analysed to measure baseline phenylalanine concentrations and to allow calculation of phenylalanine flooding levels.

Fractional protein synthesis and RNA content: sample analysis

PCA samples containing individual homogenised limpets were mixed and a 2 ml sub-sample removed for further analysis. The sub-sample was centrifuged (Hermle Z 323 K centrifuge, 3500 ***g***, 20 min, 4°C, fixed rotor) to separate the precipitated protein pellet, RNA and DNA from the intracellular free-pool (Houlihan et al., 1995a). The amount of NaOH-soluble protein in the protein pellet was measured (Lowry et al., 1951) using bovine serum albumin as the standard. Total RNA was measured by comparing the sample concentrations to known RNA standard (Type IV, calf liver, Sigma) concentrations determined spectrophotomically at 665 nm after reaction with an acidified orcinol reagent (Mejbaum, 1939). The protein pellet was washed twice in 0.2 mol l^{-1} PCA before being hydrolysed in 6 mol l^{-1} HCl for 18 h. Phenylalanine concentrations in the intracellular free-pool, hydrolysed protein pellet and injection solution were measured using a flourometric assay, after the enzymatic conversion of the phenylalanine to β-phenylethylamine (PEA); these procedures are described in detail by Houlihan et al. (1995a). Known phenylalanine standards were also enzymatically converted to PEA to assess the conversion efficiency. Specific radioactivities of the intracellular free-pools, protein pellet and injection solution were measured using scintillation counting (^{3}H counting efficiency 34%, Hionic Fluor scintillation fluid, LKB-Wallac Rack Beta scintillation counter). Intracellular free-pool, protein and injection

solution specific radioactivities were expressed as d.p.m. $nmol^{-1}$ phenylalanine. Whole-animal fractional protein synthesis rates (expressed as a percentage of the protein mass synthesised per day) were calculated using the following equation (Garlick et al., 1980):

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

where k_s = % protein mass synthesized day^{-1}, S_b = specific radioactivity of protein-incorporated radiolabel (d.p.m. $nmol^{-1}$), S_a = specific radioactivity of intracellular free-pool (d.p.m. $nmol^{-1}$), t = incorporation time from injection to death (min) and 1440 represents the number of minutes in a day. The fractional protein synthesis equation above requires the intracellular free-pool-specific radioactivities to be increased and stable throughout the time period during which protein synthesis is measured.

The absolute rates of protein synthesis (A_s) were calculated using the following equation:

$$A_s = \frac{k_s}{100} \times (\text{protein mass}) , \quad (2)$$

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

Calculation of RNA concentrations, RNA:protein ratios and RNA translational efficiencies

Tissue RNA concentrations were expressed as μg RNA mg^{-1} fresh mass, and RNA:protein ratios (μg RNA mg^{-1} protein). The translational efficiency of the RNA (k_{RNA}; expressed as mg protein mg^{-1} RNA day^{-1}) was calculated using the following equation (Preedy et al., 1988):

$$k_{RNA} = \frac{10 \times k_s}{\text{RNA:protein ratio}} . \quad (3)$$

Measurement of growth rates

12 months after protein synthesis rates were measured in *N. concinna*, *in situ* growth rates were measured. During December, limpets were collected from the same site used to collect animals for the protein synthesis measurements and were returned to the aquarium. The animals were gently dried with a paper tissue, and the mass of each limpet was measured to the nearest mg. Each animal was numbered on the shell using enamel paint (Humbrol, Hull, UK). Marked animals were returned to the site of capture within 48 h. After 64 days, as many marked limpets as possible were collected and reweighed. Wet specific-growth rates (SGR, expressed as % body $mass^{-1}$) were calculated for each individual using the following equation (Ricker, 1979):

$$\text{SGR} = \frac{\ln(W_2) - \ln(W_1)}{t} \times 100 , \quad (4)$$

where W_2 and W_1 represent the mass at the end and start of the growth period, respectively, and t is the time in days. Growth measurements were repeated in winter on a second group of limpets collected in June. Growth was measured as previously described after a 96-day growth period.

Statistical analysis

All data are expressed as means ± S.E.M. Data were tested for normality prior to statistical testing using the Anderson–Darling test (Sokal and Rohlf, 1995). If data were normally distributed, they were analysed using analysis of variance (ANOVA); otherwise, the non-parametric Kruskal–Wallis test was used.

Results

Validation of flooding dose methodology

There was no significant difference between the body masses (Kruskal–Wallis, P=0.312, H=3.56, d.f.=3) or shell lengths (Kruskal–Wallis, P=0.964, H=0.28, d.f.=3) of limpets used to measure protein synthesis at the seasonal sampling points of February, July, October or December. The overall mean body masses and shell lengths of limpets used in the four seasonal experiments were 2.05±0.66 g and 25.21±0.24 mm, respectively.

The successful application of the flooding dose technique to measure fractional protein synthesis rates requires several criteria to be met, namely that the intracellular free-pool specific radioactivities increase rapidly and are stable over the course of the protein synthesis measurement, the intracellular free-pools are flooded by the injected unlabelled phenylalanine and, finally, the increase in protein radiolabelling with time is significant and linear. In our studies, the intracellular free-pool specific radioactivities increased rapidly after the flooding dose injection at all seasonal sampling points (Fig. 1). There were no significant differences in intracellular free-pool specific radioactivities over time up to 4.5 h after injection at any seasonal sampling point (Kruskal–Wallis, all P>0.05), indicating that free-pools had increased rapidly and were stable during the time course of measurement.

The second criteria of the flooding dose technique is that the intracellular free-pools are completely flooded by the unlabelled phenylalanine. Injection of the limpets resulted in a 4.4-fold increase in whole body phenylalanine concentrations above the baseline (0.34 nmol mg^{-1} fresh mass). After the flooding dose injection of 1.35 nmol mg^{-1} fresh mass, phenylalanine concentrations should have increased to 1.69 nmol mg^{-1}. At all sampling points, phenylalanine concentrations increased to 87–100% of the theoretical post-injection concentration (February = 1.48 nmol mg^{-1}; July = 1.70 nmol mg^{-1}, October = 1.58 nmol mg^{-1}, December = 1.54 nmol mg^{-1}). The close agreement between the predicted and actual phenylalanine concentrations after injection suggests that the injected phenylalanine had equilibrated within the limpet tissues.

Intracellular free-pool specific radioactivities were pooled within each sampling point and compared with the injection solution specific radioactivity. The overall mean intracellular

free-pool specific radioactivity for all sampling points (1013±30 d.p.m. nmol^{-1} phenylalanine) was significantly lower than the injection solution specific radioactivity (1622±43 d.p.m. nmol^{-1} phenylalanine), owing to dilution by tissue baseline phenylalanine concentrations. If the total tissue phenylalanine concentrations were increased 4.4-fold after the flooding dose injection, then the specific radioactivity of the intracellular free-pool would be expected to be approximately 25% lower than the injection solution specific radioactivity. In fact, the mean free-pool specific radioactivity was 37% lower than the injection solution. The mean free-pool specific radioactivity was probably slightly lower than predicted due to

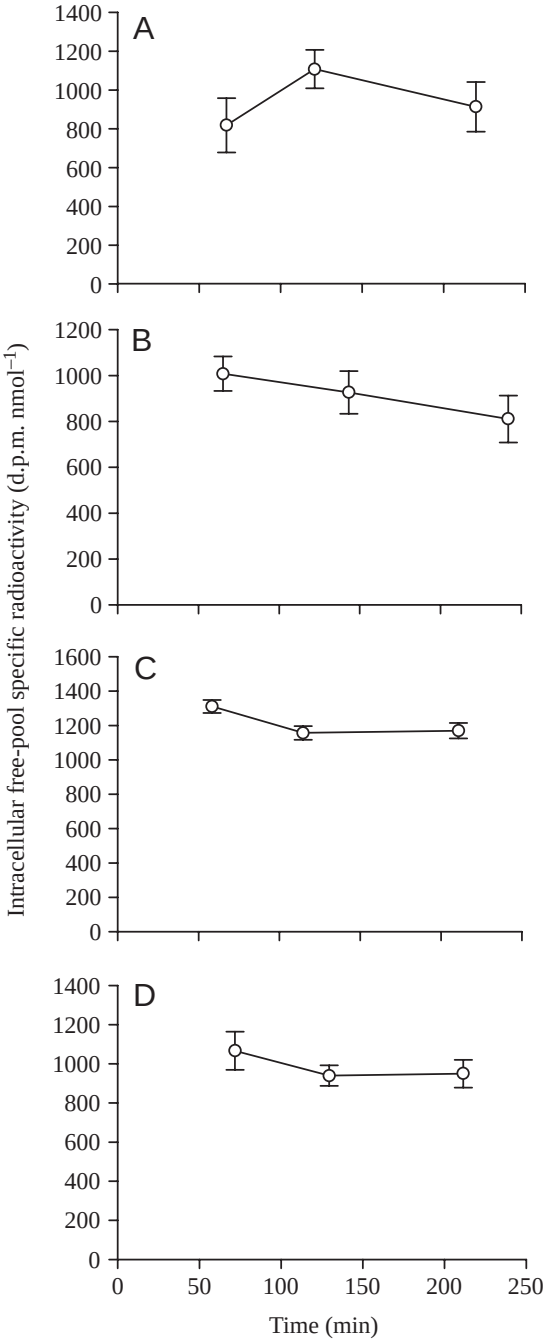

Fig. 1. Intracellular free-pool phenylalanine specific radioactivities in *Nacella concinna* at Rothera Point, Antarctica in (A) February, (B) July, (C) October and (D) December. Measurements were made after flooding dose injections at time zero and are expressed as d.p.m. nmol^{-1} phenylalanine. All data points are means ± S.E.M. N=6, except for the last two data points of (B), (C) and (D), in which N=7.

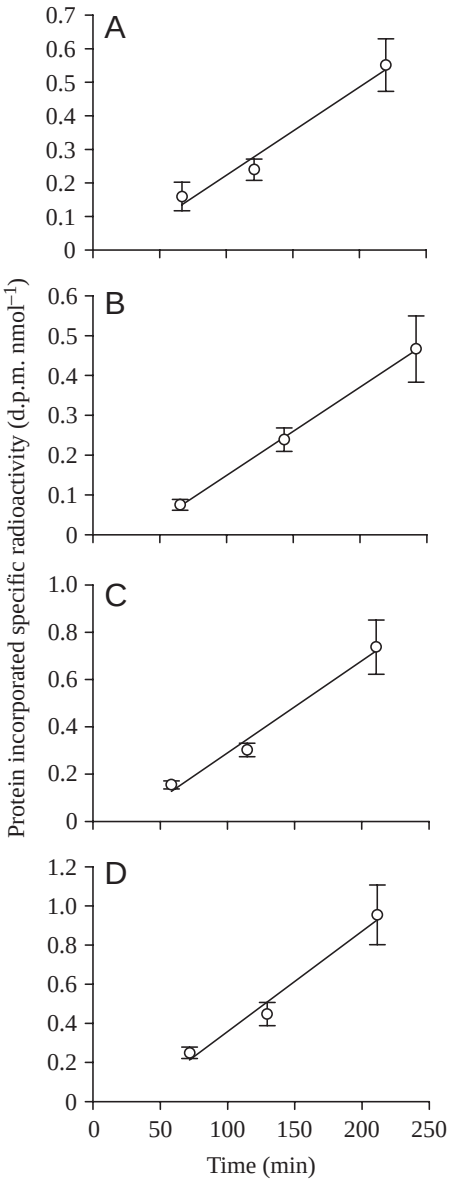

Fig. 2. Incorporation of radiolabelled phenylalanine into body protein of *Nacella concinna* at Rothera Point, Antarctica, after flooding dose injections at time zero. Data are expressed as d.p.m. nmol^{-1} phenylalanine. (A) February (y=0.0026x–0.0346, r^2=0.58); (B) July (y=0.0022x–0.0747, r^2=0.59); (C) October (y=0.004x–0.1147, r^2=0.67); and (D) December (y=0.0054x–0.1986, r^2=0.63), where y is the protein-incorporated specific radioactivity expressed as d.p.m. nmol^{-1}, and x is the time in min. Data points are means ± S.E.M. N=6, except for the last two data points of (B), (C) and (D), in which N=7. All regression slopes are highly significant (P<0.001), with intercepts not significantly different from zero.

some loss of the injection solution from the injection site after injection. The fact that both unlabelled and radiolabelled phenylalanine concentrations in the animal approximate to predicted levels after injection suggest that the intracellular free-pools have successfully flooded with phenylalanine; thus, the second criteria of the flooding dose technique is satisfied.

Regression analysis demonstrated a significant linear increase in incorporated specific radioactivity with time for all seasonal sampling points (Fig. 2). Second- and third-order regression models were also fitted to the protein radiolabelling data but these did not significantly improve the residual mean squares in any data set; the linear model was therefore used. At all seasonal sampling points, the intercepts of regression equations describing the incorporation of radiolabelled phenylalanine into protein were not significantly different from zero, suggesting that the radiolabel rapidly equilibrated with the intracellular free-pool and that incorporation of the radiolabel occurred rapidly after injection. The current work thus fulfils all the requirements of the flooding dose technique; the specific radioactivities of the intracellular free-pools increased and were stable, the intracellular free-pools were flooded with phenylalanine and, lastly, there was significant, linear incorporation of the radiolabelled amino acid into proteins. Protein synthesis rates were calculated for each animal using the animal's individual intracellular free-pool and radiolabelled protein specific radioactivity. A mean protein synthesis rate was calculated for each seasonal sampling point using the protein synthesis rate measurements for each individual animal at all three labelling time points.

Fractional protein synthesis rates, tissue RNA concentrations, RNA:protein ratios and RNA translational efficiencies

Fractional protein synthesis rates during the austral summer in December were significantly higher than during the other sampling points in February, July and October (Table 1, Kruskal–Wallis, $P<0.05$, $H=25.33$, d.f.=3). The highest rate of fractional protein synthesis (December) was twofold higher than the lowest protein synthesis rate (July).

Tissue RNA concentrations also showed a similar seasonal pattern, with maximum values in summer and minimum values in winter (Table 1). Peak RNA concentrations in February were significantly higher than at all other sampling points. The RNA concentration in July was significantly lower than in October and December [analysis of variance (ANOVA), $P<0.05$, $F=12.49$, d.f.=3].

The RNA:protein ratio also decreased significantly in winter (ANOVA, $P<0.05$, $F=40.01$, d.f.=3), with winter values falling to a third of summer values (Table 1). There was also a seasonal pattern in the absolute protein synthesis rates, with the highest rates in the austral summer, although the high variance in these measurements means that the differences were not significant (Kruskal–Wallis, $P=0.147$, $H=5.36$, d.f.=3). The seasonal pattern in RNA translational efficiencies was far less clear; the RNA translational efficiency measured in February was significantly lower (Kruskal–Wallis, $P<0.05$, $H=16.28$, d.f.=3) than in July and December but, overall, no clear seasonal pattern was evident (Table 1). In summary, this study has revealed a clear seasonal pattern in fractional protein synthesis and RNA concentration and a less-distinct seasonality in RNA translational efficiency and absolute protein synthesis.

Growth rates

During summer, the marked limpets increased in mass from 2.07±0.15 g to 2.30±0.16 g ($N=31$) with a mean SGR of 0.07±0.01% body mass day^{-1}. In winter, the marked animals decreased from an initial mass of 2.42±0.01 g to a final mass of 2.35±0.01 g ($N=44$), with a mean SGR of –0.01±0.01% body mass day^{-1}, giving a clear seasonal difference in growth rate (Student's t-test, $P<0.001$, $t=5.93$, d.f.=39).

Discussion

Validation of flooding dose methodology

When examining a new study-organism, particularly in an environment such as the polar regions where relatively few studies have been undertaken, it is important to establish the validity of the technique being used. As previously discussed, the current work fulfilled the criteria of the flooding dose methodology in that Fig. 1. demonstrates that intracellular free-pool specific radioactivities increased and were stable over the time period in which protein synthesis was measured, the predicted unlabelled and radiolabelled phenylalanine concentrations after injection were very close to measured concentrations and, finally, there was a significant linear incorporation of the radiolabelled phenylalanine into tissue

Table 1. *Seasonal protein synthesis, RNA concentration, RNA:protein ratio and translational efficiency in* Nacella concinna

	Fractional protein synthesis (k_s) (% day^{-1})	RNA concentration (μg RNA mg^{-1} fresh mass)	RNA:protein ratio (μg RNA mg^{-1} protein)	RNA translational efficiency (k_{RNA}) (mg protein mg^{-1} RNA day^{-1})	Absolute protein synthesis (A_s) (mg protein animal^{-1} day^{-1})
February	0.40±0.06^{a}	5.57±0.55^{a,b,c}	53.71±4.47^{a,b,c}	0.09±0.02^{a,b}	0.71±0.13
July	0.27±0.03^{b}	2.55±0.17^{a,d,e}	17.42±1.26^{a,d,e}	0.17±0.02^{a}	0.53±0.05
October	0.35±0.03^{c}	4.30±0.29^{b,d}	29.67±1.16^{b,d}	0.12±0.01	0.62±0.05
December	0.56±0.04^{a,b,c}	3.79±0.28^{c,e}	35.68±2.70^{c,e}	0.18±0.02^{b}	0.81±0.08

All data are means ± S.E.M. In February, $N=19$; in July, October and December, $N=20$.
Values with the same superscript letter are significantly different ($P<0.05$).

protein (Fig. 2). In whole-animal studies, it is also important that the injected radiolabel equilibrates with the peripheral organs and does not simply remain at the injection site. Individual organs in *N. concinna* are too small to allow the measurement of organ protein synthesis rates; however, previous whole-body flooding dose studies have shown that individual organs successfully equilibrated with the injected radiolabelled amino acid (Fauconneau and Arnal, 1985; Hewitt, 1992; Houlihan et al., 1994). Other studies have also shown that the individual organs of an Antarctic holothurian species successfully equilibrated with radiolabelled phenylalanine after an intraperitoneal flooding dose injection at the same water temperature as the current study (K. P. P. Fraser, A. Clarke and L. S. Peck, unpublished data). Therefore, it is highly likely that the peripheral tissues will have equilibrated successfully after the flooding dose injection in *N. concinna*.

Fractional protein synthesis rates

Seasonal variability in fractional protein synthesis rate (k_s) has previously been measured in only one other marine invertebrate: the temperate bivalve *Mytilus edulis*. Hawkins (1985) reported k_s in fed *M. edulis* of 0.29% day^{-1}, 0.40% day^{-1} and 0.38% day^{-1} in March, June and October, respectively. Seasonal differences in protein synthesis rates were also evident in *N. concinna* in the current study. It is likely that seasonal variation in food consumption is the main factor driving seasonal changes in *N. concinna* protein synthesis. Faecal egestion data, collected simultaneously with the current work in a separate group of limpets, showed that food consumption in *N. concinna* decreased by nearly an order of magnitude during winter, while the experimental water temperature in which the animals were maintained only varied by approximately 2°C from summer to winter (Fraser et al., 2002). *N.* concinna faecal egestion rates were 11.92±0.76 mg dry faeces day^{-1}, 1.41±0.58 mg dry faeces day^{-1}, 7.35±0.84 mg dry faeces day^{-1} and 10.75±1.07 mg dry faeces day^{-1} in February, July, October and December, respectively (Fraser et al., 2002). A long-term reduction in food consumption has previously been shown to decrease protein synthesis rates and tissue RNA concentrations in several species (McNurlan et al., 1979; Foster et al., 1993; Arndt et al., 1996). Although protein synthesis rates in *N. concinna* and *M. edulis* were measured at water temperatures differing by a minimum of 8°C, the protein synthesis rates were very similar (Hawkins et al., 1983; Hawkins, 1985; Table 1).

The only other comparable protein synthesis rates in molluscs are those measured in *Octopus vulgaris* at 22°C by Houlihan et al. (1990). The authors reported whole-body protein synthesis rates ranging from 2% day^{-1} to 7% day^{-1}, which are considerably higher than those in *N. concinna*, even allowing for differences in water temperature. Cephalopods are, however, notable for being semelparous and having rapid somatic growth, so direct comparison is difficult (O'Dor and Wells, 1987). Larval cephalopod RNA:DNA ratios are substantially higher than in juvenile fish, again suggesting protein synthesis rates might be unusually high in cephalopods (Clarke et al., 1989).

Whole body k_s has only been measured previously in the adult stage of one Antarctic species, *G. antarcticus*, for which Whiteley et al. (1996) reported a k_s of 0.24±0.04% day^{-1} in fed animals held at 0°C, while Robertson et al. (2001b) reported k_s values ranging between 0.16% day^{-1} and 0.38% day^{-1} in starved and fed animals at the same temperature. These results are well within the range of those measured in *N. concinna* and are very similar to those measured in *M. edulis* (Hawkins et al., 1985). Protein synthesis rates in Antarctic and temperate sea urchin embryos are also very similar (Marsh et al., 2001). It therefore appears that, despite the low water temperature and highly seasonal food supply typical of Antarctic waters, marine invertebrates are able to maintain fractional protein synthesis rates that are comparable with related temperate species.

Previous workers have suggested that liver and white-muscle protein synthesis rates in some species of Antarctic fish show a degree of temperature adaptation (Smith and Haschemeyer, 1980; Haschemeyer, 1983). *Trematomus hansoni*, *Trematomus bernacchii*, *Trematomus newnesi* and *Gymnodraco acuticeps* have protein synthesis rates approximately two times higher than would be predicted from extrapolation of temperate fish protein synthesis rates to polar water temperatures (Smith and Haschemeyer, 1980). However, caution does need to be exercised in interpreting these results, as predicted protein synthesis rates are simply calculated using estimated Q_{10} (2.5) values and do not consider ecological or scaling factors. Laboratory-based studies have shown temperate fish increasing their tissue RNA:protein ratios and decreasing their k_{RNA} in some tissues as water temperatures decrease (Foster et al., 1992). After juvenile cod were acclimated to 5°C or 15°C for 40 days, there was no significant difference in protein synthesis rates, suggesting that intra-specific protein synthesis rates are maintained at some 'optimum' level by altering RNA:protein ratios to counter temperature-induced changes in k_{RNA} (Foster et al., 1992).

RNA concentration, RNA:protein ratios and RNA translational efficiencies

There was a clear seasonal variation in both RNA concentrations and RNA:protein ratios, closely following changes in k_s (Table 1). Seasonal changes in tissue RNA concentrations and RNA:DNA ratios have been demonstrated in several species (Bulow et al., 1981; Robbins et al., 1990; Kent et al., 1992), and food consumption has been shown to influence tissue RNA concentration, which, in turn, has a large effect on k_s (Millward et al., 1973). As food consumption rates change, initial alterations in protein synthesis rates are controlled by modification of k_{RNA}, while changes in RNA concentration occur over longer time periods (Millward et al., 1973). RNA:protein ratios measured in *N. concinna* appear high in comparison with temperate species, while k_{RNA} values seem low.

Animals studied in the current work had effectively undergone a natural acclimatisation to seasonal environmental

conditions. Therefore, RNA:protein ratios were likely to have reached optimum levels for variables such as the rate of food consumption and water temperature. The lack of any consistent seasonal pattern in k_{RNA} in the current work is therefore perhaps not surprising. The significant differences in k_{RNA} that do exist probably reflect short-term nutritional variations.

Inter-specific comparison of the effect of temperature on RNA:protein ratios and RNA translational efficiencies

Comparisons of inter-specific temperature-induced changes in RNA:protein ratios and k_{RNA} are complicated by mass-specific changes in RNA:protein ratios (Goldspink and Kelly, 1984; Houlihan et al., 1995a; Tesseraud et al., 1996). To allow valid inter-specific comparisons of RNA:protein ratios, suitable data were compiled from the literature and standardised to the mean body mass of all the animals used in the analysis using a scaling coefficient of –0.24 (Fig. 3). The scaling coefficient was calculated by least-squares regression analysis of log-transformed body mass and RNA:protein data for the complete data set. Data were used only if the animals in the studies were fed and experiments were conducted at temperatures within the environmental range encountered by the species. There was a significant inverse relationship between RNA:protein ratios and temperature across a wide range of species (Fig. 3). It should be noted that the r^2 value for the relationship is low, only explaining 20% of the variation in RNA:protein ratios. Much of the unexplained variation in the relationship is probably due to nutritional differences between the studies (Millward et al., 1973). It is probable that a much stronger relationship could be demonstrated in animals maintained at differing temperatures but under the same nutritional regimes. The rat data of Goldspink and Kelly (1984) were not included within the data set used to fit the regression line, as the rat was the only non-ectotherm included in Fig. 3. However, the rat data do clearly lie very near to the regression line fitted to the ectotherm data.

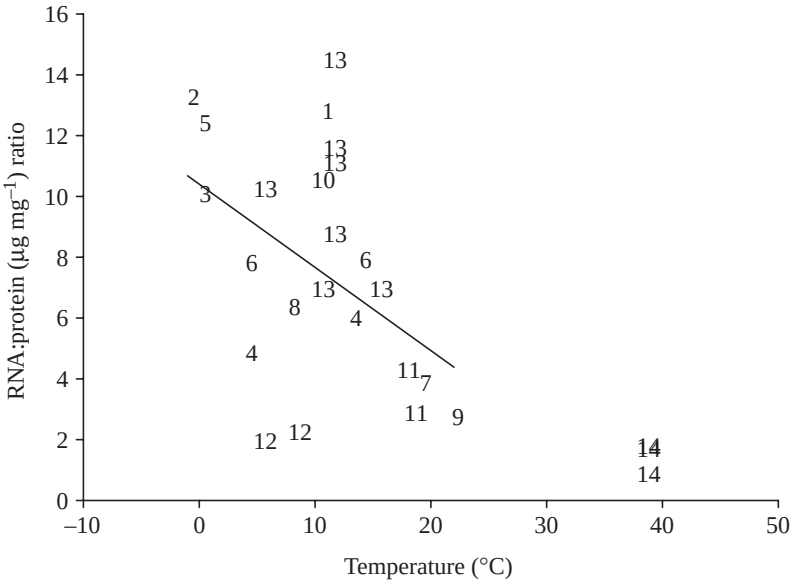

Fig 3. Mass-standardised whole-body RNA:protein ratios plotted against temperature. All values were standardised to a mean body mass of 129 g, the mean body mass of all the animals in the data set, using a scaling coefficient of –0.24. The scaling coefficient was calculated by fitting a least-squares regression model to the natural-log-transformed body mass and RNA:protein ratio data for all species. The regression line relating temperature and RNA:protein ratio was fitted by least-squares regression analysis (y=10.4–0.274x, r^2=0.20, P<0.05) to all data with the exception of points labeled 14 (rat). For sources of plotted data, see Table 2.

Table 2. *Source of data and animal species used to plot Fig. 3*

Data point label	Species	Author
1	*Limanda limanda* (teleost)	Houlihan et al., 1994
2	*Nacella concinna* (gastropod)	This paper
3	*Glyptonotus antarcticus* (isopod)	Robertson et al., 2001b
4	*Saduria entomon* (isopod)	Robertson et al., 2001a
5	*Glyptonotus antarcticus* (isopod)	Whiteley et al., 1996
6	*Idotea rescata* (isopod)	Whiteley et al., 1996
7	*Homarus gammarus* (decapod)	Mente et al., 2001
8	*Oncorhynchus mykiss* (teleost)	McCarthy et al., 1994
9	*Ctenopharyngodon idella* (teleost)	Carter et al., 1993
10	*Gadus morhua* (teleost)	Lyndon et al., 1992
11	*Dicentrarchus labrax* (teleost)	Langar and Guillaume, 1994
12	*Clupea harengus* (teleost)	Raae et al., 1988
13	*Oncorhynchus mykiss* (teleost)	Mathers et al., 1993
14	*Rattus norvegicus* (mammal)	Goldspink and Kelly, 1984

There was no significant relationship between body mass and k_{RNA} in the overall data set (P=0.187, F=1.85, N=26), and k_{RNA} was therefore not standardised to a mean body mass. Whole-body k_{RNA} did however increase significantly with temperature (Fig. 4), although the r^2 was comparatively low. The k_{RNA} is very sensitive to the nutritional state of an animal, and variations in nutrition are likely to explain the low r^2 value (Millward et al., 1973).

The limited amount of suitable data in the literature meant that several species in these analyses (Fig. 3, 4) are represented by more than one data point. This can lead to a biased analysis, which could unduly influence conclusions through overestimation of the degrees of freedom. Both RNA:protein ratio and k_{RNA} data sets were therefore re-analyzed after reducing each species to a single data point by calculation of a species mean. There was still a significant relationship between RNA:protein ratio and temperature (y=14.0–0.455x, r^2=0.53, P<0.05, F=10.29, N=11). However, the relationship relating k_{RNA} and temperature just failed to reach significance

(r^2=0.34, P=0.076, F=4.16, N=10). It is likely that a significant relationship will be obtained when data from further studies are available.

The relationship of both RNA:protein ratio and k_{RNA} with temperature is therefore similar in conspecifics acclimated to different water temperatures (Foster et al., 1992) and interspecifically across a broad range of animal groups. This suggests strongly that ectotherms living at low temperatures maintain considerably elevated tissue RNA:protein ratios to counteract very low k_{RNA} (Figs 3, 4; Robertson et al., 2001b; Whiteley et al., 1996). The elevation of tissue RNA:protein ratios in Antarctic species is thus a clear evolutionary adaptation to living at low temperatures, allowing the continued synthesis of sufficient protein at temperatures approaching the lower thermal limits of RNA translation.

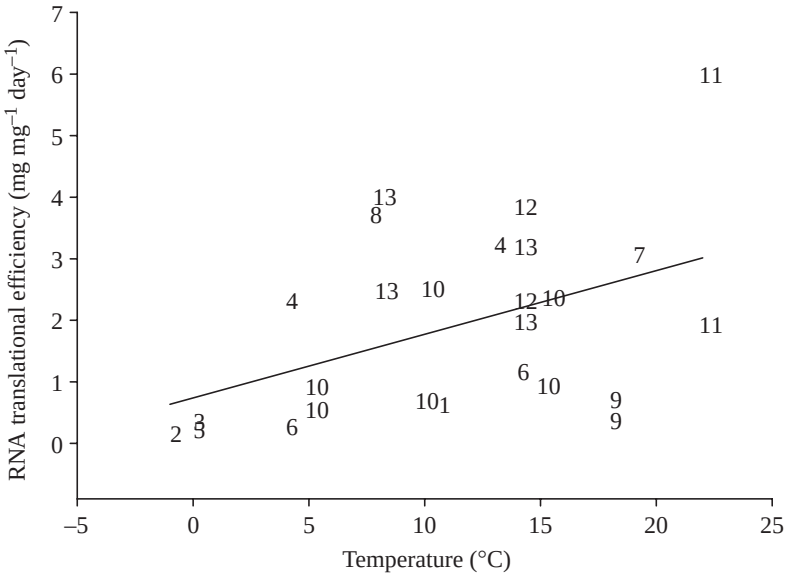

Fig 4. Whole-body RNA translational efficiencies (k_{RNA}) plotted against temperature. The plotted regression line was fitted by least-squares regression analysis (y=0.738+0.104x, r^2=0.20, P<0.05) to all data within the data set. For sources of plotted data, see Table 3.

Growth rates

Growth in Antarctic ectotherms is generally considered to be variable and reduced in comparison to temperate and tropical species with similar ecology and size (Barnes, 1995), although there are a few species that do demonstrate relatively high growth rates (Dayton et al., 1974; Dayton, 1989; Rauschert, 1991). Specific growth rates measured in the current work suggest that overall growth rates in *N. concinna* are also slow in summer but negative in winter. Previous authors have reported low shell growth rates in *N. concinna* in comparison with those in temperate and tropical limpets (Shabica, 1976). Interestingly, overall proportional growth rates (expressed as % body mass day^{-1}) in N. *concinna* are considerably lower than proportional protein synthesis rates (expressed as % protein mass synthesised day^{-1}), suggesting that protein growth rates are also likely to be low (unless very large changes occur in animal protein content). In turn, this suggests that protein degradation rates are considerable.

Table 3. *Source of data and animal species used to plot Fig. 4*

Data point label	Species	Author
1	*Limanda limanda* (teleost)	Houlihan et al., 1994
2	*Nacella concinna* (gastropod)	This paper
3	*Glyptonotus antarcticus* (isopod)	Robertson et al., 2001b
4	*Saduria entomon* (isopod)	Robertson et al., 2001a
5	*Glyptonotus antarcticus* (isopod)	Whiteley et al., 1996
6	*Idotea rescata* (isopod)	Whiteley et al., 1996
7	*Homarus gammarus* (decapod)	Mente et al., 2001
8	*Oncorhynchus mykiss* (teleost)	McCarthy et al., 1994
9	*Dicentrarchus labrax* (teleost)	Langar and Guillaume, 1994
10	*Oncorhynchus mykiss* (teleost)	Mathers et al., 1993
11	*Ctenopharyngodon idella* (teleost)	Carter et al., 1993
12	*Salmo salar (*teleost)	Houlihan et al., 1995c
13	*Oncorhynchus mykiss* (teleost)	Houlihan et al., 1995c

Estimated energetic cost of protein synthesis

Current estimates indicate that protein synthesis is energetically expensive and accounts for 25–42% of total oxygen consumption in a wide range of animal species (Houlihan et al., 1995a; Houlihan et al., 1995b). Two recent studies have reported widely differing energetic costs of protein synthesis in Antarctic marine invertebrates. Whiteley et al. (1996) calculated that protein synthesis following a satiating meal accounted for 66% of total post-prandial oxygen consumption in the giant isopod *G. antarcticus* and estimated an energetic cost of 148 mmol O_2 g^{-1} protein. In contrast, Marsh et al. (2001) reported that protein synthesis accounted for 1–53% of oxygen consumption in developing embryos of the Antarctic sea urchin *S. neumayeri* and estimated a cost of only 1 mmol O_2 g^{-1} protein (Marsh et al., 2001). This latter cost of protein synthesis in *S. neumayeri* embryos is lower than the minimum theoretical stoichiometric cost of peptide elongation by a factor of eight (Houlihan et al., 1995b; Marsh et al., 2001). It seems unlikely that this apparent difference of two orders of magnitude in the cost of protein synthesis between these two species can be real.

The reason for the widely differing costs of protein synthesis in these two Antarctic species remains to be resolved. Reported costs of protein synthesis in non-polar species also vary widely (reviewed in Houlihan et al., 1995b). The large variation in experimentally derived energetic costs of protein synthesis is probably largely due to differences in methodology and insufficient validation of techniques. For example, recent work has shown that high concentrations of cycloheximide may affect cellular processes other than protein synthesis, leading

Table 4. *The estimated percentage of oxygen consumption used for protein synthesis in* Nacella concinna

Month	Oxygen consumption (mmol day^{-1})	Absolute protein synthesis (mg day^{-1})	Percentage of oxygen consumption utilised for protein synthesis
February	16.99	0.712	34
July	11.81	0.533	36
October	13.97	0.618	35
December	16.03	0.806	40
Mean	–	–	36

Energetic costs of protein synthesis were calculated using the theoretical minimum cost of protein synthesis (8.0 mmol g^{-1}; Houlihan et al., 1995b), expressed as mmol O_2 g^{-1} protein. Oxygen consumption data was taken from Fraser et al. (2002).

to an overestimate of protein synthesis costs (Fuery et al., 1998; Wieser and Krumschnabel, 2001).

The proportion of oxygen consumption used for protein synthesis in the current work (34–40%) was estimated using the minimal stoichiometric cost of protein synthesis (Table 4, Houlihan et al., 1995b). This technique provides a minimum cost of protein synthesis, based solely on the cost of peptide-bond formation. The *N. concinna* oxygen consumption data used to make the calculations were from measurements made at the same time as the protein synthesis measurements but in a separate group of animals (Fraser et al., 2002). The proportion of oxygen consumption used for protein synthesis in *N. concinna* is fairly consistent throughout the season and falls at the upper extreme range of estimates made using the same theoretical cost of protein synthesis in a range of temperate and tropical fish species (mean 26%; range 11–42%) (Table 4; Carter and Houlihan, 2001). Measurements of the proportion of oxygen consumption used for protein synthesis by Marsh et al. (2001) and Whiteley et al. (1996) and from our calculated value suggest that the proportion of the energetic budget dedicated to protein synthesis in polar species might be higher than in temperate or tropical species. Further experimental work is required to clarify the absolute costs of protein synthesis in polar species and the proportion of the metabolic budget allocated to the synthesis of proteins. If polar species use a higher proportion of their metabolic budget for protein synthesis and for maintaining considerably elevated tissue RNA concentrations, the overall costs of growth may be considerably higher than in temperate and tropical species. In turn, the energy remaining that is available for other processes, such as ion transport, would be greatly reduced.

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