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The Metabolic Costs and Physiological Consequences to Juvenile Rainbow Trout of a Simulated Summer Warming Scenario in the Presence and Absence of Sublethal Ammonia

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Abstract.—Quantitative bioenergetic and physiological measurements were made on juvenile rainbow trout *Oncorhynchus mykiss* exposed over summer (June–September 1993) to a simulated summer warming scenario of +2°C in the presence and absence of 70 μmol total ammonia/L (nominal; equivalent to 0.013 mg NH_3-N/L at 15°C, pH = 7.6) to determine the metabolic costs and physiological consequences associated with their growth in a warmer, more polluted environment. With unlimited food, fish exposed to +2°C show better energy conversion efficiency and increased nitrogen retention at a metabolic cost equivalent to the base temperature group. Metabolic fuel use appears to have been optimized to support the bioenergetic demands imposed during maximum summer water temperatures. Low-level ammonia enhances nitrogen and energy conversion efficiency by stimulating protein retention, which ultimately results in the most cost-effective growth. However, in the +2°C ammonia treatment, the stimulatory effect of low-level ammonia is lost during mid to late summer due to the greater energy demands when fish are forced to cope with the additional stress of a small further increase in temperature.

Temperature is the single most important environmental factor affecting animal activity, and the effects of temperature change are especially pronounced for aquatic ectotherms such as fish. Aside from natural diel and seasonal elevations in water temperature, fish face increased water temperatures from the discharge of cooling water from power plants, water diversion for crop irrigation, and canopy removal from forest clear-cutting (Sprague 1985; Thomas et al. 1986). Water temperatures are also likely to rise in association with global warming. Simulation modeling suggests that a doubling of the atmospheric CO_2 concentration will increase the Earth's average temperature by 1.5 to 4.5°C in the next 50 years (Hansen et al. 1988). Schindler et al. (1990) have already reported a 2°C increase in mean air and lake temperatures over the past 20 years in northern Ontario, Canada. Despite a large body of literature addressing the effects of elevated temperature on fish, large uncertainties in the magnitude and timing of potential warming impacts still exist (Rubin et al. 1992). To date relatively few studies have addressed the effects of chronic small temperature elevations over the annual cycle on fish.

The metabolic and physiological effects on fish of small temperature elevations above present natural fluctuations in water temperatures could be large. Many species inhabit waters encompassing a wide range of temperatures, but each typically has a narrow range at which growth and survival are maximized (Fry 1967; Magnuson et al. 1979; Elliot 1982; Trippel et al. 1991). Even small changes in local temperature may alter fishes' energetic requirements (Dawson 1992; Mehner and Weiser 1994).

The goal of the present paper is to quantify the metabolic costs and physiological consequences to juvenile trout associated with living in warmer, marginally polluted environments. The thermal optimum for growth and food utilization in rainbow trout *Oncorhynchus mykiss* is ordinarily 15°C (Cho and Kaushik 1990), and the species' upper lethal limit is approximately 26°C (Bidgood and Berst 1969; Kaya 1978). Reid et al. (1995) compared juvenile rainbow trout held in hard and soft water during the summer of 1993 to fish held 2°C above the natural water temperature cycle (characteristic of the inshore region of Lake Ontario) and found an average 20% reduction in growth, appetite, gross conversion efficiency and protein turnover during peak summer water temperatures (20–24°C). In this paper, we address the energetic and physiological effects of the above thermal regime as well as the effect of a low, but environmentally realistic, level of ammonia (a 70-μmol/L dilution of total ammonia [NH_4^+ + NH_3], equivalent to 0.013 mg un-ionized ammonia/L at 15°C and pH 7.6). The thermal and elevated ammonia regimes are assessed alone and in combination,

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which requires use of some data (growth, appetite, and conversion efficiency for the ammonia-free treatments only) originally reported by Reid et al. (1995).

Ammonia is an important pollutant in aquatic environments. It is highly toxic to fish and ubiquitous in surface waters (Thurston and Russo 1983; Erickson 1985; Russo 1985). More molecules of ammonia are manufactured each year than any other industrial chemical (Atkins 1987). Ammonia also is a problem in fish culture where high-density stocking may result in an accumulation of ammonia via excretion from the fish. Larmoyeux and Piper (1973) found that growth of juvenile rainbow trout raised in hatchery water-reuse systems was significantly reduced in tanks with un-ionized ammonia loads greater than 0.01 mg/L. The lowest lethal concentration of un-ionized ammonia found for salmonids is 0.083 mg/L (Thurston et al. 1984), but sublethal effects have been reported at concentrations even lower than 0.01 mg/L (Rice and Bailey 1980; EPA 1985). This low chronic value is often exceeded in many urban and industrial areas including Hamilton Harbor (Lake Ontario) and its tributaries, where summer total ammonia concentrations have been recorded as high as 66 μmol/L at pH = 7.3, equivalent to 0.012 mg/L un-ionized ammonia (B. Crosbie, McMaster University, personal communication).

We used several physiological indices including food intake, growth, metabolic rate, nitrogen balance, and proximate composition to assess the long-term consequences and metabolic costs for juvenile trout exposed to a small increase in temperature and an environmentally relevant concentration of priority pollutant, alone and in combination. We asked three questions: (1) What physiological changes occur in juvenile rainbow trout growing under the normal, fluctuating summer thermal regime? (2) What is the effect of +2°C superimposed on this regime? (3) What is the effect of 70 μmol ammonia/L on both of the above? Our data suggest that additional temperature (2°C) and ammonia (70 μmol/L) are beneficial up to a threshold temperature above which loss of appetite and increased maintenance costs greatly reduce growth and nitrogen transformation efficiencies.

Methods

Animals.—Juvenile rainbow trout (2–5 g) were obtained from Rainbow Springs Trout Farm, Thamesford, Ontario, on 19 April 1993 and allowed to acclimatize for 6 weeks prior to testing. The trout were held in a 600 L aerated polyethylene tank receiving 2.5-L/min flow of dechlorinated Hamilton tap water ($[Ca^{2+}]$ = 0.98 ± 0.11 mmol/L, $[Na^+]$ = 0.56 ± 0.03 mmol/L, $[Cl^-]$ = 0.73 mmol/L; pH = 7.57 ± 0.26) at an ambient water temperature of 12 ± 1°C. They were fed a maintenance ration equivalent to 1% body weight per day (wet basis) of Zeigler's Trout Starter number 3 (50% protein, 15% lipid, 12% moisture) and kept under the natural photoperiod for Hamilton, Ontario, throughout the experiment.

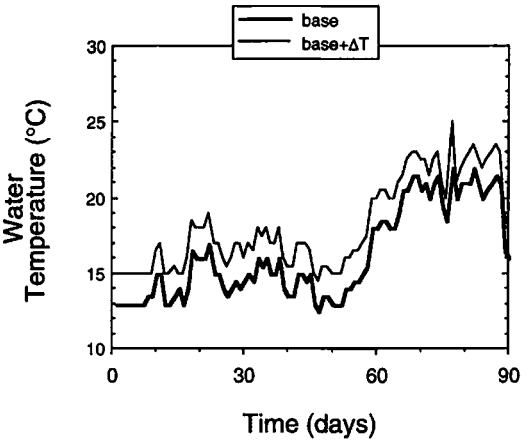

FIGURE 1.—Daily water temperature profile as measured each day over 90-d exposures from 17 June to 15 September 1993. Juvenile rainbow trout were exposed to either ambient laboratory water temperatures (base, solid line) or to ambient water temperatures + 2°C (base+ΔT, dashed line).

Experiments.—Groups of approximately 150 rainbow frout were randomly distributed to eight exposure tanks (270 L) representing four treatment conditions (N = 300 fish per treatment). The tanks received 2.5-L/min flows (95% replacement time, 4.2 h) of either ambient temperature water (base = City of Hamilton tap water taken from the inshore region of Lake Ontario) or ambient temperature water plus 2°C (base+ΔT), each with or without an additional 70 μmol total ammonia/L (T_{Amm} = NH_4^+ + NH_3; base+Am and base+ΔT+Am, respectively). Water temperatures of base + 2°C were achieved with a heat exchanger. The actual mean difference between temperature treatments was 1.90 ± 0.03°C (Figure 1). The desired T_{Amm} concentration was achieved by delivering the required amount of $(NH_4)_2SO_4$ stock solution via mariotte bottles (Mount and Brungs 1967). The mean concentrations of total ammonia in the water of each tank along with the pH and partial pressure of oxygen are listed in Table 1.

During the experiment, the fish were fed to sa-

TABLE 1.—Mean (±SE) ammonia concentrations, pH, and partial oxygen pressures in tanks during 90-d exposures of rainbow trout to +2°C (ΔT) and 70 μmol ammonia/L (Am).

Treatment	Replicate	Total ammonia: Tank mean (μmol/L)	Total ammonia: N	Total ammonia: Treatment mean (μmol/L)	pH: Mean	pH: N	P_{O_2}: Mean (torr)	P_{O_2}: N
Base+Am	1	65.8 ± 6.3	10	61.5 ± 3.8	7.6 ± 0.1	14	131.9 ± 4.5	14
	2	58.2 ± 5.6	10		7.6 ± 0.1	14	134.3 ± 4.1	14
Base	1	6.2 ± 1.6	9	6.0 ± 0.2	7.6 ± 0.1	14	132.9 ± 3.5	15
	2	5.8 ± 0.9	8		7.6 ± 0.1	13	134.6 ± 3.9	15
Base+ΔT	1	4.5 ± 1.0	9	5.8 ± 1.3	7.6 ± 0.1	14	132.2 ± 3.5	15
	2	7.1 ± 1.5	9		7.5 ± 0.1	13	124.3 ± 4.7	15
Base+ΔT+Am	1	78.6 ± 7.6	10	75.7 ± 2.9	7.5 ± 0.1	14	121.3 ± 4.1	15
	2	72.8 ± 6.6	10		7.6 ± 0.1	14	123.3 ± 4.3	15

tiation twice daily (at 0830 and 1630 hours) from preweighed bags of Zeigler Trout Starter so we could monitor appetite, following the methods of Wilson et al. (1994a). Samples of fish from each exposure were taken immediately after the test began and approximately every 30 d thereafter.

Each sampling period began by measuring routine in-tank oxygen (O_2) consumption and nitrogenous (N) waste excretion (T_{Amm} + urea) rates in one tank per treatment simultaneously. Fish were fed at their regular times throughout the sampling period. To measure O_2 consumption, samples were taken every other hour over an 8-h period beginning at 0700 and ending at 1500 hours. The tank water and air supplies were closed, the water surface was sealed with a transparent lid fitted snugly to the tank walls, and magnetic drive pumps (Little Giant, 1-EUAA-MD) were activated to recirculate and mix the water. Water samples were taken every 10–20 min for a total of 30 min to 1 h. The partial pressure of O_2 (P_{O_2}) of the water samples was measured with a temperature-equilibrated Cameron O_2 electrode connected to a Cameron OM 200 oxygen meter. After the final P_{O_2} measurement, the water and air supplies were re-opened for 1 h prior to the next sampling period to allow flushing and re-aeration. The O_2 consumption rates for each closed sample period were calculated from the mean decline in P_{O_2} over time (10–20 min readings) and the water oxygen solubility coefficients of Boutilier et al. (1984). The P_{O_2} of the water in any one tank was not allowed to drop below 90 torr. Mean O_2 consumption rates were plotted for each closed cycle and the area under the curve determined with a digitizing tablet (Sigma Scan) to give a single integrated O_2 consumption rate representative of the 8-h interval between 0700 and 1500 hours.

Nitrogenous waste excretion was measured the next day. Tank flow and aeration were maintained. Approximately 50 mL of influent and effluent water were collected simultaneously every 4 h for 24 h, and the samples were frozen immediately for future analysis of total ammonia-N and urea-N. The area under the curve was determined as above. All O_2 consumption and N-waste excretion data were corrected for differences in fish size with the weight exponent 0.824 determined for rainbow trout by Cho (1990). The contribution of bacteria to in-tank routine O_2 consumption and N-waste excretion was not estimated during the experiment; however, an estimate ("worst case scenario") was obtained following the experiment. Then, 25-g rainbow trout (the approximate size of the fish after 60 d of exposure) held at 21°C (equivalent to the highest temperature reached in the ambient temperature treatments over summer) were removed from their tank (270 L) just after they had fed to satiation, and the rates of bacterial O_2 and T_{Amm} consumption were measured over 24 h. The contribution of bacterial respiration to O_2 consumption was determined to be no greater than 5%. The influence of bacteria on T_{Amm} consumption was negligible.

Following the in-tank measurements of routine O_2 consumption and N-waste excretion, food was withheld from the fish for the next 48 h, during which blood and tissue sampling were conducted. Ten fish from each tank were killed for whole-body proximate analysis (protein, lipid, carbohydrate, and ash) and blood ions (plasma T_{Amm} and sodium) and another 10 were sacrificed for the estimation of T_{Amm}, urea, and glutamine in white muscle and liver. The fish were sampled by netting a number of fish from which one was randomly taken; the procedure was repeated 10 times at 5-min intervals. With this approach, there was no effect of sample order on any of the measured

variables. For whole-body composition and blood sampling, the fish were killed by a quick blow to the head, blotted dry, and measured for wet weight and total length. A terminal blood sample was collected via caudal severance, the plasma was separated, and the carcass was freeze-clamped with aluminum tongs precooled in liquid N_2. Both plasma and carcass were stored at −70°C until further analysis.

The fish sampled for T_{Amm}, urea, and glutamine in white muscle and liver were handled in a similar manner, but were sacrificed in a bucket of water containing an overdose of tricaine (MS-222, 1 g/L) buffered with $NaHCO_3$ (2 g/L) to avoid T_{Amm} build-up in muscle due to struggling (Wang et al. 1994). The liver 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. Each tissue was individually wrapped in aluminum foil and stored at −70°C for further analysis.

Analysis.—Ammonia concentrations were determined in water by the salicylate–hypochlorite method of Verdouw et al. (1978) and in plasma by a commercial enzymatic kit (Sigma 170-UV). The two assays were cross-validated. Water urea samples were freeze-concentrated 5-fold by lyophilization before assay by the modification of the diacetyl monoxime method described by Lauff and Wood (1996). The concentration of Na^+ in water and plasma was determined by atomic absorption spectrophotometry (Varian AA 1275).

For the determination of proximate composition, frozen whole bodies kept at −70°C were ground into a fine powder with a grinding mill (IKA-M10/M20) cooled to −40°C by a methanol–dry ice mixture, and then the powder was lyophilized for 72 h at −55°C. A small portion of the frozen ground tissue was withheld and dried in an oven at 80°C for 48 h to obtain the water content. The protein content of the lyophilized tissue was measured by the Lowry method as modified by Miller (1959); glucose, glycogen, and lactate (carbohydrate) were determined as described by Bergmeyer (1985); and lipid, after extraction in a 2:1 chloroform:methanol mixture, was measured as described by Folch et al. (1957).

Frozen white muscle and liver were ground into a fine powder in an insulated mortar cooled with liquid N_2. Subsamples (100 mg) were deproteinized in 1 mL of 8% perchloric acid (PCA) and measured for ammonia as described by Kun and Kearney (1971). Similar deproteinizing procedures were used to measure white muscle and liver urea as described by Crocker (1967). Conversely, for glutamine measurement we used approximately 50 mg of frozen white muscle and liver in 250 μL of 3% PCA and assayed as described by Bergmeyer (1985).

Calculations.—Daily food intake (g) was determined as the difference in food bag weight at the beginning and end of feeding divided by the number of fish in the tank (grams per fish per day). Cumulative food intake was calculated as the sum of the daily food intakes over each 30-d period.

Specific growth rates (SGR, %/d) were determined for 30 fish removed from each tank for monthly sampling ($N = 60$ per treatment) and calculated by the standard formula

$$\mathrm{SGR} = 100(\log_e Y_2 - \log_e Y_1)/(t_2 - t_1),$$

where Y_1 and Y_2 are the mean wet weights of fish at times t_1 and t_2. Condition factors were determined as the quotient of the wet weight of the fish and its total length cubed, multiplied by 100.

The nitrogen 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 proportion of oxygen consumption dedicated to protein catabolism was measured as the ratio of the NQ at each respective time period to the maximum aerobic value (0.27; Kutty 1972).

The nitrogen budget followed the equation summarized by Birkett (1969):

$$I - F_N = A = R + E,$$

where I is the total N consumed, F_N is the N lost in fecal and unaccounted material (mucus, etc.), A is the N absorbed from food, R is the nitrogen retained in body materials, and E is the nitrogen lost via branchial and urinary excretion. Measurements of N consumption, retention, and excretion allowed for estimation of N lost through feces (and unaccounted N) and the N absorbed. Protein was measured as a surrogate for N; the percentage N by weight in the protein of the food consumed and in the protein of the fish was taken as the standard value, 16% (Jobling 1980; Soderberg 1995).

The energy budget was derived from the equation

$$C = F_E + U + \Delta B + R_{tot},$$

where C is the total energy consumed from food, F_E is the energy lost in feces, U is the energy lost via excretion (branchial and urinary), ΔB is the energy stored in body materials, and R_{tot} is the total metabolic energy lost as heat. The following

energy values were assumed: 23.6, 39.5, and 17.2 kJ/g for protein, lipid, and carbohydrate, respectively (Braefield and Llewellyn 1982), 24.9 kJ/g for nonfecal nitrogen (Cho and Kaushik 1990), and 13.6 kJ/g for O_2 (Elliott and Davison 1975).

A new and relatively simple index, the N-cost index (the total moles of O_2 consumed per mole of nitrogen stored), was used to compare the total metabolic expenditure associated with the incorporation of nitrogen into body material under the stated conditions.

Statistics.—The values for the nitrogen and energy budgets are reported on a per-fish basis from the one experimental tank in each treatment where O_2 consumption and N-waste excretion were measured. Because $N = 1$ for these parameters, no statistical comparisons could be employed. In addition, cumulative food consumption was calculated on a per-fish basis. Here, $N = 2$ (two tanks per treatment), and again, statistics were not employed. All other data are expressed as means ± 1 SE from individual samples pooled together from the two tanks per treatment. One-way analyses of variance with SAS JMP (SAS Institute Inc., Version 2.0.5) followed by Tukey–Kramer honestly significant difference multiple-means comparison tests were used to distinguish statistically significant differences among the four treatment groups within each sample period (30, 60, and 90 d, respectively). The level of statistical significance for all analyses was $P \leq 0.05$.

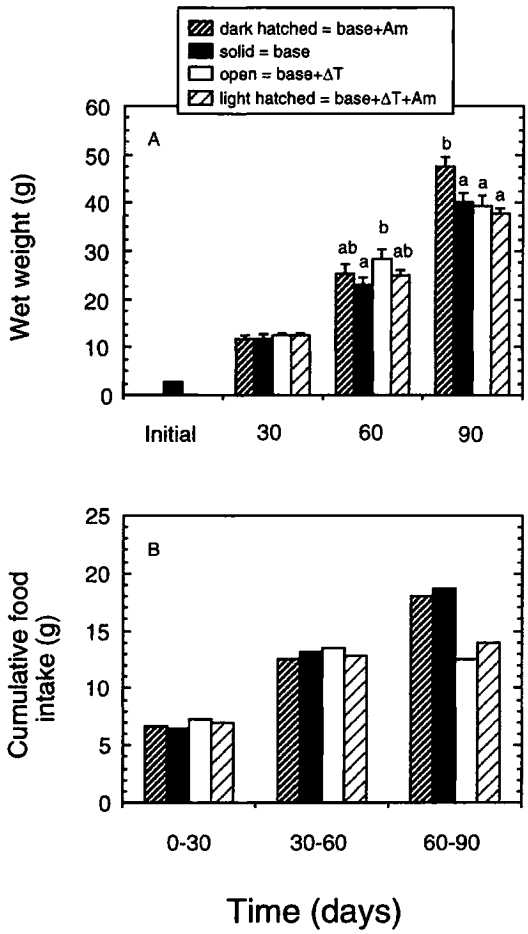

FIGURE 2.—Effects of +2°C (ΔT) and 70 μmol ammonia/L (Am) on (A) growth and (B) cumulative food intake of juvenile rainbow trout fed to satiation twice daily. Growth was measured as the wet weight of the fish sampled initially ($N = 20$) and after 30, 60, and 90 d of exposure ($N = 60$ per treatment per time period). Cumulative food intake is presented on a per-fish basis and represents the cumulative food eaten over the respective time periods; each value represents the mean of the two replicate tanks per treatment ($N = 2$). Columns with the same letter on the same sampling day are not significantly different ($P > 0.05$).

Results and Discussion

Physiological Responses Due to the Base Summer Thermal Regime

Juvenile rainbow trout (2–5 g) exposed to the natural, or base, thermal regime and fed to satiation twice a day for 90 d exhibited an overall specific growth rate of 3.02%/d. Despite several days of fluctuating water temperature about a mean of 20.5°C between days 60 and 90 (Figure 1), our fish continued to feed and grow with no apparent loss in appetite (Figure 2). Thus, maximum energy intake was greater than the maintenance energy used during this period. In contrast, Elliott (1982) showed that brown trout *Salmo trutta* do not grow at water temperatures above 19.5°C when fed maximum rations of *Tubifex* sp.

The metabolic cost associated with growth of our trout was high. Routine O_2 consumption by these fish (Figure 3A), after size correction (see methods), was approximately 75% of the maximum size-corrected O_2 consumption determined for juvenile rainbow trout held at 15°C in our laboratory (Wilson et al. 1994b). These high values were most likely associated with the high protein (50%) and lipid (15%) contents of their diet and with the intensive feeding regime (twice daily to satiation). Soofiani and Hawkins (1982) showed that for Atlantic Cod *Gadus morhua*, elevation in metabolic rate associated with feeding depends on ration size, increasing linearly as food intake increases. They also showed that the costs of food

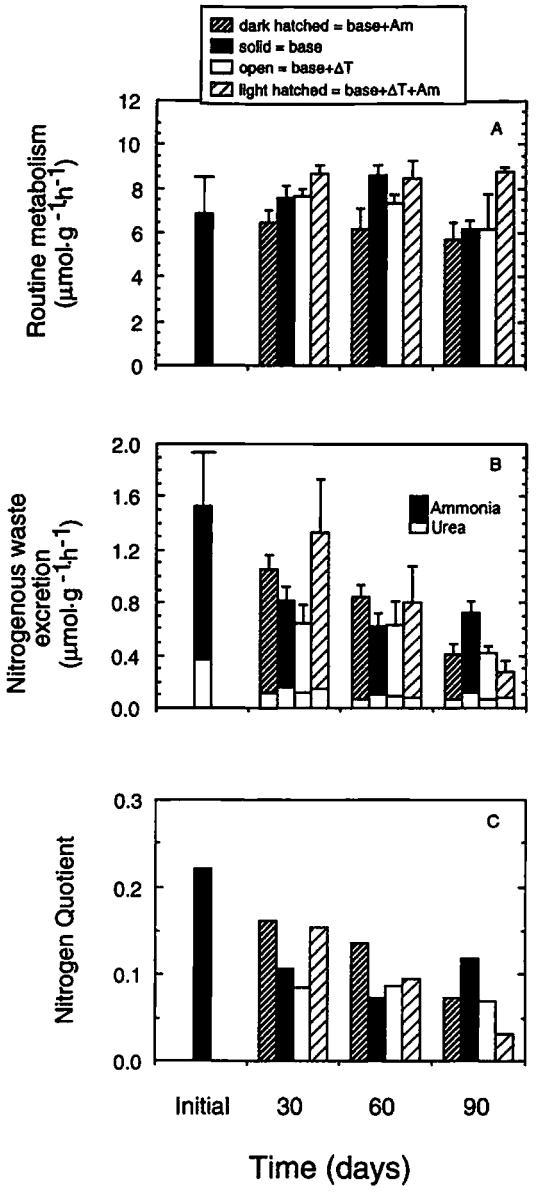

FIGURE 3.—Effects of +2°C (ΔT) and 70 μmol ammonia/L (Am) on (A) routine in-tank oxygen consumption (N = 5 measurements on the same tank), (B) routine in-tank N-waste excretion (ammonia + urea N; N = 6 measurements on the same tank), and (C) the nitrogen quotients of juvenile rainbow trout fed to satiation twice daily. In (A) and (B) the data have been scaled for fish weight. Measurements were made on one tank of individuals (N = 1) per treatment. Error bars represent measurement SE (of the mean O_2 consumption and N-waste excretion rates calculated over the respective sample intervals) and cannot be used for statistical comparisons between treatments.

assimilation were highest for fish fed to satiation and that the cost increases with increasing temperature.

The type of food and feeding regime also have considerable effect on the rate of N-waste excretion in fish. Wood (1995), in his review of the routes and rates of excretion in salmonids, concluded that the single most important factor affecting N-waste excretion is feeding. He reported that for rainbow trout fed to satiation, total N excretion rate generally ranges from 1.0 to 2.0 $\mu mol \cdot g^{-1} \cdot h^{-1}$ (0.6 to 1.4 when scaled for fish size), but pointed out from Fromm's (1963) data that it may drop to 0.5–1.0 $\mu mol \cdot g^{-1} \cdot h^{-1}$ (0.07–0.35) after 5 d of starvation. The scaled N-waste excretion rates in our satiation-fed fish dropped from 1.5 $\mu mol \cdot g^{-1} \cdot h^{-1}$ initially to 0.8 $\mu mol \cdot g^{-1} \cdot h^{-1}$ by day 90 (Figure 3B). Because appetite was not inhibited in this study, the decline in N-waste excretion rate may be the consequence of the metabolic partitioning of food energy into growth. For example, our results are consistent with the responses of other salmonids fed high-lipid diets; increased lipid in the diet acts to spare protein for growth (Atherton and Aitken 1970), thus reducing the amount of exogenous N-waste excretion (Jayaram and Beamish 1992; Agradi et al. 1995) and ultimately increasing the proportion of ingested N retained as growth (Beamish and Tandler 1990). This would explain the observed decrease in protein catabolism. Our fish showed 39–55% decreases in aerobic protein catabolism (NQ) between the initial value and those on days 30, 60, and 90 (Figure 3C), hence comparable reductions in the use of protein for energy.

Rainbow trout under base conditions showed large increases of whole-body lipid from initial values, indicating an excess of dietary energy beyond their needs for maintenance and metabolism, though lipid values declined slightly during high summer temperatures after day 60 (Table 2; Figure 1). During the 90-d exposure period, condition factors increased from 0.87 to 1.22 in accord with this observed increase in whole-body lipid level (Table 2). It is believed that deposition of energy into lipid may be one of the most critical components of fish survival (Adams and Breck 1990). Swift (1955) found that fish typically accumulate lipid in the summer and fall and use lipid in the winter when food consumption is minimal. Conversely, the percentage of whole-body carbohydrate (very small in comparison to protein and lipid) showed the opposite trend (Table 2). Carbohydrate did not change through day 30, but by

TABLE 2.—Proximate chemical composition and condition factors of juvenile rainbow trout fed to satiation and exposed to a +2°C warming scenario (ΔT) and 70 μmol ammonia/L (Am). Fish were sampled initially and after 30, 60, and 90 d of exposure. Results are expressed as means ± SE, based on wet weight. For each sampling day separately, values within a column without a letter in common are significantly different ($P \leq 0.05$). Sample sizes are in parentheses.

Sampling day and treatment	Protein (mg/100 mg)	Lipid (mg/100 mg)	Carbohydrate (mg/100 mg)	Condition factor
Initial	10.7 ± 0.3 (9)	4.7 ± 0.2 (10)	0.45 ± 0.03 (10)	0.87 ± 0.01 (20)
Day 30				
Base+Am	14.3 ± 0.2 z (20)	9.0 ± 0.2 z (20)	0.40 ± 0.02 z (20)	1.20 ± 0.01 z (20)
Base	13.1 ± 0.2 y (20)	8.4 ± 0.4 z (20)	0.43 ± 0.04 z (10)	1.17 ± 0.01 zy (20)
Base+ΔT	13.5 ± 0.2 y (20)	9.0 ± 0.3 z (20)	0.36 ± 0.03 zy (20)	1.18 ± 0.02 z (20)
Base+ΔT+Am	14.6 ± 0.3 z (19)	9.4 ± 0.2 z (18)	0.30 ± 0.02 y (19)	1.13 ± 0.01 y (20)
Day 60				
Base+Am	13.8 ± 0.2 z (20)	10.4 ± 0.2 zy (19)	0.20 ± 0.01 z (20)	1.11 ± 0.01 z (20)
Base	12.8 ± 0.3 zy (20)	9.4 ± 0.2 z (20)	0.20 ± 0.01 z (20)	1.14 ± 0.02 z (20)
Base+ΔT	12.2 ± 0.3 y (20)	10.8 ± 0.2 y (19)	0.25 ± 0.01 y (10)	1.12 ± 0.01 z (20)
Base+ΔT+Am	15.5 ± 0.6 x (20)	10.6 ± 0.4 y (20)	0.23 ± 0.02 zy (6)	1.12 ± 0.01 z (20)
Day 90				
Base+Am	15.5 ± 0.4 z (20)	8.8 ± 0.2 z (18)	0.35 ± 0.03 z (10)	1.24 ± 0.02 z (20)
Base	15.6 ± 0.4 z (19)	8.8 ± 0.3 z (19)	0.27 ± 0.01 y (13)	1.22 ± 0.02 z (20)
Base+ΔT	13.8 ± 0.3 y (15)	9.8 ± 0.3 y (16)	0.28 ± 0.02 y (4)	1.16 ± 0.02 z (20)
Base+ΔT+Am	15.0 ± 0.4 z (20)	10.5 ± 0.2 y (20)	0.25 ± 0.01 y (10)	1.19 ± 0.04 z (20)

day 60, when temperature had begin its summer increase (Figure 1), carbohydrate had decreased to less than half its the initial amount. Energy in excess of maintenance requirements can be stored as lipid or glycogen (Brett and Groves 1979; Wood 1993). However, during times of increased energy demand, such as stress, these energy stores are mobilized (Mayer et al. 1992).

Total ammonia levels were initially four times higher in liver than in white muscle (Figure 4A, 5A). However, over time, less ammonia was present in the liver and more glutamine accumulated there (Figure 5). These trends may have resulted from increased glutamine synthetase production and incorporation of ammonia into protein. Reid et al. (1995) reported an increase in liver protein synthesis rates over the duration of this experiment, the most dramatic increase occurring over the first 30 d.

Crude nitrogen and energy budgets were constructed to assess the costs of growth incurred under these conditions. Nitrogen retention efficiency for the 90-d period of growth between June and September was 25.9% (Table 3). This value is somewhat lower than those reported by Birkett (1969), who found that gross N conversion efficiency (synonymous with our N retention efficiency) ranged from 27.9 to 49% over three species. Our estimated fecal N losses, regarded as undigested or unabsorbed diet and all N not accounted for, are quite high (over 48%) (Table 3). Subsequent analysis of similar water samples in later experiments to estimate N-waste excretion by the Kjeldahl method (Skoog and West 1982) indicated that total N-waste excretion may be as much as 34% greater than we estimated from total ammonia and urea only. The missing nitrogenous waste components probably include mucoproteins and amino acids (Olson and Fromm 1971). Nevertheless, feeding to satiation may have also contributed to the low N absorption, because there was a strong correlation in the present study between the amount of N consumed and the amount of N lost in feces before the large temperature increase at day 60 ($r^2 = 0.92$; df = 7; $P = 0.0001$).

The energy budget tended to correlate with the

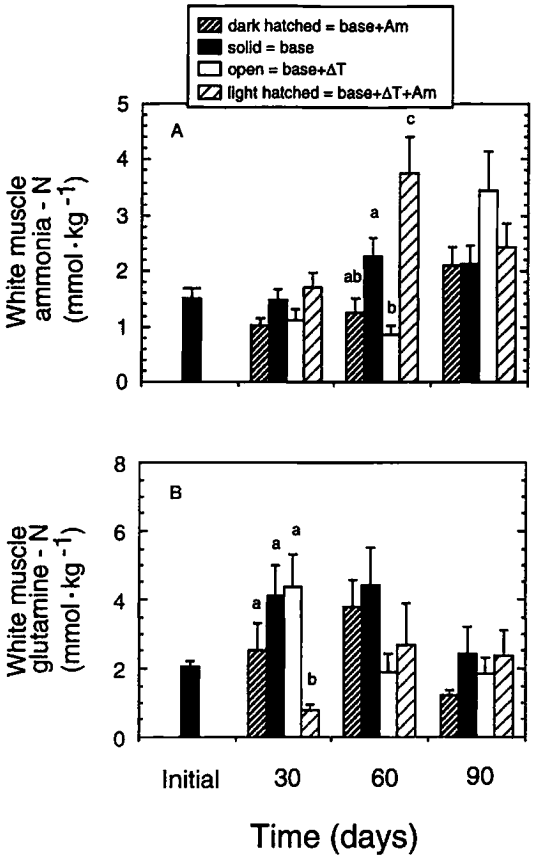

FIGURE 4.—Effects of +2°C (ΔT) and 70 μmol ammonia/L (Am) on (A) total ammonia (N = 6–20) and (B) glutamine (N = 5–10) concentrations in white muscle of juvenile rainbow trout fed to satiation twice daily. Data are means + SE. Columns with the same letter on the same sampling day are not significantly different (P > 0.05).

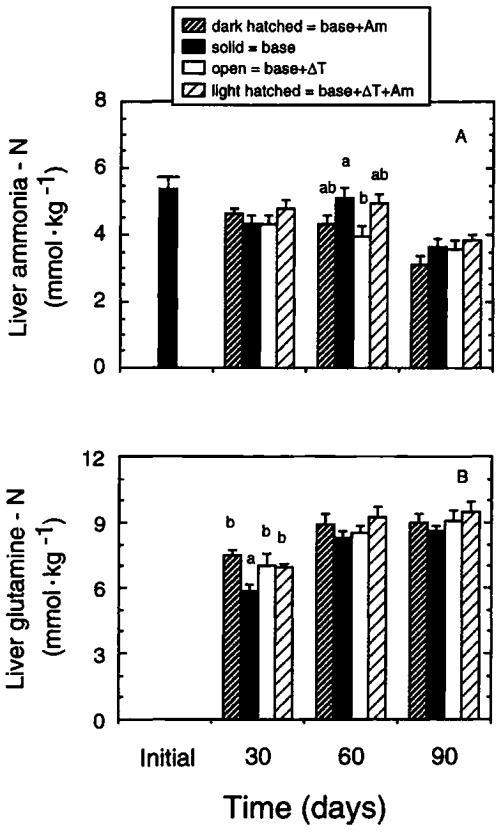

FIGURE 5.—Effects of +2°C (ΔT) and 70 μmol ammonia/L (Am) on (A) total ammonia (N = 6–20) and (B) glutamine (N = 5–10) concentrations in liver of juvenile rainbow trout fed to satiation twice daily. Data are means + SE. Columns with the same letter on the same sampling day are not significantly different (P > 0.05).

results of the nitrogen budget (Table 4). Gross conversion efficiency over the 90-d study period was 38% under base conditions. It was associated with high food intake, relatively high energy loss via excretion (F_E, 22.5%; U, 2.9%), and a high loss via heat (R, 36%). The results reported here are consistent with previous reports under comparable conditions (Brett and Groves 1979). Cho et al. (1982) report a conversion of energy into body materials of 46%, an energy loss in excreta of 23% (fecal, 15%; nonfecal 8%), and a final 30% loss via heat production. To summarize, for juvenile rainbow trout fed to satiation and growing under the base thermal regime, a relatively high proportion of gross energy intake was being stored in body materials (especially lipid) but at the expense of nitrogen retention efficiency and energy conversion efficiency. Their estimated cost of growth, or N-cost index, was 9.66 moles of O_2 consumed per mole of N stored.

Effects of an Additional 2°C

The high temperatures reached in the last 30 d of the experiment approached the upper thermal limit of juvenile rainbow trout, 26°C (Bidgood and Berst 1969; Kaya 1978). Despite the higher temperature, the 90-d growth rate of fish held at +2°C (3.01 ± 0.05 %/d) was nearly identical to that of the base temperature group. However, the growth rate of the base+ΔT fish was actually higher than that of base temperature fish during the first 60 d, before the sharp temperature increase, as indicated by the statistically significant increase in wet weight (Figure 2A), but lower during the last 30 d. The latter decline stemmed in part from a lev-

TABLE 3.—Nitrogen budgets for juvenile rainbow trout fed to satiation for 90 d and exposed to a +2°C warming scenario (ΔT) and 70 μmol ammonia/L (Am).

Interval and treatment	Nitrogen (mmol/fish)[a]					Retention efficiency[b] (%)	Absorption efficiency[c] (%)
	I	*E*	*R*	F_N	*A*		
Days 0–30							
Base+Am	34.9	14.0	15.5	5.5	29.5	44.4	84.3
Base	36.6	11.7	14.5	10.5	26.2	39.5	71.4
Base+ΔT	41.8	10.0	15.3	16.6	25.3	36.5	60.4
Base+ΔT+Am	40.4	16.4	17.5	6.5	33.9	43.4	83.8
Days 30–60							
Base+Am	61.9	23.6	17.6	20.8	41.2	28.3	66.5
Base	76.6	17.9	17.2	41.5	35.1	22.4	45.8
Base+ΔT	66.8	17.8	19.0	30.0	36.8	28.4	55.1
Base+ΔT+Am	74.8	26.2	12.7	35.9	38.9	16.9	52.0
Days 60–90							
Base+Am	84.8	24.7	48.5	40.4	73.2	57.2	86.4
Base	110.3	27.6	26.3	56.5	53.9	23.9	48.8
Base+ΔT	68.1	22.0	29.6	16.6	51.5	43.4	75.7
Base+ΔT+Am	83.1	20.4	21.9	40.9	42.3	26.3	50.9
Days 0–90							
Base+Am	181.7	62.3	81.6	37.8	143.9	44.9	79.2
Base	223.5	57.1	58.0	108.5	115.1	25.9	51.5
Base+ΔT	176.8	49.8	63.8	63.1	113.6	36.1	64.3
Base+ΔT+Am	198.3	63.0	52.1	83.3	115.0	26.3	58.0

[a] I = total N consumed; E = N lost via branchial and urinary excretion; R = N retained in body materials; F_N = N lost in fecal material and all unaccounted N; A = N absorbed from food.
[b] Retention efficiency = $100R/I$.
[c] Absorption efficiency = $100A/I$.

TABLE 4.—Energy budgets for juvenile rainbow trout fed to satiation for 90 d and exposed to a +2°C warming scenario (ΔT) and 70 μmol ammonia/L (Am).

Interval and treatment	Energy (kJ/fish)[a]					Conversion efficiency[b] (%)
	C	*U*	*ΔB*	*R*	F_E	
Days 0–30						
Base+Am	108.5	4.9	80.0	35.2	−11.6	73.8
Base	113.6	4.1	68.6	38.7	2.2	60.4
Base+ΔT	129.7	3.5	79.8	40.7	5.8	61.5
Base+ΔT+Am	125.3	5.7	86.0	44.7	−11.1	68.6
Days 30–60						
Base+Am	192.1	8.2	83.8	70.2	29.9	43.6
Base	237.7	6.2	94.6	93.5	43.3	39.8
Base+ΔT	207.4	6.2	105.9	90.8	4.4	51.1
Base+ΔT+Am	232.0	9.1	108.3	99.0	15.5	46.7
Days 60–90						
Base+Am	263.0	8.6	160.0	105.3	−10.8	60.8
Base	342.3	9.6	101.5	120.6	110.6	29.7
Base+ΔT	211.3	7.7	95.2	120.6	−12.2	45.1
Base+ΔT+Am	257.9	7.1	91.4	148.0	11.4	35.4
Days 0–90						
Base+Am	563.7	21.7	323.8	210.7	7.5	57.4
Base	693.6	19.9	264.7	252.8	156.1	38.2
Base+ΔT	548.4	17.4	280.9	252.0	−1.9	51.2
Base+ΔT+Am	615.2	22.0	285.7	291.8	15.8	46.4

[a] C = total energy consumed from food; U = energy lost via excretion (branchial and urinary); ΔB = energy stored in body materials; R = total metabolic energy lost as heat; F_E = energy lost in feces.
[b] Conversion efficiency = $100\Delta B/C$.

eling off of food intake (Figure 2B) and from a concomitant change in energy metabolism.

A 30% reduction in food intake (relative to base) during days 60-90 (mean temperature, 22°C) was perhaps the most significant effect of the 2°C warming on juvenile rainbow trout. This effect is not uncommon for salmonids; as water temperature nears the upper thermal tolerance of a species, food consumption declines precipitously (Jobling 1993). However, up until day 60, additional temperature had little effect on food consumption. Wurtsbaugh and Davis (1977) observed that for fish fed higher ration levels, elevated temperature up to 17°C increased growth rate, but that when the temperature reached 22.5°C in their summer experiment, the fish ate less than those at 19.5°C.

The additional 2°C did not affect routine O_2 consumption rates even when food intake was suppressed (Figure 3A). However, N-waste excretion was markedly reduced at this time (Figure 3B). Protein utilization depends on water temperature (Steffens 1981). Reid et al. (1995) reported a 20% decrease in protein turnover in these last 30 d and proposed that metabolic fuels (protein, lipid, and carbohydrate) may have been diverted from growth to supply energy for maintenance and other homeostatic processes. Because N-waste excretion was reduced at that time, protein probably was not a major energy source. Indeed, whole-body protein increased between days 60 and 90 (Table 2), suggesting that trout exposed to an additional 2°C retained more N for growth (Table 3). However, whole-body lipid decreased (Table 2) and may have been used to meet the increased energetic demands above 20°C, as argued earlier. We suggest that during the last 30 d, change in fuel use allowed the fish exposed +2°C to meet the increased maintenance costs associated with the higher water temperature. As a result, the overall cost associated with N gain in base+ΔT fish (N-cost index = 9.94 moles of O_2 consumed per mole of N stored) was similar to that of the base temperature group (9.66).

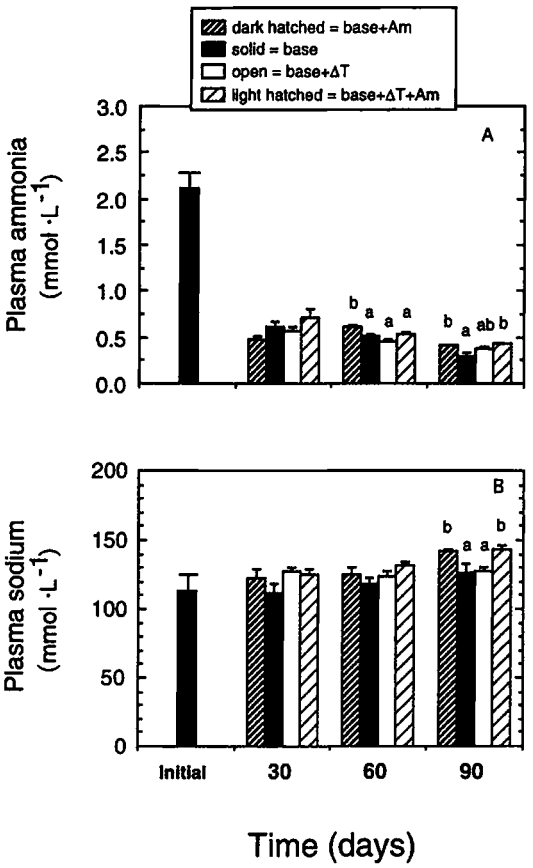

FIGURE 6.—Effects of +2°C (ΔT) and 70 μmol ammonia/L (Am) on (A) total ammonia (N = 10–20) and (B) Na^+ (N = 5–10) concentrations in blood plasma of juvenile rainbow trout fed to satiation twice daily. Data are means + SE. Columns with the same letter on the same sampling day are not significantly different ($P > 0.05$). The initial plasma ammonia value may be artificially high because the fish were small and sampling was invasive (caudal severance).

Effects of Elevated Ammonia

The physiological effects of low environmental ammonia are not well documented. The vast majority of studies concerning the chronic effects of ammonia on freshwater fish were conducted at levels considerably higher than the 70 μmol ammonia/L we used. Effects of sublethal ammonia toxicity on fish may include reduced growth (Robinette 1976; Rice and Bailey 1980; Beamish and Tandler 1990), reduced feeding (Beamish and Tandler 1990), gill damage (Smart 1976), and plasma ion disturbance (Buckley et al. 1979; Thurston et al. 1984; Twitchen and Eddy 1994). With respect to ion disturbance, fish exposed to ammonia in the present study not only exhibited significantly more plasma ammonia than base and base+ΔT fish by day 90, but significantly more plasma Na^+ as well (Figure 6). Although the mechanisms of branchial ammonia excretion in freshwater teleosts remain controversial (Randall and Wright 1987), two mechanisms are thought to be responsible for the majority of the ammonia excreted under most environmental conditions: (1) passive NH_3 diffusion along the partial pressure (P_{NH_3}) gradient from blood to water and (2) electroneutral exchange of

NH_4^+ for Na^+ (Wood 1993). Because the P_{NH_3} gradients calculated here for the ambient control and ammonia group at 14°C were 262 and 305 μtorr, respectively (based on measured total ammonia in plasma and water, measured water pH, arterial blood pH values from Randall and Cameron 1973, water and plasma p*K* values from Cameron and Heisler 1983, and equations from Wright and Wood 1985), and because a significant increase in plasma Na^+ concentration was observed over time in these fish, it is likely that both mechanisms contributed to the excretion of ammonia to water.

One effect that has not been reported previously, however, is the stimulation of fish growth by exposure to such a low level of ammonia. In the present study, juvenile rainbow trout exposed over summer to 70 μmol ammonia/L at base water temperature (base+Am) had significantly greater weight gain (Figure 2A), better N and energy conversion (Tables 3, 4), and higher N retention efficiency at a lower metabolic cost (Figure 3A) than ambient controls. The N-cost index for this group was only 6.58 moles of O_2 consumed per mole of N stored. Thus more N, in the form of protein, was being retained and put towards growth (Tables 2, 3). These results contrast with those of Beamish and Tandler (1990), who reported no change in either carcass protein or growth of juvenile lake trout *Salvelinus namaycush* exposed for 60 d to approximately 330 μmol total ammonia/L at 11°C. The increase in growth can not be explained by increased N consumption because ammonia-exposed fish ate no more food than controls (Figure 2B). Instead, the ammonia appears to have stimulated protein turnover (unpublished data from our laboratory), resulting in better N conversion efficiency. In just 30 d, hepatosomatic indices (liver weights as proportions of body weights) were elevated; by day 60, liver protein synthesis, degradation, and accretion rates were significantly increased. This increase in protein turnover may have been an indirect result of ammonia detoxification mechanisms acting in response to relatively higher levels of plasma ammonia (Figure 6A). For example, ammonia is incorporated into glutamine (Levi et al. 1974; Walton and Cowey 1977) and other amino acids (Iwata et al. 1981; Dabrowska and Wlasow 1986), which may in turn be used as substrates for protein synthesis, ultimately improving growth. Indeed, concentrations of glutamine in the livers of the ammonia-exposed fish appeared to be elevated in comparison to the base temperature group (Figure 5B).

The above arguments also hold true for those fish exposed to the ammonia at +2°C (base+ΔT+Am) up to day 30; beyond this period (above 16°C), the stimulatory effect of ammonia was lost and the combination became deleterious. Nitrogen retention and absorption efficiencies dropped sharply (Table 3) and the amount of energy lost as heat increased (*R*, Table 4) between days 30 and 90, which undoubtedly contributed to the high N-cost index (11.06 moles of O_2 consumed per mole of N stored) calculated for the 90 d of exposure. From these results, it is apparent that increased metabolic costs associated with both elevated temperature and ammonia detoxification were manifest in the high N-cost index estimated for these fish. We conclude that juvenile rainbow trout fed to satiation and exposed over summer to a simulated warming scenario of +2°C can make the necessary metabolic adjustments necessary to maintain growth. However, in the presence of sublethal ammonia, the cost of growth will increase and growth may be compromised.

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