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The effects of elevated winter temperature and sub-lethal pollutants (low pH, elevated ammonia) on protein turnover in the gill and liver of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Appetite, growth, and protein turnover (synthesis, growth and degradation) of liver and gills were measured in juvenile rainbow trout (*Oncorhynchus mykiss*) fed to satiation, and exposed for 90 days to elevated winter temperatures (+2 °C above ambient) and either low pH (5.2) in softwater or 70 μM total ammonia (T_{Amm}) in hardwater. All fish increased in weight during the experiments, but those exposed to +2°C grew significantly more than those at ambient temperature due to a stimulation of appetite. During the relatively constant temperature of the first 75 days, +2 °C caused a significant increase in the rates of protein synthesis and degradation in the liver of hardwater-acclimated fish, as a result of an increase in RNA translational efficiency (K_{RNA}). The elevated temperature also induced an increase in gill protein synthesis in softwater-acclimated fish but in this case the underlying mechanism was an increase in C_s, the capacity for protein synthesis (RNA:protein) rather than in K_{RNA}. The addition of 70 μM T_{Amm} had no effect on protein turnover in either liver or gills of hardwater-acclimated fish. Low pH inhibited protein growth in the liver of softwater-acclimated fish at day 90 under both temperature regimes. This inhibition was effected via a decrease in protein synthesis at control temperature but via an increase in protein degradation when the fish were exposed to both low pH and +2 °C. From these results we conclude that a simulated global warming scenario has potentially beneficial rather than detrimental effects on protein turnover and growth of freshwater fish during winter.

Introduction

Freshwater fish are poikilothermic and therefore their metabolic rate and many of their physiological functions are fundamentally influenced by temperature. Thus these animals are likely to be amongst those most affected by the long-term increases in environmental temperature predicted by models of global climate change or global warming (e.g. Hansen et al. 1988; Mohnen and Wang 1992). An increase in mean lake temperature of 2 °C has already been recorded over the last 20 years in the Experimental Lakes Area of Northern Ontario, Canada (Schindler et al. 1990). Although a number of studies have predicted or modelled the potential impact of global warming on freshwater fish, particularly with respect to habitat loss (Magnuson et al. 1990; Keleher and Rahel 1996), only a very few studies have been based on empirical data (e.g. Regier et al. 1990). These have however, provided evidence that temperature change of the magnitude predicted by global warming (2–5 °C) may indeed have significant consequences for freshwater fish. For example, Johnson and Evans (1990) found that the winter mortality of young-of-the-year white perch (*Morone americana*) was much lower at 4 °C as compared to 2.5 °C. As low-temperature is thought to be a major constraint

of the invasion of this species into the Great Lakes, global warming could result in significant changes in population of white perch in these waters.

Many freshwaters are also subject to chronic contamination with pollutants, the effects of which may interact with those of environmental temperature change (Schindler et al. 1996). However, virtually no data are available on the effects of combined environmental temperature change and pollution. In 1992, we undertook a project to examine the metabolic and physiological consequences to freshwater fish of a small increase (+2 °C) in water temperature, representative of global warming, imposed upon the natural thermal regime. The effects of temperature change were studied in the presence and absence of sublethal concentrations of two common pollutants in environmentally relevant media: ammonia in hardwater (HW) and acidity (low pH) in softwater (SW). A number of studies from this project have demonstrated significant physiological impacts of both a 2 °C increase in environmental temperature and the presence of chronic low-level pollutants on the physiology of rainbow trout (*Oncorhynchus mykiss*) (Reid et al. 1995, 1996, 1997; D'Cruz et al. 1997; Dockray et al. 1996; Linton et al. 1997a,b).

The aim of the present study was to examine the effects of elevated temperature, ammonia and acidity on protein turnover (synthesis, growth and degradation) in the gills and liver of juvenile rainbow trout during winter, when natural water temperatures were low (4–10 °C) and when the majority of natural acidic episodes occur (Reader and Dempsey 1989). Protein synthesis is one of the major components of metabolic energy demand in resting fish (Houlihan et al. 1993, 1995a) and has been shown to be affected by a wide range of variables, including temperature (Fauconneau and Arnal 1985; Loughna and Goldspink 1985; Watt et al. 1988) and pollutant stress (Houlihan et al. 1994). Liver and gills were chosen as the tissues to be studied as these are respectively, the principal site of ammonia metabolism (Randall and Wright 1987; Wood 1993) and of acid toxicity (McDonald 1983; Wood 1989) in freshwater fish. Liver and gills also have high rates of protein synthesis in feeding fish (Fauconneau 1985; Houlihan et al. 1986) and so any effects of environmental change might be expected to be more pronounced than in other tissues. No previous data on the effects of elevated environmental ammonia on protein turnover are available. However, low pH has been shown to inhibit gill protein synthesis in rainbow trout (Wilson et al. 1996; Reid et al. 1997), which may partially explain the lack of an acclimatory or damage-repair (McDonald and Wood 1993) response in acid-exposed fish (Audet and Wood 1988).

The present results are compared to those of similar previous studies carried out during summer when natural water temperatures were much higher (13–22 °C) (Reid et al. 1995, 1997). Other metabolic and physiological effects in the current experiments induced by elevated environmental temperature and total ammonia concentration (T_{Amm}) are considered in Linton et al. (1997b), whilst those induced by elevated temperature and acidity are discussed in D'Cruz et al. (1997).

Materials and methods

Experimental animals

Juvenile rainbow trout of 2–6 g were obtained from Rainbow Springs Trout Farm (Thamesford, Ontario). The fish were maintained indoors in polyethylene tanks (600 l capacity) supplied with flowing, aerated, dechlorinated Hamilton tapwater at ambient temperature (initially 4.0 °C). The chemical composition of the water was (mM) $[Na^+] \approx 0.5$, $[Cl^-] \approx 0.7$, $[Ca^{2+}] \approx 1.0$, $[Mg^{2+}] \approx 0.2$, $[K^+] \approx 0.05$; titratable alkalinity to pH 4.0 $\approx$ 1.0; pH 7.6 – 8.0. Photoperiod was controlled and adjusted to mimic the natural photoperiod throughout the acclimation and experimental periods. The fish were fed a ration of 1% of body weight day^{-1} (wet basis) of Zeigler Trout Starter no.3 (protein, 50%; lipid, 15%; water, 12%).

After a three-week acclimation period the fish were divided into two groups: hardwater- and softwater-acclimated fish. The hardness of the water supplied to the latter group was slowly decreased by increasing the ratio of artificially-softened Hamilton tapwater (generated by reverse osmosis) to achieve final concentrations of $[Ca^{2+}] \approx 25\mu M$ and $[Na^+] \approx 75\mu M$ (pH 6.1). The fish were allowed to acclimate to this softwater for a further three weeks prior to the start of experiments. The hardwater-acclimated fish were maintained in normal Hamilton tapwater throughout.

Experimental exposures

Approximately 130 hardwater-acclimated fish were transferred to each of eight 270 l tanks, representing four experimental treatments in duplicate. The tanks received water at either ambient temperature (base) or at ambient temperature plus an additional 2 °C

(base+2 °C), achieved via a heat exchanger. These two treatments were further split into those receiving and those not receiving an additional nominal 70μM total ammonia (T_{Amm}, equivalent to 7μg l^{-1} NH_3 at 7 °C, pH=7.6; base+Am and base+Am+2 °C) via a $(NH_4)_2SO_4$ stock solution delivered from mariotte bottles (Mount and Brungs 1967). Actual T_{Amm} concentrations in the base, base+2 °C, base+Am and base+2 °C+Am treatments were 8.3±0.3, 9.5±0.3, 76.6±1.9 and 88.8±1.8 μM respectively and water pHs were 7.46 (±0.02), 7.47 (±0.02), 7.51 (±0.02) and 7.56 (±0.02) respectively. The terminology used to describe these four treatment groups has been chosen for consistency with the report of Linton et al. (1997b) on the physiology and metabolism of these same fish.

A similar number of softwater-acclimated fish were also divided into four, replicated treatments. Fish were again exposed to ambient temperature or ambient temperature plus 2 °C, however passage of the water through the reverse osmosis unit increased the ambient water temperature such that the water temperatures for softwater-acclimated fish were 3–4 °C greater than those of hardwater-acclimated fish. Each temperature treatment was divided into two acidities: the ambient pH of the artificial softwater (≈6.1) and low pH (≈5.2). The latter was achieved by automatic titration of 0.1 M H_2SO_4 as described by Dockray et al. (1997). The four treatments are referred to as follows (actual pH ± SEM): control temperature/control pH 6.1 = 0/6.1 (6.08 ± 0.03); control temperature/sublethal pH 5.2 = 0/5.2 (5.14 ± 0.05); control temperature +2 °C/control pH 6.1 = +2/6.1 (6.06 ± 0.03); control temperature +2 °C/sublethal pH 5.2 = +2/5.2 (5.22 ± 0.04). This terminology has been chosen to avoid confusion with the slightly different thermal regime of the hardwater/ammonia experiment, and is consistent with the report of D'Cruz et al. (1997) on the physiology and metabolism of these same fish.

Both hardwater- and softwater-acclimated fish were exposed to their respective experimental treatments for 90 days, from January to April, 1994. Throughout the exposure the fish were fed by hand to satiation twice daily (08:30h and 16:30h) from a known weight of food which was re-weighed after feeding (see Wilson et al. 1996 for details). However, as only a single measurement of food consumed was made at each time for each tank (i.e. N=2 per treatment), no values of the error of this parameter were calculated.

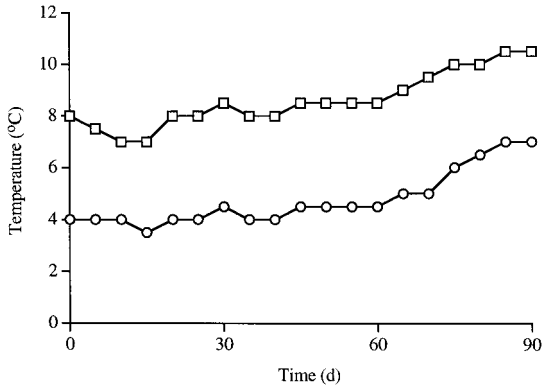

Figure 1. Ambient water temperature profiles during exposure of juvenile rainbow trout to elevated temperature and sublethal pollutants in hardwater (base, circles) and softwater (control, squares).

Measurement of protein turnover

The fractional rates of protein synthesis was measured in each of the four treatments for both hardwater- and softwater-acclimated fish immediately prior to the start of (day 0), and at 75 and 90 days of, exposure. Protein growth (accretion) and degradation were calculated for the periods 0–75 and 75–90 days. This sampling regime was dictated by the water temperature during the exposures: base temperature was relatively constant between 4 and 5 °C from day 0–day 75, then gradually increased to 7 °C by day 90 (Figure 1). Protein synthesis in the liver and gills was measured using the flooding dose technique of Garlick et al. (1980), adapted for fish as in Houlihan et al. (1995b). The precise methodology followed in the present experiments has previously been described in detail (Reid et al. 1995, 1997) so only a brief outline follows:

Fish were not fed for 24 h prior to measurement. Ten fish from each treatment were then removed individually, weighed and injected (unanaesthetized) via the caudal vein with 10 μl g^{-1} body weight of 135 mM phenylalanine solution, containing 3.7×10^6 Bq ml^{-1} ^{3}H-phenylalanine, in Cortland saline (Wolf 1963). Following injection, the fish were placed in individual, darkened chambers containing water from the respective treatment. Following a 1 h incorporation period, determined as appropriate for these tissues by Wilson et al. (1996), the fish were killed by a blow to the head and whole livers and branchial baskets were dissected out, weighed, frozen in liquid nitrogen and stored at −70 °C for later analysis.

The RNA and protein content, and fractional rate of protein synthesis (K_s) of liver and gills were determined as in Houlihan et al. (1995b): Approximately 100 mg of tissue was homogenized in ice-cold 0.2 M perchloric acid (PCA) and the precipitated proteins separated by centrifugation at 6000 g for 10 min. PCA in the supernatant was precipitated by the addition of saturated tripotassium citrate and removed by centrifugation, leaving the 'free-pool' phenylalanine in solution. Protein in the PCA-extracted pellet was solubilized in 0.3 M NaOH and aliquots taken for protein determination (Lowry et al. 1951) and ^{3}H-phenylalanine analysis (by liquid scintillation counting). This step yielded the 'rough' estimation of protein synthesis rate discussed below. Protein and DNA in the remaining solution was precipitated by 3.4 M PCA and total RNA in the supernatant was analysed by the orcinol method (Munro and Fleck 1966). The protein and DNA pellet was hydrolysed at 110 °C for 18 h with 6 M HCl, which after cooling, was removed by evaporation. The resulting protein-hydrolysate was resuspended in 0.5 M sodium citrate buffer (pH 6.3), and the phenylalanine in these and the corresponding free-pool samples was converted to ß-phenylethylamine by incubation with L-phenylalanine decarboxylase. The ß-phenylethylamine was selectively extracted with n-heptane and analysed with a ninhydrin assay (Suzuki and Yagi 1976). The ^{3}H in the hydrolysate and free-pool samples (now present as ^{3}H-ß-phenylethylamine) was measured by liquid scintillation counting.

The fractional rate of protein synthesis, K_s (% d^{-1}) was calculated as:

$$K_s = \frac{SA_b}{SA_f} \times \frac{1440}{t} \times 100$$

where SA_b is the protein bound (hydrolysate) specific activity, SA_f the free-pool specific activity (both dpm $nmol^{-1}$), t the exact ^{3}H-phenylalanine incorporation period (min.) and 1440 is the number of minutes in a day. In practice the true value of K_s (as above) was calculated for only 50% of samples (selected at random) due to the cost of the assay. However 'rough' values of K_s were calculated for *all* samples as:

$$\text{Estimated } K_s = \frac{\text{roughSA}}{1400} \times \frac{100}{t} \times 1440$$

where rough SA = ^{3}H-phenylalanine counts/275/mg protein of the pellet suspended in NaOH (above); 275 is the assumed phenylalanine content of fish proteins (nmol g^{-1}) and 1400 is the assumed free-pool specific activity (dpm $nmol^{-1}$) based on the injected dose. A simple linear regression equation was obtained by comparing calculated true and estimated values of K_s(r=0.71; P <0.001), and this was subsequently used to transform all estimated K_s to (apparent) true K_s values, in accord with the procedure of Wilson et al. (1996).

The rate of protein degradation (K_d,% d^{-1}) was calculated as the difference between the rate of protein synthesis (K_s) and the rate of protein growth (K_g,% d^{-1}); the latter calculated as the specific growth rate of tissue protein using the equation of Ricker (1979):

$$\text{Protein growth rate}, K_g(\%d^{-1}) = \frac{(\ln W_2 - \ln W_1)}{t} \times 100$$

where W_1 and W_2 are the final and initial weights of tissue protein (mg) respectively and t is the growing period (d). As a different group of fish were sacrificed at each sampling time values of W_1 and W_2 were not available from the same fish. Therefore the mean value of W_1 was subtracted from individual, measured values of W_2 to obtain individual values of K_g.

The balance between K_s, K_d and K_g is described by the term 'protein turnover'. However, protein turnover may vary markedly depending upon the nutritional state of the fish. When tissue protein is increasing i.e. K_g is positive, as in the present experiments, then the rate of protein turnover is equal to the rate of protein degradation (Houlihan et al. 1993, 1995b).

The capacity for protein synthesis (C_s) was calculated as the ratio of total tissue RNA:protein (µg:mg) and RNA translational efficiency, K_{RNA} (g protein synthesized g^{-1} RNA d^{-1}) was calculated as:

$$K_{RNA} = \frac{Ks}{Cs} \times 10 \quad \text{(Houlihan et al. 1995a)}$$

K_s, K_g and C_s have been shown to vary with body size, therefore these were corrected to a standard body size of 40 g (in order to be comparable to similar, previous studies of Reid et al. 1995, 1997) using the natural log transformation of the allometric equation $Y = aX^b$ and exponents of −0.2 for K_s and C_s (Houlihan et al. 1988) and −0.41 for K_g (Jobling 1983).

Statistical analysis

Mean values ± 1 SEM are used throughout the text. Statistical differences between means were determined by analysis of variance followed by Fisher's multiple comparison test (MINITAB). All tests were performed at the 5% level of confidence.

Table 1. Cumulative food consumption (appetite, g fish^{-1}), specific growth rates (SGR,% d^{-1}) over 0–75 and 75–90 days, and initial and final weights in juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to chronic elevated temperature and sublethal pollutants. Values are means (± SEM, except appetite) and those at each time that do not share a letter (where present) are significantly different from each other ($p<0.05$)

Treatment	Initial weight (g)	0–75 days		75–90 days		Final weight (g)
		Appetite	SGR	Appetite	SGR	
Hardwater-acclimated fish (n = 40):						
Base+Am		4.9	0.45 (0.10)^{a}	2.8	2.44 (0.85)	11.2 (0.7)^{a}
Base	5.5 (0.3)	5.1	0.70 (0.13)^{ab}	2.3	0.89 (0.67)	10.6 (0.5)^{a}
Base+2 °C		7.2	0.97 (0.14)^{bc}	3.9	1.75 (0.72)	14.8 (0.8)^{b}
Base+2 °C+Am		7.1	1.02 (0.10)^{bc}	4.1	1.34 (0.73)	14.5 (0.8)^{b}
Softwater-acclimated fish (n=30):						
0/5.2		11.7	1.13 (0.13)^{a}	9.3	1.84 (0.99)	20.2 (1.5)^{a}
0/6.1	6.5 (0.4)	9.8	1.05 (0.15)^{a}	10.4	2.39 (0.67)	20.6 (1.0)^{a}
+2/6.1		15.4	1.73 (0.38)^{ab}	17.5	2.04 (0.69)	32.4 (1.7)^{b}
+2/5.2		18.9	1.73 (0.14)^{b}	17.0	3.80 (0.73)	42.3 (2.4)^{c}

Results

General

The base (hard-) water temperature during the present experiments remained relatively constant at approximately 4–5 °C until around day 70, after which it increased steadily to reach 7 °C by day 90 (Figure 1). The control (soft-) water temperature followed a similar pattern but was consistently 3–4 °C higher throughout the exposure due to heat imparted by the reverse osmosis unit in the softening process. Both hardwater- and softwater-acclimated fish increased in weight during the experiments (Table 1). However, the fish exposed to +2 °C grew significantly more than those at base or control temperature due to a stimulation of appetite at the elevated temperature (Table 1). This whole-body growth was reflected in the two tissues analysed for protein turnover: fractional protein growth rates (K_g) of liver and gills were positive in all experiments (Figures 2–5b). Although the ambient water temperature was much lower than that in a similar study carried out during summer (13–22 °C; Reid et al. 1995, 1997), fractional rates of liver protein synthesis (K_s) and degradation (K_d) in both softwater- and hardwater-acclimated fish were broadly similar in the two studies, ranging between 4–9% d^{-1}. However, gill protein turnover was much lower than that of liver in the present experiments and indeed was much lower than in the summer experiments; ranging between 2–3% d^{-1} presently, versus 4–8% d^{-1} in the studies of Reid et al. (1995, 1997).

Hardwater–acclimated fish

Liver

Immediately prior to the start of the exposure (day 0) the rate of protein synthesis in the liver of hardwater–acclimated fish was 1.8% d^{-1} (Figure 2) By day 75, protein synthesis rate in all treatments was considerably greater; that of the base group being 4.9% d^{-1}. At this time, the rates of liver protein synthesis and degradation were significantly greater in those fish exposed to an additional +2 °C although protein growth rate was unaffected. The RNA: protein ratio or capacity for protein synthesis of the liver (C_s) was unaffected by exposure to +2 °C (Table 2) and hence the increase in protein synthesis at the elevated temperatures was due to an increase in RNA translational efficiency (K_{RNA}) (Table 3). The addition of 70 μM T_{Amm} had no effect on protein synthesis or degradation in the liver: although the values of these parameters in the base+Am group were noticeably lower than in the base group, with a similar difference in the +2 °C treatments, these differences were not significant (Figure 2). By day 90, protein synthesis rates in the fish exposed to +2 °C had decreased such that no significant differences be-

Table 2. Capacity for protein synthesis, C_s (μg RNA mg^{-1} protein) in the liver and gills of juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to chronic elevated temperature and sublethal pollutants for 75 and 90 days. Values are means (± SEM, n = 6–10) and those at each time that do not share a letter (where present) are significantly different from each other ($p<0.05$)

Treatment	Liver			Gills		
	0	75	90	0	75	90
Hardwater-acclimated fish:						
Base+Am		67.8 (2.9)	64.6 (2.5)		37.2 (1.4)	44.5 (1.1)^a
Base	64.0 (1.9)	72.2 (1.4)	68.4 (3.2)	32.4 (1.9)	39.0 (1.5)	43.4 (1.5)^a
Base+2 °C		64.9 (1.8)	65.5 (2.9)		39.2 (1.5)	45.3 (1.5)^a
Base+2 °C+Am		63.5 (3.0)	69.2 (1.5)		38.8 (1.5)	49.7 (1.7)^b
Softwater–acclimated fish:						
0/5.2		73.8 (1.5)	58.7 (3.2)		39.9 (2.5)	46.0 (1.5)^a
0/6.1	73.0 (3.0)	70.3 (2.1)	63.4 (2.0)	28.1 (0.7)	36.0 (2.3)	44.5 (2.0)^a
+2/6.1		73.5 (1.0)	65.2 (2.0)		39.8 (1.7)	51.3 (1.5)^b
+2/5.2		76.2 (1.9)	61.7 (2.5)		36.6 (1.4)	48.3 (0.9)^{ab}

tween the four treatments in either protein or RNA parameters of the liver were apparent.

Gills

The fractional rate of protein synthesis in the gills of hardwater-acclimated fish at day 0 was similar to that of liver: 1.8% d^{-1} (Figure 3). However, the gill capacity for protein synthesis at day 0 was much lower than that of liver (32.4 and 64.0 μg mg^{-1} protein respectively, Table 2) and hence gill RNA translational efficiency was much greater than that of the liver (0.63 and 0.28 g protein synthesized g^{-1} RNA d^{-1} respectively, Table 3). Gill protein synthesis did not change significantly during the exposure (Figure 3a) whilst the capacity for protein synthesis increased, and hence RNA translational efficiency decreased gradually over the 90 days. Neither +2 °C nor 70 μM T_{AMM} had any significant effect on protein or RNA parameters of the gill at day 75, but at day 90 the capacity for protein synthesis was significantly greater in the gills of fish in the base+2 °C+Am group (Table 2).

Softwater-acclimated fish

Liver

The rate of protein synthesis in the liver of softwater-acclimated fish prior to the start of the exposure was 3.7% d^{-1} (Figure 4) – approximately twice that of the liver of hardwater-acclimated fish at the same time. At day 75 no significant treatment effects were observed in either protein synthesis or degradation but +2 °C caused a significant increase in liver protein growth. By day 90, following an increase in water temperature, the rates of protein synthesis in liver in general had increased to an average of approximately 7.0% d^{-1} (Figure 4). The rate of protein growth was reduced by low pH at control temperature due to a similar reduction in protein synthesis; degradation was not affected. At the elevated temperature however, low pH stimulated protein synthesis but degradation increased to an even greater extent such that the net result was again a reduction in protein growth at low pH.

The relatively high pre-exposure rate of protein synthesis in the liver of softwater-acclimated fish was reflected in the corresponding value of the capacity for protein synthesis, which was also relatively high at 73.0 μg RNA mg^{-1} protein (Table 2). However, whilst protein synthesis generally increased during the exposure, capacity for protein synthesis showed a distinct overall decrease such that over the 90 days of exposure, the RNA translational efficiency increased by approximately 2-fold (Table 3). In spite of these overall trends however, no significant treatment effects on RNA parameters were observed, although the values of K_{RNA} at day 90 did reflect the pattern seen in protein synthesis rates at that time.

Gills

The rate of protein synthesis in the gills of softwater-acclimated fish increased from 1.6% d^{-1} at day 0 to an average of 2.9% d^{-1} at day 75, then fell somewhat to approximately 2.3% d^{-1} at day 90 (Figure 5). As

Table 3. RNA translational efficiency (K_{RNA}, g protein synthesized g^{-1} RNA d^{-1}) in the liver and gills of juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to chronic elevated temperature and sublethal pollutants for 75 and 90 days. Values are means (± SEM, n = 6–10) and those at each time that do not share a letter (where present) are significantly different from each other ($p<0.05$)

Treatment	Liver			Gills		
	0	75	90	0	75	90
Hardwater-acclimated fish:						
Base+Am		0.46 (0.09)[a]	0.60 (0.05)		0.45 (0.02)	0.37 (0.02)
Base	0.28 (0.03)	0.68 (0.04)[a]	0.54 (0.04)	0.63 (0.04)	0.48 (0.02)	0.40 (0.04)
Base+2 °C		1.17 (0.11)[b]	0.52 (0.04)		0.48 (0.02)	0.38 (0.01)
Base+2 °C+Am		0.94 (0.13)[b]	0.45 (0.05)		0.50 (0.02)	0.39 (0.02)
Softwater-acclimated fish:						
0/5.2		0.55 (0.05)	0.93 (0.10)		0.74 (0.10)	0.45 (0.02)
0/6.1	0.50 (0.04)	0.59 (0.04)	1.10 (0.06)	0.58 (0.03)	0.80 (0.08)	0.48 (0.03)
+2/6.1		0.60 (0.08)	1.04 (0.07)		0.80 (0.07)	0.50 (0.02)
+2/5.2		0.56 (0.06)	1.25 (0.17)		0.76 (0.06)	0.53 (0.01)

in hardwater-acclimated fish, the capacity for protein synthesis in the gills was much lower than that in the liver of softwater-acclimated fish (Table 2). Indeed, values of gill K_s, K_g K_d and C_s over the 90d were similar in hardwater- and softwater-acclimated fish. The capacity for protein synthesis increased gradually over the length of the exposure. No effect of either +2 °C or sublethal low pH was observed on protein or RNA parameters at day 75. At day 90 fish exposed to an additional +2 °C had a significantly greater gill protein synthesis than those at ambient temperature due to an increase in the capacity for protein synthesis (Table 2); RNA translational efficiency was not significantly affected by +2 °C (Table 2). As in the liver, protein growth in the gill was reduced at low pH, although in this case the differences were not statistically significant.

Discussion

The effects of elevated environmental temperature

The vast majority of previous studies on the effects of temperature on protein turnover in fish have found that, providing food is not limiting, fractional rates of protein synthesis (K_s) and degradation (K_d) are significantly greater at higher water temperatures (Fauconneau and Arnal 1985; Watt et al. 1988; Mathers et al. 1993). Loughna and Goldspink (1985) found that protein synthesis in the muscle of rainbow trout and carp (*Cyprinus carpio*) increased with increasing water temperature from 5–20 °C. Similarly, K_s in the heart of rainbow trout acclimated to (and tested at) 15 °C was approximately twice that of fish acclimated to 5 °C (Sephton and Driedzic 1995). A comparison of rates of protein turnover in the present, winter experiments with those from experiments carried out during the summer months (ambient temperature = 13–22 °C), but under otherwise similar conditions (Reid et al. 1995, 1997), partially supports this relationship. Gill protein synthesis and degradation rates in the winter experiments (2–3% d^{-1}) were markedly lower than those during the summer (4–8%; Reid et al. 1995, 1997). Indeed the present values of gill protein synthesis are some of the lowest recorded for rainbow trout (cf. Houlihan et al. 1986; Wilson et al. 1996). As these fish were relatively small and fed a high ration, both of which would be expected to elevate K_s (Houlihan et al. 1986), it is assumed that the very low values are a result of low environmental temperature. The greater rate of protein synthesis at day 0 in the gills of SW- compared to HW-acclimated fish may also be partly attributable to temperature; that of the former being approximately twice that of the latter (Figure 1). However, Reid et al. (1995) found that trout acclimated and maintained in softwater had higher rates of gill protein synthesis than fish in hardwater and hence the effects of temperature versus water chemistry cannot be separated in this comparison. In contrast to the gill, values of liver protein synthesis and degradation in the present study were broadly similar to those of

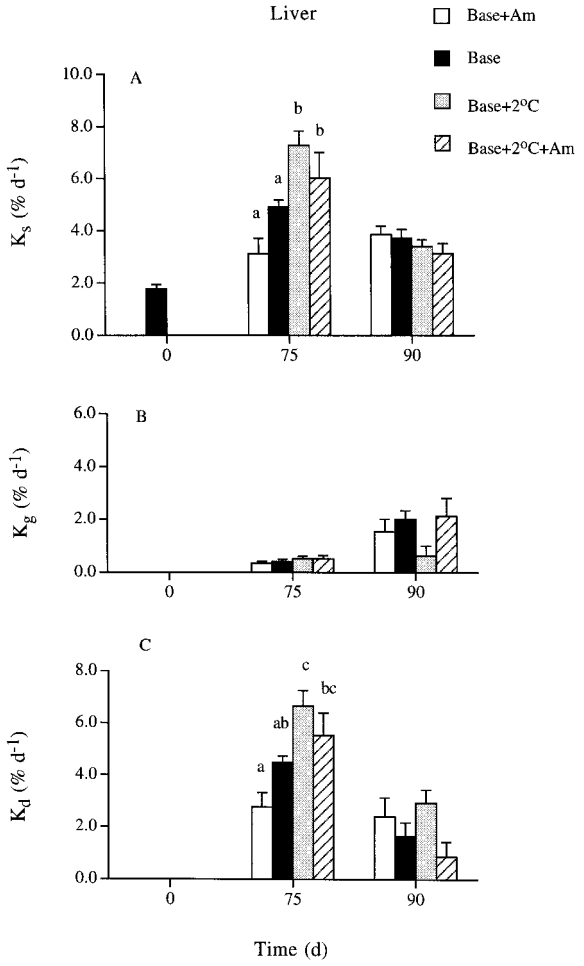

Figure 2. The effects of elevated winter temperature and an additional 70 μM total ammonia on the fractional rates (% d^{-1}) of A) protein synthesis (K_s), B) protein growth (K_g) and C) protein degradation (K_d), in the liver of hardwater-acclimated juvenile rainbow trout. Values are means (± SEM, n = 8–10) and those at each time that do not share a letter (where present) are significantly different from each other ($p < 0.05$).

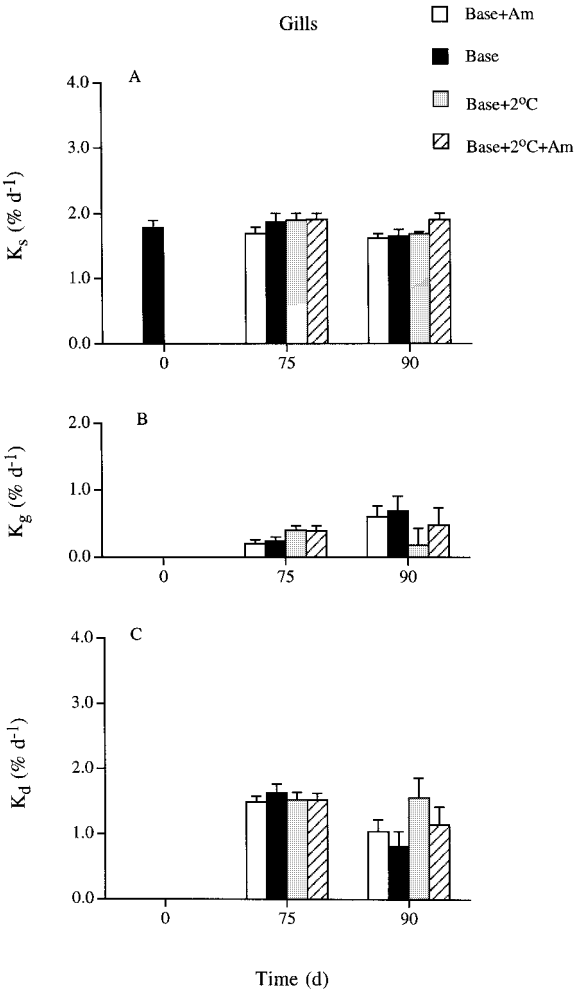

Figure 3. The effects of elevated winter temperature and an additional 70 μM total ammonia on the fractional rates (% d^{-1}) of A) protein synthesis (K_s), B) protein growth (K_g) and C) protein degradation (K_d), in the gills of hardwater-acclimated juvenile rainbow trout. Values are means (± SEM, n = 8–10) and those at each time that do not share a letter (where present) are significantly different from each other ($p < 0.05$).

Reid et al. (1995), ranging 4–9% d^{-1}. This lack of a temperature effect is not unprecedented: Foster et al. (1992) recorded no difference in the K_s of various tissues of cod acclimated to either 5 or 15 °C.

The principal purpose of the present work however, was to examine the effects on protein turnover, of a small (+2 °C) temperature change superimposed upon the natural thermal regime, rather than compare two or more constant, well-separated (≥ 5 °C) temperatures, as in the vast majority of previous studies. Despite this difference in approach, further support for the positive effect of temperature on protein turnover was obtained: rates of protein synthesis and degradation were significantly greater in the liver of HW-acclimated, and the gills of SW-acclimated trout at +2 °C. Reid et al. (1995) also demonstrated that a difference in temperature of only 2 °C superimposed on typical summer temperature was sufficient to result in a significant increase in protein synthesis.

The differences in protein parameters between base/control temperatures and +2 °C were used to calculate the changes expected with a 10 °C increase in temperature (Q_{10}) using the van't Hoff equation (data not shown). Two points of note emerged: Firstly, the Q_{10}s for protein growth (K_g) in both tissues at

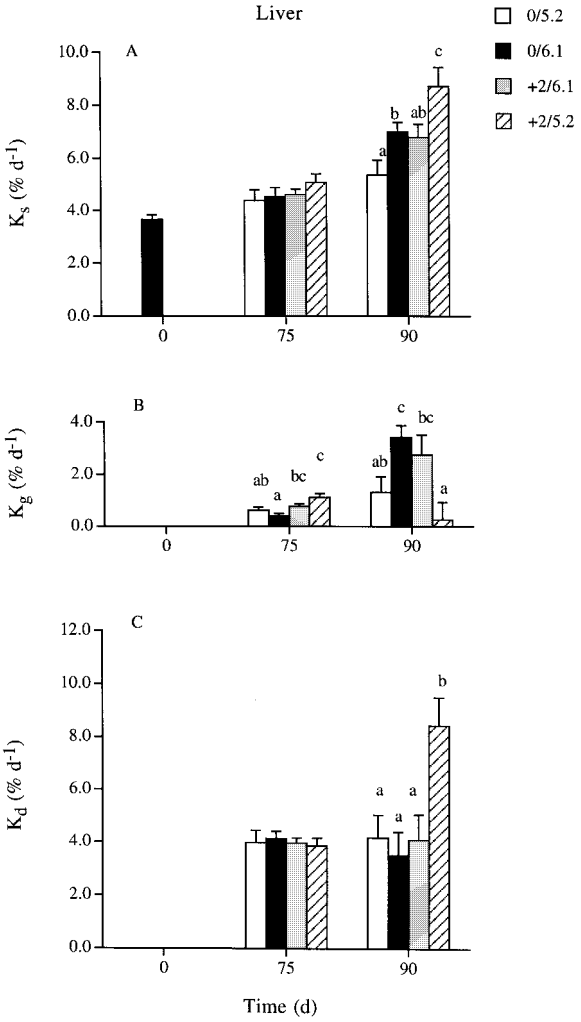

Figure 4. The effects of elevated winter temperature and low pH on the fractional rates (% d^{-1}) of A) protein synthesis (K_s), B) protein growth (K_g) and C) protein degradation (K_d), in the liver of sotwater-acclimated juvenile rainbow trout. Values are means (± SEM, n=6–10) and those at each time that do not share a letter (where present) are significantly different from each other ($p < 0.05$).

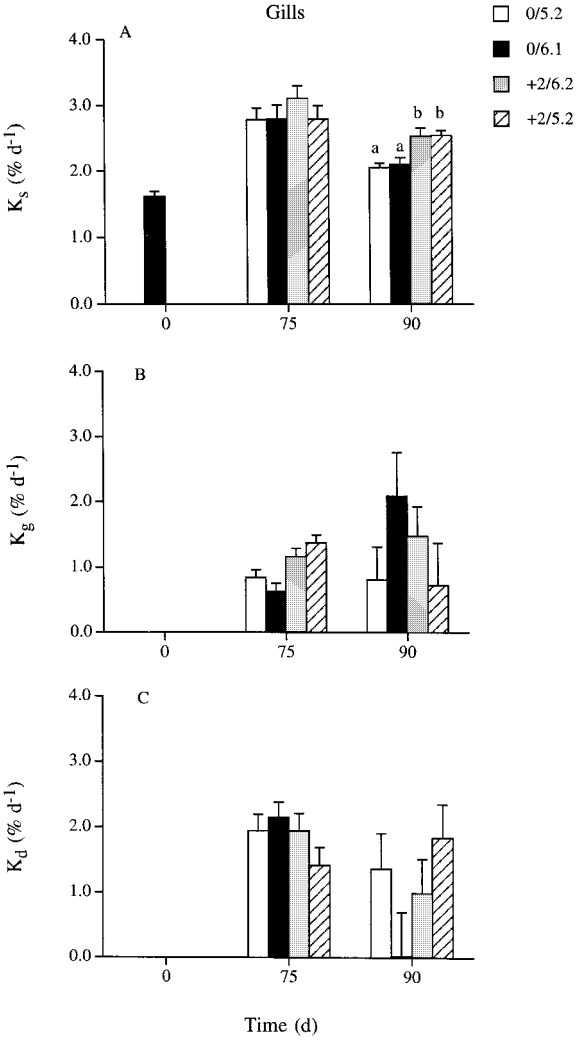

Figure 5. The effects of elevated winter temperature and low pH on the fractional rates (% d^{-1}) of A) protein synthesis (K_s), B) protein growth (K_g) and C) protein degradation (K_d), in the gills of sotwater-acclimated juvenile rainbow trout. Values are means (± SEM, n = 9–10) and those at each time that do not share a letter (where present) are significantly different from each other ($p < 0.05$).

day 75 were consistently very high, averaging 15.9. Whilst this value may seem excessive, it is in good agreement with the physiological data from these same fish of Linton et al. (1997b) who stated that '...the temperature coefficients (Q_{10}s) for appetite and other related physiological processes (growth, oxygen consumption, and nitrogenous waste excretion) over the relatively constant temperature phase during the first 75 days are extraordinarily high; 19.6.' Indeed, the Q_{10}s for whole-body specific growth rate (SGR, Table 1) at this time were 5.1 and 12.1 for HW- and SW-acclimated fish, respectively. The five-fold difference in stored energy in maximally fed brown trout (*Salmo trutta*) exposed to either 3.8 or 5.6 °C reported by Elliott (1976) would also tend to support such large temperature coefficients of (protein) growth. Whole-body growth and metabolism in the present experiments are considered in greater detail in associated papers (Linton et al. 1997b; D'Cruz et al. 1997).

The second noteworthy point from Q_{10} calculations was that although the Q_{10}s of K_s, K_g and K_d in each tissue considered separately were quite variable, both within and between sampling periods, the values

in the liver at any given time were generally very similar to the respective value in the gills for both HW- and SW-acclimated fish. This implies that previous thermal history is the dominant factor in determining the effect of elevated temperature on protein turnover.

The biochemical mechanisms of the increases in protein synthesis at +2 °C in the two tissues were, however, different. In liver the increase in K_s at elevated temperature was due to increases in RNA translational efficiency (K_{RNA}): +2 °C had no effect on the capacity for protein synthesis, C_s (RNA:protein). The majority of tissue RNA is ribosomal (Millward et al. 1973) and hence this mechanism implies a faster rate of ribosomal amino acid processing at the elevated temperature. In the gills however, the reverse was true: +2 °C caused a significant increase in the capacity for protein synthesis with no change in RNA translational efficiency ie. the treatment resulted in the production of additional ribosomal RNA. Both of these mechanisms have been observed in previous studies in which increases in protein synthesis have been measured in fish exposed to different environmental temperatures (Fauconneau and Arnal 1985; Pannevis and Houlihan 1992; Mathers et al. 1993, Sephton and Driedzic 1995). There is no indication that the alternate mechanisms are tissue specific, but in feeding studies, changes in RNA concentration and hence C_s, occur only relatively slowly, over a period of days (McLaughlin et al. 1995) or weeks (Houlihan et al. 1993), whereas changes in RNA activity, and hence K_{RNA}, may occur over only a few hours (McMillan and Houlihan 1992).

Increases in protein synthesis and degradation at +2 °C were not observed consistently throughout the experiments. Two possible explanations immediately arise. Firstly, ambient water temperature was relatively constant up to day 70 but then increased steadily to day 90: this changing temperature may have prevented thermal acclimation of rates of protein turnover. Loughna and Goldspink (1985) found that the temperature dependency of protein synthesis was modified as a result of acclimation in muscle of the carp, although no such modification was observed in rainbow trout muscle. Secondly, fish exposed to +2 °C ate more than those at ambient temperature (Table 1), and several studies have demonstrated that protein turnover increases with ration size (Houlihan et al. 1988; 1995c; Watt et al. 1988). However, both of these explanations apply equally to SW- and HW-acclimated fish and yet the stimulatory effect of +2 °C was seen only at day 75 in liver of HW-acclimated fish, and only at day 90 in gills of SW-acclimated fish. No clear explanation for this inconsistency can therefore be provided. The effects of ration on protein turnover during exposure to elevated temperature is dealt with in more detail in a subsequent study (I. J. Morgan, L. M. D'Cruz, J. J. Dockray, T. K. Linton and C. M. Wood, unpublished data).

The effects of exposure to sublethal pollutants (ammonia/low pH)

Ammonia

In the present study, exposure to 70 μM T_{Amm} in hardwater had no significant effect on protein synthesis or degradation in juvenile rainbow trout. In a similar, previous investigation carried out during the summer, 70 μM T_{Amm} caused a significant stimulation in growth of juvenile rainbow trout (Linton et al. 1997a) which was reflected in a significant increase in rates of protein synthesis (Reid et al. 1998). However, in the present experiments, whole-body growth was also unaffected by elevated environmental ammonia (Linton et al. 1997b). These differences between the results of the summer and winter studies are attributed to the difference in environmental temperature; it may be that at the low temperatures of the present study, metabolic rates were low such that any effect of 70 μM T_{Amm} on growth and/or protein turnover were not apparent.

Low pH

Environmental acidification is exclusively a softwater problem (Wood 1989) as hardwater has sufficient alkalinity to neutralize any potential toxicity to aquatic animals. The principal site of action of acidity in fish is the gills (McDonald 1983; Wood 1989) and hence it is in the gills that any effects of low pH on protein turnover were expected. Wilson et al. (1996) recorded a depression of gill K_s (-17%) and K_d (-27%) in juvenile rainbow trout exposed to pH 5.2 in softwater for 15 days and Reid et al. (1997) also observed a reduction in gill protein synthesis at low pH. However, no effects of low pH on gill protein turnover were seen in the present studies: although protein growth in the 0/5.2 group was considerably lower than that in the 0/6.2 group, the large within treatment variability rendered this difference insignificant. However, the present study was performed at markedly lower temperatures (6–9.5 °C) than either of these earlier investigation (15 °C: Wilson et al. 1996; 13–24 °C: Reid et al. 1997) and the rates of protein turnover

were correspondingly low in all treatments, perhaps to the extent that any potential effect of low pH was not apparent.

An alternative explanation for the absence of a reduction in protein synthesis in gills at low pH in the present experiments was that the increased acidity also stimulated appetite (Table 1; D'Cruz et al. 1997). Protein synthesis in gills varies with food consumption (Houlihan et al. 1988; Houlihan 1991) and therefore the acid-induced increase in appetite may have compensated for any suppression of gill protein synthesis that would otherwise have occurred. However, Reid et al. (1997) saw a reduction in gill protein tsynthesis at low pH, despite a simultaneous stimulation of appetite. The effects of low pH on protein turnover at a constant ration size are examined in a later study (I. J. Morgan, L. M. D'Cruz, J. J. Dockray, T. K. Linton and C. M. Wood, unpublished data).

The only significant effects of environmental acidity on the components of protein turnover in SW-acclimated rainbow trout were lower rates of synthesis and growth in the liver; degradation was not affected. The liver is not generally regarded as a target of acid toxicity but Reid et al. (1997) also noted a reduction in liver protein synthesis at low pH. The mechanism of this effect is uncertain but exposure to low pH has been shown to elevate plasma cortisol concentration (Marshall Adams 1985; Brown et al. 1990) and this in turn maintains a high plasma glucose concentration at the expense of growth (van der Boon et al. 1991). Indeed, the whole-body specific growth rate of juvenile rainbow trout was very slightly reduced at low pH, despite an increase in food consumption (Table 1). However, plasma glucose was not measured in the present experiments and whilst plasma cortisol concentrations were some 40–60% greater at pH 5.2 compared to pH 6.2, these differences were not significant (D'Cruz et al. 1997), and thus this hypothesis remains highly speculative.

Conclusions

In contrast to earlier studies carried out at higher temperatures, the levels of pollutants used in the present experiments did not substantially affect protein turnover in the liver and gills of rainbow trout during winter, perhaps due to low rates of metabolism. However, an increase in environmental temperature of only 2 °C was sufficient to stimulate protein synthesis and degradation in the liver and gills, and to stimulate whole-body growth. It can therefore be concluded that a simulated global warming scenario has no exacerbating effects on these parameters during the winter, and indeed could be considered to be beneficial to the growth of freshwater fish.

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