

Mini-review

The cost of living for freshwater fish in a warmer, more polluted world

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Abstract

Little of the vast literature on the temperature physiology of freshwater fish is useful in predicting the effects of global warming. In the present review a series of laboratory experiments is reviewed in which rainbow trout (*Oncorhynchus mykiss*) were exposed to simulated global warming, a 2 °C increment superimposed upon the *natural* thermal regime, in the presence and absence of two common freshwater pollutants, ammonia and acidity (low pH). Simulated global warming had little effect on the growth and physiology of trout fed to satiation over much of the summer. However, in late summer, when ambient water temperature was at its highest, the addition of 2 °C caused a marked inhibition of appetite and growth, although this impact was not exacerbated by a reduction in food availability. In winter, +2 °C stimulated metabolism, appetite and growth by approximately 30–60%.

Exposure of satiation-fed trout to low levels of pollutants produced unexpected results. Ammonia ($\text{NH}_3 + \text{NH}_4^+ = 70 \mu\text{m}$) stimulated summer growth and energy conversion efficiency, whilst acidification (pH 5.2) increased appetite and growth but caused no disturbance of electrolyte balance. These pollutant effects were additive upon, but not synergistic with, the effects of +2 °C. The ability of the fish to acclimate to the experimental conditions was tested with acute lethal temperature and/or toxicant challenges. Fish exposed to +2 °C had a slightly (0.2–1.0 °C) but significantly higher lethal temperature than those exposed to ambient temperature when fed to satiation. However, there was no evidence of acclimation to either ammonia or low pH. It is concluded that the impact of global warming on freshwater fish will vary seasonally. The additional temperature may provide growth benefits in winter, but may threaten fish populations living towards the upper end of their thermal tolerance zone in (late) summer.

Keywords: acidity, ammonia, fish, global warming, growth, temperature

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Introduction

Current global warming models predict increases in mean air temperature of 1.3–4.5 °C over the next 50–100 years (Hansen *et al.* 1988; Mohnen & Wang 1992; IPCC 1995), with similar increases in the temperature of aquatic ecosystems (Regier & Meisner 1990). The first response of many animals to climate warming may be a shift in distribution towards higher latitude or elevation (Holbrook *et al.* 1997; Thomas & Lennon 1999). In

freshwater ecosystems that often have discrete physical boundaries, such large-scale movements may not be possible, but smaller-scale spatial variation in temperature, e.g. with depth, may allow aquatic animals to migrate to cooler areas during warming (Magnuson & Destasio 1997). However, climate change will also affect the availability of thermal habitats (Meisner 1990; Rahel *et al.* 1996) and therefore exposure to increased temperature may be unavoidable (Hill & Magnuson 1990). In such situations, physiological effects of climate warming are expected to occur.

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Fish are likely to be amongst those animals most affected by climate change as, being ectothermic, their metabolic rate and physiology are fundamentally affected by environmental temperature. Indeed, there is a vast literature on the effects of temperature on fish physiology (for reviews see Brett 1979; Hazel 1993). However, much of this work has examined animals acclimated to two or more well-separated ($\geq 5^\circ\text{C}$) temperatures and is therefore of little value in predicting the effects of a small temperature increment in the natural environment. Nevertheless, there is evidence to suggest that small changes in environmental temperature may have significant consequences for freshwater fish (Regier *et al.* 1990). For example, Johnson & Evans (1990) found that the winter mortality of juvenile white perch (*Morone americanus*) was much lower at 4°C than at 2.5°C and therefore global warming could greatly affect the population structure of this species.

Many freshwaters are also subject to chronic contamination with toxic pollutants. Exposure to such toxicants induces metabolic costs (Calow 1991), and their effects may interact with those of environmental temperature change (Vitousek 1994; Schindler *et al.* 1996). Again, however, virtually no data are available on the effects of combined temperature and pollution from a global warming perspective (Reid *et al.* 1997b). Therefore, in 1992, a series of experiments was initiated to study the chronic effects of a conservative estimate of global warming ($+2^\circ\text{C}$), superimposed upon the natural thermal regime, on the growth and energetics of a reference coldwater fish (Magnuson *et al.* 1979), the rainbow trout (*Oncorhynchus mykiss*). These effects were studied in the presence and absence of two common freshwater pollutants: ammonia and acidity (low pH). Ammonia is ubiquitous in surface waters, and ammonia pollution is a steadily increasing problem. More molecules of ammonia are manufactured each year than any other industrial chemical (Atkins 1987). Therefore, the effects of $+2^\circ\text{C}$ and ammonia were studied in the natural hard water of the laboratory (see below). Environmental acidity is a widespread problem in the Northern hemisphere but is exclusive to soft water (Wood 1989), as hard water has sufficient alkalinity to neutralize any potential toxicity to aquatic animals. Therefore, the effects of $+2^\circ\text{C}$ and low pH were studied in artificially generated soft water. In order to determine the ecological implications of global warming and pollutant exposure, the effects of ambient environmental temperature (i.e. season; summer vs. winter) and food ration (satiation vs. restricted) were also examined.

This project resulted in 16 peer-reviewed publications. The present review of the work does not intend to provide an exhaustive list of the results obtained from the individual experiments; rather, it reports the most

consistent and important findings, and seeks to identify the conclusions that can be drawn about the potential effects of global climate change on freshwater fish in temperate climates.

Experimental design

A novel experimental design used heat exchangers to add a 2°C increment onto the ambient temperature of Hamilton city tap water, which follows the natural thermal cycle of the inshore region of Lake Ontario (Fig. 1). In winter, ambient water temperature remains relatively constant at around $4\text{--}6^\circ\text{C}$ for several months. Summer temperatures are more variable, typically in the range $14\text{--}18^\circ\text{C}$, but may exceed 20°C in mid-late August. The experiments were divided into two main groups based on the pollutant tested with the simulated global warming, and its appropriate water chemistry:

(i) The effects of $+2^\circ\text{C}$ in the presence/absence of a sublethal concentration ($70\ \mu\text{M}$) of total ammonia ($T_{\text{Amm}} = \text{NH}_3 + \text{NH}_4^+$) in hard water (HW = Hamilton city water, control $T_{\text{Amm}} = 6\text{--}8\ \mu\text{M}$). The chemical composition of the water before manipulation was (mM) $[\text{Ca}^{2+}] \approx 1.0$; $[\text{Na}^+] \approx 0.6$; $[\text{Cl}^-] \approx 0.7$; $[\text{Mg}^{2+}] \approx 0.2$; $[\text{K}^+] \approx 0.05$; titratable alkalinity to pH 4.0 ≈ 1.0 ; pH $7.8\text{--}8.0$.

(ii) The effects of $+2^\circ\text{C}$ in the presence/absence of sublethal acidification (H_2SO_4 , pH = 5.2) in soft water (SW, control pH = 6.2). Soft water was generated by reverse osmosis to achieve final concentrations of $[\text{Ca}^{2+}] \approx 25\ \mu\text{M}$, $[\text{Na}^+] \approx 75\ \mu\text{M}$, $[\text{Cl}^-] \approx 75\ \mu\text{M}$, [titratable alkalinity] $\approx 250\ \mu\text{M}$. This process unavoidably added $2\text{--}3^\circ\text{C}$ to ambient temperature prior to addition of the simulated global warming increment, so that the control temperature in soft water was $2\text{--}3^\circ\text{C}$ greater than the control in hard water.

The fish were allowed to acclimate to water chemistry for at least six weeks before addition of the simulated global warming and/or pollutants. The fish were exposed to the treatments for 90 days in separate tests during summer and winter and fed to satiation twice daily (≈ 2.5 and 1.5% wet body weight d^{-1} , respectively) so that the effects of the treatments on appetite could be determined. The 90-day exposures were repeated in summer, but at a limited food ration of 1% wet body weight d^{-1} . The following parameters were measured in both sets of experiments: food consumption (appetite), growth, routine (in-tank) oxygen consumption, nitrogenous waste excretion, body proximate composition (protein, lipid, carbohydrate), and tissue protein metabolism (protein synthesis, accretion and degradation), following the methods of Houlihan *et al.* (1995). Energy budgets were also constructed. In addition, several parameters relevant to the mechanism(s) of toxicity of the individual pollutants were measured. In HW, plasma ammonia and

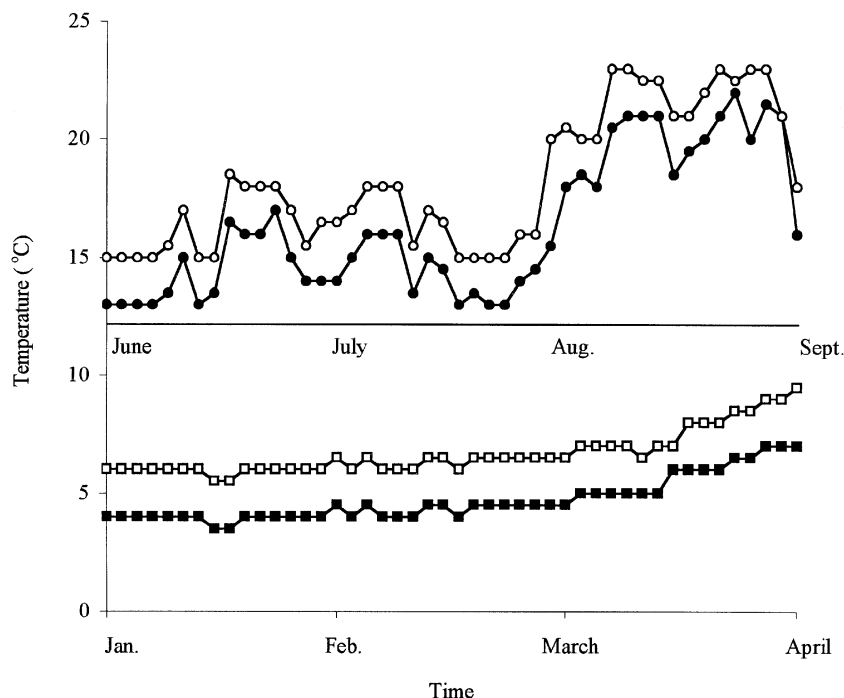


Fig. 1 Thermal profiles during summer (above, circles) and winter (below, squares) exposure of satiation-fed juvenile rainbow trout to ambient temperature (filled symbols) and simulated global warming (open symbols).

tissue ammonia and glutamine concentrations were measured, and nitrogen budgets constructed. In SW, plasma protein and plasma and whole-body ion concentrations were measured. Finally, the ability of the fish to acclimate to the simulated global warming was studied by acute challenges to lethal temperatures and/or pollutant concentrations. Full details of the experimental design and sampling methodology are described in Linton *et al.* (1997) (HW-acclimated fish) and Dockray *et al.* (1996) (SW-acclimated fish).

Effects of exposure to simulated global warming: +2 °C

Summer (June – September)

The addition of 2 °C to the natural thermal regime had little effect on the growth and physiology of juvenile rainbow trout fed to satiation over the majority of the summer, suggesting that there is compensation to the additional temperature in most physiological and energetic parameters. However, in the late summer when ambient temperature was at its highest (Fig. 1) and approached the upper thermal limit for rainbow trout (26 °C; Bidgood & Berst 1969), +2 °C caused a marked inhibition of appetite and growth in both SW-acclimated (Dockray *et al.* 1996) and HW-acclimated (Linton *et al.*

1997) rainbow trout (Table 1). The high sensitivity of growth to the experimental treatments was a consistent result throughout the project.

Even during late summer when appetite and growth were suppressed, few of the physiological parameters measured were affected by the simulated global warming (Dockray *et al.* 1996; Linton *et al.* 1997). One that was affected by +2 °C at high ambient temperature was nitrogen (NH₃ + urea) excretion rate, which was reduced in both acclimation groups (HW and SW) as a result of the reduction in food (protein) consumption. However, the most sensitive physiological indicator of the impact of simulated global warming was tissue protein metabolism, i.e. fractional rates of protein synthesis, accretion and degradation or turnover (Houlihan *et al.* 1995). For example, protein accretion in liver and white muscle was significantly reduced by approximately 20% by +2 °C in the late summer (Reid *et al.* 1995). In general, this suppression was the result of an increase in protein degradation or turnover, rather than a reduction in protein synthesis rates (Fig. 2).

A reduction in ration from satiation ($\approx 2.5\%$ wet body weight day⁻¹) to 1% d⁻¹ greatly reduced growth and protein metabolism at ambient temperature, as predicted (Dockray *et al.* 1998; Linton *et al.* 1999). Food conversion efficiency also decreased significantly as the proportion of ingested energy used for maintenance requirements

Table 1 Effects of simulated global warming (+2 °C) on appetite and growth of satiation-fed rainbow trout over the 30-day period of maximum (late) summer temperatures (20–24 °C). Values are means ± SEM. *Indicates a significant effect of +2 °C ($P < 0.05$)

Parameter	HW-acclimated fish		SW-acclimated fish	
	Ambient†	+2 °C	Ambient†	+2 °C
Appetite (g d ⁻¹)	0.66 ± 0.07	0.46 ± 0.07*	0.68 ± 0.08	0.49 ± 0.08*
Growth rate (g d ⁻¹)	0.72 ± 0.05	0.26 ± 0.03*	0.58 ± 0.04	0.07 ± 0.01*
Conversion ratio	1.10 ± 0.09	0.57 ± 0.07*	0.85 ± 0.07	0.14 ± 0.02*

†Ambient temperature in SW was approx. 2–3 °C greater than that in HW.

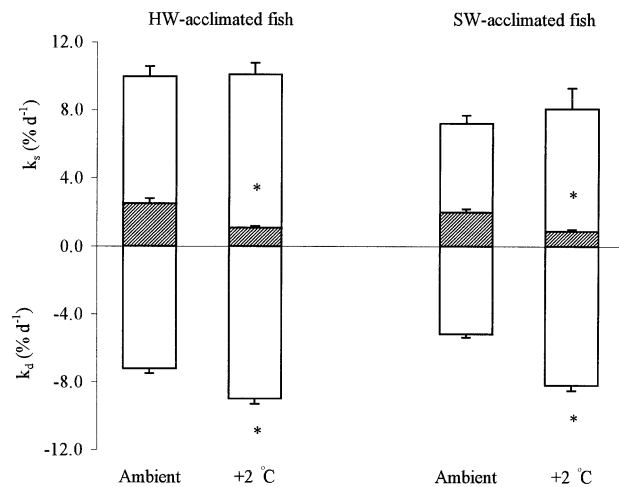


Fig. 2 Fractional rates of protein synthesis (k_s , open bars above x -axis), protein degradation (k_d , calculated from $k_g - k_s$; open bars below x -axis) and net protein accretion (k_g , hatched bars) in the liver of juvenile rainbow trout following 90 days exposure to simulated global warming (+2 °C) during summer at a satiation ration. Values are means ± SEM, $N=5$. *Indicates a significant effect of +2 °C ($P < 0.05$).

increases with decreasing ration (Mehner & Wieser 1994). Surprisingly, however, the reduction in ration did not increase the impact of simulated global warming above that seen in fish fed to satiation. Indeed, the suppression of appetite and growth seen in late summer at satiation rations (above) was not observed at the restricted ration. This may have been the result of lower peak ambient temperatures in the latter experiment, which was performed in a different year (18.5 °C vs. 22.0 °C previously). However, suppression of appetite and growth in the late summer was also seen in a subsequent year-long experiment in which the fish were fed to satiation, even though ambient temperature reached only 18 °C on that occasion (Linton *et al.* 1998a). Brett & Higgs (1970) found that the appetite and conversion efficiency of juvenile sockeye salmon *Oncorhynchus nerka* fell dramatically above 15 °C. It is

therefore likely that a high ration can itself be costly for growth at high ambient temperatures because of the high metabolic demand of food processing and digestion (the specific dynamic action, SDA; Beamish 1974).

Winter (January – April)

At the relatively low ambient temperatures in winter, +2 °C significantly stimulated appetite and growth by over 50% in both HW-acclimated (Linton *et al.* 1998b) and SW-acclimated fish (D'Cruz *et al.* 1998) (Table 2). The additional temperature caused a general stimulation of metabolism, e.g. in oxygen consumption and N-excretion and in HW-acclimated fish, the amount of energy consumed that was retained as growth approximately doubled resulting in a significant increase in conversion efficiency at +2 °C (Table 2). General rates of protein metabolism in winter were much lower than those recorded in summer, but +2 °C stimulated rates of liver protein synthesis and degradation (Morgan *et al.* 1998). The magnitude of the effect of +2 °C on physiological functions was quite remarkable: calculated Q_{10S} of oxygen consumption and nitrogen excretion averaged 5.2 and 14.3 in SW-acclimated fish, and 10.3 and 17.5 in HW-acclimated fish. It was thus concluded that, if food is not limiting, global warming '...will promote the [winter] growth of juvenile trout at no additional metabolic cost, placing these fish at an ecological advantage leading into the summer growth period' (Linton *et al.* 1998b).

It might be expected that if food were limited in winter, then the large increase in metabolism at +2 °C might reduce the energy available for other processes, resulting in significant performance costs. The only such experiment performed during the project involved SW-acclimated fish fed a maintenance ration of only 1% wet body weight every four days (1% 4d⁻¹), designed primarily to study the effects of diet on acid toxicity (below). The low ration suppressed metabolism and growth, leaving little scope for further inhibition by the experimental treatments, and although the fish exposed to +2 °C had lower food conversion efficiencies, there

Table 2 Effects of 90 days exposure to simulated global warming (+2 °C) in winter on appetite, growth and metabolism of satiation-fed juvenile rainbow trout. Ambient temperature was 4.5–7.0 °C in HW and 7.5–10.5 °C in SW. Q_{10} , the temperature coefficient or quotient is calculated as $Q_{10} = (P_1/P_2)^{10/(T_2-T_1)}$ where P_1 and P_2 are measures of a parameter, P , at temperatures T_1 and T_2 , respectively. Values of growth rate are means \pm SEM. *Indicates a significant effect of +2 °C ($P < 0.05$). All other parameters were measured on a per tank basis ($N = 2$); errors and statistical comparisons are not available

Parameter	HW-acclimated fish			SW-acclimated fish		
	Ambient	+2 °C	Q_{10}	Ambient	+2 °C	Q_{10}
Appetite (g d ⁻¹)	0.08	0.12	7.6	0.16	0.26	11.3
Growth rate (g d ⁻¹)	0.06 (± 0.02)	0.10* (± 0.02)	12.9	0.13 (± 0.03)	0.27* (± 0.04)	38.6
Conversion ratio	0.75	0.83	1.7	0.81	1.04	3.5
O ₂ consumption ($\mu\text{mol. g}^{-1}\text{h}^{-1}$)	3.4	4.5	4.1	5.3	6.8	3.5
N-excretion ($\mu\text{mol. g}^{-1}\text{h}^{-1}$)	0.57	0.80	5.4	0.51	0.72	5.6

Table 3 Effects of exposure to 70 μM total ammonia in summer ($T_{\text{Amm}} = \text{NH}_3 = \text{NH}_4^+$) on growth energetics and nitrogen metabolism of satiation-fed, HW-acclimated juvenile rainbow trout. Values are means \pm SEM. *Indicates a significant effect of +2 °C ($P < 0.05$)

Parameter	Control (7 μM T_{Amm})	Ammonia exposed (70 μM T_{Amm})
Appetite (g d ⁻¹) †	0.43	0.41
Growth rate (g d ⁻¹)	0.40 \pm 0.02	0.49 \pm 0.03*
Conversion ratio†	0.93	1.20
Energy conversion efficiency (%)†	38.2	57.4
N-retention efficiency (%)†	25.9	44.9
Plasma [T_{Amm}] (mM)	0.26 \pm 0.06	0.38 \pm 0.02*
Liver protein synthesis rate (% d ⁻¹)	9.7 \pm 0.6	11.7 \pm 0.7*

† Parameters measured on a per tank basis ($N = 2$); errors and statistical comparisons are not available.

was no significant difference in the final weight between treatments (D'Cruz *et al.* 1998).

Effects of exposure to sublethal pollutants

Ammonia

Previous studies have generally shown that exposure to sublethal ammonia inhibits growth in freshwater fish (Robinette 1976; Rice & Bailey 1980; Beamish & Tandler 1990). Other effects of ammonia toxicity include gill damage (Smart 1976) and plasma ion disturbance (Thurston *et al.* 1984; Twitchen & Eddy 1994). It was therefore a surprise to find that juvenile rainbow trout exposed to 70 μM T_{Amm} in summer and fed to satiation had higher growth and whole-body protein content than the controls (7 μM T_{Amm}) (Linton *et al.* 1997). This extra growth was achieved without an increase in appetite and so the ammonia-exposed fish had greater nitrogen absorption and retention efficiencies, and higher energy conversion efficiency (Table 3). Similar effects on body

protein and nitrogen retention efficiency were seen in ammonia-exposed fish in summer on a reduced ration (Linton *et al.* 1999), but not when fed to satiation during winter (Linton *et al.* 1998b).

Such positive effects of ammonia on growth have not been recorded previously, although the concentration of total ammonia in the present study was much lower than those used in other studies. The extra growth of ammonia-exposed fish when fed to satiation in summer may have resulted from a decrease in activity as these fish had lower routine oxygen consumption than the controls. However, the ammonia-exposed fish also had higher plasma ammonia concentrations (Linton *et al.* 1997), and greater rates of protein synthesis in liver and white muscle tissue (Reid *et al.* 1998). It is suggested that the increase in protein synthesis could be the indirect result of an ammonia detoxifying process whereby ammonia was incorporated into glutamine (Walton & Cowey 1977) and other amino acids (Iwata *et al.* 1981; Dabrowska & Wlasow 1986), which could then be used as substrates for protein synthesis.

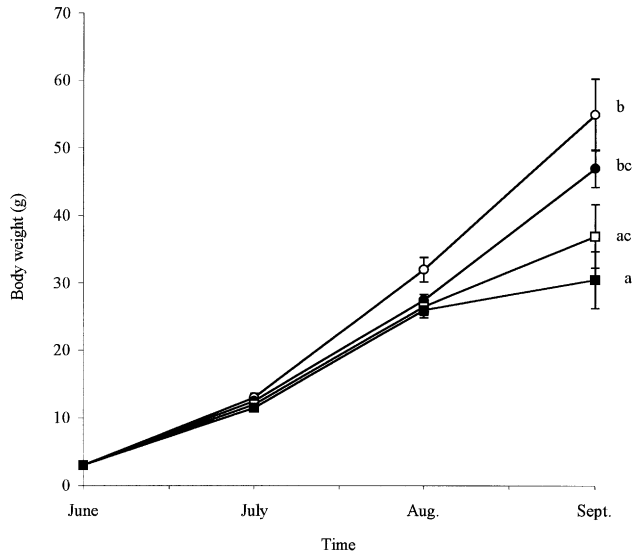


Fig. 3 Body weight of juvenile rainbow trout during exposure to ambient temperature (circles) or simulated global warming (+2 °C, squares) at control pH 6.2 (open symbols) or low pH 5.2 (closed symbols), in summer at a satiation ration. Values are means \pm SEM, $N=20$ (June), 60 (thereafter). Final weights that do not share a letter are significantly different from each other ($P < 0.05$). After Dockray *et al.* (1996).

Further investigation would be required to confirm this speculative hypothesis. However, regardless of the mechanisms involved, the positive growth effect of low-level ammonia has significant implications for the economics of freshwater aquaculture, where high water flow rates and associated heating costs are often used to ensure that ammonia does not rise above background levels.

Environmental acidity (low pH)

Exposure of freshwater fish to sublethal levels of environmental acidity has generally been reported to result in a decrease in plasma and/or whole-body ion concentrations (Lacroix 1985; Neville 1985; Audet *et al.* 1988). However, in the experiments reviewed, rainbow trout exposed to sublethal, low pH (5.2) in soft water showed few of these classical physiological symptoms, either when fed to satiation (Dockray *et al.* 1996; D'Cruz *et al.* 1998) or a ration of 1% body weight d^{-1} (Dockray *et al.* 1998). In fact, the appetite and growth of fish fed to satiation at pH 5.2 were significantly greater than those of fish at pH 6.2 regardless of season (Fig. 3). Even at the reduced ration, acid-exposed fish had a higher growth

rate than controls and hence a higher energy conversion efficiency (Dockray *et al.* 1998).

The principal site of acid toxicity is the gill (McDonald 1983; Wood 1989). Sublethal acidification (pH 5.2) inhibited gill protein synthesis in fish fed to satiation in summer (Wilson *et al.* 1996; Reid *et al.* 1997a). However, in both of these studies, gill protein degradation was also reduced at low pH, so that the net protein accretion was greater than in controls, in accordance with the whole-body growth results. It is possible that rather than being a direct toxicological effect; the reduction in protein degradation was an adaptive mechanism to maintain tissue growth and/or enzyme complement (Reid *et al.* 1997a). When protein metabolism was suppressed by reduced ration or low winter temperature, no effects of environmental acidification were observed (Morgan *et al.* 1998, 1999). Low pH also caused a decrease in liver protein accretion in fish fed to satiation, regardless of season (Reid *et al.* 1997a; Morgan *et al.* 1998). These results were unexpected, as acid toxicity is not generally associated with liver metabolism. One possible explanation is that exposure to low pH elevates plasma cortisol concentrations (Brown *et al.* 1990; Audet & Wood 1993), which in turn may maintain a high plasma glucose concentration at the expense of growth (Van der Boon *et al.* 1991). However, although plasma cortisol concentrations were generally higher at pH 5.2 than at pH 6.2, the differences were not significant (D'Cruz *et al.* 1998) and so this hypothesis cannot be confirmed.

The unexpected stimulation of appetite and growth at low pH, and the lack of any ionoregulatory impact, even at reduced ration, prompted investigation into the role of diet in mitigating the physiological effects of acid exposure. It was suggested that acid-exposed fish compensated for branchial ion losses with dietary salts, and that the resulting increase in food consumption led to increased growth rates. This idea was supported by earlier data of Sadler & Lynam (1986), who found that toxic effects of low pH were only observed if the fish were starved during acid exposure. When ration was reduced to maintenance levels (0.25% d^{-1} , delivered as a 1% meal once every four days), exposure to pH 5.2 did indeed cause ionoregulatory disturbances, such as lower whole-body Cl^{-} concentration (Fig. 4), and mortality increased threefold compared to the controls (D'Cruz *et al.* 1998). A further experiment in which the levels of salts and energy in the diet were manipulated confirmed independently that it is the salt rather than the energy in food that is responsible for preventing and/or correcting ionoregulatory disturbances (D'Cruz & Wood 1998). Indeed, a high dietary energy elevated oxygen consumption (SDA), and exacerbated the ion loss associated with a low salt diet (D'Cruz & Wood 1998).

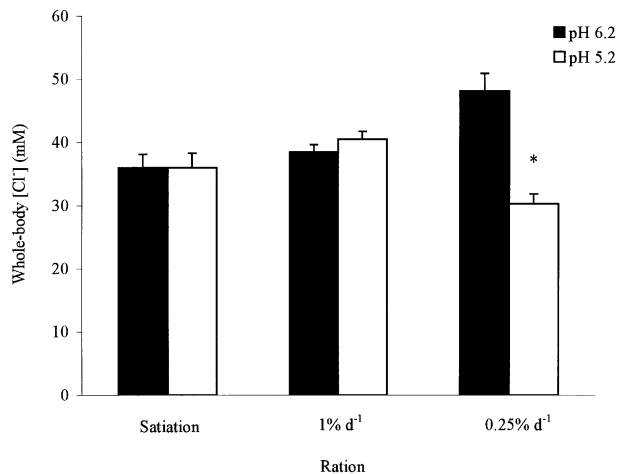


Fig. 4 The influence of food availability (ration) on the effect of exposure to environmental acidification (low pH 5.2) on whole-body chloride concentration in juvenile rainbow trout. Values are means \pm SEM, $N=6-18$. *Indicates a significant effect of low pH ($P < 0.05$).

Interactive effects of exposure to +2 °C and pollutants

+2 °C and ammonia

Under most conditions of ambient temperature and ration, exposure to either +2 °C or 70 μM T_{Amm} stimulated growth and energetics, and these separate treatment effects were additive. For example, when fed to satiation in winter, fish exposed to both treatments had the highest nitrogen absorption and retention efficiencies and highest energy conversion efficiencies (Linton *et al.* 1998b). Once again, an exception was seen at the very high ambient temperatures of late summer, when the beneficial effects of low-level ammonia were lost as a consequence of greater routine oxygen consumption, i.e. greater energy demands, of the simulated global warming (Linton *et al.* 1997). A similar inhibitory effect of the combined treatments was observed in liver protein metabolism (Reid *et al.* 1998).

+2 °C and environmental acidity

The effects of low pH were additive upon those of +2 °C, but the direction of this addition was dependent upon ration. At rations of $\geq 1\%$ d^{-1} the positive effects of low pH on growth (above) ameliorated the growth inhibition caused by simulated global warming during the high ambient temperatures of late summer (Dockray *et al.* 1996). Over this period, the food conversion efficiency of fish exposed to a combination of +2 °C and low pH was much larger than that of fish exposed to +2 °C alone

(Dockray *et al.* 1996). Similarly, when fish were fed to satiation in winter, those animals exposed to both low pH and +2 °C showed the greatest appetite, growth and food conversion efficiency (D'Cruz *et al.* 1998). However, when ration was reduced to approximately maintenance levels (0.25% d^{-1}), low pH and +2 °C had an additive, negative influence on ionoregulatory status: fish that were exposed simultaneously to +2 °C had significantly lower plasma $[\text{Na}^+]$ and $[\text{Cl}^-]$ than those exposed to either +2 °C or low pH alone.

Acclimation

The ecological impact of climate change on a coldwater fish such as the rainbow trout may depend upon the ability of the animals to adapt to the new conditions. Disagreement in the literature over the ability of freshwater fish to acclimate to environmental toxicants (e.g. acidification) can be partially explained by the use of different definitions of 'acclimation'. Some studies propose that recovery from the initial impact of the toxicant, either to the pre-exposure condition or to a new steady state, constitutes acclimation. For example, McWilliams (1980) interpreted the gradual recovery of plasma $[\text{Na}^+]$ and $[\text{Cl}^-]$ following an initial decline during exposure to low pH in brown trout (*Salmo trutta*) as evidence of acclimation. However, most toxicological studies now employ a stricter definition whereby acclimation requires '...an increased tolerance of an elevated, usually lethal concentration of a toxicant arising from chronic exposure to a sublethal concentration of that toxicant' (McDonald & Wood 1993). This definition was used in the present project to determine whether juvenile rainbow trout were able to acclimate to simulated global warming and/or environmental pollutants by challenging fish that had been chronically exposed to these conditions with acute, lethal temperature and/or pollutant concentrations.

+2 °C and ammonia

Previous studies have concluded that freshwater fish are able to acclimate to either increased temperature (Brett 1952; Elliott 1981, 1991) or ammonia (Redner & Stickney 1979; Thurston *et al.* 1981). However, as with much of the literature on temperature and pollutants, these studies have generally employed constant, well-separated temperatures and high levels of ammonia and so have little relevance to a global warming scenario. Following the 90 d exposures to +2 °C and/or 70 μM T_{Amm} in both summer and winter at a satiation ration, and in summer on a restricted ration (1% d^{-1}), juvenile rainbow trout were subjected to lethal temperature, lethal ammonia (1.8 mm T_{Amm}) and lethal temperature plus ammonia

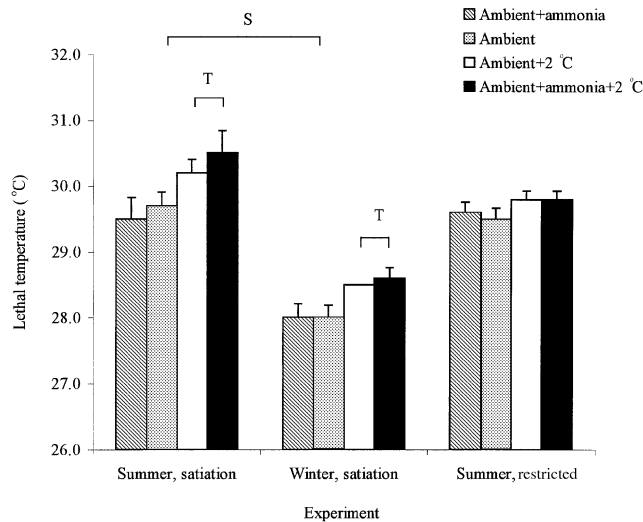


Fig. 5 The effect of exposure to simulated global warming (+2 °C) and/or 70 µm ammonia on lethal temperature of juvenile rainbow trout. Values are means ± SEM, $N=6-10$. T indicates a significant effect of +2 °C within an experiment; S indicates a significant seasonal effect between experiments ($P < 0.05$). After Linton *et al.* (1998c).

challenges. Mean lethal temperatures were 1.2–1.9 °C higher in summer than in winter, and when fed to satiation, fish exposed to +2 °C had a slightly (0.2–1.0 °C) but significantly higher lethal temperature than those exposed to ambient temperature, i.e. temperature acclimation occurred (Linton *et al.* 1998c). This difference was not seen in fish fed a restricted ration (Fig. 5). Ammonia pre-exposure did not alter temperature tolerance. However, the presence of 1.8 mm T_{Amm} reduced lethal temperature by 3–7 °C for all fish; pre-exposure to 70 µm T_{Amm} offered some protective effect. However, there was no evidence of acclimation to ammonia alone; pre-exposure to 70 µm T_{Amm} did not prolong survival during the lethal ammonia challenge. These results suggest that whilst trout may be able to adapt at least partially to climate change, this ability may be impaired by food availability and/or the simultaneous presence of high ammonia, and will not be affected by chronic pre-exposure to low level ammonia.

Environmental acidity

Previous studies that have employed the 'increased tolerance' definition of acclimation (above) have concluded that freshwater fish are unable to acclimate to environmental acidity (Falk & Dunson 1977; Wood *et al.* 1988a,b) or indeed that sublethal acid exposure results in reduced tolerance of further acidification, i.e. acid sensitization occurs (Audet & Wood 1988). However,

these studies generally employed very low or zero feeding rates. Results on the importance of diet in acid toxicity from the present authors raised the possibility that the ability of fish to acclimate to low pH may also be diet dependent. The unidirectional Na^+ fluxes were measured in juvenile rainbow trout that had previously been exposed to pH 5.2 (acid pre-exposed) or pH 6.2 (naïve) and fed either 1% d^{-1} or 1% $4d^{-1}$ for 90 days, during a 24-h challenge at pH 4.0. Although the ion losses were greater for those fish fed the lower ration, there was no difference between the response of the naïve and acid pre-exposed fish to the challenge at either ration (Morgan *et al.* 2000). It was therefore concluded that freshwater fish are unable to acclimate to sublethal acidification regardless of dietary history.

Conclusions

Despite the attention afforded to global climate change in recent years, very few experimental studies have been carried out to study the possible consequences for aquatic animals. This is likely because of the difficulty both in predicting the parameters, such as the magnitude and variation of the warming scenario, and in simulating this within the practical constraints of experimental biology. The experimental design used herein superimposed an additional, constant 2 °C—a conservative estimate of global warming—upon the natural thermal regime of inshore Lake Ontario. Whilst the utilization of a constant temperature increment may be simplistic, there is insufficient information to predict the seasonal variation in warming with any degree of certainty. Thus it is likely that the present experimental design, incorporating natural variation in water temperature, is currently the best simulation of global warming in freshwater that has been used in controlled, laboratory studies. The principal conclusions for freshwater fish from this project are:

1 The physiological effects of global warming may be minimal over much of the year and beneficial in winter, with positive effects on appetite, growth, N-metabolism, and protein accretion.

2 However, the additional temperature may threaten fish populations living towards the upper end of their thermal tolerance zone at the high ambient temperatures of (late) summer. The principal impacts at this time include suppression of appetite, growth, nitrogen metabolism, and decreases in net protein accretion principally due to increased degradation rate.

3 A reduction in food availability will not necessarily increase the impact of global warming. Indeed, unlimited food availability may have a negative impact on growth at high ambient temperatures due to the specific dynamic action (SDA) effect of food in raising metabolic demand.

4 Very low levels of sublethal ammonia promote growth, protein synthesis and nitrogen retention efficiency, suggesting that fish may be able to use ammonia as an additional substrate for protein synthesis.

5 The effect of environmental acidification is dependent upon food availability. If food is not limited, fish may compensate for branchial ion losses by an increase in intake of dietary salts, i.e. in appetite, and this will lead to an incidental increase in growth. If food availability is low, acidity causes ionoregulatory disturbance.

6 The effects of exposure to sublethal pollutants are additive upon those of simulated global warming. Where effects of the pollutant alone are beneficial, they may alleviate any impact of additional temperature. However, where the pollutant itself has detrimental effects, e.g. low pH at low ration, simultaneous exposure to global warming may invoke additional physiological costs.

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