Effects of chronic sublethal ammonia and a simulated summer global warming scenario: protein synthesis in juvenile rainbow trout (*Oncorhynchus mykiss*)

Scott D. Reid, T.K. Linton, J.J. Dockray, D.G. McDonald, and C.M. Wood

Abstract: Protein synthesis, net accretion, and degradation in liver, gill, and white muscle and ribosomal translational efficiency and protein synthesis capacity in liver and gill were measured using a flooding dose of [^{3}H]phenylalanine in juvenile rainbow trout (*Oncorhynchus mykiss*). The fish were chronically exposed (90 days) in hardwater to the presence or absence of sublethal ammonia (70 μmol total ammonia·L^{-1}) alone or in combination with a 2°C elevation in the normal temperature profile over the months of June–September 1993 (ambient temperature range 13–22°C). Chronic sublethal exposure to ammonia had little impact on gill protein synthesis and degradation (protein turnover) and even less in muscle. However, in the liver, both protein synthesis and degradation were stimulated following 60 days of the sublethal ammonia exposure. The 2°C elevation in temperature resulted in a slight increase in protein turnover in both gills and liver. However, during the period of peak water temperature, the 2°C elevation in temperature inhibited protein dynamics in these tissues. Overall, elevated environmental ammonia in combination with a summer global warming scenario would challenge the ability of fish to adapt to alterations in the quality of their environment, most notably during periods of peak temperatures.

Résumé : La synthèse des protéines, leur augmentation et leur altération nettes, dans le foie, les branchies et les muscles blancs, l'efficacité de la traduction ribosomique, et la capacité de synthèse protéinique du foie et des branchies, ont été mesurés à l'aide d'une dose massive de phénylalanine[H^3] chez des truites arc-en-ciel juvéniles (*Oncorhynchus mykiss*). Les poissons ont été exposés de façon chronique (90 jours) en eau dure à la présence ou à l'absence d'une dose sublétale d'ammoniac (70 μmol d'ammoniac total·L^{-1}), seule ou combinée à une élévation de 2°C du profil normal de température entre juin et septembre 1993 (plage de température ambiante de 13 à 22°C). Une exposition sublétale chronique à l'ammoniac a eu peu d'effet sur la synthèse et la dégradation (renouvellement) des protéines dans les branchies et encore moins dans les muscles. Toutefois, dans le foie, la synthèse et la dégradation des protéines ont été stimulées après 60 jours d'exposition à une dose sublétale d'ammoniac. La hausse de 2°C de la température s'est traduite par une légère augmentation du renouvellement des protéines dans les branchies et dans le foie. Toutefois, pendant la période où la température de l'eau était maximale, l'élévation de 2°C de la température a inhibé la dynamique des protéines dans ces tissus. En général, une concentration élevée d'ammoniac dans l'environnement, associée à un scénario de réchauffement global en été, met à l'épreuve la capacité du poisson à s'adapter aux altérations de la qualité de son environnement, surtout pendant les périodes de températures extrêmes. [Traduit par la Rédaction]

Introduction

Freshwater fish are exclusively poikilothermic, and therefore, their body temperature is essentially identical to, and set by, the temperature of the water in which they live. Temperature is the single most important factor determining their metabolic rate (i.e., cost of living), which in turn influences a variety of physiological variables ranging from reproductive rate to growth rate to swimming performance (see Reid et al. 1995). The effects of climate change on fish and fisheries are therefore likely to be substantial, and yet, little attention has been drawn towards its impact on freshwater populations (Schindler et al. 1990). Furthermore, there are little or no hard experimental data on the chronic effects of relatively small temperature elevations on freshwater fish species over the annual cycle (see Reid et al. 1997*b*). Indeed, most of the biological data available to climate change modellers do not satisfactorily reflect real-world conditions (DeAngelis and Cushman 1990; Regier et al. 1990). Furthermore, perhaps the most important water quality issue in Canada today is contamination; most aquatic organisms no longer live in a pristine environment. There are virtually no data on how the effects of incremental temperature changes will interact with the effects of anthropogenic toxicants. This was the rationale behind a project that our laboratory initiated to examine the physiological impact on freshwater fish of exposure to a reasonable proximation of the global warming scenario (Mohnen and Wang 1992). Juvenile rainbow trout (*Oncorhynchus mykiss*) were chronically exposed to a small increase (+2°C) in water temperature superimposed upon the natural temperature profile representative of

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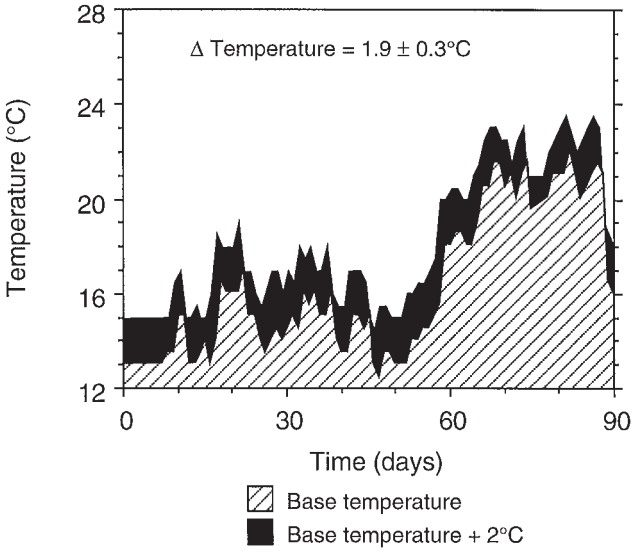
Fig. 1. Temperature profile for the hardwater exposure system during the 90-day experiment that ran over the summer of 1993 from June 18 (day 0) to September 15 (day 90). Juvenile rainbow trout were exposed to the natural daily and seasonal temperature variability representative of inshore Lake Ontario. Daily measurements were made on test tanks receiving base temperature water or the base temperature raised by 2°C.



inshore Lake Ontario. In addition, the effects of the elevation in the natural thermal regime were studied alone and in combination with sublethal, environmentally realistic concentrations of two common pollutants: low water pH (in softwater) and ammonia (in hardwater). Reid et al. (1995, 1997*a*) have provided an overview of the project as a whole, Dockray et al. (1996) have presented specific details on the physiological responses to the low pH–warming regime, and Linton et al. (1997) have presented parallel information on the physiological responses to the elevated ammonia–warming regime.

The objective of the present study was to determine the metabolic costs of a combination of increased temperature and elevated ammonia on fish as measured by tissue protein dynamics. The study parallels that of Reid et al. (1997*a*) on tissue protein dynamics of trout under the low pH–warming regime in softwater. It is generally believed that exposure to an environmental stressor and particularly adaptation to extreme environments are associated with increased metabolic costs (Calow 1991). Such shifts in the energy budget towards the defense of the internal environment suggest that less energy is available for other processes such as growth, activity, and reproduction. Protein dynamics were measured, as any change in the ability of the animal to grow, maintain electrolyte homeostasis, or increase fuel utilization might well be reflected in alterations in protein synthesis, accretion, or degradation in tissues directly or indirectly affected by the environmental stressor. In addition, it has been shown that a correlation exists between protein synthesis and metabolic rate in fish (Jobling 1985; Houlihan 1991) and that protein turnover rates are altered during and after exposure to toxicants (see Reid et al. 1997*b*).

The effects of chronic exposure to sublethal ammonia exposure are well documented and include alterations in gill morphology, reduced food uptake assimilation and growth, hypoglycaemia, increased liver glycogen and plasma Na^+ concentration, and reduced haemoglobin oxygen-carrying capacity (see Linton et al. 1997). These effects could result in or be the result of increased metabolic costs. The majority of these studies were conducted at total ammonia concentrations that were not environmentally realistic and were considerably higher than that used in the present and companion study (Linton et al. 1997). However, despite these differences, we predicted that the combination of elevated temperature and chronic sublethal ammonia would result in significant alterations in tissue protein dynamics, the cost of which could represent a serious challenge to the animals' ability to adapt to these and other changes in the quality of their environment.

Materials and methods

Experimental animals

Approximately 1600 rainbow trout of both sexes weighing initially 2–5 g were obtained April 19, 1993, from Rainbow Springs Hatchery (Thamesford, Ont.). Fish were held in two 600-L fiberglass tanks supplied at a rate of 2.5 $L \cdot min^{-1}$ with dechlorinated Hamilton tap water (moderately hard water: $[Ca^{2+}] = 1.95 \pm 0.22$ $mequiv. \cdot L^{-1}$, $[Na^+] = 0.56 \pm 0.03$ $mequiv. \cdot L^{-1}$, pH = 7.4–7.8) at the city supply temperature (inshore Lake Ontario; initially 11°C). During this initial holding period (6 weeks), fish were fed dry trout pellets (Zeigler, Salmon Starter) at a ration of 1% of body weight per day (wet weight basis). During the chronic exposures, fish were hand-fed to satiation twice daily as detailed in Dockray et al. (1996). Photoperiod was controlled and was adjusted weekly to mimic the natural photoperiod during the course of acclimation and chronic exposure.

Chronic exposure to elevated temperature and total ammonia

Fish were exposed for 90 days (starting on June 18, 1993, and ending on September 15, 1993) to one of four combinations of temperature and total ammonia in replicated tanks (eight 270-L tanks; 150 fish per tank) continuously supplied with aerated City of Hamilton dechlorinated tap water at 2.5 $L \cdot min^{-1}$. The four treatments were ambient water temperature (referred to as the "base" water temperature treatment) or base temperature raised by 2°C (base+2°C), each with or without an additional 70 μM total ammonia (base+Am and base+2°C+Am, respectively). Heat (+2°C) was added to the water using a countercurrent heat exchanger as detailed in Reid et al. (1995).

When exposures were started in mid-June, base and base+2°C water temperatures were 13 and 15°C, respectively (Fig. 1). During the initial 30 days, average water temperature rose only slightly. However, the day-to-day variability in temperature was as much as 4°C during this time, with peak temperatures reaching 17 and 19°C in the base and base+2°C treatment groups, respectively. The second 30-day period (days 30–60) was characterized by a gradual decline in water temperature in both treatment groups as the water temperature fell by approximately 2°C over the first 20 days of this period. However, the greatest increase in temperature was observed during the subsequent 10 days of this period as water temperatures rose rapidly by 5.5°C in both the base and base+2°C treatment groups. The highest water temperatures were reached during the final 30 days of this study (days 60–90), reaching peak temperatures of 22 and 23.5°C in the base and base+2°C exposure groups. Following 20–23 days of extreme temperature, water temperature fell dramatically over the final days of the experiment. During this period, fish experienced the single largest drop in water temperature as it fell by 5°C in both the base and base+2°C treatment groups.

Ammonia (total ammonia = $NH_4^+ + NH_3$) was added using mariotte bottles that were adjusted manually to deliver a 0.05 M $(NH_4)_2SO_4$ stock at an appropriate rate to achieve a nominal water

total ammonia of 70 μmol·L^{-1}. Ammonia concentrations in water were monitored by the salicylate–hypochlorite method of Verdouw et al. (1978). Ambient water total ammonia was found to be 5.9 ± 0.63 (35) μmol·L^{-1}. The ammonia concentrations in the treatment groups exposed to elevated total ammonia were within approximately 20% of the nominal concentration (70 μmol·L^{-1}), with individual tank concentrations ranging from 58.2 ± 5.6 (10) to 78.6 ± 7.6 (10) μmol·L^{-1}. The elevation of water total ammonia had no influence on water pH, and water partial pressure of O_2 (P_{O_2}) was maintained well above 100 Torr (1 Torr = 133.322 Pa), with in-tank P_{O_2}s ranging from 121.3 ± 4.1 (15) to 134.6 ± 3.9 (15) Torr. In-tank water pH was monitored manually as described in Dockray et al. (1996) and ranged from 7.4 to 7.8 (mean = 7.6 ± 0.26). A detailed analysis of in-tank water quality for all eight tanks is presented in Linton et al. (1997).

Rates of protein synthesis, accretion, and degradation

Protein synthesis, accretion, and degradation rates in branchial baskets, liver, and white muscle were determined for temperature controls and their respective treatment groups before starting (day 0) and at 30, 60, and 90 days of exposure.

Tissue protein synthesis was determined from the incorporation of radioactive phenylalanine based on the method of Garlick et al. (1980), which was modified by Houlihan et al. (1986) for use in fish. Fish were not fed 24 h before injection. On the day of injection, six fish from each tank (N = 12 per treatment) were randomly selected, quickly blotted dry, weighed to the nearest 0.01 g, and then injected via the caudal vein with a solution of 150 mM [^{3}H]L-phenylalanine (Sigma, St. Louis, Mo.) containing 37 × 10^6 Bq L-2,6-[^{3}H]phenylalanine·mL^{-1} (Sigma) in Cortland saline (Wolf 1963) at pH 7.5 (injection dose specific activity = 1480 dpm·nmol^{-1}). The dose was 1.0 mL·100 g body weight^{-1} and the fish were not anaesthetized. Following injection, the fish were placed in individual darkened 1-L containers fitted with lids and airlines and containing water taken from the treatment tank from which they had been removed. Approximately 60 min postinjection, fish were killed by a blow to the head and the branchial basket, liver, and a sample of white muscle dorsolateral to the dorsal fin were dissected out and frozen in liquid nitrogen. Individual dissections were completed within 2–4 min. Once frozen, tissues were individually wrapped in aluminum foil, temporarily stored in liquid nitrogen until all fish had been sampled, and then stored at –75°C for later weighing (organ somatic indices) and analysis (protein turnover).

Analysis

Tissue protein and RNA content and fractional rate of protein synthesis were analyzed as detailed in Houlihan et al. (1986). Briefly, tissue samples were homogenized using an electric tissue grinder (IKA A10) in ice-cold 20% perchloric acid (PCA) and the denatured proteins separated by centrifugation. PCA in the supernatant was precipitated with tripotassium citrate and centrifuged, leaving the free phenylalanine in solution. Phenylalanine was converted to β-phenylethylamine by L-tyrosine decarboxylase, extracted using *n*-heptane, and analyzed by a ninhydrin reaction. The content of [^{3}H]phenylalanine was measured using liquid scintillation counting techniques with quench correction. NaOH was used to resuspend the PCA-extracted tissue pellet and duplicate aliquots were taken for analysis of protein content (Lowry et al. 1951). The remaining suspension was acidified with PCA, centrifuged, and the resultant supernatant analyzed for total RNA by the orcinol assay of Munro and Fleck (1966). The pellet, containing protein and DNA, was washed twice with PCA and then hydrolyzed with HCl. Subsequently, the HCl was removed by evaporation. The free amino acids were resuspended in sodium citrate buffer and phenylalanine in the samples determined as described above. The protein synthesis rate (K_s, percent per day) was calculated as

$$K_s = \frac{SA_p}{SA_f} \times \frac{1440}{t} \times 100$$

where SA_p is the protein-bound specific activity (disintegrations per minute per nanomole), SA_f is the specific activity of the pool of free amino acids (disintegrations per minute per nanomole; average measured value 1678.2 ± 174.0, N = 40), 1440 is the number of minutes in a day, t is the exact time (minutes) from the [^{3}H]phenylalanine injection to tissue sampling, and 100 is the conversion to percentage.

The rate of protein degradation (K_d, percent per day) for gill and liver was calculated as the difference between the rate of protein synthesis and the net protein accretion of the tissues (K_g, percent per day), with the latter determined as the product of the average tissue growth rate and average tissue protein content. Specific growth rates were calculated using the equation of Ricker (1979):

$$\text{Growth rate } (K_g, \%\cdot\text{day}^{-1}) = \frac{(\ln W_2 - \ln W_1)}{t} \times 100$$

where W_1 and W_2 are the final and initial weights (grams), respectively, and t is the length of the growing period (days).

This method of estimating protein turnover greatly underestimates protein synthesis rate in white muscle when incorporation times are less than 2 h (Foster et al. 1992). Therefore, white muscle protein synthesis rate must be considered only a relative estimate of protein synthesis in this tissue. Further, it is for this reason that white muscle protein degradation rate could not be calculated.

Liver and gill somatic indices were calculated as the ratio of organ weight to total fish weight, expressed as a percentage of the total, before starting the exposures and at each subsequent sampling period.

As there were differences in the final body weights of the fish, protein synthesis and accretion rates were corrected for body size to a standard 40-g fish (the average weight of the fish following the 90-day exposure) using the log transformation of the allometric equation $Y = aX^b$ and exponents of –0.2 for protein synthesis and –0.41 for protein accretion in accordance with the methods of Houlihan et al. (1986) and Jobling (1983).

Protein synthesis capacity was determined as the ratio of micrograms of tissue RNA to milligrams of tissue protein and tissue translational efficiency of RNA (K_{RNA}, grams protein synthesis per gram RNA per day) calculated by dividing the protein synthesis capacity by the appropriate fractional rate of protein synthesis value multiplied by 10 (Sugden and Fuller 1991).

Statistical analysis

Values shown in figures are means ± 1 standard error of the mean (SE). Statistical differences between treatment means on a specific day were determined by analysis of variance (ANOVA) followed by Fisher's LSD multiple comparison test, using a commercial statistical software package (Statview 512+); 95% was accepted as the level of confidence.

Results

Organ somatic indices

Before exposure, the branchial baskets and liver of the hardwater-acclimated trout represented approximately 2.8 and 1.4% of the total body weight, respectively (Fig. 2). Values tended to decline slightly during the exposures, but none of the treatment effects were statistically significant. Thus, organ somatic indices were not sensitive indicators of the organ-specific impact of combinations of chronic exposure to elevated environmental temperature and water total ammonia.

Protein turnover: gill

Before exposure, gills of these hardwater-acclimated fish

Fig. 2. Effect of water temperature and total ammonia level on the (A) branchial basket and (B) liver somatic indicies of hardwater-acclimated fish. The organ somatic indicies were measured before exposure (day 0) and at 30, 60, and 90 days of exposure. Solid histograms, control water temperature and ambient total ammonia (6.0 μmol·L^{-1}); angled hatched histograms, base water temperature with elevated total ammonia (70 μmol·L^{-1}); open histograms, ambient total ammonia at base+2°C; horizontally hatched histograms, base+2°C water with elevated total ammonia. Data are presented as means ± 1 SE (N = 12).

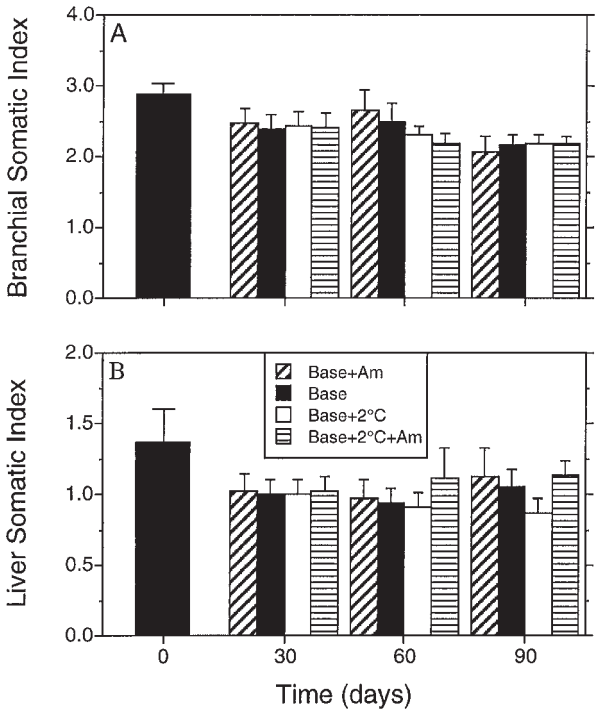
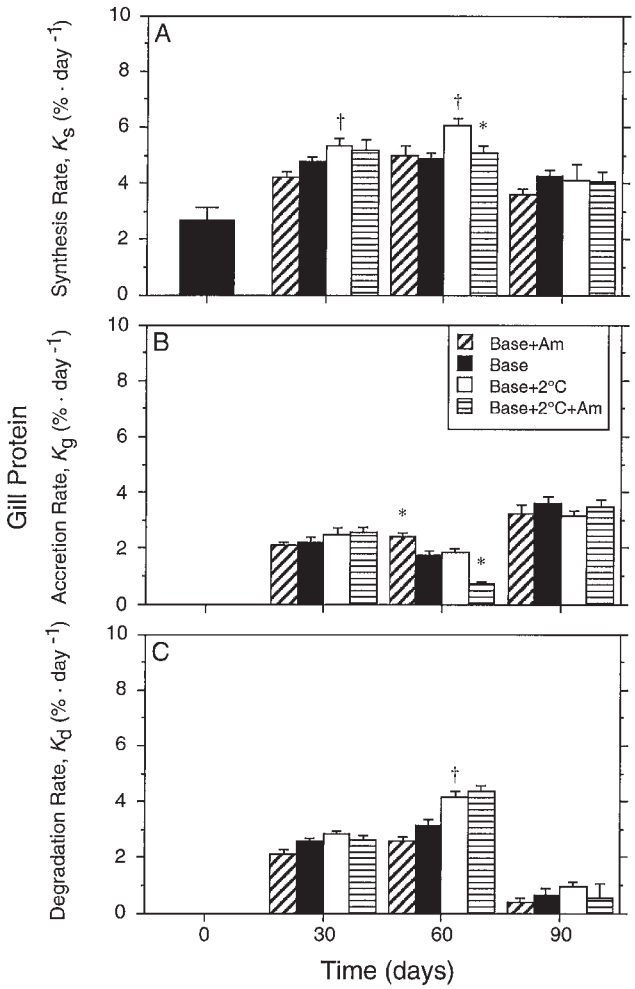


Fig. 3. Effect of water temperature and total ammonia level on gill protein (A) synthesis, (B) accretion, and (C) degradation rates in hardwater-acclimated trout. All rates were calculated on a %·day^{-1} basis and determined as outlined in Materials and methods. Data are presented as outlined in Fig. 2. Protein synthesis and accretion rates were corrected to a standard body size of 40 g (see Materials and methods for details). An asterisk indicates a statistically significant difference (P < 0.05) between the two levels of total ammonia (6.0 versus 70 μmol·L^{-1}) at the same temperature and a dagger indicates a significant difference (P < 0.05) between temperature groups at the ambient water ammonia concentration (6.0 μmol·L^{-1}).



synthesized proteins at a body size corrected rate of approximately 3%·day^{-1} (Fig. 3A). Rates increased to about 5%·day^{-1} at days 30 and 60 but fell again to about 3.5%·day^{-1} at day 90. The addition of 2°C (base+2°C) was found to influence gill protein synthesis of hardwater-acclimated trout to a greater extent than was observed in softwater-acclimated trout exposed under nearly identical conditions (Reid et al. 1997*a*). At both days 30 and 60, gill protein synthesis was greater in the base+2°C group than in the base temperature group. Also, there is some evidence to suggest that exposure to elevated total ammonia may have inhibited gill protein synthesis. However, only at day 60 and within the higher temperature treatment groups did elevated total ammonia significantly reduce gill protein synthesis. In general, it appears that ammonia had less effect on protein synthesis in this tissue than did exposure of softwater-acclimated juvenile rainbow trout to pH 5.2 under similar conditions (Reid et al. 1997*a*).

No significant influence of water temperature and ammonia on net gill protein accretion was obvious at either day 30 or 90 (Fig. 3B), although some differences were statistically significant at day 60. At this time, protein accretion rates in gills from fish at base temperature were significantly enhanced at elevated total ammonia, while the opposite response was observed at the higher water temperature (base+2°C), despite the fact that, alone, water temperature appeared to have no influence on gill protein accretion rate at this time. Interestingly, these effects at day 60 were similar to those changes in responses to temperature and pH in gills from softwater-acclimated fish at the same time (Reid et al. 1997*a*).

Protein degradation rates in gills of hardwater-acclimated fish tended to reflect changes in protein synthesis in response to elevated water temperature and total ammonia (Fig. 3C). Exposure to elevated water temperature tended to enhance rates of protein degradation, while in five of six cases, exposure to elevated total ammonia suppressed the rate at which proteins were broken down, although the differences were not statistically significant. Note the large suppression of protein

Fig. 4. Effect of water temperature and total ammonia level on gill protein content. All values were calculated on a percent basis (mg protein·mg sample^{-1} × 100%) and were determined as outlined in Materials and methods. Data are presented as outlined in Fig. 2 and statistically evaluated as in Fig. 3.

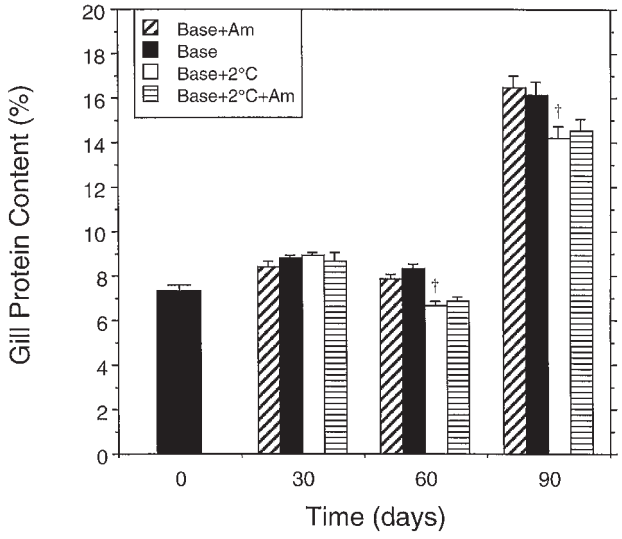
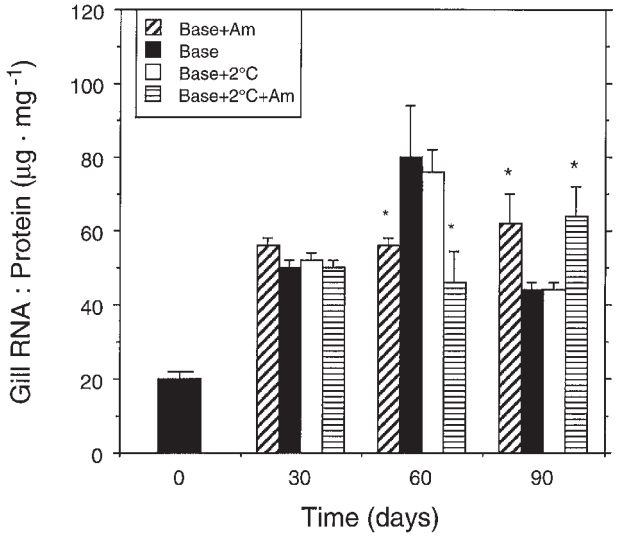


Fig. 5. Effect of total ammonia and water temperature on gill RNA:protein. The RNA:protein values (tissue protein synthesis capacity) were calculated and expressed as mg RNA·mg protein^{-1} and were determined as outlined in Materials and methods. Data are presented as outlined in Fig. 2 and statistically evaluated as in Fig. 3.



degradation rates at day 90 in all groups, shortly after peak water temperatures.

Before the start of the experiment, gill protein content was 7.4 ± 0.3% (milligrams of protein per 100 mg of tissue, Fig. 4). During the first 60 days of exposure, gill protein content changed very little, although it was slightly suppressed in the base+2°C groups at day 60. However, the mean gill protein content of all treatment groups approximately doubled at day 90, again with evidence of suppression by the addition of 2°C. There was no apparent influence of elevated water total ammonia at any time.

Gill protein synthesis capacity (RNA:protein), initially 20 ± 2.1 µg RNA·mg protein^{-1}, increased approximately 2.5 times during the first 30 days of exposure as a result of dramatic increases in gill RNA content (Fig. 5). This increase in gill RNA, relative to gill protein, resulted in a 27% reduction in gill translational efficiency (Table 1). No treatment effect was evident at this time. However, during the subsequent 30 days, the protein synthesis capacity in gills of fish held under conditions of ambient water total ammonia increased an additional 1.5 times. RNA:protein values in the gills of fish exposed to elevated water total ammonia were significantly lower than in their respective controls, and the gill protein synthesis capacity in these fish was not different from day 30 values. At the same time, gill translational efficiencies in fish exposed to the elevated water ammonia concentration tended to be greater than in the appropriate controls (Table 1).

During the final 30 days, the period of peak water temperatures, dramatic increases in gill protein content were noted. Despite the near doubling of gill protein content over this period, values for fish exposed to base+2°C water remained significantly lower than for the ambient fish. The gill protein synthesis capacity of the base and base+2°C fish dropped dramatically from the day 60 values, while, similar to the previous 30 days of exposure, little change was evident in the gill RNA:protein of those fish exposed to 70 µmol total ammonia·L^{-1}. The result was that at day 90, the gill protein synthesis capacities of fish exposed to elevated ammonia were significantly greater than in their respective controls. Despite this, gill translational efficiencies were significantly lower in the 70 µmol total ammonia·L^{-1} fish than in the appropriate controls (Table 1).

Table 1. Branchial ribosomal translational efficiency (K_{RNA}, g protein synthesis·g RNA^{-1}·day^{-1}) from juvenile rainbow trout chronically exposed to elevated water total ammonia (Am, 70 µmol·L^{-1}) and an elevated seasonal temperature profile (+2°C).

	Time			
Exposure group	Day 0	Day 30	Day 60	Day 90
Base+Am		0.86±0.09	0.88±0.06	0.58±0.15*
Base	1.33±0.23	0.95±0.07	0.61±0.18	0.97±0.08
Base+2°C		1.03±0.07	0.79±0.12	0.94±0.08
Base+2°C+Am		1.04±0.08	1.11±0.60	0.63±0.14*

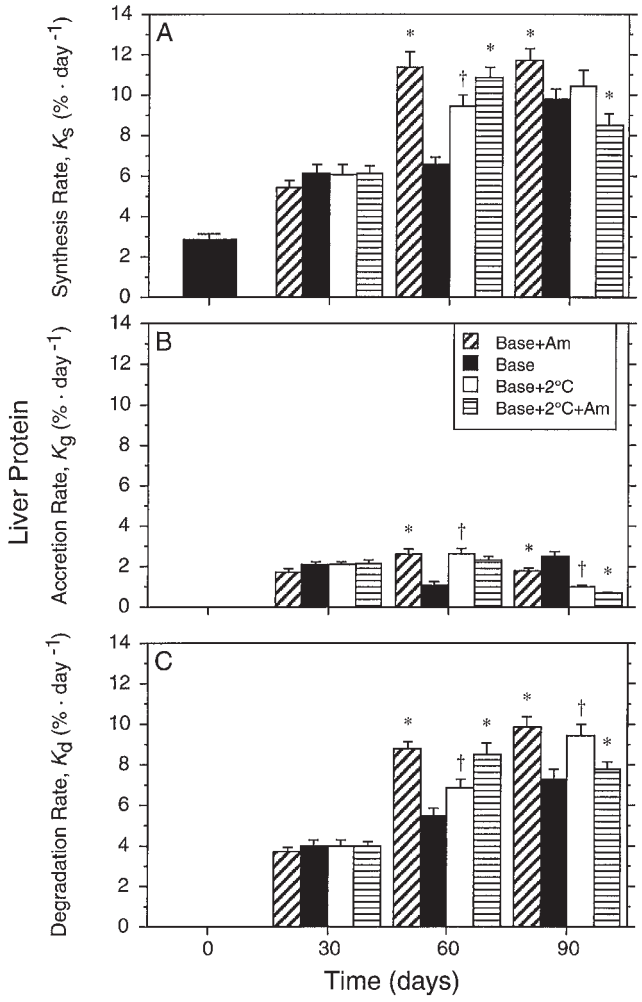
Note: Values are the means ± SE with a sample size of 12 for each group. Ribosomal translational efficiency was calculated from the RNA:protein (Fig. 5) and the fractional rate of protein synthesis (Fig. 3).

*Statistically significant difference ($P < 0.05$) between the two levels of water total ammonia at the same temperature.

Protein turnover: liver

Protein synthesis in the livers of hardwater-acclimated juvenile trout were similar to those of softwater-acclimated fish (Reid et al. 1997*a*) at approximately 3%·day^{-1} before their exposure to the treatment regime (Fig. 6A). Liver protein dynamics were more consistent than those of the gills in their response to chronic exposure to elevated total ammonia and additional temperature. At day 30, there was little influence of treatment on liver protein synthesis. After 60 days of exposure, protein synthesis was significantly increased in response to chronic elevations in total ammonia by approximately 65 and

Fig. 6. Effect of water temperature and total ammonia level on liver protein (A) synthesis, (B) accretion, and (C) degradation rates in hardwater-acclimated trout. All rates were calculated on a %·day^{-1} basis and determined as outlined in Materials and methods. Data are presented, body size corrected, and statistically evaluated as outlined in Figs. 2 and 3, respectively.



40% at base and base+2°C temperatures, respectively. In addition, liver protein synthesis was significantly enhanced by the addition of 2°C to the base temperature at ambient levels of water total ammonia. At day 90, protein synthesis in the base temperature group remained significantly enhanced in the presence of elevated total ammonia (base+Am). However, it appeared that the high water temperatures experienced by the base+2°C fish at this time resulted in a reduction in protein synthesis because there was no longer any significant enhancement in protein synthesis as a result of the additional temperature or total ammonia. Furthermore, elevated total ammonia during this time resulted in a significant inhibition of liver protein synthesis in fish at the higher water temperature (base+2°C+Am), a result opposite to that in fish at ambient water temperature (base+Am).

Liver protein accretion was insensitive to alterations in water temperature and total ammonia levels during the first 30 days of exposure (Fig. 6B). At day 60, however, water temperature and total ammonia levels induced alterations in protein accretion similar to the observed changes in protein synthesis (Fig. 6A). Both the 2°C elevation in water temperature at ambient ammonia levels and increased total ammonia at the ambient water temperature significantly enhanced net accumulation of protein in the liver. By day 90, however, the stimulatory influence of both temperature and ammonia had been lost. In fact, both the increase in water temperature and the increases in total ammonia inhibited, rather than enhanced, liver protein accretion.

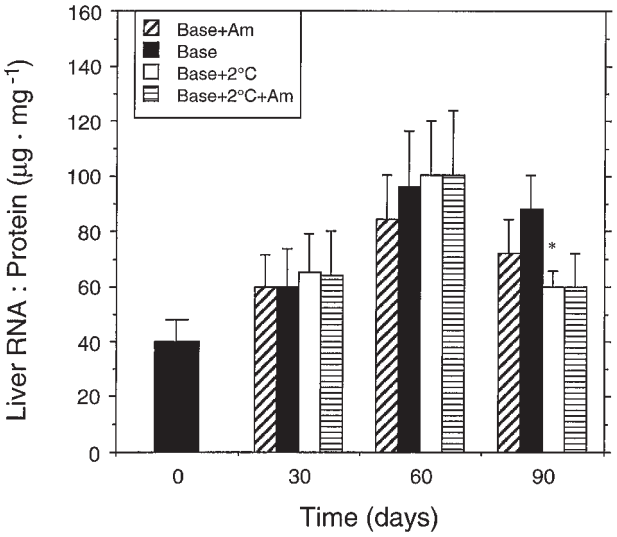
Liver protein degradation rates tended to reflect changes in protein synthesis in response to elevated water temperature and total ammonia. Exposure to elevated water temperature resulted in significantly greater protein degradation at both days 60 and 90 (Fig. 6C). At day 60, exposure to elevated total ammonia also significantly enhanced the rate at which liver proteins were broken down at both temperatures. During the final 30 days, exposure to elevated total ammonia significantly altered liver protein degradation, but in opposite ways depending on temperature. At the ambient temperature, the additional ammonia stimulated protein degradation. However, during this period of peak ambient water temperature and in the base+2°C treatment group, the elevated total ammonia (base+2°C+Am) resulted in a significant reduction in protein degradation.

Before the start of the experiment, liver protein content was 23.4 ± 2.8% (milligrams of protein per 100 mg of tissue) with little change throughout the 90-day exposure. At the 60-day period, liver protein content was reduced 8% in fish exposed to 70 μmol total ammonia·L^{-1} at the base temperature and 14% when exposed at the base+2°C temperature. At day 90 of the exposure, liver protein content of the base+2°C+Am fish was significantly greater than in their respective controls (base+2°C), 27.5 ± 0.5 and 24.5 ± 0.7 mg protein·100 mg tissue^{-1}, respectively.

The liver protein synthesis capacity (RNA:protein), initially 40 ± 6 μg RNA·mg protein^{-1}, increased gradually over the first 60 days of exposure to reach a peak of 100 μg RNA·mg protein^{-1} in the base+2°C treatment group (Fig. 7). No treatment effects were evident during this period of the exposure. Following the period of peak environmental temperatures, days 60–90, liver protein synthesis capacities in all treatment groups tended to fall. Although an influence of elevated water ammonia on liver protein synthesis capacity was not evident, the addition of +2°C caused a significant inhibition.

Liver ribosomal translational efficiency was 0.71 ± 0.13 g protein·g RNA^{-1}·day^{-1} before the start of the exposures (Table 2). At day 30, liver translational efficiency in all groups increased by an average of 35% as a result of the near doubling of protein synthesis (Fig. 6A) and an approximate 50% increase in RNA:protein (Fig. 7) in the liver. However, no treatment effect was apparent at this time. This modification in ribosomal translational efficiency was again observed during the following 30-day period. However, at day 60, a treatment effect was apparent, as the ribosomal translational efficiency of trout exposed to elevated total ammonia at the ambient temperature (base+Am) was significantly greater than that in the base temperature, ambient total ammonia fish. During the final 30 days of the experiment, ribosomal translational efficiency increased in all treatment groups, as was observed for the previous two sampling periods (Table 2). The increase in

Fig. 7. Effect of total ammonia and water temperature on liver RNA:protein. The RNA:protein values (tissue protein synthesis capacity) were calculated and expressed as μg RNA·mg protein^{-1} and were determined as outlined in Materials and methods. Data are presented as outlined in Fig. 2 and statistically evaluated as in Fig. 3.



ribosomal translational efficiency at day 90 was attributed to the overall decrease in liver protein synthesis capacity (Fig. 7) more so than to any change in liver protein synthesis (Fig. 6A). As was observed at day 30, no treatment effect was apparent at this time.

Protein turnover: white muscle

White muscle protein synthesis declined over time in all treatment groups but was not influenced by chronic exposure to elevated total ammonia or temperature (Fig. 8A).

White muscle protein accretion in hardwater-acclimated fish was also unaffected by treatment during the first month of exposure (Fig. 8B). During the second month, protein accretion was significantly greater in both elevated ammonia (at ambient water temperature; base+Am) and elevated temperature treatments due to an apparent reduction in the white muscle protein accretion rate in fish exposed to the base temperature treatment and ambient total ammonia (base). At day 90, the net accumulation of protein in this tissue was affected in a manner nearly identical to that observed during this period in this tissue of softwater-acclimated fish exposed for 90 days to combinations of water temperature and pH (Reid et al. 1997*a*). At base temperature, elevated total ammonia (base+Am) inhibited protein accretion, as did the elevation in water temperature (at ambient total ammonia; base). As mentioned in the Materials and methods section, white muscle degradation rates could not be calculated because of the short incorporation times for phenylalanine with respect to this tissue.

White muscle protein content (11.8 ± 0.7%) was not influenced by water temperature or total ammonia over the duration of the 90-day experiment. RNA content was similarly unaffected which resulted in a relatively unaltered RNA:protein of 6.0 ± 0.3 μg RNA·mg protein^{-1}. Thus, white muscle

Fig. 8. Effect of water temperature and total ammonia level on white muscle protein (A) synthesis and (B) accretion rates in hardwater-acclimated trout. All rates were calculated on a %·day^{-1} basis and determined as outlined in Materials and methods. Data are presented, body size corrected, and statistically evaluated as outlined in Figs. 2 and 3, respectively.

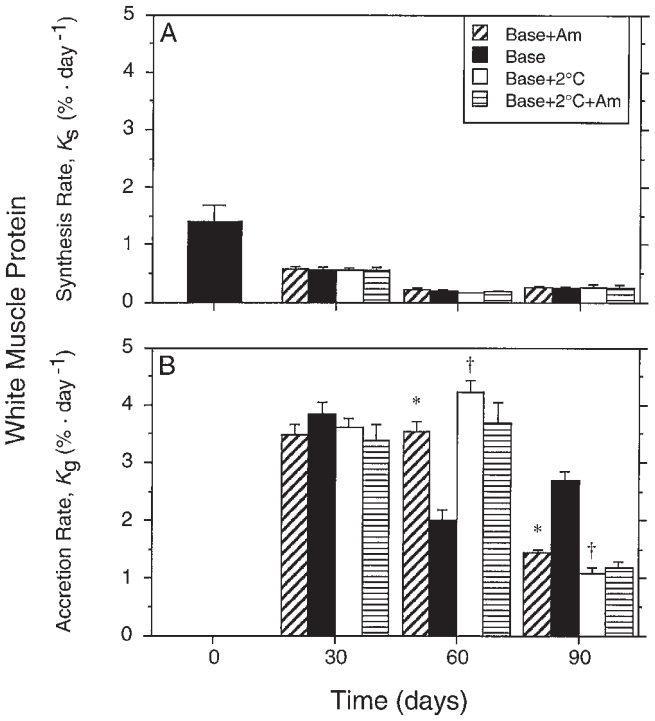


Table 2. Liver ribosomal translational efficiency (K_{RNA}, g protein synthesis·g RNA^{-1}·day^{-1}) from juvenile rainbow trout chronically exposed to elevated water total ammonia (Am, 70 μmol·L^{-1}) and an elevated seasonal temperature profile (+2°C).

	Time			
Exposure group	Day 0	Day 30	Day 60	Day 90
Base+Am		0.91±0.17	1.35±0.26*	1.62±0.27
Base	0.71±0.13	1.02±0.24	0.68±0.12	1.11±0.15
Base+2°C		0.94±0.20	0.93±0.18	1.73±0.24
Base+2°C+Am		0.96±0.24	1.08±0.25	1.41±0.29

Note: Values are the means ± SE with a sample size of 12 for each group. Ribosomal translational efficiency was calculated from the RNA:protein (Fig. 7) and the fractional rate of protein synthesis (Fig. 6).

*Statistically significant difference ($P < 0.05$) between the two levels of water total ammonia at the same temperature.

translational efficiency simply reflected the changes in protein synthesis over the duration of the exposure (data not shown).

Discussion

In the past, tissue protein turnover rates have been used successfully to provide general estimates of the costs associated with acute or chronic sublethal exposure to stressors such as waterborne metals (Zn: Hogstrand et al. 1995; Al: Wilson et al. 1996*a*), sewage sludge (Houlihan et al. 1994), temperature (Goolish et al. 1984; Fauconneau and Arnal 1985; Loughna and Goldspink 1985; Foster et al. 1992), and environmental

acidification (Wilson et al. 1996*a*; Reid et al. 1997*a*). The application of the protein synthesis assay to the present experimental design, the factors that influence it, the limitations of the assay, the interpretation of metabolic cost based on changes in protein turnover, and the unique features of the current approach have been discussed elsewhere (Reid et al. 1995, 1997*a*, 1997*b*). In the companion study (Reid et al. 1997*a*), fish were similarly exposed to two temperature profiles separated by approximately 2°C, but in the presence and absence of low water pH in softwater.

Temperature

Due to the direct link between the environmental temperature and the metabolic rate in ectotherms, it might be predicted that chronic exposure to a slightly warmer environment (i.e., +2°C) would enhance protein synthesis in fish. This is clearly the case when exposure temperatures have differed considerably more than in the present study (Δ8°C: Fauconneau and Arnal 1985; Δ15°C: Loughna and Goldspink 1985). In addition to the much larger differences in temperature, these and most similar studies have used fish that are acclimated and exposed to constant temperature. Not only was the temperature difference between our control (base) and +2°C fish quite small, this difference was superimposed upon a temperature profile that incorporates much of the natural daily and seasonal temperature variation that would be associated with the inshore region of Lake Ontario. Nonetheless, despite these unique characteristics of this and the similarly designed companion study (Dockray et al. 1996; Reid et al. 1997*a*), we demonstrated numerous physiological responses to chronic exposure to a slightly warmer environment, including changes in tissue protein turnover, the absolute sum of protein synthesis and protein degradation.

In this study, gill and liver protein turnover increased by an overall average of 18.4% as a result of exposure to water temperature only 2°C above the base temperature, with the largest change in protein turnover occurring between days 30 and 60. Protein turnover has been shown to be directly proportional to ration size (Houlihan et al. 1988, 1995); however, there was no difference in the food intake between these two groups (Linton et al. 1997). These findings are similar to the increase in gill and liver protein turnover that occurred when similarly acclimated juvenile rainbow trout were exposed to a 2.0 ± 0.2°C elevation in the winter temperature profile in our laboratory system (Morgan et al. 1998). These authors found that gill and liver protein turnover increased by an average of 20.5% as a result of the 2°C increase in water temperature during the months of January–April. Furthermore, when softwater-acclimated juvenile rainbow trout were exposed to a nearly identical temperature profile during both the summer (Reid et al. 1997*a*) and winter months (Morgan et al. 1998), gill and liver protein turnover increased by an average of 10 and 12.2%, respectively. Taken together, these findings support our earlier suggestion (Reid et al. 1995) that the decreased responsiveness of protein turnover in fish in softwater to an identical elevation in the normal temperature profiles might be attributed to the inherently greater metabolic cost associated with living in an ion-poor environment.

The mechanism through which the 2°C elevation in the base temperature profile increased protein turnover appears to have been an increase in ribosomal translational efficiency. Although not statistically significant, in 75% of the comparisons (not including day 90 rates which followed the high temperature shock), the ribosomal translational efficiencies in gill and liver were higher at the +2°C temperature than at the base water temperature (Tables 1 and 2). The rationale for not including the day 90 rates was that during this time, when the ambient temperature had recently approached nearly lethal temperatures for rainbow trout (Kaya 1978), there was a significant change in liver protein synthesis capacity (Fig. 7). Despite the influence of this period of extreme base water temperature, our findings are consistent with other studies in which elevated water temperature also stimulated protein synthesis via increases in tissue ribosomal translational efficiency with little or no change in tissue protein synthesis capacity (Goolish et al. 1984; Fauconneau and Arnal 1985; Morgan et al. 1998).

Ammonia

Ammonia is largely formed in the liver and requires no modification and therefore little energy for excretion. In contrast, urea synthesis and excretion consumes energy (Wood 1993). Urea excretion was unaffected by chronic sublethal ammonia exposure at base or base+2°C (Linton et al. 1997). However, total nitrogenous waste excretion, and therefore ammonia excretion, tended to be highest in fish exposed to elevations in water ammonia during the first 60 days of this exposure (Linton et al. 1997). Plasma ammonia levels in hardwater-acclimated fish were unaffected by 30 days of exposure to 70 μM total ammonia·L^{-1} at either temperature. However, during the subsequent 60 days, plasma ammonia levels in fish chronically exposed to elevated ammonia (base+Am) were significantly greater than in those exposed to only ambient concentrations of water total ammonia at the base temperature. Similar, although not statistically significant, alterations in plasma ammonia in ammonia-exposed fish were apparent at the base+2°C temperature at days 60 and 90 (Linton et al. 1997).

This period of elevated plasma ammonia levels corresponds to the dramatic enhancement in liver protein synthesis and degradation measured at day 90 in both the base and base+2°C treatment groups (Fig. 6). As any detoxification of plasma ammonia would involve the liver and glutamine synthetase (Wood 1993), our data indicate that these changes in liver protein turnover may have been stimulated by this period of elevated plasma ammonia and that this is a response directed at reestablishing more normal levels of plasma ammonia. Furthermore, the stimulation of protein turnover in the liver by elevated water ammonia may provide the explanation for the stimulation of growth, nitrogen and energy conversion, and higher nitrogen retention efficiency reported by Linton et al. (1997). The additional plasma ammonia load, once incorporated into glutamine (Wood 1993) and other amino acids, could be used as substrates for protein synthesis. Thus, this ammonia-based increase in available amino acids could result in the apparent increase in food conversion efficiency, nitrogen retention, and enhanced growth in the absence of any apparent increase in food consumption (Linton et al. 1997).

There was some evidence to suggest that gill protein turnover, primarily degradation, was reduced by chronic exposure to elevated ammonia (Fig. 3). The apparent reductions in protein degradation may have been sufficient to maintain or marginally enhance net protein accretion in this tissue. Interestingly, similar alterations in protein turnover were

observed in the gills of softwater-acclimated fish exposed to sublethal acidity (Reid et al. 1997*a*). The majority of nitrogenous wastes is excreted across the gills (Sayer and Davenport 1987), and this organ is also the likely tissue across which ammonia would enter the animal. Elevated water ammonia concentration might be expected to impair branchial ionoregulation through inhibition of Na^+ uptake (Wilson et al. 1996*b*) with the compensatory response being the production of additional enzymes or new Na^+ channels. Indeed, measurements of Na^+ unidirectional fluxes revealed that the fish chronically exposed to elevated total ammonia exhibited higher rates of Na^+ turnover (Linton et al. 1998). Perhaps the enhanced net protein accretion in the gills was in some way associated with this greater Na^+ transport capacity.

The mechanism by which sublethal ammonia influenced protein turnover appears to involve alterations in both tissue protein synthesis capacity and ribosomal translational efficiency, depending on the tissue and duration of the exposure at sampling. Despite only minor changes in gill protein synthesis, degradation, and accretion during the last 60 days of the exposure, the data do suggest that chronic exposure to elevated total ammonia altered gill protein synthesis capacity (Fig. 5). As a consequence, normal levels of protein turnover appear to have been maintained through compensatory adjustments in gill ribosomal translational efficiency (Table 1). However, in the liver, where significant alterations in protein turnover were observed, the elevation in protein turnover induced by ammonia resulted from enhanced ribosomal translational efficiency (Table 2). Liver protein synthesis capacity was unaffected by chronic exposure to sublethal total ammonia (Fig. 7). Evidence that protein turnover in gill and liver is affected by environmental stressors through different mechanisms has been reported previously by us, in response to chronic exposure to a low-pH environment (Reid et al. 1997*a*).

Other possible mechanisms have been suggested that may be independent of changes in protein synthesis capacity or translational efficiency. Houlihan et al. (1994) chronically exposed dab (*Limanda limanda*) to a mixture of metals contained within sewage sludge and determined monthly rates of tissue protein turnover (white muscle, liver, kidney, and spleen) using methods similar to those of the present study. Despite no impact on tissue protein synthesis, protein growth rates (accretion) were lower in fish chronically exposed to sewage sludge compared with the control group. Houlihan et al. (1994) suggested that chronic exposure to sewage sludge reduced protein accretion through enhanced protein degradation, as tissue RNA concentrations and posttranslational efficiency were largely unaffected. Similarly, Wilson et al. (1996*a*) reported no significant modification in protein synthesis capacity or ribosomal translational efficiency in gill, liver, and carcass (whole body minus gills and liver) following 15 days of exposure to pH 5.2 softwater when compared with the pH 6.5 controls. However, in response to the addition of 30 μg $Al \cdot L^{-1}$, these authors noted a significant reduction in gill protein synthesis capacity and a significant reduction in carcass ribosomal translational efficiency, with no impact on either parameter in liver following a 32-day exposure. Thus, in response to chronic stressors, it appears that there are no common mechanisms responsible for alterations in tissue protein turnover. It seems possible that modifications in protein turnover can result from pre- or post-translational modification of protein synthesis, alterations in protein degradation, or both and may differ between tissues within the same individual.

Ammonia and temperature

The impact of this combination of stressors on gill protein turnover was negligible, as the impact of ammonia was neither enhanced nor suppressed as the result of the 2°C elevation in the base temperature profile. In the case of the liver, however, our findings suggest that the combination of elevated temperature and ammonia results in a reduction in the stimulator influence that each has separately on liver protein turnover (Fig. 6). This was evident at both days 60 and 90 of the experiment, with the combination effect being more dramatic at day 90, presumably due to the high ambient temperature (Fig. 1). Linton et al. (1997) drew similar conclusions as to the impact of the combination of elevated temperature and total ammonia based on a suite of quantitative bioenergetic and physiological measurements made on fish exposed to the exact same conditions during the same season of the year. Nonetheless, these finding are somewhat contrary to those of the companion low-pH softwater study (Reid et al. 1997*a*). In that study, we reported that the impacts of the addition of 2°C to the base temperature profile and of the environmental acidification were, in the gills of softwater-acclimated trout, additive. Therefore, we suggest that the physiological impact of a warmer and polluted environment will depend on the site and mechanism of action of the pollutant of concern and whether the fish are in ion-poor or ion-rich water. However, when water temperatures approach the upper lethal temperature for a given species of fish, the additional 2°C would triggered a dramatic reduction in tissue protein turnover and thereby mask any influence of a pollutant on protein turnover.

Summary

The present study has shown that protein turnover rates in the tissues of fish exposed chronically to sublethal levels of total ammonia are altered in a manner consistent with the hepatic detoxification of accumulated internal ammonia. When chronically exposed to elevated total ammonia, liver protein turnover was enhanced, while the rates in the gill were not affected. The addition of +2°C to the environment generally increased protein synthesis in both the gills and liver; however, in combination with exposure to sublethal levels of total ammonia the ammonia-stimulated increase in protein turnover was muted. The dominant impact of the 2°C elevation was most apparent during the last 30 days of the experiment when ambient summer water temperature peaked several times at 22°C. Therefore, even if the rise in mean global temperature triggered by the doubling of atmospheric CO_2 is in the lower part of the overall range (0.5–7°C) predicted by current models, the results of this study suggest that it would increase the cost of living for fish already exposed to elevated water ammonia concentrations. The consequences of such changes in the quality of the aquatic environment would be particularly severe if these animals are living near the upper limit of their temperature tolerance.

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