Physiological Effects of Sublethal Acid Exposure in Juvenile Rainbow Trout on a Limited or Unlimited Ration during a Simulated Global Warming Scenario

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ABSTRACT

Changes in the physiology and cost of living of fish were studied during exposure to simulated global warming and environmental acidification, alone and in combination. Trout were exposed to slightly elevated water temperatures (+2°C), in the presence and absence of sublethal acidity (pH 5.2) in synthetic softwater for 90 d (8°–12°C). Fish were either fed to satiation (ca. 1%–3% of their wet-body weight daily) or fed 1% of their wet-body weight once every 4 d. Satiation-fed fish exposed to sublethal pH showed no ionoregulatory disturbances but exhibited increased appetites and growth compared to fish in control pH waters. In contrast, fish maintained on a limited ration did not grow and showed typical ionoregulatory responses to acid stress, with lower whole-body Na^+ and Cl^- concentrations and greater mortality. Detrimental effects were greater in the global warming scenario (+2°C). Overall, a slight temperature increase and sublethal pH increased the cost of living as determined by increased food consumption in satiation-fed fish and greater mortalities in fish maintained on a limited ration. Most important, these findings suggest that fish given sufficient food can compensate for increased energy expenditure or difficulties in maintaining ion balance associated with low pH exposure.

Introduction

Large numbers of softwater lakes in North America and Europe are affected by acidic precipitation from sulphur dioxide and nitrogen oxide emissions (Kelso et al. 1990; Kemp 1994). Field studies have shown that low pH increases fish mortality, decreases species diversity, and reduces reproductive success and rates of growth (Beamish 1974*b;* Beamish et al. 1975; Scheider et al. 1979; Lacroix and Townsend 1987).

Understanding the mechanisms of toxicity associated with environmental acid exposure is best achieved through laboratory experiments designed to simulate field conditions. Acute exposure to environmentally representative pHs (4.0–5.8) results in a disturbance to ionoregulation due to inhibition of active salt uptake because of competition between H^+ and Na^+ and stimulated diffusive ion losses (reviewed by Wood [1989]; Reid [1995]). Disruption of branchial ion transport probably accounts for the decrease in whole-body ions seen in fish after chronic exposure to low pH (Lacroix 1985; Neville 1985; Audet et al. 1988; Booth et al. 1988). However, in recent studies by Wilson et al. (1994*a,* 1994*b*) and Dockray et al. (1996), in which fish were fed to satiation during exposure to pH 5.2 for extended time periods, no decreases in whole-body ion concentrations were reported. These results suggest that the uptake of dietary salts may compensate for branchial ion losses incurred during low pH exposure. Further support for this hypothesis comes from Sadler and Lynam's work (1986) on brown trout, *Salmo trutta.* They demonstrated decreased growth, decreased mineral content, and lower plasma chloride, muscle water, and potassium and sodium content in acid-exposed starved fish, but no significant changes in these parameters in acid-exposed fed fish. Previous work has also revealed that the susceptibility of largemouth bass to low pH may change depending on the amount they are fed (Kwain et al. 1984; Leino and McCormick 1992). These results highlight the need to examine the role of nutrition in the response of rainbow trout to low pH.

In addition to the acidic precipitation problem, the Intergovernmental Panel on Climate Change (1995) estimated an increase in global temperature of 1.8°C by the year 2030, with larger temperature changes occurring in winter months (Mohnen and Wang 1992). This global warming may have a significant impact on fish, as they are poikilothermic, especially if food availability is also affected. Dockray et al. (1996) and Reid et al. (1997) investigated the cost of living for rainbow trout in waters of low pH and slightly elevated temperatures (+2°C) during summer when temperatures reached near lethal levels (26.2°C; Elliott 1982). Parameters such as oxygen consumption

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($\dot{M}_{O_2}$), nitrogenous waste excretion ($\dot{M}_N$), protein synthesis rates, appetite, growth, and partitioning of food energy were used as indicators of metabolic cost, while indicators such as whole-body and plasma ions were measured to determine physiological effects. Overall, exposure to low pH results in increased energy intake and weight gain and better conversion efficiency, while a temperature increase of +2°C reduces gross energy intake and increases fecal energy losses. These studies were unusual as they followed the natural, fluctuating thermal profile for inshore Lake Ontario during the summer of 1993, creating ecologically relevant studies (Reid et al. 1995). However, winter months are of particular interest in the study of environmental acidity in Ontario. The buildup of acidic snow, which then melts, causes between 36% and 77% of the annual acid export from watersheds to lakes (Semkin and Jeffries 1988; Kelso et al. 1990).

The purpose of this study was to examine the effects of two rations (satiation and limited ration) on the physiological and metabolic response of rainbow trout during chronic exposure (90 d) to low pH (5.2) at relatively low water temperatures (8°–12°C). The second, equally important purpose was to determine the effects of a slight temperature increase (+2°C) on the cost of living alone and in the presence of low pH. Methods similar to Dockray et al. (1996) were employed, with the additional measurement of plasma cortisol to monitor the stress response of fish to these warmer, acidified waters.

Material and Methods

Two 90-d exposures, both in synthetic softwater, were conducted from January to April, during the winters of 1994 and 1996. The 1994 experiment will be referred to as the "satiation exposure," and the 1996 experiment will be referred to as the "limited-ration exposure," in reference to the different feeding regimes. Methods used were similar to those described by Dockray et al. (1996) and are summarized with any deviations from this method below.

Pre-exposure Holding

Juvenile rainbow trout (*Oncorhynchus mykiss;* 7–15 g) were obtained from local hatcheries. On arrival, fish were held in two 400-L polypropylene tanks that were supplied with Hamilton dechlorinated tap water (Ca^{2+} = 0.98 ± 0.11 mmol L^{-1}, Na^+ = 0.56 ± 0.03 mmol L^{-1}, pH 7.6–8.0). They were fed dry trout pellets (Salmon Starter no. 3, Zeigler, Hazelton, Pa.) at a ration of 1% of their wet-body weight daily. This food was used for both subsequent experiments. After 3 wk, fish were acclimated to synthetic softwater by slowly increasing the amount of softwater to the tank and decreasing the amount of tap water. After experimental water chemistry conditions were achieved (Ca^{2+} = 0.025 ± 0.002 mmol L^{-1}, Na^+ = 0.076 ± 0.007 mmol L^{-1}, pH 6.1–6.2), fish were held for another 3 wk before the experiments were started. Softwater was obtained by passing Hamilton tap water, which closely follows the thermal fluctuations of inshore Lake Ontario, through a reverse osmosis unit (Anderson Water Conditioning Equipment, Dundas, Ontario). Photoperiod mimicked the natural photoperiod throughout acclimation and exposures.

Exposure System and Experimental Design

The synthetic softwater flow (~pH 6.1) was subdivided into two head tanks, with half of the water being gravity-fed through a heat exchanger that increased the temperature by 2°C. Water was further subdivided, with H_2SO_4 (0.2 N) titrated to the control and the warmer water, which resulted in four treatments: control temperature and sublethal pH; control temperature and control pH; control temperature plus 2°C and control pH; and control temperature plus 2°C and sublethal pH. Two 205-L polypropylene tanks per treatment received water at an average rate of 0.8 L min^{-1}. Sublethal pH (5.2) was maintained by pH statting; pH in the treatment tanks was continually monitored by an industrial pH electrode system (Leeds and Northrup Meridian II combination industrial electrode, Mississauga, Ontario), which controlled a solenoid valve (Cole Parmer Instrument Co., CP#01367-70, Niles, Ill.) that opened and closed to deliver H_2SO_4 to the head tank. Low pH and the 2°C temperature elevation were monitored via a Ladder Logic and Texas PLX Model TI315 programmable controller that was connected to an alarm and an automatic message dialer system (Safe House Model 49-433A, Taiwan, China; see Dockray et al. [1996] for a more detailed description). Vigorous aeration maintained the partial pressure of oxygen (P_{O_2}) above 120 Torr (where 1 Torr = 133.322 Pa). Temperature and pH were measured daily with independent probes, and [Na^+] and [Ca^{2+}] were monitored weekly using atomic absorption spectroscopy (Varian AA-1275, Mississauga, Ontario).

In the satiation-exposure experiment, approximately 142 fish were placed in each tank; thus, 284 fish were exposed to each of the four treatments. In the limited-ration-exposure experiment, 85 fish were placed in each tank; consequently, 170 fish were exposed to each of the four treatments.

Feeding Regimes

In the satiation-exposure experiment, fish were fed by hand to satiation twice daily (0830 and 1630 hours) as described by Wilson et al. (1994*a*), and appetite was monitored by weighing bags of the Zeigler diet before and after feeding. Fecal matter was removed daily, and tanks were cleaned weekly. During the limited-ration-exposure experiment, fish were fed 1% of their wet-body weight once every 4 d (~0.25% daily ration). Pellets were spread over the water surface to allow all fish access to food. This regime was chosen in order to minimize formation of a social hierarchy (McCarthy et al. 1993). Gross conversion

efficiencies were calculated as the wet weight of food eaten divided by the wet weight gained over the 90-d exposure.

Physiological Measurements

Sampling Protocol. During the satiation-exposure experiment, sampling and physiological measurements ($\dot{M}O_2$, $\dot{M}N$, blood and plasma composition, proximate composition, and whole-body ion analysis) were conducted over three 4-d periods, ending on days 0, 75, and 90. In the limited-ration exposure, fish sampling and physiological measurements were conducted over four 3-d periods ending on days 0, 30, 60, and 90. $\dot{M}O_2$ and $\dot{M}N$ measurements were made on days 0, 30, 60, and 90 for both exposures.

Growth. Whole-tank biomass was determined weekly in the satiation-exposure experiment and monthly in the limited-ration-exposure experiment. Fish were quickly netted en masse into a 10-L bucket that was lined with a plastic sieve and filled with water of the appropriate pH and temperature. The bucket and contents were weighed on a balance (GSE 450 Scale Systems, Farmington Hills, Mich.), and then the sieve and fish were lifted free of the bucket, and the fish were replaced in the appropriate tank. The bucket, sieve, and water were then reweighed. The difference in weight was the whole-tank biomass.

In addition, as there is more interindividual variability in growth when food intake is restricted (McCarthy et al. 1993), individual fish ($n = 24$ per treatment) were followed throughout the limited-ration exposure. Before the exposure began, fish were chosen using a random-number table, lightly anesthetized with MS222, which was neutralized with $NaHCO_3$, and then marked with a unique identifier using a Pan-jet needleless injector (Wright Dental Group, Dundee, Scotland). Weight and fork length were measured. The number of marked fish declined, to as low as eight individuals, over the 90 d because of mortality and loss of identifiable marking in some treatments.

Condition factor (CF), a morphological measure of health, was calculated using the weights and lengths of the individual fish sampled at each period, with the equation: $CF = 100 \times [\text{body weight (g)}]/[\text{total length (cm)}]^3$. Specific growth rate (SGR) was estimated using the equation: $SGR = 100 \times (\ln wt_{90} - \ln wt_0)/90$, where 90 represents the number of exposure days, wt_{90} is the weight at day 90, and wt_0 is the weight at day 0. Average weights of the fish killed on days 0 and 90 were used in this calculation for the satiation-exposure experiment. In the limited-ration-exposure experiment, the specific growth rate calculation used the individual weights of marked fish followed throughout the 90 d.

Metabolic Rates. All $\dot{M}O_2$ and $\dot{M}N$ data were corrected for fish size differences with the weight exponent 0.824, determined for rainbow trout by Cho (1992).

In the satiation-exposure experiment, $\dot{M}O_2$ was determined with closed-system respirometry every second hour over a 10-h period from 0700 to 1700 hours. Fecal matter was siphoned from the tanks. Each tank was then sealed with an air-tight, transparent lid, and water within the tank was recirculated with a submersible pump. Water samples were taken every 20 min for an hour, and then water flow and aeration were resumed for 1 h. PO_2 was measured with a thermostatted oxygen electrode (Radiometer E5046, Copenhagen, Denmark) and oxygen meter (Cameron Instrument Company, Port Aransas, Tex.). The rate of oxygen depletion was determined for each hour with oxygen solubility coefficients from Boutilier et al. (1984), factored by time, volume, and total fish weight to yield $\dot{M}O_2$. Mean $\dot{M}O_2$ values were graphed against time, and the curve produced was integrated for each treatment to give an overall mean $\dot{M}O_2$ value for that 10-h period.

During the limited-ration-exposure experiment, $\dot{M}O_2$ and $\dot{M}N$ were determined simultaneously over 48 h with the same methods. The measurements made in the first 24 h were of fish that had not been fed for 3 d, while measurements in the second half covered a 24-h period starting 1 h after fish had finished eating. These two different measurement regimes were used in these two exposures to best capture the different daily patterns of $\dot{M}O_2$ resulting from feeding regimes.

In the satiation-exposure experiment, in-tank $\dot{M}N$ was measured over 24 h, using six 1-h periods. Water flow to the tank was discontinued and samples were taken at the beginning and end of the 1-h periods. Flow to the tank was then resumed during the following 3 h. Water samples were frozen at −20°C for later analysis of ammonia-N by the salicylate-hypochlorite assay (Verdouw et al. 1978) and urea-N by the diacetyl monoxime method, after a fivefold concentration of the water sample by lyophilization (Lauff and Wood 1996). $\dot{M}N$ was determined by the difference between nitrogen concentration at the beginning and end of the flux period and was factored by time, volume, and total fish weight. Data over the 24-h sampling period were graphed against time, and the area under the curve was determined for each treatment to give a 24-h $\dot{M}N$.

In the limited-ration-exposure experiment, similar methods were used to determine $\dot{M}N$. Owing to the smaller biomass in this experiment, six 3-h sample periods were measured over the 48-h period. Again, this encompassed the two different stages in the feeding regime.

Blank trials to assess the importance of microbial processes in altering the measured whole-tank $\dot{M}O_2$ and $\dot{M}N$ values were not performed in the present study, because in two other laboratory studies conducted at the same time (Alsop and Wood 1997; Linton et al. 1997), under virtually identical conditions, these effects were shown to be negligible. In brief, these tests consisted of "worst case scenarios" in which tanks of trout of comparable size and density were fed to satiation for several days. Fish were removed from the tanks immediately after the final feeding, and the blank rate of $\dot{M}O_2$ and $\dot{M}N$ were measured

on the empty tank containing feces and food remnants over the following 24 h. These trials were conducted at several different temperatures encompassing the range used in the present study. The microbial contribution to $\dot{M}_{O_2}$ amounted to no more than 5% of the total, and the microbial influence on $\dot{M}_N$ was essentially zero. These values were considered within the error of the measurements, and therefore blank corrections were not applied.

Fractional protein utilization was determined for each treatment at each sampling period. This is an index of the fraction of $\dot{M}_{O_2}$ that is supported by protein metabolism, that is, the degree to which the fish depend on protein as a fuel source. Fractional protein utilization is calculated by dividing the nitrogen quotient (the ratio of the moles of nitrogen produced to moles of oxygen consumed) by the theoretical maximum nitrogen quotient in which protein supports all aerobic metabolism. This theoretical maximum for fish has been determined by Kutty (1972) to be 0.27.

Whole-Body and Blood Sampling. In the satiation-exposure experiment, 20 fish were randomly chosen from each treatment tank on each sampling day. Ten fish were rapidly killed by a blow to the head and blotted dry, and weight and length were measured. Blood was collected into ammonium heparinized capillary tubes after caudal severance. Hematocrit was determined following centrifugation at 10,000 *g*, and plasma protein was measured with a hand-held refractometer (American Optical, St. Louis, Mo.). The remaining plasma was frozen at −70°C for subsequent analysis of plasma $[Na^+]$, $[Cl^-]$, and cortisol. The other 10 fish were killed with a lethal dose of MS222. These fish were immediately freeze-clamped with aluminum tongs that were chilled in liquid nitrogen and then stored at −70°C.

During the limited-ration-exposure experiment, 10 fish at each sampling period were rapidly killed by a blow to the head; plasma was taken and then the bodies were freeze-clamped as described above. Fish with unique identifiers were not killed before day 90. To ensure that the marked fish were representative of fish in the tank, all fish were handled during the measuring of the marked fish.

Sample Processing

Whole bodies were ground frozen using a grinding mill (IKA, M10/M20, Staufen, Germany) cooled to −70°C with dry ice and methanol. Approximately 2 g of this ground tissue was oven dried at 80°C until a constant weight was achieved, to determine water content. The remaining frozen tissue was lyophilized (Labconco Lyph-Lock 6, Kansas City, Mo.) and stored desiccated at −20°C for proximate analysis. Whole-body protein, lipids, and carbohydrates were quantified on the freeze-dried tissue. Protein was measured by the Lowry assay as modified by Miller (1959), lipids were measured by the chloroform/methanol extraction method (Folch et al. 1957), and standard enzymatic analyses (Bergmeyer 1985) were used to measure glucose, glycogen, and lactate. The sum of the latter three was taken as an estimate of total carbohydrates. Whole-body ions were determined from lyophilized tissue that was digested in 8% perchloric acid at a 9 : 1 ratio. Whole-body $[Na^+]$, $[Ca^{2+}]$, and $[K^+]$ and plasma $[Na^+]$ were then determined by atomic absorption spectroscopy. Whole-body and plasma $[Cl^-]$ were determined using coulometric titration (Radiometer CMT10, Copenhagen, Denmark). Cortisol was measured using an ^{125}I Radioimmunoassay (ICN Biomedicals, Costa Mesa, Calif.).

Statistical Analysis

Values are given as the mean ±SEM (n = number of fish). There were no significant differences between replicate tanks, and thus data were combined in all analyses. Mean values were compared using one-way ANOVA (SAS JMP Version 2.0.5); when the F-value indicated significance, a Tukey-Kramer comparison of all pairs was used to determine treatment differences within a sampling period. Statistical comparisons were not performed for the metabolic rate measurements ($\dot{M}_{O_2}$ and $\dot{M}_N$) because only whole-tank measurements were made for each treatment. In these data, the reported SEMs were for repeated measurements of the same tank. To determine significant differences between the satiation and limited-ration exposures, an unpaired Student's t-test was used to compare the day-90 data of the same treatment groups. A chi-square test for distribution was used to determine whether there were any significant differences in mortality among treatments. The level of significance chosen was $P < 0.05$.

Results

Temperature Profile and pH

The average low pH regime was 5.14 ± 0.03 for the satiation exposure and 5.27 ± 0.02 for the limited-ration exposure, while the control pH was 6.10 ± 0.03 for both exposures. As the exposures deliberately used water that followed the natural thermal fluctuations of inshore Lake Ontario, and these varied in the two years, the temperature regimes were slightly different. In the limited-ration-exposure experiment, temperatures were about 2°C higher than in the satiation exposure. However, temperature profiles in the two exposures were similar, with temperatures remaining relatively constant from day 0 to day 75 and then increasing steadily until day 90 (Fig. 1).

Mortalities

During the satiation-exposure experiment, less than 10% of fish died from treatment effects (data not shown). However, in

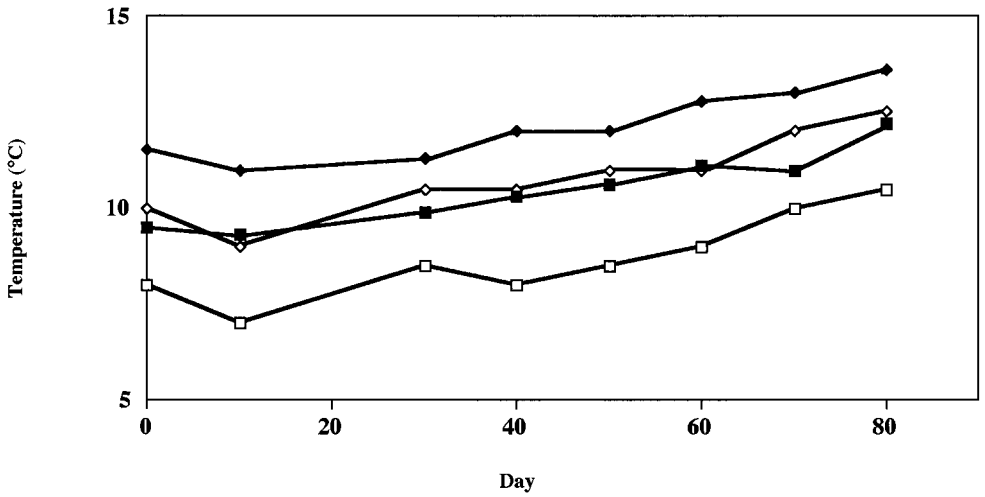

Figure 1. Water temperatures experienced by juvenile rainbow trout over the 90-d exposure periods. Control temperatures gradually increased from 8° to 12°C, with a more rapid increase in water temperatures between days 75 and 90. Open squares represent the control temperature, and solid squares represent the +2°C temperature elevation, for the satiation exposure (January to April, 1994). Open diamonds represent the control temperature, and solid diamonds represent the +2°C temperature elevation, for the limited-ration-exposure experiment (January to April, 1996).

the limited-ration-exposure experiment, significant mortality related to treatment was seen. Mortality occurred in the acid-exposed fish between days 50 and 90; cumulative mortality was 34% in the control temperature and sublethal pH treatment and 39% in the +2°C and sublethal pH treatment. These values were significantly greater than for the fish at circumneutral pH (Fig. 2).

Appetite, Feeding, Growth, and Condition Factor

Satiation Exposure. In this experiment, fish in the +2°C treatments consumed 1.5%–3% of their body weight in food daily, while fish at the control temperatures consumed 1%–2% of their wet-body weight daily. Acid-exposed fish had a slightly increased appetite compared with their respective controls. For example, the +2°C and sublethal pH treated fish consumed 29.7 g of food per fish by the end of the exposure, while the +2°C and control pH treated fish consumed only 23.2 g per fish (Fig. 3*A*). Increased food consumption was accompanied by increased growth in all treatment groups (Table 1). Specific growth rate was greatest in the fish exposed to +2°C and sublethal pH treatment at 2.07% d^{-1} (Table 1). Fish exposed to the +2°C treatments gained significantly more weight than fish at control temperatures (Fig. 3*C*) and utilized their food more efficiently (Table 2). Throughout the 90 d, gross conversion efficiency was slightly higher in the +2°C treatments, and at this elevated temperature, acid-exposed fish used their food slightly more efficiently than non-acid-exposed fish. This same pattern was not repeated in fish at control temperatures. By day 90, the fish in the +2°C and sublethal pH treatment had the highest weight. This was significantly different from those of the control temperature treatments, but not statistically different from the 2°C and control pH treatment (Fig. 3*C*).

Limited-Ration Exposure. In this experiment, in which fish were fed only approximately 0.25% of their body weight per day, fish at control temperatures were fed approximately 85% less food than fish in the same treatments in the satiation-exposure experiment, while fish in the +2°C treatments received approximately 95% less food than their respective treatments in the satiation-exposure experiment (Fig. 3*B*). Fish fed the limited ration had significantly lower condition factor and specific growth rates than did the fish in the satiation-exposure experiment, independent of treatment. Fish maintained on this limited ration had relatively constant weights (Fig. 3*D*) and no significant differences in specific growth rates (Table 1). All fish were in a catabolic state from days 0 to 30 and 60 to 90 (Table 2).

Plasma

Satiation Exposure. Plasma [Na^+] and [Cl^-] showed only a few treatment-related changes (Table 3). On day 75, the control temperature and sublethal pH treated fish had a higher plasma [Na^+] compared with the fish in the control temperature, and control pH treatment, and by day 90, +2°C and sublethal pH fish had a lower plasma [Na^+] than fish from the control temperature treatment, though this was not statistically different from the other two treatments. Plasma [Cl^-] was significantly higher in the control temperature and sublethal pH treatment compared to both control pH treatments. Hematocrit fluctuated, with transient differences at day 75. By day 90, hematocrit in the +2°C and control pH treatment was higher than in the control temperature treatments. However, this differ-

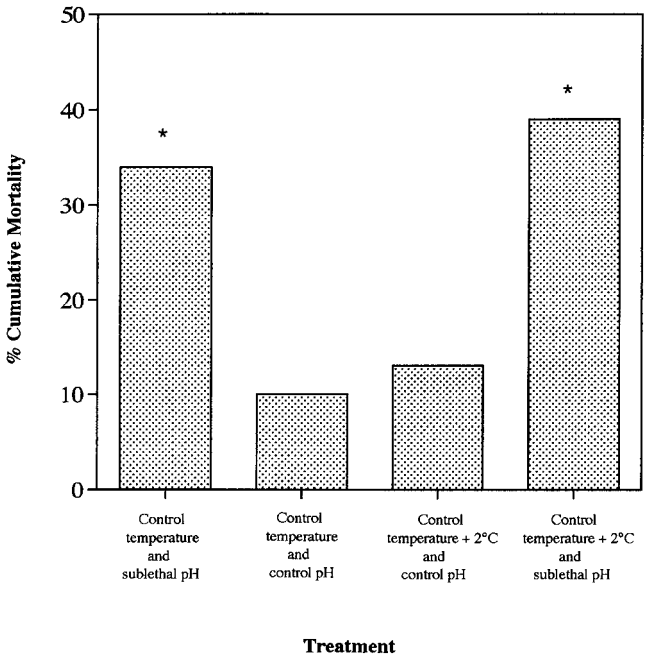

Figure 2. Cumulative mortality for the limited-ration-exposure experiment over the 90 d. Based on a chi-square analysis, the acid-exposed fish have a significantly higher mortality, denoted by an asterisk.

ence between temperature treatments did not prevail in the +2°C and sublethal pH group, which was no different from any of other three treatments. Plasma protein showed no changes over the exposure in any treatments (Table 3). Cortisol, as determined by radioimmunoassay, was high in the day-0 sampling, but this may have been an artifact of sampling such small fish (Fig. 4*A*). By day 75, values were approximately 4–7 ng mL^{-1} and increased by day 90 to approximately 8–11 ng mL^{-1}.

Limited-Ration Exposure. Plasma $[Na^+]$ and $[Cl^-]$ showed a number of transitory differences, but one trend that continued throughout the exposure was the significantly lower plasma ion concentrations in the +2°C and sublethal pH treatment (e.g., plasma $[Na^+] \cong 108$ mmol L^{-1} and plasma $[Cl^-] \cong 107$ mmol L^{-1} at day 90; see Table 3). Hematocrit and plasma protein showed a number of transitory differences (Table 3), and on day 90, the +2°C and sublethal pH treated fish had elevated levels compared with the other treatments. Cortisol levels at day 30 increased from day 0 and then surged at day 60, correlating with the increased rate of mortality. By day 90, cortisol levels had decreased from day 60 values but were still elevated, with the highest level in the +2°C and sublethal pH treatment (Fig. 4*B*).

Satiation and Limited-Ration Exposure. When comparing treatment groups between the two ration regimes on day 90 (Table 3), fish maintained on the limited diet at pH 6.1 had a higher plasma $[Na^+]$ than fish in the satiation-exposure experiment at pH 6.1. In contrast, the +2°C and sublethal pH limited-ration fish had lower ion levels than their satiation-fed counterparts. However, there were no significant differences between the control temperature and sublethal pH treatment groups for plasma $[Na^+]$ and $[Cl^-]$ based on feeding regime. This did not hold for plasma $[Cl^-]$ for fish at pH 6.1. Satiated fish in the +2°C and control pH treatment had a higher chloride level than fish in the limited-ration-exposure experiment, but this difference was not seen at control temperatures. Hematocrit was significantly higher in the satiation-exposure experiment, except between the +2°C and sublethal pH treatments, in which values for the limited-ration-exposure experiment were higher than those for the satiation-exposure experiment. Plasma protein was significantly higher, approximately twofold, in all satiation-fed fish compared with those in the limited-ration-exposure experiment, while cortisol levels were much higher, approximately sixfold, in the limited-ration-exposure experiment (Fig. 4).

Whole-Body Ions and Whole-Body Analysis

Satiation Exposure. Whole-body ions, $[Na^+]$, $[Cl^-]$, and $[K^+]$ did not show any variation among treatments (Fig. 5), except for modest differences in whole-body $[Cl^-]$ on day 75 (Fig.

Table 1: Condition factor (after 90 d of exposure) and specific growth rate over 90 d in each treatment

	Condition Factor	Specific Growth Rate (% d^{-1})
Satiation-exposure experiment:		
Control temperature and control pH	1.05 ± .01[a]	1.27
Control temperature and sublethal pH	1.05 ± .01[a]	1.25
+2°C and control pH	1.08 ± .01[a,b]	1.78
+2°C and sublethal pH	1.12 ± .01[b]	2.07
Limited-ration experiment:		
Control temperature and control pH	.87 ± .02	.03 ± .1
Control temperature and sublethal pH	.91 ± .09	.02 ± .1
+2°C and control pH	.79 ± .02	−.22 ± .1
+2°C and sublethal pH	.77 ± .02	−.17 ± .1

Note. Specific growth rates for the satiation-exposure experiment were calculated using the average weights for 30 fish at day 0 and 60 fish at day 90 and therefore could not be evaluated statistically. Specific growth rates for the limited-ration-exposure experiment were calculated using the individually marked fish ($n = 8–24$) that were monitored throughout the 90-d exposure. No significant differences for specific growth rates occurred within the limited-ration-exposure experiment. Significant differences ($P < 0.05$) only occurred for condition factor in the satiation-exposure experiment; these are indicated by treatment groups that do not share a letter.

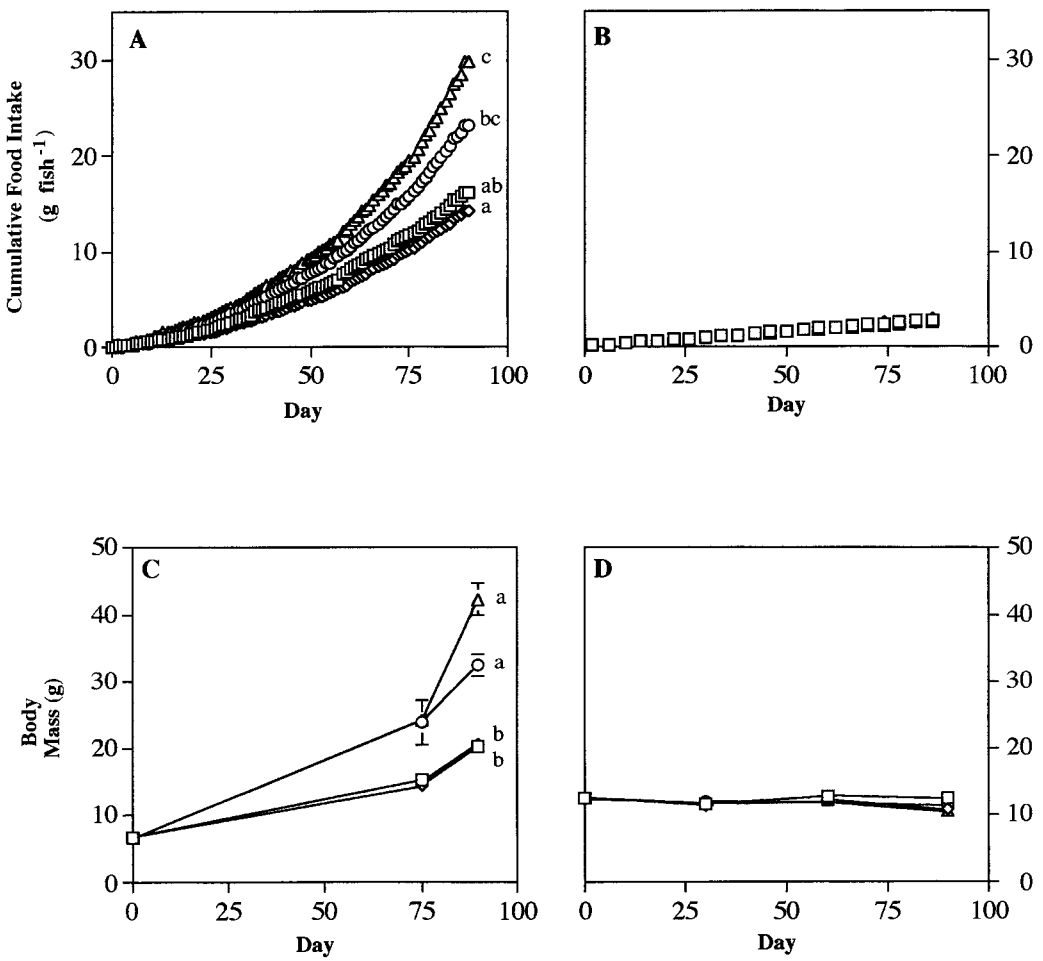

Figure 3. Cumulative food intake, expressed on a wet-weight basis (g per fish) in the satiation-exposure experiment (*A*) and the limited-ration-exposure experiment (*B*), and absolute growth expressed as wet-body mass (g) in the satiation-exposure experiment (*C*) and the limited-ration-exposure experiment (*D*). *Squares*, control temperature and sublethal pH; *diamonds*, control temperature and control pH; *circles*, +2°C and control pH; *triangles*, +2°C and sublethal pH. Cumulative food intake ("appetite") was calculated by the amount of food eaten per tank divided by the number of fish present; statistical comparisons are based on 180 daily appetite measurements. In the satiation-exposure experiment (*A*), fish in the +2°C thermal regimes consumed more food than fish at control temperatures. In comparison, in the limited-ration-exposure experiment, fish consumed 80%–95% less than the fish fed to satiation. In the satiation-exposure experiment, (n = 40, except in the +2°C and sublethal treatment, where n = 20), the fish in the +2°C treatments grew the most. Growth in the limited-ration-exposure experiment (n = 20) was minimal. Within exposures, significant differences ($P < 0.05$) are indicated by treatment groups that do not share a common letter.

5). Water content decreased from approximately 77% to 73% over the 90-d exposure in all treatments (data not shown). Although protein increased slightly, the main compensating change was from lipids, which increased from approximately 5% to 7%–9% (Fig. 6*C*). There were no treatment effects on water content, protein, or lipids during the exposure (Fig. 6*A, B, C*). The only difference among treatments was on day 90 in the amount of stored carbohydrates (glucose, lactate, and glycogen), which was higher in the +2°C and sublethal pH treatment (Table 4).

Limited-Ration Exposure. Whole-body [Na^+] and [Cl^-] decreased over the 90 d, most rapidly in the +2°C and sublethal pH group, and by day 90, both acid-exposed groups showed lower levels of whole-body [Na^+] and [Cl^-] (Fig. 5*D, E*). Whole-body [K^+] was lowest in the control temperature and sublethal pH treatment from day 60 onwards (Fig. 5*F*). Water content increased over the 90 d from approximately 75% to approximately 79%–80% in all treatments (data not shown). This increase was accounted for by a slight decrease in protein and a larger decrease in lipids. Protein showed a transient difference among treatments (Fig. 6*B*), but by day 90 there were no differences resulting from treatment. Lipids decreased by approximately 2%–4% in all treatments over the 90 d, except in the control temperature and sublethal pH group (Fig.

Table 2: Food conversion efficiencies (ratio of wet weight gained by fish to wet weight of food eaten) calculated for each 30-d period

Time Period (d)	Treatment			
	Control Temperature and Control pH	Control Temperature and Sublethal pH	+2°C and Control pH	+2°C and Sublethal pH
Satiation-exposure experiment:				
0–30	.56	.56	.82	1.07
30–60	1.13	.97	1.34	1.39
60–90	.98	.72	1.11	1.18
Limited-ration-exposure experiment:				
0–30	−.42	−.61	−.23	−.46
30–60	1.48	1.21	.55	1.21
60–90	−.25	−1.26	−.77	−1.95

Note. Measurements were determined by calculating the amount of food consumed per tank and the change in whole-tank biomass for that period and then by taking the mean of the two tanks in each treatment.

6*D*). There were no differences in stored carbohydrates among treatments on day 90 (Table 4).

Satiation and Limited-Ration Exposure. By day 90, whole-body $[Na^+]$ and $[Cl^-]$ were higher in the non-acid-exposed fish of the limited-ration-exposure experiment than in the satiation-fed fish (Fig. 5). Acid-exposed fish, regardless of feeding regime, showed no feeding-related differences in either whole-body $[Na^+]$ or $[Cl^-]$, but whole-body $[K^+]$ was lower in fish fed a limited ration. Water content was significantly lower in the satiation-fed fish than in fish fed a limited ration. Protein was higher in all the satiation-fed fish except for the control temperature, and control pH treatment, in which there were no differences in protein content between the exposures (Fig. 6). Lipids were higher in the satiation-fed fish, except for the control temperature and sublethal pH group (Fig. 6).

Metabolic Rates

While statistical comparisons could not be performed (see Material and Methods), a number of clear trends were apparent.

Satiation Exposure. At each sample period, fish exposed to the elevated temperature (at both pH levels) showed an increased $\dot{M}_{O_2}$, compared with that of the fish at control temperatures (Fig. 7*A*). By day 90, when the control temperature had increased to about 10°C, the difference between the +2°C and control temperature groups was not as marked. Acid exposure had no effect on $\dot{M}_{O_2}$.

Urea excretion accounted for approximately 11% and ammonia excretion for approximately 89% of the total nitrogenous waste, regardless of temperature or pH (Fig. 7*B*). $\dot{M}_N$ was higher throughout the exposure in the +2°C groups. Again, acid exposure had no effect on $\dot{M}_N$. Fractional protein utilization represents the degree to which the fish depend on protein as an aerobic fuel source. Fractional protein utilization increased from day 0 until day 60 in all treatments; by day 90, use had decreased in all treatments (Fig. 7*C*). The +2°C and sublethal pH treatment tended to use protein more than those in the other three treatments.

Limited-Ration Exposure. $\dot{M}_{O_2}$ for 24 h pre- and postfeeding were not dramatically different ($\sim 2.4 \pm 0.2$ μmol g^{-1} h^{-1}, compared with $\sim 2.6 \pm 0.2$ μmol g^{-1} h^{-1}, respectively), so the values were averaged together for Figure 7*D*. There were no treatment effects on $\dot{M}_{O_2}$.

Urea excretion accounted for about 21% of the total nitrogenous waste excretion and ammonia excretion for approximately 79%. Neither temperature nor low pH caused any consistent variation in $\dot{M}_N$ (Fig. 7*F*). However, $\dot{M}_N$ showed a marked difference between unfed and fed states, as it was approximately 33% higher in the 24 h after feeding. In addition, feeding resulted in increased protein utilization, while utilization decreased approximately 30%–50% after 3 d of not being fed (Fig. 7*F*). However, for the ease of comparison with the satiation-exposure experiment, pre- and postfeeding values again were averaged together for both $\dot{M}_N$ and fractional protein utilization (Fig. 7*D, E, F*). A higher fractional protein utilization in the acid-exposed fish after feeding (data not shown) indicates that these fish were using more protein than the non-acid-exposed fish throughout the 90 d.

Satiation and Limited-Ration Exposure. $\dot{M}_{O_2}$ and $\dot{M}_N$ were much lower for all groups, regardless of thermal regime or acid exposure, in the limited-ration-exposure experiment than they were in the satiation-fed fish. In addition, acid-exposed

Table 3: Measured plasma [Na^+], plasma [Cl^-], hematocrit, and plasma protein for each sampling period

	Plasma [Na^+] (mmol L^{-1})	Plasma [Cl^{-1}] (mmol L^{-1})	Hematocrit (%)	Protein (g [100 mL]$^{-1}$)
Satiation-exposure experiment:				
Day 75:				
Control temperature and control pH	124.8 ± 1.0[b]	124.5 ± 1.5	30.7 ± 1.5[a]	5.3 ± .2
Control temperature and sublethal pH	131.2 ± 1.6[a]	122.4 ± 1.9	34.7 ± 1.0[a,b]	5.4 ± .2
+2°C and control pH	128.7 ± 2.0[a,b]	124.3 ± 1.0	37.8 ± 1.3[b,c]	5.6 ± .2
+2°C and sublethal pH	131.9 ± 2.2[a,b]	127.7 ± 1.8	40.7 ± 1.9[c]	5.5 ± .4
Day 90:				
Control temperature and control pH	125.0 ± 1.4[a,b]	122.9 ± 1.8[b]	39.5 ± 2.3[a]	5.4 ± .1
Control temperature and sublethal pH	131.3 ± 1.8[a]	128.2 ± 1.4[a]	36.2 ± .8[a]	5.8 ± .2
+2°C and control pH	124.8 ± .9[a,b]	123.6 ± .6[b]	45.7 ± 1.7[b]	5.6 ± .2
+2°C and sublethal pH	117.8 ± 1.4[b]	125.1 ± 1.1[a,b]	42.0 ± 1.4[a,b]	6.2 ± .4
Limited-ration-exposure experiment:				
Day 30:				
Control temperature and control pH	133.9 ± 1.4[a]	127.9 ± 1.3[a]	37.1 ± 1.7[a,b]	2.9 ± .2
Control temperature and sublethal pH	129.2 ± 2.5[b]	123.6 ± 2.6[a]	40.5 ± 2.7[a]	2.7 ± .1
+2°C and control pH	135.2 ± 1.9[a]	126.4 ± 4.1[a]	37.0 ± 1.3[a,b]	2.9 ± .1
+2°C and sublethal pH	124.0 ± 3.6[b]	118.6 ± 4.2[b]	33.2 ± 1.3[b]	2.5 ± .2
Day 60:				
Control temperature and control pH	126.2 ± 2.4[a]	129.9 ± 1.9[a]	29.0 ± 1.3[b]	2.8 ± .2[a]
Control temperature and sublethal pH	125.2 ± 2.7[a]	120.7 ± 1.0[a]	30.0 ± 1.0[a,b]	3.0 ± .2[a]
+2°C and control pH	124.9 ± 1.4[a]	124.0 ± 2.2[a]	33.7 ± .9[a]	2.6 ± .1[a]
+2°C and sublethal pH	116.1 ± 2.2[b]	105.5 ± 4.4[b]	39.7 ± 1.2[c]	3.8 ± .2[b]
Day 90:				
Control temperature and control pH	129.3 ± .9[a]	119.4 ± 2.5[a]	30.0 ± 1.0[a,b]	2.9 ± .1[b]
Control temperature and sublethal pH	124.9 ± 3.8[a]	125.0 ± 1.3[a]	33.2 ± 1.2[a]	2.2 ± .2[a]
+2°C and control pH	130.5 ± 1.5[a]	125.6 ± 1.4[a]	31.6 ± 1.1[a]	2.4 ± .2[a,b]
+2°C and sublethal pH	108.3 ± 4.0[b]	106.8 ± 3.9[b]	36.2 ± 1.2[b]	3.0 ± .2[b]

Note. Mean values ±SEM ($n = 10–20$) are shown. Treatments that share a letter within a day and column are not significantly different from one another ($P < 0.05$).

fish had a higher fractional protein utilization, suggesting increased reliance on protein for fuel (Fig. 7).

Discussion

Thermal Profile

Temperature and food consumption are the two major influences on bioenergetics in freshwater fish (Jobling 1994). The present study examined how differences in the amount of food consumed influenced the physiological and metabolic response of rainbow trout to low pH in conjunction with a slight increase in temperature (+2°C), equivalent to the rise expected for a global warming scenario (Intergovernmental Panel on Climate Change 1995). These experiments deliberately used a natural water source, and therefore fluctuations in temperature occurred due to natural variation both within and between the two exposures.

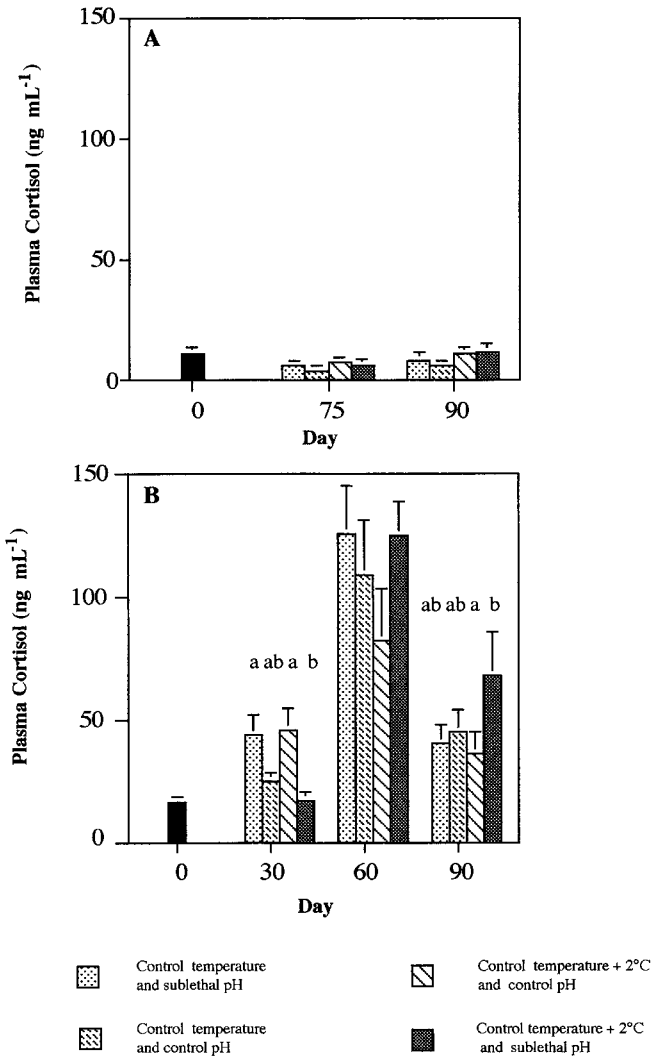

Figure 4. Plasma cortisol (ng mL^{-1}) in the satiation-exposure experiment (*A*) and limited-ration-exposure experiment (*B*). The solid bar represents values at day 0. Values are given as means + SEM ($n = 12–20$), except in the satiation-exposure experiment, in which $n = 10$ for the +2°C and sublethal pH treatment. Significant differences ($P < 0.05$) were only seen in the limited-ration-exposure experiment on days 30 and 90 and are indicated by means that do not share a common letter.

The thermal regime in the limited-ration-exposure experiment was about 2°C above the mean temperature for the satiation exposure. The fish in the +2°C treatment of the satiation-exposure experiment experienced a similar thermal regime to the fish in the control temperature of the limited-ration exposure. However, physiological measurements were not comparable, suggesting that the physiological response was influenced more by the dietary amount than by the absolute temperature. Regardless, in the two separate years, fish were exposed to a similar thermal pattern: relatively constant temperatures from days 0 to 75, with a more rapid increase from days 75 to 90. Thus, comparisons made at day 90 were between similarly treated fish but at different absolute temperatures. Therefore, we cannot eliminate the possibility that some of the differences observed between the responses in the two dietary regimes reflected the difference in temperature rather than, or in addition to, the difference in feeding status.

Low pH Effects

Effects on Ionoregulation. Dockray et al. (1996) proposed that the ability to compensate for ionoregulatory disturbances resulting from low pH may depend on the amount of food consumed by fish. Results from these current experiments clearly confirm that, given sufficient food, fish can compensate for increased energy expenditures and/or ionoregulatory disturbances. Fish on a limited ration, especially at slightly elevated temperatures, generally show typical ionoregulatory disturbances (McDonald and Wood 1981; Fugelli and Vislie 1982; Brown et al. 1984; Lacroix 1985; Audet et al. 1988; Wood 1989), with lower levels of whole-body $[Na^+]$, $[Cl^-]$, and $[K^+]$, as in this study. Analysis of the plasma showed similar results, with lower plasma $[Na^+]$ and $[Cl^-]$ and increased hematocrit in acid-exposed fish, especially in the +2°C treatment, as compared with the control temperatures. In contrast, when fish were fed to satiation (given ~1%–3% of their body weight per day) and exposed to low pH, no whole-body ionoregulatory disturbances were found by day 90, though plasma $[Na^+]$ values had declined in the +2°C and sublethal pH treatment (see below). Present results are consistent with the work of Sadler and Lynam (1986), who found greater ionoregulatory disturbances in starved fish at low pH.

These exposures also supported work by Smith et al. (1995), who found that fish could regulate transbranchial Na^+ efflux depending on dietary salt consumption in nonacid waters. In their work, fish that consumed higher amounts of NaCl have increased Na^+ efflux. Thus, in the satiation-exposure experiment, the non-acid-exposed fish probably had increased Na^+ efflux, which may explain why their whole-body Na^+ levels were lower than non-acid-exposed fish maintained on the limited ration.

Effects on Growth and Metabolic Rate. Better growth, as demonstrated by a higher specific growth and higher condition factor, was seen in the fish fed to satiation in the +2°C and sublethal pH treatment, as compared with the non-acid-exposed fish at this temperature. However, at control temperatures, there was no effect of pH on either growth or $\dot{M}O_2$. These results are contradictory to those of other investigators, who found that sublethal low pH increased $\dot{M}O_2$ (Hargis 1976; Waiwood and Beamish 1978; Butler et al. 1992), subsequently leading to reduction in growth. However, in two previous studies, when a similar satiation regime was employed, increased growth was reported in acid-ex-

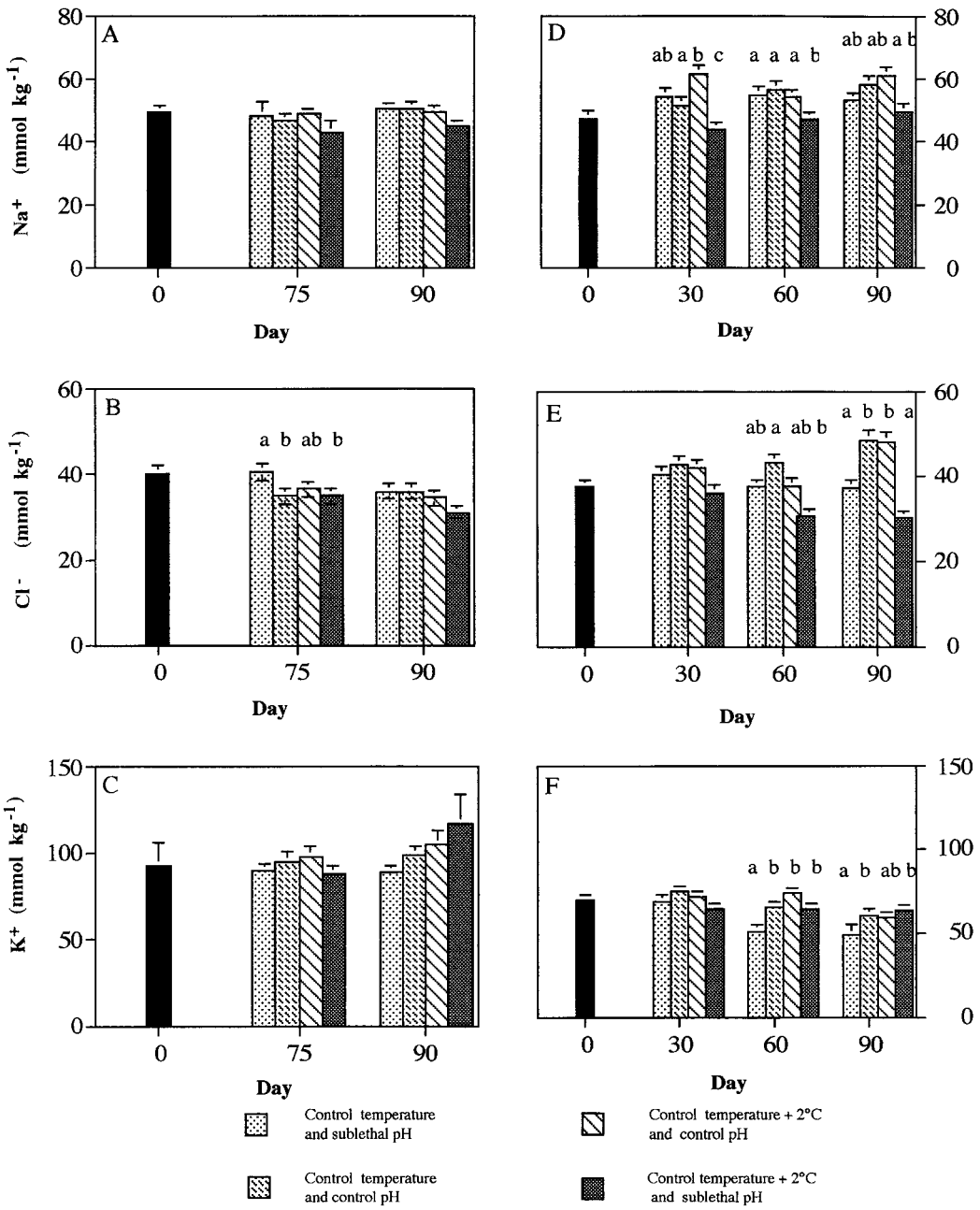

Figure 5. Whole-body ion concentrations. Satiation-exposure experiment (*A*, *B*, *C*) and limited-ration-exposure experiment (*D*, *E*, *F*). Values are given as means + SEM (n = 12–18) except for the satiation-exposure experiment, where n = 6. Significant differences ($P < 0.05$) are indicated by treatment means that do not share a common letter. If there are no letters present for the sampling period, there are no significant differences between means.

posed fish (Wilson et al. 1994*a;* Dockray et al. 1996). A possible explanation for such discrepancies may be compensation for branchial ion loss by dietary means. Recent work by Smith et al. (1995) showed that fish vary branchial Na^+ influx and efflux depending on dietary salt intake. In acid-exposed fish, appetite may have been stimulated by a Na^+ deficit (Salman and Eddy 1987), thereby replacing branchial ion loss and whole-body reserves, with a secondary effect of increased growth. For the +2°C treatments, $\dot{M}O_2$ did not increase with increased feeding in the acid-exposed fish, and food was converted more efficiently (Table 2); however, the acid-exposed and non-acid-exposed fish had a similar level of growth (Table 1). These results are different from those found in juvenile *Salmo salar,* which have depressed appetites when exposed to low pH (Waiwood et al. 1992).

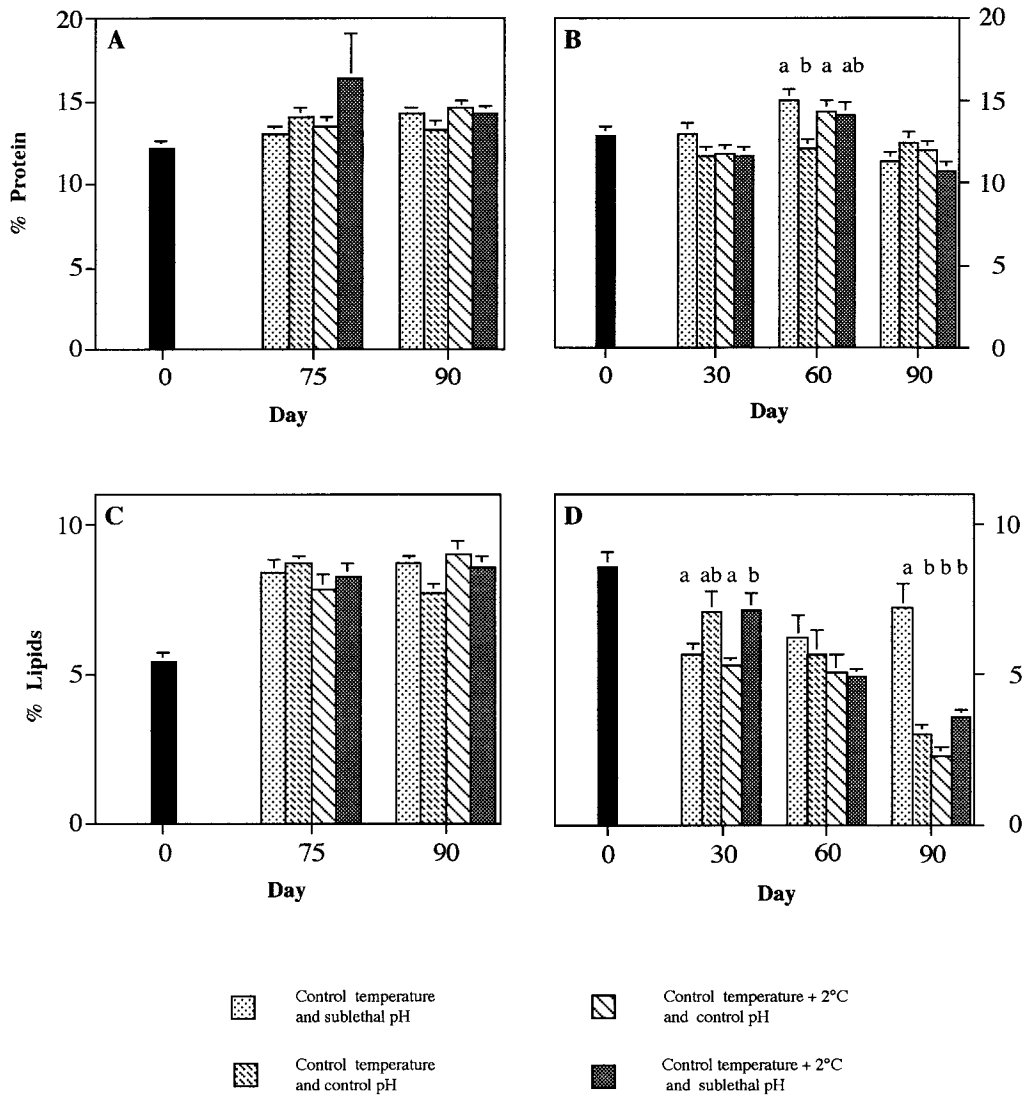

Figure 6. Whole-body protein in the satiation-exposure experiment (*A; n* = 12, except in the +2°C and sublethal pH treatment, where *n* = 6) and limited-ration-exposure experiment (*B; n* = 20), and whole-body lipids in the satiation-exposure experiment (*C; n* = 12, except in the +2°C and sublethal pH treatment, where *n* = 6) and limited-ration-exposure experiment (*D; n* = 20). Values are given as means + SEM. Significant differences ($P < 0.05$) are indicated between means that do not share a common letter. If there are no letters present for the sampling period, there are no significant differences between means.

Effects on Whole-Body Composition. Whole-body composition of fish fed to satiation and exposed to low pH showed no treatment-related differences in lipids and protein components. However, carbohydrates stores were higher in the +2°C and sublethal pH treatment by day 90. In the limited-ration-exposure experiment, there were only slight changes in weights in the four treatments, in contrast to the large weight gain in the satiation-fed fish. Whole-body lipids decreased and water content increased, except in the control temperature and sublethal pH treatment, suggesting that fish were in a state of catabolism, using their lipid stores for energy. In the control temperature and sublethal pH treatment, lipid stores were 7%, in comparison to approximately 2%–4% in the other three treatments. As water content, protein, and stored carbohydrates were similar to the other treatments, the inherent difference in this treatment may be due to significantly decreased amounts of inorganic content (ash). The feeding cycle employed in the limited-ration-exposure experiment (1% of wet-body weight every 4 d) may have helped fish conserve whole-body protein (Kaushik and Gomes 1988).

Temperature and Feeding Effects

Effects on Metabolic Rate. Routine $\dot{M}O_2$ was used as an integrative measure of the cost of energy expenditure in these

Table 4: Stored carbohydrates (the sum of glucose, lactate, and glycogen) at day 90, expressed as a percentage of whole-body composition

	Carbohydrates (%)	*n*
Satiation-exposure experiment:		
Control temperature and control pH	.30 ± .03[a,b]	9
Control temperature and sublethal pH	.39 ± .03[a]	10
+2°C and control pH	.29 ± .03[b]	9
+2°C and sublethal pH	.35 ± .03[a,b]	9
Limited-ration-exposure experiment:		
Control temperature and control pH	.29 ± .04	6
Control temperature and sublethal pH	.20 ± .03	8
+2°C and control pH	.24 ± .04	6
+2°C and sublethal pH	.25 ± .03	8

Note. Stored carbohydrates in the satiation-exposure experiment were .39 ± .03 ($n = 4$) and were .30 ± .02 ($n = 6$) in the limited-ration-exposure experiment. Significant differences were seen only in the satiation-exposure experiment and are indicated by treatments that do not share a letter ($P < 0.05$).

exposures. Routine $\dot{M}O_2$ includes not only basal metabolism but also activity-related metabolism. Temperature, food intake, and energy requirements for the biochemical and mechanical aspects of feeding and digestion (termed specific dynamic action or SDA; Cho and Kaushik 1990) may influence $\dot{M}O_2$. In the present study, food intake greatly influenced $\dot{M}O_2$. $\dot{M}O_2$ was approximately 50% less in fish fed 0.25% of their wet-body weight per day (this approximates a maintenance ration, a level of dietary intake at which animals neither gain nor lose weight; Kaushik and Gomes 1988), compared with fish fed to satiation. $\dot{M}O_2$ differences between feeding regimes were probably due to both increased SDA and increased activity in the fish fed to satiation, that is, a higher metabolic rate caused by increased food intake (Beamish 1974*a;* Brett and Groves 1979; Cho and Kaushik 1990; Alsop and Wood 1997).

Temperature also had a great impact on metabolic rate in satiated fish but had no effect on fish fed a limited ration. This may be because the latter fish tended toward a state of starvation (Brett and Groves 1979; Jobling 1994) and thus, regardless of temperature, maintained a very low metabolic rate. However, in satiated fish, a slight temperature increase caused an approximately 25%–35% increase in $\dot{M}O_2$. This increase in $\dot{M}O_2$ was probably due to increased SDA and a higher metabolic rate resulting from the increased temperature (Brown 1946; Brett 1971). These results suggest that there is a greater temperature dependence for SDA than for basal metabolism. Work by Soofiani and Hawkins (1982) indicated a similar situation in juvenile cod, in which increasing temperatures more dramatically influences SDA than basal metabolism.

Effects on Growth. Satiation feeding resulted in relatively high growth rates for all treatments, with large accumulation of lipid stores. Distinct differences in growth rates between the +2°C treatments and control temperatures occurred mainly because of increased appetite. In addition, fish in the +2°C treatments were closer to their optimal temperature for growth (between 15° and 20°C; Cho and Kaushik 1990). Fish maintained on the limited diet lost dry weight gradually. The weight loss between days 0 and 30 was probably due to adaptation to the feeding regime (Brett et al. 1969; Kaushik and Gomes 1988). Between days 30 and 60, there were improved food-conversion efficiencies; however, the +2°C treatments did not influence this conversion. From day 60 to day 90, gross food-conversion efficiency was again quite low, especially in the acid-exposed treatment. This period encompassed the most dramatic increase of water temperatures and the highest rate of mortality, suggesting that during this period, dietary energy was being utilized in maintaining ionoregulation rather than maintaining body weight. When fish were fed a limited ration and held in a more constant environment, that is, not adapting to the feeding regime or changing temperatures, food conversion efficiencies (Table 2) were higher than in fish fed to satiation. These results are similar to those reported by Paloheimo and Dickie (1966). Jobling (1980), Miglavs and Jobling (1989) and Quinton and Blake (1990) have demonstrated that starvation or restricted feeding leads to reduced lipid content and a replacement of this lipid with water in fish tissues, as seen in the present results.

Effects on Ionoregulation. The slight increase in water temperatures from approximately day 75 to day 90 seemed to have been stressful for both satiated and limited-ration fish exposed to the +2°C and sublethal pH treatments, though effects were less dramatic in the former exposure. In the limited-ration-exposure experiment, fish in the +2°C and sublethal pH treatment had greater mortality and ionoregulatory disturbances. Effects in the satiation-fed fish in the +2°C and sublethal pH treatment included increased ionoregulatory disturbance, with lowered plasma $[Na^+]$ and $[Cl^-]$. These effects were not mimicked in the control temperature and sublethal pH treatment, suggesting that the increase in temperature was more stressful for the +2°C and sublethal pH exposed fish. A possible explanation may be that satiated fish in the +2°C and sublethal pH treatment already had elevated metabolic rates, and increasing water temperatures resulted in additional ion loss. This phenomena has been coined the "osmorespiratory compromise," in which increased $\dot{M}O_2$ corresponds with increased movement of all permeable electrolytes across the gills (Randall et al. 1972; Gonzalez and McDonald 1992). As fish in the control temperature and

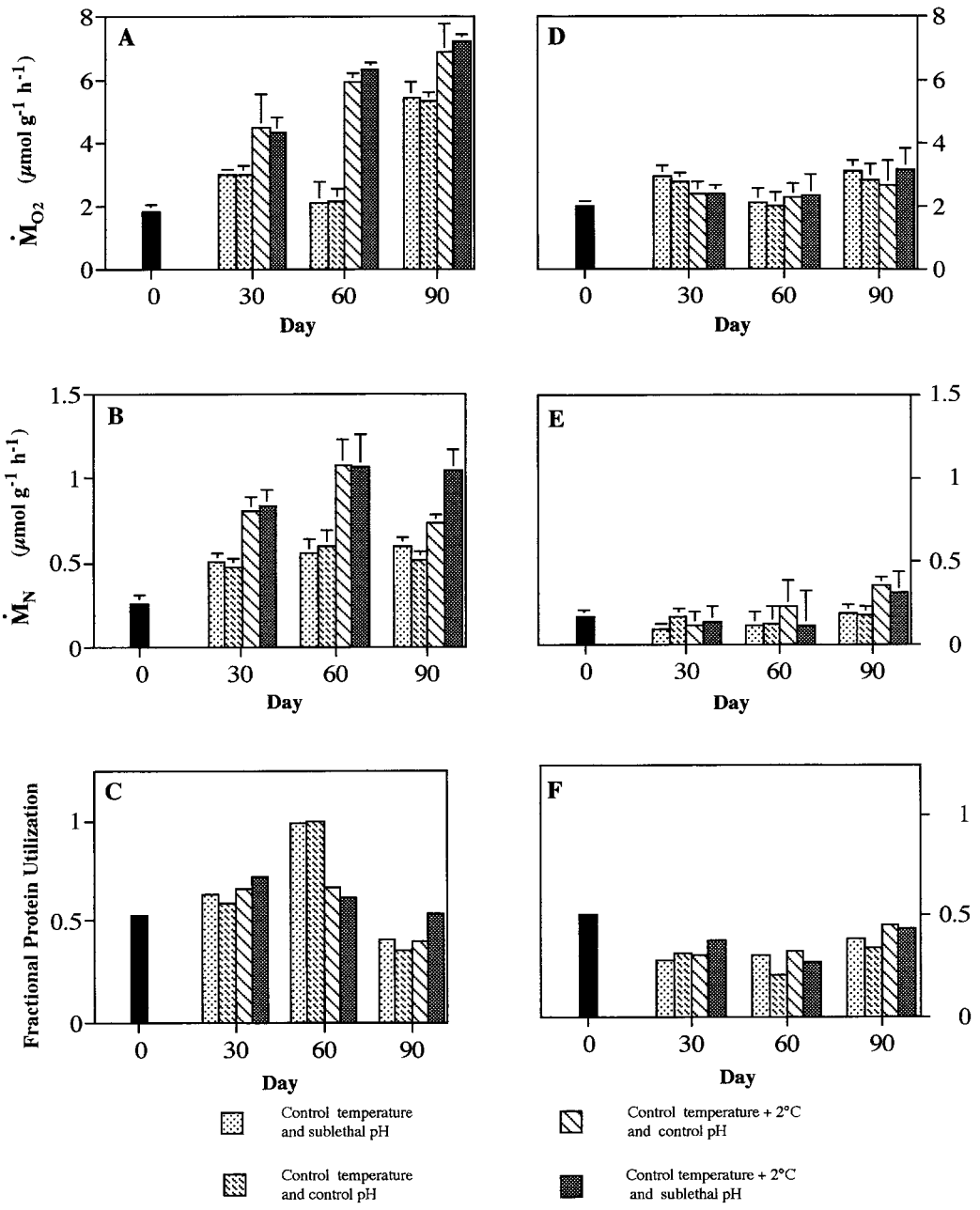

Figure 7. Metabolic rates for the satiation (*A, B, C*) and limited-ration-exposure experiments (*D, E, F*). Data for in-tank $\dot{M}_{O_2}$ and $\dot{M}_N$ were corrected for size differences using the weight exponent determined by Cho (1992). Rates for the satiation-exposure experiment were determined for the total number of fish in one duplicate tank, over a 10-h period during the day, including feeding periods. Rates for the limited-ration-exposure experiment were determined for the total number of fish in each tank, and replicate treatments were averaged together; $\dot{M}_{O_2}$ was measured over a 48-h period, including feeding periods. Error bars represent measurement standard error only, and thus no statistical comparisons can be carried out. Fractional protein utilization was calculated by dividing the nitrogen quotient (the ratio of the moles of nitrogen produced to moles of oxygen consumed) by the theoretical maximum nitrogen quotient 0.27, which was determined by Kutty (1972).

sublethal pH treatment had a lower metabolic rate, they consequently were able to meet the increasing energy demands brought about by the rising water temperatures.

L. M. D'Cruz and C. M. Wood (unpublished data) carried out a month-long experiment to determine whether it is the energy or salt provided by food that allows for better salt homeostasis. Dietary salt appears to have a dominant influence on the response of rainbow trout in maintaining ion balance.

Effects on $\dot{M}_N$ and Protein Use. $\dot{M}_N$ followed trends similar to those for $\dot{M}_{O_2}$, with rates being much higher in satiated fish. Increased feeding corresponded to increased nitrogen losses associated with the assimilation and deamination of protein (Jobling 1981). In addition, satiated fish used protein as fuel more routinely, as determined by a higher fractional protein utilization value (Fig. 7); nevertheless, endogenous protein stores were still increased because of the high dietary intake. Increased substrates from the diet also probably explain the much higher levels of plasma protein than those in the fish maintained on a limited ration. Though protein use decreased from day 60 in the satiation-fed fish, it was higher in acid-exposed fish than in control pH fish. In the limited-ration-exposure experiment before feeding, the $\dot{M}_N$ profile suggests $\dot{M}_N$ was of an endogenous source, and the increase after feeding was due to exogenous nitrogen sources (Jobling 1981). By day 90, the decreased levels of whole-body protein indicate that these fish were using protein energy stores that are usually not used (Jobling 1981).

Indicators of Stress

The generalized stress response characterized by activation of the pituitary-interrenal axis results in release of cortisol into circulation (Donaldson 1981). Elevated plasma cortisol levels have been reported in a number of longer term, low pH studies (Brown et al. 1984, 1986; Brown et al. 1989; Audet and Wood 1993). However, in shorter-term exposures to low pH, basal cortisol levels are recovered (Goss and Wood 1988) or do not increase (Balm and Pottinger 1993). In the present study, cortisol levels were elevated in the treatments experiencing greater ionoregulatory disturbances but also seemed greatly affected by ration level. Indeed, all treatments in the limited-ration-exposure experiment had elevated levels as compared with the satiation-fed fish. However, Anderson et al. (1991) found that cortisol is not elevated in starved fish, suggesting there are distinct differences in the stress response depending on nutritional status. For example, in this study, when fish were fed approximately 1.4%–1.9% of their body weight daily, the interrenal axis was not activated, while fish fed 1% of their body weight every 4 d showed a marked elevation in cortisol. Thus, either no food or an abundance of food prevented activation of the interrenal axis.

Increased mortality during the limited exposure also indicates that the fish were stressed. This mortality is probably not size related, as Toney and Coble (1980) reported that there were no differences in mortality depending on fish size in an overwintering study. Mortality in the limited-ration-exposure experiment was probably due to impaired ionoregulation, which resulted in a shift of fluid from the extracellular compartment to the intracellular compartment, thereby resulting in decreased circulatory efficiency (Milligan and Wood 1982).

The decreasing levels of protein, and generally decreased levels of lipids, in limited-ration fish suggest that at this thermal regime (8°–12°C), feeding restriction was itself stressful. Further support for this is provided by the results from the satiated fish, with high endogenous energy stores that did not change regardless of thermal or pH regime.

Effects of Diet on Ionoregulation

When fish were fed to satiation at temperatures between 8° and 12°C, they did not suffer from ionoregulatory disturbances or mortalities when exposed to low pH and increased temperature. In fact, metabolic rate, growth, and appetite were increased dramatically in the fish from the +2°C treatments. Acid exposure at this temperature stimulated appetite, possibly because of a need to replace ions lost through branchial efflux with those from dietary salts; acid exposure also improved food conversion efficiency. However, when fish from the +2°C treatments were faced with the need to acclimate to slightly higher temperatures resulting from seasonal changes, ion loss increased, though this may have been transitory. We speculate that given enough time and food, fish would have replaced this ionic loss.

When fish were maintained on a limited ration, acid exposure resulted in ionoregulatory disturbances and hematological changes, resulting in a higher mortality rate. These data support the hierarchy of energy allocation, such that fish with lower energy intakes and growth rates have proportionately greater responses to stresses (Rice 1990). A slight elevation in temperature did not increase metabolic rate or growth and tended to be detrimental, especially for the acid-exposed fish. These fish were not able to compensate for the increased energy expenditure and salts needed to maintain homeostasis during this thermal regime. The limited-ration-exposure experiment is probably more ecologically relevant than the satiation-exposure experiment, as food deprivation is a more common occurrence in winter (Smith et al. 1989; Smith and Griffith 1994). Thus, a slight increase in temperature will be detrimental to the fish population living in a marginalized environment.

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