# Effects of chronic environmental acidification and a summer global warming scenario: protein synthesis in juvenile rainbow trout (*Oncorhynchus mykiss*)

**S.D. Reid, J.J. Dockray, T.K. Linton, D.G. McDonald, and C.M. Wood**

**Abstract**: Protein synthesis ($K_s$), net accretion ($K_g$), and degradation ($K_d$) in liver, gills, and white muscle were measured using a flooding dose of [$^3$H]phenylalanine in juvenile rainbow trout chronically exposed (90 days) to softwater in the presence or absence of sublethal acidity ($H_2SO_4$, pH 5.2) alone or in combination with a 2°C elevation in the normal temperature profile over the months of June–September 1993 (control temperature range 13–24°C). Chronic sublethal exposure to low pH reduced protein synthesis and degradation in both the gill and liver with little apparent impact on white muscle. As a result, protein was increased in the affected tissues. This suggested that both liver and gill have some capacity to compensate for the effects of acid exposure. The 2°C elevation in the normal temperature profile resulted in a slight increase in protein turnover in both gills and liver. However, during the period of peak water temperature, the 2°C elevation in temperature triggered a dramatic reduction in the protein turnover rates in these tissues. The exact mechanism by which these modifications in protein turnover occurred could not be clearly established. Overall, environmental acidification in combination with a summer global warming scenario would decrease fish growth and survival, most notably during periods of peak temperatures.

**Résumé** : La synthèse protéique ($K_s$), le dépôt net ($K_g$) et la dégradation ($K_d$) dans le foie, les branchies et les muscles blancs ont été mesurés à l'aide d'une dose saturante de [$^3$H]phénylalanine chez des truites arc-en-ciel juvéniles exposées de manière chronique (90 jours) à de l'eau douce en présence ou en absence d'une acidité sublétale ($H_2SO_4$, pH 5,2) seule ou en combinaison avec une élévation de 2°C du profil de température normal au cours des mois de juin–septembre 1993 (étendue de température témoin : 13–24°C). L'exposition chronique sublétale à un faible pH a réduit la synthèse et la dégradation des protéines dans les branchies et le foie, mais très peu dans le cas des muscles blancs. Il en a résulté une augmentation des protéines dans les tissus touchés. Ces constatations indiqueraient que le foie et les branchies ont une certaine capacité pour compenser les effets d'une exposition acide. Une augmentation de 2°C du profil de température normal a entraîné une légère augmentation du renouvellement des protéines dans les branchies et le foie. Cependant, au cours de la période de température maximale de l'eau, l'élévation de 2°C a déclenché une réduction très prononcée des taux de renouvellement des protéines dans ces tissus. Le mécanisme exact par lequel ces modifications du renouvellement des protéines surviennent n'a pu être établi clairement. Globalement, l'acidification du milieu en combinaison avec un scénario de réchauffement global estival aurait pour effet de diminuer la croissance et la survie des poissons, plus particulièrement durant les périodes de températures maximales.
[Traduit par la Rédaction]

## Introduction

Environmental acidification has proven to have a significant detrimental impact on fish populations in regions sensitive to this anthropogenic toxicant (Leivestad and Muniz 1976; Beamish 1976; Kelso et al. 1990). The physiological impact on freshwater fish depends on many factors including water chemistry (e.g., hardness, alkalinity, presence of aluminum) and exposure duration. The gill has been clearly established as the target organ of acute exposure to acidified environments (see review by Reid 1995). The most frequently reported physiological impact is a disturbance in electrolyte balance, the result of which is internal hemoconcentration and subsequent circulatory collapse (Milligan and Wood 1982; Wood 1989). However, under chronic sublethal exposure conditions, Audet et al. (1988) have shown that electrolyte losses gradually decrease and plasma electrolytes stabilize. Despite the apparent reduction in physiological impact during chronic exposure, recovery did not occur. In fact, it has been shown that prior exposure to low-pH water generally exacerbates the physiological impact of subsequent acid exposures (Audet and Wood 1988; Reid et al. 1991), i.e., sensitization rather than acclimation occurs.

One could argue that any acclimation or recovery of electrolyte homeostasis in response to chronic acid exposure might be associated with alterations in gill protein turnover. For

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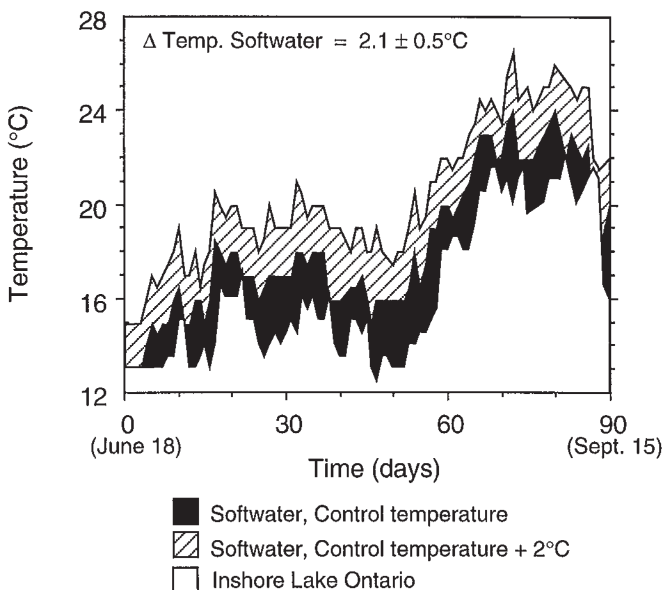
**Fig. 1.** Temperature profile for the softwater exposure system during the 90-day experiment that ran over the summer of 1993 from June 18 to September 15. Juvenile rainbow trout were exposed to the natural daily and seasonal temperature variability (control temperature; solid) representative of inshore Lake Ontario (open) or the control temperature raised by 2°C (control temperature + 2°C; cross hatched).




example, an increase in protein synthesis would be needed to build more transport sites and cells or to repair existing ones. Similarly, sensitization could be reflected in, and perhaps be caused by, increased rates of protein degradation. Wilson et al. (1996) measured protein turnover in specific tissues of juvenile trout during exposure to pH 5.2 softwater. Gill protein synthesis and degradation rates were found to be significantly reduced in fish exposed to acid conditions for 15 days when compared with control fish (6.5 softwater alone). However, the net rate of protein accumulation was not affected. Alterations in tissue protein turnover resulting from longer duration exposure (e.g., longer than 15 days) to environmental acidification have not yet been studied.

A potentially confounding influence on the physiological impact of environmental stressors is temperature. Freshwater fish are exclusively poikilothermic, i.e., their body temperature is essentially identical to, and set by, the temperature of the water in which they live. Temperature is the single most important factor determining their metabolic rate (i.e., cost of living), which in turn influences a variety of dependent physiological variables ranging from reproductive rate to growth rate to swimming performance (Brett 1964; Fry 1971; Elliot 1975; Houlihan 1991; Cho 1992). The effects of global warming, a predicted rise in global mean air temperature of 1.3–4.5°C (Hansen et al. 1988; Mohnen and Wang 1992), on fish and fisheries are therefore likely to be substantial (Regier et al. 1990; Schindler et al. 1990), and yet to date, there is a critical scarcity of hard experimental data on the chronic effects of relatively small temperature elevations over the annual cycle. Furthermore, there are virtually no data on how the effects of incremental temperature changes will interact with the effects of anthropogenic toxicants such as environmental acidification.

The goals of this study were to determine (*i*) if there are changes in tissue growth and protein turnover rates in much longer duration exposure of juvenile trout to reduced water pH, 3 months as compared with 15 days (cf. Wilson et al. 1996), and (*ii*) to determine what influence the predictions of the global warming scenario might have on protein turnover rates, alone and in combination with the impact of environmental acidification. A related study has described other metabolic and physiological responses associated with the same experimental regime (Dockray et al. 1996).

## Materials and methods

### Experimental animals

Approximately 1600 rainbow trout (*Oncorhynchus mykiss*) of both sexes weighing initially 1.5–4 g were obtained April 19, 1993, from Rainbow Springs Hatchery (Thamesford, Ont.). Fish were held in a 400-L fibreglass tank supplied with dechlorinated Hamilton tap water ($[Ca^{2+}]$ = 1.95 ± 0.22 mequiv.·L$^{-1}$ (mean ± SE), $[Na^{+}]$ = 556 ± 33 µequiv.·L$^{-1}$, pH = 7.4–7.8) at the city supply temperature (inshore Lake Ontario; initially 11°C). During this initial holding period (1 week), fish were fed dry trout pellets (Zeigler, Salmon Starter) at a ration of 1% (wet weight of feed) of wet body weight per day. Photoperiod was controlled and was adjusted weekly to mimic the natural photoperiod during the course of acclimation and chronic exposure.

### Softwater acclimation

Fish were gradually acclimated to synthetic softwater following the 1-week period of acclimation to laboratory conditions. The total period for acclimation to synthetic softwater prior to start of exposure was approximately 8 weeks, from April 25 to June 18, 1993. Synthetic softwater was produced by mixing dechlorinated Hamilton City tap water with deionized water, generated by reverse osmosis (Anderson Water Treatment Systems, Dundas, Ont.), in a ratio of 1:40. The resultant soft water had a $[Ca^{2+}]$ and $[Na^{+}]$ of 57.7 ± 5.9 and 97.6 ± 5.9 µequiv.·L$^{-1}$, respectively, and a pH of 6.27 ± 0.03 and was representative of natural softwater in eastern North America (Beamish 1974; Beamish et al. 1975). The reverse osmosis unit added 2°C to the temperature of the incoming water (from inshore Lake Ontario), so the acclimation temperature was approximately 13°C. Fish were fed during acclimation and maintenance in synthetic softwater or hardwater at a ration of 1% of body weight per day.

### Chronic exposure to elevated temperature and reduced pH

Fish were exposed for 90 days (June 18 – September 15, 1993) to one of four combinations of temperature and water pH in replicated tanks (170 per tank) continuously supplied with 1.2 L·min$^{-1}$ aerated, synthetic softwater generated as described previously. When the exposures were initiated in mid June, "control" and "+2°C" water temperatures were 13 and 15°C (Fig. 1). After 3 days the water temperature rose by 2°C because of the generation of deionized water by reverse osmosis. Therefore, control water temperatures were actually 2°C greater than true ambient (see Reid et al. 1995). Despite this unavoidable increase in water temperature, the initial 30 days of the exposure was characterized by a general increase in water temperature. Water temperature rose 3°C during this period but peaked at 18.5 and 20.5°C on day 17 for control and +2°C exposures, respectively. The second 30-day period (days 30–60) was characterized by a gradual decline in water temperature as the water temperature fell by approximately 2°C over the first 20 days of this period. However, during the subsequent 10 days of this period, control and +2°C exposure water temperatures rose rapidly from 16 to 20°C and from 17.5 to 22°C, respectively. The highest water temperatures were reached

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during the final 30 days of this study (days 60–90). The peak temperatures were 24 and 26.5°C in the control and +2°C exposures, respectively, which was near or at the upper lethal temperature for rainbow trout (Kaya 1978). Following 20–23 days of extreme temperature, water temperatures fell by 2.5–3°C over the final days of the experiment.

At these water temperatures, synthetic softwater pH fluctuated from 5.9 to 6.4 (mean 6.27 ± 0.03). Low-pH synthetic softwater (control and +2°C, pH 5.2) was generated with automatic titration of inflow water with 0.1 M sulfuric acid to a nominal pH of 5.2 (mean 5.27 ± 0.03). Acid titration was constantly monitored using Leeds Northrop Meridian II industrial electrodes (model 074383) and controlled by a custom-built system (Anderson Water Systems, Dundas, Ont.). In-tank water pH was independently verified using a hand-held pH meter (Markson, model 88).

During chronic exposure, fish were fed according to a different protocol from that used during laboratory and softwater acclimation. This protocol involved hand feeding each tank of fish to satiation twice daily (08:30 and 16:30) with the same commercial trout pellets used and described above (Zeigler Salmon Starter, 50% protein; ~12% water). With this feeding regime, the weight of food consumed provided an indicator of appetite, because the fish were able to consume as much food as desired.

### Rate of protein synthesis, accretion, and degradation

Protein synthesis, accretion, and degradation rates in branchial baskets, liver, and white muscle were determined for temperature controls and their respective treatment groups prior to the initiation of (day 0) and at 30, 60, and 90 days of exposure.

Tissue protein synthesis was determined from the incorporation of radioactive phenylalanine based on the method of Garlick et al. (1980), which was modified by Houlihan et al. (1986) for use in fish. Fish were not fed 24 h prior to injection. On the day of injection, 10 fish from each tank ($N = 10$ per treatment) were randomly selected from each treatment tank, quickly blotted dry, weighed to the nearest 0.01 g, then injected via the caudal vein with a solution of 150 mM L-phenylalanine (Sigma, St. Louis, Mo.), containing $37 \times 10^6$ $Bq \cdot mL^{-1}$ of L-2,6-[$^3$H]phenylalanine (Sigma, St. Louis, Mo.) in Cortland saline (Wolf 1963) at pH 7.5 (injection dose specific activity 1480 $dpm \cdot nmol^{-1}$). The dose was 1.0 $mL \cdot 100\ g^{-1}$ body weight, and the fish were not anaesthetized. Following injection, the fish were placed in individual darkened 1-L containers fitted with lids and airlines and containing water taken from the treatment tank from which they had been removed. Approximately 60 min after injection (exact time noted), fish were killed by a blow to the head, and the branchial basket, liver, and a sample of white muscle dorsolateral to the dorsal fin were dissected out and frozen in liquid nitrogen. Dissections were completed within 2–4 min. Once frozen, tissues were individually wrapped in aluminum foil, temporarily stored in liquid nitrogen until all fish had been sampled, then stored at –75°C for later weighing (organ–somatic indices, tissue growth rates) and analysis (protein turnover).

### Analysis

Tissue protein and RNA content, and fractional rate of protein synthesis ($K_s$) were analyzed as detailed in Houlihan et al. (1986). Briefly, tissue samples were homogenized using an electric tissue grinder (IKA A10) in ice-cold 20% perchloric acid (PCA), and the denatured proteins were separated by centrifugation. The PCA in the supernatant was precipitated with tripotassium citrate and centrifuged, leaving the free phenylalanine in solution. Sample phenylalanine was converted to β-phenylethylamine by L-tyrosine decarboxylase, extracted using *n*-heptane, and analysed by a ninhydrin reaction. The content of [$^3$H]phenylalanine was measured using liquid scintillation counting with samples corrected for quench. Sodium hydroxide was used to resuspend the PCA-extracted tissue pellet, and duplicate aliquots were taken for analysis of protein content (Lowry et al. 1951). The remaining suspension was acidified with PCA, centrifuged, and the resultant supernatant was analyzed for total RNA by the orcinol assay of Munro and Fleck (1966). The pellet, containing protein and DNA, was washed twice with PCA and then hydrolyzed with HCl. Subsequently, the HCl was removed by evaporation. The free amino acids were resuspended in sodium citrate buffer and phenylalanine in the samples was determined as described above. The protein synthesis rate, $K_s$ ($\% \cdot day^{-1}$), was calculated as

$$K_s = \frac{SA_p}{SA_f}\left(\frac{1440}{t}\right)100$$

where $SA_p$ is the protein-bound specific activity ($dpm \cdot nmol^{-1}$), $SA_f$ is the specific activity of the pool of free amino acids ($dpm \cdot nmol^{-1}$; average measured value $1678.2 \pm 174.0$, $N = 40$), 1440 is the number of minutes in a day, $t$ is the exact time (min) from the [$^3$H]phenylalanine injection to tissue sampling, and 100 is the conversion to percent.

The rate of protein degradation ($K_d$; $\% \cdot day^{-1}$) for gill and liver was calculated as the difference between $K_s$ and the net protein accretion of the tissues ($K_g$), with the latter determined as the product of the average tissue growth rate and average tissue protein content. Specific tissue growth rates ($K_g$; $\% \cdot day^{-1}$) were calculated using the equation of Ricker (1979):

$$K_g = \frac{(\ln W_2 - \ln W_1)}{t}\,100$$

where $W_1$ and $W_2$ are the final and initial tissue weights (g), respectively, and $t$ is the length of the growing period (days). For white muscle, total white muscle content of individual fish was estimated assuming that this tissue represents 40% of total fish weight (Houlihan and Laurent 1987).

This method of estimating protein turnover greatly underestimates $K_s$ in white muscle when incorporation times are less than 2 h (Foster et al. 1992); the most frequently used incubation for such assays is 1 h. Therefore, white muscle $K_s$ is only a relative estimate of protein synthesis in this tissue. Further, it is for this reason that white muscle $K_d$ could not be calculated.

Liver–somatic and gill–somatic indices were calculated as the ratio of organ weight to total fish weight, expressed as a percentage of the total, prior to the initiation of the exposures and at each subsequent sampling period.

As there were differences in the final body weights of the fish, protein synthesis and accretion rates were corrected for body size to a standard 40-g fish (the average weight of the fish at the end of the 90-day exposure) using the log transformation of the allometric equation $Y = aX^b$ and exponents of 0.2 for protein synthesis and 0.41 for protein accretion in accordance with the methods of Jobling (1983) and Houlihan et al. (1986).

Protein synthesis capacity was determined as the ratio of micrograms of tissue RNA to milligrams of tissue protein and tissue translational efficiency of RNA ($K_{RNA}$, g protein $synthesis \cdot g^{-1}\ RNA \cdot day^{-1}$) calculated by dividing the protein synthesis capacity by the appropriate $K_s$ value multiplied by 10 (Sugden and Fuller 1991).

### Statistical analysis

Values shown in the figures are means ± 1 SE. Statistical differences amongst means were determined by analysis of variance (ANOVA) followed by Fisher's LSD multiple comparison test, using a commercial statistical software package (Statview 512$^+$); 95% was accepted as the level of confidence.

## Results

### Organ–somatic indices

Prior to exposure, the branchial baskets and livers of the softwater-acclimated trout represented 2.9 and 1.3% of the total body weight, respectively (Fig. 2). However, as the treatment

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**Fig. 2.** The effect of water temperature and pH on the (A) branchial basket – somatic and (B) liver–somatic indices of softwater-acclimated fish. The organ–somatic indices were measured prior to (day 0) and at 30, 60, and 90 days of exposure. Data from fish exposed to control water temperature at pH 6.2 are represented by the solid histograms; control water temperature at pH 5.2, by the heavily hatched histograms; control water pH at +2°C, by the open histograms; and +2°C water at pH 5.2, by the lightly hatched histograms. Data are means + 1 SE (N = 10).

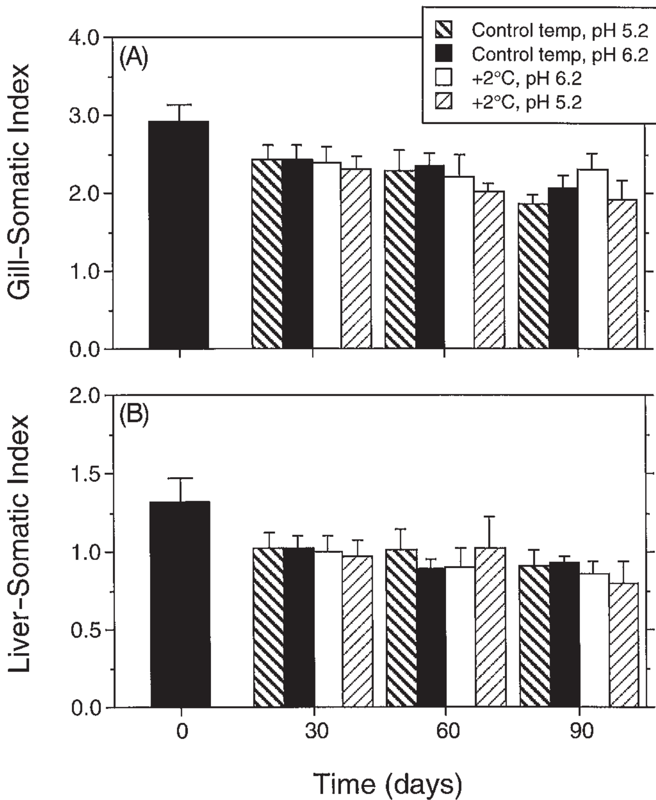
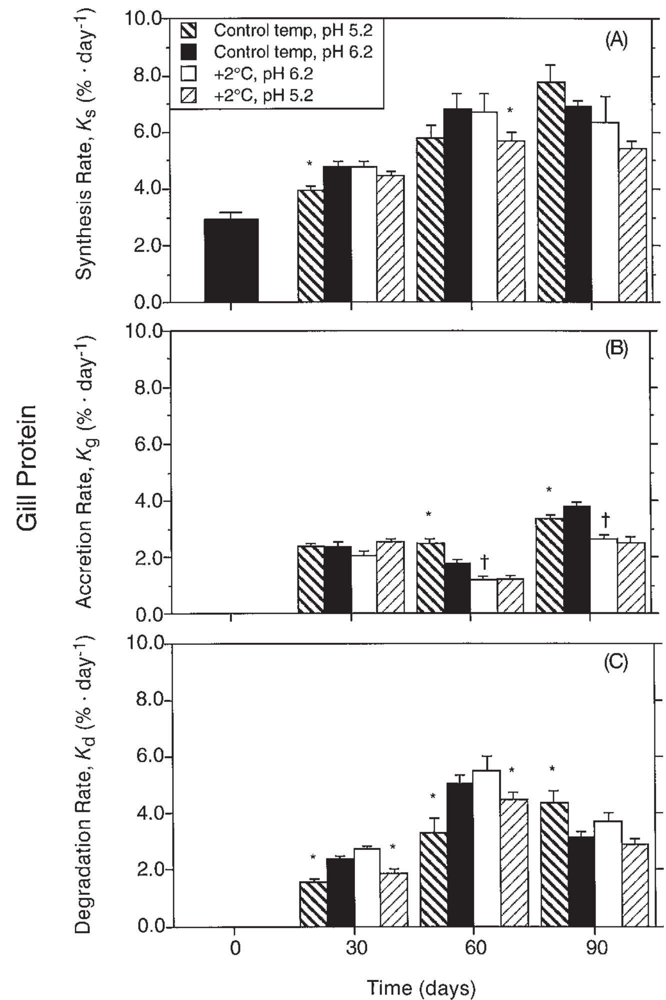



**Fig. 3.** The effect of water temperature and pH on gill protein (A) synthesis ($K_s$), (B) accretion ($K_g$), and (C) degradation ($K_d$) rates in softwater-acclimated trout. All rates were calculated on a %·day$^{-1}$ basis and determined as outlined in the Materials and methods. Data are presented as in Fig. 2. Protein synthesis and accretion rates were corrected for a standard body size of 40 g (see Materials and methods for details). *, Statistically significant difference ($P < 0.05$) between the two levels of acidity (pH 6.5 vs. 5.2) at the same temperature; †, significant difference ($P < 0.05$) between temperature groups at the control pH (pH 6.5).




effects were not statistically significant, the organ–somatic indices were not sensitive indicators of the organ-specific impact of combinations of chronic exposure to low pH and elevated environmental temperature.

**Protein turnover: gill**

Prior to the initiation of the exposure, fish acclimated for over 4 weeks to synthetic softwater synthesized proteins in the gills at a body size corrected rate of approximately 3%·day$^{-1}$ (Fig. 3A). At 30 and 60 days of exposure, a consistent relationship between $K_s$ and treatment became apparent. Gills of fish held at control + 2°C synthesized protein at a rate similar to that of fish exposed at control temperature. However, the gills of low pH treated fish synthesized proteins at a lower rate than those of their respective controls, and in two of four cases, this was statistically significant. During the final 30 days of exposure, a period during which water temperature rose to between 24 and 26°C (see Fig. 1A), the apparent influence of acidified synthetic softwater on gill protein synthesis was inconsistent.

Although significant differences in gill protein synthesis were seen after only 30 days of exposure, at this time the gill $K_g$ was not influenced by treatment (Fig. 3B). At day 60, protein accretion in gills from fish at control + 2°C was lower than the rate measured in gills from fish exposed to control temperature. Further, protein accretion in gills of fish exposed to pH 5.2, control temperature water was significantly greater than in gills of fish exposed to control pH (pH 6.2) at the same temperature. However, this pH-attributed stimulation (or lack of inhibition) of protein synthesis in gills was not evident at the elevated water temperature (+2°C; Fig. 3B) at this time. After 90 days of exposure, it was again evident that water temperature altered the net accumulation of protein in the gills of fish. However, at this time the reduction in water pH at the control temperature led to a significant inhibition of protein accretion, while at the raised temperature, reduced pH had no effect.

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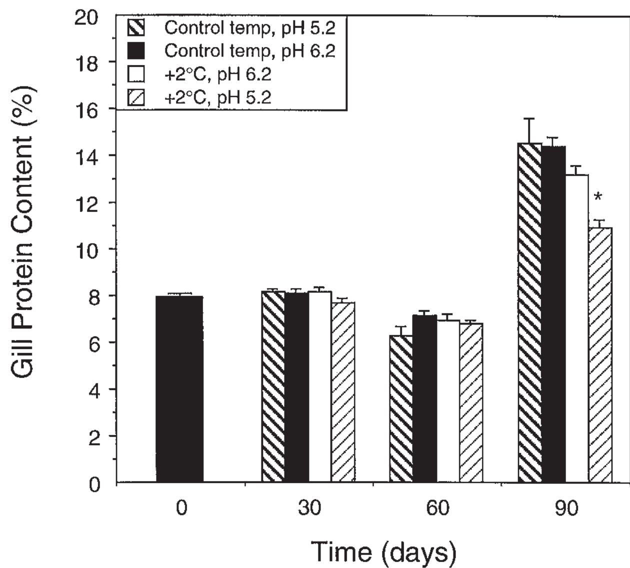


**Fig. 4.** The effect of pH and water temperature on branchial protein content. All values were calculated on a percent basis (mg protein·mg$^{-1}$ sample) and were determined as outlined in Materials and methods. Data are presented as in Fig. 2.

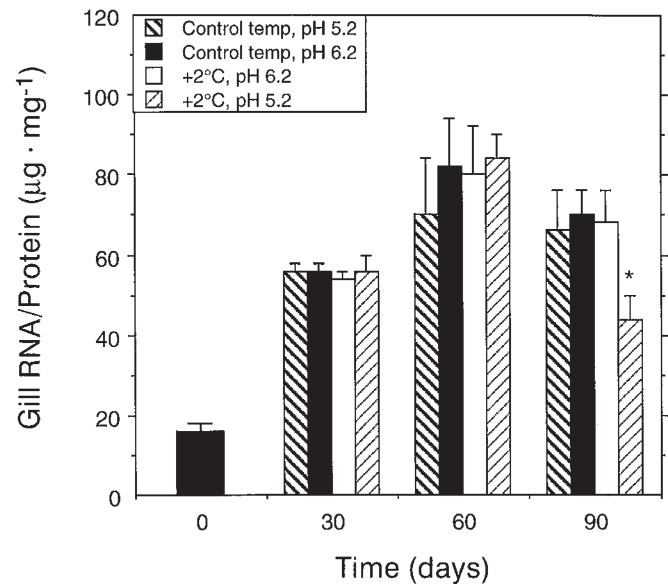


**Fig. 5.** The effect of pH and water temperature on branchial RNA/protein ratio. The RNA/protein ratios (tissue protein synthesis capacity) were calculated and expressed as μg RNA·mg$^{-1}$ protein and were determined as outlined in Materials and methods. Data are presented as in Fig. 2.

In general, $K_d$ in the gills reflected the relative rates of synthesis in this tissue, i.e., a treatment group with high rates of synthesis also possessed relatively high rates of degradation. Like the rates of synthesis for the gills of softwater-acclimated fish, after only 30 days, significant alterations in gill protein degradation were observed in association with water temperature and pH (Fig. 3C). At both days 30 and 60, exposure of fish to pH 5.2 water significantly reduced the rate of protein degradation in the gills at both temperatures. At day 90, the treatment effects were no longer consistent with those at previous sampling times. At the higher temperature, low pH had no significant effect. However, at this time, pH 5.2 at control temperature significantly stimulated the rate of protein breakdown, which was contrary to the impact of this treatment on $K_d$ on days 30 and 60.

Branchial protein content remained almost constant throughout the first 60 days of exposure at 7.5 ± 0.23% (mg protein·100 mg$^{-1}$ tissue; Fig. 4). During this time, branchial RNA steadily rose resulting in an apparent increase in protein synthesis capacity (RNA/protein; Fig. 5), which as with branchial protein content, was not influenced by water pH, the addition of 2°C to the control temperature profile, or the combination of both. Translational efficiency ($K_{RNA}$) changed only slightly during the first 60 days of exposure (Table 1). These data mimic the trends noted in gill protein synthesis rates measured during this time (Fig. 3A).

During the final 30 days of exposure (i.e., fish sampled at day 90), average branchial protein content was found to be 13.3 ± 0.56%, nearly double that during the previous samplings (Fig. 4). There was no significant pH effect at control temperature, but there was at the raised temperature, where low pH decreased protein content compared with the control. Branchial protein synthesis capacity (RNA/protein) was not significantly lower at 90 days except for fish exposed to the combination of increased temperature and reduced pH (Fig. 5). During this time of peak water temperature, the alterations in branchial protein and RNA content corresponded to a slight increase in translational efficiency (Table 1). After 90 days of exposure, branchial translational efficiencies were highest in fish exposed to pH 5.2 water, the opposite effect of that observed earlier in the exposure period (day 30) when the water temperatures were well below the upper lethal temperature for rainbow trout. The highest branchial translational efficiencies at day 90 were from fish exposed to the combination of +2°C and pH 5.2 water, although the differences in the branchial translational efficiencies among all treatment groups at this time were not statistically significant.

### Protein turnover: liver

Prior to the initiation of the exposure, livers of fish acclimated to synthetic softwater for over 4 weeks synthesized proteins at a rate corrected for body size of approximately 2.7%·day$^{-1}$ (Fig. 6A). Protein synthesis by the liver, in general, appeared to be less influenced by treatment than protein synthesized by the gills. Nevertheless, exposure to a higher temperature apparently enhanced the rate of protein synthesis, while exposure to reduced pH inhibited liver protein synthesis only after 90 days, when water temperature reached a maximum of 26°C (see Fig. 1) for the +2°C group. Despite the general insensitivity of liver protein synthesis to the treatments when compared with protein synthesis in the gills, protein synthesis in the liver appeared to be more sensitive than gill protein synthesis to the specific combination of higher temperature and low pH; liver $K_s$ was reduced by nearly 80% while gill $K_s$ was reduced by only about 20% (Fig. 6A vs. Fig. 3A) in this treatment group.

Net accretion of protein in the livers of softwater trout was not impacted by 30 days of exposure to the treatment regime (Fig. 6B). However, by day 60, liver protein accretion was significantly enhanced by low pH at both water temperatures,

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**Table 1.** Branchial ribosomal translational efficiency ($K_{RNA}$; g protein synthesis·g$^{-1}$ RNA·day$^{-1}$) from juvenile rainbow trout chronically exposed to acidified softwater and and elevated seasonal temperature profile (global warming scenario).

| | Time (days) | | | |
|---|---|---|---|---|
| Exposure group | Day 0 | Day 30 | Day 60 | Day 90 |
| Control temperature, pH 5.2 | | 0.71±0.071 | 0.83±0.061 | 1.17±0.085 |
| Control temperature, pH 6.2 | 1.82±0.274 | 0.85±0.059 | 0.83±0.060 | 0.987±0.101 |
| +2°C, pH 6.2 | | 0.89±0.056 | 0.80±0.063 | 0.93±0.107 |
| +2°C, pH 5.2 | | 0.79±0.063 | 0.68±0.074 | 1.23±0.081 |

**Note**: Values are mean ± SE with a samples size of 10 for each group. $K_{RNA}$ was calculated from the RNA/protein ratio (Fig. 5) and the fractional rate of protein synthesis ($K_s$; Fig. 3).

**Fig. 6.** The effect of water temperature and pH on liver protein (A) synthesis ($K_s$), (B) accretion ($K_g$), and (C) degradation ($K_d$) rates in softwater-acclimated trout. All rates were calculated on a %·day$^{-1}$ basis and determined as outlined in Materials and methods. Data are presented and body size corrected as in Figs. 2 and 3, respectively.

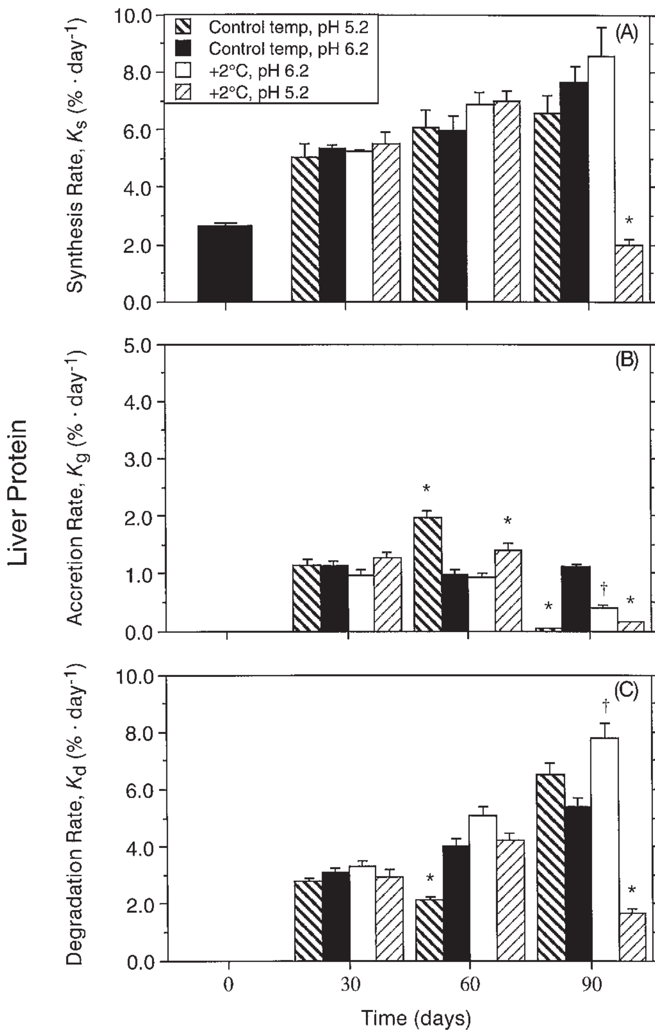
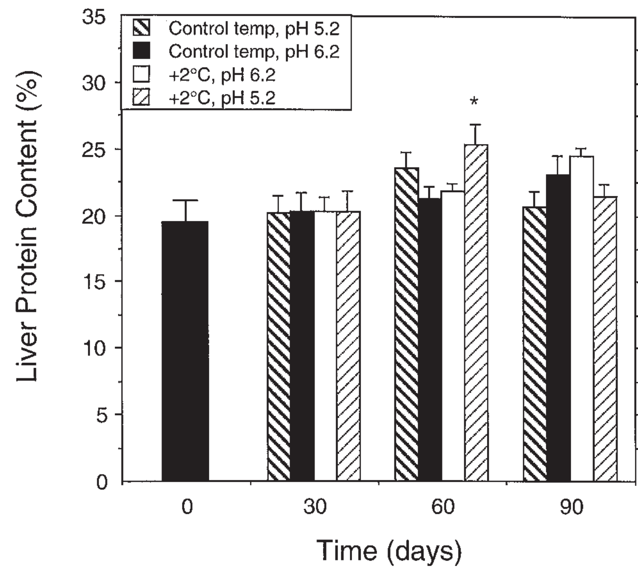



**Fig. 7.** The effect of pH and water temperature on liver protein content. All values were calculated on a percent basis (mg protein·mg$^{-1}$ sample) and determined as in Materials and methods. Data are presented as in Fig. 2.




with no difference in $K_g$ found between the temperature groups at control pH. The stimulatory influence of low water pH was no longer apparent at day 90. To the contrary, at this time the chronic exposure to pH 5.2 softwater significantly inhibited liver protein accretion as did the addition of 2°C to the control temperature profile. These influences of water temperature and pH on net liver protein accumulation were reflected in the liver–somatic indices (Fig. 2A), particularly at day 60.

Liver protein degradation was significantly altered as a result of exposure of softwater-acclimated trout to elevated water temperature and reduced pH (Fig. 6C). Similarly to the effect on gill protein degradation, liver protein degradation was suppressed at the control temperature by the reduction in pH at day 60. At day 90, there was no longer any impact of low pH on protein degradation in the livers of fish exposed at control temperature. However, exposure of fish to low pH at the raised (+2°C) temperature profile resulted in a significant 77% reduction in the liver protein degradation rate.

Liver protein content (average value of 21.7 ± 0.51%) changed only slightly over the course of the 90-day exposure (Fig. 7). Minor differences were apparent at day 60 with the treatment effects at 30 and 60 days mirroring quite closely the observed changes in liver $K_g$ (Fig. 6B). At day 60, liver protein content was highest in acid-exposed fish at the raised temperatures. As was observed in branchial tissue, the liver RNA content tended to increase as the duration of the exposure increased until day 60 when no further increase or reductions

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**Table 2.** Liver ribosomal translational efficiency ($K_{RNA}$; g protein synthesis·g$^{-1}$ RNA·day$^{-1}$) from juvenile rainbow trout chronically exposed to acidified softwater and and elevated seasonal temperature profile (global warming scenario).

| Exposure group | Time (days) Day 0 | Day 30 | Day 60 | Day 90 |
|---|---|---|---|---|
| Control temperature, pH 5.2 | | 0.85±0.071 | 0.66±0.051 | 0.75±0.066* |
| Control temperature, pH 6.2 | 0.74±0.274 | 0.86±0.068 | 0.65±0.055 | 1.06±0.087 |
| +2°C, pH 6.2 | | 0.89±0.073 | 0.69±0.052 | 1.13±0.090 |
| +2°C, pH 5.2 | | 0.89±0.075 | 1.09±0.081* | 0.22±0.018* |

**Note**: Values are mean ± SE with a samples size of 10 for each group. $K_{RNA}$ was calculated from the RNA/protein ratio (Fig. 8) and the fractional rate of protein synthesis ($K_s$; Fig. 6).

*Significantly different ($P < 0.05$) from pH 6.2 at the same temperature.

**Fig. 8.** The effect of pH and water temperature on liver RNA/protein ratio. The RNA/protein ratios (tissue protein synthesis capacity) were calculated and expressed as μg RNA·mg$^{-1}$ protein and were determined as outlined in Materials and methods. Data are presented as in Fig. 2.

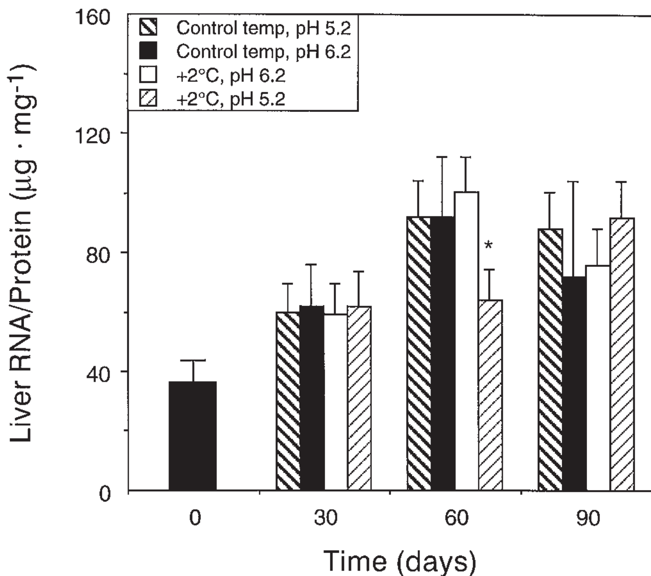
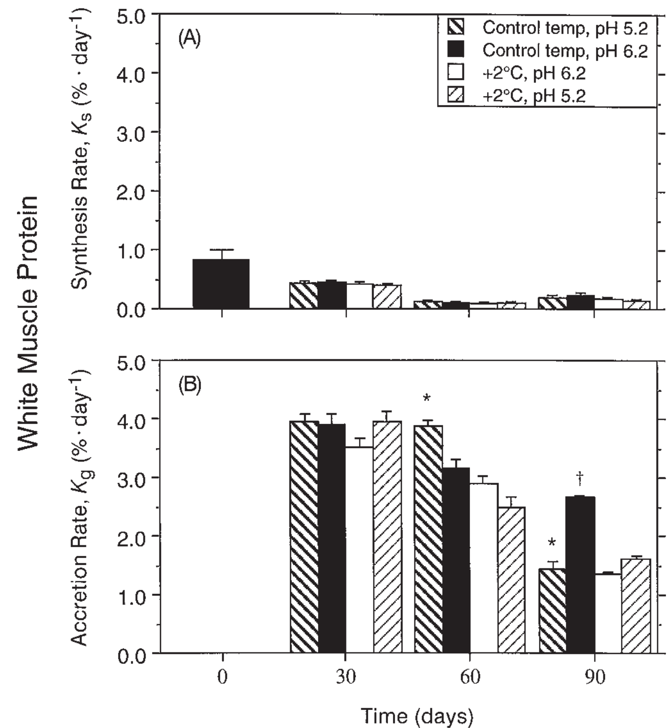



**Fig. 9.** The effect of water temperature and pH on white muscle protein (A) synthesis ($K_s$) and (B) accretion ($K_g$) rates in softwater-acclimated trout. All rates were calculated on a %·day$^{-1}$ basis and determined as outlined in Materials and methods. Data are presented and body size corrected as in Figs. 2 and 3, respectively.




in liver RNA content were apparent. The significant reduction in liver RNA/protein in fish exposed to the combination of elevated control temperature and pH 5.2 at 60 days (Fig. 8) resulted from both a slight increase in protein content and a reduction in RNA content relative to the other treatment groups.

Liver translational efficiency ($K_{RNA}$) did not differ between groups except it was lower at low pH than control pH on day 90 at both temperatures (Table 2). However, translational efficiency in the +2°C, pH 5.2 group was significantly greater than all other treatment groups at day 60.

### Protein turnover: white muscle

White muscle protein synthesis rates are considered relative rather than absolute rates for methodological reasons (see Matherials and methods). Protein synthesis in the white muscle of softwater-acclimated trout appeared to be less affected by combinations of higher temperature and lower pH when compared with gills or liver (Fig. 9A).

White muscle protein accretion was not influenced by water temperature or pH after only 30 days exposure (Fig. 9B). Differences appeared at day 60 with low pH at control temperature significantly enhancing protein accretion. At day 90, however, exposure to pH 5.2 water was inhibitory in this group, while white muscle protein accretion was also apparently inhibited at the raised temperature (pH 6.5) at a time when the control temperature was already 24°C.

White muscle protein content (11.02 ± 0.65%) was not influenced by water temperature or pH over the duration of the 90-day experiment. RNA content was similarily unaffected which resulted in a relatively unaltered RNA/protein ratio of 6.41 ± 0.09 μg RNA·mg$^{-1}$ protein. Therefore, white muscle $K_{RNA}$ simply reflected the changes in protein synthesis over the duration of exposure (data not shown).

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## Discussion

The present study differs significantly from the majority of previous studies of this type in the areas of temperature exposure and feeding regime as discussed in some detail by Reid et al. (1995, 1996) and Dockray et al. (1996). Briefly, fish were not acclimated and exposed to two constant, yet different temperatures (e.g., 15 vs. 17°C) as is typical for such studies (i.e., Fauconneau and Arnal 1985; Foster et al. 1992). In the present study, the temperature regime mimicked the inshore temperature of Lake Ontario over the summer months (see Fig. 1). This natural seasonal variation in water temperature resulted in the fish experiencing a range in water temperature with the control and +2°C being separated by approximately 2°C throughout; our interpretation of the global warming scenario. As for the feeding regime, Houlihan et al. (1988) have shown that in vivo rates of protein synthesis and degradation in Atlantic cod (*Gadus morhua*) increase linearly with ration size based on a feeding regime of 1–4% body weight·day$^{-1}$. In the present study, fish were fed twice daily to satiation to quantify appetite, which could then be used as an additional estimate of cost of living or metabolic rate (Dockray et al. 1996). As a result, fish were not fed controlled or equal ration size. The implications are that changes in protein synthesis, accretion, and degradation are likely to reflect changes in food consumption, conversion efficiency, and amino acid homeostasis in addition to changes associated with links between environmental temperature and metabolic rate. Thus, the observed changes in tissue protein turnover provides a general estimate of the cost associated with chronic sublethal exposure to toxicant stress under a global warming scenario.

### Acid

The gill has been clearly established as the primary site of action of environmental acidification (McDonald 1983; McDonald et al. 1991). Branchial ion loss is central to the acid toxicity syndrome in fish and typically the first disturbance upon exposure to acid water (see reviews by Leivestad 1982; Wood and McDonald 1982, 1987; Wood 1989; McDonald and Wood 1993; Reid 1995). Furthermore, Wilson et al. (1996) demonstrated that gill protein synthesis and degredation were significantly inhibited following acute exposure to low pH softwater at constant water temperature. Therefore, it is not surprising that we observed an alteration in gill protein turnover as a result of chronic exposure to low pH, despite the natural variability in the temperature regime of this study. However, the finding that the liver was similarly affected was unexpected.

After only 30 days exposure to pH 5.2 softwater, gill protein synthesis was significantly reduced (Fig. 3A). However, the net accumulation of protein in the gill was not affected at this time, because there was a similar significant reduction in the degradation rate. These findings are similar to those reported by Wilson et al. (1996), in which softwater-acclimated rainbow trout were exposed to pH 5.2 softwater for only 15 days. Based on these findings, it could be suggested that reduced rates of protein degradation may be the only mechanism available to maintain tissue growth or the normal enzyme complement when an increase in protein synthesis is not possible. Such was apparently the case in this study for both the liver and gill tissues as the reductions in degradation rates were sufficient to result in a greater rate of protein accretion than in the controls. These findings suggest that both tissues retained some capacity through the net accumulation of protein to compensate for the effects of chronic sublethal acid exposure. However, on a relative basis, it could be argued that the compensatory capacity of the gill is limited compared with that of the liver, as liver protein synthesis was not adversely affected by water pH. Therefore, changes in protein accretion would not be limited to only modulation in the rate of protein degradation, as it appears to have been in the gill. This suggested difference in recovery or compensatory capacity in these two tissues may result from the physical separation between the liver and the acidified softwater, which is unlike the situation between the gill and the low-pH environment, more so than from unique differences in tissue metabolism.

In similar studies involving the exposure of trout to elevated concentrations of metals, it has been suggested that recovery from acute toxicant exposure is associated with an increase in tissue-specific protein synthesis. Hogstrand et al. (1995) exposed juvenile rainbow trout to 150 μg·L$^{-1}$ zinc. Within 1 week of exposure, depressed plasma [$Ca^{2+}$] was corrected concurrent with an increase in the affinity of a gill $Ca^{2+}$ transporter and increased gill protein turnover ($K_s$ and $K_d$). Based on these changes and alterations in $Ca^{2+}$-transport kinetics, the authors interpreted the increased gill protein synthesis as an enhanced rate of $Ca^{2+}$ carrier regeneration and an integral part of the zinc-induced gill repair mechanism. In addition, Wilson et al. (1996) reported that gill protein turnover ($K_s$ and $K_d$) was greatly stimulated after 7 days exposure to aluminum (30 μg·L$^{-1}$) in acidified soft water (pH 5.2) and attributed this to gill repair and (or) acclimatory processes. However, the same authors reported that the only effects observed for exposure to acid alone was the depression of gill protein turnover after 15 days. Therefore, it is tempting to speculate that the continued reduction in gill protein synthesis reported by Wilson et al. (1996) and the inhibition of protein synthesis observed in this study, at comparable temperatures, adds further support to the suggestion that rainbow trout are unable to acclimate to sublethal exposure to low pH softwater.

According to the damage-repair hypothesis (McDonald and Wood 1993) acclimation cannot occur without prior damage. Acclimation results from the subsequent repair, which occurs in such a manner as to provide increased tolerance or resistance to the damage-inducing toxicant. In the case of the gills of salmonids, chronic sublethal acid exposure has been shown to result in little structural damage. Mueller et al. (1991) exposed juvenile brook trout (*Salvelinus fontinalis*) to pH 5.2 soft water for 42 days and observed only slight hyperplasia of undifferentiated cells and some proliferation and hypertrophy of chloride cells. Similarly, Audet and Wood (1993) reported only minor changes in gill structure in rainbow trout exposed to pH 4.8 soft water for 81 days. Chloride cell numbers and the lamellar water–blood diffusion distance were not affected. These data suggest that, while the extent of damage done to the gills by chronic sublethal exposure is enough to result in serious impairment in electrolyte homeostasis and minor alterations in morphology and reductions in protein turnover, it is insufficient to induce a repair phase and the associated stimulation of gill protein synthesis. In fact, the work of Audet et al. (1988) and Audet and Wood (1988) show that rainbow trout are unable to acclimate to sublethal environmental

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acidification and that prior low pH exposure does not confer increased tolerance to subsequent acid exposure.

It is difficult to interpret the changes in liver protein turnover that occurred in response to chronic sublethal acid exposure. Chronic exposure to environmental acidification has been shown to chronically elevate plasma cortisol (Brown et al. 1984). As a glucocorticoid, cortisol will likely induce alterations in carbohydrate metabolism. In mammals, it is well documented that cortisol stimulates the catabolism of muscle, resulting in an elevation of circulating plasma amino acids (Leung and Munck 1975; Kraus-Freidmann 1984). Elevated plasma amino acids have been reported in rainbow trout following chronic elevations in plasma cortisol (Andersen et al. 1991). However, in the same study, cortisol treatment had no effect on the flux of the gluconeogenic precursors, lactate and alanine, to glucose. Furthermore chronic elevations in plasma cortisol failed to alter activities of key metabolic enzymes or glucose turnover in the liver. Because of the difficulties associated with measuring protein synthesis in muscle (Foster et al. 1992), the present study provides no evidence for or against catabolism of muscle during chronic sublethal acid or temperature stress. It is possible, however, that the reduction in protein degradation in the liver observed in the acid-exposed fish may reflect a reduction in the turnover of gluconeogenic enzymes.

Only at the end of the study (day 90), after the fish experienced the highest water temperatures, were there any significant differences in gill protein content (Fig. 4) and the gill protein synthesis capacity (Fig. 5), both of which were reduced. Prior to day 90, from the protein turnover data (Fig. 3), chronic exposure to environmental acidification inhibited both protein synthesis and degradation. It is tempting to suggest that not only does chronic exposure to a low pH environment inhibit the ability of gill to generate proteins, but it does so not by reducing the efficiency of translation but rather by deleteriously modifying transcription through changes in RNA production, stability, or half-life. This is apparently not the case in liver, which is not in intimate contact with the altered environment. The alterations in liver protein accretion observed after 60 and 90 days of acid exposure might be the result of metabolically driven modifications in ribosomal translational efficiency. This is particularly evident at day 90, a time at which both net protein accumulation (Fig. 6B) and ribosomal translational efficiency (Table 2) were significantly reduced. In a study by Houlihan et al. (1994), dab (*Limanda limanda*) were exposed to sewage sludge (predominantly a mixture of cadmium, chromium, copper, mercury, nickel, lead, and zinc) for 3 months with tissue (white muscle, liver, kidney, spleen) protein turnover determined monthly using methods similar to those of the present study. Despite no impact on tissue protein synthesis ($K_s$), protein growth rates (accretion, $K_g$) were lower in fish chronically exposed to sewage sludge compared with the control group. Houlihan et al. (1994) suggested that chronic exposure to sewage sludge reduced protein accretion through enhanced $K_d$ as tissue RNA concentrations and post-translational efficiency were largely unaffected. Similarly, Wilson et al. (1996) reported no significant modification in protein synthesis capacity (RNA/protein ratio) or $K_{RNA}$ in gill, liver, and carcass (whole body minus gills and liver) following 15 days exposure to pH 5.2 softwater when compared with the pH 6.5 controls. However, in response to the addition of 30 $\mu g \cdot L^{-1}$ aluminum to the pH 5.2 softwater, Wilson et al. (1996) noted a significant reduction in gill protein synthesis capacity, a significant reduction in carcass ribosomal translational efficiency with no impact on either parameter in liver following a 32-day exposure. Thus, in response to chronic stressors, it is possible that modifications in protein turnover can result from pre- or post-translational modification of protein synthesis, alterations in protein degradation, or both and may differ between tissues within the same individual. The specific mechanism by which environmental stressors alter tissue-specific or whole-animal protein turnover requires further study.

## Temperature

Because of the direct linkage between the environmental temperature and the metabolic rate in ectotherms, it might be predicted that chronic exposure to a slightly warmer environment (i.e., +2°C) would enhance protein synthesis in fish. In the study of Fauconneau and Arnal (1985), acclimation to elevated temperature resulted in greater rates of tissue protein synthesis in trout. Fauconneau and Arnal (1985) found that by increasing acclimation temperature from 10 to 18°C, liver and digestive tract protein synthesis increased 3.4- and 2.3-fold, respectively. In the present study, although much less dramatic due to the natural temperature profile, a 2°C increase over control temperature led to an increase in liver and gill protein synthesis in 50% of all comparisons, while protein degradation was enhanced in these tissues in all but one situation. This slight increase in the natural temperature profile had no apparent influence on ribosomal translational efficiency (Tables 1 and 2) or protein synthesis capacity (Figs. 5 and 8) in gill, liver, or white muscle. A temperature quotient, or $Q_{10}$, independent of the influence of water pH was determined to be approximately 2.1 based on the control to +2°C temperature range (Reid et al. 1995). Loughna and Goldspink (1985) reported that rainbow trout initially acclimated to 15°C showed a $Q_{10}$ of nearly 3.6 between 5 and 20°C. The differences in temperature sensitivity reported for this species are likely accounted for by the differences in prior temperature history, temperature range (2°C vs. 15°C), and the fact that the $Q_{10}$ for the data of the present study, and reported in Reid et al. (1995), was a cumulative estimate, incorporating temperature-related changes in appetite, weight gain, and tissue protein turnover. Both translational efficiency and protein synthesis capacity in all tissues assayed were insensitive to the 2°C increment in the natural temperature profile.

Nevertheless, larger changes in seasonal temperature resulted in changes in both protein synthesis capacity and translational efficiency. As the temperature rose over the initial 60 days of the study, protein synthesis capacity increased (Figs. 5 and 8), while translational efficiency was reduced, at least in liver (Table 2). These results tend to contradict the literature pertaining to the influence of environmental temperature on fractional rates of protein synthesis. In general, it is suggested that, as a result of a reduction in ribosomal translational efficiency, cold-acclimated fish compensate via an increase in tissue RNA/protein ratios or protein synthesis capacity (Goolish et al. 1984; Fauconneau and Arnal 1985; Foster et al. 1992). However, the apparent discrepancy may be the result of the temperature profile used in the present study. Trout were not acclimated to any set temperature (e.g., 5 vs. 15°C for 40 days; Foster et al. 1992) but rather experienced

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the natural daily and seasonal variation in temperature (see Fig. 1) with the 2°C increment in temperature superimposed upon this highly variable temperature profile.

In general, it appeared that the effects of both temperature and toxicant stress on tissue growth and protein turnover were apparent, but there were times during the study when the effects of temperature, particularly on protein turnover, overshadowed the influences of the chronic reduction in pH. Between days 60 and 90, the water temperature in the soft-water exposure system peaked at either 24 or 26°C depending on the treatment group (Fig. 1A). It is apparent that temperature was the dominant influence on protein turnover at this time, resulting in dramatic reductions in gill and liver protein synthesis, accretion, and degradation (Figs. 3 and 4). Although these alterations in protein turnover were not evident in the organ–somatic indices, such alterations in protein turnover are suggestive of dramatic and rapid reductions in metabolism between 24 and 26°C. At this time, food conversion efficiency, appetite, growth, nitrogenous waste excretion, and oxygen consumption were depressed (Dockray et al. 1996). These data also suggest that, at the very extreme range of their thermal tolerance between 24 and 26°C, metabolism is dramatically suppressed, possibly in an attempt to conserve energy or in response to elevated metabolic end products. Therefore, for a trout that may at some time experience an environmental temperature of 22–24°C, even an increase in the mean annual water temperature of only 2°C would be deleterious.

**Summary**

The present study has shown that protein turnover rates in the tissues of fish exposed to chronic sublethal levels of acidity are altered in a manner consistent with the known toxic mechanisms of environmental acidification. Protein turnover in both the gill and liver were significantly altered, which also altered tissue growth. The exact mechanism by which these modifications in protein turnover occurred could not be elucidated by this study, yet it does appear that it depends on whether the tissue is directly (gill) or indirectly (liver and muscle) impacted by environmental acidification. The addition of +2°C to the control temperature profile generally increased protein synthesis, the impact of which was additive with that of chronic exposure to an acidified environment. However, at temperatures near the upper lethal temperature for trout, the slight temperature increase triggered a dramatic reduction in the protein turnover rates of the exposed fish. Therefore, even if the rise in mean global temperature triggered by the doubling of atmospheric $CO_2$ is in the lower range predicted by current models (0.5–7°C), the results of this study suggest that it would decrease fish growth and survival, particularly of fish living near their upper limit of temperature tolerance.

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## References

Andersen, D.E., Reid, S.D., Moon, T.W., and Perry, S.F. 1991. Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **48**: 1811–1817.

Audet, C., and Wood, C.M. 1988. Do rainbow trout (*Salmo gairdneri*) acclimate to low pH? Can. J. Fish. Aquat. Sci. **45**: 1399–1405.

Audet, C, and Wood, C.M. 1993. Branchial morphological and endocrine responses of rainbow trout (*Oncorhynchus mykiss*) to a long-term sublethal acid exposure in which acclimation did not occur. Can. J. Fish. Aquat. Sci. **50**: 198–209.

Audet, C., Munger, R.S., and Wood, C.M. 1988. Long-term sublethal acid exposure in rainbow trout (*Salmo gairdneri*) in soft water: effects on ion exchanges and blood chemistry. Can. J. Fish. Aquat. Sci. **45**: 1387–1398.

Beamish, R.J. 1974. Loss of fish populations from unexploited remote lakes in Ontario, Canada, as a consequence of atmospheric fallout of acid. Water Res. **8**: 85–95.

Beamish, R.J. 1976. Acidification of lakes in Canada by acid precipitation and the resulting effects on fishes. Water Air Soil Pollut. **6**: 501–514.

Beamish, R.J., Lockart, W.L., Van Loon, J.C., and Harvey, H.H. 1975. Long-term acidification of a lake and resulting effects on fishes. Ambio, **4**: 98–102.

Brett, J.R. 1964. Respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. **21**: 1183–1226.

Brown, S.B., Eales, J.G., and Evans, R.E. 1984. Interrenal, thyroidal, and carbohydrate responses of rainbow trout *Salmo gairdneri* to environmental acidification. Can. J. Fish. Aquat. Sci. **41**: 36–45.

Cho, C.Y. 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. Aquaculture, **100**: 107–123.

Dockray, J.J., Reid, S.D., and Wood, C.M. 1996. Effects of elevated summer temperatures and reduced pH on metabolism and growth of juvenile rainbow trout (*Oncorhynchus mykiss*) on unlimited ration. Can. J. Fish. Aquat. Sci. **53**: 2752–2763.

Elliot, J.M. 1975. Growth rate of brown trout on reduced rations. J. Anim. Ecol. **44**: 805–821.

Fauconneau, B., and Arnal, M. 1985. *In vivo* protein synthesis in different tissues and the whole body of rainbow trout (*Salmo gairdneri* R.). Influence of environmental temperature. Comp. Biochem. Physiol. **82A**: 179–187.

Foster, A.R., Houlihan, D.F., Hall, S.J., and Burren, L.J. 1992. The effects of temperature acclimation on protein synthesis rates and nucleic acid content of juvenile cod (*Gadus morhua* L.). Can. J. Zool. **70**: 2095–2102.

Fry, F.E.J. 1971. Effects of environmental factors. *In.* Fish physiology.Vol. 6. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, New York.

Garlick, P.J., McNurlan, M.A., and Preedy, V.R. 1980. A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [$^3$H]phenylalanine. Biochem. J. **192**: 719–723.

Goolish, E.M., Barron, M.G., and Adelman, I.R. 1984. Thermoacclimatory response of nucleic acid and protein content of carp muscle tissue: influence of growth rate and relationship to glycine uptake by scales. Can. J. Zool. **62**: 2164–2170.

Hansen, J., Fung, I., Lacis, A., Rind, D., Lebedeff, S., Ruedy, R., Russell, G., and Stone, P. 1988. Global climate changes as forecast by Goddard Institute for Space Studies three-dimensional model. J. Geophys. Res. **93**: 9341–9364.

Hogstrand, C., Reid, S.D., and Wood, C.M. 1995. Calcium versus zinc transport in the gills of freshwater rainbow trout, and the cost of adaptation to waterborne zinc. J. Exp. Biol. **198**: 337–348.

Houlihan, D.F. 1991. Protein turnover in ectotherms and its relation

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to energetics. *In* Advances in comparative and environmental physiology. Vol. 7. *Edited by* R. Gilles. Springer-Verlag, Berlin. pp. 1–44.

Houlihan, D.F., and Laurent, P. 1987. Effects of exercise training on the performance, growth, and protein turnover in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. **44**: 1614–1621.

Houlihan, D.F., McMillan, D.N., and Laurent, P. 1986. Growth rates, protein synthesis, and protein degradation rates in rainbow trout: effect of body size. Physiol. Zool. **59**: 482–493.

Houlihan, D.F., Hall, S.J., Gray, C., and Noble, B.S. 1988. Growth rates and protein turnover in Atlantic cod, *Gadus morhua*. Can. J. Fish. Aquatic Sci. **45**: 951–964.

Houlihan, D.F., Costello, M.J., Secombes, C.J., Stagg, R., and Brechin, J. 1994. Effects of sewage sludge exposure on growth, feeding and protein synthesis of dab (*Limanda limanda* (L.)). Mar. Environ. Res. **37**: 331–353.

Jobling, M. 1983. Growth studies with fish—overcoming the problems of size variation. J. Fish Biol. **22**: 153–157.

Kaya, C.M. 1978. Thermal resistance of rainbow trout from a permanently heated stream, and of two hatchery strains. Prog. Fish-Cult. **40**: 138–142.

Kelso, J.R.M., Shaw, M.A., Minns, C.K., and Mills, K.H. 1990. An evaluation of the effects of atmospheric acidic deposition on fish and the fishery resources of Canada. Can. J. Fish. Aquat. Sci. **47**: 644–655.

Kraus-Freidmann, N. 1984. Hormonal regulation of hepatic gluconeogenesis. Physiol. Rev. **64**: 170–259.

Leivestad, H. 1982. Physiological effects of acid stress on fish. *In* Acid rain/fisheries. *Edited by* R.E. Johnson. American Fisheries Society, Bethesda, Md. pp. 157–164.

Leivestad, H., and Muniz, I.P. 1976. Fish kill at low pH in a Norweigian river. Nature (London), **259**: 391–392.

Leung, K., and Munck, A. 1975. Peripheral actions of glucocorticoids. Annu. Rev. Biochem. **37**: 245–272.

Loughna, P.T., and Goldspink, G. 1985. Muscle protein synthesis rates during temperature acclimation in euryhaline (*Cyprinus carpio*) and a stenothermal (*Salmo gairdneri*) species of teleost. J. Exp. Biol. **118**: 267–276.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the Folin reagent. J. Biol. Chem. **193**: 265–275.

McDonald, D.G. 1983. The effects of $H^+$ upon the gills of freshwater fish. Can. J. Zool. **61**: 691–703.

McDonald, D.G., and Wood, C.M. 1993. Branchial mechanisms of acclimation to metals in freshwater fish. *In* Fish ecophysiology. *Edited by* C. Ranking and F.B. Jensen. Chapman & Hall, London. pp. 295–319.

McDonald, D.G., Freda, J., Cavdek, V., Gonzalez, R., and Zia, S. 1991. Interspecific differences in gill morphology of freshwater fish in relation to tolerance to low-pH environments. Physiol. Zool. **64**: 124–144.

Milligan, C.L., and Wood, C.M. 1982. Disturbances in hematology, fluid volume distribution, and circulatiory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. J. Exp. Biol. **99**: 397–415.

Mohnen, V.A., and Wang, W.-C. 1992. An overview of global warming. Environ. Toxicol. Chem. **11**: 1051–1059.

Mueller, M.E., Sanchez, D.A., Bergman, H.L. McDonald, D.G., Rhem, R.R., and Wood, C.M. 1991. Nature and time course of acclimation to aluminum in juvenile brook trout (*Salvelinus fontinalis*). II. Gill histology. Can. J. Fish. Aquat. Sci. **48**: 2016–2027.

Munro, H.N., and Fleck, A. 1966. The determination of nucleic acids. *In* Methods of biomedical analysis. Vol. 14. *Edited by* D. Glick. Wiley Interscience, New York. pp. 113–176.

Regier, H.A., Holmes, J.A., and Pauly, D. 1990. Influence of temperature changes on aquatic ecosystems: interpretation of empirical data. Trans. Am. Fish. Soc. **119**: 374–389.

Reid, S.D. 1995. Adaptation to and effects of acid water on the fish gill. *In* Biochemistry and molecular biology of fishes. Vol. 5. Analytical techniques. *Edited by* P.W. Hochachka and T.P. Mommsen. Elsevier, Amsterdam, The Netherlands. pp 213–228.

Reid, S.D., Rhem, R.R., and McDonald, D.G. 1991. Acclimation to sublethal aluminum: modifications of metal – gill surface interactions of juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **48**: 1995–2004.

Reid, S.D., Dockray, J.J., Linton, T.K., McDonald, D.G., and Wood, C.M. 1995. Effects of a summer temperature regime representative of a global warming scenario on growth and protein synthesis in hardwater- and softwater-acclimated juvenile rainbow trout (*Oncorhynchus mykiss*). J. Therm. Biol. **20**: 231–244.

Reid, S.D., McDonald, D.G., and Wood, C.M. 1996. Interactive effects of temperature and pollutant stress. *In* Global warming—implications for freshwater and marine fish. *Edited by* D.G. McDonald and C.M. Wood. Soc. Exp. Biol. Semin. Ser. No. 61. Cambridge University Press, Cambridge, U.K.

Ricker, W.E. 1979. Growth rates and models. *In* Fish physiology. Vol. 8. *Edited by* W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. pp. 677–742.

Schindler, D.W., Beaty, K.G., Fee, E.J., Cruikshank, D.R., Debruyn, E.R., Findlay, D.L., Shearer, J.A., and Turner, M.A.. 1990. Effects of climatic warming on lakes of the central boreal forest. Science (Washington, D.C.), **250**: 967–970.

Sugden, P.H., and Fuller, S.J. 1991. Regulation of protein turnover in skeletal and cardiac muscle. Biochem. J. **273**: 21–37.

Wilson, R.W., Wood, C.M., and Houlihan, D.F. 1996. Growth, feeding, and protein turnover during acclimation to acid and aluminum in juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. **53**: 802–811.

Wolf, K. 1963. Physiological salines for freshwater teleosts. Prog. Fish-Cult. **25**: 135–140.

Wood, C.M. 1989. Physiological problems of fish in acid water. *In* Acid toxicity and aquatic animals. *Edited by* R. Morris, E.W. Taylor, D.J.A. Brown, and J.A. Brown. Soc. Exp. Biol. Semin. Ser. No. 34. pp. 125–152.

Wood, C.M., and McDonald, D.G. 1982. Physiological mechanisms of acid toxicity to fish. *In* Acid rain/fisheries. *Edited by* R.E. Johnson. American Fisheries Socitey, Bethesda, Md. pp. 197–226.

Wood, C.M., and McDonald, D.G. 1987. The physiology of acid/aluminum stress in trout. Ann. Soc. R. Zool. Belg. **117**: 399–410.

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