A comparative study of tissue protein synthesis rates in an Antarctic, *Harpagifer antarcticus* and a temperate, *Lipophrys pholis* teleost

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ABSTRACT

The affect of temperature on tissue protein synthesis rates has been reported in temperate and tropical, but not Antarctic fishes. Previous studies have generally demonstrated low growth rates in Antarctic fish species in comparison to temperate relatives and elevated levels of protein turnover. This study investigates how low temperatures effect tissue protein synthesis and hence tissue growth in a polar fish species. Groups of Antarctic, *Harpagifer antarcticus* and temperate, *Lipophrys pholis*, were acclimated to a range of overlapping water temperatures and protein synthesis was measure in white muscle (WM), liver and gastrointestinal tract (GIT). WM protein synthesis rates increased linearly with temperature in both species (*H. antarcticus* 0.16–0.23%.d^{-1}, *L. pholis*, 0.31–0.76%.d^{-1}), while liver (*H. antarcticus* 0.24–0.27%.d^{-1}, *L. pholis* 0.44–1.03%.d^{-1}) and GIT were unaffected by temperature in *H. antarcticus* but increased non-linearly in *L. pholis* (*H. antarcticus* 0.22–0.26%.d^{-1}, *L. pholis*, 0.40–0.86%.d^{-1}). RNA to protein ratios were unaffected by temperature in *H. antarcticus* but increased weakly, in *L. pholis* WM and liver. In *L. pholis*, RNA translational efficiency increased significantly with temperature in all tissues, but only in liver in *H. antarcticus*. At the overlapping temperature of 3 °C, protein synthesis (WM 26%, Liver, 39%, GIT, 35%) and RNA translational efficiency (WM 273%, Liver, 271%, GIT, 300%) were significantly lower in *H. antarcticus* than *L. pholis*, while RNA to protein ratios were significantly higher (WM 270%, Liver 170%, GIT 186%). Tissue specific effects of temperature are detectable in both species. This study provides the first evidence, that tissue protein synthesis rates are constrained in Antarctic fishes.

1. Introduction

The Antarctic is characterised by low, stable water temperatures and strong seasonal variation in sea ice, light and primary productivity (Clarke et al., 2008). In the shallow marine environment, annual temperature variation ranges from 0.2 °C in the high Antarctic coastal regions to 3.5 °C along the western Antarctic Peninsula and in the Scotia Sea (Barnes et al., 2006; Peck et al., 2006). Growth rates of Antarctic marine ectotherms are low in comparison to warmer water species, suggesting a possible thermal constraint to growth (Fraser et al., 2002a, 2002b; Clarke et al., 2004), and growth has even been shown to be slower in Antarctica than would be predicted from considerations of standard temperature effects on biological systems (Peck, 2018). Although high growth rates have been reported in a limited number of Antarctic invertebrates (Dayton, 1989; Rauschert, 1991; Barnes, 1995), these are still significantly lower than the faster rates in temperate and tropical relatives (Peck, 2018). A recent whole animal comparative

study of ecologically similar Antarctic, *Harpagifer antarcticus*, and temperate, *Lipophrys pholis*, fishes has highlighted the specific challenges of synthesising and retaining proteins as growth, at low temperatures (Fraser et al., 2022). Whole animal protein synthesis rates are comparatively low and protein degradation rates high in both Antarctic fish (Fraser et al., 2022) and invertebrates (Fraser et al., 2007). It appears that animals living at Antarctic water temperatures experience problems with post-translational protein folding, necessitating high constitutive levels of members of the 70 kDa heat shock protein family (HSP70) (Clark and Peck, 2009) which play key roles in successful folding of synthesised proteins. They also have high levels of ubiquitinated proteins, an indication of denatured proteins (Todgham et al., 2007, 2017) and higher proteasome activity, resulting in protein degradation rates 2–5 times higher in Antarctic than temperate fish (Todgham et al., 2017). In addition, Antarctic species, examined to date, have extremely high concentrations of RNA, to counteract low RNA activity (Whiteley and Fraser, 2008). Taken together, these data suggest that species living

at Antarctic water temperatures have reduced protein synthesis rates, in spite of elevated RNA concentrations and much higher rates of protein degradation, in turn resulting in reduced capacity for growth (Peck, 2016; Fraser et al., 2022).

A recent study has reported whole animal protein synthesis rates in an Antarctic fish species (Fraser et al., 2022), however, few studies to date have examined tissue fractional protein synthesis rates in Antarctic fish and in those cases, either only a single tissue was examined (Lewis et al., 2015), or older, less robust methodologies were used (Smith and Haschemeyer, 1980; Haschemeyer, 1983). Therefore, detailed knowledge of the tissue specific fractional protein synthesis rates underlying whole-animal fractional protein synthesis rates is lacking for Antarctic species, as is the effect of temperature. The aim of the current study was to investigate whether tissue protein synthesis rates followed a similar pattern to those seen at the whole animal level in an associated study (Fraser et al., 2022). It is not currently clear whether protein synthesis rates are low in all major tissues in Antarctic fishes, or if elevated rates are maintained in some tissues. The tissues selected for study were white muscle, liver and gastrointestinal tract (GIT). White muscle is the dominant tissue by mass in fish, with liver and GIT the next largest tissues and the only other two tissues of a large enough mass, to provide sufficient tissue for analysis in these species (Carter and Houlihan, 2001).

Here we investigate the effect of biologically relevant temperatures on underlying tissue protein synthesis rates, RNA to protein ratios and RNA translational efficiency in a temperate fish (the shanny, *Lipophyrs pholis*) and an Antarctic fish (the spiny plunderfish *Harpagifer antarcticus*). A phylogenetic approach was not used for the study due to the lack of an accurate phylogeny relating the two study species (for a discussion on the use of phylogenetic approaches see Garland et al. (2005). The authors are aware of the limitations of undertaking two species comparative studies (Garland and Adolph, 1994), however, the practical issues of including a wide range of fish species in a study design such as this and the extreme difficulty in obtaining sufficient Antarctic fish of any species, returning them to a suitable laboratory and undertaking the study precluded the incorporation of more species. From an ecological perspective the two fish species are ecologically similar, both are crypto-benthic, feed on small invertebrates, are found intertidally, or in very shallow water and are of a similar maximum body length. *H. antarcticus* is only found in Antarctica and at Rothera Research Station, experiences water temperatures ranging between −1.89 °C and approximately 2 °C (Clarke et al., 2008). *L. pholis* is distributed from Southern Norway to Spain and inhabits a range of water temperatures from as low as 2 °C in the winter in Norway, to average sea surface temperatures exceeding 20 °C in the summer in Spain (AquaMaps, 2019; Sabatés et al., 2006; Saetre and Ljoen, 1972;). In this study *L. pholis* were kept at temperatures from 3 to 18 °C, whilst *H. antarcticus* were maintained at temperatures from −1-3 °C. Critically these experiments included a cross over temperature between the two species at 3 °C.

2. Material and methods

2.1. Collection and husbandry of fish

The methods used in the current study were similar to those used in Fraser et al. (2022), but utilised a separate group of fish. *H. antarcticus* were collected by hand from sublittoral sites around Rothera Point, Antarctic Peninsula (67°34'07" S, 68°07'30" W) during the austral summer by SCUBA divers and held in a through-flow aquaria before being returned to the UK in a refrigerated transport system. In the UK, fish were maintained in a recirculating flow aquarium, under a 12 h light: dark regime (water temperature 0 °C ± 1.0 °C, salinity 34 – 36PSU), for at least one month before experimental temperature acclimation started. The *H. antarcticus* were fed twice weekly to satiation on shelled *Euphausia superba* (krill) and L. *pholis* on *Macruronus novaezelandiae* (New Zealand Hoki), as the latter refused krill. *L. pholis* were

collected subtidally in Weymouth (UK), purchased from a commercial supplier and transported to the experimental facility by vehicle, in aquaria. The mean water temperature of the L. *pholis* stock tank was 12.5 °C ± 1.1 °C, salinity 34-36PSU and a 12 h light: dark regime. All fish were held, and experimental work carried out at the British Antarctic Survey, Cambridge, UK. Experimental work was carried out between July and December for *H. antarcticus* and February to May for *L. pholis*, effectively winter through to spring for each species in its respective natural habitat. In both species, mature adults were used in all experiments.

2.2. Temperature acclimation

Experimental animals were weighed in water (± 0.1 g) and digitally photographed to allow individual identification. The fish were maintained in tanks and fed as reported in Fraser et al. (2022) and following Peck et al. (2009). The fish were placed in pairs, in jacketed aquaria ($18.8 \times 20.3 \times 22.0$ cm) containing aerated seawater at their stock tank temperature. Water temperatures were maintained (± 0.1 °C) using a thermocirculator (Grant Instruments, Cambridge, UK). Once each day the aquaria were divided in half using a perspex insert, thereby allowing fish to be fed individually to satiation.

Adjustment of aquaria to the required experimental water temperatures (*H. antarcticus*, −1 °C (number of fish per temperature treatment, $n = 8$), 1 °C ($n = 8$) and 3 °C ($n = 16$), and *L. pholis*, 3 °C ($n = 16$), 8 °C ($n = 8$), 13 °C ($n = 8$) and 18 °C ($n = 12$)) was carried out at a rate of 0.1 °C h^{-1} (*H. antarcticus*), or 0.5 °C h^{-1} (*L. pholis*), up to maximum of 0.8 °C d^{-1} (*H. antarcticus*) or 4 °C d^{-1} (*L. pholis*). Fish were acclimated to the experimental temperature for 28 days prior to the measurement of protein synthesis, as Antarctic fish can take three to four weeks to acclimate to elevated temperatures (Bilyk and DeVries, 2011), and are the slower of the two species to acclimate. The fish were weighed on the first and penultimate day of the 28-day acclimation period at the experimental temperature (± 0.1 g).

2.3. Protein synthesis measurement

Tissue protein synthesis rates were measured using a modification of the flooding dose method (See following references for detailed methodology; Garlick et al., 1980; Fraser et al., 2022). Fish were not fed on the day that protein synthesis was measured, but had been fed on the preceding day.

Groups of fish were injected in the peritoneum with a flooding dose of unlabelled and ^{3}H labelled phenylalanine (10 μl.g^{-1} fish wet mass of 135 mmol.l^{-1} L-[2,6-^{3}H] phenylalanine at 3.6 MBq.ml^{-1} (GE Healthcare, Little Chalfont, UK)). After injection, pairs of fish were placed into aquaria containing 4 l of aerated seawater maintained at the same temperature as the acclimation. At a pre-determined time-period after injection, the fish were killed by a sharp blow to the head and subsequent destruction of the brain. Immediately following this the liver, GIT and white muscle were sampled, weighed and frozen in liquid nitrogen. By squeezing the GIT under the back of a scalpel blade, the contents were removed and discarded prior to freezing. *H. antarcticus* acclimated to 3 °C, and the groups of *L. pholis* acclimated to 3 °C and 18 °C, were killed after time periods of 1 h, 2 h and 3 h, to allow validation of the flooding dose time-course. *H. antarcticus* that had been acclimated to −1 °C and 1 °C, and *L. pholis* that had been acclimated to 8 °C and 13 °C were killed after 2 h. Baseline, pre-injection phenylalanine levels were measured in liver, GIT and white muscle of both species ($n = 10$). All tissues were stored at −80 °C prior to analysis.

2.4. Protein synthesis and RNA content: sample analysis

Frozen tissue samples of 100 mg were homogenized in 2 ml of ice-cold, 0.2 M perchloric acid (PCA). The samples were then centrifuged (Eppendorf 5810R, swing bucket rotor, 3980 x g, 10 min, 4 °C) to

separate the protein precipitate and RNA from the intracellular free-pool (Houlihan et al., 1995). The pellet was washed twice with 0.2 M PCA which has been found to be sufficient to remove unbound labelled-phenylalanine (Houlihan et al., 1995). The supernatant, was decanted and the NaOH soluble protein in the pellet measured using bovine serum albumin (Sigma-Aldrich, Poole, UK) as the standard (Lowry et al., 1951). 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). Subsequently, the pellet was washed twice with 0.2 M PCA and hydrolysed in 6 M HCl for 18 h at 110 °C. The acid was removed from the hydrolysed protein residue using repeated washes of distilled water and rotary evaporation to dryness between washes, before the residue was re-suspended in 0.5 M sodium citrate buffer (pH 6.3). The phenylalanine concentration of the hydrolysed protein residue, injection solution and the intracellular free-pools was measured flourometrically, after enzymatic conversion of the phenylalanine to β-phenylethylamine (PEA) [Fraser et al., 2002a; Houlihan et al., 1995).

The specific radioactivity of the free pools, protein pellet and injection solutions were measured by scintillation counting (Wallac 1409 LSC, Packard Bioscience Hionic-Fluor scintillant, 34% ^{3}H counting efficiency) and expressed as disintegrations per minute (d.p.m.) $nmol^{-1}$ phenylalanine.

The fractional (k_s) rate of protein synthesis was calculated from:

$$k_s = \frac{S_b}{S_a} \times \frac{100}{t} \times 1440,$$

where k_s = % protein mass synthesised per day (% d^{-1}), S_b = specific radioactivity of protein-incorporated radiolabel (d.p.m. $nmol^{-1}$ phe), S_a = specific radioactivity of intracellular free-pool (d.p.m. $nmol^{-1}$ phe), t = incorporation time from injection of radiolabel to death in minutes and 1440 is the number of minutes in a day (Garlick et al., 1980).

2.5. Calculation of RNA translational efficiencies

Tissue RNA to protein ratios were expressed as μg RNA mg^{-1} protein. The translational efficiency of RNA (k_{RNA}, mg protein mg^{-1} RNA day^{-1}) was calculated following Preedy et al. (1988):

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

2.6. Ethics

All experimental work was carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines. The project was approved by the British Antarctic Survey Ethical Review Committee and carried out under Home Office Project Licence PIL 80/8523.

2.7. Statistical analysis

All data are expressed as mean ± standard error (s.e.m.). Statistical analysis was carried out using Minitab version 19 (Minitab, Coventry, UK). Prior to statistical analysis, data were checked for normality and homogeneity of variances using the Anderson-Darling and Levene's tests (Sokal and Rohlf, 2012). Parametric data were analysed using ANOVA and Tukey honestly significant difference (HSD) tests, while non-parametric data were analysed using the Kruskal-Wallis test. The student's *t*-test was used to assess differences in measures between species. Two-tailed significance was accepted at $p < 0.05$.
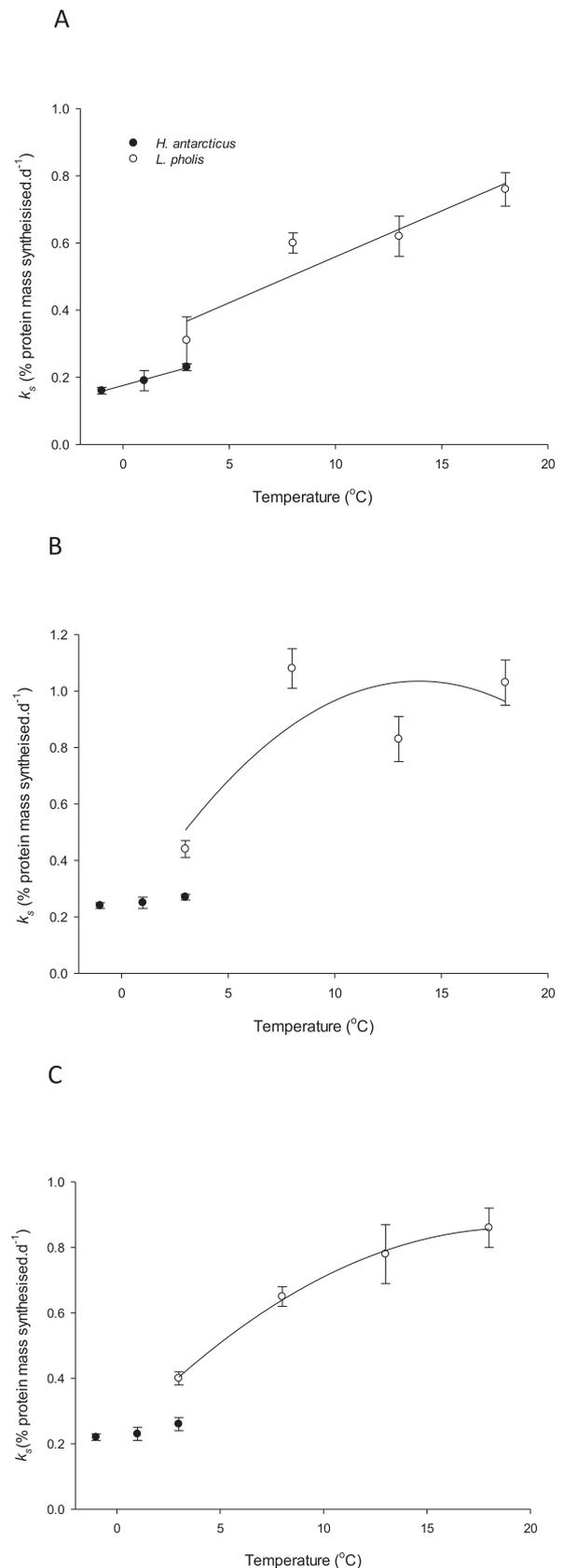
Fig. 1. The fractional rate of protein synthesis (k_s) in the white muscle (A), liver (B) and GIT (C) of groups of *H. antarcticus* acclimated to −1 (8), 0 (8) and 3 °C (16) and *L. pholis* acclimated to 3 (16), 8 (8), 13 (8) and 18 °C (12). Numbers in parenthesis are n for each data point. All data points are mean ± s. e.m. For significant regression equations see Table 1.

Table 1

Regression equations for significant relationships between the fractional rate of protein synthesis (k_s) and temperature in the white muscle, liver and GIT of *H. antarcticus* and L. *pholis* acclimatised to a range of water temperatures. Each statistical result represents the relationship between temperature groups for a specific tissue within a species.

Species	Tissue	Water temp.	$k_s \pm$ SEM s.e.m.	n	p	Regression	r^2
		−1 °C	0.16 ± 0.01	8			
	White muscle	1 °C	0.17 ± 0.03	8	0.001	0.1739 + 0.01926×	30.2
		3 °C	0.23 ± 0.01	16			
		−1 °C	0.24 ± 0.01	8			
H. antarcticus	Liver	1 °C	0.25 ± 0.02	8	0.207	NS	
		3 °C	0.27 ± 0.01	16			
		−1 °C	0.22 ± 0.01	8			
	GIT	1 °C	0.23 ± 0.02	8	0.213	NS	
		3 °C	0.26 ± 0.02	16			
		3 °C	0.31 ± 0.07	16			
	White Muscle	8 °C	0.60 ± 0.03	8	<0.001	0.1739 + 0.04498×	72.80
		13 °C	0.62 ± 0.06	8			
		18 °C	0.76 ± 0.05	12			
		3 °C	0.44 ± 0.03	16			
L. pholis	Liver	8 °C	1.08 ± 0.07	8	<0.001	$0.1364 + 0.1268× − 0.004427 ×^2$	52.8
		13 °C	0.83 ± 0.08	8			
		18 °C	1.03 ± 0.08	12			
		3 °C	0.40 ± 0.02	16			
	GIT	8 °C	0.65 ± 0.03	8	<0.001	$0.1964 + 0.06934× − 0.001815 ×^2$	60.2
		13 °C	0.78 ± 0.09	8			
		18 °C	0.86 ± 0.06	12			

NS = not significant.

3. Results

There were no significant differences in mean fish masses at the end of the acclimation period when protein synthesis was measured, for temperature treatments, or species; therefore, the data were not mass standardised prior to analysis (mean fish masses in g ± s.e.m. *H. antarcticus*, −1 °C, 12.96 ± 1.06, +1 °C, 13.69 ± 0.80, 3 °C, 13.20 ± 0.60; *L. pholis*, 3 °C, 13.38 ± 0.62, 8 °C, 15.07 ± 0.86, 13 °C, 13.74 ± 0.48, 18 °C, 14.80 ± 0.87; ANOVA, p = 0.200, F = 1.48).

3.1. Validation of the flooding dose

As tissue protein synthesis rates have not been measured in either study species, validation of the flooding dose methodology using accepted criteria was carried out (Fraser and Rogers, 2007). In both species, and for each temperature where the flooding dose was validated (*H. antarcticus*, 3 °C; *L. pholis*, 3 and 18 °C), the intracellular free-pool specific radioactivity increased rapidly after the flooding dose injection and remained elevated and stable over the course of the experiment (see supplementary materials Fig. S1–3, Table S1). There were no significant differences in free-pool specific radioactivity over time, within a temperature treatment, for up to 3 h after injection. For all incorporation time courses, incorporation of radiolabelled phenylalanine into protein over time was best described by a linear model (Supplementary materials, Fig. S1–3, Table S2). The intercepts of the regression equations describing radiolabel incorporation did not differ significantly from zero for any treatment, demonstrating that the incorporation of radiolabel commenced rapidly after injection.

3.2. Fractional protein synthesis

There was a significant linear relationship between fractional protein synthesis (k_s) and temperature in the white muscle of *H. antarcticus* (Fig. 1A, Table 1). There was no significant relationship between temperature and k_s in *H. antarcticus* liver and GIT, although there were small non-significant increases (Figs. 1 BCE, Table 1). In contrast, in *L. pholis* there was a linear relationship between k_s and temperature in white muscle and a quadratic relationship for both liver and GIT (Fig. 1, Table 1). There was no significant difference between the slopes describing white muscle k_s and temperature in the two species (Fig. 1A;

F = 2.99, p = 0.087). The fractional rate of protein synthesis was significantly different in *H. antarcticus* and *L. pholis* at the overlapping temperature of 3 °C in all tissues (Fig. 1, white muscle, $T = −4.63$, p < 0.001; liver, $T = −6.21$, p < 0.001; GIT, $T = −4.14$, p < 0.001).

3.3. RNA to protein ratio

The RNA to protein ratio was independent of temperature in *H. antarcticus* in all tissues (Fig. 2, Table 2), although in white muscle and GIT the RNA to protein ratio did decrease at 3 °C. In *L. pholis*, there was a slight linear increase in white muscle RNA to protein ratio with temperature, a quadratic relationship in liver and no effect of temperature in GIT (Fig. 2, Table 2). The RNA to protein ratio was significantly different between *H. antarcticus* and *L. pholis* in all tissues at the overlapping temperature of 3 °C (white muscle, $T = 11.93$, p < 0.001; liver, $T = 5.45$, p < 0.001; GIT, $T = 5.82$, p < 0.001).

3.4. RNA translational efficiency (k_{RNA})

There was a significant linear increase in k_{RNA} with temperature in the liver of *H. antarcticus* (Fig. 3, Table 3) and a slight, but non-significant, increasing trend in k_{RNA} in white muscle and GIT. In *L. pholis*, there was a linear relationship between temperature and k_{RNA} in all three tissues (Fig. 3, Table 3). k_{RNA} was significantly different between *H. antarcticus* and *L. pholis* in all tissues at the overlapping temperature of 3 °C (white muscle, $T = −8.35$, p < 0.001; liver, $T = −9.26$, p < 0.001; GIT, $T = −6.68$, p < 0.001).

4. Discussion

Methodological validation was not carried out for both species at all experimental temperatures to minimise the number of animals that were utilised in the study. Therefore, there is a very small risk that validation requirements may not have been met at -1 °C and 1 °C in *H. antarcticus* and 8 °C and 13 °C in *L. pholis*. However, this does seem very unlikely, as acceptable validation criteria at the other temperatures utilised was fully met. In addition, the protein synthesis methodology has previously been validated in the Antarctic limpet, *Nacella concinna* at water temperatures as low as −1.1 °C (Fraser et al., 2002a).

The relationship between protein synthesis, growth and degradation
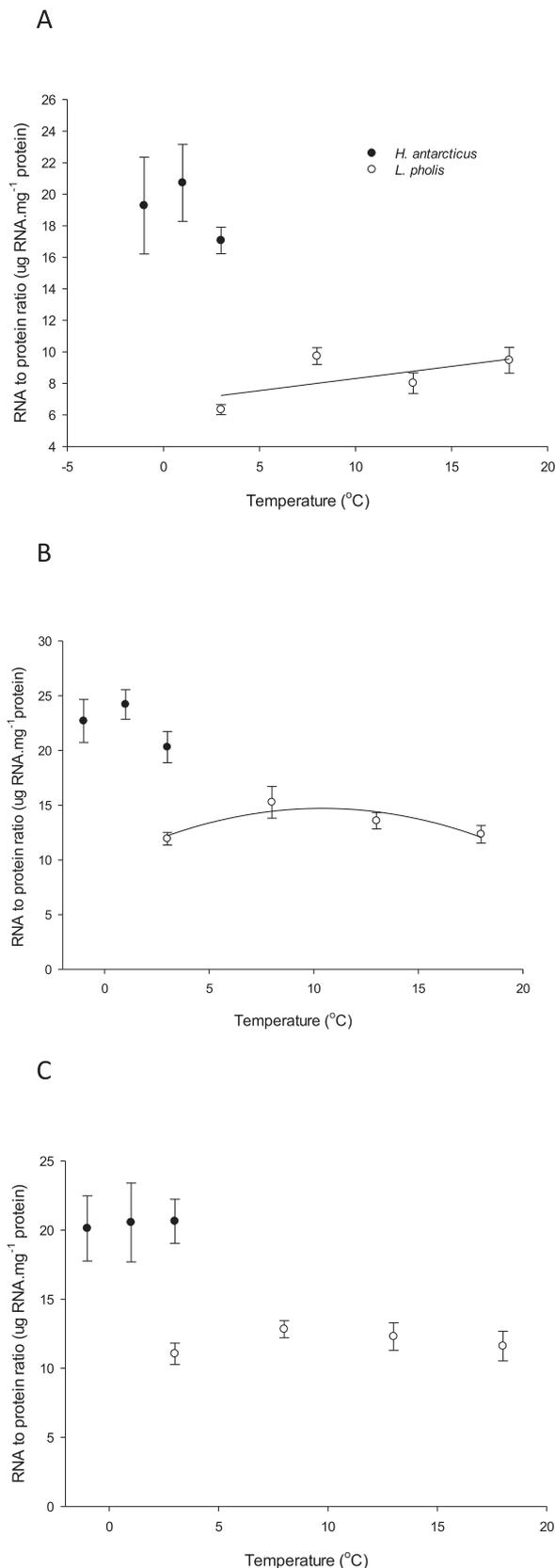
Fig. 2. The RNA to protein ratio in the white muscle (A), liver (B) and GIT (C) of groups of *H. antarcticus* acclimated to −1 °C (8), 0 °C (8) and 3 °C (16) and *L. pholis* acclimated to 3 °C (16), 8 °C (8), 13 °C (8) and 18 °C (12). Numbers in parenthesis are n for each data point. All data points are mean ± s.e.m. For significant regression equations see Table 2.

is critical in determining soft tissue growth rates (Houlihan et al., 1995; Fraser and Rogers, 2007; Calabrese et al., 2022). Temperature and food consumption are the major drivers of protein synthesis, and hence growth (Owen et al., 1999; Morgan et al., 2000; Katersky and Carter, 2010). Whole animal protein turnover data have recently been reported for *H. antarcticus* and *L. pholis* exposed to the same range of temperatures used in the current study (Fraser et al., 2022). However, whole animal data do not provide any detail on tissue specific protein synthesis rates. Some early studies did examine tissue protein synthesis rates in Antarctic fish, but only held at a single temperature (eg. Smith and Haschemeyer, 1980; Haschemeyer, 1983), and more recently in notothenioid hearts (Lewis et al., 2015), but no studies have investigated the effect of a range of temperatures on tissue protein synthesis in Antarctic fish, or directly investigated whether responses differ in Antarctic and temperate species. Our understanding of tissue specific fractional protein synthesis rates in Antarctic species is therefore poor. Hence, the current comparative study, aimed to investigate the effect of biologically relevant temperature acclimation on tissue protein synthesis rates in an Antarctic and an ecologically similar temperate teleost.

Previous studies of tissue fractional protein synthesis in ectotherms have generally examined protein synthesis rates at a single, or narrow range of temperatures, in a single species, e.g. in fish (Haschemeyer et al., 1979; Pocrnjic et al., 1983; Foster et al., 1992; Lewis and Driedzic, 2007; Saulnier et al., 2021) or invertebrates (eg. Fraser et al., 2004; Bowgen et al., 2007; Carter et al., 2009; Lamarre et al., 2016). Very few studies have examined tissue protein synthesis across a wide range of temperatures, approximating to the range of natural habitat water temperatures (McCarthy et al., 1999; Katersky and Carter, 2007; Lamarre et al., 2009).

4.1. The effect of temperature on tissue fractional protein synthesis

The current study demonstrates subtle, tissue-specific, differences in the effect of temperature on tissue fractional protein synthesis, that were not detectable at the whole animal level (Fraser et al., 2022). In *H. antarcticus* there was a small, but non-significant increase in whole animal fractional protein synthesis rates with temperature (Fraser et al., 2022). Liver and GIT protein synthesis rates were similar in exhibiting a small but non-significant increase with temperature (Fig. 1). In contrast, white muscle (WM) protein synthesis rates increased significantly (Table 1). It is likely that the significant increase in WM fractional protein synthesis rates with temperature reported in this study, was not detectable at the whole-animal level (Fraser et al., 2022) due to masking by other tissues, even though overall whole-animal fractional protein synthesis are likely to be dominated by WM rates. White muscle is by far the largest tissue by mass in fish and contains the largest amount of protein, for example, in rainbow trout, *Oncorhynchus mykiss*, 42% of the total body protein is within white muscle, while GIT and the liver only account for 3.4% and 1.4% respectively (Carter and Houlihan, 2001).

In contrast, in all tissues in *L. pholis*, there was a significant effect of temperature on tissue fractional protein synthesis, as reported with whole body rates (Fraser et al., 2022). In WM, *L. pholis* fractional protein synthesis rates increased with temperature linearly, in liver maximal protein synthesis rates occurred at 13 °C before decreasing, in GIT it appeared that the rate of increase in protein synthesis with temperature was slowing by 18 °C (Fig. 1). Intriguingly, these data suggest tissue-specific differences in the response of protein synthesis to increasing temperature, a feature that has previously been demonstrated in fish and reptiles on exposure to anoxia, and when undergoing seasonal metabolic depression (Smith et al., 1996; Fraser et al., 2001; Lewis and Driedzic, 2007). The slopes of the regression lines describing the increase in WM fractional protein synthesis rates with temperature did not differ between species (Fig. 1A).

Previous studies have generally shown an increase in protein synthesis rates with temperature up to the thermal limits of the species; providing the animals were fed to satiation and not maintained on a

Table 2

Regression equations for significant relationships between the RNA to protein ratio and temperature in the white muscle, liver and GIT of *H. antarcticus* and *L. pholis* acclimatised to a range of water temperatures. Each statistical result represents the relationship between temperature groups for a specific tissue within a species.

Species	Tissue	Temperature	RNA to protein ± s.e.m.	n	P	Regression	r^2
H. antarcticus	White muscle	−1 °C	19.28 ± 3.07	8			
		1 °C	20.72 ± 2.44	8	0.873	NS	
		3 °C	17.07 ± 0.84	16			
	Liver	−1 °C	22.70 ± 1.96	8			
		1 °C	24.21 ± 1.36	8	0.222	NS	
		3 °C	20.31 ± 1.42	16			
	GIT	−1 °C	20.13 ± 2.36	8			
		1 °C	20.56 ± 2.85	8	0.986	NS	
		3 °C	20.64 ± 1.60	16			
L. pholis	White Muscle	3 °C	6.34 ± 0.32	16			
		8 °C	9.73 ± 0.53	8	<0.001	$y = 6.330 + 0.1820 \times$	22.3
		13 °C	8.02 ± 0.66	8			
		18 °C	9.47 ± 0.82	12			
	Liver	3 °C	11.93 ± 0.58	16			
		8 °C	15.27 ± 1.45	8	0.012	$y = 9.533 + 0.9783 \times - 0.04634 \times^2$	14.4
		13 °C	13.58 ± 0.75	8			
		18 °C	12.34 ± 0.80	12			
	GIT	3 °C	11.05 ± 0.78	16			
		8 °C	12.84 ± 0.62	8	0.159	NS	
		13 °C	12.30 ± 1.00	8			
		18 °C	11.61 ± 1.08	12			

NS = not significant.

fixed ration (Foster et al., 1992; McCarthy et al., 1999; Treberg et al., 2005). Fractional tissue protein synthesis rate data from other species exposed to as wide a range of temperatures as in the current study are sparse. In the wolffish, *Anarhichas lupus,* WM and whole body fractional protein synthesis rates increased linearly with temperature between 5 °C and 14 °C (McCarthy et al., 1999). In a second study in the same species, WM fractional protein synthesis increased up to 8 °C before decreasing (Lamarre et al., 2009), while in the barramundi, *Lates calcarifer*, there was no significant difference in whole body k_s at temperatures between 21 °C and 33 °C, and WM k_s was only significantly lower at the lowest acclimation temperature of 21 °C (Katersky and Carter, 2007). It should, however, be noted that *L. calcarifer* has a thermal tolerance range of 15–40 °C (Katersky and Carter, 2007), which is significantly wider than the temperature range used in this study and therefore the species here may not have been exposed to a high enough water temperature to negatively impact protein synthesis rates. From the current study it would appear that in *L. pholis*, there are tissue-specific differences in how tissue fractional protein synthesis rates vary across a range of temperatures that approximate those that the species experiences in the field. In contrast, in the highly stenothermal *H. antarcticus*, even when exposed to temperatures near to the upper thermal limit of the species, with the exception of the small but significant increase in WM fractional protein synthesis rates, protein synthesis is relatively unaffected by temperature. The lack of a thermal response may be because of the narrow range of temperatures *H. antarcticus* has evolved in and could, therefore, be safely acclimated to in this study, although it has previously been suggested that Antarctic fishes have a lower thermal sensitivity of whole animal protein metabolism than temperate and tropical species (Fraser et al., 2022). It is also notable that in *H. antarcticus* protein synthesis rates were very similar in all tissues, at all temperatures, with the tissue means ranging between 0.19 and 0.24%.d^{-1}. While in *L. pholis* the tissues means ranged between 0.57 and 0.85%.d^{-1} with liver protein synthesis rates considerably higher than in white muscle, or GIT. Typically, in fish, liver protein synthesis rates are considerably higher than in tissues such as white muscle (Houlihan et al., 1994; Martin et al., 1993). The similar protein synthesis rates measured in all *H. antarcticus* tissues, could indicate an inability to maintain high liver protein synthesis rates.

Bilyk and DeVries (2011) demonstrated that in three species of Antarctic fish, acclimated to 4 °C, but which normally live at ∼ − 1.8 °C, Critical Thermal Maximum (CTmax) rapidly increased over the first 7 days of acclimation, but subsequently did not change. In turn, this suggests that acclimation of CTmax to increased temperature occurred in around 7 days. It is possible that the lack of an increase in *H. antarcticus* liver and GIT protein synthesis rates with temperature is due to incomplete acclimation even after 4 weeks. However, short-term changes in protein synthesis rate with temperature, are usually mediated by rapid changes in k_{RNA}, with longer term changes a result of changes in tissue RNA concentrations (Fraser and Rogers, 2007). It is also worth noting that white muscle did demonstrate a small but significant increase in protein synthesis rate with temperature. It therefore seems unlikely, that the weak thermal response of protein synthesis to increasing temperature in *H. antarcticus* was due to incomplete acclimation.

In all tissues, fractional protein synthesis rates were significantly lower in *H. antarcticus* than *L. pholis* when both species were acclimated to 3 °C. In turn, this further suggests that there is a fundamental thermal constraint on the synthesis of proteins in species that have evolved to live at very low temperatures (Fraser and Rogers, 2007; Whiteley and Fraser, 2008; Fraser et al., 2022). There is a great deal of evidence for there being a fundamental constraint on synthesis of proteins at low temperatures from other areas of research, including high, or very high, levels of ubiquitination and constitutive chaperones, rapid functioning of the proteasome, and very slow rates of embryonic development and growth in polar species (see Peck, 2018 for review).

In both species studied here, fractional protein synthesis rates ranked WM < GIT < Liver, similar to those reported in *Opsanus tau*, the oyster toadfish, *Sufflamen verres* the orange-side triggerfish, and *Notothenia coriiceps*, the Antarctic cod (Haschemeyer et al., 1979; Haschemeyer, 1983; Pocrnjic et al., 1983) and reviewed in fish generally, in Carter and Houlihan (2001). It should be noted that Haschemeyer (1983), has previously reported significantly higher tissue fractional rates of protein synthesis in Antarctic fish than those reported here (e.g. *N. coriiceps*, liver 10.4% d^{-1}, white muscle 0.37% d^{-1}), however, it appears that the flooding dose methodology applied was not robustly validated and the absolute rates reported in this study should be treated with caution (Fraser and Rogers, 2007; Lewis et al., 2015).

The relative fractional protein synthesis rates in the tissues of *H. antarcticus* and *L. pholis* seem intuitively realistic; the metabolically active liver has an important role in amino acid metabolism and export of synthesised proteins, whilst the GIT of fish is essential to the

A

B

C
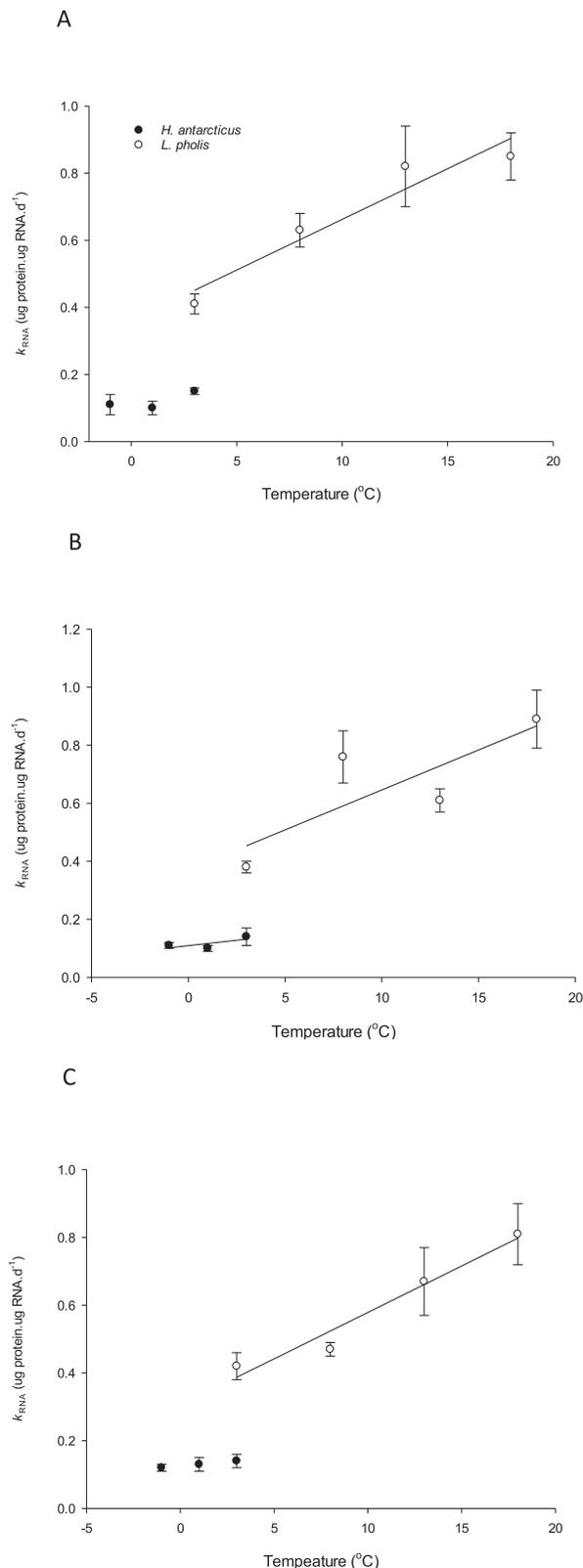
Fig. 3. RNA activity (k_{RNA}) in the white muscle (A), liver (B) and GIT (C) of groups of *H. antarcticus* acclimated to −1 °C (8), 0 °C (8) and 3 °C (16) and *L. pholis* acclimated to 3 °C (16), 8 °C (8), 13 °C (8) and 18 °C (12). Numbers in parenthesis are n for each data point. All data points are mean ± s.e.m. For significant regression equations see Table 3.

production of carbohydrates such as mucopolysaccharides and a range of enzymes. In contrast muscle does not synthesize and export large amount of proteins, therefore protein synthesis will occur primarily for tissue growth and maintenance (Houlihan et al., 1990; Hewitt, 1992; Carter and Houlihan, 2001). Only one study has reported k_s in the tissues of an Antarctic invertebrate. Fraser et al. (2004) showed that body wall fractional protein synthesis rate of the Antarctic holothurian, *Hetero-cucumis steineni ,* which comprises largely white muscle, ranged from a maximum of 0.35% day^{-1} in the austral summer, to 0.23% day^{-1} in winter, very similar values to the rate reported here in *H. antarcticus* WM.

4.2. The effect of temperature on tissue RNA to protein ratios and RNA translational efficiency

Alterations in tissue RNA to protein ratios, RNA translational efficiency and translational initiation are important mechanisms in controlling rates of protein synthesis (McMillan and Houlihan, 1989; Carter and Bransden, 2001; Jefferson and Kimball, 2001). Previous research has shown that RNA to protein ratios generally increase with decreasing temperature to offset a reduction in RNA translational efficiency (Houlihan, 1991; McCarthy et al., 1999; Fraser et al., 2002a, 2022; Storch et al., 2003; Treberg et al., 2005), although Robertson et al. (2001)) did not find any correlation between RNA to protein ratios and temperature in the Antarctic isopod, *Glyptonotus antarcticus*.

The relationship between tissue RNA to protein ratios and temperature in this study was significantly less consistent than at the whole organism level (Fraser et al., 2022). There was no significant relationship between RNA to protein ratio and temperature in *H. antarcticus* although there was a weak but non-significant decrease in WM and liver. At 3 °C, RNA to protein ratios were 70–169% higher in *H. antarcticus*, than in *L. pholis,* but even with these elevated RNA to protein ratios, fractional tissue protein synthesis rates were 26–39% lower and the Antarctic species synthesised 63–67% less protein per unit of RNA (Figs. 2, 3). A previous study has demonstrated that 24–35% of respiratory oxygen consumption is utilised by combined DNA and RNA synthesis in Antarctic fish hepatocytes, suggesting that the energetic cost of maintaining such elevated tissue RNA concentrations is high (Mark et al., 2005). The effect of temperature on RNA to protein ratios in *L. pholis* was fairly inconsistent, with a linear increase in WM, curvilinear increase then decrease in liver and no effect in GIT. It should further be noted here that the changes with temperature in RNA to protein ratio in *L. pholis* are small and on relatively small amounts of data and hence should be interpreted with caution.

In summary, our data provide the first robustly validated tissue protein synthesis rates for an Antarctic fish and demonstrate that at the level of tissues, protein synthesis rates are considerably constrained, in spite of greatly elevated tissue RNA concentrations. Our data add to the growing literature that has demonstrated that making proteins at Antarctic water temperatures is problematic (Clark and Peck, 2009; Fraser et al., 2002a; Todgham et al., 2007, 2017). The low protein synthesis rates reported in this study will fundamentally restrict the ability of Antarctic fish to grow quickly in comparison to warmer water species. In addition, a lower capacity for growth will have implications for life history characteristics such as embryonic development and the ability to respond to warming polar seas, or potentially competing with any future non-native fish species colonising warming Antarctic waters. As noted in the Introduction, a phylogenetic approach was not taken in this study due to the lack of an accurate phylogeny relating the chosen species (Garland et al., 2005). Therefore, the conclusions of this study must be considered in the light, that the some of the inter-specific physiological differences could be the result of phylogenetic divergence (*H. antarcticus*, Order Perciformes, *L. pholis*, Order, Blenniiformes) rather than environmentally driven physiological adaptations per se. A multi-species study utilising a wider larger range of species, with a well understood phylogeny, would be beneficial in more fully understanding

Table 3

Regression equations for significant relationships between RNA translational efficiency (k_{RNA}) and temperature in the white muscle, liver and GIT of *H. antarcticus* and *L. pholis* acclimatised to a range of water temperatures. Each statistical result represents the relationship between temperature groups for a specific tissue within a species.

Species	Tissue	Temperature	$k_{RNA} \pm$ s.e.m.	n	p	Regression	r^2
H. antarcticus	White muscle	−1 °C	0.11 ± 0.03	8			
		1 °C	0.10 ± 0.02	8	0.099	NS	
		3 °C	0.15 ± 0.01	16			
	Liver	−1 °C	0.11 ± 0.01	8			
		1 °C	0.10 ± 0.01	8	0.033	y = 0.1081 + 0.009205×	15.9
		3 °C	0.14 ± 0.03	16			
	GIT	−1 °C	0.12 ± 0.01	8			
		1 °C	0.13 ± 0.02	8	0.813	NS	
		3 °C	0.14 ± 0.02	16			
L. pholis	White Muscle	3 °C	0.41 ± 0.03	16			
		8 °C	0.63 ± 0.05	8			
		13 °C	0.82 ± 0.12	8	<0.001	y = 0.2058 + 0.07193× - 0.002000 × ^{2}	48.2
		18 °C	0.85 ± 0.07	12			
	Liver	3 °C	0.38 ± 0.02	16			
		8 °C	0.76 ± 0.09	8			
		13 °C	0.61 ± 0.04	8	<0.001	y = 0.3299 + 0.03064×	38.8
		18 °C	0.89 ± 0.10	12			
	GIT	3 °C	0.42 ± 0.04	16			
		8 °C	0.47 ± 0.02	8			
		13 °C	0.67 ± 0.10	8	<0.001	y = 0.3227 + 0.02635×	35.0
		18 °C	0.81 ± 0.09	12			

NS = not significant.

the limitations of growth in polar waters.

CRediT authorship contribution statement

Keiron P.P. Fraser: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Lloyd S. Peck:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization. **Melody S. Clark:** Writing – review & editing, Writing – original draft, Project administration, Investigation, Funding acquisition, Conceptualization. **Andrew Clarke:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2024.111650.

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