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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*)

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Abstract—1. Growth, appetite, gross conversion efficiency and protein turnover rates of liver, gills and white muscle were measured in juvenile rainbow trout chronically exposed (90 days) to soft and hardwater at two temperatures (ambient, ambient temp. +2°C). The temperature regime followed that of inshore Lake Ontario over the months of June–September 1993 as temperature rose from ~13 to 24°C.

2. Over the initial 60 days of exposure, the addition of 2°C to the ambient temperature increased growth, appetite, gross conversion efficiency and protein turnover by an average of 16%. However, further exposure during the period of peak ambient temperatures, led to an average 20% reduction in growth, appetite, gross conversion efficiency and protein turnover.

3. Increased rates of gill protein turnover and arguably lower rates of growth indicate that the cost of living for a trout acclimated and maintained in synthetic softwater is higher than that of hardwater fish. In addition, lower appetite in softwater fish suggest that life in softwater is itself a mild form of environmental stress.

Key Word Index: Fish; protein synthesis; protein turnover; growth; elevated temperature; appetite; global warming; softwater; gills; liver

INTRODUCTION

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; Cho, 1990; Houlihan, 1991). The effects of global warming on fish and fisheries are therefore likely to be substantial; however as Schindler *et al.* (1990) have recently pointed out, the influence of climatic change on freshwaters has been largely disregarded in major global change programs. To date, there is a critical scarcity of experimental data on the chronic effects of relatively small temperature elevations over the annual cycle. Such information is essential for process models, but most have used laboratory data obtained at "static" temperatures or field data indexed to "mean annual temperature" (DeAngelis and Cushman, 1990; Regier *et al.*, 1990). At best these are approximations—"better than nothing" according to Regier *et al.*, 1990; cycling temperature-versus-effect relationships are not simple, because biological complexities (e.g. acclimation processes, changes in appetite) and physical complexities (e.g. changes in water viscosity, density, O_2 capacity, pH of neutrality) are superimposed on basic Arrhenius relationships.

For "coldwater" species such as salmonids, it seems likely that the most dramatic effects of global warming will occur during the summer months. The rainbow trout, *Oncorhynchus mykiss*, is a typical coldwater species endemic to the north-western region of North America, and now widely introduced for recreational fishing throughout the Northern Hemisphere. In many parts of its present range, including south-eastern Ontario, summer temperatures (16–24°C) are at present well above optimum (15°C) and occasionally approach the upper lethal temperature (26°C; Kaya, 1978). Current climate change scenarios suggest that mean annual temperature may increase by 0.5–5°C over the next 50 years in this region (Mohnen and Wang, 1992). In the

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present study, our primary objective was to assess changes in the cost of living of juvenile rainbow trout subjected to a natural summer water temperature regime, and one to which 2°C was added throughout to represent a realistic global warming scenario. The 3 month period studied encompassed the hottest time of the year (June–September).

A second objective was to evaluate possible interactions between temperature stresses and a marginal environment. For the latter we chose a naturally occurring stressor, low water hardness. Natural softwaters low in calcium and other electrolytes are widespread in eastern North America and Scandinavia, providing an environment in which salmonids can live but with lower productivity than in hardwater (Howells *et al.*, 1983; Hunn, 1985; Mount *et al.*, 1988; Ingersoll *et al.*, 1990). It is generally believed that adaptation to such a marginal environment is associated with increased metabolic costs (e.g. Calow, 1991). Shifts in the energy budget towards the defense of internal homeostasis would suggest that less energy is available for other processes such as growth, activity, and reproduction.

Changes in the cost of living during these exposures have been assessed in the traditional way by measuring appetite, whole body growth and gross conversion efficiency, and also by measuring organ-specific protein synthesis, net accretion and degradation. Recent studies have shown that protein synthesis is correlated with both growth and metabolic rate and indeed may account for a large portion of the latter in fish (Jobling, 1985; Houlihan, 1991; Houlihan *et al.*, 1993). Protein dynamics appear to be a sensitive integrator and indicator of environmental impacts. A unique aspect of the present study is that measurements have been made under a "natural situation" whereby as the fish grew and increased in size, water temperature was rising for much of the period. Previous studies have evaluated the effects of body size alone (at constant temperature—e.g. Houlihan *et al.*, 1986) and temperature alone (at constant individual body size—e.g. Fauconneau and Arnal, 1985), but not their interaction.

METHODS AND MATERIALS

Experimental animals

Approximately 3200 rainbow trout (*Oncorhynchus mykiss*) of both sexes weighing initially 1.5–4 g were obtained April 19, 1993 from Rainbow Springs Hatchery (Thamesford, Ontario). Fish were held in two 400 liter fiberglass tanks supplied with dechlorinated Hamilton tap water ($[Ca^{2+}] = 1.95 \pm 0.22$ mequiv·l^{-1}, $[Na^{+}] = 556 \pm 33$ 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% of 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

One tank of trout, containing approximately 1600 fish, were gradually acclimated to synthetic softwater following the 1 week period of acclimation to laboratory conditions. The total period of acclimation to synthetic softwater prior to start of exposure was approximately 8 weeks; from April 25 to June 18, 1993. The other 1600 fish were maintained in Hamilton tap water for this same period for "hardwater acclimation". Synthetic softwater was produced by mixing dechlorinated Hamilton City tap water with deionized water, generated by reverse osmosis (Anderson Water Treatment Systems, Dundas, Ontario), at 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 6.28 and was representative of natural softwater in Eastern North America (Beamish, 1974; Beamish *et al.*, 1975). An unavoidable consequence of reverse osmosis was the addition of 2°C to the softwater supply; consequently, softwater-acclimated trout were held at 13 rather than 11°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 water hardness

Fish were exposed for 90 days (starting on June 18, 1993 and ending on September 15, 1993) to one of four combinations of temperature and water hardness in replicated tanks (170 per tank) continuously supplied with 1.2 l·min^{-1} aerated, synthetic softwater generated as described previously. Treatment temperatures (Figs 1A and B) were "ambient" (equivalent to the inshore water temperature of Lake Ontario) and water heated by 2°C ("ambient temp. +2°C") using manually controlled counter-current heat exchangers supplied with domestic hot water. Ambient temperature increased from about 13°C to 24°C over this period, peaking in late August, and then declining rapidly to about 16°C in early September. At these water temperatures, synthetic softwater pH fluctuated from 5.9–6.4 (mean = 6.28) and hardwater (tap water) from 7.4–7.8 (mean = 7.60). Throughout the 90 day exposure, water temperature in the softwater exposure system

was unavoidably 2°C greater than in the hardwater system due to the reverse osmosis process. Therefore "ambient" temperature in the softwater system was approximately equal to ambient +2°C in the hardwater system; comparison of these two treatments allowed a direct assessment of the influence of water hardness. "Ambient +2°C" in the softwater system was elevated by approximately 4°C relative to hardwater "ambient".

During chronic exposure, fish were fed according to a different protocol from that used during hardwater and softwater acclimation. This protocol involved hand-feeding each tank of fish to satiation twice daily (8:30 a.m. and 4:30 p.m.) 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 in each treatment group prior to the initiation of treatment (day 0) and at 30, 60 and 90 days of exposure.

Tissue protein synthesis was determined from the incorporation of radioactive phenylalanine, according to the method of Garlick *et al.* (1980) modified by Houlihan *et al.* (1986) for use in fish. Fish were not fed 24 h prior to injection. On the day of injection, fish were randomly selected from each treatment (five fish from each tank, $N = 10$ per treatment), 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), containing 37×10^6 Bq·ml^{-1} of L-2,6-[^{3}H]-phenylalanine (Sigma, St. Louis) in Cortland saline (Wolf, 1963) at pH 7.5. The dose was 1.0 ml·100 g body weight^{-1} 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 post-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) and analysis (protein turnover). Following this, an additional 20 fish were randomly selected from each treatment for analyses not presented as part of this study. However, the weight data was used to increase the sample size of this measure from 10 to 30 per treatment.

Analysis

Tissue protein 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 separated by centrifugation. PCA in the supernatant was precipitated with tripotassium citrate and centrifuged, leaving the free phenylalanine in solution. Phenylalanine in the supernatant was then converted to β-phenylethylamine, by L-tyrosine decarboxylase, extracted using n-heptane, and analyzed by a ninhydrin reaction. The content of [^{3}H]-phenylalanine was measured using liquid scintillation counting techniques with quench correction. 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 pellet, containing protein and DNA, was washed twice with PCA and then hydrolyzed with 6 M hydrochloric acid (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 (%·day^{-1}) was calculated as:

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

where SA_p is the protein-bound specific activity (dpm·nmol^{-1}), SA_f the specific activity of the pool of free amino acids (dpm nmol^{-1}), 1440 the number of min in a day, "t" the exact time (min) from the [^{3}H]-phenylalanine injection to tissue sampling and 100 the conversion to percent.

The rate of protein degradation (K_d, %·day^{-1}) for gill and liver was calculated as the difference between the rate of protein synthesis (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 growth rates were calculated using the equation of Ricker (1979):

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

where W_1 and W_2 are the final and initial weights (g), respectively, and t is the length of the growing period (d).

This method of estimating protein turnover greatly underestimates K_s in white muscle when incorporation times are less than 2 h yet the optimum and most frequently used time for other tissues is 1 h, the incorporation period chosen in the present study (Foster *et al.*, 1992). Therefore, white muscle K_s must be considered only a relative estimate of protein synthesis in this tissue and white muscle K_d could not be calculated.

Liver 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.

Body weight changed during the course of the exposure, and to different extents in different treatments. Therefore to assess the effects of temperature (and hardness) independent of body weight, protein synthesis and accretion rates were standardized to 40 g body mass (the average weight of the fish following the 90 day exposure) using the allometric equation $Y = aX^b$ (Jobling, 1983) and exponents of -0.22 for protein synthesis and -0.44 for protein accretion in accordance with Jobling (1983) and Houlihan *et al.* (1986).

Growth of fish was calculated as the daily weight gain (wet weight) on a per fish basis. Appetite was determined by measuring the daily food intake (wet weight) of a tank of fish and expressing it on a per fish basis. Gross conversion efficiency was calculated by dividing the daily wet weight gain (growth) by the daily wet food intake (appetite) and expressed on a percent basis.

Statistical analysis

Values shown in figures are means $\pm$ 1 standard error of the mean (SE). Statistical differences between 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

Temperature profile

When the exposures were initiated in mid June, "ambient" and "ambient +2°C" water temperatures in both the hardwater and softwater systems were 13 and 15°C, respectively (Fig. 1). However after 3 days the water temperature rose by 2°C in the softwater system due to the generation of deionized water by reverse osmosis. During the initial 30 days of exposure water temperature rose in all treatments, with the increase in the softwater system occurring more rapidly and due to the initial 2°C rise observed after day 3. During this period, the cumulative thermal exposures for hardwater-acclimated fish were 424.5 (ambient) and 446.5 (ambient +2°C) degree-days while softwater-acclimated fish were exposed to 479.5 (ambient) and 513.5 (ambient +2°C) degree-days.

The second 30 day period (day 30 to 60) was characterized by a gradual decline in water temperature as the water temperature in all treatments fell by approximately 2°C over the first 20 days of this period. During the subsequent 10 days of this period,

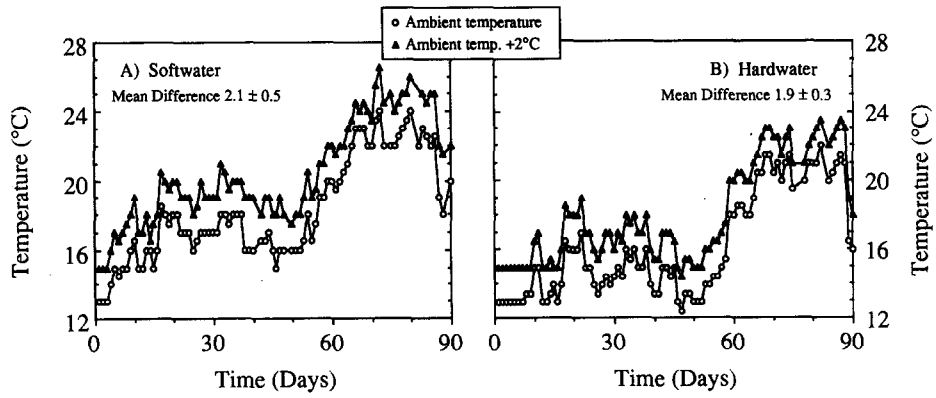

Fig. 1. Temperature profile for the synthetic softwater (A) and hardwater (B) exposure system during 90 day experiment, which ran over the summer of 1993 from June 18 to September 15. Juvenile rainbow trout were held at temperatures representative of inshore Lake Ontario (ambient temp., open circles) or at a temperature 2°C higher (ambient temp. +2°C, filled triangles). The softwater system "ambient" temperature is 2°C higher than the hardwater ambient due to the pressurization of the water necessary to generate synthetic softwater by reverse osmosis. Thus, the softwater ambient temp. +2°C is equivalent to the hardwater ambient temp. +4°C.

water temperature rose rapidly and by 4 and 6°C in the softwater and hardwater systems, respectively. During this second 30 day period, the cumulative thermal exposures for hardwater-acclimated fish were 483.0 (ambient) and 498.5 (ambient +2°C) degree-days while softwater-acclimated fish were exposed to 540.5 (ambient) and 580.5 (ambient +2°C) degree-days.

The highest water temperatures were reached during the final 30 days of this study (day 60 to 90). In the softwater system, the peak temperatures were 24 and 26.5°C in the ambient and ambient temp. +2°C exposures while peak temperatures of 22 and 24.5°C were reached the hardwater ambient and ambient temp. +2°C exposure groups. Following 20–23 days of extreme temperature, water temperature fell dramatically over the final days of the experiment; by 5°C in the hardwater system, 2.5–3°C in the softwater system. During this period of peak temperatures, the cumulative thermal exposures for hardwater-acclimated fish were 601.5 (ambient) and 653.0 (ambient +2°C) degree-days while softwater-acclimated fish were exposed to 655.5 (ambient) and 718.5 (ambient +2°C) degree-days.

Growth, appetite and conversion efficiency

There was no difference in weight gain (growth, Fig. 2A) amongst treatments, with all fish gaining approximately 0.31 g per day over the initial 30 day period. During the second 30 days of exposure, weight gain nearly doubled in all groups except the hardwater fish held at ambient temperature, which experienced growth at rate not different from the initial 30 days. However, between days 60 and 90 of the experiment, the time of highest temperature, there were dramatic influences on the growth of fish in both hardwater and synthetic softwater. While the weight gain of the hardwater fish doubled and that of the softwater fish remained unchanged, growth in fish held at a water temperature of ambient +2°C was significantly reduced by 63 and 87% in hardwater and softwater-acclimated trout, respectively. In addition, when comparing hard- and softwater-acclimated fish at identical or near identical water temperatures (hardwater, ambient temp. +2°C vs softwater, ambient temp.), growth of fish acclimated to synthetic softwater was significantly greater than of hardwater-acclimated fish during this time.

The amount of food consumed by these fish generally mirrored the amount of growth that occurred during the 90 day experiment (Fig. 2B). For the initial and subsequent 30 days of the experiment, no difference in appetite was found between treatment groups despite the near doubling of appetite during the second 30 day period. During the final 30 days of exposure, appetite tended to increase in the two ambient temperature groups, particularly that of the hardwater fish. At this time of highest temperature, the addition of 2°C to the ambient water temperature inhibited appetite in both softwater- and hardwater-

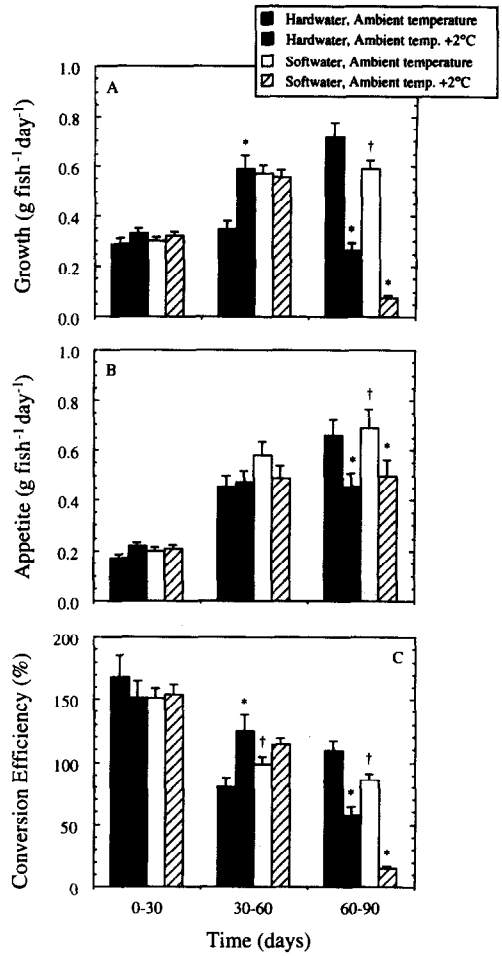

Fig. 2. The effect of water temperature on (A) growth, (B) appetite and (C) food conversion efficiency of softwater- and hardwater-acclimated fish. Growth was measured as the weight gained (g) by the fish over a 30 day period ($N = 30$). Appetite was the daily average amount of feed consumed by the two replicate tanks of fish as fish were fed to satiation ($N = 2$). Conversion efficiency was calculated as the ratio of the weight gain to food consumed, both in wet weight. All data are presented on a per fish basis. Data from hardwater-acclimated fish exposed to the ambient water temperature profile hatched are represented by the hatched solid histograms and to ambient water temperature +2°C by the solid histogram. Data from fish acclimated to synthetic softwater exposed to the ambient water temperature profile are represented by the open histograms and to ambient water temperature +2°C by hatched open histograms. An * indicates a statistically significant difference ($P < 0.05$) between the ambient and ambient +2°C water temperature while a † indicates a significant difference ($P < 0.05$) between data from hardwater- and softwater-acclimated fish at the same water temperature (hardwater, ambient vs softwater, ambient +2°C).

acclimated fish by approximately 30%. However, at the same time, and at nearly the same temperature, fish acclimated to artificial softwater had a significantly greater appetite than the hardwater-acclimated fish.

The ability of these fish to convert consumed food into body weight (conversion efficiency) declined significantly over the duration of the experiment (Fig. 2C), during which time the fish were growing and water temperature was rising. During the first 30 days of the experiment, gross conversion efficiency was approximately 155% and not influenced by temperature or water hardness. During the subsequent 60 days, differences amongst groups were noted. Between days 30 and 60, the addition of 2°C tended to enhance conversion efficiency, although only the difference in the hardwater-acclimated fish was statistically significant. In addition, conversion efficiency in the softwater-acclimated fish was significantly lower than in fish acclimated to hardwater. However, during the final 30 days of exposure, the influence of water hardness on conversion efficiency was reversed. During this time, conversion efficiency was now significantly greater in fish acclimated to synthetic softwater compared to hardwater-acclimated fish at the same or nearly the same water temperature. Conversion efficiency was significantly reduced by the addition of 2°C to the ambient temperature profile, by approximately 47 and 83% in the hardwater- and softwater-acclimated groups, respectively. These findings were consistent with the observed changes in growth and appetite of these fish during this specific phase of the exposure regime; the period of peak water temperatures (day 60–90).

Organ-somatic indices

There was a significant reduction in the somatic indices for both gill and liver over the 90 day exposure period indicating a lower rate of growth of the organs relative to the entire animal (Fig. 3). Prior to exposure to the temperature regime, the branchial baskets and livers of both hardwater- and softwater-acclimated trout represented 2.9 and 1.3% of the total body weight, respectively. Neither the addition of 2°C, nor acclimation to synthetic softwater influenced the organ-somatic indices at any of the sampling times. Although we have shown that whole body growth was influenced both by temperature and water hardness, these data suggest that the growth of the branchial baskets and livers, relative to the whole body weight, was influenced more by standard allometry (i.e. changing body weight) than by water hardness or the addition of 2°C to the natural temperature profile.

Organ-specific protein dynamics

Gills. Prior to the initiation of the specific feeding and temperature regime (day 0), gill protein synthesis (K_s) was approximately 4.6%·day^{-1} in hardwater-acclimated fish and 5.1%·day^{-1} in softwater-acclimated trout, values which were not significantly different (Fig. 4A). After 30 days of exposure to increasing temperature and growth of the fish, gill protein synthesis rates (K_s) in all treatment groups were greater than determined at day 0. At this time, K_s in gills of softwater-acclimated fish was significantly lower than in the gills of hardwater-acclimated fish, exposed at the same temperature (hardwater, ambient temp. +2°C vs softwater, ambient temp.). Furthermore, the addition of 2°C to the ambient temperature profile tended to enhance K_s, an effect which was also apparent at day 60 but statistically significant in the hardwater-acclimated fish. However, by this time water hardness had no significant impact on gill protein synthesis. After 90 days, K_s in gills of fish acclimated to synthetic softwater was nearly 1.6 times greater than the K_s in gills of hardwater fish at the same water temperature. The addition of 2°C to the ambient temperature profile during this time, the time of peak water temperature, had no effect on gill K_s.

Net accumulation of protein (accretion, K_g), by the

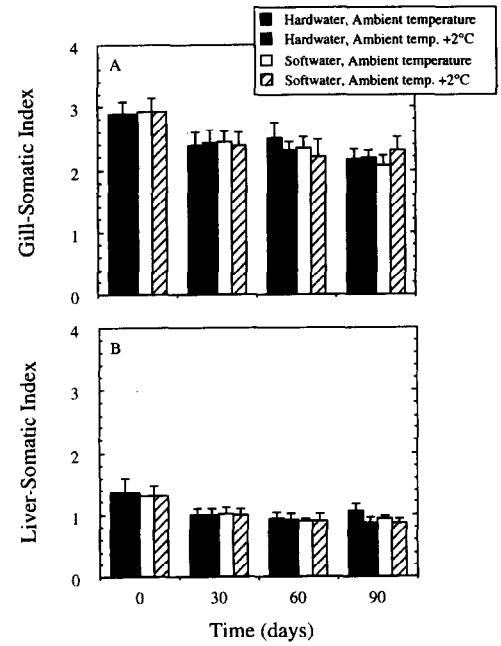

Fig. 3. The effect of water temperature on the (A) branchial basket- and (B) liver-somatic indices of softwater- and hardwater-acclimated fish. The organ-somatic indices were measured prior to (day 0) and at 30, 60 and 90 days of exposure. Data presented as outlined for Fig. 2. Data presented as means ± 1 SE (N = 10).

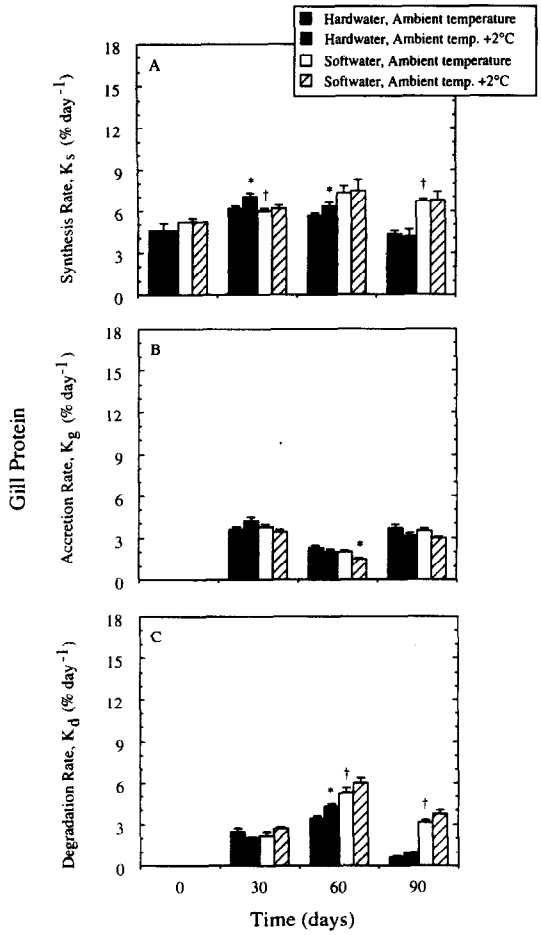

Fig. 4. The effect of water temperature on gill protein (A) synthesis (K_s), (B) accretion (K_g) and (C) degradation (K_d) rates in softwater- and hardwater-acclimated trout. All rates were calculated on a % day^{-1} basis and determined as outlined in the Methods. Data presented as outlined for Fig. 2. Data presented as means ± 1 SE ($N = 10$).

gills of hard and softwater-acclimated fish were not significantly different, at approximately 3.8% · day^{-1}, after 30 days of exposure (Fig. 4B). During the subsequent 30 days, gill K_g was significantly reduced in all treatment groups. Water hardness did not affect the net accumulation of protein in this tissue, while the addition of 2°C to the ambient temperature profile tended to reduce gill protein accretion, with the reduction in gills of softwater-acclimated fish being statistically significant. The effect of additional temperature was identical during the final 30 days of exposure (day 60–90) although, in general, rates of protein accretion had increased to rates similar to those measured after only 30 days of exposure.

Rates of protein degradation in the gills (K_d, Fig. 4C) tended to be positively correlated with gill protein synthesis (Fig. 4A); at a given time, those tissues with higher rates of synthesis possessed higher rates of degradation. Gill protein degradation after only 30 days exposure was approximately 2.4% · day^{-1} and not different amongst treatments. During the subsequent 30 days, gill protein K_d was significantly elevated in all groups. The addition of 2°C to the ambient temperature profile tended to increase the rate of gill protein degradation independent of water hardness, although only the difference in the gills of hardwater-acclimated fish was statistically significant. In addition gill K_d was significantly greater in softwater fish than in hardwater fish at or near the same water temperature. During the final 30 days of the experiment, gill K_d was reduced from those rates determined over the period of day 30 to 60. At the time of highest water temperature, the influence of water hardness on gill protein degradation was enhanced while that of the addition of 2°C to the ambient temperature profile was no longer significant.

Livers. Prior to the exposure of juvenile rainbow trout to the temperature profile and specific feeding regime, protein synthesis rates (K_s) in livers was approximately 4.8% · day^{-1} and independent of water hardness (Fig. 5A). Over the duration of the experiment, liver K_s tended to increase in all treatment groups, the most dramatic increase occurring over the initial 30 days of the experiment when liver protein K_s increased by approximately 62 and 44% in fish acclimated to hard and synthetic softwater, respectively. At this time, there was no difference in liver protein synthesis associated with the addition of 2°C to the ambient temperature profile, however, the rate of synthesis in this tissue was significantly lower in softwater-acclimated fish compared to that of livers from hardwater-acclimated fish at or near the same water temperature. The significant reduction in liver protein K_s associated with softwater-acclimation was noted at all subsequent sampling periods. By day 60 and 90, the addition of 2°C tended to elevate liver protein K_s in tissues from both hardwater- and softwater-acclimated fish, with the former being statistically significant at day 60 and the latter at day 90.

Over the initial 60 days of the experiment, the net liver protein accretion (K_g) was not influenced by either water hardness or the addition of 2°C to the ambient temperature profile (Fig. 5B). During this time, liver K_g was approximately 3.5% · day^{-1}, a rate which tended to decline as the fish grew and the water temperature increased. However, during the final 30 days of exposure, liver K_g was significantly reduced in both softwater- and hardwater-acclimated fish exposed to ambient temperature +2°C. In addition, the rate of net accumulation of liver protein was significantly higher in fish acclimated to synthetic softwater when compared to liver K_g in hardwater fish exposed at or near the same water temperature.

The changes in the degradation rates (K_d) of livers from softwater- and hardwater-acclimated juvenile trout (Fig. 5C) were similar to the changes in liver protein synthesis (Fig. 5A) over the duration of this experiment. After only 30 days exposure, protein degradation rates in livers of fish acclimated to synthetic softwater were significantly lower than the K_d in livers from hardwater fish acclimated at a nearly identical temperature. This apparent influence of water hardness on liver K_d remained evident during the subsequent two 30 day periods. At all times, in both acclimation groups, the rate of liver protein degradation was found to be slightly higher at the elevated water temperature (ambient temp. +2°C). However, these differences were only statistically significant after 60 days in the softwater-acclimated fish and after 90 days in both treatment groups.

White muscle. Initial "nominal" protein synthesis rates (see Methods) in the white muscle were approximately 2.4% · day^{-1} and 1.5% · day^{-1} in juvenile trout acclimated to hardwater and softwater, respectively, and were not significantly different (Fig. 6A). Over the duration of the experiment, as fish were growing and the ambient temperature rising, white muscle K_s declined with no significant impact of either water hardness or the addition of 2°C. In most cases, however, white muscle K_s tended to be lower in muscle from fish acclimated to synthetic softwater than in hardwater-acclimated trout.

The net accumulation of protein (K_g) in white muscle occurred at a rate of approximately 6.1% · day^{-1} over the first 30 days (Fig. 6B). During the subsequent exposure period the addition of 2°C to the ambient temperature profile significantly increased white muscle K_g in hardwater fish. This was not evident in fish acclimated to synthetic softwater, nor was there any significant difference associated with water hardness, despite the lower rate of protein accretion in the softwater fish compared to those acclimated to hardwater. During the final 30 days, the time of highest water temperature, white muscle K_g was significantly lower in fish exposed at ambient temperature +2°C in both acclimation groups. In addition, when compared at the same temperature, white muscle from fish acclimated to synthetic softwater had a significantly higher K_g than white muscle from hardwater-acclimated trout.

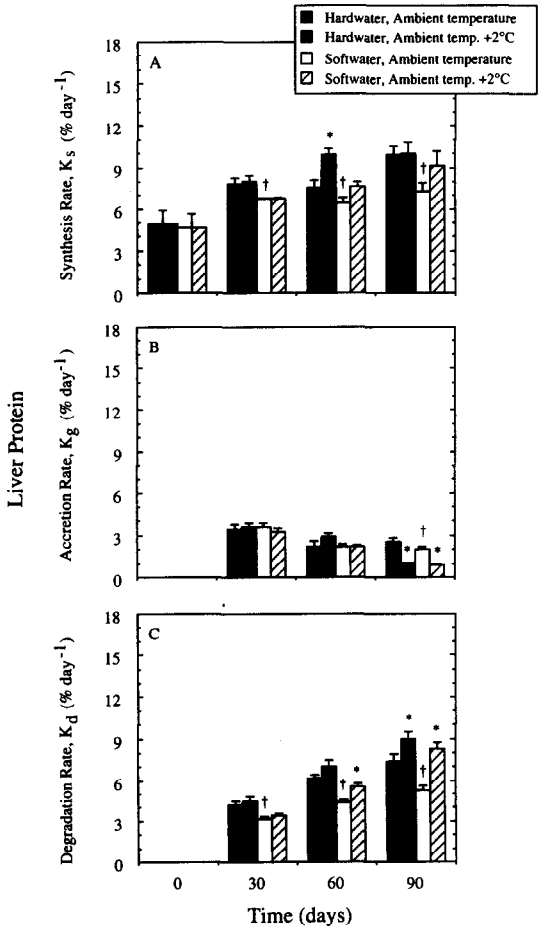

Fig. 5. The effect of water temperature on liver protein (A) synthesis (K_s), (B) accretion (K_g) and (C) degradation (K_d) rates in softwater- and hardwater-acclimated trout. All rates were calculated on a % day^{-1} basis and determined as outlined in the Methods. Data presented as outlined for Fig. 2.

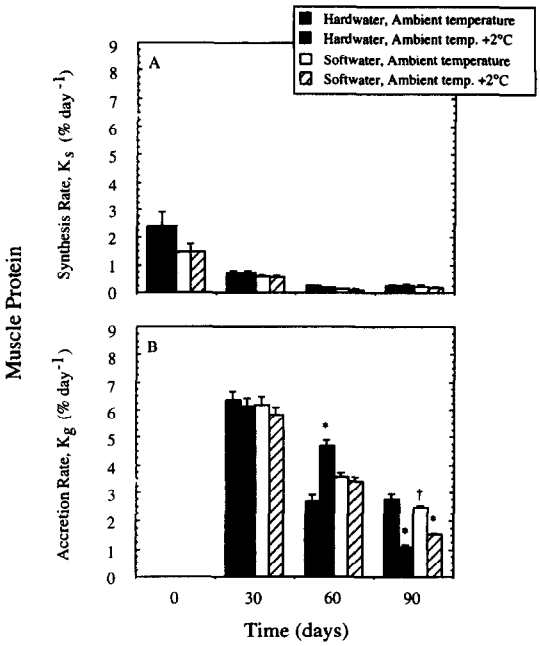

Fig. 6. The effect of water temperature on white muscle protein (A) synthesis (K_s) and (B) accretion (K_g) rates in softwater-acclimated trout. All rates were calculated on a % day^{-1} basis and determined as outlined in the Methods. Data presented as outlined for Fig. 2.

Tissue protein dynamics corrected for differences in body weight

Both size and temperature have profound influences on protein turnover in fish, and both were variables in this study. Houlihan *et al.* (1986) clearly demonstrated a negative correlation between body size and protein turnover (synthesis and degradation) in rainbow trout. While the fish grew over the course of this 90 day study from approximately 3 to 40 g, the temperature also rose from ~13 to ~25°C thus complicating the interpretation of these data. Therefore, gill and liver protein dynamics data were standardized to a common final body weight (40 g) to eliminate the effect of differing body size and thereby elucidate the influence of temperature and water hardness.

As the differences between treatment body weights were minor compared to the changes in weight due to the growth of the fish over the duration of the experiment, the scaling of the data had little or no influence on the treatment effects (i.e. hardness and +2°C) detailed in Figs 4 and 5. What is apparent is the profound overall influence of temperature on protein synthesis in both the liver and gills of both hardwater- and softwater-acclimated trout. The monthly thermal exposures experienced by each treatment group was quantified using degree·days (inserts in Figs 7A and 8A). Over the first 60 days, protein synthesis in these tissues correlated positively with the monthly thermal exposures (degree·days), the regressions of which were not significantly different amongst treatments. By day 90, gill protein synthesis in both hardwater- and softwater-acclimated fish no longer followed this relationship; gill K_s in the hardwater fish significantly declined (Fig. 8A) while remaining unchanged in softwater fish compared to day 60 rates (Fig. 7A). Liver protein synthesis was affected in a similar manner as there was no apparent break in the relationship between environment and temperature and the rate of liver protein synthesis in either softwater- or hardwater-acclimated fish (Figs 7A and 8A).

Scaling the protein accretion data to a standardized body weight eliminated the apparent reduction in gill, liver and muscle protein accretion rates which occurred between the first subsequent 30 days of the study (Figs 7B and 8B) but did not alter any treatment effects. Similarly, rates of protein degradation in these tissues were not obviously affected by the removal of the influence of body size (Figs 7C and 8C).

Using the size-corrected protein turnover data in combination with the growth, appetite, and food conversion efficiency permits the quantification of the

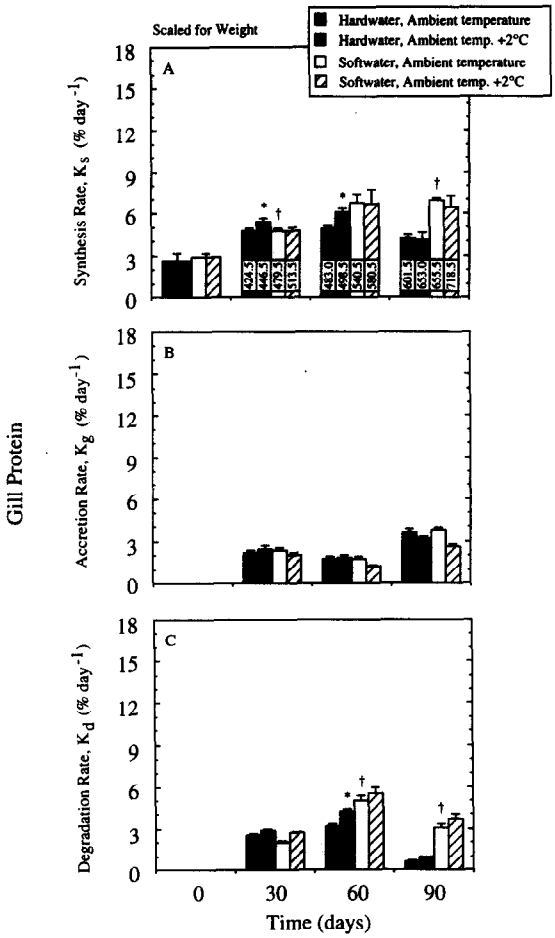

Fig. 7. The effect of water temperature on gill protein (A) synthesis (K_s), (B) accretion (K_g) and (C) degradation (K_d) rates in softwater- and hardwater-acclimated trout. All rates were calculated on a % day^{-1} basis and determined as outlined in the Methods. Protein synthesis and accretion rates were body size corrected to a standard size of 40 g (see methods for details). Numbers inserted into the histograms represent the degree·days over that 30 day exposure period for that treatment. Data presented as outlined for Fig. 2.

effect of our global warming scenario (+2°C). During the first 60 days while water temperature was rising, rates of growth, appetite, food conversion efficiency, tissue protein synthesis, net accretion and degradation were enhanced in fish (both softwater- and hardwater-acclimated) by $16.0 \pm 5.18\%$ ($N = 39$). The temperature quotient for these indicators of cost of living equates to a Q_{10} of approximately 2.1. However, during the final 30 days, the period of peak water temperatures, +2°C resulted in a $19.8 \pm 6.92\%$ reduction in these parameters, a Q_{10} of approximately 0.4.

DISCUSSION

In the present study, we utilized the flooding dose of a radiolabelled phenylalanine technique to assess

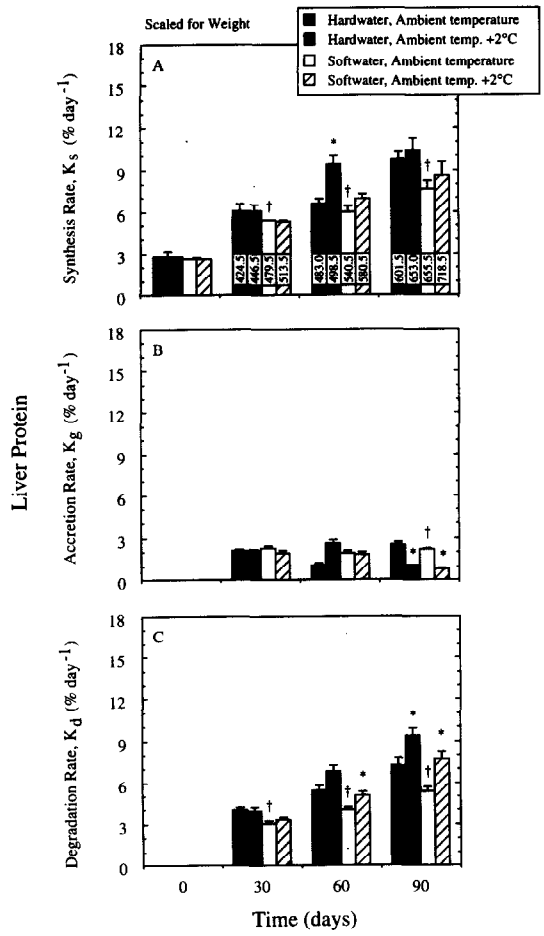

Fig. 8. The effect of water temperature on liver protein (A) synthesis (K_s), (B) accretion (K_g) and (C) degradation (K_d) rates in softwater- and hardwater-acclimated trout. All rates were calculated on a % day^{-1} basis and determined as outlined in the Methods. Protein synthesis and accretion rates were body size corrected to a standard size of 40 g (see Methods for details). Numbers inserted into the histograms represent the degree·days over that 30 day exposure period for that treatment. Data presented as outlined for Fig. 2.

the cost of living for fish living in a warming environment for 90 days, in the presence or absence of additional temperature (+2°C) and a marginal environment (synthetic softwater). Estimates of protein turnover based on a flooding dose of radiolabelled amino acids (e.g. phenylalanine or leucine) have been used to answer a variety of question related to temperature effects on protein synthesis (Fauconneau and Arnal, 1985; Loughna and Goldspink, 1985; Foster *et al.*, 1992), the relationship between metabolic rate and the rate of protein synthesis (Pannevis and Houlihan, 1992), the influence of body, tissue and ration size on rates of protein synthesis, accretion and degradation (Decken and Leid, 1992; Houlihan *et al.*, 1986; Houlihan *et al.*, 1988) and the metabolic cost associated with zinc-induced tissue damage and subsequent repair (Hogstrand *et al.*, 1994a, b).

There are two design issues that highlight the uniqueness of the present study, the temperature and feeding regimes. The temperature regime mimics 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 ambient and ambient +2°C being separated by 2°C throughout. Therefore, there was a considerable overlap in their thermal histories, though with temporal separation. For example, softwater-acclimated trout exposed at ambient water temperature experienced temperatures ranging from 13 to 24°C over the duration of the 90 day experiment; a total exposure of 1675.5 degree·days. The ambient +2°C water temperature covered a range of 15 to 26°C which resulted in a cumulative exposure of 1812.5 degree·days. Therefore, only at the extreme high and low range of the temperature regime were there absolute differences in temperature experience. However, cumulative temperature exposures, expressed as degree·days, were different among treatments over the duration of this experiment. As a consequence of the attempt to mimic a natural temperature regime, the present study differs significantly from the majority of previous investigations into the relationship between temperature or temperature acclimation and protein synthesis or metabolic rate. Typically, two (9 vs 19°C, Fauconneau and Arnal, 1985; 5 and 15°C, Foster *et al.*, 1992) or more (Loughna and Goldspink, 1985) discrete and constant acclimation temperatures are chosen and rates of protein synthesis are determined.

The feeding regime was one in which fish were fed twice daily to satiation in order to quantify appetite which could then be used as an additional estimate of cost of living, or metabolic rate (Cho, 1990; Cho, 1992). As a result, fish were not feed controlled or equal rations. This deviation in feeding regime, although useful for estimating appetite, complicates the interpretation of the protein synthesis data as 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 to 4% body weight day^{-1}.

Growth, appetite and conversion efficiency

Appetite increased as a function of both increased fish size and increased environmental temperature over the duration of this study (Fig. 2B). Temperature profoundly influences metabolic rate and there-

fore food consumption (Cho *et al.*, 1982; Cho, 1992). Thus appetite in itself is an idirect indicator of metabolism or cost of living. However, as evident by the progressive reduction in gross conversion efficiency over the 90 days (Fig. 2C), growth (weight gain per fish) did not increase with temperature at the same rate as appetite. Transit time for food through gut decreases with increased temperature but efficiency of digestion increases with temperature (Cho, 1990) which implies that conversion should not change simply as a result of changes in environmental temperature. However, Cho (1992) has shown that conversion efficiency declines with increased growth in rainbow trout held at fixed temperature. Martinez *et al.* (1992) reported similar findings.

During the final 30 days of this study, growth of both hardwater- and softwater-acclimated fish at ambient +2°C water temperature was dramatically reduced (Fig. 2A). Conversion efficiency was also reduced more than would be predicted based on temperature, as appetite, although significantly reduced, was not affected to the same extent as was growth in these fish (Fig. 2B). At this time, these fish experience water temperatures (24–26°C) at or near the upper lethal temperature (26°C) for rainbow trout (Kaya, 1978). The discrepancy in the appetite and growth data suggests that during this time, fuel is diverted from growth to other maintenance or homeostatic processes. One explanation for this apparent energy repartitioning is the possible induction of heat shock proteins within the tissues of these fish. Heat shock proteins are highly conserved proteins encoded by the hsp70 and hsp90 gene families (Lundquist and Craig, 1988) that are thought to play an important role in heat-induced thermal resistance (Heikkila *et al.*, 1982; Mosser *et al.*, 1987).

Protein dynamics

Due to 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 present study, a 2°C increase in ambient temperature led to an increase in liver and gill protein synthesis in 83% of all comparisons, while protein degradation was enhanced in these tissues in every situation. During the first 60 days of this experiment, the overall influence of +2°C (including protein turnover, whole body growth, appetite and gross conversion efficiency) was to increase those rates by approximately 16% or a 2.1 fold increase with a 10°C increase in temperature (Q_{10}). These findings agree with those of both Fauconneau and Arnal (1985) and Loughna and Goldspink (1985) in as much as acclimation or exposure to elevated temperature resulted in greater rates of tissue protein synthesis in trout. Fauconneau and Arnal (1985) found that by increasing acclimation temperature of rainbow trout from 10 to 18°C, liver and digestive tract protein synthesis increased 3.4 and 2.3 fold, respectively. Similarly, Loughna and Goldspink (1985) reported a Q_{10} for rainbow trout white muscle protein synthesis of approximately 3.6 over a temperature range of 5 to 20°C. However, contrary to these findings are those of Foster *et al.* (1992) who reported no differences in either weight-specific growth rate or weight-specific tissue protein synthesis (ventricle, gill, stomach and intestine) rates in Atlantic cod acclimated to 5 and 15°C. The explanation for discrepancy may be due to interspecific variations in protein metabolism temperature compensation. Loughna and Goldspink (1985) determined that although prior thermal history has no modifying influence on temperature effects on protein synthesis in rainbow trout, carp (*Cyprinus carpio*) have the ability to modify their rate of protein synthesis at different temperatures which was not altered by prior thermal history.

Between day 60 and day 90, the water temperature in the softwater exposure system peaked at either 24 and 26°C depending on the treatment group (Fig. 1A) and resulted in dramatic reductions in gill and liver protein synthesis, accretion and degradation (Figs 4 and 5). these alterations in protein turnover were reflected in the other indicators of metabolic activity in these fish (food conversion efficiency, appetite and growth) and implicate that metabolism is dramatically and rapidly reduced at temperatures between 24 and 26°C. The average reduction of all of these estimates of cost of living being approximately 20% in all treatment groups. Softwater fish held at ambient temperature and hardwater-acclimated fish in the ambient +2°C group experienced a peak temperature of only 24°C (Fig. 1B). Only appetite and food conversion efficiency of the softwater fish were affected at this temperature, although other metabolic indicators (nitrogenous waste excretion and nitrogen quotients) were depressed in the hardwater-acclimated fish at 24°C (Linton *et al.* unpublished results). 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 as a last resort prior to heat-induced mortality. Heat shock proteins production appears to occur at the expense of the production of other proteins (DiDomenico *et al.*, 1982). However, one would not expect total protein synthesis and net accretion to be so dramatically reduced if the tissues are switching from the

production of one set of proteins to another, unless heat shock protein production represents only a minor percentage of the synthesis which is shut-down. Only with quantification of heat-shock protein production can this question of heat shock protein production and partitioning of metabolism be adequately addressed. Nonetheless, for a trout which 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.

Water hardness

In order to acclimate to a stressful condition, such as softwater, it has been proposed that the fish must undergo a cost directly attributable to the required physiological/biochemical adjustments (Calow, 1991). Therefore, one would predict that fish living in softwater would already have a greater cost of living and that any subsequent increase in their cost of living would be of greater consequence to them than a fish living in a hardwater environment.

Regardless, at the start of this experiment there were no differences in gill and liver protein synthesis between fish acclimated to softwater or hardwater. These results were obtained despite fish having been held in synthetic softwater or natural hardwater for approximately 8 weeks prior to the initiation of experiment. Only after 30 days exposure to different thermal environments were differences in tissue protein turnover apparent. At this time other indicators of cost of living, namely growth and appetite (Fig. 2A, B), were not different between softwater- and hardwater-acclimated fish. Nonetheless, gross conversion efficiency was significantly lower in softwater fish (Fig. 2C). The most dramatic difference between these two groups of fish, however, was the rate of liver protein synthesis which was significantly greater in the hardwater-acclimated fish. This was unexpected but could be related to apparent differences in appetite and the relationship between ration size and protein synthesis (Houlihan *et al.*, 1988). One would have predicted differences in gill, not liver protein dynamics, based on the reduction of water ion content and alkalinity and the fact that many of compensatory mechanisms invoked involve modifications in protein turnover. It is well established that acclimation of trout to softwater invokes hormonally driven compensatory responses which counteract changes in ion gradients and gill permeability, e.g. increased transport activity for Na^+, Cl^- (Maetz, 1974) and Ca^{2+} (Perry and Wood, 1985), increased epidermal thickness and density of mucocytes (Wendelaar Bonga, 1978), proliferation of chloride cells (Laurent *et al.*, 1985) and increased gill surface Ca^{2+} binding activity (Reid, 1990). Such expected differences in gill protein dynamics were seen in the final 30 days of this study. Additional evidence implicating a difference in the cost of living based on water hardness becomes apparent when appetite and growth examined in relationship to monthly cumulative temperature exposure (i.e. degree·days). Taking into account thermal history, appetite and growth were apparently 52 and 32% lower in softwater-acclimated than in hardwater-acclimated trout, respectively. Further, gross conversion efficiency was approximately 14% higher in softwater fish compared to hardwater fish. Therefore, it could be suggested that softwater is, in itself, a mild stress which suppresses food consumption. In order to support an adequate rate of growth despite a reduction in appetite and enhanced rates of gill protein synthesis, a compensatory increase in the efficiency by which food is converted to weight must take place. Yet, despite the alteration in conversion efficiency, growth remains limited in the softwater fish. Therefore, it could be argued that the cost of living for fish acclimated to (not acclimating to) synthetic softwater is greater than for fish acclimated to hardwater.

SUMMARY

The addition of +2°C to the normal inshore Lake Ontario temperature profile generally further enhanced the rates of these metabolic cues by approximately 16% during the first 60 days of exposure. However, at temperatures near the upper lethal temperature for trout, this slight temperature increase triggered a dramatic 20% reduction in growth, appetite, food conversion efficiency, protein turnover and accretion of the exposed fish. Therefore it could be stated that 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 (0.5–6°C), the results of this study suggest that it would increase the cost of living for fish, such as the trout, and have serious consequences if these animals were living near the upper limit of their temperature tolerance or living in an already marginal and energetically demanding environment such as softwater.

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