**Supplementary material**

**Material & Methods**

All experimental work was approved by the British Antarctic Survey Ethical Review Committee and carried out under Home Office Project Licence PIL 80/8523. Fish were killed by a sharp blow to the cranium and subsequent destruction of the brain an approved Schedule 1 method.

Data were not mass standardised prior to statistical analysis as there was no significant differences in the masses of fish used for experimental groups (Table 1s, GLM, F = 1.03, P = 0.314)

Table 1s. Body masses of *Harpagifer antarcticus* and *Lipophrys pholis* used for experimental treatments.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | *Harpagifer antarcticus* | | *Lipophrys pholis* | |
| Temperature (oC) | Mean (g) | SE | Mean (g) | SE |
| -1 | 13.73 | 1.35 |  |  |
| 1 | 14.83 | 0.98 |  |  |
| 3 | 13.24 | 0.51 | 15.25 | 1.01 |
| 8 |  |  | 16.83 | 1.78 |
| 13 |  |  | 15.28 | 2.23 |
| 18 |  |  | 17.63 | 2.02 |

*Methodology: validation of the flooding dose*

In both species, and for each temperature where the flooding dose was validated, the intracellular free-pool specific radioactivity increased rapidly after the flooding dose injection and did not change significantly for up to 3h after injection (Fig. S1, ANOVA, P>0.05). The time-course of radiolabelled phenylalanine incorporation into protein, was best described by a linear model for all time-courses (Fig. S1). The intercepts of the regression lines describing radiolabel incorporation did not differ significantly from zero for any treatment, demonstrating that incorporation of the radiolabel occurred rapidly after injection.

Injection of the flooding dose resulted in a mean 3.7-fold increase in phenylalanine concentrations of the free-pool above the measured baseline free-pool concentration (0.40nmol phe.mg-1 fresh mass). After a flooding dose injection of 1.35nmol phe.mg-1 fresh mass, phenylalanine levels should

A D

 

B E  

C F

 

Fig. 1s. Intracellular free-pool (FP) specific (A, B, C) and protein incorporated specific radioactivity (D, E, F) in groups of fish (*H. antarcticus*, 3°C, A, D; *L. pholis*, 3°C, B, E; *L. pholis*, 18°C, C, F) measured at set time points after injection with a flooding dose of phenylalanine at time zero. Within each trial, there were no significant differences in FP specific radioactivity at any time point (ANOVA, P> 0.05). All data are mean ± SEM. n = 5, except for the final data point in each figure, where n = 6. Error bars that are not visible lie within the symbol.

Table 2s. Regression equations for time course of specific radioactivity of protein-incorporated radiolabel. All regression relationships are P<0.001 and slopes that do not differ from zero.

|  |  |  |
| --- | --- | --- |
| Timecourse | r2 | Equation |
| *Harpagifer antarcticus* 3oC | 88.2 | y = 0.02112 + 0.001274x |
| *Lipophyrs pholis* 3oC | 92.6 | y =-0.00844 + 0.002326x |
| *L. pholis* 18oC | 77.1 | y = -0.0972 + 0.007094x |

have increased to 1.75nmol phe.mg-1. Phenylalanine levels increased to between 1.38 and 1.72 nmol phe.mg-1 (79-98%) of the theoretical post-injection concentration, suggesting that the flooding dose was successful, the injected phenylalanine had equilibrated within the tissues and flooding levels were similar to those previously reported in Antarctic invertebrates [1,2, 3].

It should be noted that the method used to measure protein degradation (*k*d) in the current study, subtracting the fractional protein growth rate from the protein synthesis rate (*k*s-*k*g), effectively calculates an average degradation rate over the 28 day study, on the basis of the difference between protein synthesis and growth. If post-translational folding after synthesis is an increased problem at Antarctic water temperatures and an even larger proportion of synthesised proteins are rapidly degraded within minutes of synthesis than in human cells, they would be unlikely to be detected in this study, which could in turn potentially lead to an underestimation of both protein synthesis and degradation [4].

**References**

1. Fraser KPP, Clarke A, Peck LS. 2002 Low-temperature protein metabolism: seasonal changes in protein synthesis and RNA dynamics in the Antarctic limpet *Nacella concinna* Strebel 1908. *J. Exp. Biol.* **205**, 3077-3086.
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4. Schubert U, Antón LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR. 2000 Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* **404**, 770-774. doi: 10.1038/35008096. PMID: 10783891.