Effects of elevated summer temperatures and reduced pH on metabolism and growth of juvenile rainbow trout (*Oncorhynchus mykiss*) on unlimited ration

Jacqueline J. Dockray, Scott D. Reid, and Chris M. Wood

Abstract: Juvenile trout (*Oncorhynchus mykiss*) were exposed to a simulated global warming – acidic water scenario over a 90-day summer period (control temperature range 13–24°C). The addition of 2°C to the fluctuating summer cycle of inshore Lake Ontario and H_2SO_4 to synthetic soft water resulted in four treatments: control, acidification of control, simulated global warming alone, and global warming plus acidification. The twice-daily feeding regime raised metabolic rates to ~75% of $\dot{M}O_2$(max). Large increases (from 4.5 to 11.5%) in whole-body lipid, smaller increases (from 12.0 to 15.5%) in protein, and compensating decreases in water content (from 77 to 71%) occurred in all treatments over time. The addition of 2°C resulted in depressed appetites and growth, particularly after the period of peak temperature (days 60–90; 26°C). Metabolic rate and nitrogenous waste excretion were also depressed. Overall, exposure to low pH resulted in increased appetites and growth, the increase of 2°C reduced gross energy intake and increased fecal energy losses, and exposure to low pH resulted in increased energy intake and gain and better conversion efficiency. The lack of ionoregulatory disturbance in trout chronically exposed to pH 5.2 suggested that dietary NaCl may have compensated for branchial ion losses.

Résumé : Des truites juvéniles (*Oncorhynchus mykiss*) ont été exposées à une simulation de réchauffement global et d'acidification de l'eau pendant une période de 90 jours en été (plage de température témoin de 13–24°C). Le relèvement de 2°C du cycle fluctuant de la température estivale des eaux côtières du lac Ontario et l'ajout de H_2SO_4 à de l'eau douce de synthèse ont servi à pratiquer quatre traitements : témoin, acidification du témoin, simulation de réchauffement global seulement, et réchauffement plus acidification. Le régime d'alimentation biquotidien a fait monter le métabolisme à 75% de $\dot{M}O_2$(max). De fortes augmentations (de 4,5 à 11,5%) des lipides du corps entier, des augmentations plus faibles (de 12,0 à 15,5%) des protéines et des baisses compensatoires de la teneur en eau ont été observées dans tous les traitements (de 77 à 71%) au fil du temps. Le relèvement de 2°C a causé une diminution de l'appétit et de la croissance, particulièrement après le pic de température (jours 60 à 90; 26°C). Le métabolisme et l'excrétion de déchets azotés ont également baissé. Globalement, l'exposition à un pH faible a occasionné une augmentation de l'appétit et de la croissance. Le relèvement de 2°C a réduit l'absorption d'énergie brute et accru les pertes d'énergie par les fèces, tandis que l'exposition à un faible pH a causé une augmentation de l'absorption et du gain d'énergie, et une meilleure efficacité de conversion. L'absence de perturbation de la régulation ionique chez les truites chroniquement exposées à un pH de 5,2 permet de penser que le NaCl contenu dans l'aliment peut avoir compensé les pertes ioniques au niveau branchial.
[Traduit par la Rédaction]

Introduction

The Intergovernmental Panel on Climate Change (IPCC; 1991) has forecast that with a doubling of atmospheric CO_2, which will occur over the next 50 to 100 years if current production levels continue, and increases in other "greenhouse" gases, such as methane, nitrous oxide, and chlorofluorocarbons, global air temperatures may increase 1.3–4.5°C.

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J.J. Dockray, S.D. Reid,[1] and C.M. Wood.[2] Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON L8S 4K1, Canada.

[1] Present address: Department of Biology, Okanagan University College, 3333 College Way, Kelowna, BC V1V 1V7, Canada.

[2] Author to whom all correspondence should be addressed. e-mail: woodcm@mcmail.cis.mcmaster.ca

Increased air temperatures will result in increased temperatures of freshwater bodies, which will directly affect the fisheries associated with these habitats. Regier and Meisner (1990) discuss the effects of increased temperature on water quantity and quality in the Great Lakes using an iterative assessment process. Schindler et al. (1990) described an increase in air temperature at the Experimental Lakes Area in northwestern Ontario of 2°C over the last 20 years and provided data indicating that evaporation has increased, precipitation has decreased, and consequently, water renewal rates have decreased. These changes have directly affected stenothermal fish species by decreasing available cold water habitats. From these observations, they concluded that fresh waters should be considered in major global change programmes because of the fisheries' importance as a food source and their scarcity worldwide.

Anthropogenic production of pollutants has many other environmental impacts that should be considered in concert with projected climate change. For example, the burning of coal and oil and the smelting of metallic ores result in the production of

sulphur dioxide (SO_2), and internal combustion engines produce nitrogen oxides (NO_x). Release of SO_2 and NO_x into the atmosphere results in acid precipitation, which has decreased lake pHs by between 0.5 and 1.5 units in many softwater lakes in North America and Europe over the last 140 years (Kemp 1994). Particularly sensitive lakes, with alkalinities below 50 μequiv·L^{-1}, include those found in the eastern provinces of Canada (Schindler 1988). In Ontario, lakes found on the Canadian Shield have a highly resistant granitic base, which results in low water-buffering capacity. Acidification of these lakes has been extensively studied by Scheider et al. (1979), Schindler et al. (1980), and Kelso et al. (1982, 1986), and the deleterious effects on fish have been described by Beamish et al. (1974*a*, 1975), Kelso and Gunn (1984), and Kelso et al. (1986, 1990).

Because fish are poikilotherms, their body temperature is set by the external temperature of the water they inhabit. Of all environmental factors that affect fish, temperature is considered to be the ecological master factor (Brett 1971) and has direct influence on metabolic rates, feeding rates, and activity levels. Metabolic rate, as measured by oxygen consumption, is a composite measure of the cost of living for a fish in a particular environment. Important costs include maintenance metabolism, energy expended in feeding, nutrient assimilation, subsequent waste excretion, growth, and locomotion. All of the latter requirements for living have a requisite cost associated with them, the amount of which is set by temperature.

In fish, exposure to low pH has been shown to either reduce growth or have no effect on it (Mount 1973; Leivestad et al. 1976; Menendez 1976; Jacobsen 1977). Decreased growth may be a consequence of decreased feeding under acid conditions (Beamish 1972; Swarts et al. 1978). Low pH has also been shown to increase resting oxygen uptake (M_{O_2}), indicating increased metabolic cost, and decrease critical swimming speeds (U_{crit}), thereby reducing the scope for activity (Butler et al. 1992).

Protons exert their toxic effect at the gills by disturbing electrolyte balance. Ionoregulatory failure and subsequent fluid shifts, hemoconcentration, and circulatory collapse are the result of lethal levels of low pH (Milligan and Wood 1982; Wood 1989). Chronic sublethal levels of low pH, however, have been shown to result in stabilization of electrolyte balance and blood plasma parameters (Audet et al. 1988), and recovery of whole-body [NaCl] has been shown to occur in one study (Wilson et al. 1994*a*). The physiological changes associated with the original ionoregulatory disturbance, and adjustment to the new conditions, are considered deleterious and do not result in acclimation to more severe acid stress in rainbow trout (Audet and Wood 1988).

The physiological adjustments required by fish exposed to low pH and the increased rate of physiological processes caused by higher temperatures presumably would result in increased metabolic costs. Our goal in the present study was to assess the effects of chronic exposure to sublethal low pH (5.2) and elevated (+2°C) summer water temperatures on metabolic costs. The experiment was carried out over the summer (June–September 1993), when the summer peak temperature occurred, using juvenile rainbow trout (*Oncorhynchus mykiss*) that were fed to satiation. A suite of metabolic cost indicators was measured, including appetite, growth, metabolic rate, and the partitioning of food energy as protein, lipid, and carbohydrate. Other potential indicators of increased metabolic cost and stress that were monitored include whole-body and plasma ion levels, hematocrit, plasma protein, and lactate levels.

Materials and methods

Animal holding

Experiments were conducted on 1146 juvenile rainbow trout (2–3 g), transported from Rainbow Springs Hatchery, Thamesville, Ont., on April 19, 1993. At McMaster University, they were placed in a 400-L polypropylene tank and acclimated to and maintained in continuously flowing Hamilton dechlorinated tap water ($[Ca^{2+}] = 1.95 \pm 0.22$, $[Na^+] = 0.556 \pm 0.33$ mequiv·L^{-1}; pH = 7.4–7.8) at 11°C for 1 week after arrival. The water hardness was reduced gradually over the 2nd week by increasing the proportion of artificially softened water generated by reverse osmosis (Anderson Water Conditioning Equipment, Dundas, Ont.). When the desired water quality was achieved, the trout were acclimated for another 7 weeks. Fish were fed ~4% (dry weight of food) of their dry body weight·day^{-1} with Zeigler Trout Starter No. 3 (water, 11.66%; protein, 50%; fat, 15%; fibre, 2%; sodium, 0.50%; calcium, 2.30%; phosphorus, 1.80%) and maintained on this diet for the 8-week acclimation period before the start of the experiment. Photoperiod was controlled and adjusted to mimic the natural photoperiod throughout the acclimation and experimental periods.

Exposure system

The temperature of Hamilton city tap water closely follows the natural thermal regime of the inshore region of Lake Ontario, from which it is collected. Passage through the reverse osmosis unit unavoidably added 2°C to the water temperature; therefore, the ambient regime was consistently 2°C above the natural regime. Hereafter, the ambient regime will be referred to as the control temperature. After softening and deionization by reverse osmosis, the product water was pumped to a main head tank where hard water was added in a ratio of 1:40 to achieve $[Ca^{2+}]$ and $[Na^+]$ of ~50 and 100 μequiv·L^{-1}, respectively. This synthetic soft water (pH 6.3) was then gravity fed into two subhead tanks. The gravity feed to one of these two subhead tanks passed through a heat exchanger where 2°C was added to the control temperature. The water from each of these two tanks was further split into two more subhead tanks, resulting in four treatment head tanks. H_2SO_4 (0.2 M) was metered into one each of the control and +2°C temperature head tanks, resulting in the following four treatments: control temperature – control pH 6.3 (0/6.3); control temperature – sublethal pH 5.2 (0/5.2); control temperature + 2°C – control pH 6.3 (+2/6.3); and control temperature + 2°C – sublethal pH 5.2 (+2/5.2).

The water from the treatment head tanks flowed into duplicate 211-L polypropylene exposure tanks at an average rate of 1.2 L·min^{-1}, which allowed for 90% replacement in 7 h. Sublethal pH 5.2 was maintained by monitoring the pH in the treatment tanks using an industrial pH electrode (Leeds & Northrup Meredian II Combination). The electrode reading controlled a solenoid valve (Cole Parmer Instrument Co., CP No. 01367–70) that opened and closed according to the tank pH and the high and low pH control points, adding H_2SO_4 to the treatment head tank. Temperature and low pH were monitored via a programmable controller (Ladder Logic and Texas PLC model TI315), which was connected to an alarm and an automatic message dialer system (Safe House model 49-433A). The alarm system was triggered by a drop or increase in temperature of 1.0°C and (or) pH of 0.8 pH units. All tanks and head tanks were aerated, and water partial pressure of O_2 (P_{O_2}) was determined once a week using a thermostatted radiometer – P_{O_2} electrode (Copenhagen E5046) connected to an oxygen meter (Cameron Instrument Co.). P_{O_2}s averaged 115 Torr (1 Torr = 133.322 Pa) and did not drop below 80 Torr. Temperature and pH were measured daily. Ambient pH averaged 6.27 ± 0.03, and low pH averaged 5.27 ± 0.03. $[Na^+]$ and $[Ca^{2+}]$ were monitored twice a week using atomic absorption spectroscopy (Varian AA-1275) and

averaged 97.5 ± 2.23 and 57.7 ± 2.28 μequiv·L^{-1}, respectively. Titratable alkalinity was determined once a week by titration to an end point of pH 4.2, using 0.02 M HCl. Mean values were 269.5 ± 18.8 and 120.3 ± 15.3 μequiv·L^{-1} for the control pH and low pH treatments, respectively. Water chloride (69.9 ± 14.5 μequiv·L^{-1}; spectrophotometric determination; Zall et al. 1956) and total chlorine (10–12 μg·L^{-1}; Hach, Loveland, Colo.) were measured every 30 days.

Feeding regime

At the end of the 8 weeks of acclimation, 142 trout were selected at random and placed in each of the eight treatment tanks, with 152 fish being placed in one of the 0/6.3 treatment tanks to allow 10 extra fish for day 0 sampling. Fish were hand-fed Zeigler's Trout Starter No. 3 to satiation twice daily at 08:30 and 16:30. The food was changed to Trout Starter No. 4 (of identical composition) halfway through the exposure because of fish growth. An aliquot of food from preweighed bags was sprinkled on the water surface of each replicate tank. If all of the food was consumed within 1 min, more food was placed on the water surface. When food was left on the surface at the end of the 1-min period, a second minute was allowed. If food remained after this 2-min period, the fish were assumed to be satiated and feeding was stopped. If not, the process was repeated until feeding stopped. At the end of each day, the bag was reweighed to determine the amount of food consumed. Fish consumed ~10–11% of their dry body weight·day^{-1} of dry food on this feeding regime. Fecal material was removed from the tanks by siphon every day, and tanks were scrubbed clean once a week.

Physiological measurements

Sampling protocol

Sampling and physiological measurements were conducted over a 4-day period every 30 days, including the first day of the experiment. The tests done on each of the 4 days are outlined below. Trout were not fed on the 3rd and 4th days of the sampling period, when whole-body and blood samples were collected. Methods were modified for nitrogenous waste excretion and oxygen consumption measurements on days 30, 60, and 90 relative to day 0. These changes were primarily dictated by the need for longer periods of aeration between sampling because of the higher summer temperatures present at these times.

Nitrogenous waste excretion

Day 0 (experiment initiation): In-tank routine nitrogenous waste excretion was measured over a 24-h period on the 1st day of each 30-day exposure period by stopping water flow to the tank. Aeration ensured mixing, and tests demonstrated no loss of ammonia as a result of aeration. Initial and final water samples were taken at time 0 and 1 h. The flow to the tank was then resumed for 1 h, and the sampling and closure continued at times 3 h and 4 h. This cycle was continued over the 24-h period. Samples were frozen at –20°C for later analysis of ammonia by the salicylate–hypochlorite assay (Verdouw et al. 1978) and of urea by the diacetyl monoxime method (Rahmatullah and Boyd 1980), as modified by T.P. Mommsen (see Lauff and Wood 1996). For the latter, the samples were first freeze-concentrated 5-fold by lyophilization. Nitrogen production (N; total ammonia and urea nitrogen) was determined by the difference between N concentration at the beginning and end of each closed-tank period, factored by time, volume, and fish weight.

Days 30, 60, and 90: Nitrogenous waste excretion was measured on a flow-through basis on the 1st day of each 30-day exposure period by taking an inflow and outflow water sample for each treatment every 4 h over a 24-h period. Inflow water rate was measured for each sampling period. Samples were frozen and later analyzed for urea and ammonia nitrogen as above. The difference between inflow and outflow total N concentration, factored by flow rate and fish weight, gave the nitrogen production rate in μmol·g^{-1}·h^{-1}. For each of the six sampling periods, data were graphed against time and the area under the curve was determined for each treatment to give an overall daily nitrogen production rate.

Fractional protein utilization (FPU) was determined for each treatment at each sampling period. FPU is an index of the fraction of M_{O_2} that supports protein metabolism. It is calculated by dividing the nitrogen quotient (NQ = the ratio of moles of nitrogen produced to moles of oxygen consumed) by the theoretical maximum NQ in which protein supports all aerobic metabolism. This theoretical maximum for fish was determined by Kutty (1972) to be 0.27. In effect, the FPU represents the degree to which the fish depend on protein as an aerobic fuel source.

Metabolic rates

Day 0 (experiment initiation): Metabolic rates were determined on the 2nd day of the exposure period by measuring in-tank routine oxygen consumption rates (M_{O_2}) every other hour (alternating open and closed periods) over a 10-h period from 07:00 to 17:00. Fecal matter was first siphoned from the tanks. Aeration and inflow water were removed, the tanks were then sealed with a clear, air-tight lid, and water was recirculated within each tank using a submersible pump (Little Giant, 1 EUAA-MD). A water sample was removed from mid-tank every 20 min using a 5-mL syringe, and this sample was injected into a jacketed radiometer – P_{O_2} electrode (Copenhagen E5046) with ambient temperature water circulating through the jacket. The P_{O_2} was recorded on an oxygen meter (Cameron Instrument Co.) and was never allowed to decrease below 80 Torr. Mean M_{O_2} values, determined for each hour using oxygen solubility coefficients from Boutilier et al. (1984), were graphed against time. The curve produced was integrated, for each treatment, to give an overall mean M_{O_2} value for that 10-h period.

Days 30, 60, and 90: The same methods as above were used on the 2nd day of each 30-day exposure period, except that the O_2 electrode was suspended directly in the tank rather than in an external thermostatted cuvette.

All nitrogen excretion and M_{O_2} data were corrected for fish size differences using the weight exponent 0.824, determined for rainbow trout by Cho (1992).

General procedures for blood and tissue analysis

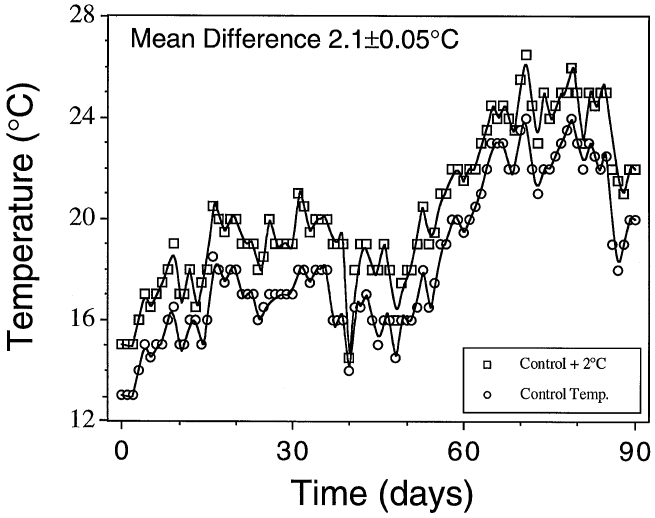
Blood analysis

Ten fish were randomly chosen from each duplicate treatment tank (10 fish were taken from the control tank (0/6.3) only for day 0 sampling). Each fish was quickly killed by a blow to the head and blotted dry, and weight and total length were determined. Blood was collected from the dorsal aorta by caudal severance into ammonium heparinized capillary tubes. The blood samples were spun in an IEC MB micro hematocrit centrifuge (Damon/IEC Division) for 5 min at 10 000 × *g*. Hematocrit was determined as the ratio of the length of packed red blood cells to the total sample length and multiplied by 100 to give a percentage value. Because of the small size of the fish, it was important to conserve blood; the plasma in the capillary tube was removed, using a 50-μL Hamilton syringe, and frozen for subsequent ion analysis. Plasma Na^+ and Ca^{2+} levels were determined by atomic absorption spectroscopy, and Cl^- levels were determined by coulometric titration (Radiometer CMT10). When volume permitted, plasma protein was measured using a hand-held refractometer (American Optical) (Alexander and Ingram 1980).

Whole body and muscle samples

Ten fish were selected, without conscious choice, from each duplicate treatment tank and placed in terminal anaesthetic (1.0 g·L^{-1} MS-222 and 2.0 g·L^{-1} $NaHCO_3$). Each fish was blotted dry, weight and total length were determined, and the fish was rapidly frozen using aluminum tongs frozen in liquid N_2. Fish were wrapped individually in

Fig. 1. Thermal cycle (June 18 – September 15, 1993) experienced by juvenile rainbow trout over the 90-day experimental period. Control temperatures peaked between days 60 and 90 at 24°C (26°C in the +2°C treatments).



labelled aluminum foil and stored at –80°C for later whole-body analysis. Whole fish were ground frozen, using an IKA (M10/M20) grinding mill cooled to approximately –72°C. A sample of the tissue was weighed and then dried to constant weight at 80°C to determine water content. The remainder of the tissue was lyophilized (Labconco Lyph-Lock 6), desiccated, and stored at –20°C. Whole-body Na^+, Ca^{2+}, K^+, and Cl^- levels were determined as above for plasma ions after 100 mg of tissue was digested for 48 h in 900 µL of 1 M H_2SO_4 at 80°C. Subsamples of freeze-dried tissue were used to determine whole-body protein levels, using the Lowry assay as modified by Miller (1959). Lipids were extracted and quantified using the chloroform–methanol (2:1) method (Folch et al. 1957). Glycogen, glucose, and lactate levels were determined as an estimate of whole-body carbohydrate, using standard enzymatic analyses (Bergmeyer 1985). Percentage inorganic content (ash) was determined by burning a subsample of whole-body tissue at 550°C in a muffle furnace until a constant weight was achieved.

A portion of the white muscle was removed from the dorsal section posterior to the operculum, and anterior to the dorsal fin, from the fish used for blood sampling above. The skin and fin rays were removed, and the white muscle was frozen in liquid nitrogen, wrapped in labelled aluminum foil, and stored at –80°C for later analysis. The samples were weighed and then dried at 80°C to a constant weight to determine water content.

Mortalities

Few mortalities occurred as a result of the treatments over the 90-day period; they amounted to less than 10% overall. Unfortunately, because of a laboratory mishap, the +2/5.2 treatment duplicate tank was lost at day 23.

Energy budget

Because we determined the quantity of food consumed by trout in each treatment, the weight gain, the proximate components of that weight gain (protein, lipid, and carbohydrate), metabolic expenditure, and excretory losses, we have calculated a crude energy budget on an individual fish basis for each treatment over the 90-day period. It is crude in that these measurements were made on whole tanks of fish rather than on individuals, although corrected to an individual fish basis. However, the energy budget allows for examination of each of the inputs and outputs of energy in common units (Soofiani and Hawkins 1985). The transformation of each of the inputs and outputs into energy units (kilojoules) allows for a more accurate assessment of growth and energy conversion as it relates to energy gain because each of the components of new tissue (protein, lipid, carbohydrate) have different energy values. The energy budget, therefore, should show the effects of treatments on the allocation of energy in the present study and give a composite measure of the cost of living for trout in each treatment.

The basic energy budget equation is $C = P + M + U + F$, where C equals the total energy intake, P is the total energy gain, M is metabolic expenditure, U is the excretory nitrogen energy loss, and F is fecal loss. It is important to note that there are other energy losses for which we are unable to account, such as energy lost in the excretion of mucus sloughed from the surface of the body and the production of mucus tubes that envelop the feces (Shehadeh and Gordon 1969). Because fecal losses were estimated by difference, these unaccountable energy losses are included in the estimated value for fecal energy losses. We refer to F, then, as fecal and unaccounted energy losses. The sum of the energy gains, expenditures, and losses should equal the energy consumed. If the energy source is not adequate to meet the expenses of daily living, then endogenous stores will be mobilized and the fish should have a negative P value. To determine the energy equivalences of the nutrient components of food and fish, conversion factors from Jobling (1994) were used.

Statistical analysis

Values are given as the mean ± SEM (n = number of fish). Individual data from the replicate tanks were combined in all analyses. Mean values were compared using one-way ANOVA, and in cases where the F value indicated significance, the Tukey–Kramer comparison of all pairs test was applied to determine treatment differences within a sampling period. The accepted probability level for significance was $p < 0.05$.

Results

Experimental temperature regime

The control temperature ranged from 13 to 24°C, and the +2°C treatments ranged from 15 to 26°C (Fig. 1). Control temperature rose from 13 to 16–18°C over the first 20 days, after which it was fairly stable until day 55. Thereafter, the temperature rose to a maximum of 24°C at day 70 and fluctuated around this temperature until day 82. At this point, the temperature started to decrease and reached 20°C on day 90.

Consumption, growth, and condition

From day 0 (experiment initiation), the appetites of the trout in all treatments averaged ~10% body weight·day^{-1} (dry food/dry weight) until days 60–90. At ~day 70, appetites started to fall, and trout consumed about 5% body weight·day^{-1}. Overall, the trout exposed to control temperatures tended to consume more than the trout exposed to +2°C, and those exposed to low pH tended to eat more than their respective controls (Fig. 2A). Appetites began to differ at ~day 45, with the fish treated with control temperatures tending to consume more than the fish in the +2°C treatments. By day 90, it was apparent that the trout exposed to sublethal low pH were consuming more than their respective controls.

At day 90, growth was greatest in the control temperature treatments (Fig. 2B). The 0/5.2 fish grew significantly more than either of the +2°C treatments but not more than 0/6.3. The +2/6.3 treatment trout grew significantly less than the 0/6.3 trout.

Fig. 2. (A) Cumulative appetites, expressed on a dry weight of food basis (grams per fish), and (B) absolute growth (grams), expressed as wet body mass, over each 30-day period. Significant differences are indicated by treatment groups that do not share a common letter.

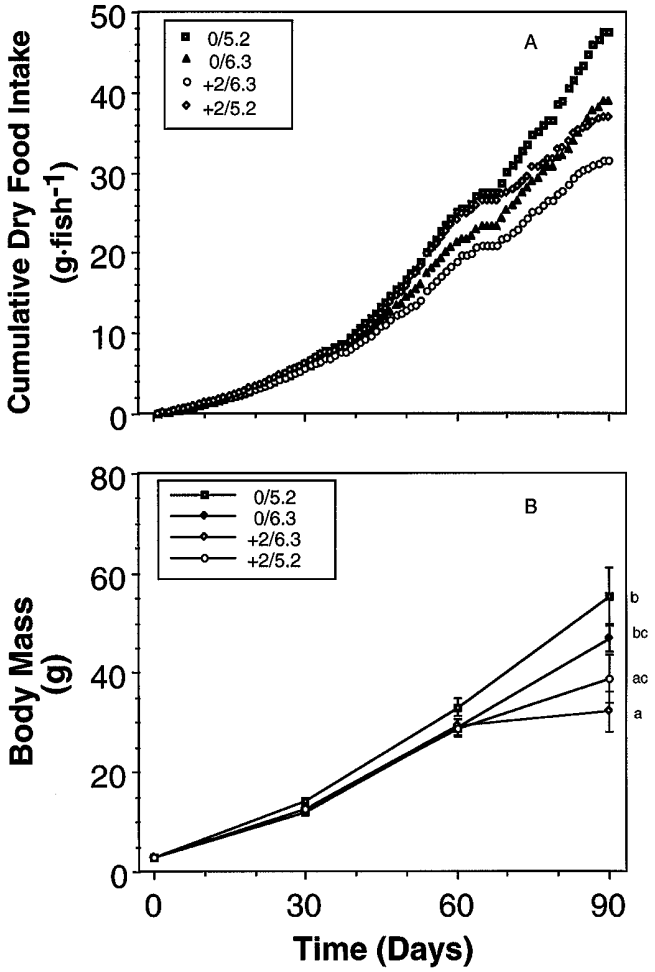


Table 1. Food conversion efficiencies for each 30-day period.

	Treatment			
Time period (days)	0/6.3	0/5.2	+2/6.3	+2/5.2
0–30	42.4	46.6	45.6	40.3
30–60	29.5	27.2	33.8	24.5
60–90	27.3	28.3	6.2	20.9

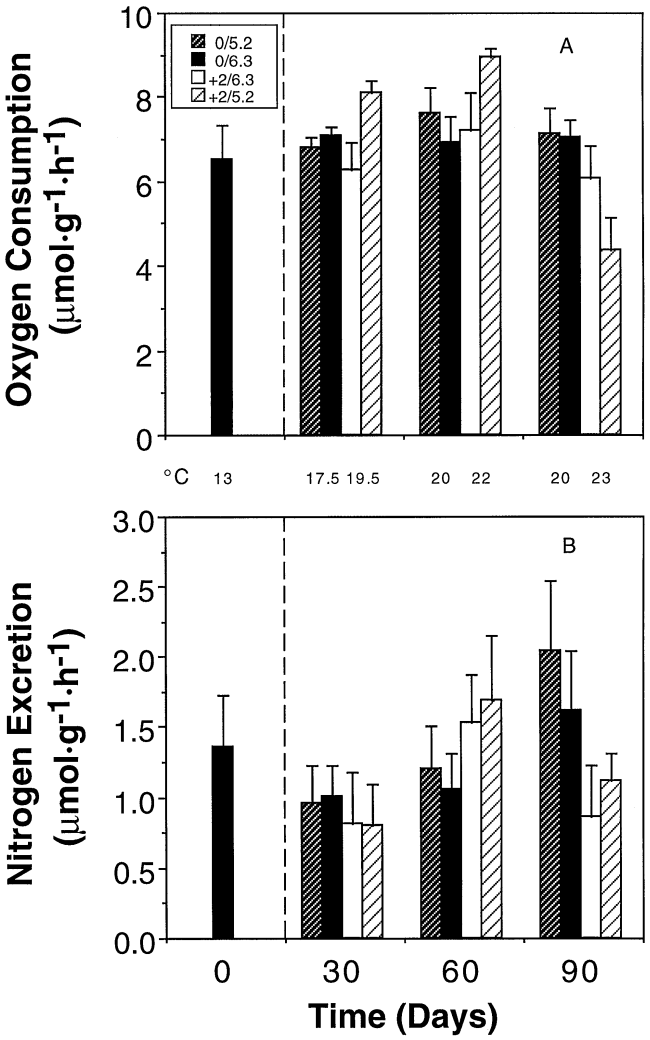
Note: Food conversion efficiencies calculated as ratio of dry weight of food to dry weight of fish (%·day^{-1}). Treatment regimes are described in the Materials and methods.

The condition factor (weight/length^3; Bagenal and Tesch 1978) of the trout in all treatments increased over the 90-day exposure period from ~0.9 to 1.2, although the efficiency with which dry weight of food was converted to dry weight of tissue decreased over the 90 days from ~40 to 25% (Table 1). At day 90, conversion efficiencies were lowest in both +2°C treatments. We have no explanation for the unusually low conversion efficiency in the +2/6.3 treatment between days 60 and 90.

Metabolic and nitrogenous waste excretion rates

Oxygen consumption measurements indicated that routine

Fig. 3. (A) Routine in-tank oxygen consumption (M_{O_2}) and (B) nitrogen excretion (total ammonia + urea nitrogen) rates (μmol·g^{-1}·h^{-1}). Rates were determined for the total number of fish in one duplicate tank, over a 10- and 24-h period, respectively, during the day, including feeding periods. Numbers between the graphs are the temperatures at which the rates were determined. The broken line separates day 0 (initial values) from the other measurements because satiation feeding did not begin until day 0. Errors bars represent measurement standard error only, and thus no statistical comparisons can be carried out.



values for these satiation-fed fish were about 7.0 μmol·g^{-1}·h^{-1} (Fig. 3A). Notably, there was a lack of increase in M_{O_2} with increasing temperatures over the summer exposure. Among treatments, there was a trend towards a higher M_{O_2} in the +2/5.2 treatment until day 60 and day 90 M_{O_2} values were depressed.

Nitrogenous waste excretion rates decreased initially; thereafter they steadily increased to about 1.75 μmol·g^{-1}·h^{-1} at day 90 (Fig. 3B). About 10–20% of this nitrogenous excretion was represented by urea nitrogen. Over the 90-day period, this fraction decreased from ~20 to 10% as a result of increased ammonia nitrogen excretion and relatively constant urea nitrogen excretion. At days 60 and 90, the fish in the low pH treatments

Table 2. Fractional protein utilization (FPU) in four treatment regimes.

	Day 0,	Day 30				Day 60				Day 90			
	0/6.3	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2
FPU	0.78	0.52	0.52	0.48	0.37	0.59	0.56	0.78	0.70	1.07	0.85	0.52	0.96

Note: FPU is calculated as (moles N produced/moles O_2 consumed)/0.27, where the denominator is the theoretical maximum nitrogen quotient as determined by Kutty (1972). Treatment regimes (0/5.2, 0/6.3, +2/6.3, and +2/5.2) are described in the Materials and methods.

Table 3. Measured hematocrit and plasma protein for each treatment at 30-day periods.

	Day 0,	Day 30				Day 60				Day 90			
	0/6.3	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2
Hematocrit (%)	46.2	33.1	35.5	33.1	32.8	39.0	38.8	41.2	38.7	33.1	33.2	31.8	30.9
±SEM	1.5	1.2	1.4	2.1	1.2	0.8	1.1	0.9	1.2	1.9	1.4	0.7	1.1
n	20	20	19	19	10	20	17	18	10	9	12	11	6
Plasma protein ($g \cdot 100\ mL^{-1}$)	5.6	5.6	5.3	5.7	5.5	5.6	5.2	5.5	5.6	5.3*b*	4.6*ab*	4.4*a*	3.8*a*
± SEM	0.1	0.1	0.1	0.2	0.3	0.1	0.1	0.2	0.2	0.3	0.1	0.2	0.3
n	10	10	13	10	5	10	10	12	8	9	12	11	6

Note: Mean values ± SEM and sample size (*n*) are shown. Significant differences between treatments occurred for plasma protein at day 90 only, and at this time, treatments that share a letter are not significantly different from each other ($p < 0.05$). Treatment regimes (0/5.2, 0/6.3, +2/6.3, and +2/5.2) are described in the Materials and methods.

tended to excrete more nitrogen than their respective controls, and excretion rates were depressed in the +2°C treatments at day 90.

Fractional protein utilization values indicate that protein use generally increased in all treatments over the 90-day period. At day 90, protein use was lowest in the +2/6.3 treatment (Table 2).

Blood analysis

Initial values of hematocrit on day 0 were high at 46% (Table 3), perhaps reflecting the difficulty that we experienced in obtaining blood from such small fish (3 g). On days 30, 60, and 90, hematocrits were between 35 and 40% in all treatment groups, and no treatment differences were evident. Plasma protein levels remained unchanged at ~5.5 $g \cdot 100\ mL^{-1}$ until day 90 (Table 3). At this point, plasma protein was significantly higher in the 0/5.2 treatment than in the two +2°C treatments, although none was different from the 0/6.3 treatment.

Plasma Na^+ generally increased over time in all treatment groups from the initial value of ~105 to 150 $mequiv \cdot L^{-1}$ (Fig. 4A). Between days 60 and 90, the +2/5.2 treatment had significantly lower levels of plasma Na^+ than both control temperature treatments. Plasma Cl^- did not mirror this trend (Fig. 4B). Because plasma was limited at day 0, there is no initial value for plasma Ca^{2+}. At subsequent sampling times, plasma Ca^{2+} was ~4.5 $mequiv \cdot L^{-1}$ with no apparent influence of treatment (data not shown).

Proximate analysis

Whole-body analysis indicated that the percentage of protein by weight (wet) generally increased from ~12.0 to 15.5% over the 90-day period (Fig. 5A). Protein content was significantly greater in all treatments than in the control (0/6.3) at day 30. At day 60, protein content was significantly greater in the control temperature treatments than in the +2°C treatments. However, neither of these trends was maintained at day 90.

There was a substantial increase (~2.5-fold) in whole-body lipid content over the 90-day period from ~4.5 to 11.5% in all treatments (Fig. 5B). Among treatments, lipid content was significantly greater in the 0/5.2 treatment and lower in the +2/6.3 treatment than in the 0/6.3 treatment, at day 60 only.

Total carbohydrate (estimated as glucose + glycogen + lactate) did not show any consistent trend among treatments or over time and constituted only ~0.36% of whole-body composition (data not shown). Percentage whole-body water decreased over time in all of the treatments from 77 to 71% (data not shown), compensating for the large increase in lipid. There was no change in total inorganic content (ash; data not shown).

Whole-body Na^+, Cl^-, Ca^{2+}, and K^+ levels indicated that treatment effects were minor (Table 4). $[Na^+]$ and $[Cl^-]$ tended to decrease over the 90-day period, $[Ca^{2+}]$ increased, and $[K^+]$ was not affected. At day 90, the 0/5.2 treatment fish had significantly lower whole-body $[Cl^-]$ than the control treatment, and whole-body $[Ca^{2+}]$ was significantly elevated in the +2/5.2 fish.

Energy budget

A greater total amount of energy was consumed by the fish in the 0/5.2 treatment than in all other treatments (Table 5). That food energy was also converted more efficiently into energy gained. Metabolic expenditure was similar in all treatments, although slightly more energy was lost, in the form of nitrogen, by fish in the 0/5.2 and +2/5.2 treatments. Fecal and unaccounted energy losses were greatest in the +2/6.3 treatment fish and generally greater in the two +2°C treatments than in the control temperature treatments.

Discussion

The present study is the first to assess the effects of chronic sublethal low pH and an elevated summer thermal cycle on freshwater fish. Our original hypothesis was that the cost of living in a polluted environment, such as anthropogenically acidified soft water, would be further increased by the addition

Fig. 4. (A) Plasma Na^+ and (B) Cl^- levels (mequiv·L^{-1}). Values are given as means ± SEM. Significant differences ($p < 0.05$) are indicated by treatment groups that do not share a common letter.

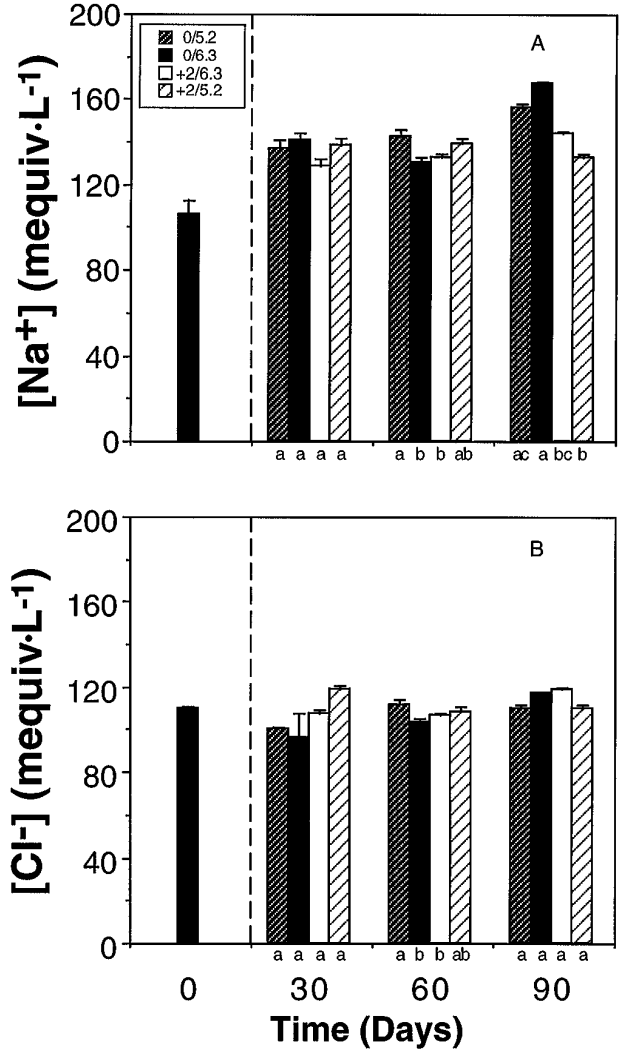
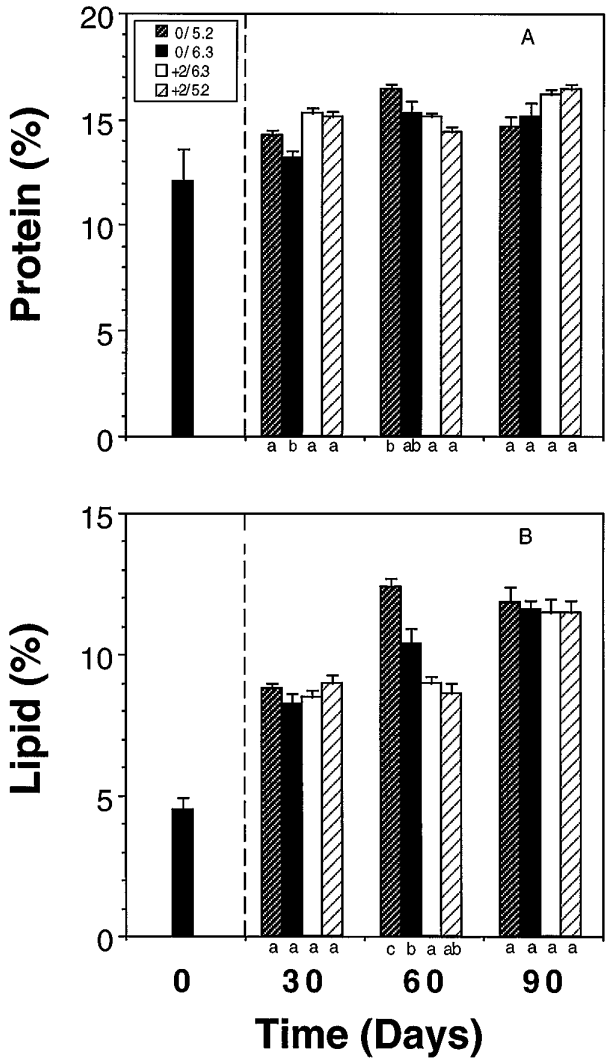


Fig. 5. (A) Whole-body protein and (B) lipid (%, wet weight). Values are given as means ± SEM. Significant differences ($p < 0.05$) are indicated by treatment groups that do not share a common letter.



of 2°C to the natural thermal cycle. As a result of the high pressure required for reverse osmosis, an additional 2°C was added to the ambient thermal cycle, resulting in an overall increase in temperature of 4°C. It is also one of the few studies (Kwain 1975; Menendez 1976; Leino and McCormick 1992; Wilson et al. 1994*a*) to examine the effects of these environmental stressors on fed fish. Often, studies investigating environmental stressors, such as pollutants, are carried out on starved or weight-maintained fish (Neville 1985; Audet et al. 1988; Booth et al. 1988; Butler et al. 1992). In the wild, and particularly in the summer months, fish actively feed as a consequence of both increased temperatures and food availability.

Temperature effects

Metabolic rate is a measure of the integrated cost of whole-body physiological and biochemical processes. In fish, it is affected by temperature, fish size, and food intake and assimilation (the heat increment of feeding (Cho and Kaushik 1990), also known as apparent specific dynamic action (SDA) (Beamish 1974*b*)). After correcting for weight increases, the most striking aspect of the routine $\dot{M}O_2$ data in the present study was that they were about 75% of $\dot{M}O_2$(max) values (Wilson et al. 1994*b*; also corrected for weight). This general effect was likely due to the feeding regime used. The increased cost of metabolism as a result of feeding is well documented (Beamish 1974*b*; Brett 1976; Soofiani and Hawkins 1982; Cho and Kaushik 1990). Ration size and composition and temperature determine the extent of the increase in oxygen consumption (Cho et al. 1982). Soofiani and Hawkins (1982) found that for cod fed to satiation, $\dot{M}O_2$ almost equalled the active metabolic rate. This dramatic increase in $\dot{M}O_2$ has important consequences for the scope for activity (difference between routine and active metabolic rates; Brett 1956) in fish. The authors found that this effect reduced the scope for activity in cod (*Gadus morhua* L.) by 83–97%, depending on the temperature. However, Brett (1976) found that the increase in $\dot{M}O_2$ as a result of feeding had less of an impact on the scope for activity in fingerling sockeye salmon (*Oncorhynchus nerka*).

The rates of most physiological processes increase with

Table 4. Whole-body [Na^+], [$C1^-$], [Ca^{2+}], and [K^+] (mequiv·kg^{-1}) for each treatment at each 30-day period.

	Day 0,	Day 30				Day 60				Day 90			
	0/6.3	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2	0/5.2	0/6.3	+2/6.3	+2/5.2
n	10	19	20	18	10	20	18	19	10	12	18	12	6
[Na^+]	45.4	43.6	46.5	42.2	44.3	37.5	37.8	39.7	38.5	36.2*a*	40.9*ab*	48.7*b*	37.7*ab*
±SEM	0.4	2.8	4.0	0.7	0.6	1.1	0.9	0.6	1.1	2.0	0.7	5.9	1.1
[$C1^-$]	40.1	35.8	38.7	33.8	35.7	30.2	29.7	30.2	29.6	26.5*b*	31.9*a*	33.7*a*	27.9*ab*
±SEM	0.6	1.8	3.4	0.6	0.4	0.9	0.6	0.5	1.0	2.0	0.6	1.1	1.1
[Ca^{2+}]	73.3	85.2	84.9	76.8	77.3	84.6	86.1	88.7	87.5	81.4*a*	84.6*ab*	79.3*a*	92.0*b*
±SEM	1.4	4.7	6.9	1.0	1.9	1.7	1.2	1.1	2.1	1.9	1.3	2.6	1.9
[K^+]	83.2	86.6	105.1	92.9	87.1	87.4	93.5	88.9	87.1	84.9*a*	85.8*a*	84.5*a*	84.6*a*
±SEM	2.3	4.3	9.6	1.3	1.7	2.3	2.0	1.7	3.0	1.8	1.2	1.7	2.9

Note: Mean values ± SEM and sample size (*n*) are shown. Significant differences occurred between treatments at day 90 only. During this period, treatments that share a letter are not significantly different from each other ($p < 0.05$). Treatment regimes (0/5.2, 0/6.3, +2/6.3, and +2/5.2) are described in the Materials and methods.

Table 5. Energy budget for each treatment for the 90-day period.

	Total energy consumed (*C*)		Total energy gained	Conversion efficiency	Total metabolic expenditure (*M*)		Total N energy lost (*U*)		Fecal and unaccounted energy lost (*F*)	
Treatment	kJ	%	(*P*), kJ	(*K*), %	kJ	%	kJ	%	kJ	%
0/5.2	963.9	100	480.6	49.9	288.7	30.0	51.8	5.4	142.9	14.8
0/6.3	837.1	100	369.7	44.2	249.5	29.8	38.3	4.6	179.6	21.5
+2/6.3	785.8	100	254.4	32.4	218.9	27.9	29.0	3.7	283.5	36.1
+2/5.2	723.0	100	283.6	39.2	224.0	31.0	34.5	4.8	180.9	25.0

Note: The general energy budget equation is $C = P + M + U + F$, where *C* is the energy consumed, *P* is the energy gained, *M* is metabolic expenditure, *U* is excretory energy loss, and *F* is fecal and unaccounted energy loss. Fecal and unaccounted losses are estimated by the difference ($C - (P + M + U)$). Each component of the budget is expressed as a percentage of *C* to determine allocation differences. *K* is the efficiency with which food energy is converted into total energy gain ($C/P \times 100$). Treatment regimes (0/5.2, 0/6.3, +2/6.3, and +2/5.2) are described in the Materials and methods.

increasing temperature (Schmidt-Nielsen 1987), the rate increase often expressed as a Q_{10}. For example, Mehner and Wieser (1994) found that $M\dot{O}_2$ values in juvenile perch (*Perca fluviatilis*) acclimated to 20°C had a Q_{10} of 2.43 when compared with perch acclimated to 15°C. Claireux et al. (1995) found that resting cod, acclimated to 5°C, exhibited a Q_{10} of 2 in $M\dot{O}_2$ when the temperature was increased to 7.5°C. Calculations based on the data of Fig. 3A indicated that Q_{10} values averaged only ~1.0 in the present study. Fish acclimate more rapidly to increases in temperature than to decreases in temperature (Jobling 1994). Because the temperatures gradually increased over the 90-day test period, the fluctuating and increasing thermal regime involved in the present study may have allowed for continuous acclimation, thereby permitting complete metabolic compensation and thus no apparent increase in metabolic rates. Thermal history, therefore, is an important parameter to be considered when determining metabolic expenditures, especially when predictions of growth are important.

Temperatures approaching the upper lethal temperature for salmonids have been shown to depress appetite and metabolic rates (Brett 1971; Elliott 1982) and, subsequently, growth (Brett et al. 1969). The upper incipient lethal temperature for rainbow trout (26.2°C; Elliott 1982) was reached in the +2°C treatments. However, the increasing temperatures over the previous 60 days allowed for gradual acclimation and may well have increased thermal tolerance in that no mortalities occurred over the 10-day peak temperature period. The depression in appetite and growth in the +2°C treatments, notably at day 90, and the decline in gross conversion efficiency in the +2°C treatment over the period of days 60–90 indicate that 2°C added to the control thermal cycle significantly affected trout, particularly after peak temperatures were reached. A 20% reduction in protein turnover was also evident in these fish during this period of peak temperatures (Reid et al. 1995) and is further evidence of the suppressive effects of high temperature on metabolic processes.

Endogenous energy catabolism did not increase in these fish, as indicated by stable whole-body protein, lipid, and carbohydrate levels, even though food intake was reduced. The suppressive effects of high temperature on appetite and metabolic rates discussed above may be extended to endogenous energy catabolism in an overall effort to conserve energy by reducing the energy expended on SDA. Although this could be considered a pathological response, it may also be an adaptive strategy used by the fish in the face of high temperature conditions.

Addition of 2°C tended to increase nitrogen excretion rates, until the peak in temperature was reached. At day 90, nitrogen excretion rates in the +2°C treatments were reduced. Because endogenous nitrogen use did not increase, as indicated by the maintenance of whole-body protein levels, this reduction was due to the suppression of appetite during the 60- to 90-day period. A reduction in exogenous protein intake, and no reliance on endogenous protein sources, is further supported by the reduction in use of protein, as indicated by the low fractional protein utilization (FPU) value in the +2/6.3 treatment.

In general, addition of 2°C to the naturally fluctuating control

thermal cycle appeared beneficial to rainbow trout until their optimal temperature for growth was surpassed. This temperature has been determined by Cho and Kaushik (1990) to be between 15 and 20°C. Control temperatures, for the first 50 days, fluctuated between 15 and 18°C, and a separation in appetites was evident at day 50, just as the temperature began its incline to the peak temperature at day 70. It was over this period that the inhibitory effects of the additional 2°C on appetite and growth became most evident.

Low pH effects

The greater appetites and better growth exhibited by trout exposed to sublethal acidity than their respective controls were contrasted with those of many previous studies showing deleterious effects of acidic pH. For example, some investigators have reported that sublethal low pH increases M_{O_2} (Hargis 1976; Waiwood and Beamish 1978; Butler et al. 1992), indicating increased metabolic cost, and reduces growth in fish. Menendez (1976) and Sadler and Lynam (1987) found that growth in brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) was impaired at pH levels below 5.2. The important difference in the present study was that trout were fed to satiation. In this regard, Wilson and Wood (1992) reported no difference in growth for rainbow trout fed 1% body weight·day^{-1} and concluded that chronic exposure to pH 5.2 did not deleteriously affect the energy budget of fed fish. Indeed, in a separate study, Wilson et al. (1994*a*) reported increased growth in trout that were exposed to pH 5.2 and fed to satiation for 35 days, a result similar to that of the present study. Conversion efficiencies of acid-exposed trout were not, in general, different from the control, indicating that the subsequent increased growth was purely a result of increased appetite. It is noteworthy, then, that chronic exposure to pH 5.2 should apparently stimulate appetite. This was also evident in the +2/5.2 treatment discussed below, although growth was not significantly different from control growth.

Proximate analysis indicated that increased appetites in the 0/5.2 treatment resulted in deposition of more protein and lipid at days 30 and 60, respectively. This trend was not maintained at day 90, as a result of the peak in temperature over the period of days 60–90. Also, a tendency for higher nitrogen excretion rates denoted increased protein catabolism (as a result of increased food intake rates). It is clear from the above discussion that chronic exposure to low pH in trout does not reduce growth when trout are simultaneously fed to satiation in the laboratory environment.

Combination treatment effects

Trout exposed to both low pH and increased temperature generally expended more metabolic energy. The greater depression in M_{O_2} in the +2/5.2 treatment than in the +2/6.3 treatment, after the day 70 peak in temperature, indicated greater physiological impact of the peak temperatures on the former. These trout had appetites greater than those of the fish exposed to +2°C alone and equal to those of the 0/6.3 treatment. However, they did not exhibit better growth than the trout in the +2/6.3 treatment. This result implies that the increased cost of living in this +2/5.2 environment was being paid in the form of greater immediate use of the exogenous energy, resulting in less energy available for deposition as growth. This is supported by the lower conversion efficiencies exhibited by these fish at all periods.

The amount of energy taken in was sufficient to maintain endogenous energy stores as indicated by the similar protein and fat deposition in all treatments to the control. After the period of peak temperature, it is evident that the trout in the +2/5.2 treatment were reducing both food intake and M_{O_2}. Consequently, nitrogen excretion rates were reduced, arguing that endogenous sources were not used. Energy conservation was clearly of paramount importance during this period.

Indicators of stress

Exposure to chronic sublethal low pH did not result in decreases in plasma Na^+ and Cl^- concentrations (cf. Audet et al. 1988), nor did it cause fluid shifts, increased hematocrit, or increased plasma protein concentrations. There was, however, a reduction in whole-body chloride in the 0/5.2 treatment relative to its control at day 90 only. The general absence of ionoregulatory disturbance in this experiment contrasts with other chronic studies, such as that carried out by Leino and McCormick (1992) on juvenile largemouth bass, where mean blood osmolalities declined at pH 5.0 and 4.5. Audet et al. (1988) showed conclusively that the main toxic effect of chronic exposure to sublethal low pH (pH 4.8) in adult rainbow trout was ionoregulatory disturbance, manifested by a partial inhibition of Na^+ and Cl^- influx and a reduction of Na^+ and Cl^- efflux. In their study, plasma Na^+ and Cl^- concentrations were reduced but stabilized at a lower level, while plasma protein concentration was increased. However, the fish in their study were fed only once per week. Wilson et al. (1994*a*) demonstrated that juvenile rainbow trout, exposed to pH 5.2 for 35 days, recovered whole-body [Na^+] and [Cl^-] to control levels, after a nadir at day 17. This recovery was attributed to "enough time"; these fish were fed to satiation twice a day, a feeding regime duplicated in the present study.

As pointed out by Smith et al. (1989), very little research has been directed at accounting for ions taken in as part of the diet and the role that they play in ionoregulation. We have calculated the dietary ion budget for our fish on the basis of food ion concentrations and whole-body ion pools. Trout in the low pH treatments were consuming, on average, 10% of their body pool of Na^+ per day in a food ration amounting to 10% of the dry body weight per day. If indeed there was an initial loss of ions, as described by Wilson et al. (1994*a*), the availability of ions in the food in combination with enough time might have allowed the observed recovery in their study and would account for the observed lack of ionoregulatory disturbance in the trout exposed to low pH in the present study. Sadler and Lynam (1987) found similar effects in yearling brown trout that were exposed to low pH conditions (4.4–5.2) and fed 2% of their body weight per day. In brown trout that were starved, however, reductions in plasma chloride concentration, increased muscle water content, and losses of body sodium and potassium were significant. The authors suggest that the fed trout may be making use of dietary ions to ameliorate the effects of low pH.

Energy budget

The data in Table 5 support the preceding discussion of greater appetites and thus weight gain in the 0/5.2 treatment. Energy intake (*C*) was greater in both control temperature treatments

than in their respective +2°C treatments. The value beside each gross energy value indicates the percentage of the total energy intake allocated to each component of the energy budget. It is clear that the fish in the 0/5.2 treatment not only took in more energy, but also allotted a greater percentage of that energy intake to growth (energy gain; 49.9%). There were almost equal metabolic energy expenditures over the 90-day period, and the 0/5.2 trout lost more energy in the form of nitrogen than those in the other treatments, particularly the +2/6.3 treatment. However, lower estimated fecal and unaccounted energy losses (14.8%) indicated that not only were the 0/5.2 trout taking in a greater gross amount of energy, but they were also converting that energy more efficiently into gross energy gain (*K*). In contrast, and in agreement with the previous discussion, the +2/6.3 trout showed the least energy gain and the least energy expenditure, but the greatest fecal and unaccounted energy losses. It is also apparent from the data that the fish in the +2/6.3 treatment had a greater gross energy intake over the 90-day period than those in the +2/5.2 treatment. This is explained by a slightly greater absolute food intake over the period of days 60–90 than those in the +2/5.2 treatment, although appetites at all other periods were less than those in the +2/5.2 treatment fish. Nonetheless, the trout in the +2/6.3 treatment converted food less efficiently into energy gain than in all other treatments. In general, the fish in the two control temperature treatments consumed more food, gained more energy as growth, and converted the energy taken in into energy gain more efficiently than in the +2°C treatments. Also, fish in both low pH treatments exhibited better conversion efficiency and greater overall energy gain than in their respective controls.

Concluding remarks

The results of this 90-day exposure of juvenile rainbow trout to sublethal low pH, +2°C, and a combination of these two treatments indicate that, averaged over the entire exposure period, those trout exposed to +2°C exhibited a greater cost of living than those exposed to the control thermal regime. This is supported by the decreased food intake rates of trout exposed to higher temperatures, lower conversion efficiencies, and subsequent depressions in growth (energy gain). Metabolic rates were especially costly for these treatments over the period of days 60–90 when temperatures peaked, close to the incipient lethal temperature. It is evident, then, that a global temperature increase of 2°C, as a consequence of "greenhouse gas" production, may be deleterious to temperate cold-water fish species, particularly when temperatures reach the summer high.

Exposure of juvenile rainbow trout to sublethal levels of acidity resulted in greater food intake and growth rates (energy gain), notably at control temperatures. This may be an indication that sublethal levels of this particular toxicant stimulate increased energy intake to compensate for increased costs associated with living in an acidic environment. These higher costs could be a consequence of ionoregulatory disturbance. If so, appetite might be driven by the requirement for NaCl through the diet. This stimulus, coupled with an unlimited food supply, may result in an overcompensation for the increased cost of living in an acidic environment in the form of increased growth. Smith et al. (1989) stated that under conditions of impairment of branchial sodium uptake, such as under acidic conditions, dietary ions may play a critical role in body ion homeostasis. Sadler and Lynam (1987) indicated that compensation for increased energy expenditure and ionoregulatory imbalance, as a result of moderately low levels of pH, may be occurring when fish are given sufficient food. It is uncertain how much food juvenile salmonids consume in the wild, but an unlimited ration is not realistic. It is also uncertain how much energy a fish expends searching for food and avoiding predators. However, it is unlikely that the unlimited food supply available to the trout in the present study is representative of food availability in the wild, because M_{O_2} values were at 75% of M_{O_2}(max) as a consequence, we propose, of SDA. We suggest that future studies on global warming – low pH scenarios should examine possible effects of food limitation. Limiting food may elucidate whether there are actually increased costs associated with living in a sublethally stressful environment and what role food intake rates play in compensation.

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