Transactions of the American Fisheries Society 123:824–829, 1994

Nitrogen Excretion in Four Species of Fish from an Alkaline Lake

JAMES C. MCGEER

Department of Biological Sciences, University of Dundee
Dundee, Scotland DD1 4HN, UK

PATRICIA A. WRIGHT

Department of Pathology, Ontario Veterinary College
University of Guelph, Guelph, Ontario N1G 2W1, Canada

CHRIS M. WOOD AND MICHAEL P. WILKIE

Department of Biology, McMaster University
1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada

CARL F. MAZUR AND GEORGE K. IWAMA

Department of Animal Science and Canadian Bacterial Diseases Network
University of British Columbia
Room 248, 2357 Main Mall, Vancouver, British Columbia V6T 1K8, Canada

Abstract.—In this study, we examined the ammonia and urea excretion rates in four species of fish native to Pyramid Lake, Nevada (pH 9.4): tui chub *Gila bicolor*, Tahoe sucker *Catostomus tahoensis*, Lahontan cutthroat trout *Oncorhynchus clarki henshawi*, and cui-ui *Chasmistes cujus*. The activities of liver enzymes associated with the ornithine urea cycle and uricolysis were also measured in all but cui-ui. As in other teleost fish, most of the nitrogen was excreted as ammonia. Ammonia excretion rates ranged from 48 to 342 μmol N$\cdot$kg$^{-1}\cdot$h^{-1}. Urea excretion rates varied from 17 to 139 μmol N$\cdot$kg$^{-1}\cdot$h^{-1}. The proportion of nitrogenous waste excreted by fish as urea in the alkaline Pyramid Lake water was greater than by fish in neutral water; however, ornithine urea cycle enzyme activities, with the exception of arginase (range, 5.2–41.0 μmol$\cdot$g$^{-1}\cdot$min^{-1}), were relatively low. All three of the uricolytic enzymes were present in significant activities. Therefore, it seems that urea is produced in these fish by uricolysis, as it is in other teleost fishes. Although the fish native to Pyramid Lake experience conditions that would inhibit branchial ammonia excretion in other fish, they seem to be able to excrete ammonia and have no unique urea production capabilities.

Pyramid Lake, in northwest Nevada, is one of the largest remnants of Pleistocene Lake Lahontan (Minckley et al. 1986). Pyramid Lake is about 50 km long and up to 16 km wide, and it has a maximum depth of 100 m. It is an endorheic alkaline–saline lake with a pH of 9.4, a salinity of 4.3‰, and a total dissolved solids concentration of 5,500 mg$\cdot$L^{-1}. The lake supports a relatively productive biological community (Galat et al. 1981), and 10 species of fish have been reported (Snyder 1916; La Rivers and Trelease 1952; Moyle 1976; Galat et al. 1981; Sigler and Sigler 1987). The four most abundant species are tui chub *Gila bicolor*, Tahoe sucker *Catostomus tahoensis*, Lahontan cutthroat trout *Oncorhynchus clarki henshawi*, and cui-ui *Chasmistes cujus* (Vigg 1978, 1980). Each of these four species is native to the Lahontan basin. The tui chub is the most abundant fish species and exists as two variants (Galat and Vucinich 1983), both of which spend all phases of their life cycle in the lake. The Tahoe sucker, cutthroat trout, and cui-ui are anadromous and migrate up the Truckee River to spawn. Earlier in this century, diversions of the Truckee River lowered lake levels and blocked migration routes, which contributed to a depletion of cui-ui stocks and the extinction of the native Pyramid Lake cutthroat trout (Sumner 1940; Scoppettone et al. 1986). The current population of Lahontan cutthroat trout in Pyramid Lake derives from successfully planted stocks that originated in other alkaline–saline lakes in the Lahontan basin (Coleman and Johnson 1988). Both the cui-ui and Lahontan cutthroat trout populations in Pyramid Lake are now maintained artificially.

Fish native to the Lahontan basin became geographically isolated from other North American fishes in the Pleistocene era (Behnke 1981; Minckley et al. 1986) and have evolved in isolation since then. Cui-ui are found only in Pyramid Lake and are the sole surviving pure species of their genus (Miller and Smith 1981; Sigler et al. 1985). Lahontan cutthroat trout have the greatest morphological divergence of any cutthroat trout subspecies (Behnke and Zarn 1976; Behnke 1981), and Vigg and Koch (1980) suggested that their temperature tolerance differs from that of other subspecies. Although many of the native cutthroat trout populations in western North America have been displaced by nonnative introductions (Griffith 1988), this has not occurred in the alkaline-saline lakes of the Lahontan basin, probably because only the native fish are adapted to the conditions in these lakes (Behnke and Zarn 1976).

We hypothesized that some of those physiological adaptations might include nitrogenous waste excretion. Most fish excrete nitrogen wastes primarily as ammonia through the gills (Smith 1929; Forster and Goldstein 1969; Randall and Wright 1987). The branchial excretion of ammonia is severely inhibited in high-pH water (≥ 9.5) because there is a reduction of the blood-to-water NH_3 gradient and an attenuation of Na^+–NH_4^+ exchange; death from ammonia toxicity may result from such an inhibition (Wright and Wood 1985; Randall and Wright 1989; Lin and Randall 1990; Wilkie and Wood 1991; Yesaki and Iwama 1992). One dramatic adaptation to living in highly alkaline waters can be seen in the Magadi tilapia *Tilapia alcalica* of Lake Magadi, Kenya (pH 10). Those fish produce and excrete urea as the main nitrogenous end product of metabolism and have a complete complement of ornithine urea cycle (OUC) enzymes in their liver (Randall et al. 1989; Wood et al. 1989). In most teleost fish the OUC is not active, and a small amount of urea is produced by uricolysis (Mommsen and Walsh 1991).

The objective of the present study was to quantify the relative excretion of ammonia nitrogen and urea nitrogen in four fish species native to Pyramid Lake: cutthroat trout, Tahoe sucker, tui chub, and cui-ui. In particular, we focused on their enzymatic capacity for urea production by the OUC and uricolysis.

Methods

Fish.—Experiments were conducted in May 1991 with juvenile cutthroat trout, adult tui chub, adult Tahoe suckers, and cui-uis (including adults and yolk-sac fry). The juvenile cutthroat trout and the cui-ui yolk-sac fry were offspring of adults living in Pyramid Lake and were being reared as part of the enhancement efforts of Pyramid Lake Fisheries. The cutthroat trout (mean weight ± SE, 202.3 ± 6.6 g) were netted from a rearing pond fed with lake water at the David Dunn Hatchery, where they had been held for about 6 weeks. Before that, they had been reared indoors in well water. The cui-ui yolk-sac fry (about 0.02 g) were taken from rearing troughs fed with well water inside the David L. Koch Cui-ui Hatchery. The adult cui-uis (1,616.2 ± 51.2 g), tui chub (269.6 ± 23.8), and Tahoe suckers (357.7 ± 22.5) were seined directly from Pyramid Lake. The adult cui-uis, which were collected for use as broodstock in the Pyramid Lake Fisheries cui-ui enhancement program, were held in well water for about 1 week prior to testing. Tests with cutthroat trout, tui chub, and Tahoe suckers were done in Pyramid Lake water, and those with cui-uis were done in well water. Pyramid Lake water had a pH of 9.4 and a temperature of 9.5°C; Koch Hatchery well water had a pH of 8.6 and a temperature of 13°C. The chemical composition of well and lake water is given by Wright et al. (1993).

Nitrogen flux tests.—Excretion rates (flux) of total ammonia nitrogen and urea nitrogen were measured on each of the five groups of fish. Fish were starved for at least 1 week prior to testing. The individuals were transferred to a container and held there for a minimum of 12 h; water flow was then stopped, and nitrogen flux was measured over a 3- or 6-h period. Water samples (15 mL) were taken at 0, 1, 2, 3 and 6 h. In the tests with cui-ui yolk-sac fry, 50 individuals were placed in each container, and water samples were taken immediately (0 h) and at 3 h.

The containers in which flux was measured were chosen to provide a water volume:fish wet weight ratio of about 15:1. Black 4-L perspex boxes were used for the cutthroat trout, tui chub, and Tahoe sucker trials. In the adult cui-ui trials, we used 40-L aquaria; in the fry cui-ui flux tests, we used modified 25-mL plastic scintillation vials. The flux containers were aerated, and the temperature was controlled by placing boxes in tanks with flowing water.

At the end of flux tests, liver samples were taken from cutthroat trout, tui chub, and Tahoe suckers, immediately frozen, and stored in liquid nitrogen. Liver samples were not taken from the adult cui-uis because the species is listed as endangered and fish could not be killed. The small size of the yolk-sac fry cui-ui did not permit tissue collection. Liver samples were transported to the University of Guelph in liquid nitrogen and stored at −80°C.

Assays.—Water samples were assayed for total ammonia (combined NH_3 and NH_4^+) concentra-

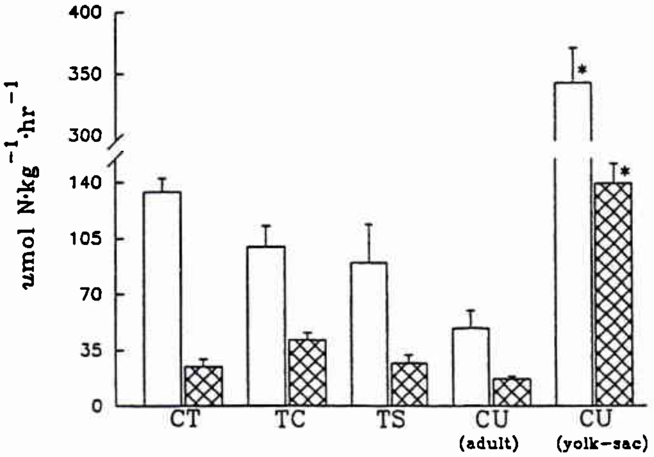

FIGURE 1.—Mean weight-adjusted hourly excretion of total ammonia (open bars) and urea (crosshatched bars) nitrogen in five groups of fish from Pyramid Lake. Abbreviations: CT = cutthroat trout, (N = 8); TC = tui chub (N = 11); TS = Tahoe sucker (N = 11); and CU = cui-ui, adult (N = 6) and yolk-sac fry (N = 10 groups of 50 individuals). The standard error is included on each bar and asterisks indicate means significantly different from others ($P < 0.05$).

tion and urea concentration. Total ammonia concentration in water samples was measured spectrophotometrically at 640 nm with the salicylate–hypochlorite assay described by Verdouw et al. (1978). Water urea concentration was measured spectrophotometrically at 530 nm by the diacetyl monoxime method (535A, Sigma Diagnostics, St. Louis).

The frozen liver samples were used to measure the activity of the enzymes associated with the OUC and uricolysis. The OUC enzyme activities measured were carbamoyl-phosphate synthase III, ornithine carbamoyltransferase, glutamine–ammonia ligase, arginase, and argininosuccinate synthase. The uricolysis enzyme activities measured were urate oxidase, allantoinase, and allantoicase. A complete description of the methodology for the enzyme assays is given in Wilkie et al. (1993).

Calculations and statistics.—The information from the water analysis was combined with container volume, fish weight, and time to calculate nitrogen fluxes (μmol nitrogen excreted per hour per kilogram body weight) for total ammonia (J_{amm}) and urea (J_{urea}). Activity of enzymes was measured in μmol of substrate converted to product per gram of liver tissue per minute (μmol·g^{-1}·min^{-1}) at 22°C under saturation conditions, except for carbamoyl-phosphate synthetase III, which was measured as μmol converted per gram of isolated mitochondria per hour. A one-way analysis of variance (ANOVA) was used to test for significant difference among the means for J_{amm}, J_{urea} and each of the liver enzymes. Where appropriate, means were compared by means of Tukey's test.

Results and Discussion

The physiological effects of exposure to alkaline water on trout have been described by Wright and Wood (1985), Heming and Blumhagen (1988), Lin and Randall (1990), Wilkie and Wood (1991), Yesaki and Iwama (1992), and Wilkie et al. (1993). These studies and others showed that the elimination of nitrogenous wastes, which occurs primarily at the gill as ammonia, is inhibited under alkaline conditions. Wilkie and Wood (1991) and Yesaki and Iwama (1992) showed that in fish challenged with high-pH water, normal rates of J_{amm} can be reestablished after an initial inhibition. In spite of this, an alkalotic condition persists and death, caused primarily by ionoregulatory failure, can occur within a few days. However, some natural alkaline lakes, including Pyramid Lake, have thriving fish populations.

The rates of nitrogen excretion for ammonia and urea differed among the five fish groups tested (Figure 1). Rates were significantly higher for yolk-sac cui-ui than for all other groups, and there were no significant differences among the cutthroat trout, tui chub, and Tahoe sucker for either J_{amm} or J_{urea}. The adult cui-ui had the lowest rates of both J_{amm} and J_{urea}. Ammonia excretion of adult cui-ui was less than values recorded for cutthroat and rainbow trout in neutral water, and J_{urea} was similar. The J_{amm} for trout in neutral water are

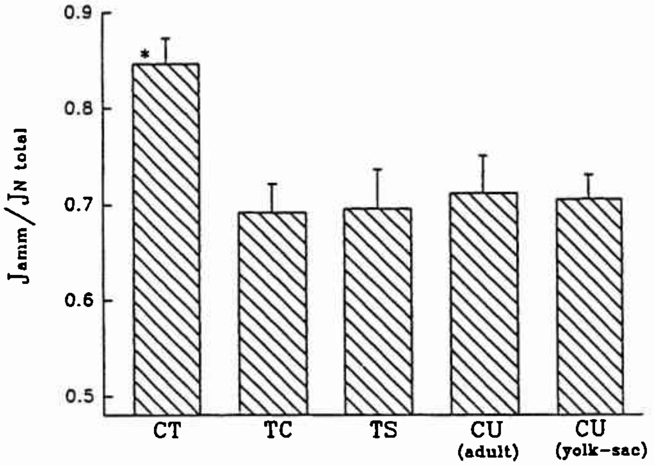

FIGURE 2.—Mean ratio of hourly excretion rate of ammonia nitrogen (J_{amm}) relative to total nitrogen ($J_{N\ total}$) in five groups of fish from Pyramid Lake. Abbreviations: CT = cutthroat trout ($N = 8$); TC = tui chub ($N = 11$); TS = Tahoe sucker ($N = 11$); and CU = cui-ui, adult ($N = 6$) and yolk-sac fry ($N = 10$ groups of 50 individuals). The standard error is included on each bar and an asterisk indicates a mean significantly different from other means ($P < 0.05$).

typically 200 $\mu mol \cdot h^{-1} \cdot kg^{-1}$ or more (Wilkie and Wood 1991; Wilkie et al. 1993; Wright et al. 1993; G.K. Iwama unpublished). Wright et al. (1993) concluded that cutthroat trout in Pyramid Lake have slightly higher plasma pH and ammonia, lower J_{amm}, higher renal ammonia excretion, and slightly higher J_{urea} than trout in neutral water.

Our results, like those of Wright et al. (1993), show that there were relatively low levels of total nitrogen excretion ($J_{N\ total} = J_{amm} + J_{urea}$), primarily due to reduced J_{amm} in all four species tested. The ratio of J_{amm} to $J_{N\ total}$, which is usually about 80% (Goldstein and Forster 1970) or less in freshwater teleosts, was low in three of the species tested (Figure 2). The proportion of J_{amm} was higher for cutthroat trout (85%) than for the other three species studied (about 70%). Wright et al. (1993) showed J_{amm} to be about 66%, and one of us (G.K.I., unpublished data) measured it at 87% of $J_{N\ total}$, for cutthroat trout in Pyramid Lake. A recent study examining ammonia and urea excretion of the tarek *Chalcalburnus tarichi* in the alkaline Lake Van, Turkey (pH 9.8), showed ammonia excretion to be 63% of total nitrogen excretion (Danulat and Kempe 1992). In other fish species J_{urea} tends to remain fairly constant, and the changes in $J_{N\ total}$ brought about by factors such as nutritional status or diurnal influences are due mostly to changes in J_{amm} (Brett and Zala 1975; Guerin-Ancey 1976).

Although there were significant differences among liver enzyme activities of the species (Table 1), the results show that the OUC does not play a significant role in the three species tested.

TABLE 1.—Mean activity (±SE) of liver enzymes from three species of fish in Pyramid Lake. Activity is given in $\mu mol \cdot min^{-1} \cdot g^{-1}$ of liver tissue for all enzymes except carbamoyl-phosphate synthase III, which is given as $\mu mol \cdot h^{-1} \cdot g^{-1}$ of isolated mitochondria. For each enzyme, means followed by the same letter are not significantly different from each other ($P \geq 0.05$).

Hepatic enzyme	Cutthroat trout	Tui chub	Tahoe sucker
Ornithine urea cycle			
Carbamoyl-phosphate synthase III	0.024 z (±0.01)	0.007 z (±0.004)	0.025 z (±0.016)
Ornithine carbamoyltransferase	0.027 z (±0.003)	0.012 y (±0.001)	0.006 y (±0.003)
Glutamine–ammonia ligase	0.348 z (±0.039)	0.445 z (±0.039)	0.370 z (±0.107)
Arginase	40.96 z (±4.19)	5.18 y (±1.24)	4.38 y (±2.40)
Argininosuccinate synthase	0.040 z (±0.006)	0.065 zy (±0.009)	0.082 y (±0.014)
Uricolysis			
Urate oxidase	1.834 z (±0.248)	0.558 y (±0.191)	0.215 y (±0.107)
Allantoinase	1.608 z (±0.232)	2.538 zy (±0.402)	3.018 y (±0.457)
Allantoicase	0.479 z (±0.083)	0.622 z (±0.044)	0.448 z (±0.088)

The activities of OUC liver enzymes were low and similar to other ammoniotelic teleost fish species (Randall et al. 1989). A few unique teleosts which excrete urea at relatively high rates, such as the gulf toadfish *Opsanus beta* (Read 1971; Mommsen and Walsh 1989), Magadi tilapia (Randall et al. 1989), and some of the freshwater air-breathing fishes (Saha and Ratha 1989), have much higher OUC enzyme activities. The ureotelic Magadi tilapia, for example, had carbamoyl-phosphate synthase III and ornithine carbamoyltransferase activities that were more than 15 and 270 times higher than those of the cutthroat trout in this study. The results of the present study agree with the findings of Danulat and Kempe (1992), who measured no OUC enzyme activity in the tarek of Lake Van (pH 9.8).

In most teleost fishes, urea arises from catabolism of purines by uricolysis or from dietary arginine by arginase (Forster and Goldstein 1969). Because fish were starved for a week or more in the present study, direct production of urea from arginine was probably not substantial. It is more likely that urea was derived from uric acid by means of uricolysis. Activities of uricolysis enzymes, urate oxidase, allantoicase, and allantoinase, were within the range of other teleost fish whose primary method of urea production is uricolysis (Brown et al. 1966; Forster and Goldstein 1969; Randall et al. 1989). As urea excretion rates in all species of fish were similar, the explanation for the higher level of urate oxidase in cutthroat trout is unknown.

In this study we demonstrated that the physiological processes for nitrogenous waste excretion in the four fish species native to Pyramid Lake were similar to those of other teleost fishes. Ammonia was the dominant nitrogenous waste product, although excretion rates were slightly lower than in cutthroat trout in neutral water. Urea was produced in slightly higher proportions than usually observed, and its source was from uric acid via the uricolysis pathway. There was no evidence of significant OUC activity in any of the three fish species in which liver enzymes were measured.

Acknowledgments

Financial support was provided by operating grants and an International Collaborative Research Grant from Natural Sciences and Engineering Research Council of Canada to G.K.I. and C.M.W. We thank the Paiute People of Pyramid Lake for the invitation and opportunity to work with the fish of the lake and the use of their facilities. We recognize the help of the personnel of Pyramid Lake Intertribal Enterprises; Pyramid Lake Fisheries; Department of Biology, University of Nevada, Reno; and the U.S. Fish and Wildlife Service, Reno. Special thanks are extended to G. Wedemeyer, U.S. Fish and Wildlife Service; Lee Carlson, Nancy Vucinich, and Dan Mosley, Pyramid Lake Fisheries Lake Operations; Paul Wagner, Pyramid Lake Fisheries; Beverly Houten, Koch Cui-ui Hatchery; and Carol Ort, University of Nevada, Reno.

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Received April 2, 1993
Accepted February 24, 1994