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Temperature-dependent protein synthesis capacities in Antarctic and temperate (North Sea) fish (Zoarcidae)

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Summary

For an evaluation of effects of seasonal cold acclimation and evolutionary cold adaptation on protein synthesis capacity, the protein synthesis apparatus was isolated from the gills and white muscle of Antarctic eelpout *Pachycara brachycephalum* and North Sea eelpout *Zoarces viviparus*. Both species had been acclimated to 0°C (control) and 5°C (Antarctic) and 5°C and 10°C (North Sea control). The translational capacities of the protein synthesis machineries were determined in an optimised cell-free *in vitro* system. The results demonstrate that tissues from the polar zoarcid possess cold-adapted protein synthesis machineries, indicated by low activation energies and, especially, high RNA translational capacities at similar RNA:protein ratios when compared to temperate zoarcids at 10°C. When both species were brought to 5°C, the temperate species displayed cold compensated protein synthesis capacities caused by elevated RNA:protein ratios. Warm exposure (from 0 to 5°C) of the Antarctic zoarcid revealed a capacity for thermal acclimation indicated by a reduction in protein synthesis capacities associated with lower RNA:protein ratios.

Key words: RNA translational capacity, RNA:protein ratio, *Zoarces viviparus*, *Pachycara brachycephalum*, cold acclimation, cold adaptation.

Introduction

It is commonly accepted that polar marine invertebrates and fish have slow annual growth rates compared to animals from lower latitudes. The first intuitively appealing explanation is that temperature slows down physiological rates in the cold, but this explanation ignores the possibility that over the course of evolution, polar species may have achieved some degree of compensation for the rate-limiting effects of low temperature (Clarke and North, 1991). Recent evidence demonstrates, in fact, that cold-adapted invertebrates and fish of the Southern Ocean reach growth rates during the short Antarctic summer similar to those of their counterparts from temperate regions (Brey and Clarke, 1993; Arntz et al., 1994; Kock and Everson, 1998; Peck, 2002). More recently, interest has focused on protein synthesis in polar species because growth and cellular functioning in tissues are closely related to protein turnover. These studies provided evidence that whole body protein synthesis rates *in vivo* (Whiteley et al., 1996; Robertson et al., 2001; Marsh et al., 2001; Fraser et al., 2002) are similar to those observed in temperate species.

Enabling of the protein synthesis machinery to function at very low 'operating temperatures' *in vivo* has been suggested to be brought about by elevated tissue RNA:protein ratios (milligrams RNA per gram protein). Accordingly, this parameter is commonly used as an indirect measure of the *in vivo* protein synthesis capacity of a tissue (Waterlow et al., 1978; Sugden and Fuller, 1991; Houlihan, 1991). Increased RNA:protein ratios have been found upon cold acclimation or during winter in various tissues of fish, and for the last two decades this finding has been interpreted to reflect cold compensation of RNA translational activities ($k_{\mathrm{RNA\ in\ vivo}}$, defined as grams protein synthesized *in vivo* per gram RNA per day, also known as RNA translational efficiency), at low temperatures (Goolish et al., 1984; Foster et al., 1992; Foster et al., 1993; Mathers et al., 1993; McCarthy and Houlihan, 1997; McCarthy et al., 1999). Accordingly, it seems very tempting to extrapolate this interpretation to cold-adapted stenotherms from polar regions. The increase in RNA:protein ratios in Antarctic species reflected by increased RNA levels in cold stenotherms from the Southern Ocean (Whiteley et al., 2001; Robertson et al., 2001; Marsh et al., 2001; Fraser et al., 2002) has in fact been suggested to counteract a thermally induced reduction in RNA translational efficiency *in vivo*.

These interpretations imply that *in vivo* translational efficiency falls in the cold because of a reduction in individual biochemical processes involved in protein synthesis. Such a decrement has never been demonstrated. Synthesis and maintenance of higher RNA levels to counteract the negative effect of cold temperatures on translational activity may further imply higher costs of protein synthesis in the cold. Fraser et al. (2002) proposed that maintaining considerably elevated tissue

RNA concentrations causes high metabolic costs of growth in the cold compared to temperate and tropical species. However, increased RNA stability may also lead to enhanced RNA levels at no extra costs and, in the light of energy savings commonly observed in Antarctic species, the protein synthesis machinery should rather be energy efficient with a cold adapted RNA translation apparatus that operates at enhanced catalytic efficiency. In fact, similar *in vitro* protein synthesis capacities in gills of the Antarctic scallop *Adamussium colbecki* measured at 0°C and in the temperate scallop *Aequipecten opercularis* measured at 25°C, were explainable by the ninefold higher and, thus, cold-compensated RNA translational capacity ($k_{\mathrm{RNA\ in\ vitro}}$, defined as grams protein synthesized *in vitro* per gram RNA per day) on top of a twofold elevated RNA content (Storch et al., 2003) at similar energetic costs (Storch and Pörtner, 2003). Elevated RNA contents in cold adapted ectotherms may therefore be the result of enhanced RNA stability resulting from low RNA turnover rates, and may not reflect enhanced energy costs. Elevated levels of mRNA and protein synthesis sites, in turn, would support short diffusion pathways for newly synthesized proteins to their final usage sites.

In the present study we tested this hypothesis in two confamilial fish species adapted to different temperature regimes, the Antarctic eelpout *P. brachycephalum* and the temperate eelpout *Z. viviparus*, which are both members of the cosmopolitan fish family Zoarcidae. We determined the translational capacity of the protein synthesis machineries using an *in vitro* cell-free system isolated from gills and white muscle. The *in vitro* amino acid incorporation system is a far more sensitive and immediate indicator of RNA translational capacity than the *in vivo* RNA:protein ratio (Lied et al., 1985; Houlihan, 1991). It has the inherent advantage that both the actual protein synthesis capacities of the tissue (expressed as mg protein synthesized *in vitro* per mg fresh mass per day) and, considering the RNA concentration within the tissue, the translational capacities of the RNA ($k_{\mathrm{RNA\ in\ vitro}}$) can be quantified accurately under optimized physiological conditions, i.e. at unrestricted energy and amino acid supply in the assay (Storch and Pörtner, 2003).

To our knowledge, this is the first such comparison of confamilial fish species from polar as well as temperate waters and should allow access to potential differences between cold acclimation effects and features of evolutionary cold adaptation to Antarctic conditions. If a capacity for thermal acclimation still exists in polar fishes, such an analysis should also reveal to what extent thermal acclimation may occur differently in polar compared to temperate fish. Based on the findings in the pectinids we hypothesize that the protein synthesis apparatus may be permanently cold adapted in the stenotherms, linked to enhanced RNA translational capacities in excess of effects of higher RNA:protein ratios.

Materials and methods

Animals

Antarctic eelpout *Pachycara brachycephalum* (Pappenheim), (total length: 20±4 cm, 34±11 g) were caught near King George Island (62°11.2′S, 58°20.7′W), Antarctica, by a fish trap at a depth of 397–455 m in 1998. Animals were returned to the Alfred Wegener Institute (AWI), Bremerhaven, Germany, by the research vessel ‘MS Polarstern’ and were then kept in well-aerated, re-circulating seawater at 0.0±0.5°C and 34±1 practical salinity units (PSU). Common eelpout *Zoarces viviparus* (Linné) (total length: 22±2 cm, 43±14 g) were collected from 5 m water depth around the German offshore island Helgoland, North Sea, using a fish trap in 2001. *Z. viviparus* were transported to the AWI by the research cutter ‘FS Uthörn’. They were maintained in well-aerated, re-circulating seawater at 10±1°C and 34±1 PSU. Photoperiod was adjusted to a 12 h:12 h light:dark cycle.

The long-term acclimation experiment was conducted for 10 months between February and December 2002. Both eelpout species were acclimated to habitat (control) temperatures and a common temperature of 5°C, which is well within the thermal tolerance window of both species (Van Dijk et al., 1999; Mark et al., 2002). Control animals were kept at 0.0±0.5°C for *P. brachycephalum* and 10±1°C for *Z. viviparus*. The second group of both species acclimated at 5.0±0.5°C and 34±1 PSU were held in the same large basin, separated by a grid. Throughout the 10-month acclimation period, control animals (0°C for *P. brachycephalum* and 10°C for *Z. viviparus*), warm-acclimated *P. brachycephalum* (5°C) and cold-acclimated *Z. viviparus* (5°C), were fed live sand shrimp (*Crangon crangon*) *ad libitum* once per week. The validity of this approach is supported by the observation that Antarctic eelpout displayed positive growth under these conditions. After 10 months *in vitro,* experiments were conducted within 2 months in January/February 2003.

Preparation of cell-free translation systems

Prior to tissue dissection, the fish were killed by a sharp blow to the head and ensuing transection of the spine. The fish were immediately placed on ice and tissues were quickly excised. Gills were dissected by cutting the branchial arches at their sites of attachment. Only the soft gill filaments without arches were used for lysate preparation. White muscle was taken from the dorsolateral aspect along the backbone after the skin had been removed. *P. brachycephalum*, unlike *Z. viviparus*, exhibits a subcutaneous thick layer of fat tissue, which had to be removed along with the skin. White muscle was freeze-clamped in liquid N_2 while the gill lysate was freshly prepared. The cryo-preservation did not affect the protein synthesis system as has also been shown for lysates prepared from epaxial muscle of the cod *Gadus morhua* (Lied et al., 1982).

The preparation of lysates from *P. brachycephalum* and *Z. viviparus* followed the same procedure. Gill filaments of one to two animals were pooled to provide enough tissue for all measurements. Half of the gill tissue was freeze-clamped in liquid N_2 for later examination of amino acid composition in tissue protein, and of RNA and protein content. Approximately 150–350 mg of gill tissue was transferred to a chilled, hand operated, loosely fitting 2 ml glass homogenizer and

Table 1. *Concentrations of amino acids in protein obtained from white muscle and gill tissue of* P. brachycephalum *and of* Z. viviparus

	White muscle protein (g 100 g^{-1})		Gill protein (g 100 g^{-1})	
Amino acids	*P. brachycephalum*	*Z. viviparus*	*P. brachycephalum*	*Z. viviparus*
Cysteine	0.01‡	0.01‡	0.07±0.03	0.07±0.05
Aspartate	1.70±0.15	1.71±0.12	0.89±0.07	1.09±0.27
Hydroxy-proline	ND	ND	0.10±0.05	0.15±0.05
Threonine	0.76±0.08	0.76±0.03	0.53±0.06	0.59±0.16
Serine	0.63±0.08	0.64±0.07	0.47±0.05	0.51±0.13
Glutamate	2.40±0.20	2.52±0.17	1.23±0.09	1.43±0.33
Proline	0.53±0.15	0.59±0.03	0.47±0.12	0.58±0.23
Glycine*	0.71±0.07	0.82±0.09	0.66±0.08	0.79±0.30
Alanine†	0.89±0.08	0.96±0.08	0.49±0.04	0.67±0.16
Valine	0.90±0.05	0.89±0.10	0.57±0.05	0.64±0.20
Cysteine†	0.16±0.08	0.15±0.01	0.02±0.01	0.05±0.02
Methionine†	0.59±0.09	0.64±0.05	0.09±0.03	0.23±0.11
Isoleucine	0.86±0.07	0.86±0.06	0.42±0.03	0.43±0.14
Leucine	1.44±0.15	1.38±0.15	0.81±0.07	0.86±0.26
Tyrosine	0.44±0.07	0.49±0.06	0.23±0.04	0.27±0.09
Phenylalanine	0.74±0.09	0.74±0.04	0.42±0.07	0.48±0.11
Hydroxy-lysine†	ND	ND	0.02±0.01§	0.04±0.01
Ornithin	0.04±0.02	0.03±0.01	0.01±0.00	0.01±0.00
Lysine	1.54±0.12	1.56±0.13	0.74±0.08	0.82±0.21
Histidine	0.38±0.05	0.43±0.09	0.31±0.06	0.27±0.05
Arginine	1.07±0.12	1.07±0.08	0.57±0.10	0.70±0.17
Total	16.2±1.1	15.8±1.4	10.7±2.8	9.1±0.8
% Fraction of Phe	4.6±0.3	4.7±0.1	4.5±0.3	4.7±0.5

The ratio of each amino acid to Phe in protein of gill and muscle from both species was calculated to convert nmol of Phe into grams of protein synthesized. The percentage fraction of Phe within the protein was calculated from the ratio of phenylalanine content over the sum of amino acids multiplied by 100.

Data are given as means ± S.D. (N=6, except for gill of *Z. viviparus* N=5).

*A significant difference (P<0.05) between species in white muscle.

†A significant difference (P<0.05) between species in gill.

ND=not detectable; ‡only one detectable value out of six determinations; §only five detectable values out of six determinations.

Digestion with HCl converts asparagine and glutamine to aspartate and glutamate.

homogenized with five strokes in 1 vol. ice-cold extraction buffer containing 30 mmol l^{-1} Hepes, 250 mmol l^{-1} sucrose, 120 mmol l^{-1} potassium chloride, 10 mmol l^{-1} magnesium chloride, 7 mmol l^{-1} 2-mercaptoethanol, 20 mmol l^{-1} dithiothreitol (DTT), adjusted to pH 7.1 at 25°C, and supplemented with 107 U ml^{-1} RNasin ribonuclease inhibitor (Promega, GmbH, Mannheim, Germany). The homogenate was immediately transferred to 1.5 ml Eppendorf tubes and centrifuged at 16 000 ***g*** for 30 min at 0°C. The freshly prepared post-mitochondrial supernatant was used as the lysate and kept on ice until the start of *in vitro* translation assays. Samples of the lysates were frozen in liquid nitrogen for later determination of RNA and of free endogenous phenylalanine (Phe). White muscle lysates were prepared in the same way as gill lysates except that muscle tissue (750–1250 mg) was ground to a fine, homogeneous powder under liquid nitrogen using a pre-cooled mortar and pestle before homogenization in 0.6 volumes of ice-cold extraction buffer. This preliminary step was essential for a better mechanical pulping of the stringy muscle tissue.

Cell-free in vitro *translation assays*

Protein synthesis rates were determined *in vitro* at four different temperatures (0, 5, 10, 15°C). Buffer composition and osmolarity of the cell-free system were set to mimic the intracellular fluid of marine fish and to compensate for dilution of cellular components occurring upon lysis of the gill or of white muscle tissue. A refinement of assay conditions was achieved by preliminary experiments with variable Phe, ATP, GTP and phosphocreatine (PCr) concentrations measured in gill and muscle lysates of the North Sea eelpout at 10°C. Previous analyses of temperate versus Antarctic scallop muscle had demonstrated that such optimized conditions are also effective in studies of the same parameters in polar confamilials (REF). For measurements of protein synthesis capacity, translation assays were conducted under optimized physiological conditions. Incorporation of [2,3,4,5,6-^{3}H]Phe into protein was measured as a function of time in 11-µl samples from an incubation medium containing 22.5 µl lysate 75 µl^{-1} and final concentrations of 30 mmol l^{-1}

Table 2. *Free endogenous phenylalanine in lysates prepared from white muscle and gills of* P. brachycephalum *maintained at control temperature (0°C) or acclimated to 5°C and of* Z. viviparus *acclimated to 5°C or maintained at control temperature (10°C)*

		Phe (mmol l^{-1} lysate)	
Species	*N*	White muscle	Gill
P. brachycephalum			
0°C†	5	0.32±0.05*	0.14±0.05
5°C†	5	0.21±0.03*	0.08±0.01
Z. viviparus			
5°C	4	0.10±0.03	0.11±0.02
10°C	3	0.08±0.01	0.08±0.02

Incorporation of [2,3,4,5,6-^{3}H]Phe was corrected for free endogenous Phe.

Values are means ± S.E.M.

*A significant difference (P<0.05) of free endogenous Phe concentration in lysates prepared from white muscle between species and within species at varying acclimation temperatures.

†A significant difference (P<0.05) of free endogenous Phe concentration in lysates prepared from different tissues of the same species acclimated to the same temperature.

Hepes buffer (sodium salt, adjusted to pH 7.1 at 25°C), 120 mmol l^{-1} potassium chloride, 7 mmol l^{-1} magnesium chloride, 5 mmol l^{-1} DTT, 0.2 mmol l^{-1} spermidine, all amino acids except Phe at 0.1 mmol l^{-1}, 6 µmol l^{-1} Phe (including 30 µCi of [2,3,4,5,6-^{3}H]Phe, Amersham; 116 Ci mmol l^{-1}; 1 mCi=37 MBq), 52 units RNasin ribonuclease inhibitor (Promega), 1 mmol l^{-1} ATP, 1 mmol l^{-1} GTP and 30 mmol l^{-1} PCr and 2.25 U creatine phosphokinase as an ATP regeneration system to avoid a limitation of energy supply. The contribution of low molecular mass components from the lysate to the final concentration was not included in these numbers. Before starting translation with 22.5 µl lysate the reaction mixture was allowed to equilibrate in a water bath set to the experimental temperature of 0°C, 5°C, 10°C or 15°C for 5 min. After the addition of lysate, the assay was quickly subdivided into aliquots of 11 µl and returned to the water bath set at the experimental temperature. Reactions were run for given time periods and were terminated by 2 µl pancreatic RNAse (25 U ml l^{-1}). Subsequently, 11 µl samples were pipetted onto Phe-saturated, semi-wet Whatman GF/C filters to minimize non-specific adhesion of radioactive Phe to the filter. Filters were immersed in ice-cold 10% TCA, containing 5 mmol l^{-1} Phe as carrier, then washed once in 10% and twice in 5% ice-cold TCA. After a final rinse in 95% ethanol the filters were allowed to dry in air before dissolving them in 5 ml scintillation cocktail (Packard, 57% tritium counting efficiency). Radioactivity in the precipitated protein was determined by liquid scintillation counting. A zero control, which contained RNAse right from the beginning of the assay to prevent protein synthesis, was used to correct for background due to non-specific binding of [2,3,4,5,6-^{3}H]Phe to components of the lysate. Results were expressed as [2,3,4,5,6-^{3}H]Phe incorporated into trichloroacetic acid-precipitable protein (as d.p.m. 11 µl^{-1} assay) and was later converted to µg protein mg^{-1} fresh mass day^{-1} (see below).

Analytical methods

Total RNA and free endogenous Phe levels in lysates were determined as previously described (Storch et al., 2003; Storch and Pörtner, 2003). For the determination of RNA and protein contents, tissues were ground to a fine, homogeneous powder under liquid nitrogen using a pre-cooled mortar and pestle. Two 100 mg sub-samples of the resultant power were homogenized in ice-cold 0.2 mol l^{-1} perchloric acid (PCA). Homogenates were centrifuged at 16 000 ***g*** for 1 min at 0°C and the remaining precipitate was washed twice in 0.2 mol l^{-1} PCA. Subsequently, the pellet was resuspended in 0.3 mol l^{-1} NaOH and incubated for 1 h at 37°C. Samples (15 µl) were taken for the determination of protein levels using a modified Lowry technique with bovine serum albumin as a standard (Sigma procedure no. P5656). Protein and DNA were then precipitated from the remaining alkaline digest by the addition of ice-cold 20% PCA, and after centrifugation the resultant acid-soluble fraction was removed for the estimation of RNA levels by ultraviolet absorption at 232 nm and 260 nm (Storch et al., 2003). In addition to animals used for lysate preparation, further control specimens and those acclimated to 5°C were taken for measurements of RNA and protein content. Total amino acid composition of tissue protein was determined by acidic hydrolysis followed by HPLC analysis (Dr Wiertz, Dipl. Chem. Eggert and Dr Joerissen GmbH, Hamburg, Germany).

Derived parameters and statistics

Protein synthesis rates in gill and white muscle tissues at experimental temperatures were determined using the initial, linear intercept of time-dependent [2,3,4,5,6-^{3}H]Phe incorporation curves. Protein synthesis rates were converted from d.p.m. 11 µl^{-1} assay min^{-1} *via* nmol Phe 11 µl assay^{-1} min^{-1} into µg protein mg^{-1} fresh mass day^{-1}. Rate of phenylalanine incorporation (nmol Phe 11 µl assay^{-1} min^{-1}) = [2,3,4,5,6-^{3}H]Phe incorporated into protein (d.p.m. 11 µl^{-1} assay min^{-1}) / specific activity (d.p.m. $nmol^{-1}$ Phe). The specific radioactivity, expressed as Bq nmol Phe^{-1} (1 d.p.m.=1/60 Bq), of each assay was calculated from the amount of added radioactive and non-radioactive Phe in the assay plus the measured free endogenous Phe of the lysates.

For the conversion from nmol Phe to grams of protein, one approach is to simply assume that the ratio of the incorporated radiolabelled amino acid to any of the 20 other common amino acids is 1:1 and that the average molecular mass of an amino acid is 110. Using these assumptions, each nmol of Phe incorporated into protein is equivalent to 2200 ng of protein. In the present study, however, we were concerned about potential differences in amino acid composition of protein from the polar and temperate species. Therefore, the concentration

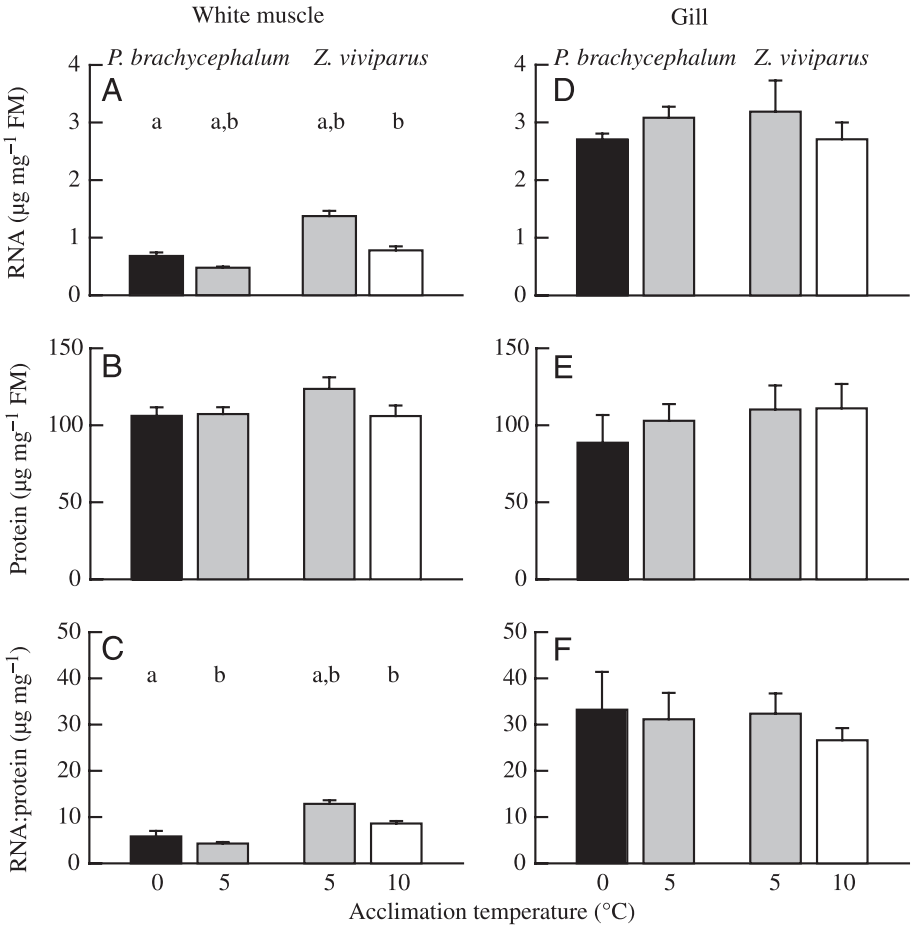

Fig. 1. RNA (A, white muscle; D, gill), protein (B, white muscle; E, gill) concentrations and RNA:protein ratios (C, white muscle; F, gill) in *P. brachycephalum* under control conditions (0°C; left, black bars), in warm-acclimated *P. brachycephalum* (5°C; left, grey bars), in cold-acclimated *Z. viviparus* (5°C; right, grey bars) and in control *Z. viviparus* (10°C; right, white bars). Values are means ± S.E.M. (N=6). Only the same tissues were tested for the significance of differences. Values with the same letter are significantly different. FM, fresh mass.

ratio of each individual amino acid to Phe was measured in protein of white muscle and gills from both species. These data and the molecular masses of the respective amino acids were used to calculate the correct relationship between nmol Phe incorporated and grams of protein synthesized.

The translational capacity of RNA (expressed as mg protein mg^{-1} RNA day^{-1}) determined in the *in vitro* system was calculated using the equation: $k_{RNA\ in\ vitro}$ = (mg protein synthesised 11 μl^{-1} assay day^{-1}) / (mg RNA 11 μl^{-1} assay). Arrhenius activation energy (E_a) and Q_{10} were determined from an Arrhenius plot of $\log V_{protein\ synthesis}$ *vs* $1/T(K^{-1})$. E_a values were expressed as E_a (in kJ mol^{-1}) = $-\boldsymbol{R}m\times 1000$, where $\boldsymbol{R}$ is the gas constant (J mol^{-1} K^{-1}) and m is the slope of the Arrhenius plot.

All data are expressed as means ± standard error (± S.E.M.) unless stated otherwise. Numbers (N) of determinations are given in parentheses or figure legends. Prior to analysis, assumptions of normal distribution of the studied variables and homogeneity of variances were tested. If any of the assumptions was violated, the data were transformed by x^x, which resulted in significantly improved normality and homogeneity of variances. Statistical differences at the 5% level were tested using analysis of variance (ANOVA) followed by the Student–Newman–Keuls *post hoc* test.

Results

Characterization of tissue composition and lysates

The amino acid composition of white muscle protein and of gill protein from *P. brachycephalum* and *Z. viviparus* is given in Table 1. Acclimation to 5°C elicited no major effect on amino acid composition in either species. Therefore, amino acid composition values determined in differently acclimated *P. brachycephalum* and *Z. viviparus*, were summarized to one mean ± S.D. for white muscle and one mean ± S.D. for gills. In muscle tissue only glycine differed significantly between species at the same protein content, whereas in gill tissue of *P. brachycephalum* alanine, cysteine, methionine and hydroxylysine levels were significantly lower than in gill tissue of *Z. viviparus* at the same level of protein content. The ratio of each amino acid to Phe in protein of white muscle and gill from both species was calculated from Table 1 and later used to convert nmol of Phe into grams of protein synthesized. The Phe percentage of total protein was equal in muscle and gill of both species (Table 1).

Despite identical protocols used for both species free endogenous Phe levels in white muscle lysates of warm-acclimated *P. brachycephalum* (5°C) were significantly reduced compared to control animals (0°C) but were still significantly higher than in lysates prepared from white muscle of cold-acclimated (5°C) or control (10°C) *Z. viviparus* (Table 2). There was no detectable difference in free endogenous Phe levels in gill lysates, either between species or within species maintained at various temperatures. The free endogenous Phe levels in the lysates were used to correct for the resulting differences in specific radioactivity.

RNA and protein concentrations as well as RNA:protein ratios, as traditional indicators of the *in vivo* capacity of protein synthesis, were measured in tissues of control animals, and of warm-acclimated *P. brachycephalum* and cold-acclimated *Z. viviparus*, respectively, for an evaluation of effects of long-term acclimation and evolutionary cold adaptation (Fig. 1).

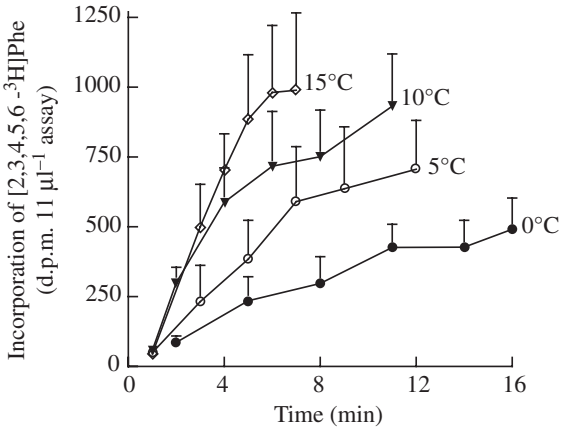

Fig. 2. Time course of incorporation of [2,3,4,5,6-^{3}H]Phe into trichloroacetic acid-precipitable protein at different temperatures by cell-free lysates prepared from gills of control *P. brachycephalum* maintained at 0°C. filled circles: 0°C; open circles: 5°C; filled triangles: 10°C; open diamonds: 15°C. Values are means ± S.E.M. (*N*=5).

RNA levels in white muscle of control *P. brachycephalum* (0°C: 0.68±0.06 µg RNA mg l^{-1} fresh mass, *N*=6) and of control *Z. viviparus* (10°C: 0.78±0.07 µg RNA mg l^{-1} fresh mass, *N*=6) were similar (Fig. 1A). In warm-acclimated *P. brachycephalum* (5°C) white muscle RNA was significantly reduced to 0.48±0.02 µg RNA mg^{-1} fresh mass (*N*=6), whereas cold-acclimated *Z. viviparus* (5°C) showed a significant elevation of white muscle RNA to 1.37±0.09 µg RNA mg^{-1} fresh mass (*N*=6). Thus, there was a significant difference in RNA content of white muscle between species at 5°C. RNA:protein ratios of white muscle tissue (Fig. 1C) followed the patterns of RNA content (Fig. 1A) because of equal protein contents (Fig. 1B) in both species. Gill tissues of both eelpout species displayed very similar values and exhibited no significant changes in RNA and protein concentrations and in RNA:protein ratios upon long-term acclimation to 5°C (Fig. 1D–F). Gill RNA content in the Antarctic eelpout at 0°C was 2.71±0.10 µg RNA mg^{-1} fresh mass (*N*=6) and at 5°C was 3.08±0.19 µg RNA mg^{-1} fresh mass (*N*=6). These values were very similar to 3.19±0.54 µg RNA mg^{-1} fresh mass (*N*=6) and 2.71±0.29 µg RNA mg^{-1} fresh mass (*N*=6) determined in gills of cold-acclimated and of control *Z. viviparus*, respectively.

The comparison of RNA contents in gills and white muscle revealed fourfold higher levels in gills than in white muscle of control *P. brachycephalum* and 6.5-fold higher values in gills than in white muscle of warm-acclimated *P. brachycephalum*. Values were around 2.3-fold higher and 3.5-fold higher in gills than in white muscle of cold-acclimated and control *Z. viviparus*, respectively.

In vitro *phenylalanine incorporation*

Phenylalanine incorporation into protein requires the complete protein synthetic machinery of the cell. The system used here resembles the buffering, osmotic and ionic conditions of the tissues. One goal of this study was to compare protein synthesis capacities in *P. brachycephalum* and *Z. viviparus* acclimated to various temperatures. By using the post-mitochondrial supernatant in an optimized cell-free system the capacities of white muscle and gill lysates of both species could be studied under their *in vivo* physiological conditions. Fig. 2 shows the time course for the incorporation of [2,3,4,5,6-^{3}H]Phe into trichloroacetic acid-precipitable material at different temperatures by lysates prepared from gills of *P. brachycephalum* (Fig. 2). The time course of *in vitro* translation in lysates prepared from both tissues were essentially the same principal pattern, regardless of acclimation temperature and species, as exemplified by the

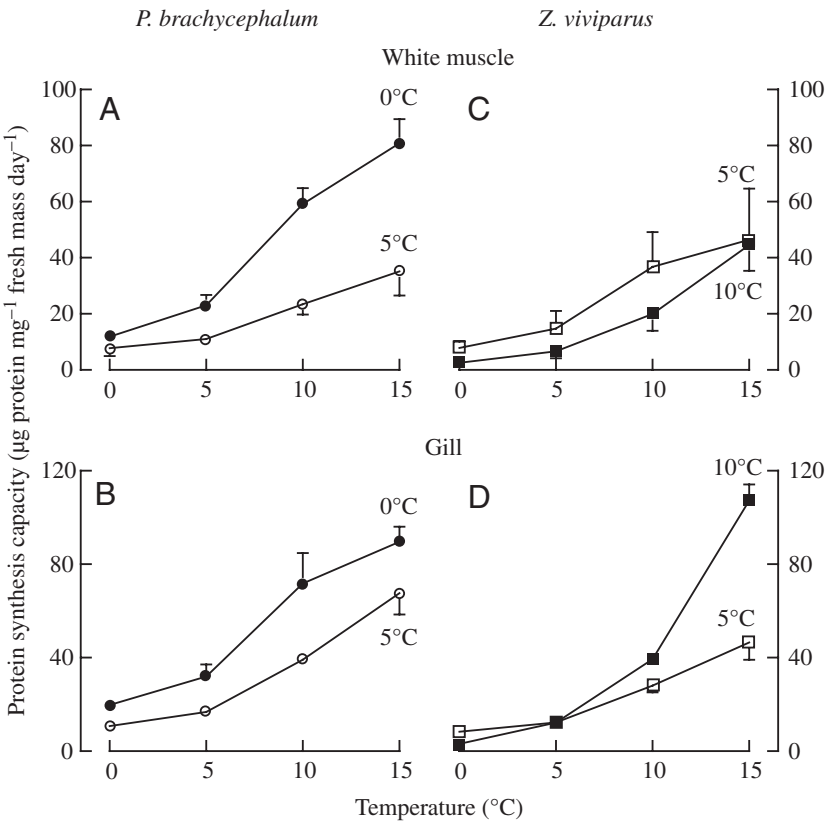

Fig. 3. Temperature-dependent protein synthesis capacities in lysates prepared from white muscle (A,C) and gills (B,D) of *P. brachycephalum* (A,B) and *Z. viviparus* (C,D) acclimated to various temperatures. Closed circles: control *P. brachycephalum* maintained at 0°C; open circles: warm-acclimated *P. brachycephalum* (5°C); closed squares: control *Z. viviparus* maintained at 10°C; open squares: cold-acclimated *Z. viviparus* (5°C). Values are means ± S.E.M. (control and warm-acclimated *P. brachycephalum N*=5; control *Z. viviparus N*=3; cold-acclimated *Z. viviparus N*=4).

gill lysate in Fig. 2: a linear period of incorporation was followed by a progressive reduction of reaction velocity. The reaction approached completion in an asymptotic manner thereafter. A higher degree of incorporation of Phe into protein was achieved at higher temperatures, combined with an earlier relative slowing of the reaction. Short periods of linear incorporation in lysates are typical when compared to other non-reticulocyte cell-free systems (Hofmann and Hand, 1994; Kim and Swartz, 2000).

In vitro *protein synthesis and RNA translational capacity*

Protein synthesis capacities (μg protein mg^{-1} fresh mass day^{-1}) and RNA translational capacities (mg protein mg^{-1} RNA day^{-1}) were calculated from the initial linear Phe incorporation rates (Fig. 2) considering Phe concentrations in the lysates (Table 2), as well as amino acid composition of tissue protein and the RNA concentration in tissues (Fig. 1A,D) and lysates.

P. brachycephalum *(0°C) compared with* Z. viviparus *(10°C)*

In vitro protein synthesis capacities in white muscle (Fig. 3A,C) and in gill lysates (Fig. 3B,D) displayed significant temperature dependence in control *P. brachycephalum* maintained at 0°C (filled circles) and in control *Z. viviparus* maintained at 10°C (filled squares). Protein synthesis capacities were significantly higher over the total temperature range in white muscle of *P. brachycephalum* (Fig. 3A) than in white muscle of *Z. viviparus* (Fig. 3C), although the same RNA contents were found in white muscle tissues of both species (Fig. 1A). An exponential increase in protein synthesis capacities was observed in white muscle with rising temperature in control *P. brachycephalum* and in control *Z. viviparus.* However, protein synthesis capacities of white muscle followed different Q_{10} values in control *P. brachycephalum* (Q_{10}=3.8±0.5, *N*=5) and control *Z. viviparus* (Q_{10}=7.0±0.2, *N*=3) (Table 3). The same findings were even more distinct in gill tissues (Fig. 3B,D) despite equal RNA contents in the gills of both species (Fig. 1D). In the temperature range 0 to 10°C protein synthesis capacities were considerably higher in gills of control *P. brachycephalum* (Fig. 3B) with a significantly lower temperature dependency compared to control *Z. viviparus* (Fig. 3D) (Q_{10}=3.0±0.3 *vs* Q_{10}=10.6±0.8). At 15°C, protein synthesis capacities of *P. brachycephalum* were below those of gill tissues of control *Z. viviparus*. Activation energies of protein synthesis were lower in both tissues of control *P. brachycephalum* (muscle: E_a=85±9 kJ mol^{-1}; gill: E_a=71±6 kJ mol^{-1}) than in tissues of control *Z. viviparus* (muscle: E_a=127±2 kJ mol^{-1}; gill: E_a=155±5 kJ mol^{-1}).

The *Z. viviparus* cell-free system had a very low rate of protein synthesis at 0°C in both tissues (muscle: 2.5±0.6 μg protein mg^{-1} fresh mass day^{-1}; gill: 3.1±0.7 protein mg^{-1} fresh mass day^{-1}) when compared to the much higher protein synthesis capacities in tissues of *P. brachycephalum* at 0°C (muscle: 11.8±2.2 μg protein mg^{-1} fresh mass day^{-1}; gill: 19.7±2.7 μg protein mg^{-1} fresh mass day^{-1}). These four- to fivefold higher *in vitro* protein synthesis capacities in tissues of *P. brachycephalum* can be explained by the four- to fivefold higher RNA translational capacity in both tissues (control *P. brachycephalum*: white muscle 4.68±0.83 mg protein mg^{-1} RNA day^{-1}, *N*=5; gill 3.09±0.34 mg protein mg^{-1} RNA day^{-1}, *N*=5, *vs* control *Z. viviparus:* white muscle 1.16±0.36 mg protein mg^{-1} RNA day^{-1}, *N*=3; gill 0.57±0 mg protein mg^{-1} RNA day^{-1}, *N*=3). At its respective habitat temperature of 10°C, protein synthesis capacities were twofold higher in both tissues of control *Z. viviparus* compared to control *P. brachycephalum* at its habitat temperature of 0°C.

Table 3. *Q_{10} values and Arrhenius activation energies (E_a) of the protein synthesis machinery isolated from white muscle and gill of* P. brachycephalum *maintained at control temperature (0°C) or acclimated to 5°C, and of* Z. viviparus *acclimated to 5°C or maintained at control temperature (10°C)*

		White muscle		Gill	
Species	*N*	Q_{10}	E_a (kJ mol^{-1})	Q_{10}	E_a (kJ mol^{-1})
P. brachycephalum					
0°C	5	3.8±0.5	85±9	3.0±0.3	71±6
5°C	5	3.0±0.4	70±9	3.2±0.2	75±5
Z. viviparus					
5°C	4	3.8±0.0	87±1	3.6±0.5	81±11
10°C*	3	7.0±0.2	127±2	10.6±0.8	155±5

Data are given as means ± S.E.M.

*A significant difference (P<0.05) of Q_{10} and E_a in lysates prepared from white muscle and gill between species and within species at varying acclimation temperatures.

Control P. brachycephalum *(0°C)* vs *warm-acclimated* P. brachycephalum *(5°C) and control* Z. viviparus *(10°C)* vs *cold-acclimated* Z. viviparus *(5°C)*

As expected protein synthesis capacities (Fig. 3) and RNA translational capacities (Fig. 4) in lysates prepared from tissues of 5°C acclimated specimens (unfilled symbols) were temperature dependent as already specified for the protein synthesis capacities of control animals (see above). The different modes of data depiction in Figs 3 and 4 reveal whether higher protein synthesis capacities in tissues can be explained by higher translational capacities of the RNA independent of the RNA content within the tissue. Fig. 4 depicts the effect of long-term acclimation to 5°C compared to control temperature on the *in vitro* RNA translational capacities in white muscle (Fig. 4A,C) and in gill (Fig. 4B,D) of both species. In this long-term acclimation experiment we found significant differences between the two species:

(1) The protein synthesis capacities of *P. brachycephalum* showed no change in Q_{10} and E_a upon acclimation in either tissue. In contrast, cold acclimation caused the protein synthesis capacities of both tissues of *Z. viviparus* to develop a lower temperature dependency and a drop in activation

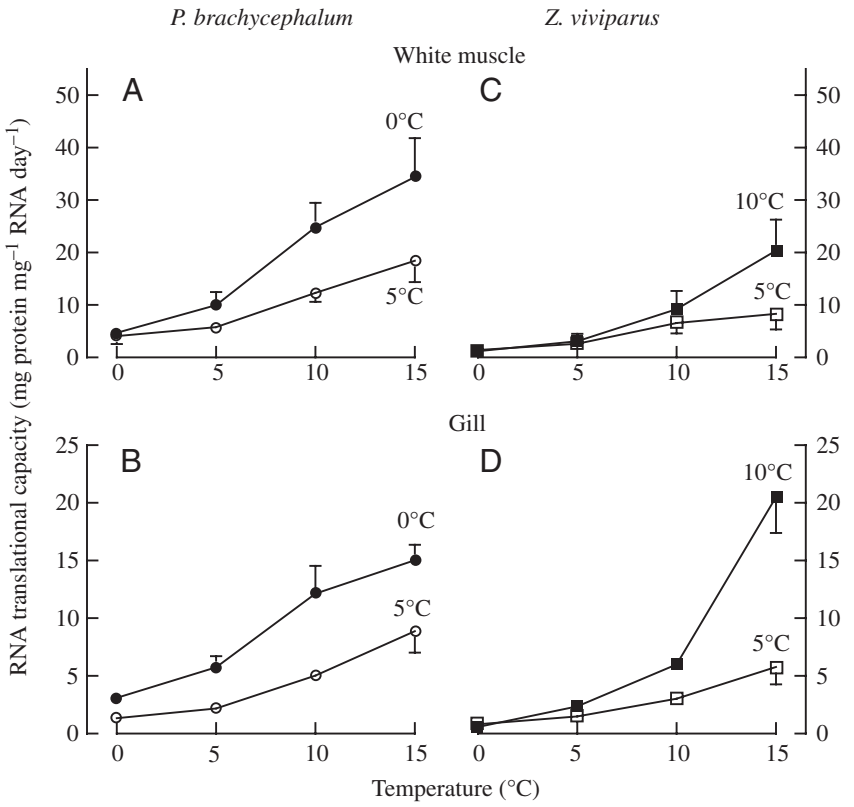

Fig. 4. Temperature-dependent RNA translational capacities in lysates prepared from white muscle (A,C) and gills (B,D) of *P. brachycephalum* (A,B) and *Z. viviparus* (C,D) acclimated to various temperatures. Closed circles: control *P. brachycephalum* maintained at 0°C; open circles: warm-acclimated *P. brachycephalum* (5°C); closed squares: control *Z. viviparus* maintained at 10°C; open squares: cold-acclimated *Z. viviparus* (5°C). Values are means ± S.E.M. (control and warm-acclimated *P. brachycephalum* *N*=5; control *Z. viviparus* *N*=3; cold-acclimated *Z. viviparus* *N*=4).

energies to values identical to those of *P. brachycephalum* (Table 3).

(2) A significant reduction in RNA translational capacities was observed in lysates prepared from tissues of warm acclimated *P. brachycephalum* compared to the 'cold' control animals at 0°C (Fig. 4A,B) over the measured temperature range. In contrast, the RNA translational capacities in lysates of tissues of *Z. viviparus* declined significantly upon cooling (Fig. 4C,D). This means that RNA translational capacities increased in *P. brachycephalum* at lower temperatures whereas *Z. viviparus* experienced a decrease in RNA translational capacities upon cold acclimation when measured in the *in vitro* cell-free system under the same physiological conditions.

In the light of varying RNA contents upon acclimation the effect of RNA translational capacity on protein synthesis in white muscle was especially interesting (Fig. 3A,C). High RNA contents amplify high RNA translational capacities and support high protein synthesis, as observed in white muscle lysates of control *P. brachycephalum*. This contrasts with low RNA contents and low RNA translational capacity in white muscle lysates of warm-acclimated *P. brachycephalum*, which result in significantly lower protein synthesis capacities. In contrast, in *Z. viviparus* similar protein synthesis capacities result at both acclimation temperatures, indicating compensation upon cold acclimation. White muscle lysates of cold-acclimated *Z. viviparus* display high RNA contents, which counteract low RNA translational capacities and bring protein synthesis capacity to the same level as seen under control conditions.

Warm-acclimated P. brachycephalum *(5°C)* vs *cold-acclimated* Z. viviparus *(5°C)*

The comparison of both species acclimated to the same temperature is interesting, especially in white muscle because of the large difference in RNA levels (Fig. 1A) due to warming of *P. brachycephalum* versus cooling of *Z. viviparus*. Therefore, in Fig. 5A,B we contrasted the RNA translational capacities and protein synthesis capacities of white muscle from warm-acclimated *P. brachycephalum* and cold-acclimated *Z. viviparus*. At an acclimation temperature of 5°C, RNA translational capacity in white muscle lysates of *P. brachycephalum* was significantly above the RNA translational capacities found in *Z. viviparus in vitro* (Fig. 5A) over the whole range of assay temperatures. This was also valid for gill tissues. Despite the much higher RNA contents in white muscle of *Z. viviparus* at 5°C the observed significantly higher RNA translational capacity in *P. brachycephalum* yielded similar protein synthesis capacities in both species (Fig. 5B).

Discussion

Characteristics of the cell-free protein synthesis system

The cell-free system was developed in the 1960s but, for a long time, low activities compared to *in vivo* protein synthesis rates have cast doubt onto its applicability to *in vivo* situations. The subsequent 40 years of intensive studies have made the cell-free translation system a valuable tool to study protein synthesis capacities under physiological conditions. For capacity estimates they should result in excess of *in vivo* protein synthesis rates (see below, Jackson, 1982; Kurland, 1982; Simon, 1987; Nakano et al., 1994; Pavlov and Ehrenberg, 1996; Patnaik and Swartz, 1998; Madin et al., 2000; Storch et al., 2003). An earlier study of an unsupplemented cell-free system prepared from liver of the Antarctic fish, *Trematomus bernacchii*, showed significant protein synthesis activity at 0°C (Haschemeyer and Williams, 1982), however, without adequate optimization the protein synthesis rate at 0°C was a factor of more than 1000 (0.004% liver protein synthesized per day) below that observed *in vivo* (5.3–6.9% tissue protein synthesized per day in liver; Smith and

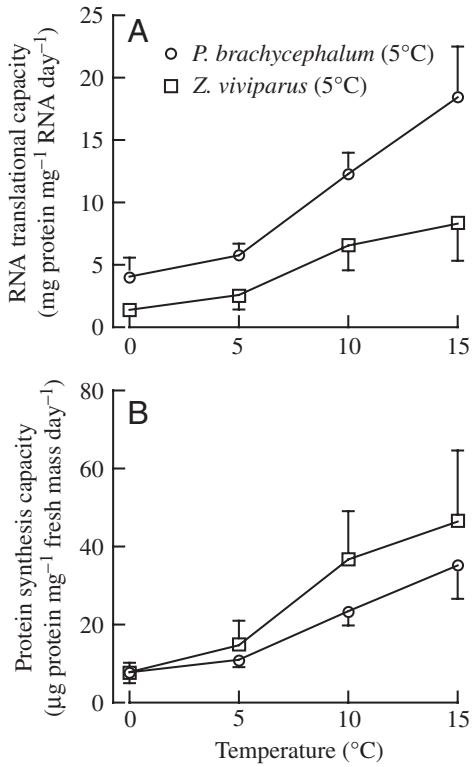

Fig. 5. Temperature-dependent RNA translational capacities (A) and protein synthesis capacities (B) in lysates prepared from white muscle of warm-acclimated *P. brachycephalum* and of cold-acclimated *Z. viviparus*. RNA translational capacities were from data in Fig. 4 and protein synthesis capacities from Fig. 3. Open circles: warm-acclimated *P. brachycephalum* maintained at 5°C; open squares: cold-acclimated *Z. viviparus* maintained at 5°C. Values are means ± S.E.M. (warm-acclimated *P. brachycephalum N*=5; cold-acclimated *Z. viviparus N*=4).

Haschemeyer, 1980). High-speed protein synthesis systems are mainly attained by providing sufficient energy and amino acids (Spirin et al., 1988; Kawarasaki et al., 1995; Kawarasaki et al., 1998; Kang et al., 2000; Storch and Pörtner, 2003) and by preventing RNA degradation by addition of RNasin ribonuclease inhibitor (Lopo et al., 1989; Moreno et al., 1991; Hofmann and Hand, 1994; Storch and Pörtner, 2003).

The optimized cell-free system developed in the present study allows direct comparison of protein synthesis capacities in white muscle and gills of *P. brachycephalum* and of *Z. viviparus* acclimated to various temperatures. The gill and white muscle lysates prepared from *P. brachycephalum* and from *Z. viviparus* in the present study had between 800- and 3000-fold higher protein synthesis rates than earlier *in vitro* determinations in Antarctic fish liver (control *P. brachycephalum*: muscle 3.5%, gills 11.8% protein synthesized per day, measured at 0°C; control *Z. viviparus*: muscle 5.3%, gills 18.7%, measured at 10°C). The 1000-fold lower *in vitro* than *in vivo* protein synthesis rates in liver of *Trematomus bernacchii* (Haschemeyer and Williams, 1982) and the order of magnitude by which the eelpout cell free systems exceed the activity of the '*Trematomus*' cell-free system corroborate the view that, because of improved methodology, values obtained here reflect the full *in vitro* capacity of the protein synthesis machinery. Capacity results above *in vivo* rates found in temperate and polar ectotherms emphasise the validity of our approach, despite the admittedly artificial nature of the *in vitro* system.

RNA translational capacity and RNA translational efficiency

These two terms should be carefully defined, before comparing *in vitro* capacity and *in vivo* translational efficiency. The *in vitro* translational capacity reflects the topmost capacity of the ribosomes to synthesize protein and can be determined by optimizing the *in vitro* assay as described above ($k_{\mathrm{RNA\ in\ vitro}}$ indicated by the rate of *in vitro* protein synthesis per unit RNA; Storch et al., 2003). The RNA:protein ratio in freshly collected tissues is commonly used as an indirect indicator of this capacity *in vivo*. For example gills are among the most active tissues with respect to protein turnover, which is reflected in a high RNA:protein ratio in both eelpout species at all temperatures when compared to white muscle (Fig. 1C,F). Muscle naturally shows very low protein turnover rates associated with high protein retention times. This was indicated by virtually identical protein concentrations in white muscle and gills (Fig. 1B,E) at significantly lower RNA contents in muscle (Fig. 1 A,D). The translational efficiency, in turn, is the extent to which the RNA translational capacity is utilized *in vivo* ($k_{\mathrm{RNA\ in\ vivo}}$ indicated by the rate of *in vivo* protein synthesis per unit RNA; Waterlow et al., 1978; Houlihan, 1991).

With adequate precaution, we attempt, in the following, to compare the measured *in vitro* capacities with *in vivo* protein synthesis rates and translational efficiencies found in literature. *In vivo* rates of protein synthesis and translational efficiency measured in gills and white muscle of various fish species in relation to their ambient temperature were compiled (Table 4) for comparison with the *in vitro* data obtained in both zoarcid species acclimated to their ambient temperature and measured *in vitro* at the same temperature. The *in vitro* RNA translational capacities obtained in gill and white muscle of both zoarcids exceed the values of *in vivo* efficiency determined in tissues of other fish species inhabiting various temperature environments and this trend is more distinct in white muscle compared to gill. This global comparison suggests that actual *in vivo* protein synthesis rates remain far below capacity. For a confirmation of this conclusion, *in vivo* protein synthesis should certainly be measured in the two zoarcids, however, their sluggish mode of life and low rate of energy turnover compared to other teleosts included in Table 4 supports the validity of this conclusion.

Furthermore, our data strongly indicate that the protein synthesis machinery is cold-compensated in the Antarctic *P. brachycephalum* at 0°C. Enhanced capacities of the protein synthesis apparatus especially in the cold resemble high enzyme capacities of aerobic metabolism, which are cold compensated too, despite reduced standard and maximum metabolic rates. Such excess capacities in metabolic and protein synthesis functions may be relevant for rapid adjustment of metabolic and functional equilibria and for full metabolic flexibility in response to external and internal stimuli in the

Table 4. *Fractional rates of protein synthesis (k_s) and translational efficiency (k_{RNA}) in gills and white muscle of various fish species adapted to different ambient temperatures*

Species	Temperature (°C)	Gill		Muscle		Reference
		k_s	k_{RNA}	k_s	k_{RNA}	
Antarctic fish						
Pachycara brachycephalum	0	11.8	3.1	3.5	4.7	This paper (*in vitro*)
	5	6.1	2.2	3.6	5.8	This paper (*in vitro*)
Trematomus bernacchii	–1.5	5.3	–	0.23	–	1
Trematomus hansoni	–1.5	3.2	1.3	0.22	0.23	1
Trematomus newnesi	–1.5	1.5	–	0.12	–	1
Gymnodraco acuticeps	–1.5	1.3	–	0.12	0.24	1
Notothenia corriceps	2	1.6	–	0.37	–	2
Chaenocephalus aceratus (icefish)	2	0.85	–	0.13	–	2
Temperate fish						
Zoarces viviparus	5	5.0	1.2	3.1	2.6	This paper (*in vitro*)
	10	18.7	5.9	5.3	9.1	This paper (*in vitro*)
Anarhichas lupus	5	–	–	0.55	0.74	3
Carassius carrasssius (FW)	10	–	–	0.3	1.9	4
Salmo gairdneri (FW)	12	9.1	–	0.49	–	5
Gadus morhua	5	7.8	1.3	–	–	6
	15	8.8	1.5	–	–	
Oncorhynchus mykiss (FW)	15	13.96	2.13	0.69	0.81	7
Opsanus tau	22	–	–	0.71	–	8
Anguilla anguilla	25	–	–	0.49	–	9
Tropical fish						
Sufflamen verres	26	15	–	0.69	–	10
(Triggerfish)	30	14	–	1.14	–	

k_s fractional protein synthesis rate (% protein synthesized day^{-1}); k_{RNA} (mg protein synthesized mg^{-1} RNA day^{-1}); FW, freshwater.
All data were measured *in vivo* unless stated otherwise.
[1]Smith and Haschemeyer, 1980; [2]Haschemeyer, 1983; [3]McCarthy et al., 1999; [4]Smith et al., 1996; [5]Houlihan et al., 1986; [6]Foster et al., 1992; [7]McMillan and Houlihan, 1988; [8]Pocrnjic et al., 1983; [9]De la Higuera et al., 1999; [10]Haschemeyer et al., 1979.

permanent cold. The extremely high capacity of the protein synthesis system in the white muscle and also in the gill of the Antarctic eelpout strongly supports these conclusions.

Temperature acclimation and adaptation of the protein synthesis machinery

The isolated translational machinery and its endogenous mRNA levels are a snapshot of the cell, which mirrors the previous history of the *in vivo* protein synthesis system. Therefore, *in vitro* protein synthesis capacities were compared in animals of equal body size that had experienced long-term acclimation to standard laboratory conditions. This minimizes the effects of fluctuating environmental parameters such as food availability, light:dark regimes and temperature, which is especially important when comparing animals in a latitudinal cline.

Thermal acclimation apparently has an effect on the temperature dependence and activation energies of the temperate, but not of the Antarctic species. Arrhenius activation energies are altered in response to the thermal history in cell-free systems prepared from the eurythermal *Z. viviparus*. This is the case for gill and white muscle, and data in the literature also indicate that this may be a common feature. Significantly reduced activation energies of protein synthesis rates were found in eel hepatocytes upon cold acclimation (Jankowsky et al., 1981). Many enzymes of cold-adapted species display reduced activation enthalpies to counterbalance the expected decrease in reaction velocity during cooling (Hochachka and Somero, 1984; Marshall, 1997; D'Amico et al., 2002). Protein synthesis requires a variety of enzymes and the overall activation energy mirrors that of the whole protein synthesis machinery.

The protein synthesis apparatus from white muscle and gill of the cold adapted *P. brachycephalum* also displays the expected cold-induced drop in activation energy, when compared to the synthesis apparatus of the respective tissues of temperate *Z. viviparus* maintained at 10°C. However, the protein synthesis complexes of *P. brachycephalum* did not exhibit a rise in activation energy during warming. Considering the long-term pattern of evolutionary cold adaptation, the cold-adapted protein synthesis machinery of *P. brachycephalum* might have lost (some of) the ability to adjust to warmer temperatures. Nonetheless, RNA translational capacity was reduced in the Antarctic species upon warming within the measured temperature range, reflecting a clear capacity for thermal

adaptation. This observation is somewhat unexpected for an Antarctic stenotherm but is in line with the apparent well being and net growth of warm acclimated *P. brachycephalum* during long-term maintenance.

Ectotherms living at high latitudes display cold compensated enzyme capacities despite reduced metabolic rates. These patterns have been interpreted to reflect a downward shift of oxygen-limited thermal tolerance windows (Pörtner, 2002). *P. brachycephalum*, naturally adapted to environments with low, constant annual mean temperatures, have higher RNA translational capacities at levels of RNA in gills and white muscle that are similar to those in their confamilial counterparts from temperate habitats. This trend may reflect cold compensation of enzyme capacities involved in protein synthesis in both high and low turnover tissues such as gills and white muscles.

The present data also allow a re-examination of whether higher RNA:protein ratios are suitable to compensate for lower RNA translational efficiencies at lower temperatures in general in cold acclimation and adaptation. The RNA concentrations of gill and white muscle did not differ between the species maintained at their habitat temperatures. Similar RNA levels in gills regardless of acclimation temperature and species emphasizes that the RNA:protein ratio does not exclusively reflect cold compensated protein synthesis capacity. This leaves upregulation of RNA translational capacity as a crucial component in the cold compensation process.

However, major changes in RNA:protein ratios were found upon acclimation in white muscle of both zoarcid species. As already mentioned, protein synthesis rates of white muscle in fish are known to correlate very well with growth rates, and react sensibly to temperature changes upon acclimation (Houlihan et al., 1988; McCarthy and Houlihan, 1997; McCarthy et al., 1999). Therefore, the following discussion will be confined to white muscle. A role for RNA:protein ratio in cold compensation can only be confirmed for cold-acclimated eurythermal eelpout *Z. viviparus*, whereas it does not apply to the cold-adapted Antarctic eelpout *P. brachycephalum*. Reduced RNA translational capacities in white muscle of *Z. viviparus* were clearly counteracted by elevated RNA:protein ratios resulting in similar protein synthesis capacities in both acclimation groups of *Z. viviparus*.

In contrast to the original hypothesis, cold adapted *P. brachycephalum* exhibited no increase in RNA:protein ratios compared to temperate eelpout, but displayed increased RNA translational capacities instead. Nonetheless, RNA levels are temperature dependent in the Antarctic eelpout, evidenced by a decrease in RNA levels during warm acclimation. At the same time, warming of the Antarctic eelpout caused a drop in translational capacities in white muscle (Figs 1, 4). Accordingly, both the levels of tissue RNA and of RNA translational capacity are thermally dependent in similar ways in both eelpout species, however, the range of RNA levels seen in *Z. viviparus* between 5 and 10°C is the same as in the Antarctic species at lower temperatures, between 0 and 5°C. This emphasizes the role of adjustments in translational capacity (per unit RNA) during thermal adaptation.

Changing translational capacities may also be involved in other species. In different organs of rainbow trout, increased specific elongation rates were measured upon cold acclimation in cell-free systems (Simon, 1987). Simon did not determine RNA levels in the assay, however, he concluded that the increased specific elongation rates in 4°C-acclimated trout are due to an effective enhancement of enzymatic elongation factor activities.

We conclude that *P. brachycephalum* evolved a cold-adapted RNA translation machinery with reduced Arrhenius activation energies to function efficiently at low temperatures, however, at lower RNA:protein ratios than seen in the temperate eelpout when cold acclimated. An increased RNA:protein ratio to counteract the temperature induced reduction of translational efficiency does not hold for the highly adapted protein synthesis machineries of Antarctic fish. This contrasts with observations of increased RNA:protein ratios in many eurythermal fish (Houlihan, 1991; McCarthy and Houlihan, 1997). It also contrasts with reduced *in vitro* translational capacities as seen in eurythermal *Z. viviparus* upon cold acclimation (this study). Increased RNA:protein ratios observed in cold-adapted invertebrates (Marsh et al., 2001; Fraser et al., 2002; Storch et al., 2003) also contrast with our present observations in Antarctic fish. As a corollary, further comparative *in vitro* and *in vivo* studies are needed in Antarctic ectotherms to evaluate whether these patterns and phenomena and the differentiation between fish and invertebrates reflect unifying mechanisms of cold compensation in protein synthesis operative in the permanent cold.

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