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Swimming and ammonia toxicity in salmonids: the effect of sub lethal ammonia exposure on the swimming performance of coho salmon and the acute toxicity of ammonia in swimming and resting rainbow trout

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Abstract

This study tested the hypothesis that swimming exacerbates ammonia toxicity in fish. Both sub-lethal and acute toxicity testing was conducted in a swim tunnel on swimming and resting coho salmon and rainbow trout, respectively. The sub lethal tests on coho salmon also considered the compartmentalization of ammonia within the fish. Coho salmon showed a significant linear decrease in U_{crit} both with increasing water ammonia (0, 0.02, 0.04 and 0.08 mg per l NH_3) and increasing plasma ammonia. Data collected included plasma pH and ammonia, muscle pH and ammonia and muscle membrane potential. Based on results found in these experiments it was concluded that the reduction in swimming performance was due to both metabolic challenges as well as depolarization of white muscle. Acute toxicity testing on swimming and resting rainbow trout revealed that swimming at (60% U_{crit} or approximately 2.2 body lengths/s) decreased the LC_{50} level from 207 ± 21.99 mg N per l in resting fish to 32.38 ± 10.81. The LC_{50} for resting fish was significantly higher than that for swimming fish. The acute value set forth by the US EPA at the same pH is 36.1 mg N per l and may not protect swimming fish. In addition the effect of water hardness on ammonia toxicity was considered. It was found that increased water calcium ameliorates ammonia toxicity in fish living in high pH water.

Keywords: Ammonia; Swimming; Coho salmon; Rainbow trout; Acute toxicity; Sub-lethal

1. Introduction

Ammonia is a common toxicant in aquatic environments and enters natural waterways by means that include sewage effluent, industrial waste and agricultural run-off. It is also an unusual toxicant in that all organisms produce ammonia as metabolic waste and must excrete it to avoid self-intoxification. Fish can readily excrete ammonia as NH_3 across the gill membranes into water providing there is a concentration gradient. Some specialized species of fish have evolved

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physiological mechanisms to cope with ammonia excretion under adverse environmental conditions. Some examples include the mudskipper, *Periophthalmus scholosseri* (Randall et al., 1999) and the lake Magadi tilapia, *Oreochromis alcalicus grahami* (Randall et al., 1989). However, if a fish encounters elevated ambient ammonia and has no physiological mechanism to cope, it will rapidly accumulate toxic levels. If internal ammonia levels become elevated fish can experience both chronic and acute toxic affects. Under laboratory conditions some noted effects include alterations of the central nervous system function (Hillaby and Randall, 1979) energy metabolism (Arillo et al., 1981b), and ionic balance (Soderberg and Meade, 1992) as well as morphological changes such as fusion of the gill lamellae (Burrows, 1964).

As ammonia is toxic in aquatic systems water quality standards have been established by some countries. The US Environmental Protection Agency (EPA) has the most comprehensive data available on ammonia toxicity and as such, other countries often refer to the US standards when establishing their own. When determining ammonia water quality criterion the US EPA follow standard toxicity protocols that require toxicity tests be conducted under static conditions on resting, unfed and unstressed fish (ASTM, 1993). These standardized tests allow researchers to make useful recommendations that cover a broad range of species. Although, this is an adequate policy for most toxicants, the nature of ammonia is such that it becomes elevated internally under exactly the conditions that the EPA is trying to avoid, swimming, feeding and stress.

Under conditions of elevated ambient ammonia, the plasma ammonia in fish will increase under resting conditions (Knoph and Thorud, 1996). Swimming fish have also shown increased internal ammonia levels when compared with resting fish. Mommsen and Hochachka (1988) reported that the ammonia level increased in the white muscle of rainbow trout following exercise, due to a breakdown of adenylates to inosine monophosphate (IMP) and NH_4^+. Beaumont et al. (1995b) noted a correlation between plasma ammonia levels and decreased swimming performance in brown trout exposed to copper. The decrease in swimming performance could be due to ammonia decreasing muscle membrane potential or by affecting muscle metabolism (Beaumont et al., 1995a). Additionally, during the migration of anadromous fish, feeding ceases. Under these conditions not only are fish swimming for prolonged periods, they experience an increase in structural protein catabolism to meet energetic demands which results in an increase in plasma ammonia (French et al., 1983). Based on the previous studies it is hypothesized that swimming fish are more susceptible to ammonia toxicity than resting fish.

The following experiments were conducted to test the hypothesis that a swimming fish is more susceptible to environmental ammonia than a resting fish and also considered how well the established criteria protects swimming fish. The first study examined the effect of elevated ammonia on the swimming performance of coho salmon and considered the compartmental distribution of ammonia in white muscle following exercise. The second study was a 96-h acute toxicity test on swimming and resting rainbow trout at ammonia levels that cover the range set forth by the US EPA. The effect of water hardness was also considered.

2. Methods

2.1. General fish husbandry

Experiments were carried out on coho salmon, *Oncorhynchus kisutch* (fork length 32.2 ± 1.5 cm, body mass 350 ± 65 g; mean $\pm$ 1 S.D.) and rainbow trout, *Oncorhynchus mykiss* (fork length 17.8 ± 2.4 cm, body mass 40 ± 11 g) and rainbow trout fry (0.15 g). Coho salmon were obtained from West Vancouver laboratories (Vancouver, BC, Canada) and rainbow trout from Spring Valley Trout Farm (Langley, BC, Canada). Prior to the experiments, fish were held in separate circular outdoor tanks supplied with aerated dechlorinated Vancouver city water. The larger coho and rainbow trout were fed a maintenance ration (0.5% body weight per day) of commercial fish food. Rainbow trout fry were fed a maintenance ration of 7% body weight every other day.

Swimming experiments that used the larger fish were carried out in a 120 l Brett swim tunnel (Gerkhe et al., 1990). Following standard protocol fish were fasted for 24 h before being placed in the swim tunnel. Fish were acclimated in the swim tunnel for at least 12 h prior to the onset of each protocol. The rainbow trout fry experiments were carried out in 2 l plastic cylinders placed in flow through water baths.

2.2. Study 1: swimming performance in coho salmon exposed to elevated ambient ammonia

During the 12-h acclimation period the water velocity in the swim tunnel was 15 cm/s and each fish was swum individually. Following the acclimation period the water velocity was increased to 30 cm/s for 30 min and then by 7.5 cm/s increments every 30 min until the fish was exhausted. Exhaustion was noted when a fish was no longer able to move away from the electrical current generated at the rear of the chamber. The critical swimming velocity (U_{crit}), a measure of swimming performance (Beamish, 1978) was recorded as the highest position-maintaining velocity plus the fraction of the time interval of the velocity in which they became exhausted. The following equation was used to make the calculations:

$$U_{crit} = U_h + \left(\frac{\Delta t}{30 \text{ min}}\right)^* 7.5 \text{ cm/sec}$$

where U_h is the highest position maintaining velocity (cm/s) and Δt is the time (min) the fish swam at that speed before becoming exhausted. U_{crit} was expressed in body lengths per second (bl/s) by dividing the swimming velocity by the fork length of each fish.

After the 12 h acclimation, groups of fish were exercised under the following conditions: a control group ($n = 10$) in dechlorinated tap water and three treatment groups ($n = 5$ per treatment) at ammonia levels of 0.02, 0.04 and 0.08 mg per l NH_3. In addition, another group ($n = 5$) was maintained at rest for 24 h in black persplex boxes containing dechlorinated tap water.

Ammonia levels were increased in the swim tunnel immediately prior to the onset of exercise by the addition of a stock solution of ammonia chloride (NH_4Cl). Ammonia levels were maintained with a peristaltic pump. The pH in the swim tunnel was held constant between 5.99 and 6.02 using a Radiometer pH stat (1.0 M KOH). Water samples were collected from the swim tunnel at the time of fish transfer, 16 h following transfer and at U_{crit}. Total water ammonia was determined using a modification of the Verdouw et al. (1977) method; nitroprusside (0.2 g per l) was substituted for ferrocyanide. The water temperature in the swim tunnel was between 9 and 12 °C.

Exhausted fish were removed from the swim tunnel and killed by a sharp blow to the head. Three ml of blood were collect by caudal puncture using a heparinized syringe. Plasma pH (pHe) was measured immediately upon collection using a microelectrode (micro Electrode Inc) thermostated at the same temperature as the swim tunnel water. Plasma was then separated by centrifugation at 4 °C and then aliquots frozen at − 80 °C for ammonia analysis. Plasma total ammonia was determined using a L-GLDH/NADH kit available from Sigma (171-UV). These values were corrected for water content using values obtained from lemon sole, *Parophyrus vetulus*, by Wright et al. (1988) (at rest 97.4% and, following exercise 95.9%). The NH_3 concentration in plasma was calculated from the Tamm ($Tamm = NH_3 + NH_4^+$) and pHe using the Henderson–Hasselbach equation with pK values given by Cameron and Heisler (1983).

A sample of white muscle was excised from the expaxial posterior region of the fish within 3 min of death and immediately frozen in liquid nitrogen.

Muscle tissue was homogenized according to Tang et al. (1992) and the ammonia concentration determined using the L-GLDG/NADH kit (Sigma 171-UV). Intracellular total ammonia was determined by first accounting for ammonia trapped in the extracelluar fluid volume (ECFV) and then corrected for intracellular water (ICFV), as follows:

$$\text{Intracellular Tamm} = \left(\frac{(\text{tissue Tamm}^*\text{total } H_2O) - (\text{plasma Tamm}^*\text{ECFV})}{\text{ICFV}}\right)$$

Values for total H_2O, ICFV and ECFV in white muscle both from rested fish (0.783, 0.697 and 0.086 ml per g) and exercised fish (0.786, 0.735 and 0.050 ml per g) were obtained from Milligan and Wood (1986). Intracellular pH was determined by direct measurement according to Portner et al. (1991).

To determine whether the distribution of ammonia between intracellular and extracellular muscle fluids was according to the pH gradient, tissue pHi was predicted from the measured ammonia concentration using the following equation:

$$\text{Predicted intracellular pHi} = \left(\frac{\text{pK} + \log(\text{intracellular}[\text{NH}_3]}{\text{intracellular}[\text{NH}_4^+]}\right)$$

assuming intracellular $[NH_3]$ = plasma $[NH_3]$.

The predicted pHi values were compared with the measured pHi values.

To determine whether the distribution of ammonia between intracellular and extracellular muscle fluids could alternatively be a function of membrane potential, $E_{NH_4^+}$ (the potential within the muscle relative to the potential of the extracellular fluids) was calculated from the Nernst equation:

$$\text{E}_{\text{NH}_4^+} = \left(\frac{RT}{zF}\right)^* \ln\left(\frac{[\text{NH}_4^+]e}{[\text{NH}_4^+]\text{i}}\right)$$

where R, T, z and F have their usual values, and $[NH_4^+]$e and $[NH_4^+]$i represent plasma and intracellular levels, respectively, based on measured extracellular and intracellular pH and Tamm values.

Data are presented as means $\pm$ 1S.E.M. and statistical differences are at $P < 0.05$ unless otherwise noted. ANOVA tests were done using Sigma Stat software (v. 2.03 Jandel Scientific) and differences between means were tested using a Tukey's test. Correlation was determined between independent variables using the Pearson product moment correlation method, while linear regression was used when dependencies occurred between variables.

2.3. Study 2: 96-h acute ammonia toxicity in rainbow trout

2.3.1. Part A: 96-h acute ammonia toxicity in resting and swimming fish

The rainbow trout (40.0 ± 11.2 g) used in this experiment were placed in groups in the swim tunnel for the acclimation period. Ammonia toxicity testing on swimming fish was conducted at 60% U_{crit}. This swimming speed was selected as it is not a level of exercise associated with fatigue under normal conditions. To determine this value a preliminary swimming performance test was conducted using a single group of six fish. U_{crit} for this group of fish was determined according to the method described in study one with the value being 59.2 cm per s. A single 96-h preliminary swimming test conducted at 60% U_{crit}, 35.5 cm per s, using dechlorinated tap water ($n = 10$) resulted in no mortality.

Again, after the 12-h acclimation period groups of 15 rainbow trout were exposed to various 96-h treatments. The treatments included: swimming fish exposed to a range of ammonia levels from 0 to 58 mg N/l and resting fish in swim tunnel exposed to a range of ammonia levels from 0 to 378 mg N per l. At the onset of the swimming treatments, the water velocity and ammonia level was simultaneously increased. Resting fish were also exposed to elevated ammonia in the swim tunnel but the velocity remained zero as the ammonia level was quickly increased. Ammonia was added to the swim tunnel as described previously. To avoid natural increases in ammonia due to the fish's metabolism the turnover rate of water in the swim tunnel was 24 l per h.

Water samples were collected at 0, 24, 48, 72 and 96 h for analysis. Total ammonia was determined using the method previously described. Water pH and temperature was also monitored at these times.

During each 96-h exposure, fish were monitored constantly. Exhausted (previously described) and morbid fish (individuals that were no longer upright and responsive to being touched) were immediately removed from the chamber and killed by a sharp blow to the head.

The 96-h LC_{50} values were calculated using the Spearman-Karber method on software provided by the US EPA. The 95% confidence intervals for the LC_{50} values were also calculated. Analysis of covariance for the slopes and intercepts from the regressions of mortality rate on water ammonia in swimming and resting fish were done using SAS.

2.3.2. Part B: the effect of calcium on acute 96-h ammonia toxicity in free-swimming rainbow trout fry

Hatchery reared rainbow trout fry (0.15 g) were used for these toxicity experiments. Acute ammonia tests were conducted under the following conditions: three calcium levels (0, 2 and 5 mM) and three pH levels (6.5, 7 and 9.0). For each treatment combination (pH and calcium) four ammonia levels and a dechlorinated water control were tested in triplicate. Ten fish were placed in each cylinder and allowed to acclimate for 24 h. The dechlorinated water was replaced with 2 l of aerated test solution prior to the onset of the experiment. All cylinders were placed in a flow-through water bath at 12 ± 1 °C with a 16-h light: 8-h dark photoperiod. Test solutions were renewed daily during the 96 h exposure. A 0.01 M Bis–tris propane solution was added to the test water and 1 M HCl and 1 M NaOH was used to keep the pH within 0.3 U of the required pH. Fish were observed constantly with dead fish being removed continually. Mortality rates were recorded over the 96-h test period. The pH and dissolved oxygen were recorded daily for the duration of the test. Ammonia concentration was also measured daily using the above-described method.

The 96-h LC_{50} values and the 95% confidence limits were calculated using the Spearman–Karber method on software provided by the US EPA.

3. Results

3.1. Study 1: swimming performance in coho salmon exposed to elevated ambient ammonia

In study one, which considered the effect of ambient ammonia on swimming performance in

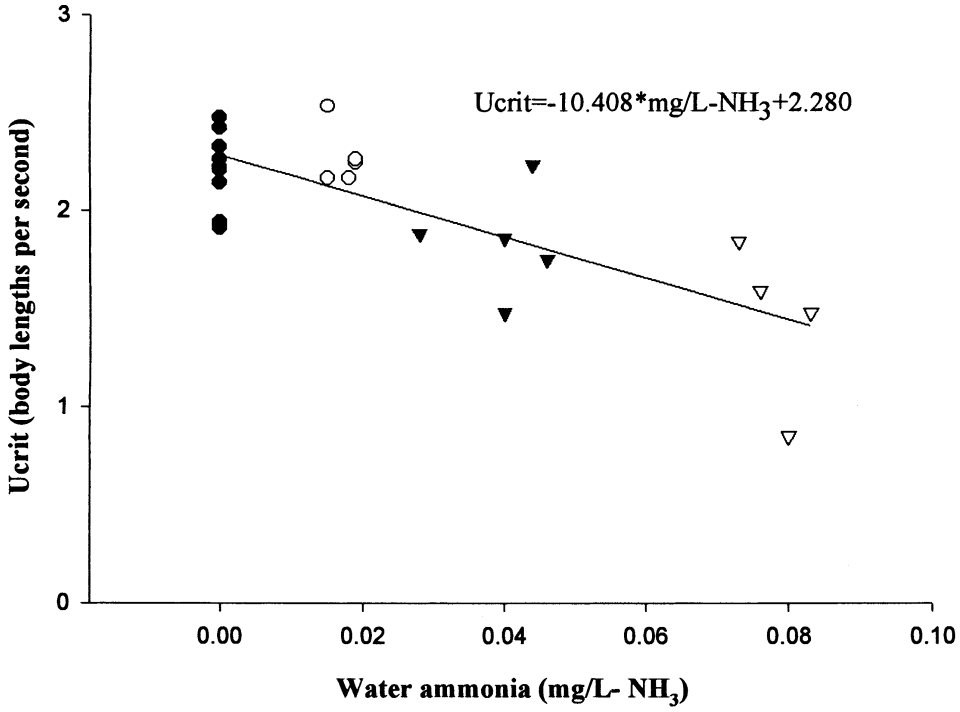

Fig. 1. Linear relationship between critical swimming velocity (U_{crit}) of coho salmon and the ambient ammonia levels. Increasing water ammonia (mg/l, NH_3 is related ($r^2 = 0.60$) to a decrease in U_{crit}. Symbols indicate groups as follows, solid circles, control; hollow circles, low ammonia; solid down triangles, medium ammonia and hollow down triangles, high ammonia.

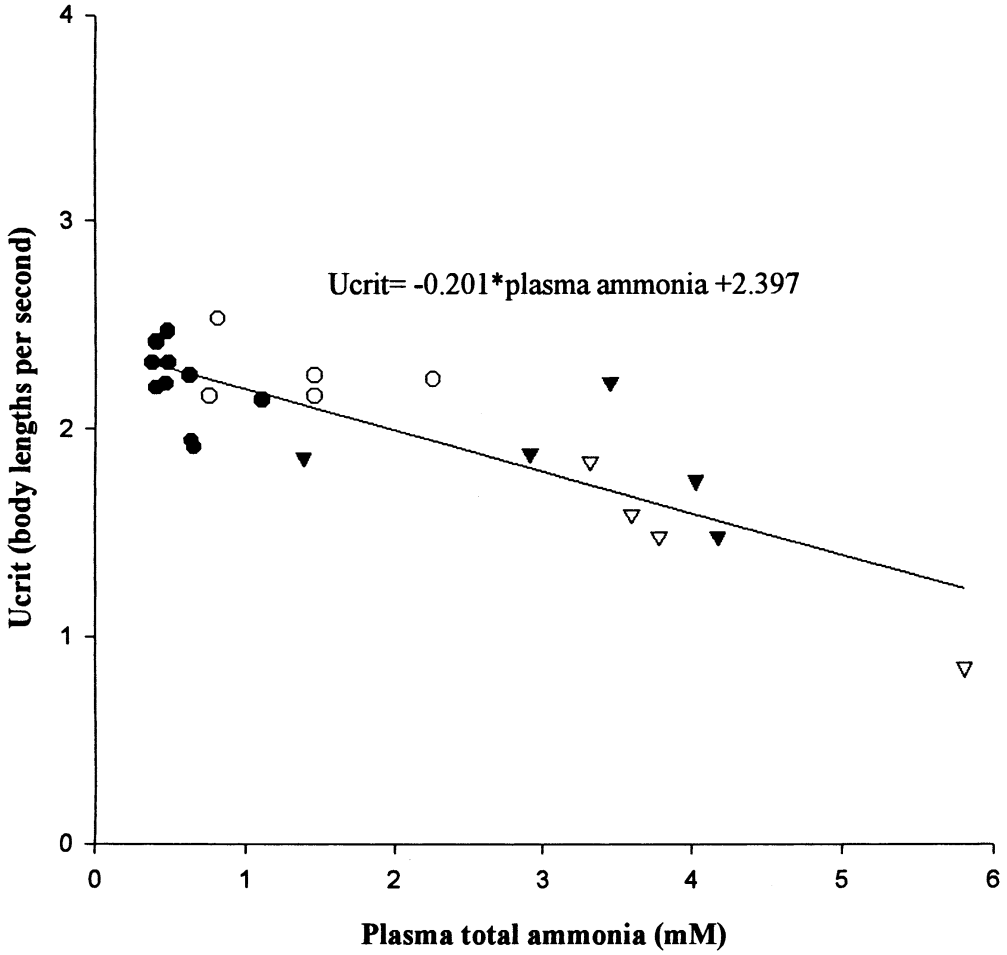

Fig. 2. Relationship between the critical swimming velocity (U_{crit}) for coho and plasma ammonia (mM) is illustrated. Increasing plasma ammonia is correlated ($r^2 = 0.69$) with a decrease in U_{crit}. Symbols indicate groups as follows, solid circles, control; hollow circles, low ammonia solid triangles-medium ammonia and hollow down triangles, high ammonia.

coho salmon, there was a linear ($r^2 = 0.60$, $P < 0.001$) decrease in U_{crit} with increasing water ammonia (Fig. 1). There was no significant difference between the U_{crit} of the control and 0.02 mg per l NH_3 exposed fish, however, swimming performance was significantly reduced at 0.04 and 0.08 mg per l NH_3. Fig. 2 shows the correlation between the plasma ammonia level and the U_{crit} of individual fish. The trend indicates that as plasma ammonia increases the U_{crit} decreases ($r^2 = 0.69$, $P < 0.001$). Only one mortality was noted during this set of exercise experiments and this was in the 0.08 mg per l NH_3 treatment group. Measured water ammonia levels are given in Table 1.

Table 1 indicates the values obtained for ammonia and pH in both the plasma and muscle tissue. The total plasma ammonia was significantly different ($P < 0.0001$) between exercised and resting fish not exposed to ammonia. Resting fish had a mean ($\pm$ S.E.M.) plasma total ammonia level of 0.227 ± 0.03 mM while swimming fish had higher mean plasma ammonia levels at 0.502 ± 0.04 mM. In exercised coho exposed to ammonia, plasma ammonia levels were both significantly elevated and correlated to the incremental increases in ambient ammonia (Fig. 3). A significant decrease in plasma pH was noted with exercise from 7.64 ± 0.03 in resting to 7.32 ± 0.03 in swimming fish. Water ammonia, however did not have a significant effect on the plasma pH of exercising fish.

Total ammonia in white muscle significantly increased, 0.9 ± 0.1–5.3 ± 0.7 mM, between resting and exercising fish (Table 1). Ambient ammo-

nia did not affect tissue ammonia concentration in exercising fish except at the highest ammonia level, 0.08 mg per l NH_3. Measured muscle pH decreased from 7.29 ± 0.05–6.71 ± 0.07 in resting versus exercising fish, however, ambient ammonia had no effect on tissue pH at any treatment level. Fig. 4 illustrates the relationship between plasma and tissue pH and total ammonia levels under a variety of conditions.

Calculated levels for muscle $E_{NH_4^+}$ were significantly more negative in control exercised than resting fish, -53 ± 3 to -33 ± 6 mV, respectively, (Table 1). Muscle $E_{NH_4^+}$ in fish exposed to ammonia was significantly lower than the exercise control, but not different from resting fish (Table 1). Exposure to ammonia reduced the calculated muscle membrane potential in exercising fish compared with that of control swimming fish.

3.2. Study 2: 96-h acute ammonia toxicity in rainbow trout

3.2.1. Part A: 96-h acute ammonia toxicity in resting and swimming fish

Water parameters measured in the swim tunnel over all treatments are as follows: mean water temperature, 16.6 ± 1.3 °C, pH 6.97 ± 0.05 and P_{O_2}, 143.8 ± 12.0 Torr. Mean total ammonia in mg N per l was calculated for each individual LC_{50} test (Fig. 4). Ammonia levels over the 96-h tests did not fluctuate by more than 4% around the mean.

The mortality rates for both swimming and resting fish exposed to ammonia in the swim tunnel increased linearly with increasing ammonia concentrations (Fig. 5). The relationship between mortality of resting and of swimming fish and ammonia in the swim tunnel is described by the following equations: mortality of resting fish = 0.269*(ammonia mg N per l) -8.727 ($r^2 = 0.74$, $P = 0.001$) and mortality of swimming fish = 1.121*(ammonia mg N per l) $+14.288$ ($r^2 = 0.91$, $P < 0.001$). The slopes of the two lines were significantly different ($P < 0.01$), thus mortality rate increased much more quickly with increasing ammonia in swimming fish than in resting fish (Fig. 5). The intercepts of the regressions were not significantly different.

The calculated LC_{50}'s ($\pm$ the 95% confidence intervals) for swimming fish and resting fish were 32.38 ± 10.81 and 207 ± 21.99 mg N per l, respectively. The LC_{50} for resting fish was significantly higher than that for swimming fish by 174.62 mg N per l.

3.2.2. Part B: the effect of calcium on acute 96-h ammonia toxicity in free-swimming rainbow trout fry

Water pH over the duration of the experiments ranged from 6.1 to 6.8, 7.5 to 7.9 and 8.6 to 9.1 for each pH treatment. The ammonia concentration consistently decreased by no more than 15% during the 24 h prior to water renewal.

Table 1
Values for water NH_3 (ug/l), measured levels for plasma and white muscle, total ammonia (mM), and pH and predicted muscle pH_i and $E_{NH_4^+}$ (mV) from coho salmon

	Resting $N=5$	Control $N=10$	Low $N=5$	Medium $N=5$	High $N=4$
Water					
NH_3[ug/l]	0 ± 0^a	0 ± 0^a	17 ± 1^b	40 ± 3^c	80 ± 2^d
Tamm[mM]	0.23 ± 0.03^a	0.50 ± 0.04^b	1.35 ± 0.27^c	3.19 ± 0.50^d	3.94 ± 0.39^{de}
Plasma					
PHe	7.64 ± 0.03^a	7.32 ± 0.03^b	7.24 ± 0.03^b	7.22 ± 0.03^b	7.31 ± 0.02^b
Tamm[mM]	0.9 ± 0.1^a	5.3 ± 0.7^b	6.8 ± 0.9^{bc}	8.5 ± 1.1^{bc}	9.5 ± 1.5^c
Muscle					
pHi (measured)	7.29 ± 0.05^a	6.71 ± 0.07^b	6.71 ± 0.05^b	6.75 ± 0.05^b	6.95 ± 0.10^b
pHi (predicted)	6.99 ± 0.15^a	6.31 ± 0.09^b	6.51 ± 0.06^b	6.77 ± 0.04^b	6.92 ± 0.10^{ab}
$E_{NH_4^+}$-[mV]	33 ± 6^{ac}	53 ± 3^b	41 ± 4^a	25 ± 3^c	20 ± 4^c

Samples were collected from fish at rest; control exercising fish and exercising fish at low, medium and high ambient ammonia. Mean values are given $\pm$ S.E.M. and significant differences noted at $P<0.05$ are indicated by changing alphabetical letters.

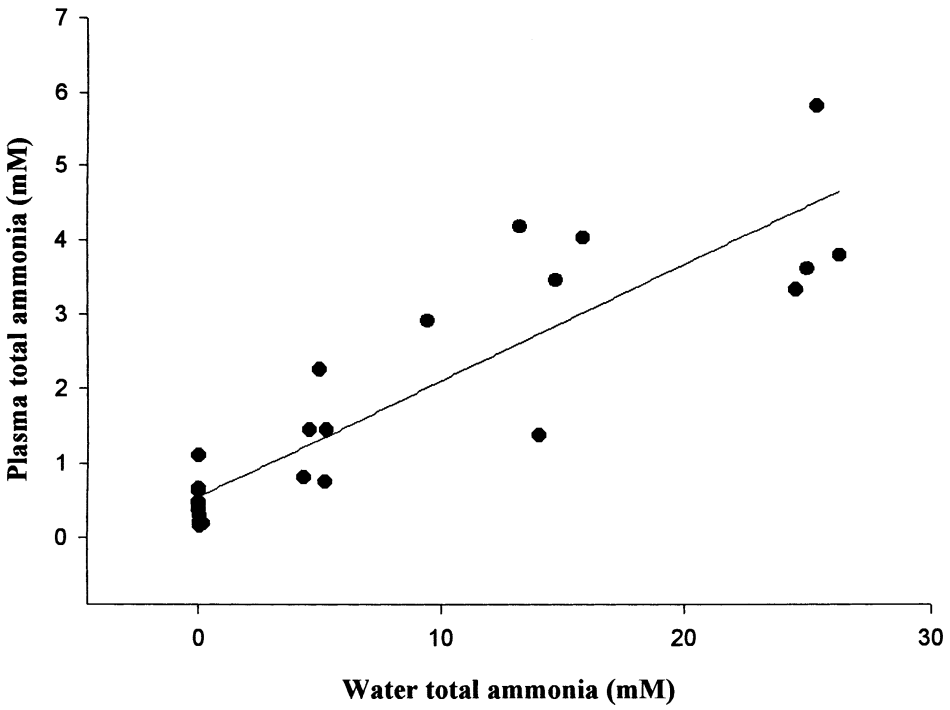

Fig. 3. Linear relationship between water total ammonia (mM) and coho plasma total ammonia (mM) exercised at 60% U_{crit} in a swim tunnel until exhaustion. Regression equation is plasma ammonia = 0.156 × water ammonia + 0.55($r^2 = 0.81$, $P = 0.0001$).

Fig. 6 summarizes the toxicity of ammonia to rainbow trout fry in 96-h tests at pH's of 6.5, 7.8 and 9.0 with varying calcium addition. Ammonia toxicity expressed in mg N per l increased with pH. Addition of calcium at each pH caused an increasing trend in ammonia LC_{50} values. At the lowest pH, 6.5, there was no significant difference between the LC_{50} ammonia concentrations with increasing calcium addition. At a pH of 7.8 the addition of calcium caused a non-significant increase in the LC_{50} level, however, only with 5 mM calcium addition was the LC_{50} value significantly increased. Tests done at pH 9 showed a similar increase with both 2 and 5 mM calcium additions causing increased LC_{50} concentrations.

4. Discussion

4.1. The effect of ammonia on the swimming performance of coho salmon

It is commonly known that a substantial number of biotic and abiotic factors affect the swimming performance of fish. Hatchery raised fish have been shown to have lower critical swimming velocity than wild fish (Brauner et al., 1994) and size of fish also correlates to swimming performance (Brett, 1964). Some of the abiotic factors that have been shown to affect swimming performance include, temperature, dissolved oxygen, pH and salinity (Brett, 1964; Jones, 1971; Glova and McInerney, 1977; Ye and Randall, 1991; Brauner et al., 1994). Many toxicants have been shown to decrease the swimming performance of salmonids. Some of these include: copper (Beaumont et al., 1995b), bleached kraft mill effluents (Howard, 1975), fenitrothion (Peterson, 1974) and hydrogen sulphide (Oseid and Smith, 1972). In the present study it was evident that ambient ammonia levels greater than 0.04 mg per l NH_3, (which is equivalent to 185 mg per l N at pH 6.0) can reduce swimming performance in coho salmon. This decrease in swimming performance is not consistent between all fish species. Walsh et al., (1993) reported that the swimming performance of Lake Magadi tilapia was unimpeded at ammonia concentrations as high as 1.5 mg per l NH_3. The

tolerance of this species of fish to high ambient ammonia is likely explained by their ability to excrete ammonia waste as urea.

Many studies have found significant relationships between plasma and tissue ammonia and swimming performance in fish. Beaumont et al.,

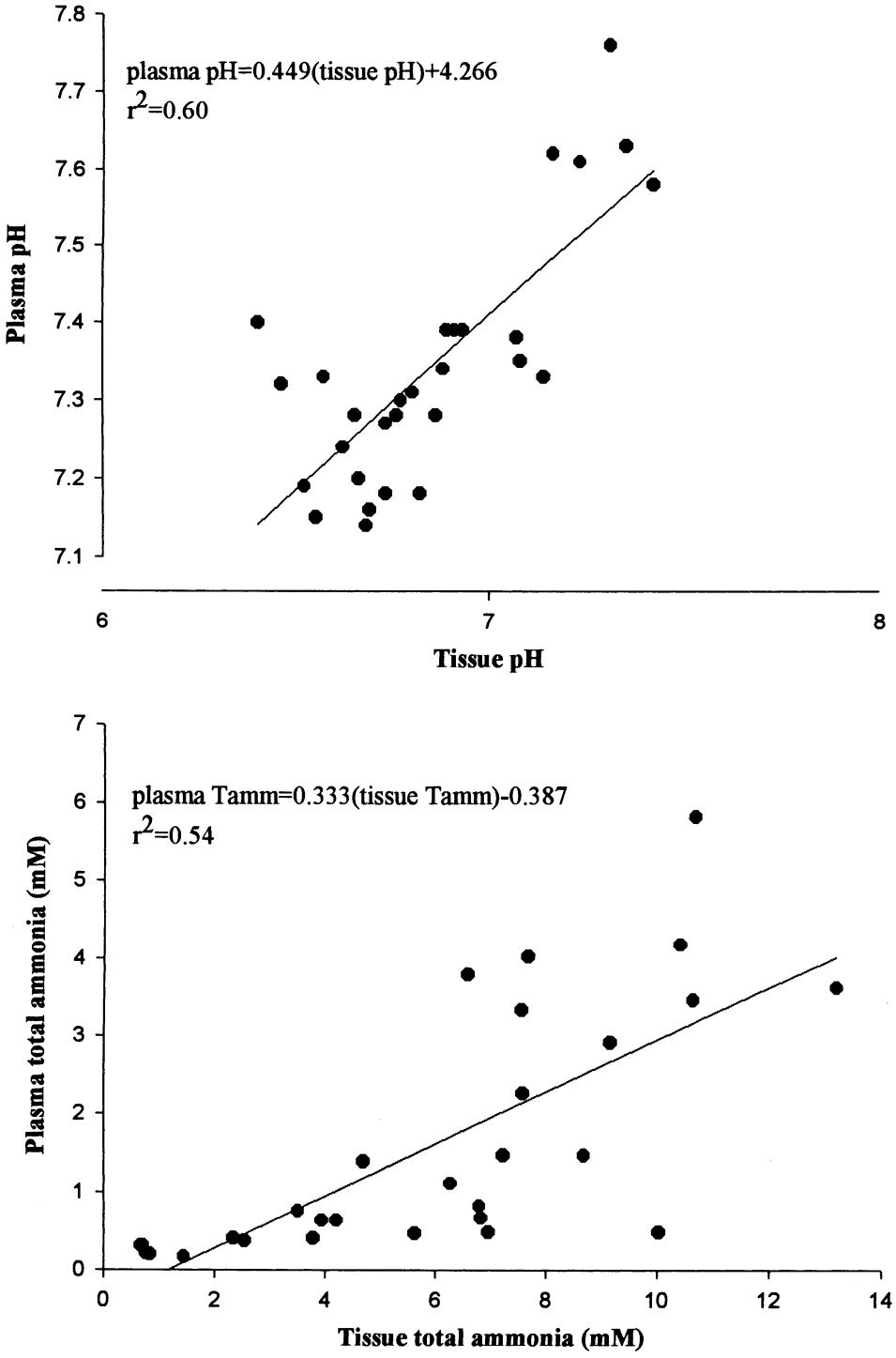

Fig. 4. Relationship between the tissue and plasma pH and tissue and plasma -total ammonia in control exercised and resting coho salmon exposed to elevated ambient ammonia.

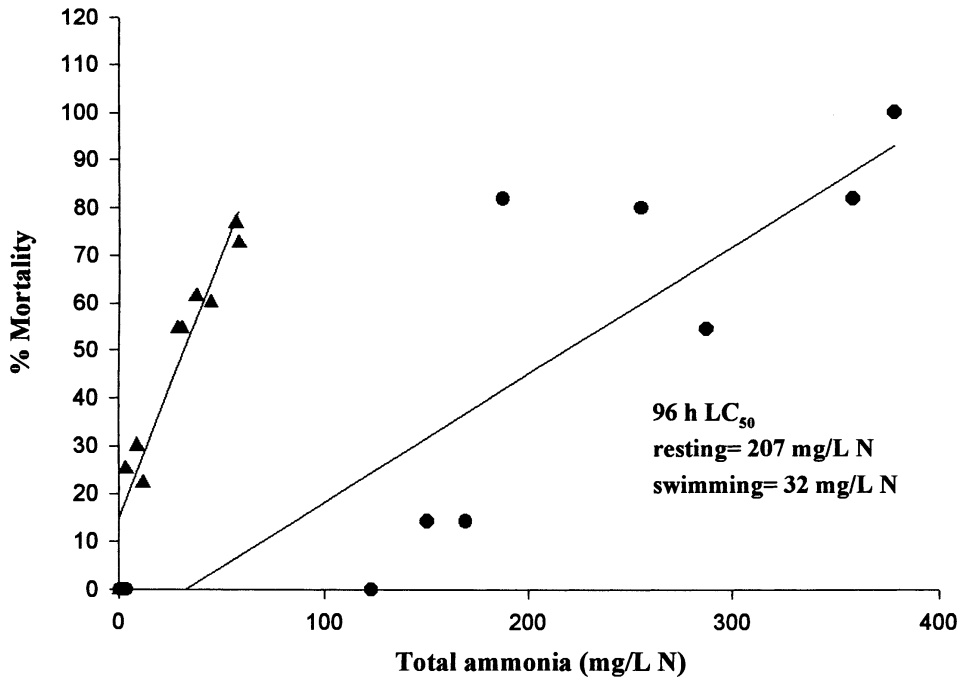

Fig. 5. Effect of total ammonia (mg/l N) on rainbow trout mortality under resting and exercise conditions (60% U_{crit}). Triangles and circles represent swimming and resting fish, respectively. The 96 h LC_{50} levels were calculated using the trimmed Spearman–Karber method.

(1995b) observed a decreased swimming performance in brown trout exposed to copper under acidic conditions that was correlated, as in this study, with increased plasma ammonia. The elevation in plasma ammonia was, however, not related to swimming but to copper exposure and low pH simultaneously. The present study found a similar relationship between plasma ammonia and U_{crit} to that of Beaumont et al. (1995b) with a significant linear model that predicts that 69% of the variation in the U_{crit} values in swimming coho salmon that are exposed to elevated ambient ammonia can be explained by the concentration of total ammonia in the plasma.

Ammonia is also accumulated in the white muscle tissue of fish during swimming and ammonia exposure. Several studies have found that exercise in fish causes a depletion of the white muscle adenylate pool (Mommsen and Hochachka, 1988) resulting in increased inosine monophosphate (IMP) and ammonia levels (Mommsen and Hochachka, 1988; Wang et al., 1994). The present study found a six-fold increase in white muscle ammonia concentration after exercise. A similar increase in tissue ammonia levels was noted by Wang et al., (1994), Tang et al., (1992) in rainbow trout and by Day and Butler (1996) in brown trout under exercise conditions. In addition, the present study determined that tissue ammonia increased with elevated ambient ammonia. Although white muscle ammonia concentrations were related to the swimming performance of coho salmon, a much stronger relationship was found between plasma ammonia and swimming performance as previously discussed. Beaumont et al., (2000a) found that although tissue ammonia increased between resting and swimming fish under control conditions that, when the fish were exposed to low pH and copper simultaneously both groups had elevated tissue ammonia, but only the resting group was different from the control. Similar results were noted by Day and Butler (1996) who also reported a decreased swimming performance associated with increased tissue ammonia levels.

It is clear from the present study and previous work by Beaumont et al. (1995a,b), Day and Butler (1996) that accumulation of ammonia in the fish contributes to a reduction in swimming performance. Many theories have been postulated

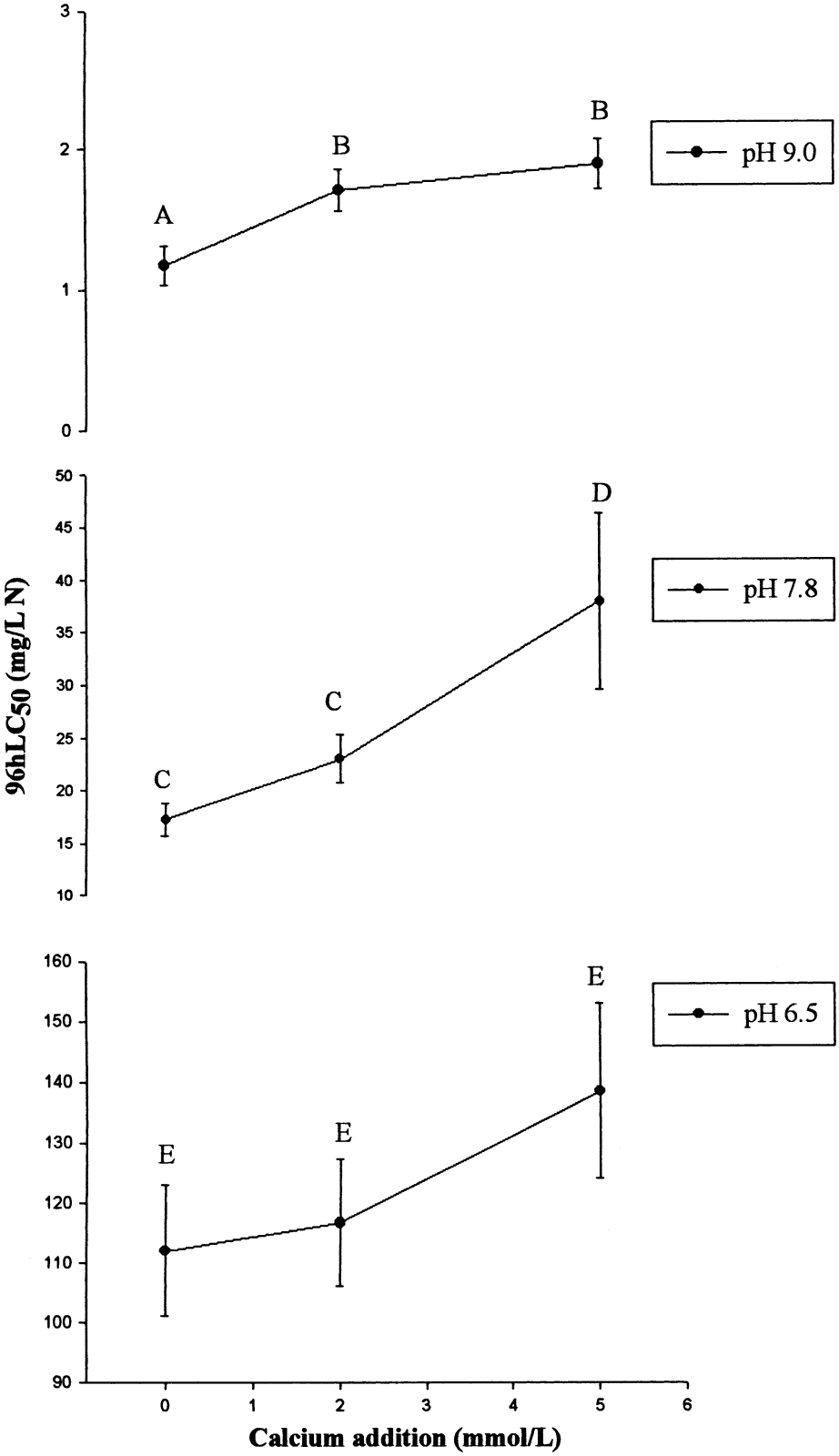

Fig. 6. Trimmed Spearman–Karber 96 h LC_{50} for total ammonia (mg/l N) in rainbow trout at pH 6.5,7.8, and 9.0 with varying calcium addition (0, 2 and 5 mmol/l). Significant differences in the 95% confidence intervals are indicated by a change in letters.

regarding the reason for ammonia decreasing swimming performance. The first theory suggested by Beaumont et al. (1995a) is that elevated ammonia interferes with muscle energy metabolism. Studies in fish have demonstrated depletion of glycogen stores, NADH and highly phosphated adenylates in fish exposed to ammonia (Arillo et al., 1981a,b; Wang et al., 1994). This would result in decreased anaerobic capacity of white muscle. Increased hepatic and cerebral activity of glutamate dehydrogenases and glutamine synthetase, both enzymes involved in converting NH_4^+ to the less toxic glutamate and glutamine, have been noted in trout exposed to ammonia (Arillo et al., 1981a,b). It is interesting to note that extremely high concentrations of these enzymes are also found in the mudskipper (Iwata, 1988) a fish well adapted to living in high ammonia environments. Vedel et al., (1998) noted increased plasma glutamate oxaloacetate transaminase (GOT) in the plasma of rainbow trout exposed to elevated ambient ammonia and in the same study brain glutamate decreased while glutamine concentrations increased with increased ammonia. Again, the reactions that convert NH_4^+ to glutamate and glutamine require energy and thus ATP and NADH supplies are being depleted. Interestingly, channel cat fish, *Ictalurus punctatus*, exposed to elevated ammonia showed no increase in brain or plasma glutamine (Weirich and Tomasso, 1995) similar to the mudskipper, suggesting a species-specific ability to deal with toxic levels of ammonia. Arillo et al., (1981a) also suggests that ammonia interferes with the citric acid cycle in rainbow trout exposed to ammonia. The combination of depleting available anaerobic energy together with the deactivation of or decreased aerobic metabolism may be a partial explanation for decreased swimming performance following elevation in ambient and plasma ammonia in this study.

The second, more controversial, theory as to why swimming performance is reduced with increased ammonia is the depolarization of muscle membrane potential, by the replacement of K^+ with NH_4^+. The mechanism responsible for white muscle ammonia distribution remains to be fully elucidated. Randall and Wright (1987), Heisler (1990) suggested that ammonia distribution is dependant on the pH gradient between white muscle and plasma. In contrast, Wright et al. (1988), Wright and Wood, (1988), Tang et al. (1992) have all proposed that the distribution of ammonia between plasma and muscle followed membrane potential. Tang et al. (1992), injected ammonia into the bloodstream of trout and observed a net influx of ammonia into the intracellular compartment. Calculation of the predicted pHi and membrane potential suggested that the fish muscle was permeable to NH_4^+.

In the present study, the conclusion is equivocal as to whether ammonia is distributed over the membrane according to pH gradient or membrane potential. No significant difference was detected between measured and predicted values of intracellular pH, and calculation of resting membrane potential was clearly lower than the range of reported values of -85–80 mV (Hagiwara and Takahashi, 1967; Hidaka and Toida, 1969; Yamamoto, 1972). Beaumont et al. (2000b) considered both ammonia distribution theories and found that the best model based on their study was the distribution of ammonia according to the muscle membrane potential.

In summary, the present study shows that swimming performance is reduced in coho salmon exposed to ammonia levels higher than 0.04 mg per l NH_3. It is probable that this reduction is due to a combination of both metabolic challenges in the white muscle as well as depolarization of the muscle itself.

4.2. 96-h acute ammonia toxicity in rainbow trout

4.2.1. Part A: 96-h acute ammonia toxicity in resting and swimming fish

At neutral pH the CMC (criterion maximum concentration = half the value of the mean acute value) value promulgated by the US EPA is 36.1 mg per l N. The acute criteria, 36.1 mg per l N will not protect swimming fish according to this study, which had an LC_{50} value of 32 mg per l N at pH 7.The explanation for the lack of protection for swimming fish based on these standards is that the US EPA requires that all toxicity testing be conducted under static conditions on unfed fish.

This ensures that all studies are consistent and comparable; however, the nature of ammonia is that it is produced naturally under conditions that are avoided by standardized toxicity testing. This is a situation where the methodologies employed to establish standards are unrealistic and fail to acknowledge the nature of the toxicant. It is well known that both plasma ammonia and tissue ammonia increase under exercise conditions and that this increase impairs swimming performance (see above Section 4). We showed that ammonia levels as low as 0.04 mg per l NH_3 reduced U_{crit}. Fish that are exposed to elevated ambient ammonia and are in situations or life history stages when swimming is critical, for example escaping predators or migratory fish, must deal with not only internal ammonia accumulation due the deamination of adenylates (Mommsen and Hochachka, 1988), but also a reduced ability to excrete ammonia. The present research demonstrated that salmonids swimming at 60% U_{crit} (about 2.2 bl per s), a speed often exceeded by migrating sockeye salmon whose swim speeds through river constrictions can range from 5.8 to 11.7 bl per s (Hinch and Bratty, 2000), exposed to the ammonia levels promulgated by the EPA may not only reduce swimming performance, but could be lethal. Based on the present study it is important to re-evaluate present ammonia standards to ensure that they protect critical fish stocks, including the economically important migratory salmon.

4.2.2. Part B: the effect of calcium on acute 96-h ammonia toxicity in free-swimming rainbow trout fry

Ammonia toxicity is based on not only pH and temperature as already discussed, but ionic strength as well. Several studies have indicated that the hardness of ambient water can affect ammonia toxicity (Soderberg and Meade, 1992). The present study on free-swimming rainbow trout fry reveals that increased calcium protects fry exposed to elevated ammonia at pH of both 7.8 and 9.0. Wilson et al. (1998) reviewed the possible mechanisms by which calcium could reduce ammonia toxicity and concluded that a reduction in the elevation of plasma cortisol which is thought to increase ammonia production (Mommsen et al., 1999) is the probable reason that elevated calcium reduces ammonia toxicity.

4.2.3. General summary

Ammonia is an unusual toxicant in that, although it is highly toxic to fish, it is produced naturally as a metabolic waste product. The amount of ammonia produced internally has been shown to be dependent on many factors, including exercise. This study has shown that fish, which are exercising and exposed to ammonia, have decreased swimming performance as well as increased susceptibility to acute ammonia exposure. In fact, the levels set forth by the US EPA will not protect swimming fish and may endanger annual migrations of anadromous fishes. It is also clear from this study that water chemistry is an important factor in ammonia toxicity and that high levels of calcium may ameliorate acute toxicity in fish.

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