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The effects of elevated summer temperature and sublethal pollutants (ammonia, low pH) on protein turnover in the gill and liver of rainbow trout (*Oncorhynchus mykiss*) on a limited food ration

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Abstract

Protein synthesis, degradation and growth of the liver and gills were determined in juvenile rainbow trout (*Oncorhynchus mykiss*) fed a limited ration and exposed for 90 days to normal or elevated summer temperatures (+ 2°C above ambient) and either low pH (5.2) in softwater or 70 μM total ammonia in hardwater. The limited ration resulted in low rates of growth (< 0.80% per day) and protein synthesis in all fish. In softwater, whole-body growth was significantly inhibited by elevated temperature but stimulated by low pH, although tissue protein metabolism was generally unaffected by these treatments. There was no significant difference in final size between the groups of fish in hardwater, but liver protein synthesis and degradation were significantly lower at + 2°C, the reduction in synthesis being due to an inhibition of both the capacity for protein synthesis, C_s and the RNA translational efficiency, k_{RNA}. Gill protein metabolism was unaffected by the experimental treatments in trout in hardwater. The authors conclude that a global warming scenario would be detrimental to protein synthesis and growth in freshwater fish under conditions of food limitation in summer, and when late summer temperatures approached the upper thermal limit of the species, regardless of food availability.

Keywords: Ammonia; *Oncorhynchus mykiss*; pH; Protein synthesis; Rainbow trout; RNA; Ration; Temperature

1. Introduction

The importance of environmental temperature in determining the metabolic rate of fish, with profound effects on physiology and growth, is well known [2,6,21]. The majority of experimental studies on temperature have examined fish acclimated to two or more constant and well-separated temperatures, but whilst this approach is useful to demonstrate the effects of temperature unequivocally, it may be of limited predictive value to field situations in which both spatial and temporal variations in temperature may occur [7,22,8]. This situation arises in what is perhaps the most important issue in environmental studies of recent years, namely global climate change or global warming. Current global warming models predict increases in air temperature of 1.3–4.5°C over the next 50–100 years [20,41], with similar increases in the temperature of aquatic ecosystems [46]. Whilst fish are expected to be amongst the animals most affected by global warming [34] there is little empirical evidence to suggest precisely what such effects might be [37].

Previous studies in the laboratory have demonstrated that one of the physiological functions in freshwater fish to be affected by a small (2°C), chronic increase in temperature is protein turnover, i.e. the balance be-

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tween protein synthesis and degradation: Morgan et al. [42] demonstrated that an additional 2°C during the winter, when natural water temperatures ranged from 4–7°C, resulted in a significant increase in protein synthesis and degradation in the liver and gills of juvenile rainbow trout (*Oncorhynchus mykiss*). A similar stimulation of protein metabolism by simulated global warming was observed in spring and early summer (ambient temperature 13–20°C) [47]. However, in the late summer when ambient temperature reached 24°C, close to the upper thermal limit of juvenile rainbow trout (26°C [1]), the additional 2°C resulted in an inhibition of protein synthesis and whole-body growth of $\approx 20\%$ [47].

Another major factor affecting both growth [12,13,28,59] and protein turnover [24,25,36] is food consumption or ration. The studies of Reid et al. [47] and Morgan et al. [42] were carried out on fish fed to satiation twice daily such that the effects of the additional temperature on appetite could also be measured. Food consumption of fish in the wild has proved difficult to assess accurately [56] but low food availabilty during the summer may occur [4,16]. Moreover, the effects of ration and temperature may interact. As ration is reduced, super-optimal temperatures have an increasingly negative effect on growth [3,14] and lower growth rates during the warmer, summer months, compared to winter or spring, have been observed in natural populations of salmonids [15,60].

The authors' novel experimental design, which allowed additional temperature to be superimposed upon the natural thermal regime of the inshore region of Lake Ontario, Canada, was therefore used to examine the effects of simulated global warming on protein metabolism in the liver and gills of juvenile rainbow trout (*O. mykiss*) fed a limited food ration of 1% per day (wet weight food/wet weight fish). Many freshwaters are also subject to chronic contamination with pollutants, and current climate change scenarios predict important interactions with pollutants such as acidity (low pH) and ammonia [48,53,57]. The effects of temperature and ammonia were studied in the natural hardwater of Lake Ontario. However, environmental acidification is exclusively a softwater problem [64] as hardwater has sufficient buffering capacity to neutralize any potential acidity, and therefore the effects of temperature and low pH were studied in artificially-generated softwater. The liver was chosen for study as it is the major center of ammonia metabolism [45,65] whilst the gills are the principal sites of acid toxicity [38,64]. These tissues are also metabolically active and have high rates of protein synthesis in feeding fish [17,23] and so any effects of environmental change might be expected to be more pronounced than elsewhere.

The results of the present study are compared to those of similar studies in which the fish were fed to satiation [47,49,50]—a ration of 2.5–3.0% per day under similar environmental conditions. The reduced ration therefore represented a marked decrease in energy availability whilst still being sufficient for some growth to occur, based on other studies in the laboratory [61]. The authors predicted that the restricted ration would shift the optimum temperature for protein growth, and hence the temperature above which protein synthesis and growth would be inhibited, to a lower level. Furthermore, the authors predicted that the reduction in energy availability might exacerbate any inhibitory effects of pollutant stress on protein metabolism compared to fish fed to satiation.

Other metabolic and physiological effects of the present experimental treatments have been presented by Dockray et al. [10] (SW) and Linton et al. [30] (HW).

2. Materials and methods

2.1. Experimental animals

Juvenile rainbow trout (*O. mykiss*) of 2–5 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 which was drawn from the inshore region of Lake Ontario, at ambient temperature (initially 12.0°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 ≈ 1.0; pH 7.6–7.8. Photoperiod was controlled and adjusted weekly to mimic the natural photoperiod throughout the acclimation and experimental periods (April–September). The fish were fed a ration of 1% of body weight per day (wet basis) of Zeigler Trout Starter no. 3 (protein 50%; lipid 15%; water 12%, fibre 2%, calcium 2.3%, sodium 0.5%).

After a 2-week acclimitization period the fish were divided into two groups: hardwater (HW)- and softwater (SW)-acclimated fish. The hardness of the water supplied to the latter group was slowly decreased by increasing the proportion of artificially-softened water (generated by reverse osmosis) to achieve final concentrations of $[Ca^{2+}] \approx 25$ μM and $[Na^+] \approx 100$ μM (pH 6.2). The fish were allowed to acclimate to this softwater for a further 4 weeks prior to the start of experiments. The hardwater-acclimated fish were maintained in normal Hamilton tapwater throughout.

2.2. Experimental exposures

Groups of 150 HW-acclimated fish were transferred to each of eight 270 l tanks, representing four experimental treatments in duplicate as described in Linton et al. [30]. The treatments were: ambient temperature (re-

ferred to as 'base'); ambient temperature plus an additional 2°C (base + ΔT); ambient temperature plus an additional 70 μM total ammonia ($T_{Amm} = NH_4^+ + NH_3$; base + Am) and ambient temperature plus an additional 2°C and 70 μM T_{Amm} (base + Am + ΔT). Concentrations of T_{Amm} in the base and base + ΔT treatments, were 6.0 and 5.8 μM, respectively. These treatments had very little effect on water pH which ranged 7.5–7.6 in all tanks. The terminology chosen to describe these four treatment groups has been chosen for consistency with the report of Linton et al. [30] on the physiology and metabolism of these same fish, as well as with the earlier paper [42].

The same number of SW-acclimated fish were also divided into four, replicated treatments. The 'ambient' temperature in these treatments was 3–4°C greater than those in hardwater due to heat added by the reverse osmosis unit, and is referred to as the 'control' temperature [10]. The four treatments were: control temperature/control pH 6.2 (0/6.2); control temperature/sublethal pH 5.2 (0/5.2); control temperature + 2°C/control pH 6.2 (+2/6.2); control temperature + 2°C/sublethal pH 5.2 (+2/5.2). The terminology here has been chosen to avoid confusion with the different thermal regime of the hardwater/ammonia trials, and as consistent with the reports of Dockray et al. [10] on the physiology and metabolism of these same fish, and Morgan et al. [42].

Both HW- and SW-acclimated fish were exposed to their respective experimental treatments for 90 days. During this time, fish were fed daily at ≈1% body weight per day of the wet biomass of the fish in the base (hardwater) and 0/6.2 (softwater) tanks: biomass was measured weekly as described by Dockray et al. [10] and ration maintained at 1% of this value in all treatments until the next biomass measurement. As ration was calculated and administered on a 'per tank' basis, no errors and hence no statistical analyses were available for measurements of total food consumed (C) and food conversion ratio (FCR).

2.3. Measurement of protein turnover

Fractional rates of protein synthesis and growth were measured, and potein degradation calculated, in the liver and gills of 10 fish from each of the four treatments for both HW- and SW-acclimated fish immediately prior to the start of (day 0), and at 30, 60 and 90 days of, exposure. The methodology is described fully in Reid et al. [47] with modifications as in Morgan et al. [42]. Briefly: fish were not fed for 24 h prior to measurement. Protein synthesis (k_s) was measured using the flooding dose technique of Garlick et al. [19], adapted for fish as described in Houlihan et al. [26]. Fish were injected via the caudal vein with 10 μl g^{-1} body weight of 150 mM phenylalanine solution, containing 3.7×10^6 Bq ml^{-1} ^{3}H-phenylalanine. Following a 1 h incorporation period the fish were killed by a blow to the head and whole livers and branchial baskets (gill filaments and cartilage have very similar rates of protein turnover [33]) were removed, weighed and stored at −70°C for later analysis.

The fractional rate of protein synthesis, k_s (% per day), in the tissues 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 and SA_f the free-pool specific activity (both dpm $nmol^{-1}$) and t is the ^{3}H-phenylalanine incorporation period in minutes [26]. The rate of protein degradation (k_d, % per day) was calculated as the difference between the rate of protein synthesis (k_s) and the rate of protein growth (k_g, % per day); the latter calculated as the specific growth rate of tissue protein using the equation of Ricker [51]:

Protein growth rate, k_g (% per day)

$$= (\ln W_2 - \ln W_1) \cdot 100/t$$

where W_1 and W_2 are the initial and final weights of tissue protein (mg), respectively, and t is the growing period (days).

The RNA content of the tissues was measured using the orcinol method [43] and the total protein concentration by the method of Lowry et al. [32]. 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 per day) was calculated as [26]:

$$k_{RNA} = \frac{k_s}{C_s} \times 10$$

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 [42,47,49,50] using the natural log transformation of the allometric equation $Y = aX^b$ and exponents of −0.2 for k_s and C_s [23] and −0.41 for k_g [27].

2.4. Statistical analysis

Mean values ± 1 standard error of the mean (S.E.M.) are used throughout the text. Statistical differences between means were determined by analysis of variance followed by Fisher's multiple comparison test. Whole-body growth curves, using both linear and logarithmic models, were analysed using analysis of covariance to determine differences between slopes. All tests were performed at the 95% level of confidence.

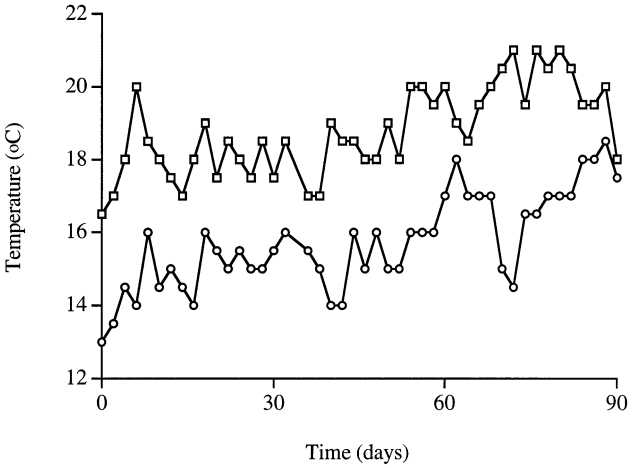

Fig. 1. Ambient water temperature profiles during exposure of juvenile rainbow trout on a limited ration to elevated temperature and sublethal pollutants in hardwater (base, circles) and softwater (control, squares).

3. Results

3.1. General, thermal regimes and whole-body growth

The natural water temperature of inshore Lake Ontario (base) increased slowly over the duration of the experiments; from 13°C at day 0 to 18°C around day 90 (Fig. 1). The control (soft-) water temperature followed a similar pattern but was consistently 3–4°C higher throughout due to heat imparted by the reverse osmosis unit in the softening process. Mortalities were generally very low in all treatments, however due to equipment failure, the entire +2/5.2 treatment in softwater was lost at day 77 and hence no data are available for these fish at day 90.

Growth rates and food conversion ratios in both HW- and SW-acclimated fish in the present experiments were much lower than those recorded in a previous summer experiments in which the fish were fed twice daily to satiation (Table 1). Significant differences were observed in growth between the groups of SW-acclimated fish fed a restricted ration. Fish at control temperature grew faster than those at +2°C and that those at pH 5.2 grew faster than those at pH 6.2 (Table 1). In HW-acclimated fish those exposed to +2°C grew slightly less over the first 60 days of the experiment (data not shown), but by day 90 had accelerated in growth to a size greater than those at base temperature, such that over the full 90 days, no significant differences in growth were observed (Table 1).

3.2. Protein turnover

3.2.1. Hardwater-acclimated fish

The fractional rates of protein synthesis (k_s) and degradation (k_d) in the liver of HW-acclimated fish showed a general increase over the duration of the experiment (Fig. 2 A, C). The fractional rate of protein synthesis in the liver of fish exposed to the base treatment was 2.2% per day at day 0 but reached 6.4% per day by day 90 (Fig. 2A). Protein growth (k_g) was relatively high ($\approx$1.5% per day) in all treatments initially (day 0–30) but much lower thereafter (Fig. 2B). Both k_s and k_d were significantly lower in those fish exposed to an additional +2°C. The inhibition of protein synthesis rates at the elevated temperatures was due to a decrease in RNA translational efficiency (k_{RNA}) at days 30 and 60 (Table 2) as the capacity for protein synthesis of the liver (C_s) was unaffected by exposure to +2°C (Table 3). However, at day 90, the

Table 1
Food consumption and growth in juvenile rainbow trout (*Oncorhynchus mykiss*) over 90 day exposure to chronic elevated temperature and sublethal pollutants at two levels of ration*

Treatment	Satiation ration ($\approx$2.5% per day)**, $n=60$					Restricted ration (=1% per day), $n=30$				
	W_i	C	W_f	SGR	FCR	W_i	C	W_f	SGR	FCR
HW-acclimated fish										
Base+Am	2.6 (0.1)	37.3	47.4 (2.2)b	3.23 (0.1)	1.40	4.7 (0.3)	5.4	6.9 (0.4)	0.45 (0.1)	0.43
Base	2.6 (0.1)	38.2	40.2 (1.9)a	3.04 (0.1)	0.98	4.7 (0.3)	5.4	7.0 (0.4)	0.47 (0.1)	0.43
Base+2°C	2.6 (0.1)	33.4	39.2 (1.7)a	3.01 (0.1)	1.10	4.7 (0.3)	5.8	6.9 (0.4)	0.45 (0.1)	0.40
Base+2°C+Am	2.6 (0.1)	33.6	38.1 (1.1)a	2.97 (0.1)	1.06	4.7 (0.3)	5.7	7.4 (0.4)	0.53 (0.1)	0.48
SW-acclimated fish										
0/5.2	3.0 (0.1)	53.9	55.5 (3.7)b	3.24 (0.2)	96.7	4.0 (0.1)	5.2	8.0 (0.3)c	0.77 (0.1)	76.9
0/6.2	3.0 (0.1)	44.1	47.0 (1.7)bc	3.06 (0.1)	100.7	4.0 (0.1)	5.2	7.8 (0.4)b	0.74 (0.1)	73.0
+2/6.2	3.0 (0.1)	35.7	32.3 (1.9)a	2.64 (0.1)	80.1	4.0 (0.1)	5.2	7.7 (0.4)a	0.73 (0.1)	71.2
+2/5.2	3.0 (0.1)	41.9	38.8 (4.8)ac	2.84 (0.3)	83.8	4.0 (0.1)				

* W_i and W_f, initial and final weight (g), respectively; C, total food consumed (g); SGR, specific growth rate (% per day) and FCR, food conversion ratio. Values of W_f and SGR that do not share a letter (where present) are significantly different from each other ($P<0.05$).

** Data from Linton et al. [30] for HW-acclimated fish on satiation ration, and from Dockray et al. [9] for SW-acclimated fish on satiation ration.

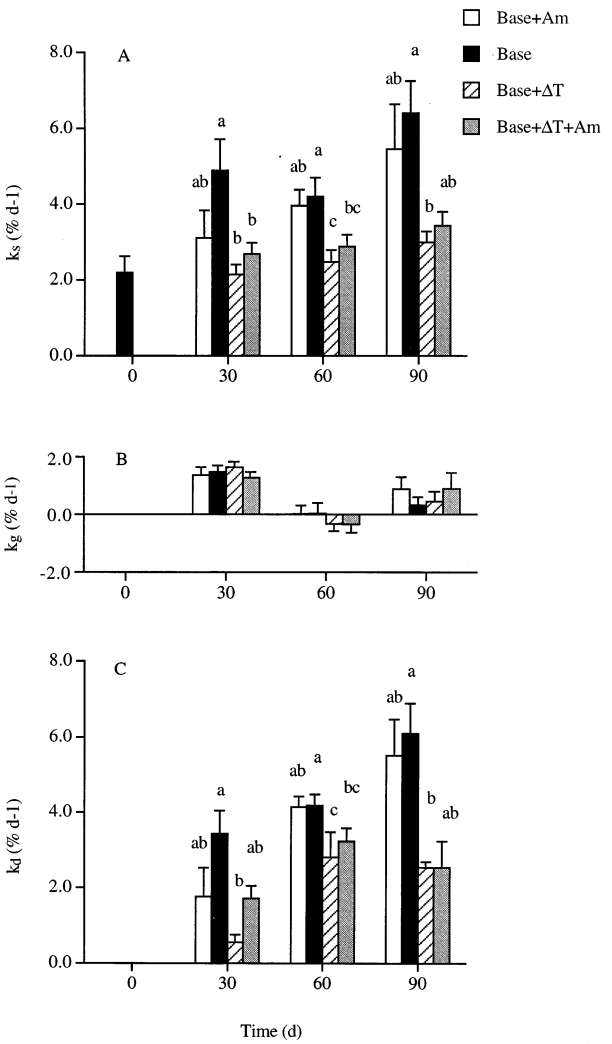

Fig. 2. The effects of elevated summer temperature and an additional 70 μM total ammonia on the fractional rates (% per day) of: (A) protein synthesis (k_s); (B) protein growth (k_g) and (C) protein degradation (k_d), in the liver of hardwater-acclimated juvenile rainbow trout on a limited ration. Values are means ($\pm$S.E.M., $n = 5$) and those at each time that do not share a letter (where present) are significantly different from each other ($P < 0.05$).

mechanism of k_s inhibition was a decrease in C_s; k_{RNA} was not affected by $+2$°C at this time. The addition of 70 μM T_{Amm} resulted in consistently lower values of k_s and k_d at base temperature, but higher values at the elevated temperature (ΔT), although these differences were not statistically significant.

The fractional rate of protein synthesis in the gills (Fig. 3A) of HW-acclimated fish at day 0 was similar to that of liver: 2.4% per day. However, unlike liver k_s, gill k_s remained relatively constant over the duration of the experiments. The capacity for protein synthesis of the gills was much lower than that of liver throughout the experiments (Table 3) and hence the gill RNA translational efficiency was markedly greater than that of the liver at day 0 (0.98 and 0.39 g protein synthesized g^{-1} RNA per day, respectively, Table 2), but this difference was lost with time. Fractional rates of protein degradation (Fig. 3C) were very similar to those of synthesis and therefore protein growth rates in gills were generally very low ($< 0.5\%$ per day) (Fig. 3B). Neither $+2$°C nor 70 μM T_{Amm} had any significant effect on either protein metabolism or RNA parameters of the gills.

3.2.2. Softwater-acclimated fish

The fractional rate of protein synthesis in the liver of SW-acclimated fish at day 0 was 2.2% per day (Fig. 4A); very similar to that in the liver of HW-acclimated fish at that time. By day 30 liver k_s had increased approximately twofold in three of the four treatments, and nearly 3-fold, to 6.0% per day, in the 0/5.2 group. The RNA translational efficiency, k_{RNA} showed similar increases from day 0 to 30 whereas the capacity for protein synthesis, C_s remained relatively constant (Table 2, Table 3). However, these apparent effects of exposure to low pH were not statistically significant. Indeed, liver protein metabolism and RNA parameters were unaffected by exposure to low pH and/or $+2$°C throughout the experiments (Fig. 4, Tables 2 and 3). Protein growth in the liver of SW-acclimated fish was relatively low (between 0.6 and -0.6% per day) from day 0 to 60 but was noticeably greater ($> 1.0\%$ per day) at day 90 in all three remaining treatment groups (Fig. 4B). The rate of protein degradation, k_d, showed a general, steady decline from $\approx 4.5\%$ per day at day 30 to $\approx 1.5\%$ per day by day 90 (Fig. 4C). Both k_g and k_d were unaffected by the experimental treatments at all sampling times.

The fractional rate of protein synthesis in the gills of SW-acclimated fish in three of the four treatment groups decreased between day 0 and day 30; from 2.1% per day to $\approx 1.5\%$ per day (Fig. 5A). The exception was the $+2/5.2$ treatment which maintained a pre-exposure rate of protein synthesis due to a significantly greater k_{RNA} (Table 2). By day 60, k_s in all treatment groups had fallen further to $\approx 1\%$ per day and the significant effect of low pH and $+2$°C had been lost. Some recovery of k_s was seen by day 90 to values of between 1.5 and 2.0% per day. The low values of k_s at day 60 were reflected in the corresponding rates of protein growth; whereas k_g was positive for all groups at other times, three of the treatment groups lost gill protein from days 30 to 60, particularly at the control temperature (Fig. 5B). Relatively high rates of gill protein degradation in the 0/5.2 and 0/6.2 groups also contributed to the loss of protein from day 30 to 60 (Fig. 5C).

Table 2
RNA translational efficiency (k_{RNA}, g protein synthesized g^{-1} RNA per day) in the liver and gills of juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to chronic elevated temperature and sublethal pollutants*

Treatment	Liver ($n=5$)				Gills ($n=10$)			
	0	30	60	90	0	30	60	90
HW-acclimated fish								
Base+Am		0.58 (0.05)a	0.69 (0.03)a	0.89 (0.06)		0.69 (0.10)	0.59 (0.04)	0.63 (0.05)
Base	0.39 (0.04)	0.93 (0.07)b	0.68 (0.02)a	1.02 (0.06)	0.98 (0.08)	0.62 (0.05)	0.54 (0.02)	0.65 (0.03)
Base+2°C		0.42 (0.02)a	0.43 (0.03)b	0.68 (0.01)		0.65 (0.06)	0.55 (0.03)	0.68 (0.05)
Base+2°C+Am		0.53 (0.02)a	0.55 (0.03)ab	0.94 (0.06)		0.69 (0.09)	0.56 (0.03)	0.66 (0.03)
SW-acclimated fish								
0/5.2		1.16 (0.06)	0.54 (0.04)	0.53 (0.04)		0.41 (0.03)a	0.38 (0.02)	0.47 (0.04)
0/6.1	0.38 (0.03)	0.73 (0.03)	0.65 (0.03)	0.66 (0.03)	0.90 (0.09)	0.44 (0.03)a	0.40 (0.03)	0.50 (0.02)
+2/6.1		0.76 (0.07)	0.74 (0.05)	0.70 (0.02)		0.53 (0.05)ab	0.41 (0.02)	0.58 (0.03)
+2/5.2		0.78 (0.02)	0.48 (0.02)			0.61 (0.05)b	0.39 (0.02)	

* Values are means ($\pm$ S.E.M.) and those at each time (i.e. within columns only) that do not share a letter (where present) are significantly different from each other ($P<0.05$).

4. Discussion

4.1. Global warming simulation

Although global climate change or global warming is perhaps the most important issue in environmental studies of recent years, very few experimental studies have been carried out to study the possible consequences. This is likely due to the difficulty both in predicting the parameters, such as magnitude and variation, of the warming scenario and in simulating this within the practical constraints of experimental biology. The present experimental design superimposed an additional 2°C, a conservative estimate of global warming [20,41], upon the thermal regime of Lake Ontario. Whilst the utilization of a constant temperature increment may be simplistic, there is insufficient information to predict accurately the temporal variation of the global warming effect over the time-scale of the present experiments. Therefore, the authors believe that at the time of writing, the experimental design used in the present and related studies [9,10,29,30,42,47,49,50], incorporating natural variation in water temperature, is the best simulation of global warming in freshwater that has been used in controlled, laboratory studies. The novelty of this design however, means that there are few data in the literature to which the authors' results can be directly compared. Therefore, where necessary, comparison has been made with data from the literature obtained at differing but constant temperatures.

4.2. The effects of ration

The fractional rates of protein synthesis, growth and degradation in the gills and liver of juvenile rainbow trout in the present study were generally much lower than those recorded in previous simulations of global warming [47,49,50] despite similar environmental conditions. For example, fractional rates of protein synthesis, k_s in the liver and gills of HW-acclimated fish were 2–6% per day and 1.0–2.5% per day, respectively in the present experiments, but 3–10% and 3–6% per day, respectively in the studies of Reid et al. [47,49,50]. This difference is likely due to the respective rations fed to the fish; 1% body weight per day (present experiments) versus satiation ($\approx$2.5–3.0% per day [47,49,50]). At a fixed temperature, whole-body protein synthesis increases with food consumption and is linearly related to protein intake [5,24,36], due either to an increase in ribosomal RNA or an increase in ribosomal RNA activity [25]. Within individual tissues the effect of ration on protein synthesis is less certain. Some studies have found that k_s in tissues that are considered to be relatively metabolically active, including liver and gills, is unaffected by starvation [44,55] whereas others have recorded a fall in k_s at reduced ration or following starvation [23,24]. The present results support the latter relationship and both C_s (the RNA:protein ratio or capacity for protein synthesis) and k_{RNA} (the RNA translational efficiency) were much lower in the fish fed a restricted ration (present experiments) than in those fed to satiation [49,50].

Table 3
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*

Treatment	Liver ($n=5$)				Gills ($n=10$)			
	0	30	60	90	0	30	60	90
HW-acclimated fish								
Base+Am		52.5 (1.1)	62.5 (2.9)	59.8 (2.3)a		33.7 (2.0)	34.2 (2.7)	30.4 (1.4)
Base	57.7 (1.2)	53.0 (0.4)	62.0 (1.8)	62.5 (0.6)a	22.1 (1.0)	40.4 (4.0)	36.5 (2.6)	31.9 (1.6)
Base+2°C		50.6 (0.8)	57.4 (0.7)	46.5 (1.7)b		33.8 (2.4)	32.0 (2.2)	28.0 (1.5)
Base+2°C+Am		50.1 (0.8)	52.9 (0.8)	43.9 (0.7)b		35.9 (3.1)	33.3 (2.3)	29.0 (2.0)
SW-acclimated fish								
0/5.2		51.2 (0.8)	46.9 (1.6)	51.4 (2.2)		28.0 (1.3)	26.9 (1.7)	31.5 (1.1)
0/6.1	61.0 (1.9)	52.7 (0.8)	47.1 (1.5)	45.0 (1.2)	24.0 (2.0)	32.3 (1.1)	27.1 (1.4)	31.7 (1.7)
+2/6.1		52.7 (0.9)	46.3 (0.9)	45.4 (1.1)		30.3 (1.5)	28.0 (2.0)	33.8 (1.8)
+2/5.2		52.3 (1.2)	42.8 (0.9)			34.9 (2.8)	25.2 (1.9)	

* Values are means (±S.E.M.) and those at each time (i.e. within columns only) that do not share a letter (where present) are significantly different from each other ($P<0.05$).

The fractional rates of protein degradation (k_d) and growth (k_g) of liver and gills of juvenile rainbow trout were also substantially reduced by a two- to 3-fold decrease in ration. For example, k_g was typically 0.5–1.5% per day, and occasionally <0 (i.e. negative), whereas the values from satiation-fed fish were always positive and typically 2–3% per day [47,49,50]. Most studies agree that a decrease in ration results in a corresponding decrease in k_g [5,23,36]. However, the results of previous studies on the effect of ration on k_d are contradictory. Houlihan et al. [24] found that increased food consumption stimulated whole-body protein degradation but others have found protein degradation to be independent of consumption [5,36].

4.3. The effects of elevated environmental temperature (global warming)

Several previous studies have found that protein synthesis in fish increases with environmental temperature, both when fish are acclimated to constant, well-separated (≥ 5°C) temperatures [17,31,58] and when a small temperature increase has been superimposed upon the natural, fluctuating thermal regime, as might occur in a global warming scenario [42,47,49,50]. An exception to this general relationship however, was observed by Reid et al. [47] when the addition of 2°C to ambient in late summer resulted in water temperatures of 24°C, approaching the upper thermal limit of rainbow trout (26°C [1]), and caused a significant inhibition of protein synthesis and whole-body growth of $\approx 20\%$. Reid et al. [47] therefore concluded that global warming could have serious, negative consequences for fish living near the upper limits of their temperature tolerance or in other energetically-demanding environments.

In the present experiments the effects of the simulated global warming were also inhibitory. Softwater-acclimated fish exposed to +2°C grew slightly less than those at control temperatures, and as all fish were fed the same ration, also had lower food conversion efficiencies. In HW-acclimated fish, the rates of protein synthesis and degradation in the liver were inhibited by additional temperature by ≈ 35–55%, although neither liver protein growth nor whole-body growth were affected. Although these experiments were carried out during the summer, the water temperatures did not approach lethal levels. Inhibitory effects of the simulated global warming were observed at ambient temperatures of only 14–20°C in the present study whereas at similar temperatures in previous studies on fish fed an unlimited ration, +2°C stimulated protein turnover [47,49,50].

These results confirmed the initial prediction that the restricted ration would lower the temperature above which protein synthesis would be inhibited by global warming. Many of the studies that have recorded the opposite trend i.e. an increase in protein synthesis with temperature did not control ration [31,35,54]. Fish exposed to elevated temperature in such studies may also have consumed more food [17] and therefore the effects of temperature versus ration are difficult to separate [37]. Few studies have examined the effects of both ration and temperature on protein turnover per se in fish, and none of these have been in the context of global warming. Foster et al. [18] found no difference in protein synthesis in cod exposed to 5 and 15°C when both groups were fed 3% body weight per day. However, the relationships between temperature (albeit constant), ration and whole-body growth, which is effected via protein synthesis, are better known. The specific growth rate of sockeye salmon (*Oncorhynchus nerka*) on fixed rations decreased as temperature increased above the optimal level for growth [2]. Similarly, for rainbow trout, Wurtsburgh and Davis [66] found that at feeding rates close to maintenance, elevated temperatures decreased growth. The ambient

temperatures of the present study were likely above optimal throughout the experiments; the optimal temperature of growth for sockeye salmon fed a ration of 1.5% per day, slightly above that of the present study, was only 5°C [2] and optimal temperature for growth decreases as ration decreases [2,14]. In the present experiments therefore, little energy was available for protein synthesis and values of k_s were generally low. The additional temperature of the simulated global warming increased maintenance costs and hence the energy available for growth was reduced still further, particularly in the warmer softwater, resulting in the observed inhibition of whole-body growth in SW-acclimated fish. In hardwater however, protein degradation was also lower at $+2$°C. It is possible that this reduction in k_d was a compensatory mechanism to maintain protein growth rather than a direct inhibition by additional temperature. Nevertheless, although fractional rates of protein growth were generally low, they were not significantly inhibited by simulated global warming.

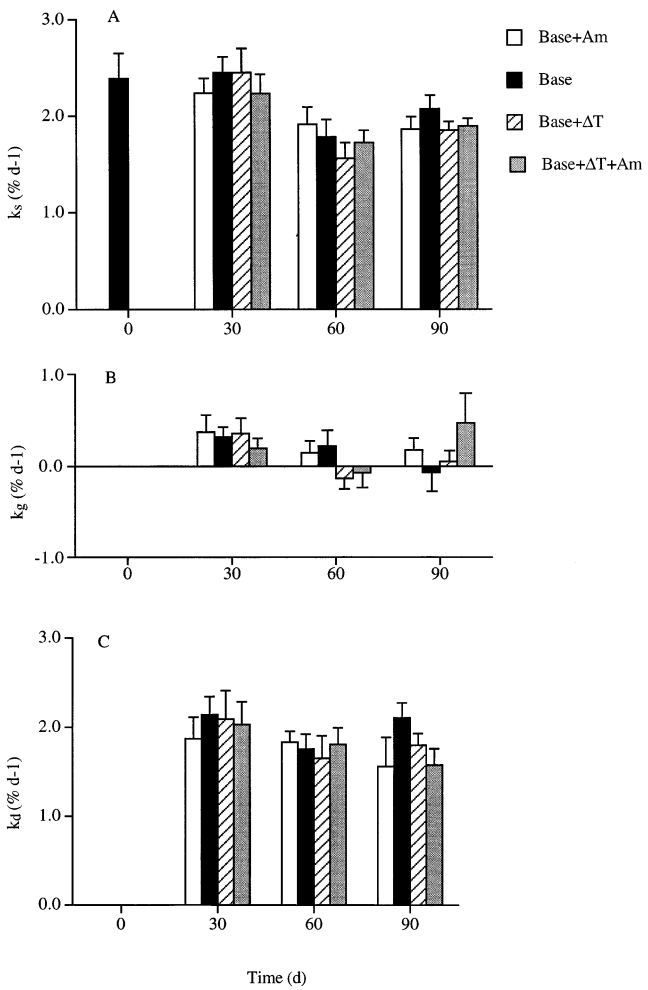

Fig. 3. The effects of elevated summer temperature and an additional 70 μM total ammonia on the fractional rates (% per day) of: (A) protein synthesis (k_s); (B) protein growth (k_g) and (C) protein degradation (k_d), in the gills of hardwater-acclimated juvenile rainbow trout on a limited ration. Values are means ($\pm$S.E.M., $n=10$) and those at each time that do not share a letter (where present) are significantly different from each other ($P<0.05$).

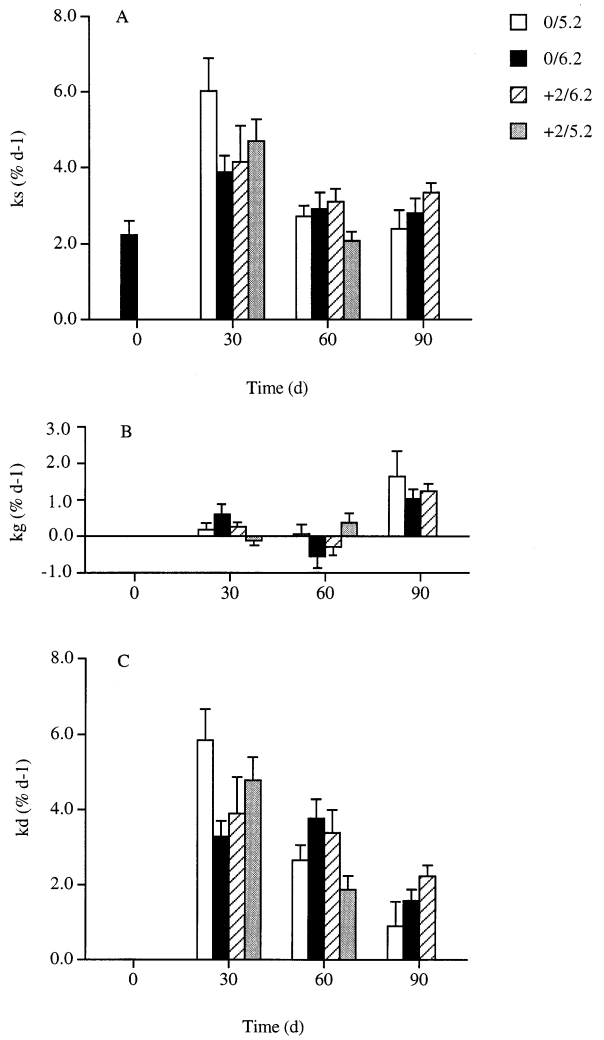

Fig. 4. The effects of elevated summer temperature and low pH on the fractional rates (% per day) of: (A) protein synthesis (k_s); (B) protein growth (k_g) and (C) protein degradation (k_d), in the liver of softwater-acclimated juvenile rainbow trout on a limited ration. Values are means ($\pm$S.E.M., $n=5$) and those at each time that do not share a letter (where present) are significantly different from each other ($P<0.05$).

4.4. The effects of exposure to sublethal pollutants

4.4.1. Ammonia

In a previous study performed at similar temperatures, but in which fish were fed to satiation, juvenile rainbow trout exposed to 70 μM total ammonia in hardwater (equivalent to 0.8 μM NH_3 at 15°C and pH 7.6) had a greater growth rate than controls [29] due to an apparent stimulation of protein synthesis [50]. The ammonia-exposed fish analysed in the present experiments showed no increase in whole-body growth. How-

ever, these fish did have a higher retention of protein nitrogen, albeit at an increased metabolic cost [30], for which an increase in liver protein synthesis, as an ammonia detoxifying process, was again suggested as a possible mechanism by Linton et al. [30]. However, the present results show that no such increase in k_s occurred in the base + Am group; indeed there was a consistent, though non-significant trend towards reduced protein metabolism (both k_s and k_d) in the liver of these fish. Moreover, this trend was not affected by the global warming scenario, contrary to the authors' initial prediction. Although the liver is generally considered to be the principal organ involved in nitrogen-metabolism in fish [45,66], it is possible that the increased whole-body nitrogen retention in ammonia-exposed fish was due to enhanced protein synthesis in other tissue(s), such as white muscle.

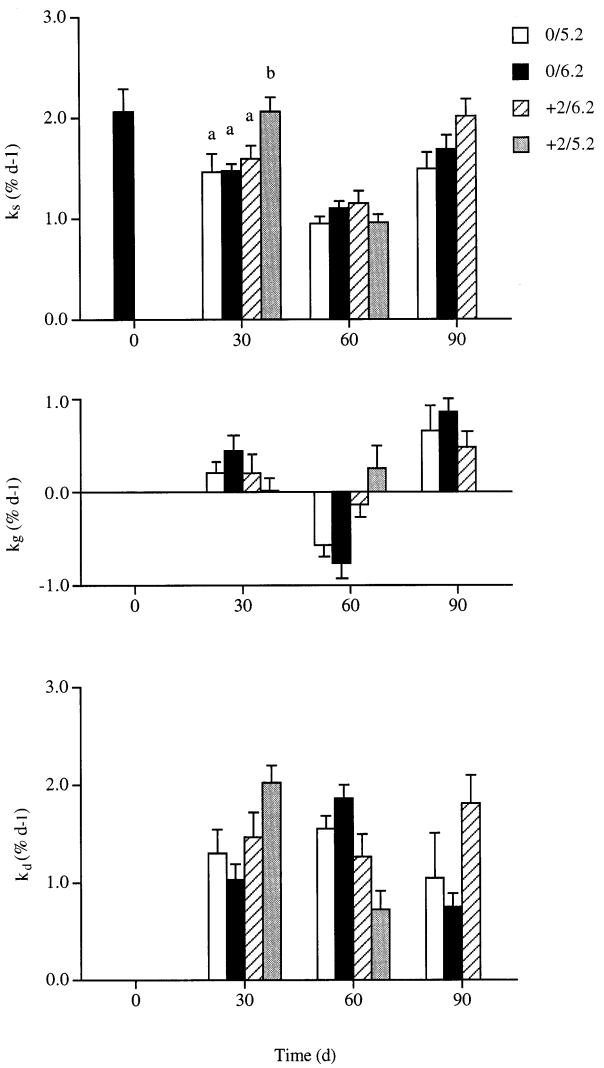

Fig. 5. The effects of elevated summer temperature and low pH on the fractional rates (% per day) of: (A) protein synthesis (k_s); (B) protein growth (k_g) and (C) protein degradation (k_d), in the gills of softwater-acclimated juvenile rainbow trout on a limited ration. Values are means (± S.E.M., $n = 10$) and those at each time that do not share a letter (where present) are significantly different from each other ($P < 0.05$).

4.4.2. Low pH

Environmental acidification is exclusively a softwater problem as the high alkalinity of hardwater buffers any potential change in pH [64]. The effect of low pH on whole-body growth in freshwater fish may be dependent upon the severity of the acidification. The whole-body growth of satiation-fed fish exposed to pH 5.2 was significantly greater than that of controls (pH 6.2) in the studies of both Wilson et al. [62] and Dockray et al. [9], as a result in the latter of an acid-induced increase in appetite. In contrast, several earlier studies in which the acid challenge was more severe recorded an inhibition of growth at low pH [11,40,52]. Exposure to pH 5.2 compared to pH 6.2 also caused an increase in growth in the present study even though food consumption was equal, and relatively low, for both groups. Dockray et al. [10] concluded that this maintenance of performance under acid conditions and food limitation was achieved via a decrease in activity. The routine metabolic rate of the SW-acclimated fish in the present experiments was some 40% lower than fish exposed to similar conditions but fed to satiation, and was not stimulated by the global warming scenario, and hence a greater proportion of available energy was available for growth [10].

Low pH exerts its toxicity primarily via disturbance of gill ionoregulatory physiology [38,64]. Wilson et al. [63] and Reid et al. [49] found that low pH inhibited protein synthesis in the gills of rainbow trout fed to satiation. Protein degradation was also inhibited such that turnover was suppressed and no net change in protein growth occurred. In the present experiments low pH had no effect on gill protein metabolism, despite the reduced ration and hence, presumably, a decrease in dietary amino acids available for protein synthesis. This result was contradictory to the authors' initial prediction that the reduced ration would exacerbate the inhibitory effects of environmental acidification on protein metabolism. Instead, the low rates of protein metabolism seen in all tissues due to the low ration may have prevented the possibility of any further inhibition by increased acidity. The fractional rate of protein synthesis in the gills in the present study was even lower than values in the literature for starved rainbow trout [39,55]. A similar lack of impact of pH 5.2 was seen in rainbow trout in which gill protein turnover had been suppressed by low environmental temperature [42].

5. Conclusions

The effect of a global warming scenario on protein metabolism (synthesis, degradation, growth) in freshwater fish depends upon environmental temperature (i.e. season) and food availability. In winter the additional temperature potentially stimulates protein synthesis and growth if sufficient food is available to fulfill that potential. However, summer temperatures are likely to be above optimal for all but satiation fed fish and hence maintenance of growth would require a much greater ration. If extra food is not available then additional temperature would inhibit protein synthesis and growth performance. At maximum summer temperatures, additional temperature will likely be inhibitory irrespective of food availability. The presence of sublethal toxicants would appear to be inhibitory only at times when protein synthesis is otherwise maximized by conditions of high ambient environmental temperature and high food availability.

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