Chronic exposure of rainbow trout (*Oncorhynchus mykiss*) to simulated climate warming and sublethal ammonia: a year-long study of their appetite, growth, and metabolism

Tyler K. Linton, I.J. Morgan, P.J. Walsh, and Chris M. Wood

Abstract: This study was conducted to assess, over the thermal cycle of an entire year, the effects (on appetite, growth, and metabolism) of a chronic small temperature increase (+2°C) and sublethal ammonia (70 μmol·L^{-1}) on rainbow trout (*Oncorhynchus mykiss*). Juvenile rainbow trout (≈11 g initially) were exposed for 14 months to four treatments: the natural water temperature cycle of the inshore region of Lake Ontario, this cycle +2°C to simulate a global warming scenario, and these temperature cycles in the presence of an additional 70 μmol total ammonia·L^{-1} (NH_3 range: 0.005–0.013 mg·L^{-1}). The additional +2°C substantially increased appetite over winter, significantly elevating specific growth rates. These gains were lost, however, over summer due to suppression of appetite and growth at high temperature. Ammonia alone tended to elevate growth, but the combination of +2°C and ammonia resulted in a general decrease in the activity of enzymes involved in nitrogen metabolism (alanine aminotransferase, aspartate aminotransferase, glutamate dehydrogenase, and glutamine synthetase). These results document the dramatic influence of a +2°C warming scenario on the growth and feeding metabolism of juvenile rainbow trout. Moreover, the data indicate that a chronic small temperature increase, together with low-level ammonia pollution, substantially alters protein dynamics, and hence growth, in juvenile freshwater fishes; juvenile rainbow trout without thermal refuge will experience an increase in t a warmer, more polluted environment.

Résumé : Cette étude a été menée dans le but d'évaluer, au cours d'un cycle thermique de 1 an, les effets (sur l'appétit, la croissance et le métabolisme) d'une petite augmentation chronique de la température (+2°C) et d'une concentration sublétale d'ammoniac (70 μmol·L^{-1}) sur la truite arc-en-ciel (*Oncorhynchus mykiss*). Des truites arc-en-ciel juvéniles (initialement ≈11 g) ont été exposées durant 14 mois à quatre traitements : le cycle naturel de la température de l'eau dans la zone riveraine du lac Ontario, ce même cycle avec une augmentayion de la température de 2°C pour simuler un scénario de réchauffement planétaire et ces deux cycles de température avec l'ajout d'une concentration d'ammoniac total de 70 μmol·L^{-1} (fourchette de NH_3 : 0,005 à 0,013 mg·L^{-1}). Les 2°C additiheir cost of living in onnels ont substantiellement fait augmenter l'appétit durant l'hiver, ce qui a eu pour effet d'accroître les taux de croissance spécifiques. Ces gains ont cependant été perdus durant l'été, où il y a eu suppression de l'appétit et arrêt de la croissance en raison de la température élevée. L'ammoniac seul tendait à faire augmenter la croissance, mais la combination des 2°C additionnels et de l'ammoniac a eu pour effet une diminution générale de l'activité des enzymes du métabolisme de l'azote (alanine aminotransférase, aspartate aminotransférase, glutamate deshydrogénase et glutamine synthétase). Ces résultats nous renseignent sur l'effet dévastateur d'un réchauffement planétaire de 2°C sur la croissance et le métabolisme alimentaire des truites arc-en-ciel juvéniles. De plus, nos données montrent qu'une légère élévation chronique de la température, couplée à une faible pollution ammoniacale, altère substantiellement la dynamique des protéines chez les poissons d'eau douce juvéniles; vivre dans un milieu plus chaud et plus pollué imposera des coûts supplémentaires aux truites arc-en-ciel juvéniles privées de refuge thermique.
[Traduit par la Rédaction]

Introduction

Growing concern over the incremental increase in ambient air temperature in the past few decades, whether from natural climatic variation or anthropogenic emissions of "greenhouse gases," has prompted an international initiative to gain a better understanding of how temperature increases of only 1.5–4.5°C could affect the basic physiological and biochemical functions of aquatic organisms (IPCC 1992; Wood and McDonald 1997). Fish are particularly susceptible to environmental temperature changes because their body temperatures, and hence their feeding, metabolic, growth, and excretion rates, are

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governed by the temperature of the water in which they live (Fry 1971; Elliott 1976). Based on studies conducted at constant acclimation temperatures, increased temperatures within the preferred thermal range of temperate fish species lead to greater growth rates provided that food is not limiting (Elliott 1982). Beyond these boundaries, however, growth potential decreases. This is due to the influence of temperature directly, and indirectly via dissolved O_2, on appetite, growth, and energetics (Brett 1979). Therefore, understanding the effect of temperature change on growth and food consumption is fundamental to predicting the potential effects of climate change on the growth performance of fish (McCarthy and Houlihan 1997).

Chronic temperature increase, however, is not the only factor predicted to influence the health and distribution of our fisheries. The effective zone of temperature tolerance, and hence growth and survival, in fish can be restricted in nature by other environmental factors such as pollution (Reid et al. 1997; Rombough 1997). Given our present demographic situation, there can be little doubt that temperature change will occur against a backdrop of increasing water pollution (Kennedy and Walsh 1997). One such pollutant is ammonia. Whether from agricultural runoff, industrial discharge, sewage effluent, N fixation, or production by the fish themselves, transient increases in water ammonia levels are damaging to fish populations (EPA 1985). Only small elevations in water ammonia are needed to induce severe physiological problems in some freshwater fishes (Randall and Wright 1987).

To date, most data regarding the response of fish to temperature have been collected under conditions of controlled temperature (usually 5–10°C apart) and food availability. There is currently a need to examine how short-term and seasonal fluctuations in water temperature affect the basic physiology and biochemistry of fish, especially in the face of low-level environmental contamination. The present study was conducted to determine the potential effects of a warmer (+2°C) and polluted (+70 μmol total ammonia (T_{amm})·L^{-1}, $T_{Amm} = NH_4^+ + NH_3$) scenario on juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to naturally fluctuating temperature throughout the entire year. A warming scenario of +2°C year-round was chosen because it represents a reasonable median in the range predicted by current climate models (see IPCC 1992). Likewise, +70 μmol T_{Amm}·L^{-1} is an environmentally "realistic" level of ammonia representative of some polluted areas in the inshore region of Lake Ontario (see Linton et al. 1997). The feeding regime (hand-fed once a day to satiation) was not one in which the fish were fed a controlled or equal ration, but rather one that was allowed to fluctuate in correspondence to the needs of the fish, thereby providing a measure of appetite.

This study on a reference cold-water species, the rainbow trout, was designed to corroborate and extend the results from three previous experiments (90 days) conducted over summer and winter without food limitation (Reid et al. 1995; Linton et al. 1997, 1998) and during summer with food limitation (unpublished data from our laboratory). In particular, we tested two predictions about the likely impacts of small chronic temperature increase: (*i*) that +2°C superimposed above the natural fluctuating thermal regime would have profound effects on the feeding physiology, growth, and energetics of juvenile trout, especially at the seasonal temperature extremes, where the temperature elevation would exert opposite effects and (*ii*) that the influence of this small temperature increase, under environmentally realistic water quality scenarios, would be large enough to alter the protein dynamics of the fish. Together, these data confirm that the levels of temperature increase predicted by some global warming models will have direct and significant effects on the metabolic costs associated with the growth of freshwater fish. Moreover, these costs will be increased in the face of low-level environmental ammonia pollution.

Methods and materials

Experimental animals

Juvenile rainbow trout (≈11 g) were obtained from Humber Springs Trout Club, Orangeville, Ont., in June 1995 and allowed to acclimatize for 2 weeks prior to testing. They were fed a maintenance ration equivalent to 1% body weight·day^{-1} of Zeigler Bros., Inc (Gardner, Pa.) Floating Fish Nuggets (40% protein, 10% lipid, manufacturer's specification) and held under conditions similar to Linton et al. (1997, 1998). Of particular note are water quality (City of Hamilton, Ont., tap water from Lake Ontario: $[Ca^{2+}] = 0.95$ mmol·L^{-1}, $[Na^+] = 0.60$ mmol·L^{-1}, $[Cl^-] = 0.75$ mmol·L^{-1}, pH = 7.65, hardness = 140 ppm as $CaCO_3$, ambient water temperature = 17–19°C) and a photoperiod representative of Hamilton.

Chronic exposure to temperature and elevated ammonia

Sixteen groups of about 80 fish each were randomly distributed to 16 exposure tanks (270 L) representing four treatment conditions (N = 320 fish per treatment): ambient temperature water (base = Hamilton tap water representative of nearshore water temperatures of Lake Ontario) or ambient temperature water plus 2°C (base+2°C), each with or without an additional 70 μmol T_{Amm}·L^{-1} (Am) (base+Am and base+2°C+Am, respectively). Within each group, 11 fish were marked with a Panjet fish marker (Wright Medical Inc., Dundee, Scotland) for individual identification. The tanks received test water at an average of 1.8 L·min^{-1} (95% replacement = 5.8 h). Water quality was monitored weekly. Mean concentrations of T_{Amm} in the water were 20.5 ± 3.1, 82.5 ± 2.0, 18.3 ± 0.5, and 66.1 ± 3.1 μmol·L^{-1} for base, base+Am, base+2°C, and base+2°C+Am, respectively. Water pHs and partial pressures of O_2 ranged from 7.58 ± 0.05 to 7.66 ± 0.03 and from 120.7 ± 1.3 to 125.5 ± 2.3 Torr (1 Torr = 133.322 Pa). The background levels of T_{Amm} in the base and base+2°C treatments of 15–20 μmol·L^{-1} resulted from the metabolism of the fish themselves. Thus, the exposure levels in the nominal 70 μmol T_{Amm}·L^{-1} base+Am and base+2°C+Am treatments were three to four times background levels. During the experiment, the fish were hand-fed to satiation daily (09:00) from preweighed bags of food, so as to monitor appetite, following the methods of Wilson et al. (1994).

Measurement of O_2 consumption and nitrogenous (N) waste excretion

Routine O_2 consumption rates over a 24-h period were measured initially (–2 days) and then monthly thereafter on whole tanks of fish using a stop-flow method similar to that of Brett and Zala (1975). Rates were determined for all four tanks per treatment, two each on consecutive days, and the fish were fed at their regular time during the measurements. Mean rates of O_2 consumption were determined for each closed period (40 min without aeration at 10:00, 15:00, 20:00, 01:00, 06:00, and 09:00, respectively) using the O_2 solubility coefficients of Boutilier et al. (1984), factored by time, volume, and total fish biomass (explicit details in Linton et al. 1997).

Routine N-waste excretion was measured at the same time as O_2 consumption initially (–2 days) and on days 60 (September 1995), 150 (December 1995), 240 (March 1996), 360 (June 1996), and 420

(August 1996). An initial 50-mL water sample was collected just after the water flow was turned off for the O_2 consumption measurements. After 40 min (the end of the O_2 consumption measurement), aeration was resumed, and after 3 h, a final sample was collected and the water flow turned back on to allow flushing (≈2 h) before the next O_2 consumption measurement. The water samples were frozen immediately for later analysis of total N on a Antek 7000 total N analyzer. The mean N-waste excretion rate was determined from the difference in N concentration of the water, factored as above. All O_2 consumption and N-waste excretion data were averaged over 24 h and corrected for differences in fish size using the weight exponent 0.824 determined for rainbow trout by Cho (1990).

Measurements of the contribution of bacteria to O_2 consumption and N-waste excretion were estimated on a tank of fish that had been receiving additional temperature and ammonia (base+2°C+Am treatment) in September (water temperature = 23°C, biomass = 1.8 kg) representing a "worst-case scenario." The fish were removed and placed in a separate tank while measurements were made over the next hour. Bacteria were estimated to contribute only 7% to the total O_2 consumed and did not alter total N excretion. However, they reduced ammonia-N and urea-N levels by up to 38%, mainly by converting excreted ammonia into nitrate or nitrite. Therefore, we elected to use the Antek nitrogen analyzer which measures all the N in the water sample, e.g., 100% recovery of ammonia-N, urea-N, nitrate-N, and nitrite-N. We assumed that all N accumulated over the 3-h closed period (no water flow, see above) came originally from the fish, regardless of the form of N measured.

Measurement of growth increment

Several times throughout the year, marked fish were measured for their growth increment. Fish were netted from the tanks, and each marked fish was identified, anaesthetized with MS 222 (0.1 g·L^{-1} adjusted to pH 7.5 with 1 N NaOH), and their wet weights and total lengths measured. The fish were then allowed to recover in fresh water and returned to their original holding tanks. At test termination, the marked fish were handled in a similar manner but were sacrificed with an overdose of MS 222 (1 g·L^{-1}, pH = 7.5). Growth was measured as before, but afterwards, the peritoneal cavity was opened for sex determination.

Whole-body, blood, and tissue sampling

At days 0 (initial sample, 10 July 1995), 25 (4 August 1995), and 418 (30 August 1996), whole bodies, individual tissues, and blood samples were collected 24 h after the O_2 consumption and N-waste excretion rates were measured. Food was withheld from the fish during this period. Five unmarked fish per tank (i.e., 20 per treatment) were randomly selected for determination of whole-body protein and water content and plasma Na^+, while another five were selected for the determination of N-enzyme activity: alanine aminotransferase (ALAat), aspartate aminotransferase (ASPat), glutamate dehydrogenase (Gdh), and glutamine synthetase (GSase) in liver, gill, and white muscle.

Fish were sampled for whole-body protein content and blood as in Linton et al. (1997, 1998). The fish sampled for tissue N-enzyme activity were first sacrificed with an overdose of MS 222 (1 g·L^{-1}) at pH 7.5 to minimize metabolic disturbance due to struggling (Wang et al. 1994). Their livers, gill baskets, and a small portion of white muscle anterior to the dorsal fin and above the lateral line were excised and freeze-clamped immediately in liquid N_2.

Analytical techniques

Whole-body protein was determined on fish blended into a homogenous mixture in three parts distilled water and assayed using a modification to the Lowry method (Miller 1959). A small portion of this mixture was withheld and dried in an oven at 80°C for 48 h to obtain the water content. The concentration of Na^+ in the plasma was determined using atomic absorption spectrophotometry (Varian AA 1275).

Frozen livers, gills, and white muscle were ground into a fine powder in an insulated mortar cooled with liquid N_2. A volume (millilitres) equal to four times the tissue weight, i.e., 100 mg of liver and gill or 200 mg of white muscle, of homogenization buffer (50% glycerol, 20 mM K_2HPO_4, 10 mM HEPES, 0.5 mM EDTA, and 1 mM dithiothreitol, pH 7.5) was added to each sample of frozen tissue in 1.5-mL microcentrifuge tubes. The tissue samples were then sonicated (Fisher sonic dismembrator model 300) for 30 s (35% maximum) and kept on ice before centrifugation (Eppendorf centrifuge 5415C) for 2.5 min at 14 000 rpm at 5°C. The ALAat, ASPat, and Gdh assays were performed on supernatants of the three tissue homogenates by continuous measurement of the decrease in absorbance at 340 nm as NADH was converted to NAD using a LKB Biochrom Ultrospec Plus spectrophotometer. Specific cocktails (1 mL·tissue sample^{-1}) consisted of the following made in 50 mM HEPES and adjusted to pH 7.5. ALAat: 0.12 mM NADH, 200 mM alanine, 0.025 mM pyridoxal 5′-phosphate, 12 units lactic dehydrogenase (in glycerol)·mL^{-1}, 10.5 mM α-ketoglutarate; ASPat: 0.12 mM NADH, 40 mM aspartate, 0.025 mM pyridoxal 5′-phosphate, 8 units malic dehydrogenase (in glycerol)·mL^{-1}, 7 mM α-ketoglutarate; Gdh: 0.12 mM NADH, 250 mM ammonium acetate, 0.1 mM EDTA, 1 mM ADP, 14 mM α-ketoglutarate. Control activity without α-ketoglutarate was less than 1% of activity with α-ketoglutarate. The millimolar extinction coefficient of NADH (6.22) was used to calculate rates of substrate disappearance.

GSase was measured by the arsenolysis of glutamine to γ-glutamyl hydroxamate at 540 nm. One millilitre of cocktail (60 mM glutamine, 15 mM hydroxylamine, 0.4 mM ADP, 20 mM KH_2AsO_4, 50 mM HEPES, 3 mM $MnCl_2$, pH 6.7) was required to convert 50 µL of sample supernatant. Liver and gill tissue samples were incubated for 1 h, while muscle samples were incubated for 4 h. A 300-µL aliquot of ferric chloride reagent (50% HCl – 24% trichloroacetic acid – 10% $FeCl_3$ in 0.2 M HCl) was added to control samples (i.e., a duplicate sample spiked with ferric chloride reagent at time $t = 0$) and incubated samples to stop the reaction. Control and incubated samples were then centrifuged for 1 min (14 000 rpm), transferred to cuvettes, and read at 540 nm. Micromoles of product was calculated using the slope of γ-glutamyl hydroxamate standards similarly prepared. All enzymatic assays were conducted at room temperature (23°C), and all reagents were from Sigma Chemical Co. (St. Louis, Mo.). Enzyme activities are expressed as units (micromoles product per minute) per gram tissue wet weight.

Calculations

Appetite was calculated as the daily average wet weight of food consumed per fish by the four replicate tanks as fish were fed to satiation. Conversion efficiencies were calculated as the ratio of the wet weight gain (marked fish) to food consumed. Specific growth rates (SGR) and condition factors were determined from the 11 marked fish measured for growth increment ($N \approx 44$ per treatment) as described in Linton et al. (1997*b*). The N quotient (NQ), or the extent of aerobic protein catabolism, was calculated as the ratio of moles of N produced to moles of O_2 consumed (Kutty 1972). The N-cost index, or the total moles of O_2 consumed per mole of N stored, was used to compare the total metabolic expenditure associated with the incorporation of N into body material, i.e., the metabolic "cost of growth" (see Linton et al. 1997, 1998). Protein was measured as a surrogate for N. The percentage N by weight in the protein of the fish was taken as the standard value, 16% (Soderberg 1995). The temperature quotient, Q_{10}, of a given rate process was calculated using the van't Hoff equation:

$$Q_{10} = (K_{\text{base+2°C}}/K_{\text{base}})^{10/2}$$

where $K_{\text{base+2°C}}$ and K_{base} are velocity constants obtained in the two temperature regimes, respectively.

Table 1. Food intake (total consumed per fish between each period), wet weight, total length, and condition factor during 14 months (420 days) of exposure of juvenile rainbow trout (N = 20–40) to a +2°C warming scenario and 70 μmol T_{Amm}·L^{-1} (Am).

	Time (days) and calendar date								
Parameter measured and treatment	0 8 July 1995	25 4 Aug. 1995	71 18 Sept. 1995	155 13 Dec. 1995	198 25 Jan. 1996	275 11 Apr. 1996	339 14 June 1996	380 15 July 1996	420 29 Aug. 1996
Food intake (g·$fish^{-1}$)									
Base+Am	8.0±0.4	21.6±0.7*a*	65.3±1.5	16.7±0.5c	39.3±1.1*a*	108.7±5.1*a*	132.8±9.3	177.6±7.2*a*	
Base	8.7±0.2	4±1.1*a*	67.2±1.5	23.2±0.5*a*	42.9±2.3*a*	99.8±5.7*a*	119.6±8.6	178.4±8.3*a*	
Base+2°C	8.8±0.7	15.6±0.5*b*	68.4±2.2	24.6±1.7*ab*	54.9±2.4*b*	156.7±8.7*b*	142.4±2.9	140.1±7.5*b*	
Base+2°C+Am	9.0±0.4	15.6±0.1*b*	75.5±3.9	28.7±1.2*b*	57.6±2.4*b*	139.4±8.3*b*	142.1±8.6	169.5±3.9*ab*	
Wet weight (g)									
Base+Am	11.8±0.6	19.8±1.1	35.3±1.8*b*	83.9±3.5	93.6±3.9	123.0±4.8	196.2± 7.4*a*	284.7± 9.8*a*	361.3±13.0
Base	10.3±0.7	18.1±1.3	27.7±2.1*a*	81.3±5.5	95.1±6.1	128.1±8.7	198.2±15.1*a*	281.2±22.3*a*	361.2±28.8
Base+2°C	12.1±0.6	22.3±1.4	27.1±1.7*a*	82.9±4.1	99.5±4.9	147.9±7.2	254.8±11.4*b*	337.1± 16.6*ab*	364.9±17.5
Base+2°C+Am	11.2±0.8	18.9±1.4	24.5±1.8*a*	84.6±5.6	102.5±6.5	147.7±9.2	253.4±12.9*b*	338.3±16.5*b*	391.8±18.6
Total length (cm)									
Base+Am	10.8±0.2	12.1±0.2	14.1±0.3*b*	19.4±0.2	20.1±0.3	21.9±0.2*a*	25.6±0.3*a*	27.9±0.3*ab*	30.3±0.4
Base	10.6±0.3	11.8±0.3	13.5±0.3*a*	19.3±0.5	20.0±0.4	21.7±0.5*a*	25.3±0.5*a*	27.7±0.6*a*	30.1±0.8
Base+2°C	10.9±0.2	12.4±0.2	13.3±0.3*a*	18.8±0.3	20.0±0.3	23.1±0.3*b*	27.0±0.4*b*	29.5±0.5*b*	30.5±0.4
Base+2°C+Am	10.7±0.2	11.8±0.3	12.8±0.3*a*	19.1±0.4	20.2±0.4	23.0±0.4*ab*	27.3±0.4*b*	29.4±0.5*b*	31.5±0.5
Condition factor									
Base+Am	0.89±0.01	1.09±0.01	1.11±0.01	1.13±0.01*ab*	1.14±0.02*b*	1.16±0.02	1.21±0.03	1.29±0.02	1.28±0.02
Base	0.84±0.02	1.07±0.02	1.10±0.03	1.12±0.03*a*	1.17±0.01*a*	1.22±0.02	1.19±0.03	1.28±0.03	1.28±0.02
Base+2°C	0.93±0.04	1.14±0.03	1.10±0.02	1.22±0.04*b*	1.21±0.01*ab*	1.18±0.02	1.28±0.02	1.31±0.04	1.26±0.02
Base+2°C+Am	0.87±0.01	1.09±0.02	1.09±0.02	1.17±0.02*ab*	1.21±0.02*ab*	1.18±0.02	1.20±0.02	1.32±0.03	1.22±0.02

Note: For each sampling day separately, values within a column without a letter in common are significantly different ($P \leq 0.05$). Where there are no letters, there were no significant differences.

Statistical analyses

All whole-body, blood, and tissue enzyme data are expressed as means ± 1 SE from individual samples pooled together from the four tanks per treatment. There were no substantial differences among individuals from different replicate tanks within a treatment. One-way analysis of variance (ANOVA) using SAS Jmp (SAS Institute Inc., version 2.0.5) followed by a Tukey–Kramer HSD multiple comparison test was used to distinguish statistically significant differences in food intake, wet weights, total lengths, and condition factors among treatment groups within each sample period. Multiple factor analyses with leverage plots were used to distinguish statistically significant temperature, ammonia, and interactive effects on whole-body protein and water content, plasma Na^+, and N-enzyme activity among treatment groups within each sample period. One-way ANOVA followed by Dunnett's test (Dunnett 1955), where N represented the number of tanks per treatment, was used to compare differences in appetite, gross conversion efficiencies, O_2 consumption rates, N-waste excretion rates, and NQs of the respective treatment groups with the "base" control group; an exception was with the analysis of SGRs, where N represented the total number of marked fish. The level of statistical significance for all analyses was $P < 0.05$.

Results

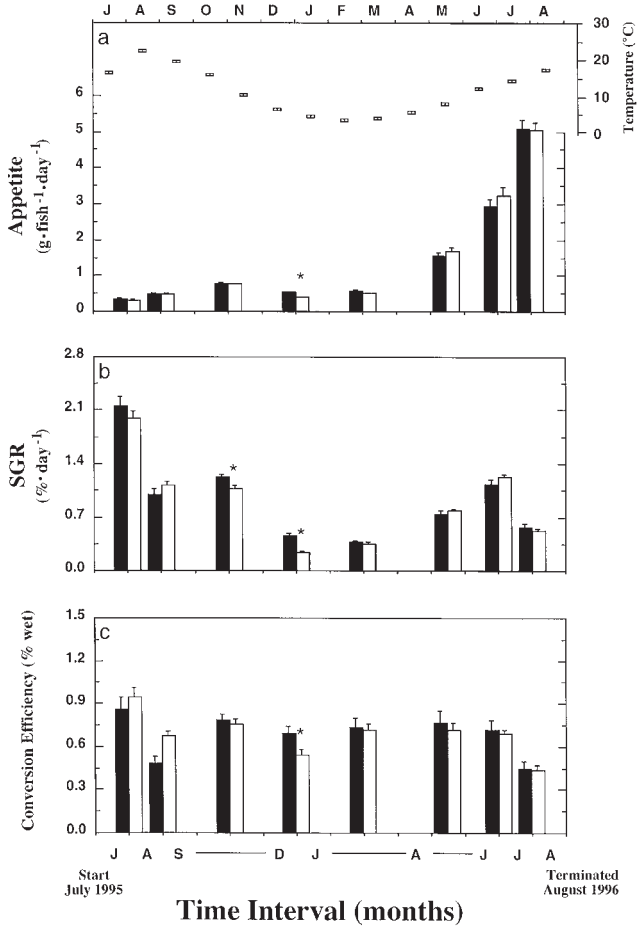
Temperature profiles and mortality

The temperature profiles for the base and base+2°C temperature regimes (characteristic of nearshore Lake Ontario) show that the annual water temperature cycle varies year to year by more than just a few degrees (see top panels in Figs. 1–6). For example, the mean monthly water temperature during August 1995 was 23°C whereas in August 1996, it was only 18°C. The extraordinarily high temperature during the first 25 days of exposure (July–August 1995) may have contributed to an increased sensitivity of juvenile rainbow trout to the warmer and polluted scenarios, i.e., total mortality was 4.6, 10.8, 13.9, and 8.9% for the base, base+Am, base+2°C, and base+2°C+Am groups, respectively, during the first 25 days. During the following 45 days (days 25–70), total mortality had dropped to 1.4, 2.3, 5.4, and 6.8%. After this initial 2.5-month period (July–September 1995), mortality was virtually nonexistent. Rainbow trout grown under the base thermal regime spent at least 6 months at temperatures below 10°C versus only 5 months for rainbow trout at base+2°C. Rates of fall cooling and spring warming were about 5.5 and 4.5°C·$month^{-1}$, respectively.

Growth, feeding, and metabolism

Over the course of the entire exposure, juvenile rainbow trout fed to satiation daily had gained 30–35 times their original wet weight and about three times their total length (Table 1). Those fish exposed to the combination of +2°C and ammonia (base+2°C+Am) outgrew the three other groups by at least 30 g in weight and an extra 1–1.5 cm in length. Substantial gains in growth were achieved by rainbow trout exposed to the additional +2°C (base+2°C) over winter and spring, only to be lost in the final 30 days (mean water temperatures at base+2°C = 20°C) of the experiment (Table 1). The increased growth was associated with an elevation in food intake through winter and spring which ultimately contributed to the consumption of an additional 60 and 75 g of food per fish, for the

Fig. 1. Comparison of effects on (*a*) appetite, (*b*) SGR, and (*c*) gross food conversion efficiency of juvenile rainbow trout fed to satiation daily and exposed to base temperature (black bars) or base + 70 μmol T_{Amm}·L^{-1} (white bars). Exposures lasted 14 months from 8 July 1995 to 30 August 1996. Appetite was the daily average amount of food consumed by the four replicate tanks of fish as fish were fed to satiation. Specific growth rates were determined from the 11 marked fish measured for growth increment ($N = 44$ per treatment). Gross food conversion efficiency was calculated as the ratio of the weight gain (marked fish) to food consumed, both in wet weight. Columns with a star are significantly different from the base control group ($P > 0.05$). The base thermal regime is depicted at the top on a monthly basis.



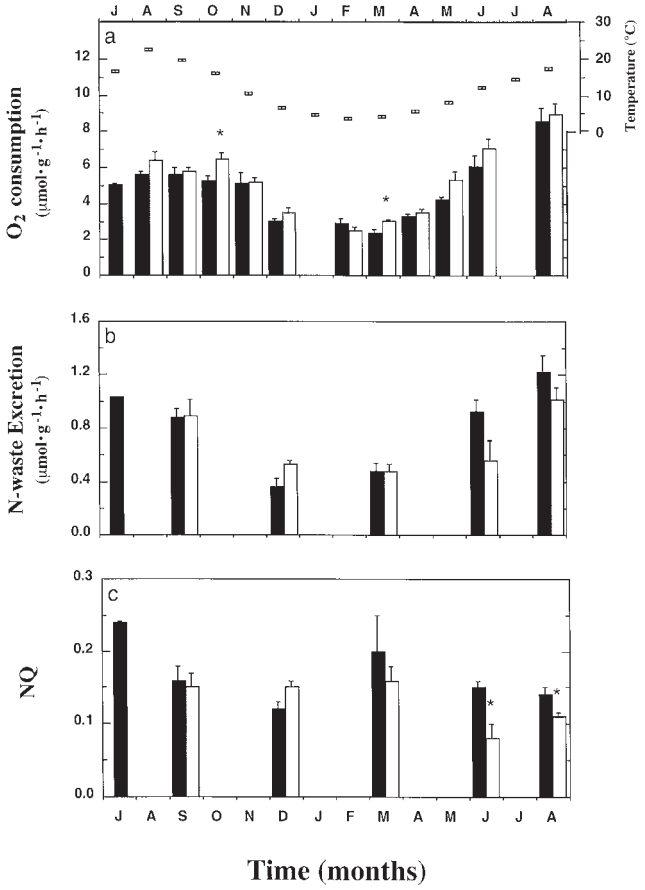
base+2°C and base+2°C+Am groups, respectively, compared with the base group (Table 1). Condition factors fluctuated seasonally over the course of the year but, in general, rose steadily from an initial mean of 0.88 to 1.26 (Table 1). An overall sex ratio (males to females) of 1.7 existed among marked fish at test termination. Gender had no significant influence on final weight of the fish (data not shown).

Specific treatment effects

Base versus base+Am

The addition of +70 μmol T_{Amm}·L^{-1} to the base thermal regime did not affect appetite, SGR, or gross food conversion efficiency of rainbow trout with an exception between 15

Fig. 2. Comparison of effects on (*a*) routine O_2 consumption, (*b*) total N-waste excretion, and (*c*) the NQs of juvenile rainbow trout fed to satiation daily and exposed to base temperature (black bars) or base + 70 μmol T_{Amm}·L^{-1} (white bars). Exposures lasted 14 months from 8 July 1995 to 30 August 1996. In Figs. 2*a* and 2*b* the data have been scaled for weight using the weight exponent 0.824 (Cho 1990). Measurements were made over a 24-h period on all four tanks per treatment. Columns with a star are significantly different from the base control group ($P > 0.05$). The base thermal regime is depicted at the top on a monthly basis.



December 1995 and 25 January 1996 (Fig. 1). Here, appetite and SGR were significantly depressed in the rainbow trout exposed to the additional ammonia compared with the base group (Figs. 1*a* and 1*b*). O_2 consumption, however, tended to be elevated in the ammonia-exposed fish, although this was significant only in October and March (Fig. 2*a*). Little difference in N-waste excretion existed throughout much of the year, although N-waste excretion of the base+Am group declined in the second summer (Fig. 2*b*), which was apparent in the significantly lower NQs (Fig. 2*c*).

Base versus base+2°C

The effects of an additional +2°C on growth and metabolism of rainbow trout were much more dramatic than additional ammonia alone owing to substantial temperature-dependent changes in appetite. The extremely high water temperatures reached during August 1995 resulted in a significantly depressed appetite (Fig. 3*a*) and a greater than 50% reduction in SGR of the warmed fish (25°C+) in comparison with the base

Fig. 3. Comparison of effects on (*a*) appetite, (*b*) SGR, and (*c*) gross food conversion efficiency of juvenile rainbow trout fed to satiation daily and exposed to base temperature (black bars) or this regime + 2°C (white bars). Exposures lasted 14 months from 8 July 1995 to 30 August 1996. Other details as in Fig. 1.

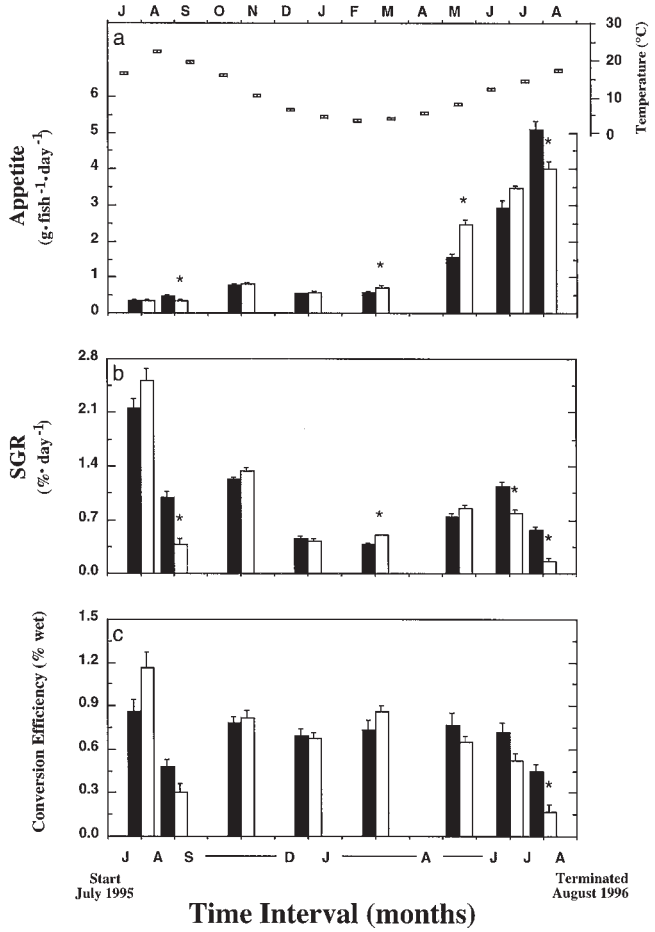
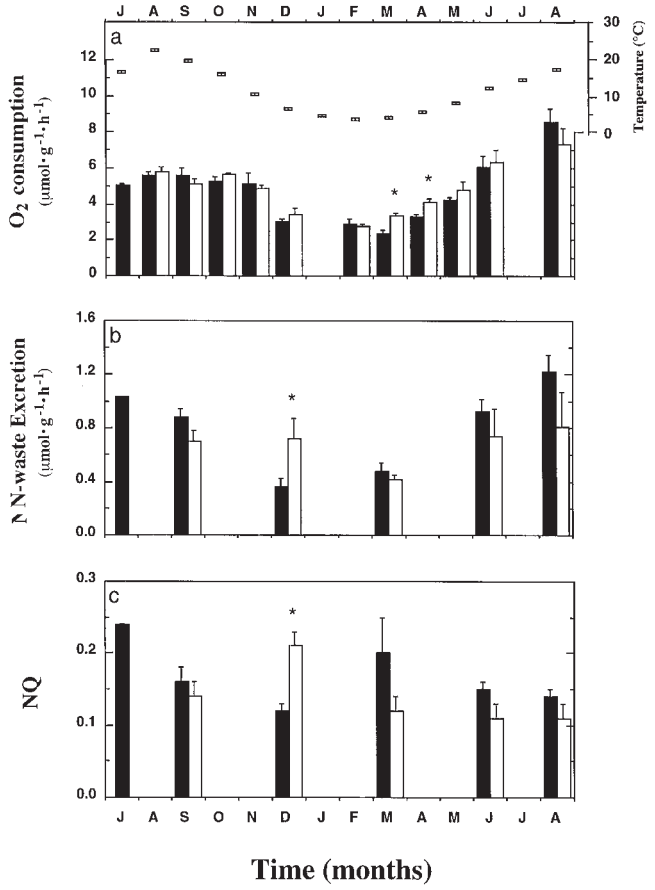


Fig. 4. Comparison of effects on (*a*) routine O_2 consumption, (*b*) total N-waste excretion, and (*c*) the NQs of juvenile rainbow trout fed to satiation daily and exposed to base temperature (black bars) or this regime + 2°C (white bars). Exposures lasted 14 months from 8 July 1995 to 30 August 1996. Other details as in Fig. 2.



group (Fig. 3*b*). A similar, and even more pronounced, negative temperature-related effect on appetite and growth occurred the next summer when the fish were larger, this despite lower mean temperatures. Through winter and spring, the additional +2°C stimulated appetite leading to slightly greater growth. However, the greater appetites and growth were also associated with significant elevations in the rates of O_2 consumed (Fig. 4*a*). There was little effect of the additional +2°C on N-waste excretion (Fig. 4*b*), except toward the end of fall where an elevated NQ indicated the greater reliance of these fish on protein as a metabolic fuel (Fig. 4*c*).

Base versus base+2°C+Am

The combination of +2°C and +70 μmol $T_{Amm}·L^{-1}$ added to base water led to similar types of effects as with the addition of +2°C alone (Figs. 5 and 6). However, appetite was less dramatically altered in the presence of +70 μmol $T_{Amm}·L^{-1}$ despite very pronounced reductions in SGRs during the high water temperatures of late summer (Figs. 5*a* and 5*b*); there were no significant effects on gross food conversion efficiency (Fig. 5*c*). The additional temperature and ammonia also helped stimulate growth through fall as well as winter, although at an additional metabolic cost, i.e., O_2 consumption rates were significantly elevated in October and from February through April compared with the base group (Fig. 6*a*). On the other hand, high temperatures in combination with the additional ammonia during late summer tended to suppress N-waste excretion leading to lower NQs (Figs. 6*b* and 6*c*). The opposite effect was observed at the low winter temperatures, indicating a greater reliance on protein as a metabolic fuel at this time.

Whole-body protein, water content, and plasma [Na^+]

Aside from an initial decline in whole-body protein content, which fell to 10% after the first 25 days (August 1995), the initial whole-body protein content of 15% was maintained at the end of the experiment. However, whole-body protein contents of fish grown at +2°C were significantly lower (two-way ANOVA, $P \leq 0.05$) than fish grown at base water temperatures at this time (data not shown). Similarly, water content, which fell from an initial 77 to about 70% (data not shown), was also reduced in the base+2°C fish after 420 days of exposure. Plasma [Na^+] increased nearly 25% from 110 to 130 mequiv.·L^{-1}, but no clear treatment effects were exhibited (data not shown).

Table 2. Activities ($\mu mol \cdot min^{-1} \cdot g^{-1}$) of ALAat, ASPat, Gdh, and Gsase in liver, gill, and muscle of juvenile rainbow trout initially and after about 1 month (day 25) and 14 months (day 420) of exposure to a +2°C warming scenario and 70 $\mu mol\ T_{Amm} \cdot L^{-1}$ (Am).

Test day and treatment	ALAat			ASPat		
	Liver	Gill	Muscle	Liver	Gill	Muscle
Initial	17.86±2.23 (4)	0.57±0.05 (3)	2.58±0.22 (9)	15.66±1.64 (7)	6.45±0.19 (10)	12.61±0.38 (8)
Day 25						
Base+Am	14.02±1.24 (11)	0.57±0.10 (5)	2.53±0.19 (18)	15.47±1.51 (13)	8.08±0.89 (16)	11.07±1.00 (8)
Base	11.07±0.81 (14)	0.62±0.03 (12)	2.60±0.23 (16)	13.95±1.43 (18)	8.83±0.80 (19)	10.50±0.72 (11)
Base+2°C	13.27±1.38 (12)	0.68±0.04 (8)	2.83±0.32 (16)	13.79±1.84 (17)	7.62±0.41 (18)	10.80±0.55 (7)
Base+2°C+Am	14.16±1.18 (16)	0.72±0.08 (7)	2.61±0.24 (18)	12.28±1.11 (15)	7.12±0.34 (18)	12.22±0.65 (11)
Day 420						
Base+Am	61.35±2.95 (18)	1.13±0.09 (11)	0.87±0.04 (18)	51.16±3.50 (19)	7.75±0.38 (19)	16.61±0.56 (15)
Base	59.03±3.28 (20)	0.91±0.08 (14)	1.12±0.09 (14)	39.63±2.15 (20)	7.99±0.37 (20)	15.32±0.41 (11)
Base+2°C	75.68±5.16 (10)	0.93±0.10 (9)	1.16±0.07 (12)	43.49±2.69 (11)	8.56±0.67 (13)	17.34±0.97 (8)
Base+2°C+Am	51.96±3.51 (11)	0.79±0.09 (4)	0.97±0.06 (15)	31.22±3.00 (14)	6.91±0.35 (17)	15.32±0.28 (13)
	I		A	I	A	I

Note: For each sampling day separately, statistically significant temperature (+2°C treatments = T), ammonia (+70 $\mu mol\ T_{Amm} \cdot L^{-1}$ treatments = A), and interactive effects (I) are denoted underneath the respective columns (two-way ANOVA, $P \leq 0.05$). Where there are no letters, there were no significant differences.

Tissue N-enzymes

The activities of ALAat and ASPat were initially greatest in liver, although differences in activities between tissues (liver, gill, and muscle) were smaller for the latter enzyme (Table 2). After 25 days of exposure, the activity of these enzymes remained fairly constant in all three tissues. By the end of the experiment, however, there was an approximate three- to fivefold increase in liver ALAat and ASPat activities (Table 2) and interactive effects such that, overall, their activities were depressed in the base+2°C+Am-treated fish. In muscle, significantly lower ALAat activity was exhibited by the ammonia-exposed fish at both temperature regimes whereas in gills, significantly lower ASPat activity was exhibited (Table 2).

The highest levels of Gdh activity were exhibited in the liver of rainbow trout, but high levels were also present in the gills that were double the activities in muscle (Table 2). The addition of +2°C caused a rapid (25 days) stimulation of Gdh activity in muscle and interacted with +70 $\mu mol\ T_{Amm} \cdot L^{-1}$ in the gills to enhance activity even further. Levels of Gdh activity in liver were relatively unaltered at this time. After 420 days, however, a dramatic increase in the activity of liver Gdh, similar in magnitude to ALAat and ASPat, was measured (Table 2). Gdh activities in liver, gill, and muscle were reduced in the ammonia-exposed fish (Table 2), and a significant interactive effect was observed in the liver such that Gdh activity was depressed in the base+Am group, but this may be an artifact due to the low sample size. On the other hand, additional temperature depressed Gdh activity in the gills at this time.

Of the four enzymes measured, GSase was by far the lowest in activity (≈1/50th). Initial GSase activities in liver and gills were the same, but GSase activity in muscle was two orders of magnitude lower (Table 2). A significant negative effect after 25 days of exposure was seen in the liver and gills of fish exposed to an additional +2°C. The activity of this enzyme increased the least in rainbow trout by the end of the experiment, e.g., liver GSase activity increased only about twofold. As with the other enzymes, liver GSase activity was significantly depressed in fish coping with the interaction of +2°C and ammonia. Muscle GSase activity changed very little over time, but the base+2°C group had significantly higher GSase activity overall.

Discussion

Effects of +2°C during summer maximum temperatures

The challenge of a warmer and more polluted environment is expected to be substantial for cold-water fish living without thermal refuge (see Wood and McDonald 1997), but to date, there has been a general lack of hard experimental evidence to support this. The present data indicate that one of the most dramatic effects of an additional +2°C superimposed above the naturally fluctuating annual thermal water cycle was on the appetite of "warmed" fish during late summer. Suppression of appetite induced by high temperature ($Q_{10} \approx 0.4$) caused a cascade of whole-animal responses that tended to decrease O_2 consumption, N-waste excretion, and the NQ (extent of protein used to fuel metabolism). This ultimately led to a substantial decline (>50%) in growth rate of warmed fish compared with those at base temperature. These results confirm earlier findings that +2°C superimposed above the annual cycle depresses appetite and protein turnover of rainbow trout fed an unlimited ration during peak summer water temperatures (see Reid et al. 1995; Linton et al. 1997).

The mechanism(s) of appetite suppression in fish is(are) largely unknown, but may be related to the increased metabolic demands of feeding fish at high temperature (Jobling 1997), thus requiring a general decrease in feeding activity. The metabolic rates of feeding fish, for example, are up to severalfold higher than those of fish on low ration (Brett 1979; Jobling 1993; Alsop and Wood 1997). This increase, along with the increased energetic demands at higher temperatures due to both general metabolism and perhaps stress resistance above a certain "species-dependent" temperature threshold, may impose greater demands on the respiratory and circulatory system, leaving insufficient energy available in the short term for

Table 2 (*concluded*).

Gdh			Gsase		
Liver	Gill	Muscle	Liver	Gill	Muscle
13.83±1.48 (7)	4.02±0.30 (9)	2.65±0.26 (10)	0.27±0.05 (10)	0.30±0.02 (10)	0.0038±0.0014 (10)
13.53±0.88 (15)	5.14±0.36 (20)	3.95±0.21 (17)	0.23±0.02 (18)	0.45±0.03 (19)	0.0023±0.0003 (20)
12.26±0.89 (16)	6.05±0.40 (20)	3.31±0.31 (19)	0.19±0.01 (16)	0.44±0.02 (17)	0.0018±0.0002 (20)
11.03±0.65 (13)	5.20±0.25 (17)	4.40±0.36 (17)	0.15±0.07 (15)	0.39±0.02 (16)	0.0017±0.0003 (18)
12.53±0.84 (12)	5.77±0.31 (19)	4.06±0.28 (18)	0.13±0.01 (14)	0.38±0.03 (14)	0.0022±0.0003 (20)
34.56±0.84 (3)	7.72±0.58 (19)	2.62±0.21 (19)	0.73±0.05 (19)	0.84±0.07 (19)	0.0011±0.0001 (17)
54.97±2.67 (20)	8.68±0.33 (20)	2.88±0.13 (20)	0.60±0.03 (20)	1.10±0.03 (20)	0.0020±0.0003 (19)
40.46±1.28 (6)	8.44±0.51 (13)	3.10±0.26 (12)	0.57±0.06 (13)	1.00±0.05 (13)	0.0038±0.0006 (13)
44.61±4.26 (13)	5.73±0.41 (17)	2.50±0.12 (17)	0.34±0.02 (16)	0.70±0.03 (17)	0.0015±0.0004 (16)
I	T, A	A	I	T, A	T, A

digestion and specific dynamic action. In the present study, routine O_2 consumption rates of fish at base temperature late in the second summer approached 90% of maximum O_2 consumption (V_{O_2max}) measured at maximum swimming speeds (U_{crit}) in fasting juvenile rainbow trout (Alsop and Wood 1997) whereas O_2 consumption rates were only 75% of V_{O_2max} in the base+2°C fish. Alsop and Wood (1997) also show that although there is no ration effect on V_{O_2max} at U_{crit}, U_{crit} is reduced in satiation-fed fish as compared with fasted fish. Thus, the authors concluded that V_{O_2max} "is limited by the capacity to take up O_2 at the gills and (or) deliver O_2 through the circulatory system, rather than the capacity to consume O_2 at the tissues." Jobling (1997) recently used Brett's (1995) data to show that appetite suppression may be related to limitations in the capacity of the respiratory and circulatory systems to deliver O_2 to respiring tissues at temperatures above threshold.

The threshold temperatures for the cessation of feeding, and subsequently growth, differed between summers (>20 versus <20°C), and thus with fish size and age. For example, despite the considerable negative impact on appetite and growth of rainbow trout in late summer, it was only in the second summer (August 1996) when the fish were bigger that the additional +2°C had any significant effect on the efficiency with which food is converted to body material (Fig. 3*c*). This is in apparent contradiction to other analyses (Elliott 1981; Rombough 1997). These other studies, however, were conducted on fish acclimated to constant temperatures.

Effects of +2°C during winter minimum temperatures

The effects of the +2°C warming scenario were equally noticeable at low temperatures. The period from January to April 1996 averaged about 4°C at base temperature. During this time, fish exposed to +2°C consumed about 8 g more food and gained an extra 20 g in weight; conversion efficiencies were elevated, although not significantly, by about 15%. The increased metabolism associated with feeding, defined as the apparent heat increment of feeding (see Beamish and Trippel 1990), had a large influence on O_2 consumption rate. Linton et al. (1998), who showed similar results, attributed the phenomenon to the increased metabolic costs of protein synthesis. The present results are perhaps not surprising, given the known influence of temperature on appetite and growth. However, the large Q_{10} (mean of 5.2) derived from the growth and O_2 consumption rates of juvenile rainbow trout at 4 and 6°C deserves further attention, especially in view of the fact that during the warmer water temperatures of late spring, appetite was significantly elevated in fish exposed to +2°C, but growth and metabolic rates were not different from those of the base fish.

Growth and metabolic modulation in relation to temperature need not only be imperative for fish at the upper thermal extreme. Decreased temperature directly affects protein content and rates of enzyme-catalyzed reactions (Clarke 1987). It might be that rainbow trout down-regulate metabolism below a certain threshold temperature, or that this process is triggered by the falling temperature itself. This would explain the lower N-waste excretion rate and NQ in base fish leading into winter. Patterns of temperature compensation such as this (type 5: inverse compensation; see Hazel and Prosser 1974) may be induced at extreme lows in an animal's thermal range to conserve energy reserves, which would increase the length of time the animal could survive winter. A similar cold-induced temperature compensation has been observed in American eel (*Anguilla rostrata*), although the mechanisms are probably quite different (Walsh et al. 1983). Down-regulation has great advantages for fish because all of the other major adaptive strategies for counteracting the effects of extreme low temperature, i.e., increase in enzyme activity, production of new enzyme variants, and alteration in cellular environment (Clarke 1987), impose additional cost. Thus, for juvenile temperate fishes such as rainbow trout, survival during a severe winter could be compromised if metabolic energy conservation did not occur.

It is apparent from the present results that a +2°C warming scenario has a substantial effect on the appetite, growth, and metabolism of juvenile rainbow trout at the temperature extremes. The consequences of these effects were considerable gains in growth of fish exposed to +2°C during winter, which

Fig. 5. Comparison of effects on (*a*) appetite, (*b*) SGR, and (*c*) gross food conversion efficiency of juvenile rainbow trout fed to satiation daily and exposed to base temperature (black bars) or this regime + 2°C + 70 μmol $T_{amm}·L^{-1}$ (hatched bars). Exposures lasted 14 months from 8 July 1995 to 30 August 1996. Other details as in Fig. 1.

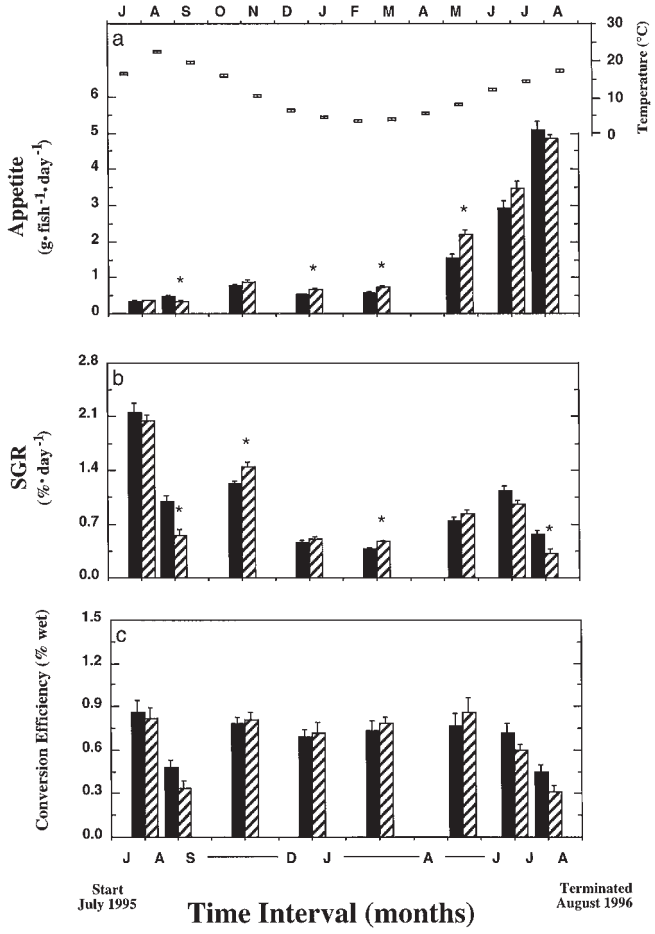


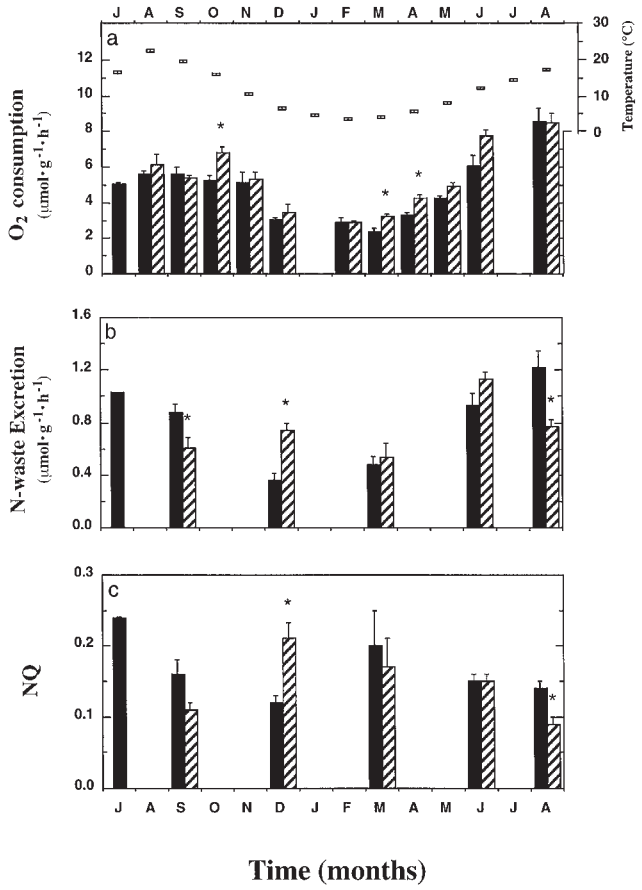
Table 3. Total O_2 consumed, total N retained (from whole-body protein measurements and factored by 6.25), and N-cost indexes (see Calculations section under Methods and materials for definition) of juvenile rainbow trout fed to satiation for 14 months from 8 July 1995 to 30 August 1996.

Treatment	Total O_2 consumed (mol)	Total N retained (mol)	N-cost index
Base+Am	10.09	0.56	18.18
Base	9.25	0.55	16.82
Base+2°C	9.72	0.52	18.69
Base+2°C+Am	11.40	0.58	19.55

Note: Values are expressed on a per fish basis.

were eventually lost towards the end of summer. The total moles of O_2 consumed for fish exposed to +2°C was estimated to be about 5% higher than in the base group, but the total moles of N that they retained was about 5% lower (Table 3). This resulted in an increase in cost of living (i.e., O_2 consumption per unit protein growth or N-cost index) of about 10% (Table 3).

Fig. 6. Comparison of effects on (*a*) routine O_2 consumption, (*b*) total N-waste excretion, and (*c*) the NQs of juvenile rainbow trout fed to satiation daily and exposed to base temperature (black bars) or this regime + 2°C + 70 μmol $T_{amm}·L^{-1}$ (hatched bars). Exposures lasted 14 months from 8 July 1995 to 30 August 1996. Other details as in Fig. 2.



Influence of environmental ammonia

The low levels of ammonia used in the present study had more of a stimulatory effect rather than an inhibitory effect on the overall appetite, growth, and metabolism of juvenile rainbow trout, except at the temperature extremes. In fact, the fish exposed to both additional ammonia and temperature achieved the greatest gain in weight by the end of the experiment. This occurred despite a considerable decline in SGR in the last month (August 1996) due to suppression of appetite associated with high temperature, as discussed above.

There is little precedence for the stimulatory effect of sublethal ammonia on fish growth in the literature. In the majority of cases, fish, and salmonids in particular, exposed to ammonia at equivalent concentrations exhibit gross physiological or even growth impairment, but these effects may have been complicated by other biotic and abiotic factors (see Meade 1985 for review). Linton et al. (1997), in a separate study, reported a stimulation of growth in juvenile rainbow trout exposed to +70 μmol $T_{Amm}·L^{-1}$ at base temperatures during summer. These fish also exhibited higher rates of protein synthesis and turnover in their livers (unpublished data from our laboratory). By the end of the present experiment, rainbow trout exposed to ammonia had retained up to 10% more N than

fish not exposed to ammonia (Table 3). This phenomenon also has been reported on at least two other occasions in feeding juvenile rainbow trout (see Linton et al. 1997, 1998).

Although appetite and growth were generally depressed in rainbow trout exposed to additional ammonia at base temperatures during winter and fall relative to the base group, they were stimulated in the ammonia-exposed fish at +2°C. O_2 consumption, on the other hand, tended to be elevated in both groups throughout most of the year. This latter response resulted in elevated N-cost indexes, 8 and 17% for the base+Am and base+2°C+Am groups, respectively, as compared with the base group (Table 3). Brown (1968) reported that ammonia was twice as toxic to rainbow trout at 3°C than at 10°C, and in an earlier study (Linton et al. 1998), growth of juvenile rainbow trout exposed to +70 μmol $T_{Amm}{\cdot}L^{-1}$ was slightly reduced at 4°C (although not significantly) as was their efficiency of N retention. These data confirm that N retention is not stimulated by sublethal ammonia at very low temperature (<5°C), which could be a threshold temperature below which these seemingly anabolic ammonia effects are negative or nonexistent. Above this threshold temperature, ammonia appears to directly stimulate N retention, either through a detoxification mechanism or as additional substrate. Since most enzymes function optimally within narrow temperature and pH ranges, it is entirely possible that ammonia acts to stimulate N retention only within the temperature range necessary for optimal growth.

Temperature, ammonia, and N-enzymes

The stimulation of N retention by fish exposed to very low levels of ammonia over summer (see Linton et al. 1997) prompted our investigation of some of the key enzymes involved in N metabolism and ammonia detoxification in freshwater teleost fish. Although the same pronounced stimulation of N retention did not appear to take place during the present experiment (see discussion above), the change in activities of some of these enzymes (albeit small changes) did indicate the potential sensitivity of N metabolism to chronic environmental change. This was particularly evident in those fish exposed to the combination of +2°C and 70 μmol $T_{Amm}{\cdot}L^{-1}$.

The additional temperature itself had negligible effect on N-enzyme activity. However, in nearly all cases, fish exposed to ammonia at +2°C had significantly reduced enzyme activities. On the other hand, the activities of at least ALAat and ASPat in fish exposed to ammonia at base temperature tended to be elevated. The exception was Gdh, where enzyme activity was significantly reduced in all tissues of fish exposed to additional ammonia. This may explain the reduced N-waste excretion rates and NQs of these fish at this time (Figs. 3*b* and 3*c*). Taken together, these results suggest that the added cost of maintaining high energy levels through amino acid catabolism at higher temperature may be too great for fish already coping with an additional demand (i.e., ammonia) on metabolism.

Metabolic "down-regulation," particularly in biosynthesis and macromolecular turnover, is an effective energy conservation mechanism induced by animals facing environmental insult (Hand and Hardewig 1996). For example, Prasad et al. (1991) suggested that oxidative deamination of glutamate is reduced in Mozambique tilapia (*Tilapia mossambica*) exposed to atrazine to combat "atrazine-induced" ammonia toxicity and possibly to minimize extra energy expenditure through protein catabolism. The large increase, after 415 days, in the activities (in the oxidative direction) of ALAat, ASPat, and Gdh, which are all highly involved in aerobic ammonia production in fish (Van Waarde 1981), is indicative of fish catabolizing excess amino acids for energy (Walton and Cowey 1977). Transamination of alanine and aspartate by ALAat and ASPat generates oxaloacetate and pyruvic acid, respectively, as well as copious amounts of glutamate. All three of these can enter the Krebs cycle for direct energy production or act as precursors for lipid and carbohydrate synthesis (Wood 1993). However, the large difference in tissue enzyme activity levels between sampling periods observed here, especially in the liver, is counter to the scaling of oxidative enzyme activity (e.g., citrate synthetase) with size noted previously in rainbow trout (Somero and Childress 1990). In fact, Somero and Childress (1990) observed only "positive" scaling for lactate dehydrogenase and creatine phosphokinase, which are enzymes indicative of the potential for anaerobic glycolysis or the maintenance of stable ATP concentration during muscular activity, respectively. The examination of changes in N-enzyme activity with water temperature, fish size, water quality, and feeding activity is a relatively unexplored area in fish physiology and deserving of much further attention. The present data suggest the potential sensitivity of these pathways to environmental change.

Acknowledgments

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